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MECHANISTIC AND SYNTHETIC STUDIES WITH ALKYLCOBALOXIMES

A new method for preparing oxygen-labelled aldehydes and ketones

by

AH KEE WONG

B.Sc. Applied Chemistry, Liverpool Polytechnic, England

M.Sc. Molecular Enzymology, Warwick, England

In partial fulfilment of the requirements for the degree of Doctor of Philosophy at the University of Warwick, Department of Chemistry and Molecular Sciences.

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Declaration and acknowledgements

This thesis is a record of research work carried out in the Dept. of Chemistry and Molecular Sciences at the University of Warwick, during the period from January 1978 to January 1981. The work described is believed to be original in the opinion of the author, except where due references are made.

I wish to thank the following persons and institutions who have provided help in the preparation of this thesis:-

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ABBREVIATIONS

Ac	acetyl
ADH	alcohol dehydrogenase
aq.	aqueous
AR	analytical reagent grade
b.p.	boiling point
Bu	butyl
c	concentration
CNDO	complete neglect of differential overlap
CoA	coenzyme A
conc.	concentrated
COSCoA	coenzyme A thioester
dec	decomposition
DEGS	diethylene glycol succinate polyester
DMF	dimethylformamide
$DMGH_2$	dimethylglyoxime
$HmgH^-$	dimethylglyoxime monoanion
DMSO	dimethyl sulphoxide
DNP	2,4-dinitrophenylhydrazone
Eq	equation
equiv.	equivalents
Et	ethyl
F.I.D.	flame ionisation detector
GLC	gas-liquid chromatography

HLADH	horse-liver alcohol dehydrogenase
HMPA	hexamethylphosphorictriamide
IR	infrared
IUPAC	International Union of Pure and Applied Chemistry
Me	methyl
m.p.	melting point
m.s.	mass spectrum
MW	molecular weight
NADH	nicotinamide-adenine dinucleotide, reduced form
NCS	N-chlorosuccinimide
NMR	nuclear magnetic resonance
O.D.	optical density
Ph	phenyl
Pr	propyl
r.t.	room temperature
temp.	temperature
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin-layer chromatography
TMS	tetramethylsilane
Ts	tosyl, p-$MeC_6H_4SO_2$-
uv	ultraviolet
YADH	yeast alcohol dehydrogenase

Other abbreviations

(40-60)/ (100-120)	boiling point range of petroleum ether

Publications

Parts of the work described in this thesis has been published, and a further part is being prepared for publications as follows:

1. B. T. Golding and A. K. Wong, "Preparation of Labelled Aldehydes and Ketones from Enamides", Angew. Chem. Internat. Edn., 1981, 20, 89.

2. E. H. Curzon, B. T. Golding, and A. K. Wong, "Studies of Reactions of Cobaloximes by ^{17}O NMR Spectroscopy", Gregynog, Wales, May 1981.

3. E. H. Curzon, B. T. Golding, and A. K. Wong, "Direct studies of Reactions of ^{17}O-labelled Cobaloximes by ^{17}O N.M.R. Spectroscopy: Hydrolysis of 2-Acetoxyethyl(pyridine)cobaloxime and Hydration of Formylmethyl(pyridine)cobaloxime ", J. C. S. Chem. Comm., 1982, 63.

4. A. K. Wong and B. T. Golding, "Preparation of Labelled Aldehydes and Ketones", full paper in preparation, January 1982.

Abstracts

1. There is a lack of suitable methods for enriching aldehydes and ketones at the oxygen position. We have shown that imine (-C=N-) derivatives (iminium salts, N-1-haloalkylamides and enamides) are readily hydrolysed with 1 mol. equiv. of [^{17}O]/[^{18}O]water to yield the parent [^{17}O]/[^{18}O]-labelled carbonyl compound. We prepared as examples 31 atom-% [^{17}O]BuCHO, 23 atom-% [^{18}O]cyclopentanone and [^{18}O]PhCHO. A sterically hindered ketone (camphor) or a branching ketone (e.g. pentan-2-one) may also be prepared by our methods. Enamides, as we have found, are generally applicable for labelling many aldehydes and ketones and hence provide a convenient route to many oxygen-enriched alcohols.

2. Our methods were extended to prepare 7 atom-% [^{17}O]$BrCH_2CHO$ and [^{17}OAc]-2-acetoxyethyl(pyridine)cobaloxime. A highly enriched 31 atom-% [^{17}O-formyl]formylmethyl(pyridine)cobaloxime was also prepared.

3. Solvolysis experiments on these highly enriched compounds indicated that: (i), in phosphate buffer (pH 6.8), the hydration of [^{17}O]pentanal at 0 ^{0}C has a half-life of 1 min, whereas the cobalt complex [^{17}O-formyl]formylmethyl(pyridine)cobaloxime has a half-life of 2 h at 50 ^{0}C; (ii), in aqueous dioxan, the hydrolysis of [^{17}OAc]-2-acetoxyethyl(pyridine)cobaloxime at 50 ^{0}C has a half life of 3 h and this hydrolysis proceeds by an unusual $B_{AL}1$ route. That the hydration of formylmethylcobaloxime is slow relative to aliphatic or even aromatic aldehydes or ketones is due to a interaction between the cobalt atom and the neighbouring -C=O bond (σ to π hyperconjugation). The preference for an AL route over an AC route is consistent with the intervention of a π-complex intermediate.

4. Although 25 atom-% [^{18}O]BuCHO lost its ^{18}O-label rapidly in citrate-phosphate buffers (pH 4.2) compared to 23 atom-% [^{18}O]PhCHO, there is evidence to suggest that the former can be rapidly reduced to [^{18}O]$BuCH_2OH$ in the presence of an excess of NADH and HLADH enzyme at this pH. These results implied that had a cobaloxime model such as $HOCHRCH(^{18}OH)(CH_2)_3Co(dmgH)_2Py$ (R = H or Ph), been prepared, then photolysis in aqueous acetic acid to give the ^{18}O-labelled aldehyde, followed by trapping and detection of the extent of ^{18}O-content in the alcohol product, would have measured the amount of 1,2-OH shift.

5. 4,4-Diethoxycarbonylpentyl(pyridine)cobaloxime was photolysed in aqueous acetic acid (pH 3) to induce radical species. However, we d'd not detect the radical rearrangement product diethyl propylsuccinate by GLC analysis with an authentic sample. Thus, preliminary findings show that this cobaloxime is not a suitable model to parallel the 1,2-carbon skeletal shift catalysed by methylmalonyl-CoA mutase.

6. We have shown that the photolysis of octyl(pyridine)cobaloxime in ethanol gives > 98 % oct-1-ene by GLC analysis. Similarly, photolysis of 2-hydroxyhexyl(pyridine)cobaloxime in water gave a quantitative yield of hexan-2-one, whereas the acid-catalysed fragmentation in $CDCl_3$ occurs readily to form hex-1-ene. Alkylcobaloximes are readily prepared from an alkylhalide or tosylate and thus provides a simple route to an alk-1-ene, an efficient process, for which, as a literature survey shows, is generally lacking.

Chapter 1

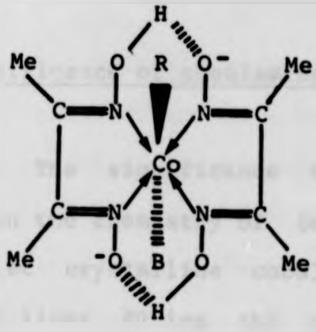
1. CHEMISTRY AND BIOCHEMISTRY OF COBALOXIMES AND COBALAMINS

1.1 Cobaloximes

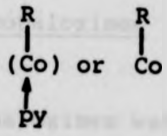
Cobaloximes [1a, 1b] are organocobalt complexes with an octahedral structure (1). The four nitrogen atoms of the ligand are sp^2 hybridised. Consequently, the bidentate ligands can form a square planar or "equatorial" arrangement around the cobalt atom. In the complex, the dimethylglyoxime ligands are monoanionic and are linked intramolecularly by two symmetrical hydrogen bonds. The coordination sphere is completed by two unidentate "axial" ligands. The group R can be an alkyl, aryl, alkenyl or an acid anion (e.g. Cl, OH). In cobaloximes, the Lewis base (B) can be e.g. pyridine, triphenylphosphine, dimethyl sulphide, water, etc. Thus alkyl(pyridine)cobaloximes are bis(dimethylglyoximato)-cobalt (III) complexes and may be formulated as $RCo(dmgH)_2py$, where dmgH is a monoanion of dimethylglyoxime and py is pyridine. The cobaloxime may also be represented as in (1a), where the parenthesis refers to the bis(dimethylglyoximato) ligand. The oxidation state of such cobalt complexes may be specified by writing e.g. $Co^{II}(dmgH)_2py$. Schrauzer stressed the name "Cobaloxime" because this type of cobalt complex resembles the cobalamins.

1.2 Cobalamin [2a-i]

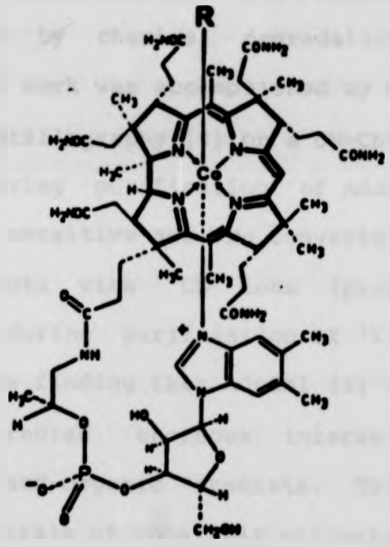
Cobalamin **(2)** belongs to a family of compounds called corrinoids. All corrinoids have the corrin nucleus, a macrocyclic tetradentate ligand. The corrin nucleus forms the core of the cobalamin complex. The four pyrroline rings form the basic unit of a corrin and are labelled A, B, C and D. The two pyrroline rings, A and D are linked directly at C-1 and C-19. The side chains of the corrin nucleus have amide functional groups and are assigned letters a to g. The base consists of e.g. an imidazole joined to a ribose moiety. In cobalamins the imidazole is 5,6-dimethylbenzimidazole. The group R in cobalamin may be cyano (CN), hydroxyl (OH), methyl (CH_3) or adenosyl (Ado) **(3)**. Cobalamins may be written as e.g. AdoCbl (adenosylcobalamin). CN-Cbl (cyanocobalamin) is formally known as Vitamin B_{12}. The alkylcobalamins may be represented as in **(4)** where Bzm is an abbreviation for 5,6-dimethylbenzimidazole. The rectangle represents the corrin ring together with the four equatorial co-ordination positions and the nucleotide side chain is attached at the left hand end. This is convenient as most of the chemistry of cobalamins to be discussed involves only variations at the organocobalt bond.



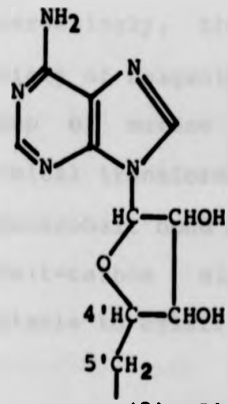
(1)



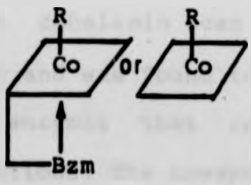
(1a)



(2)



(3) 5'deoxyadenosine (Ado = ACH_2)



(4)

1.3 Significance of cobalamins and cobaloximes

The significance of cobaloximes was only realised when the chemistry of cobalamins was established. The first crystalline cobalamin (CN-Cbl) was isolated from raw liver during the search for the anti-pernicious anaemia factor [3]. The structural features were determined by chemical degradation. The definitive structural work was accomplished by Dorothy Hodgkin using X-ray crystallography [4] on a CN-Cbl artefact, which was formed during purification of AdoCbl. AdoCbl and MeCbl are light sensitive and are converted to OH-Cbl, which then reacts with ^{-}CN ions (present as impurities in charcoal) during purification of 'Vitamin B_{12}' to form CN-Cbl. The finding that AdoCbl [5] contains a Co-C sigma bond attracted enormous interest from biochemists, inorganic and organic chemists. The challenge of the total synthesis of cobalamin attracted the best talent in the world, Eschenmoser in Switzerland, Cornforth in Britain and Woodward in the United States [2g]. Interestingly, the cobalamin can interact with a wide variety of reagents and was found to be required by a group of mutase enzymes that can perform remarkable chemical transformations. The unexpected discovery of the organocobalt bond was surprising because compounds with cobalt-carbon sigma bonds were believed to be too unstable to exist.

The unusual organocobalt bond in AdoCbl and MeCbl was believed to be a consequence of specific electronic effects of the corrin ligand system on the metal atom. It was soon realised that the tendency of the ligands to stabilise the cobalt-carbon bond was independent of the detailed nature of their vertical π-electronic system [1a]. The corrin ring itself provides a clue to the type of ligand system that might stabilise the cobalt-carbon bond. The stabilising factor required is a strong planar ligand field with a moderate amount of unsaturation.

Work was initiated to find cobalt complexes other than corrins capable of forming stable organometallic derivatives which could simulate the principal reactions of the cobalt atom in the complicated corrins. Many model ligands fall into this class, for example, the dimethylglyoxime ligands, despite the great disparity in the nature of the corrin ring.

1.4 Why cobaloxime models?

Many other cobalt chelates have been proposed as models for cobalamins, but simple cobaloximes of type (1) simulate the reactions of cobalamin quantitatively and qualitatively [1a]. The cobaloximes are easily prepared [6] and are much more stable compared to the cobalamins. The required reagents for preparation of cobaloximes are readily available. None of the other cobalt chelates to date have challenged the leading position of the cobaloximes in the field of cobalamin

model chemistry. The cobaloximes are easily characterised by chemical analysis, IR and NMR spectra. Cobalamins are much harder to characterise satisfactorily e.g. their combustion analyses are unreliable because of variable hydration. Their ^{1}H NMR spectra are exceedingly complex, whereas the ^{1}H NMR spectra of alkylcobaloximes are extremely simple: the dmgH groups show a singlet signal at 2.1 ppm (12H, Me x 4). Thus reactions of the sigma alkyl group in cobaloximes are convienient to monitor, but this is not true for the alkylcobalamins.

1.4.1 Physical structures of cobaloximes and cobalamins

Molecular orbital calculations [1a] and crystallographic studies [7] showed that the regions surrounding the cobalt ions in cobaloximes and cobalamins are comparable, e.g. for $MeCo(dmgH)_2py$: Co-C = 199.8 pm, Co-N(py) = 206.8 pm, average Co-N(dmgH) = 189.7 pm. The X-ray structure of isopropyl(pyridine)cobaloxime indicates an unusually long Co-C bond of 208.5 pm [8]. Corresponding values in AdoCbl are : 205, 223, and 194 pm [5]. The cobalt ions of $MeCo(dmgH)_2py$ and AdoCbl are not appreciably displaced from the plane of their ligands.

1.5 CHEMISTRY OF COBALOXIMES AND COBALAMINS

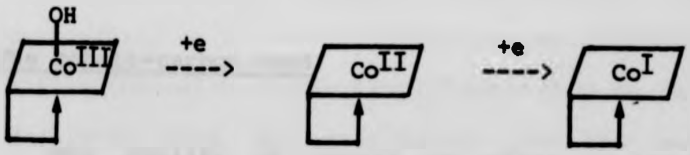
The chemistry of cobaloximes is mainly concerned with the cobalt-carbon bond; that is, how the cobalt-carbon bond is made, broken, modified and how it may be used for preparative purposes or be involved in

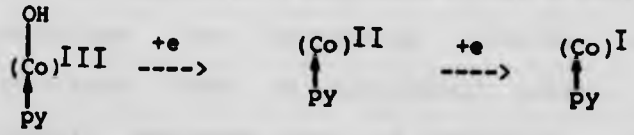
catalytic reactions. The examples given below for cobaloximes have parallel reactions for cobalamins.

1.5.1 Oxidation states of cobalt

An important biological role is attributed to the cobalt ion in cobalamins, because its state can vary from trivalent to divalent and to monovalent (cf **Scheme** 1). The cobaloximes behave analogously. Alkylcobalt complexes have formally a +3 charge and are diamagnetic. Reduction to a paramagnetic cobaloxime(II) complex occurs readily on photolysis of a deaerated aqueous solution of (1). The cobaloxime(I) anion is formed by the reduction under deaerated conditions of cobaloxime(II)/(III) with zinc in acetic acid/aq. NH_4Cl, $NaBH_4$ in ethanol, or by electrochemical reduction. The methods commonly used to generate a cobaloxime(I) complex at Warwick are shown in **Scheme** 2 & 3. The diamagnetic cobaloxime(I) complexes have a strongly nucleophilic lone pair of electrons and is an important intermediate in the synthesis of alkylcobaloximes. Some of the reagents it reacts with are shown in **Scheme** 4. Most of the alkylating agents react with the cobaloxime(I) complex by a S_N2 mechanism. However, with 2-allyloxyethyl halides, evidence for an electron transfer mechanism via 2-allyoxyethyl radicals is known [9].

The order of reactivity of alkyl halides giving alkylcobaloximes is $1^0 > 2^0 > 3^0$. The stability of alkylcobaloximes also follows that order. Cobaloxime(I) reacts with activated alkenes by nucleophilic addition.





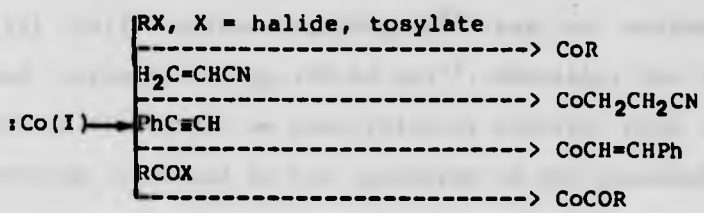
Scheme 1

$$BrCo(dmgH)_2py \xrightarrow[\text{or } KBH_4/DMSO]{NaBH_4/EtOH} :Co^{I}(dmgH)_2py$$

Scheme 2

$$CoCl_2.6H_2O \xrightarrow[\text{py in MeOH}]{2DMGH_2/2NaOH} Co^{II}(dmgH)_2py \xrightarrow{NaBH_4} :Co^{I}(dmgH)_2py$$

Scheme 3



Scheme 4

1.5.2 The cobalt-carbon bond

The ability of cobalt to exist in the three relatively stable oxidation states Co(I), Co(II), and Co(III) in appropriate complexes offers a variety of possibilities for generating reactive organic intermediates from an alkylcobalt moiety. Thus, alkylcobalt complexes can, in principle, undergo homolysis or heterolysis of their Co-C bond. Homolysis leads to a cobalt(II) - radical pair, whereas heterolysis affords either cobalt(III) and a carbanion or cobalt(I) and a carbocation. These considerations may be important for the enzymic reactions requiring AdoCbl. Some substrates can be envisaged as rearranging via intermediate carbocations, associated w'th cob(I)alamin, whereas others may prefer radicals or carbanions, associated with cob(II)alamin or cob(III)alamin, respectively. How cleavage of the Co-C bond of AdoCbl occurs during the enzymatic reactions is unknown. In general, metal-carbon bonds [10] are relatively weak (e.g. the Mn-C bond in $PhCH_2$-$Mn(CO)_5$ has a bond dissociation energy of ca 84 - 105 kJ mol^{-1}) and the Me-Co bond of a model cobalamin complex

[11a] $Co([14]tetraeneN_4)(OH_2)Me^{2+}$ has an estimated bond strength of ca 200 kJ mol^{-1}. Possibly, the Co-C bond in AdoCbl can be homolytically cleaved when the coenzyme is bound to the apoenzyme in the presence of a substrate molecule. The energy required to cleave the Co-C bond is regained from improved binding energy between cob(II)alamin, adenosyl radical, substrate and enzyme [11b]. It is believed that the presence and proper orientation of the propionamide side chains on the corrin ring are responsible for the ease of cleavage of the Co-C bond in the enzymatic system, possibly by some distortion of the corrin. Support for this hypothesis comes from the fact that hydrolysis of a side chain to the corresponding acid results in an inactive analogue of AdoCbl. Homolytic cleavage of the Co-C bond can also be induced non-enzymatically by thermal or photochemical processes as described in the following Section.

1.5.3 Photochemistry of the cobalt-carbon bond

The alkylcobalt bond in cobalamins and cobaloximes is homolytically cleaved by u.v. and visible light. The main factors of interest in the photochemical process are: (i) the character of the light absorption process and the causes of the cobalt-carbon bond fission; (ii) the nature of the organic and inorganic radicals formed on homolysis; and (iii) the fate of these species. Alkylcobaloximes have intense bands at 380 nm and 440 nm assigned to charge-transfer transitions [12]. Photolysis of

methylcobaloxime at a wavelength of 480 nm in deaerated aqueous solution, gave no change in the absorption spectrum, implying a quantum yield of zero for photodecomposition. In deaereated aqueous solution (440 nm), $\phi_{dec} = 5.4 \times 10^{-4}$. Similarly, Pratt [13, 14] showed that methylcobalamin in deaerated aqueous solution is apparently photostable. Under aerated conditions $\phi_{dec} = 0.35 \pm 0.04$ at 490 nm. The transient intermediates generated following irradiation of MeCbl and AdoCbl by the flash photolysis technique have been studied [15]. The results from methylcobaloxime and MeCbl indicated that the primary step in the photolysis is the formation of a caged radical-pair (**Scheme** 5). The reaction is reversible, with equilibrium far to the left. The recombination efficiency was about 0.999, unless scavengers such as oxygen [13], alcohols [16], p-benzoquinone [16] etc., were present.

The products from the photolysis of (1) in $CHCl_3$, acetone, or MeOH in the presence of dioxygen are the corresponding peroxocomplexes $RCOOCo(dmgH)_2py$ [17]. Some of these complexes where R is a benzyl or allyl ligand, can also form peroxide complexes in air at 40 °C by a thermal reaction [18]. The production of alkyl radicals was demonstrated by direct observation of ESR signals [19], by spin trapping [20] or by inference from the secondary reaction products.

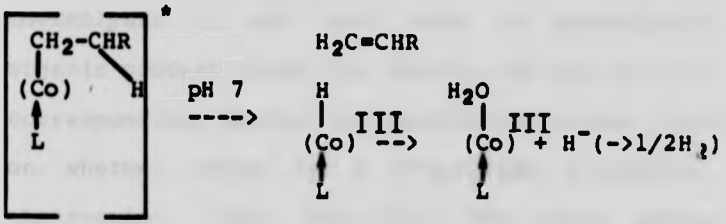
Golding and Kemp <u>et al</u> [21] carried out anaerobic photolyses of alkyl(aquo)cobaloximes under

acidic and neutral conditions. The fate of both the organic and inorganic radical was well characterised. At pH 2, photoreaction was fast and efficient, giving a quantum yield $\phi > 0.1$, which was independent of wavelength (313 - 440 nm). The radical intermediates gave products typical of abstraction and dimerisation. At pH 7, photoreaction is about 10-fold less efficient, $\phi > 0.01$, giving exclusively alk-1-ene from the axial ligand. The H atom in the abstraction product is thought to come from the hydroxyl group in the bis(dimethylglyoximato) ligands. The mechanism of the acid-independent and acid-catalysed routes and the fate of the alkylcobaloxime when photochemically excited are shown in **Scheme** 6. The alk-1-ene may be formed by a concerted β-elimination.

$$[RCo^{III}(dmgH)_2L] \overset{h\nu}{\rightleftharpoons} (R\cdot + [Co^{II}(dmgH)_2L])$$

Scheme 5

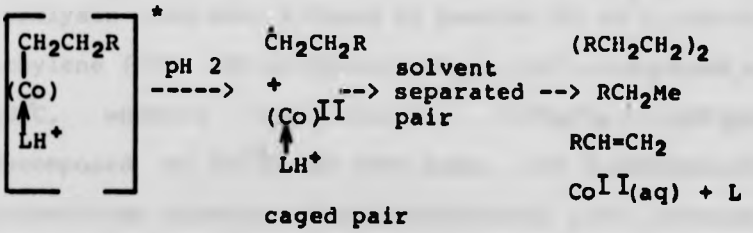
Acid-independent route



excited states

cotd...

Acid-catalysed route



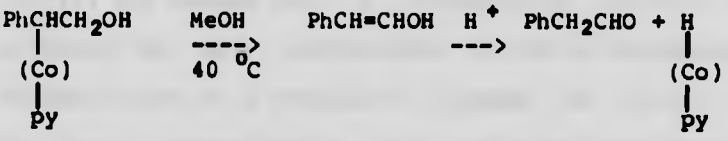
caged pair

Scheme 6

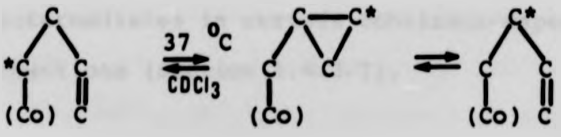
1.5.4 Thermolysis of the cobalt-carbon bond

The nature of the products obtained from the thermolysis of alkylcobalt complexes appeared similar to the products derived from the photochemical process. The temperature required to cause homolysis of the Co-C bond appeared to be variable among the alkylcobalt complexes, but generally homolysis occurred readily with allyl, benzyl or related compounds which could give stable radicals. An accurate measurement of the activation energy for the thermolysis of the Co-C bond is unavailable. The organic products from the thermolysis are usually the corresponding olefin or substituted alkane depending on whether there is a β-hydrogen available for abstraction. The alk-1-ene can arise either by concerted β-elimination or via a radical/cobalt(II) pair [2i]. The kinetic preference for β-elimination under neutral deaerated conditions was demonstrated by the alk-1-ene being the exclusive product obtained from the photolysis of alkylcobaloxime [2l]. When a β-hydrogen atom is unavailable, for example with

methylcobalamin, thermolysis gave methane and ethane (via radical dimerisation) [22]. Ethylcobalamin homolysis has been induced by heating at 60 °C, giving ethylene [23]. Ph(Me)CHCo(dmgH)$_2$py [24] decomposed at 90 °C, whereas its isomer PhCH$_2$CH$_2$Co(dmgH)$_2$py decomposed at 175 °C. In each case, the decomposition product was styrene and a hydridocobalt (III) complex. The thermolysis of Ph(CH$_2$OH)CHCo(dmgH)$_2$py [25] in MeOH at 40 °C was shown to proceed by a concerted β-elimination to give a hydridocobalt (III) complex and an enol product, which then rearranged to the aldehyde (**Scheme 7**). Golding *et al* [26] using a carbon-13 atom or a Me group as marker showed that H$_2$C=CHCH$_2$CH$_2$Co(dmgH)$_2$py rapidly isomerises via a cyclopropylcarbinylcobaloxime at room temperature as in **Scheme 8**.



Scheme 7



12 %

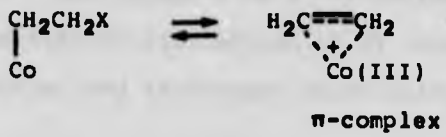
88 %

Scheme 8 [* ^{13}C or MeCH]

1.5.5 Sigma-pi rearrangement of the cobalt-carbon bond

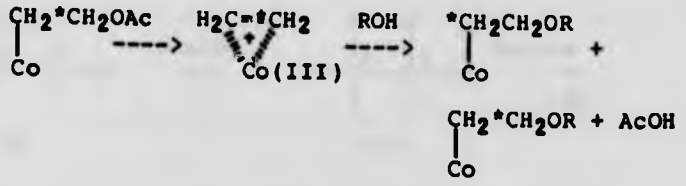
Sigma-pi interconversions are observed among organometallic compounds with a nucleophilic leaving

group β to the cobalt-alkyl bond. In this reaction, a π-complex (cf Scheme 9) is formed when the β-leaving group (X) departs as an anion. That an organocobalt complex can undergo a sigma-pi rearrangement was first shown by Golding et al [27] with a specifically deuterated cobaloxime: $AcOCD_2CH_2Co(dmgH)_2py$. This result was soon confirmed by Dolphin et al [28], using a carbon-13 labelled cobalt complex (cf Scheme 10). The deuterium and carbon-13 labels were found to be equally distributed in the ethyl group of the 2-alkoxyethylcobaloxime after the alcoholysis of the specifically labelled 2-acetoxyethylcobaloxime. The results from the labelling experiments indicated that alcoholysis of the 2-acetoxyethylcobaloxime proceeds by way of a symmetrical intermediate, possibly a π-complex of Co(III) (cf Scheme 10). A π-complex of Co(III) was proposed as an intermediate in the acid-catalysed decomposition of a cobalamin (Scheme 11) [29], and in the isomerisation of $Me(CH_2OH)CHCo(dmgH)_2py$ (Scheme 12). They have also been proposed as intermediates in certain cobalamin-dependent enzymatic reactions (Section 1.6.2.1).

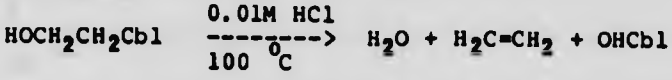


π-complex

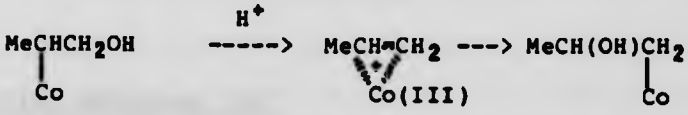
Scheme 9



Scheme 10 * = carbon-13



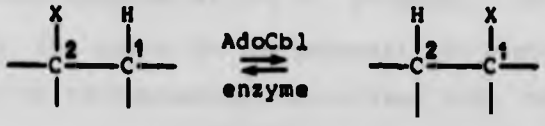
Scheme 11



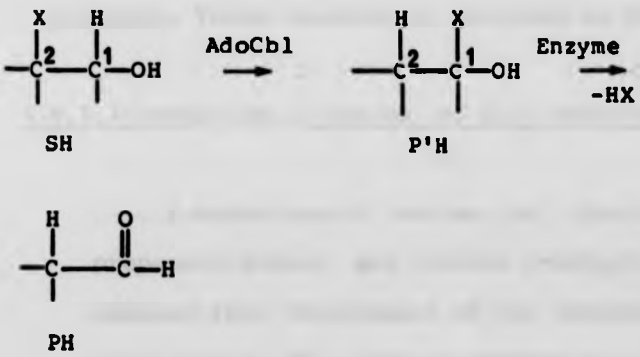
Scheme 12

1.6 ADENOSYLCOBALAMIN-DEPENDENT ENZYMIC REACTIONS [2g]

Adenosylcobalamin is a cofactor required by a group of mutase enzymes. These enzymes can catalyse the interchange of a group X on one carbon atom with the hydrogen atom of the other carbon atom. The migration of this group can be either reversible or irreversible. In the reversible reaction (Scheme 13), the group X bears a carbon fragment, for example, -COSCoA, $-CH(NH_2)COOH$, $-C(C=CH_2)COOH$. In the irreversible reaction (Scheme 14), the group X is a heteroatom group, OH or NH_2. The last step is elimination of HX, which may be water or ammonia, and is thought to be catalysed by the enzyme.



Scheme 13



Scheme 14

1.6.1 Diol Dehydrase [2g]

This enzyme converts the substrate 1,2-diol via a 1,1-diol to an aldehyde. The substrates are, for example, ethane-1,2-diol, propane-1,2-diol and butane-2,3-diol. The stereochemistry of the diol dehydrase catalysed dehydration of the enantiomers of $MeCHOHCH_2OH$ to propanal has been determined from isotopic labelling studies with propane-1,2-diol and AdoCbl. The results showed that the ^2H or ^3H in (R)-propane-1,2-diol rearranged by migration of the pro-(R)-hydrogen, while the pro-(S)-hydrogen migrated during the rearrangement of the (S)-isomer. The deuterium or tritium at C-1 of the substrate appeared in the 5'-CH_2 of AdoCbl and at C-2 of propanal. The deuterium or tritium was delivered opposite to the departing hydroxyl group. The tritium in the 5'-CH_2 of AdoCbl appeared at C-2 of propanal. Solvent protons did not appear in the propanal. An oxygen-18 label at C-2 of (R)-propane-1,2-diol was lost to the water, whereas that of the (S)-isomer appeared in the

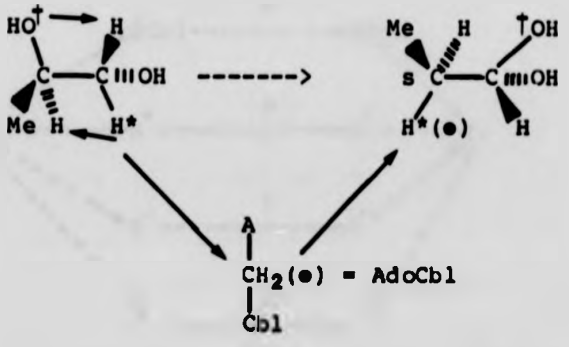
propanal. These results are outlined in **Scheme 15.**

1.6.1.1 Mechanism of action of diol dehydrase

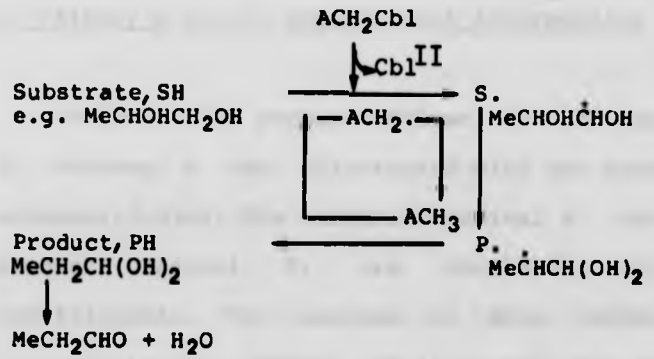
A mechanism of action of diol-dehydrase on propane-1,2-diol and AdoCbl (=ACH_2Cbl) can now be deduced from the results of the isotopic labelling experiments. The hydrogen removed from C-1 appears at the 5'-CH_2 of adenosylcobalamin. This implies that the mediator (ACH_2Cbl) must be homolytically cleaved to give cob(II)alamin and the active adenosyl radical ($ACH_2\cdot$), which can then accept the C-1 hydrogen. The adenosyl radical ($ACH_2\cdot$) becomes 5'-deoxyadenosine (ACH_3), whereas the substrate having lost the C-1 hydrogen becomes a substrate radical, $MeCHOH\dot{C}HOH$ (S.). Next, the fact that the oxygen-18 label at C-2 appears at C-1 implies that the hydroxyl group has migrated to give the intermediate specie, $Me\dot{C}HCH(OH)_2$. The reappearance of the hydrogen at C-2, implies that ACH_3 gives up the hydrogen it acquired from C-1. The ACH_3 then becomes an adenosyl radical again and can combine with the cob(II)alamin or attack another substrate molecule. Finally, propane-1,1-diol undergoes stereospecific, enzymatic dehydration (loss of the pro-R hydroxyl group) to give propanal.

The mechanistic pathway for a diol dehydrase [30] catalysed reaction is simplified in **Scheme 16.** The pathway in **Scheme 16** was proposed by Abeles in 1972 with possible application of this

Scheme to many other AdoCbl dependent enzymes. If the pathway of Scheme 16 is correct, then the proposed intermediates may be isolable. Abeles and Babior et al have isolated the 5'-deoxyadenosine intermediate from ethanolamine ammonia lyase and diol-dehydrase catalysed reactions [31]. The paramagnetic species ACH_2., cob(II)alamin and organic radical, have been detected by ESR techniques during diol-dehydrase catalysed reaction, after freezing the reaction mixture [32]. What is not clear from Scheme 16 is how does the proposed substrate radical, for example, $MeCHOH\dot{C}HOH$, enable the C-2 hydroxyl group to migrate and eventually to be converted to the product radical $Me\dot{C}HCH(OH)_2$? To summarise, the migration of the group X is a mystery and the pathway by which S. rearranges to P. is a subject of controversy among academics and is discussed in the next Section.



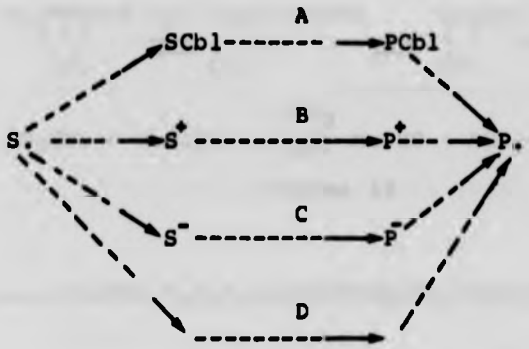
Scheme 15 [$^2H/^3H$ (* or ●), ^{18}O (†)]



Scheme 16

1.6.2 Hypothetical pathways for S. -----> P. [2i]

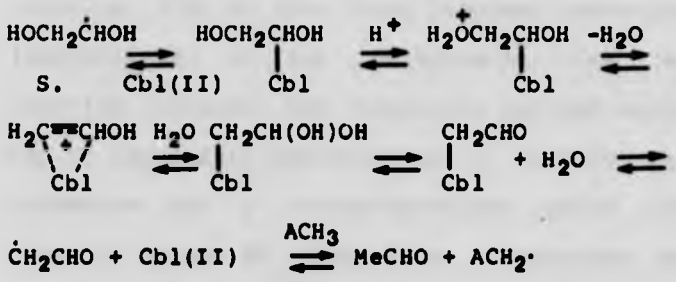
There are at least four hypothetical pathways in which the substrate S. is converted into P.. These pathways are outlined in Scheme 17 and show that S. goes to P. either by a mechanism which involves the formation of an organocobalt intermediate (pathway A), or a charge-transfer mechanism (pathway B or C), or an unaided radical rearrangement (pathway D).



Scheme 17

1.6.2.1 Pathway A via an organocobalt intermediate

Dolphin [28] proposed **Scheme 18** for pathway A. Pathway A was illustrated with the substrate ethane-1,2-diol. The substrate radical S. and the product radical P. are combined with the cob(II)alamin. The process is also known as transalkylation. **Scheme 18** shows that the group X (OH) at the β-carbon of the Co-C bond can now migrate if protonated, because the protonated nucleophilic leaving group can generate a π-complex. In **Scheme 18**, it is also required that the water must not be lost to the medium, but must be recaptured by the π-complex at the new carbon position to account for the oxygen-18 labelling experiment of Arigoni et al [33].

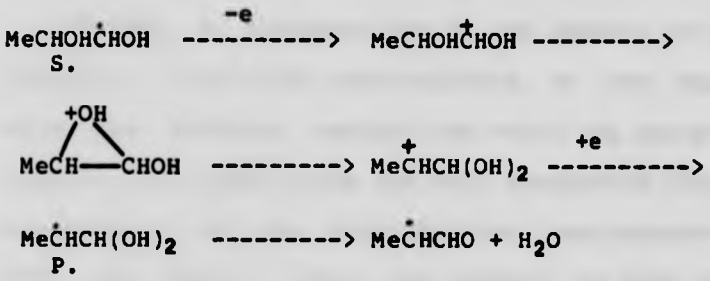


Scheme 18

1.6.2.2 Pathway B via a carbocation intermediate [34]

Pathway B requires electron transfer steps, which interconvert radicals S. and P. with carbocations S^+ and P^+ respectively. The pathway

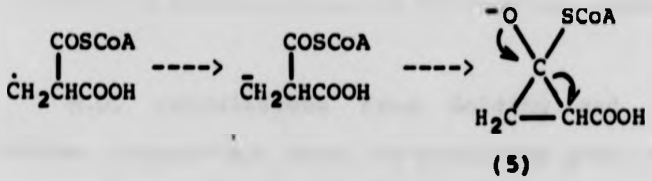
for the substrate, propane-1,2-diol, is outlined in **Scheme 19.** The hydroxyl group can migrate by a 1,2-carbonium ion rearrangement via a protonated hydroxyepoxide. Finally, the electron is recaptured and appears in the product radical P..



Scheme 19

1.6.2.3 Pathway C via a carbanion intermediate

The carbanion rearrangement via pathway C is not plausible for diol dehydrase substrates. Ingraham [35] in 1964 first proposed carbanions as intermediates in the methylmalonyl-CoA mutase reaction because the substrate derived carbanion can be reasonably converted to a product related carbanion via a cyclopropanolate anion **(5)** as shown in **Scheme 20.** Since then, cobaloxime models have been developed to support this proposal.



(5)

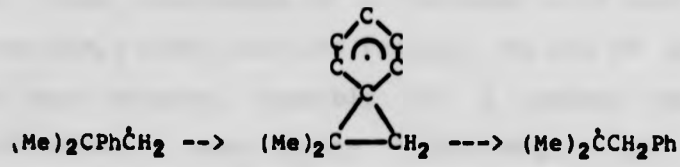
Scheme 20

1.6.2.4 Pathway D via a radical intermediate

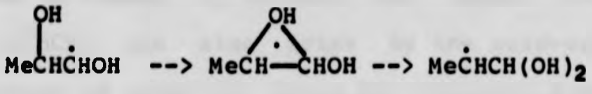
Pathway D suggests that S. can convert to P. unaided. A 1,2-radical rearrangement of the type where the adjacent carbon atom bears the phenyl, chlorine or sulphur group are well documented [36]. For example, in the Urry-Kharasch rearrangement [37], the phenyl group can migrate to give the more stable radical (**Scheme** 21). This finding led Cockle and co-workers [38] to propose that the conversion of propane-1,2-diol to propionaldehyde catalysed by glycerol dehydrase proceeded by a similar 1,2 shift of the OH group (**Scheme** 22) via a radical intermediate. However, M.O. calculations by Golding and Radom [39], showed that the pathway of **Scheme** 23, where the migrating group bears a saturated O, N or C atom, was unfavourable because the cyclic radical intermediates were highly energetic. The β-arylalkyl radicals can rearrange, because, along the reaction coordinate, a bridged intermediate can arise, in which the loss in resonance energy of the aromatic ring is offset by the gain in stabilisation of the radical center.

M.O. calculations from Golding and Radom further showed that when the migrating group X, for example hydroxyl, was protonated, hydroxyl group

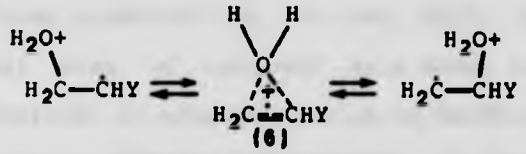
migration by way of an epoxide cation radical (6) (intermediate or transition state) had an energetically favourable pathway because the open forms (α and β) and the bridged form (6) were energetically comparable (cf Scheme 23). The mechanism proposed is an attractive one for diol dehydrase and some other AdoCbl dependent enzymic reactions, where X = COSCoA, NH_2, for example. This was the first serious attempt to calculate how group X might migrate. Pathways A and D indicate that the hydroxyl group must first of all be protonated before it can leave the carbon atom. The protonation probably occurs via the enzyme, although experimental evidence for this is as yet unavailable. Althought the 3-dimensional structure of an AdoCbl-dependent enzyme has not yet been elucidated, preliminary investigations indicate that most if not all, of their active sites contain a thiol functional group [40].



Scheme 21



Scheme 22



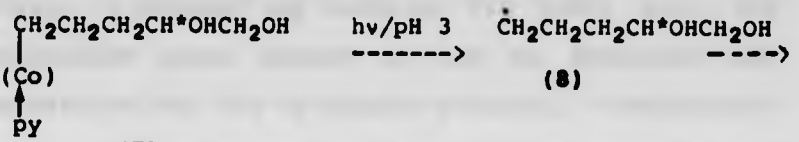
open-α-form bridge form open-β-form

Scheme 23 [Y = OH or Me]

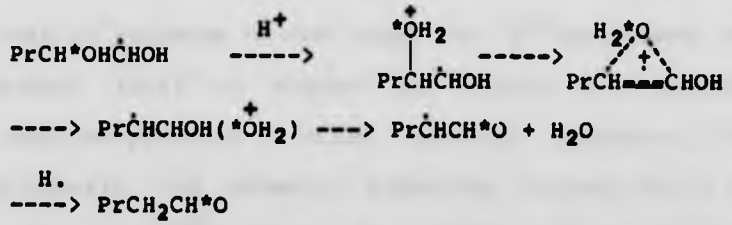
1.6.3 A cobaloxime model for group X transfer

Biochemical evidence gave a clear picture on how hydrogen atoms are transferred, whereas the nature of group X migration is still speculative. Lacking enzymological data, workers had to rely on chemical models to explain how this group X migrated. For example, Golding et al sought experimental evidence to support their M.O. calculation in which the substrate radical S. on protonation converts to the product radical P.. They synthesised the cobaloxime (7), [41]. This cobaloxime, when photolysed in aqueous acetic acid released aquated Co(II), dimethylglyoxime and a 4,5-dihydroxylpentyl radical (8), which rearranges by a favoured 1,5-H shift to $PrCHOH\dot{C}HOH$, a substrate-like radical. The C-2 OH group can then migrate, possibly via a radical cation intermediate, thus giving $Pr\dot{C}HCH(OH)_2$. The end product will be pentanal. The proposed pathway is outlined in **Scheme 24**. However, one cannot rule out that $Pr\dot{C}HCHO$ can also arise by the acid-catalysed elimination of water as shown for ethane-1,2-diol in **Scheme 25**. The substrate radical S. in **Scheme 25** was produced by the action of Fenton's reagent on ethane-1,2-diol and is thought to proceed by the

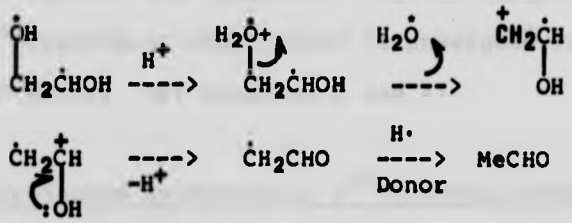
pathway suggested by Walling [42]. This reaction might also be regarded as a model reaction for the conversion of ethane-1,2-diol to MeCHO. Which of the pathways (Scheme 24 or 25) is correct can, in principle, be established by an oxygen-labelling experiment. In the former, an oxygen label at C-2 of 1,2-dihydroxypentyl will be transferred to C-1 and therefore half retained in the product pentanal. In the latter, the label will be lost to the water rapidly.



(7) (8)



Scheme 24 * = oxygen-18



Scheme 25

1.7 ALKYLCOBALOXIMES - SYNTHETIC AND MECHANISTIC ASPECTS

This section summarises the contents of the remaining Chapters of this thesis.

1.7.1 Isotopically enriched pentanal and cobaloxime

We required $HOCH_2CHOH(CH_2)_3Co(dmgH)_2py$ isotopically enriched with oxygen-17 at C-4. Equally important was to prepare a [^{17}O]pentanal. For example, if the pathway in Scheme 24 is correct, then the product will be a labelled pentanal. Compounds bearing the carbonyl functional group are readily hydrated reversibly by water. Thus, the isotopically enriched oxygen in pentanal may exchange its label with the unlabelled water before it can be detected. The objectives are: (i) to prepare pentanal, isotopically enriched at the oxygen position; (ii) to determine the rate of exchange of the label in [^{17}O]pentanal with water; (iii) to extend the method for preparing labelled pentanal to other carbonyl compounds, i.e. aliphatic and aromatic aldehydes, ketones etc.; (iv) to prepare [^{17}O]alcohols by reduction of the labelled aldehydes and ketones; (v) to trap the label in [^{17}O]pentanal even though it undergoes rapid hydration in water, (cf Chapters 2 and 4).

1.7.2 Projected synthesis of [^{17}O-formyl]formylmethylcobaloxime

The pathway of Scheme 18 showed that (S.) combined with cob(II)alamin to give an organocobalt complex, which then rearranged via a π-complex to formylmethylcobalamin. The rate of hydration of such a carbonyl functional group can be determined from a ^{17}O-labelled cobaloxime such as the title cobaloxime. The rate of hydration of this cobaloxime is measured

by observing the decaying oxygen-17 signal. Kinetic measurements for hydration at three different temperatures would allow the activation parameters to be determined (cf Chapter 3).

1.7.3 Projected synthesis of a [^{17}OAc]-2-acetoxyethylcobaloxime

2-Acetoxyethyl(pyridine)cobaloxime is readily hydrolysed to 2-hydroxyethyl(pyridine)cobaloxime in aqueous dioxan. To prove that this process goes via an alkyl-oxygen fission and involves a π-complex intermediate (see Section 1.5.5) it was proposed to synthesize the cobaloxime acetate specifically labelled with ^{17}O at the alkyl oxygen of the ester grouping. Now, hydrolysis via acyl-oxygen fission would generate a labelled alcohol and an unlabelled acid. An alkyl-oxygen fission would result in the ^{17}O label appearing in the acetic acid, implying an intermediate π-complex as a plausible pathway in the cobaloxime ester hydrolysis (cf Chapter 3).

1.7.4 4,4-Diethoxycarbonylpentyl(pyridine)cobaloxime

From the model studies of cobaloxime (7), it was demonstrated that the substrate radical S. can convert to product radical P., when photolysed in acidic buffer. Similarly, S. of $\dot{C}H_2CH(COSCoA)COOH$ could rearrange to $CH_2(COSCoA)\dot{C}HCOOH$ aided by protonation. Thus, the title cobaloxime was initially prepared. This cobaloxime will be photolysed in aqueous acetic acid and the products from the

photolysis will be be analysed. If the rearrangement product were $PrCH(CH_2COOEt)COOEt$, this cobaloxime reaction would provide an alternative model for the methylmalonyl-CoA mutase catalysed reaction (cf Chapter 5).

1.7.5 Projected synthesis of alk-1-enes

Because there is a lack of good synthetic methods for preparing alk-1-enes from alkyl halides, tosylates etc., a study has been made of the neutral thermolysis and photolysis of alkylcobaloximes with the intention of developing a preparatively useful reaction of alk-1-enes. The alkylcobaloximes are, of course, readily available from alkyl halides and tosylates (cf Chapter 6).

Chapter 2

2. INTRODUCTION

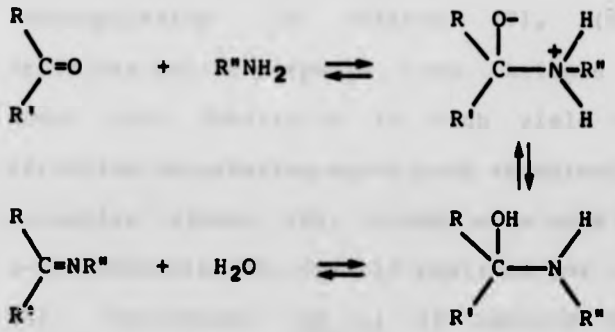
This chapter describes a new method for the preparation of [^{17}O] and [^{18}O]-labelled aldehydes and ketones. The method developed exploits some published chemistry of azomethines (imines) and enamides. The chemistry of these compounds is first reviewed. There follows a brief description of oxygen isotopes and existing methods for preparing oxygen-labelled aldehydes and ketones. Finally the results obtained are described and discussed.

2.1 AZOMETHINES (-C=N-) [1 a-c]

Aldehydes and ketones condense with a variety of amino compounds, for example, ammonia, amines, hydroxylamines and hydrazines, with elimination of the elements of water to give the corresponding azomethines (imines, oximes, hydrazones), (Scheme 26). The imines (RR'C=NR"), are often referred to as Schiff's bases, anils or azomethines. Aldimines refer to compounds where R' is a hydrogen, and R is alkyl or aryl, while ketimines refer to compounds where both R and R' are alkyl or aryl. In naming specific imines, the nomenclature of Chemical Abstracts is followed. Thus, PrC(Me)=NEt is named as N-1-methylbutylideneethylamine and PhCH=NPh as N-benzylideneaniline. In cases where R" is H, the compound is given an imine name. For example, MeCH=NH is named ethylideneimine. Where R" is an alkyl/aryl group, the amine nomenclature is used.

The demonstration that amine/carbonyl condensation is an essential step in a number of enzyme mediated processes has stimulated interest in the detailed mechanism of the amine/carbonyl condensation [2, 3]. For example, the cofactor pyridoxal-5-phosphate is required in enzyme-mediated transformations of α-amino acids. These processes involve reactions of Schiff base intermediates [4]. Azomethine hydrolysis features a tetrahedral carbinolamine intermediate which may be isolated in some cases, e.g. in the reaction of chloral with hydroxylamine.

In general, azomethine formation is acid-catalysed and the rate of condensation broadly parallels the nucleophilicity of the amino component. It is a pH-dependent process being optimal at pH 4. In neutral media, dehydration of the carbinolamine is rate-limiting, whereas in acidic media carbinolamine formation is the rate-determing step. The equilibrium for ammonia- and amine-carbonyl condensations lies largely to the left, necessitating azeotropic distillation to permit significant imine formation. In hydroxylamine and hydrazine condensations, the equilibrium is well to the right. Thus, oximesand hydrazones are readily obtained without prior removal of water.



R, R' = H, alkyl, aryl

R" = alkyl, aryl, OH, OR, NHR, Hal

Scheme 26

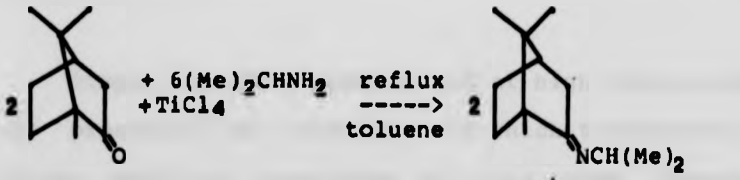
2.2 PREPARATION OF IMINES [1 a-c]

There are several methods for preparing imines: (i) condensation of an aldehyde or ketone with a primary amine, (ii) from other compounds with multiple bonds (e.g. reduction of nitriles), and (iii) by a [1,2] C ⟶ N migration of an alkyl azide.

2.2.1 Condensation methods

Campbell *et al* [5] and Tiollias [6] have prepared several aldimines by reacting primary aldehydes and amines at $0^{o}C$, with KOH added for dehydration. Aromatic aldehydes give such stable products that prior removal of water is not necessary. Ketones, especially hindered or aryl ketones required a longer reaction time and the application of condensation catalysts: protonic acids, $ZnCl_2$, BF_3 etherate etc. N-Isopropylcamphorimine was prepared in 67 % yield by refluxing camphor with

isopropylamine in toluene [7], (Scheme 27). Ketimines may be prepared from ketones and amines under mild conditions in high yield by using an effective dehydrating agent such as molecular sieves. Molecular sieves (4A, Linde) were used to prepare a n-butylketimine at the 17-position of androsterone [8]. Westheimer et al [9] reported that Linde 5A sieves were not only a dehydrating agent but also a catalyst. d-Camphor-anil (9), however, required 2 weeks at room temperature to prepare from camphor and aniline in the presence of 5A sieves. Subsequently, intensive studies by Bekkum et al [10] on several types of sieves, that is 3A, 4A, 5A, in pellet or powder forms were conducted. They concluded that the catalytic activity was not due to the sieve, but to the .mount of binding agents, added accordingly by the manufacturers. By adding such a binding agent (silica-alumina) as catalyst, d-camphor-anil was obtained in 95 % yield in 70 h at r.t.. Cyclic imines were obtained by an intramolecular amino-carbonyl condensation [11].



Scheme 27

(9)

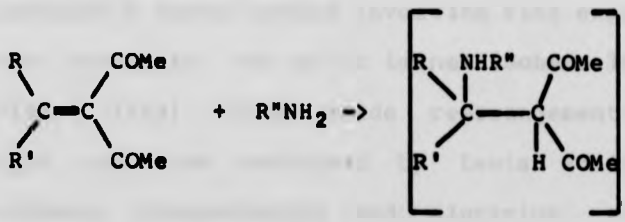
2.2.2 Imines from other compounds with multiple bonds

Double-bonds activated by electron-withdrawing groups are cleaved by reaction with primary amines to give imines (Scheme 28). Grignard reagents react with imidoyl chlorides (10) with replacement of the Cl atom to give C-substituted imines (11). Anilides (12) [tautomeric with the unstable imidic acids (13)] react similarly (Scheme 29). Replacement of the Cl in (10) by hydrogen to give isolable imines is a key step in the preparation of aldehydes by the method of Stephen [12] and Sonn-Müller [13]. Reduction of the quaternary salts of hydrazones with Grignard reagents and of oximes with $TiCl_3$ is also reported to give the corresponding imines [14]. Aldehydes and ketones react with thionylamines [1b], sulphurdi-imides [1b], isocyanates [15] and phosphinamines [1b] (Scheme 30), where X is S=O, S=NR, C=O, and PPh_3 respectively, to give the corresponding imines.

Simple acetylenes add amines at high temperatures and pressures to give enamines which tautomerise to imines. Addition reactions of this type, occurred readily with triple bonds, activated by an electron-withdrawing substituent.

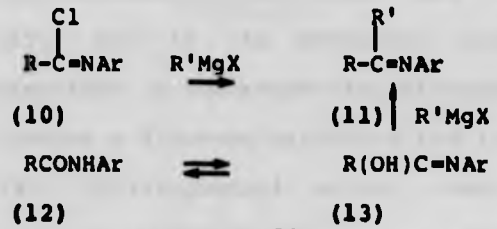
Addition reactions of nitriles [16] provide a wide variety of imine derivatives, imidoyl chlorides,

imidates, amidines and amidoximes (Scheme 31), where X is Cl, OR, NR_2 or NHOH, respectively, as well as simple imines, where X is H, alkyl or aryl. The reduction of nitriles using complex metal hydrides can be controlled sufficiently well to give good yields of imines. Reduction at low temperatures and the use of modified reagents such as sodium triethoxyaluminium hydride were particularly effective. The addition of both alkyl and aryl Grignard reagents followed by careful decomposition of the imine complex at low temperature gave ca 70 % of the corresponding ketimine [16]. Alkyl and aryl-lithium reagents also reacted readily with nitriles to give ketimines. Fry and Ott [17] reported that triethylsilane efficiently reduced N-alkylnitrilium ions to N-alkylaldimines. Mild hydrolysis of the aldimines gave aldehydes in high yield.

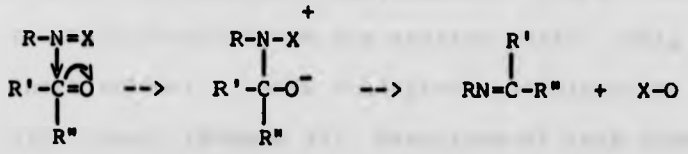


---> RR'C=NR" + $CH_2(COMe)_2$

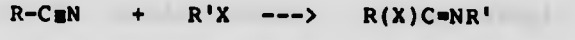
Scheme 28



Scheme 29



Scheme 30

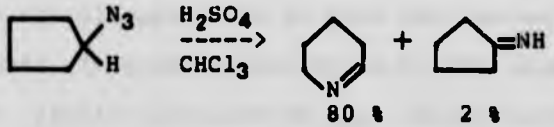


Scheme 31

2.2.3 By a route involving a [1,2] C --> N migration [18, 19]

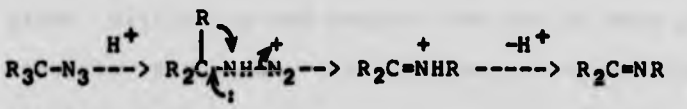
A [1,2]-migration of a substituent such as hydrogen, alkyl or aryl from a carbon position to a nitrogen atom is known to afford aldimines or ketimines. These processes are either catalysed by acid and base, or, can be induced thermally, or photochemically, without a catalyst. For example, the acid-catalysed rearrangement of cycloalkyl azides provides a useful method involving ring expansion for the synthesis of cyclic imines [**Scheme 32**, (14) --> (15) + (16)]. Alkyl azide rearrangements of this type are also catalysed by Lewis acids such as antimony pentachloride and aluminium trichloride. Kinetic and mechanistic studies indicate that rearrangement takes place not in the free azide (17), but in its conjugate acid (18) and that migration is concerted with nitrogen loss and does not involve a discrete nitrenium ion intermediate (**Scheme 33**). Triarylmethyl azides, tend to be inert to acid-catalysed rearrangement, whereas alkyl azides give products (amine and carbonyl compound) derived from the fission of imine. Closely related C --> N

shifts (Stieglitz rearrangement [20]) occur when t-alkylhydroxylamines are treated with PCl_5 in an inert solvent or when t-alkyl-N-haloamines are treated with base (Scheme 34). Reactions of this type can be of preparative use for ketimines. In contrast to their stability in acidic media, triarylmethyl azides undergo rearrangement on thermolysis at 200-300 °C to give acceptable yields of benzophenone anils. Tertiary alkyl azides undergo similar thermal rearrangements. The application of triazoline thermolysis to the synthesis of cyclic imines is exemplified by the pyrroline synthesis in (Scheme 35). Photolysis of t-alkyl azides also results in rearrangement of N-substituted ketimines and these rearrangements are cleaner in a preparative sense than the corresponding acid-catalysed rearrangements.



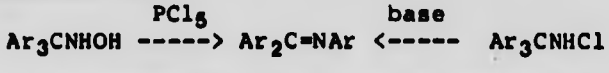
(14) (15) (16)

Scheme 32

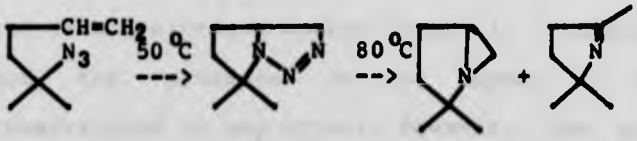


(17) (18)

Scheme 33



Scheme 34



Scheme 35

2.3 REACTIONSOF IMINES [1 a-c, 21]

2.3.1 Thermal Stability

The imines derived from formaldehyde and aliphatic aldehydes tend to polymerise readily, the latter by way of an aldol-type condensation. Imines from aromatic aldehydes and ketones are much more stable.

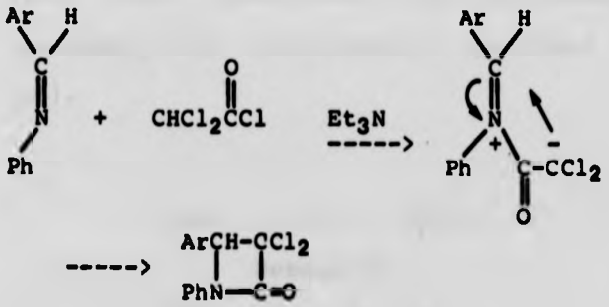
2.3.2 Reactions with electrophiles

Imines form stable iminium salts with protic acids, for example perchloric acid [22]. Direct alkylation of N-alkyl-aldimines and ketimines occurs at the nitrogen atom to give the corresponding iminium salts. In general, processes of this type are subject to steric hindrance so that imines having bulky alkyl groups or N-aryl substituents N-alkylate only with great difficulty and require the use of more powerful reagents such as trialkyloxonium salts ($R_3O^+X^-$). Alkylation of cyclohexanone imine with CH_2=C(CN)Cl and triethylamine provides a facile route to 2-substituted indoles [23].

Halogens are reported to add in a 1,2-fashion to the carbon-nitrogen double bond in N-arylaldimines, but the products do not appear to have been investigated to any extent. However, the product of the reaction of benzylideneaniline with bromine in

CCl_4 is formulated as an N-bromoiminium bromide [$PhCH=\overset{+}{N}(Br)PhBr^-$] [22].

Acylation of imines by acid anhydrides, acid chlorides, and acyl cyanides is initiated by attack at the nitrogen atom and leads to net addition of the acylating agent to the carbon-nitrogen double bond [22]. N-1-Haloalkyl amides are formed by acid halide addition to aldimines. Generally these are compounds having the characteristics of covalent compounds, although in reaction mixtures N-acyliminium ions may serve as intermediates. For example, addition of PhCOCl to PhCH=NMe yields PhCH(Cl)-N(COPh)Me **(19)** as a liquid that can be distilled [24]. Hydrolysis of **(19)** gives equimolar amounts of HCl, PhCHO and NH(COPh)Me. The base catalysed condensations of acetyl chlorides bearing an electron withdrawing group and at least one hydrogen atom at the α-position with N-arylaldimines occurs by initial acylation at the nitrogen atom, and lead to β-lactams [25] (**Scheme 36**).



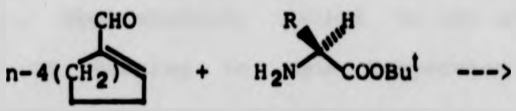
Scheme 36

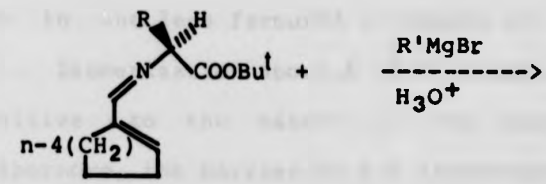
2.3.3 Reactions with nucleophiles

By analogy with the carbon-oxygen double bond, the $-\overset{|}{C}=N-$ double bond may be considered as a resonance hybrid of the canonical forms (cf **Scheme 37**) . The bipolar character of the $-\overset{|}{C}=N-$ bond enables electrophiles to react predominantly or exclusively at the nitrogen atom, whereas nucleophilic reagents react at the imidyl carbon atom. Addition of alkoxides to imines gives the corresponding α-alkoxyamino compounds. Reaction of imines with primary amines results in adducts which tend to decompose to a new imine and primary amine, the overall process corresponding to imine exchange. The rate of imine exchange increases with increase in the basicity of the primary amine effecting displacement. Chiral cyclic α,β-unsaturated aldimines (22), prepared by the condensation of an aldehyde (20) and an optically active compound (21), reacted by a 1,4-addition of a Grignard reagent, and were found to give, after hydrolysis, the trans-aldehydes (23) in reasonably high enantiomeric purities (**Scheme 38**) [26].

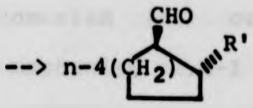
$$-\overset{|}{C}=N- \quad \longleftrightarrow \quad -\overset{|}{\overset{+}{C}}-\overset{-}{N}-$$

Scheme 37





(22)



(23)

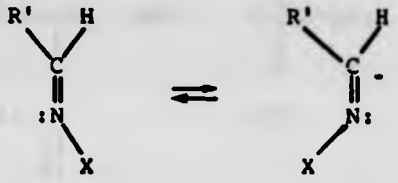
n = 5, 6; R = i-Pr, t-Bu; R' = Ph, $CH=CH_2$

Scheme 38

2.4 Geometrical (E-Z) isomers in azomethines

Because of restricted rotation about their $-\overset{|}{C}=N-$ double bond, N-substituted azomethines, for example, Schiff's bases, oximes and hydrazones, can exist as geometrical isomers represented by the structures (24) and (25) (Scheme 39). The configuration (E or Z) of imine derivatives such as hydrazones and oximes can be established via ^{13}C [27] or ^{15}N [28] NMR spectroscopy. ^1H NMR spectroscopy has been used to detect and estimate the E-Z isomer ratios of ketimines [29a] and aldimines in solution [29b] at equilibrium. Generally, for most simple aliphatic imines, the more stable E-isomer is predominant in solution at equilibrium. Z-Aldimines, which may be observed as transient intermediates in solution at -72 ^0C, after u.v. irradiation of the corresponding E-isomers, spontaneously revert to the original stable E-isomer on warming to room temperature [30]. This photochemical technique had been used to obtain solutions

rich in the less favoured Z-isomers of N-alkylaldimines [31]. Isomerization about a $-\overset{|}{C}=N-$ double bond is very sensitive to the nature of the substituents [32]. Furthermore, the barrier to E-Z interconversion in imines may be lowered by either reversible imine-enamine tautomerism [33] or by reversible acid catalysis [34]. The mechanism of E-Z isomerisation in imines is generally considered in terms of either rotation around the $-\overset{|}{C}=N-$ bond or planar inversion at nitrogen [35].



X = R, OH, NHR etc.

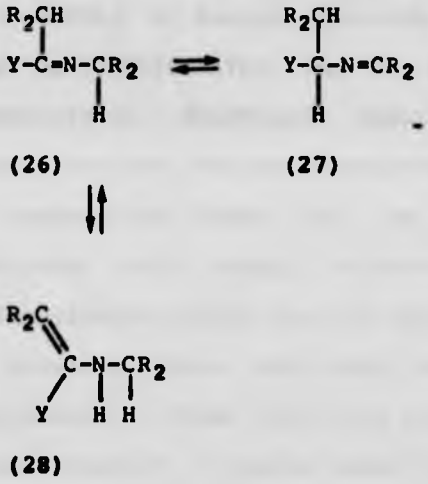
(E) (24) (Z) (25)

Scheme 39

2.5 Prototropic tautomerism in azomethines [1]

Azomethines bearing hydrogen atoms on either side of the $-\overset{|}{C}=N-$ bond can give rise to tautomers via a [1,3] - proton shift. These prototropic tautomerisms are described as azomethine - azomethine (26) ⇌ (27) or azomethine - enamine rearrangements (26) ⇌ (28) (Scheme 40). A [1,3] - proton migration of the azomethine-azomethine type is relatively slow and normally requires catalysis by heating with a strong base, for example, NaOEt in EtOH [36]. Azomethine - enamine prototropy (26) ⇌ (28) is normally a much more favourable process than its competing azomethine -

azomethine tautomerism. The azomethine form is usually the more stable structure in the solid state [11, 37], but in solution, especially polar solvents such as DMSO [38], the azomethine - enamine equilibrium is demonstrated by physical and chemical methods. The position of the azomethine - enamine equilibrium is also dependent on the substituents around the -C=N- bonds [39].



(26) (27) (28)

Scheme 40

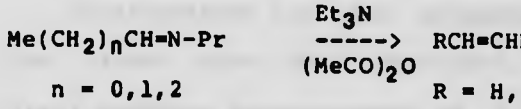
2.6 METHODS FOR PREPARING ENAMIDES [40]

Enamides are a class of compounds in which a -C=C- double bond occurs next to the nitrogen atom of an amide group. They are acylated enamines RCH=CH-N(COR)R. The enamides are prepared from several functional groups : imines, amines, oximes, nitriles etc.

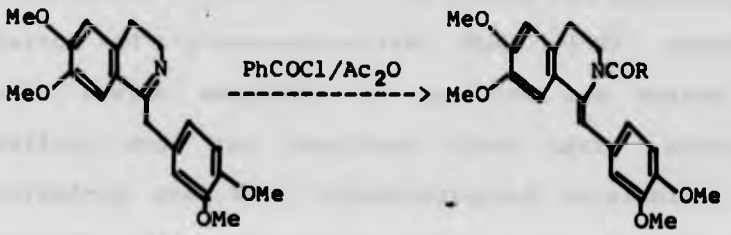
2.6.1 Imines

The simplest, most general, and most used method for the synthesis of enamides is the acylation of an imine with an acid halide or an acid anhydride. The reaction of aldimines with both acetic anhydride and acid chlorides was described by Breederveld [41] (**Scheme 41**). The reaction of N-propylpropanaldimine with MeCOCl or benzoyl chloride gave MeCH=CHN(COMe)Pr and MeCH=CHN(COPh)Pr in 64 % and 82 % yield, respectively. Equivalent results were obtained with imines derived from cyclohexanone [42]. A further work by Maujean and Chuche [43] on the condensation of ketimines with acetyl chloride in the presence of triethylamine showed that in addition to the formation of enamides, there was also obtained a oxazinone derivative. Bross and his co-workers described the acetylation of a cyclic imine [44 a-b]. The reaction of dihydropapaverine **(29)** with PhCOCl, acetic anhydride or mixed formic acetic anhydride gave mainly the (Z)-isomer [45] (**Scheme 42**). Direct acylation of N-alkyl-1-imino-2-butenes **(31)**, readily prepared from crotonaldehyde **(30)** and primary amines without isolation, with carboxylic acid anhydrides in the presence of triethylamine or sodium acetate failed to give the dienamides **(32)**. However, treatment of the conjugated imines with strong bases, such as sodium methylsulfinylmethide or sodium hexamethyldisilazane at -40^{0} to $-60\,^{0}C$ in toluene, followed by addition of an acyl chloride gave

stereoselectively the trans-N-acyl-1-amino butadienes (32), in good yield. Compound (32) undergoes elimination to give (33) at > 200°C (Scheme 43) [46].



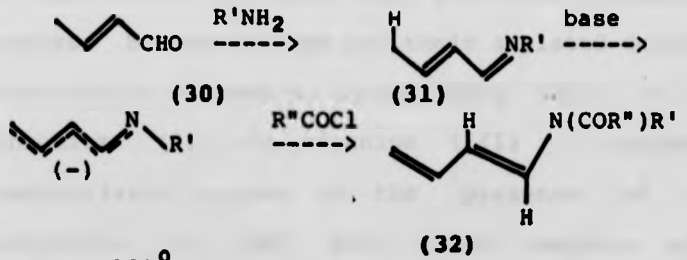
Scheme 41



(29)

R = H, Me, Ph

Scheme 42



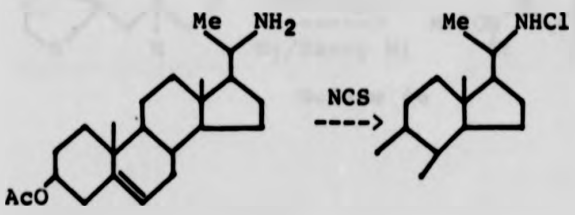
Scheme 43

2.6.2 Amines and Oximes

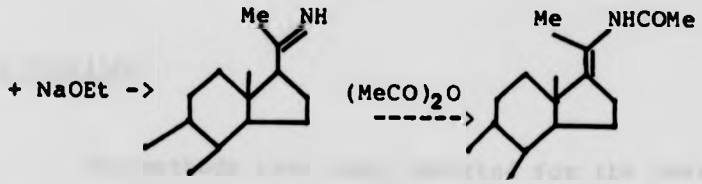
Most of the examples of enamide synthesis using amines and oximes as starting materials have occurred with steroids. Chlorination of the 20-amino group in the pregnane derivative (34) with NCS gave a high yield of N-chloroamine (35). Then, loss of HCl gave

the imine (36), which upon acetylation formed the 17(20)-enamide (37) as an E/Z mixture in 46 % overall yield based on (34) (Scheme 44) [47].

Three methods for the preparation of enamides from oximes have been described. The first was the direct Beckmann rearrangement of a steroidal α,β - unsaturated oxime [48]. The second was described by Barton and his co-workers [49]. When (38) together with acetic anhydride in pyridine was heated to reflux, and the resultant black tarry solution worked-up and then chromatographed on alumina, the enamide (39), containing about 10 % of the Δ^{8}-isomer, was obtained in 93 % yield (Scheme 45). A third method involved the reductive acylation of oximes. Because oximes and their acylated derivatives are readily reduced to imines using salts of either chromium (II) or titanium (III) , treatment of pre-acylated oximes in the presence of excess anhydride in DMF with these reagents afforded aliphatic, alicyclic, and steroidal enamides in fair yields [50]. Djerassi had earlier on reported the reduction of steroidal thiazolidines to enamides (Scheme 46) [51].

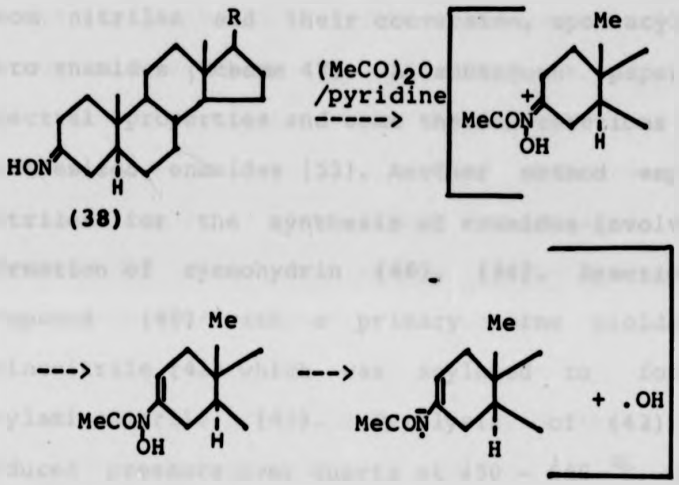


(34) (35)



(36) (37)

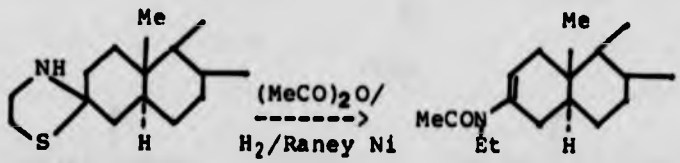
Scheme 44



(38) (39)

R = $MeCH(CH_2)_3CH(Me)_2$

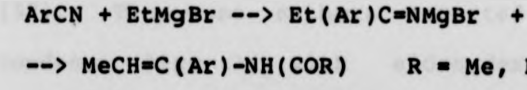
Scheme 45



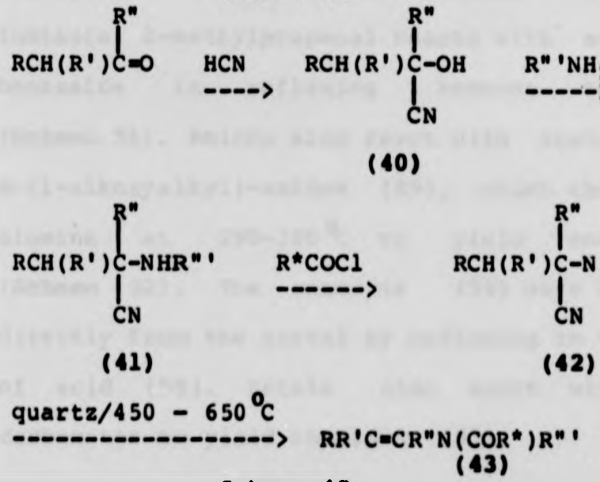
Scheme 46

2.6.3 Nitriles

Two methods have been reported for the conversion of nitriles into enamides. Kagan and co-workers [52] reported on the formation of iminomagnesium bromides from nitriles and their conversion, upon acylation, into enamides (Scheme 47). A subsequent paper gave spectral properties and some thermal reactions of the synthesized enamides [53]. Another method employing nitriles for the synthesis of enamides involved the formation of cyanohydrin (40), [54]. Reaction of compound (40) with a primary amine yielded the aminonitrile (41) which was acylated to form the acylaminonitrile (42). Pyrolysis of (42) under reduced pressure over quartz at 450 - 650 °C, yielded the enamide (43) in about 95 % yield (Scheme 48).



Scheme 47

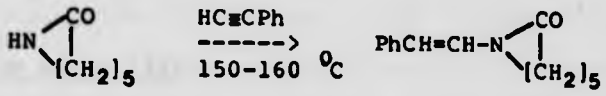


Scheme 48

2.6.4 Amides

A considerable amount of research has been reported for converting amides into enamides. Reppe *et al* described the vinylation of a wide variety of amides by acetylene using heat and pressure [55]. In agreement with Reppe *et als'* findings, Ziegenbein and Franke found that reaction of ε-caprolactam (44) and phenylacetylene at 150-160 °C in the presence of sodium gave (Z)-N-styryl-ε-caprolactam (45) (Scheme 49) [56]. Heating to higher temperatures caused isomerization to the (E)-isomer.

Ben-Ishai and Giger discovered that amidals (46), prepared from primary amides and aldehydes, eliminated an equiv. of amide upon heating, to form the enamides (47) in yields of 40-70 % (Scheme 50) [57]. The same authors reported that amides would condense directly with aldehydes, under acidic catalysis, to yield the enamides directly. For instance, 2-methylpropanal reacts with acetamide and benzamide in refluxing benzene to form (48) (Scheme 51). Amides also react with acetals to form N-(1-alkoxyalkyl)-amides (49), which thermolyse over alumina at 290-300 °C to yield enamides (50) (Scheme 52). The enamides (50) were also obtained directly from the acetal by refluxing in the presence of acid [58]. Ketals also react with amides and carbamates to yield enamides [59].



(44) (45)

Scheme 49

$RCH_2CH(NHCOR')_2$ ----> $RCH{=}CHNHCOR' + H_2NCOR'$

(46) (47)

R = H, Me, Ph R' = Me, PH

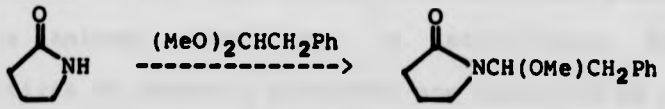
Scheme 50

$(Me)_2CHCHO$ $RCONH_2$ $(Me)_2C{=}CHNHCOR$

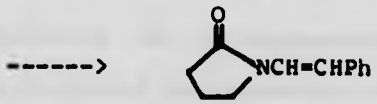
benzene (48)

R = Me :89% R = Ph :62%

Scheme 51



(49)



(78 %)

(50)

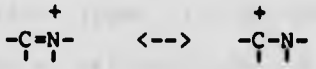
Scheme 52

2.7 Iminium salts [22]

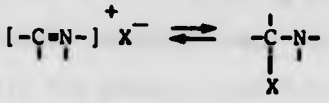
The electronic distribution in iminium cations can be shown by resonance structures (Scheme 53). An equilibrium of the ionic tautomers (51) and (52) is possible with anions which are good nucleophiles (Scheme 54). Spectroscopic evidence of this tautomerism is given for (53), by following fluorine exchange by ^{19}F NMR spectroscopy.

Iminium compounds show varying reactivity. Stabilisation comes from aliphatic substitution (hyperconjugation and steric protection) and from conjugation with π-systems and with lone-pair electrons. Complex anions also have a stabilising effect. Derivatives of aromatic aldehydes are described as stable salts, for example (54), which can be prepared from PhCH=NMe in chloroform with perchloric acid [60] or the hydrochloride of benzophenoneimine (55), which can be sublimed without decompositon at 230-250 °C [61]. Iminium salts are very rapidly hydrolysed by water with the formation of the corresponding carbonyl compound and a secondary ammonium salt (Scheme 55). The iminium salts from N-benzylidene derivatives were used to prepare N-deuterated alkylamines [62]. Iminium salts may decompose on melting even at relatively low temperatures and care is needed in their physical investigation and characterisation. Alkylation of aldimines to the iminium salt followed by hydrolysis is used in a synthesis of secondary amines. Iminium salts are useful intermediates

for the synthesis of α-alkoxyamino compounds (formed by a 1,2 - addition reaction). Iminium salts also readily add primary, secondary and tertiary amines with the formation of aminals (gem-diamino compounds) or their quaternary salts. In the Mannich reaction, an iminium salt is believed to be the electrophile which reacts with ketones to form synthetically useful Mannich bases (β-dialkylaminoketones) [63].

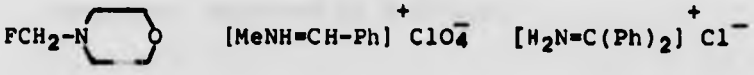


Scheme 53

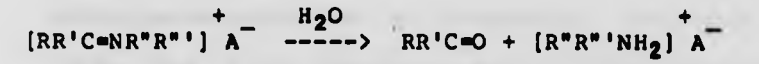


(51) (52)

Scheme 54



(53) (54) (55)



Scheme 55

2.7.1 Structures of iminium salts by NMR spectroscopy

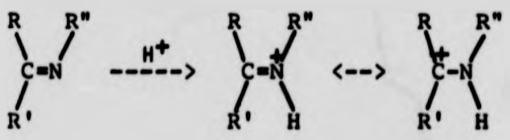
The structure of protonated aldimines and ketimines (Scheme 56) in strongly acid solutions [64] such as FSO_3H, FSO_3H-SbF_5 and D_2SO_4-SbF_5 with SO_2 or $CDCl_3$, as diluent, was determined from their ^1H NMR spectra [65, 66]. Neat N-propylidenemethylamine shows a long range coupling from the C-methyl to the N-methyl group, $J_{H\cdot H}$ trans = 1.35 Hz, $J_{H\cdot H}$ cis = 0.7

Hz, which is analogous to homoallylic coupling. The ^1H NMR spectrum of the protonated $Me_2C{=}NMe$ in SO_2 at $-20\,^{o}C$ shows the C-methyl groups to be non-equivalent, indicating that there is a significant barrier to rotation around the $-\overset{|}{C}{=}N-$ bond.

The ^1H NMR spectrum of pure anti-benzylidene methylamine in SO_2 at $-30\,^{o}C$ showed the following shifts (ppm) : 3.33 [3H, d, Me, $J_{H\cdot H}$= 1.6 Hz], 7.54 [5H, s, Ar] and 8.04 [1H, q, CH, $J_{H\cdot H}$= 1.6 Hz]. In the protonated imine ($HSO_3F/SO_2/30\,^{o}C$) the signal from the methyl protons was shifted to 3.84 ppm ($J_{H\cdot H}$ = 4.5 Hz); the phenyl protons appeared at 8.03 ppm and the methine proton at 8.8 ppm (broad doublet, $J_{H\cdot H}$ = 17 Hz). When the anion was chloride, the methine resonance appeared at 8.38 ppm.

The ^1H NMR data for the protonated E-benzylidene methylamine indicated an E-immonium structure (56) (N.B. the 17 Hz coupling), with limited contribution from the aminocarbocation form (57). The ^1H NMR and IR spectral data for several iminium salts has been listed in tabular form by Merenyi [67]. Simple aliphatic iminium salts appear to have been very little studied. One such salt that has been fully characterised is $[MeCH{=}NHEt]^{+}\ BF_4^{-}$ (C=N 1720 cm^{-1}) [68]. We observed that an aliphatic imine (BuCH=NEt) and a sterically hindered imine (N-ethylcamphorimine) readily form iminium salts (provided strict precautions were taken to minimise contact with moisture) on titrating the imines with one equiv. of

HCl prepared in dry ether. The ^{1}H NMR spectra of BuCH=$\overset{+}{N}$Et(HCl) in $CDCl_3$ (Fig. 1) shows the following chemical shifts (ppm): 0.95 [3H, t, $MeCH_2CH_2$], 1.2 - 1.8 [4H, m, $MeCH_2CH_2$], 1.50 [3H, t, $MeCH_2N$], 2.95 [2H, q, CH_2CH], 3.91 [2H, q, $CH_2\overset{+}{N}H$], 6.1 [1H, broad signal, NH] and 8.72 [1H, pair of triplets, CH=NH]. The methine proton of BuCH=NHEt, which has a chemical shift of 8.72 ppm, is trans-coupled (17 Hz) to the NH proton, and consequently is further split by the adjacent methylene protons (J 5 Hz) into a pair of triplets. The methylene group (-C=NCH_2Me) in N-ethylcamphorimine appears at 3.1 ppm. On protonation of this ketimine at the nitrogen atom, the methylene showed a quartet at 3.78 ppm (J 7 Hz) (cf Fig. 2). Interestingly, when BuCH=NEt was protonated with 1 equiv. of TFA in $CDCl_3$, ^{1}H NMR spectroscopy (cf Fig. 3) showed the occurrence of a clean aldol-type condensation. The chemical shifts of the aldol-type product are in accordance with the structure BuCH(Pr)CH=NEt: 0.92 [6H, t, J 7 Hz, Me of Pr and Bu], 1.26 [3H, t, J 7 Hz, $MeCH_2\overset{+}{N}H_3$], 1.38 [3H, t, J 7 Hz, $=NCH_2Me$], 1.3 - 1.5 [6H, m, CH_2CH_2Me, $CH_2CH_2CH_2Me$], 2.3 - 2.5 [4H, m, CH_2Pr, CH_2Et], 2.98 [2H, q, J 7 Hz, $MeCH_2\overset{+}{N}H_3$], 3.8 [2H, q, J 7 Hz, $=NCH_2Me$], 6.1 [1H, t, 7 Hz, CH=C], and 8.1 [1H, s, CH=N].



(56) (57)

R, R', R" = Me, N-isopropylidenemethylamine

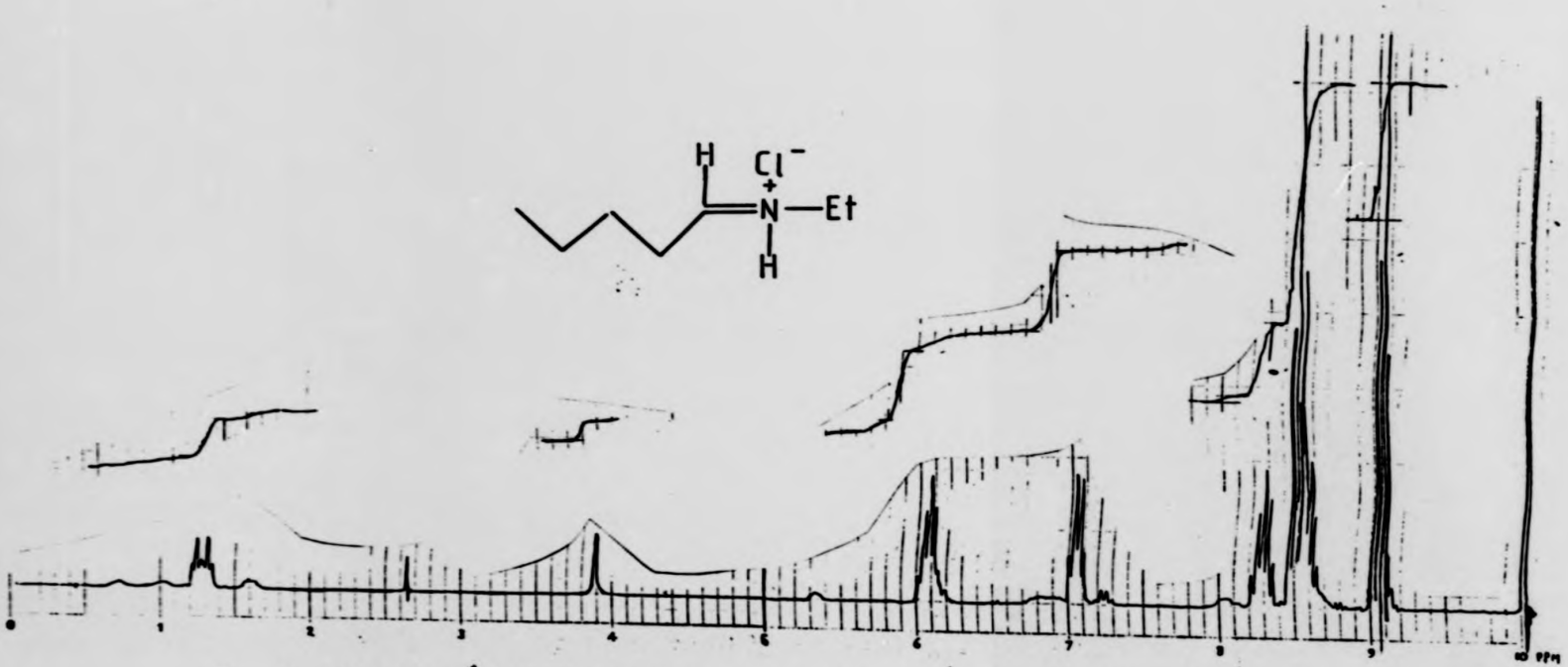


Fig. 1 220 MHz ^{1}H NMR SPECTRUM OF BUCH=$\overset{+}{N}$(HCl)Et ($CDCl_3$)

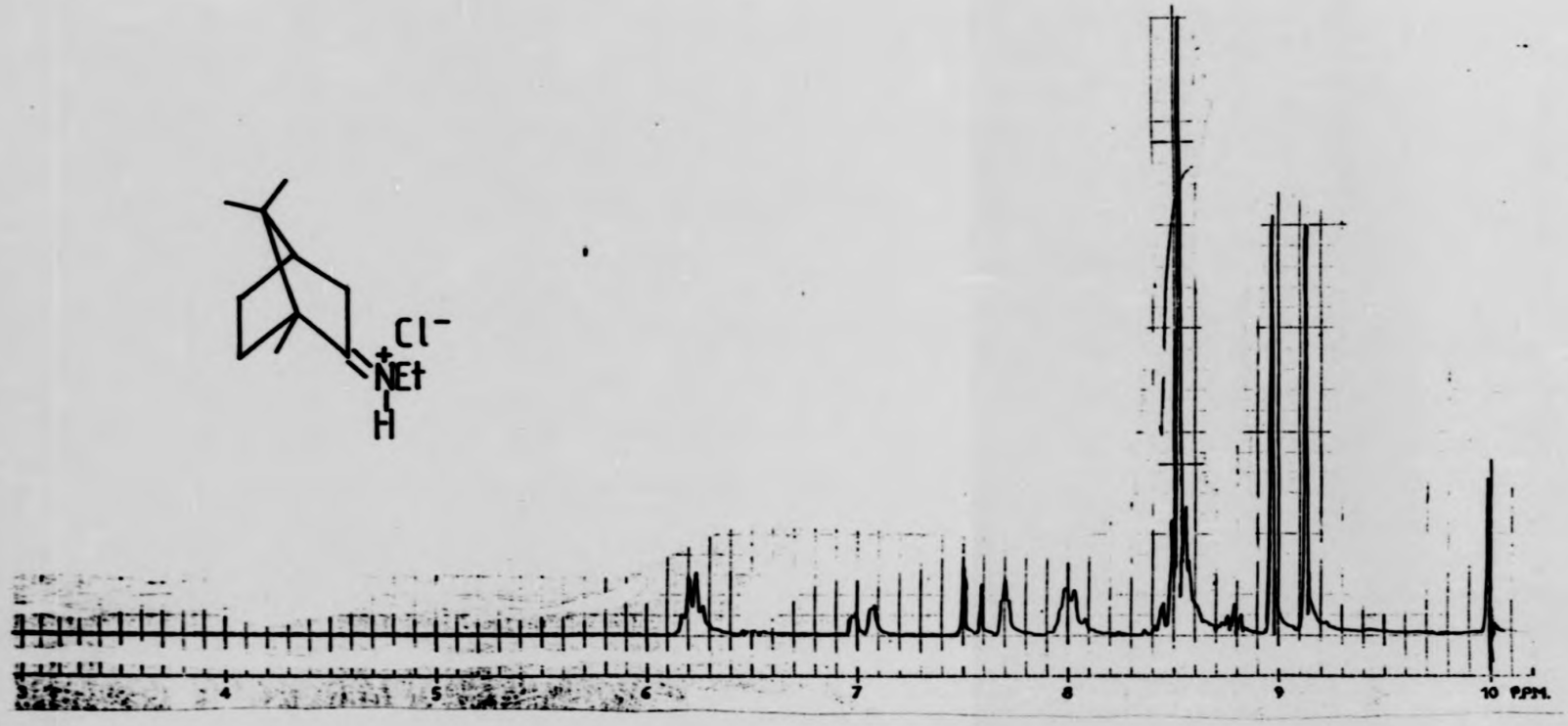


Fig. 2 220 MHz ^{1}H NMR SPECTRUM OF N-ETHYLCAMPHORIMINE HYDROCHLORIDE

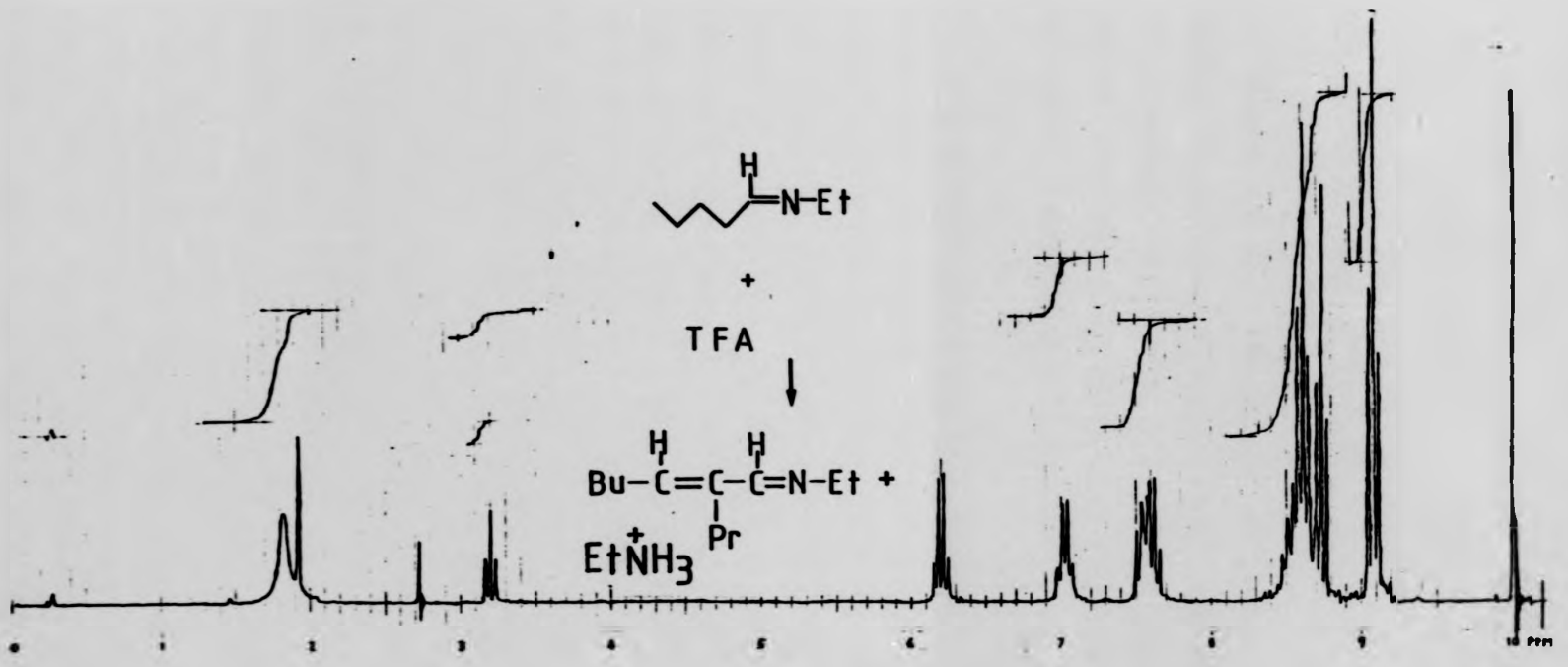


FIG. 3 220 MHZ ^{1}H NMR SPECTRUM OF BUCH=C(Pr)CH=NEt

R = H, R' = Ph, R" = Me, anti-benzylidenemethylamine

Scheme 56

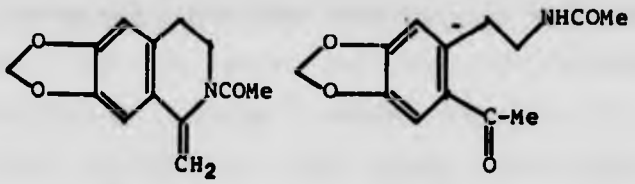
2.8 SYNTHETIC APPLICATIONS OF ENAMIDES

Simple enamides undergo an efficient photo-Fries reaction to generate vinylogous amides, RNHHC=CHCOR [40]. With dienamides, photocyclizations provide many types of natural products, particularly in the benzylisoquinoline and indole alkaloid families [40].

Enamides are stable compounds in neutral or basic conditions, cf. amides. They do however, show varying sensitivity to aqueous acid, hydrolysing by hydration of the double bond to form ketones and amides. This has been described by Brossi et al for the isoquinoline enamide (58), which hydrolyses to the acetophenone derivative (59) in dilute aqueous acid (Scheme 57) [44]. The by-product of hydrolysis is an amide which can be removed easily, and thus enamides, as we have found, provide a useful route to [^{17}O]/[^{18}O]-labelled carbonyl compound.

The synthetic usefulness of enamides was examined in comparison to the analogous enamines. Enamines (Scheme 58) [69] are useful in syntheses because some negative charge on the β-carbon atom can act as a nucleophile in reactions with alkyl and acyl halides and with electrophilic olefins. Reactions with alkyl halides, for example, lead irreversibly to C-alkylated and N-alkylated products. Subsequent hydrolysis of the C-alkylated

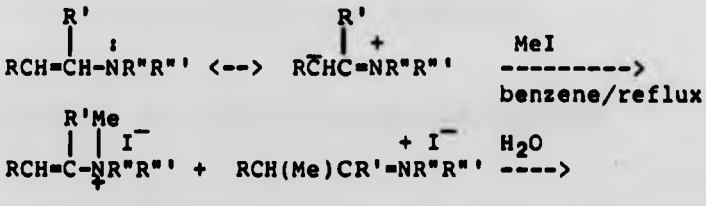
iminium salt gave the alkylated ketone; the N-alkylated product was usually water-soluble and unaffected by the hydrolysis (Scheme 59). This procedure avoids base or other catalyst and hence self-condensation reactions of carbonyl compound were minimized, providing good yields of alkylated and acylated products. Enamines, when hydrolysed in D_2O, have provided a convenient method for the preparation of 2-deuterated ketones (Scheme 60) [70]. Under certain conditions more than one atom of deuterium was introduced, which suggests that β-protonation is reversible.



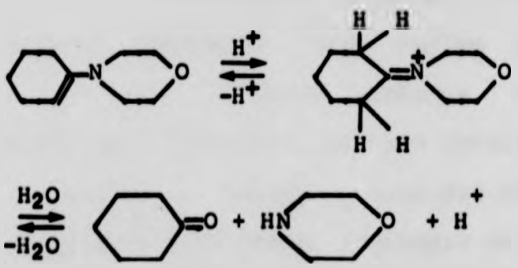
(58) (59)

Scheme 57

Scheme 58



Scheme 59



Scheme 60

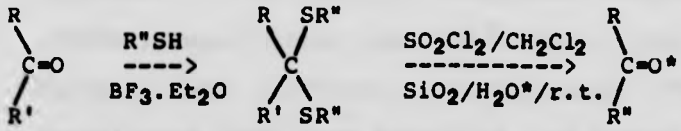
2.9 STABLE ISOTOPES OF OXYGEN [71, 72]

Natural oxygen consists of 3 stable isotopes ^{18}O (99.7587 atom %), ^{17}O (0.0374 atom-%) and ^{18}O (0.2039 atom-%). The unstable isotopes ^{14}O, ^{15}O, ^{19}O and ^{20}O are radioactive (^{15}O has the longest half-life, 2.1 min). [^{18}O]Water for example, is prepared by fractional distillation of ordinary water and is highly enriched in deuterium. The exact concentration of deuterium for each ^{18}O concentration depends on the design of the separation plant [73]. The nomenclature and formulae for a variety of isotopically modified compounds has been specified by IUPAC [74]. To write and name the structure of a specifically labelled oxygen compound, for example ethanol enriched in the oxygen atom, square brackets enclose the nuclide, e.g. Me-CH_2-[^{18}O]-H, [^{18}O]Me-CH_2-OH, [^{18}O]ethanol or ethan[^{18}O]ol. We have used the ^{17}O isotope as a tracer to study isotopic exchange reactions and the investigation of bond fission in some ^{17}O-enriched compounds (cf Chapter 3).

2.10 SYNTHESIS OF [^{17}O]/[^{18}O]-LABELLED COMPOUND

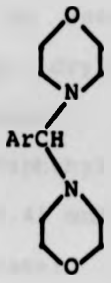
Dostrovsky and Samuel have described methods for preparing [^{18}O]-inorganic compounds [75]. A large number of organic syntheses with oxygen isotopes have been described [76]. Isotopic exchange between organic compounds and [^{18}O]water has been reviewed [77]. A review on methodology primarily intended for biochemists has been published [78]. Many inorganic or organic compounds [79 a-d] enriched in one or more positions with ^{18}O are

prepared by simple exchange processes between the normal compound and [^{18}O]water. Many deuterio-compounds are also made simply by exchanging with D_2O. A mathematical treatment of the problem of obtaining deuterated [80] and [^{18}O]-enriched compounds [78] by repeated exchange indicated that a large excess of heavy water or [^{18}O]water is required in order to obtain a highly enriched samples. Several derivatives of carbonyl compounds such as acetals [81], dithioketals (**Scheme 61**) [82], and aminals (60) [83] may be hydrolysed with a stoichiometric amount of [^{18}O]water with acidic catalysis. Such derivatives have been used to prepare MeCH[^{18}O] [81] and [^{18}O]aromatic aldehydes [83]. The methods explored in this thesis for preparing various [^{17}O]/[^{18}O] carbonyl compounds include the stoichoimetric hydrolysis of nitrogen-based derivatives such as imines, iminium salts and enamides.



R" = Me, * = Oxygen-18, e.g. p-Chlorobenzaldehyde (99 %)

Scheme 61



(60)

2.11 MATERIAL AND METHODS

2.11.1 Dry solvents [84]

AR ether was distilled from $LiAlH_4$ and stored over molecular sieves 3A/4A. CH_2Cl_2 and CCl_4 were distilled from P_2O_5. DMSO was distilled and the first 20 % was discarded.

2.11.2 Chemicals [85]

36.3 atom-% [^{17}O]water (0.1 g), 31.0 atom-% [^{17}O]water (0.250 g) and 99.8 atom-% [^{18}O]water (0.500 g) were supplied by Prochem, B.O.C. Limited. Diisopropylamino-polystyrene was a gift from Dr. J. Schreiber, ETH Zurich. Anhydrous ethylamine SLR (FISONS), was stored at -20 °C over KOH pellets. Hunig's base (Aldrich). Triethylamine (FISONS) and benzylamine (BDH) were distilled and stored over KOH pellets. CF_3COOH SLR (FISONS) was stored over P_2O_5 and distilled. MeCOCl was distilled from PCl_5. PhCOCl was purified by washing a benzene solution with aqueous $NaHCO_3$, drying with $CaCl_2$ and distilling. 5α-Cholestan-3-one & 5α-Androstan-17β-ol-3-one (Sigma). 1-Naphthyl isocyanate was distilled, b.p.70 °C at 0.45 mmHg. $TiCl_4$ (FISONS). Acetaldehyde (FISONS). Pentanal (Aldrich). d-Camphor (BDH). Aluminium oxide (ICN Pharmaceuticals). Molecular sieves (Type 3A, 1/16 inch pellets, Hopkin & Williams)

2.11.3 Instruments

^{1}H NMR spectra were recorded on a Perkin-Elmer (model R-34) at 220 MHz (probe at ambient temp.). Peaks were assigned chemical shift values (δ) in parts per million (ppm), relative to an internal standard (TMS), followed in brackets by the integral of the protons, the number of peaks in the multiplet (d = doublet, t = triplet, q = quartet, qt = quintet, sx = sextet, sp = septet and m = multiplet), the structural assignment of the proton resonance, and the spin coupling constant J in Hz.

IR spectra were recorded on a Perkin-Elmer grating infrared spectrophotometer (model 257/457 or 580B). All spectra were calibrated with the absorption spectrum of polystyrene at 1601.4 cm^{-1}. Peaks were recorded by their wave-number (cm^{-1}) and the IR intensities are classified as strong (s), medium (m), and weak (w).

GLC analyses were conducted with a Honeywell F and M (model 720) gas chromatograph using helium as the carrier gas.

Mass spectra were recorded on a Kratos MS 80 spectrometer. Elemental analyses - CHN analysis Ltd., South Wigston, Leicester. M.P.s were determined on a REICHERT (SHANDON) instrument.

2.12 EXPERIMENTAL

2.12.1 PREPARATION OF IMINES

2.12.2 N-Pentylideneethylamine

Ethylamine (10.00 g, 0.22 mol) and solid KOH (2.0 g, 0.036 mol) in a round-bottom flask, was cooled in a dry-ice bath. Pentanal (17.0 g, 0.19 mol) was added gradually to the amine. The dry-ice bath was removed when all the pentanal had been added. The reaction was allowed to warm up to room temperature, whereupon an aqueous layer separated after ca 0.5 h. The organic layer was removed and stored over fresh KOH at $4^{0}C$ overnight. Distillation of the crude product afforded N-pentylideneethylamine (9.46 g, 42 %), b.p. $67-68^{0}C$ at 95 mmHg.

^{1}H NMR (CCl_4) : 0.94 [3H, t, $Me(CH_2)_3$], 1.13 [3H, t, $MeCH_2$], 1.29 - 1.6 [4H, m, CH_2CH_2], 2.18 [2H, q, $CH_2CH_2CH{=}N$], 3.3 [2H, q, CH_2N], and 7.55 [1H, t, $CH{=}N$].

IR (CCl_4) : 2960 s, 2930 s, 2880 s, 2840 s, 1675 s (C=N), 1470 m, 1450 m, 1380 m and 1340 cm^{-1}.

The above procedure similarly afforded RCH=NEt, where R is Bu, $Bu(CH_2)_3$, Ph. Also, a ketimine, N-cyclopentylideneethylamine, was obtained.

2.12.3 N-Acetylideneethylamine

^{1}H NMR (CCl_4) : 1.14 [3H, t, $MeCH_2$], 1.89 [3H, d, MeCHN], 3.3 [2H, q , CH_2N], and 7.6 [1H, q, CH=N].

2.12.4 Octylideneethylamine

^{1}H NMR (CCl_4) : 0.9 [3H, t, $Me(CH_2)_6$], 1.13 [3H,t, $MeCH_2$], 1.3 - 1.5 [10H, m, $(CH_2)_5$], 2.18 [2H, q, CH_2CH_2CH=N], 3.3 [2H, q, CH_2N], and 7.55 [1H, t, CH=N].

2.12.5 Benzylideneethylamine

^{1}H NMR (CCl_4) : 1.25 [3H, t, Me], 3.57 [2H, q, CH_2], 7.2 - 7.7 (5H, m, Ar H], and 8.14 [1H, s, CH].

IR (CCl_4) : 3080 w, 3060 m, 3020 m, 2970 s, 2940 m, 2870 m, 2840 s, 1650 (C=N), 1580 s, 1490 m, 1470 w, 1450 s, 1370 m, 1340 s, 1310 s, 1290 w, 1210 w, 1180 w, 1100 m, 1040 m, 960 s, 900 cm^{-1}.

^{1}H NMR showed only one isomer. Note that N-benzylidenemethylamine [65] was obtained as a mixture of two geometric isomers. The ^{1}H NMR spectrum in SO_2 showed two quadruplets for the methine proton at 8.38 ppm for the anti-form and at 8.15 ppm for the syn-isomer.

2.12.6 N-Cyclopentylideneethylamine

^{1}H NMR (CCl_4) : 1.14 [3H, t, Me], 1.76 [4H, m, $-CH_2CH_2-$], 2.09 [2H, t, $CH_2C=N$], and 2.24 [2H, t, $CH_2C=N$]. The H-1 and H-4 methylene protons are magnetically non-equivalent.

IR (CCl_4) : 2960 s, 2930 m, 2880 m, 2870 m, 1680 s (C=N), 1450 w, 1420 w, 1375 w, 1340 w, and 1185 w cm^{-1}.

2.12.7 N-Ethylcamphorimine

d-Camphor (15 g, 0.1 mol) was mixed with ethylamine (27 g, 0.6 mol) in toluene (50 cm^3). The solution was stirred with cooling in a dry-ice bath. $TiCl_4$ (11.0 cm^3, 0.1 mol) dissolved in toluene (50 cm^3) was added gradually to the solution. The reaction mxture was then heated for 4.5 h at reflux and then left standing overnight at room temperature. The material was filtered through Celite after adding petroleum ether (40 - 60). Filtration removed the unwanted amine hydrochloride salt. The toluene and petroleum ether solution was evaporated, and the residue distilled to give a colourless liquid, b.p 88 °C at 9 mmHg. ^{1}H NMR showed contamination with some toluene. Redistillation afforded a purer fraction (10 g, 58 %), b.p. 93 °C at 13 mmHg. Its ^{1}H NMR spectrum showed a similar absorption pattern for the ring Me groups as found for camphor.

^{1}H NMR (CCl_4) : 0.7, 0.86, and 0.9 [each 3H, s, 3 x C-Me], 1.1 [3H, t, NCH_2Me], 1.15 - 1.9 [6H, m, $-CH_2CH_2CH-$ plus H-3 endo], 2.25 [1H, pair of t, H-3 exo] and 3.1 [2H, m, NCH_2].

IR (CCl_4) : 2960 s, 2880 m, 2280 w, 1686 s (C=N), 1550 s, 1250 m, 1220 m, 1010 m, and 980 m cm^{-1}.

2.12.8 N-Cyclohexylidenebenzylamine

Cyclohexanone (7.3 cm^3, 70 mmol) and benzylamine (7.6 cm^3, 70 mmol) were mixed in CH_2Cl_2 (21 cm^3). Molecular sieves 3A (26 g) - activated by preheating over a Bunsen flame, then cooling in a desiccator - were added to the solution. The reaction was kept at room temperature overnight. Distillation afforded a colourless liquid (6 g, 46 %), b.p. 76-80 ^oC at 0.04 mmHg.

^{1}H NMR (CCl_4): 1.5 - 1.8 [6H, m, $-(CH_2)_3-$], 2.29 [4H, distorted t, $CH_2(CH_2)C=N$], 4.41 [2H, s, NCH_2], and 7.19 [5H, m, Ar].

IR (CCl_4): 3080 w, 3060 w, 3030 w, 2928 s, 2860 s, 1665 s (C=N), 1500 s, 1450 s, 1350 m, 1030 m, and 698 s cm^{-1}.

2.12.9 N-1-Methylbutylidenebenzylamine

This was prepared from the condensation of pentan-2-one with benzylamine in CH_2Cl_2 and activated

sieves 3A. The compound was obtained as a clear liquid (50 %), b.p. 46°C at 13 mmHg. The title compound was shown to be a mixture of syn - anti geometric isomers in $CDCl_3$ at ambient temp., in a ratio of 3 : 1, from its ^1H NMR spectrum. The integrals for the MeC=N proton signals at 1.88 ppm and 2.06 ppm gave a ratio of 3 :1, respectively. The height of the benzylic protons peak at 4.47 ppm is ca 3 x that of its isomer at 4.50 ppm.

^1H NMR ($CDCl_3$): 0.94 [3H, t, $Me(CH_2)_2$], 1.62 [2H, sextet, $MeCH_2CH_2$], 1.88 [3H, s, MeC=N], its isomer 2.06 [3H, s, MeC=N], 3.0 [2H, t, $CH_2C=N$], 4.47 [2H, s, NCH_2Ph], its isomer 4.50 [2H, s, NCH_2Ph], and 7.28 [5H, m, ArH].

IR (CCl_4): 3080 w, 3060 m, 3030 m, 1662 s (C=N), 2960 s, 2930 s, 2870 s, 1610 w, 1500 s, 1458 s, 1420 m, 1380 m, 1350 m, 1300 w, 1250 w, 1030 w, 720 m, and 698 s cm^{-1}.

2.12.10 Cholestan-3-one N-benzylimine

Cholestan-3-one (100 mg, 0.258 mmol), benzylamine (0.086 cm^3, 0.792 mmol) and sieves 3A (100 mg) in CH_2Cl_2 (0.160 cm^3) were kept at room temperature. After 24 h , IR spectroscopy showed 75 % imine formation (C=N at 1650 cm^{-1} compared to the C=O absorption at 1700 cm^{-1}). ^1H NMR spectroscopy showed the benzylic protons of the imine at 4.5 ppm, as a doublet signal. The imine product completely formed

after standing the reaction for 5 days. TLC : alumina F-254 (Merck); uv; ether ; Rf 0.78. In iodine, three spots were observed: Rf 0.73, 0.84 and (benzylamine). The solution was filtered through Grade 3 alumina (3 g) to removed benzylamine, eluting with CH_2Cl_2 (10 cm^3). ^1H NMR spectroscopy, after removal of solvent, showed the residue to be starting material, suggesting that hydrolysis of the imine occurred during work-up. We have attempted to prepare a ketimine from 5α-androstan-17βol-3-one and benzylamine. After a 5 days reaction, ^1H NMR spectroscopy showed benzylic imine protons appearing as a doublet at 4.5 ppm.

2.13 PREPARATION OF ENAMIDES

2.13.1 N-Pent-1-enyl-N-ethylbenzamide [Pr=CHN(COPh)Et]

To N-pentylideneethylamine (4.00 g, 35.4 mmol) was added a solution of triethylamine (4.90 cm^3, 35.4 mmol) in ether (10 cm^3). The resulting solution was cooled in an ice-bath. Benzoyl chloride (4.10 cm^3, 35.4 mmol) in ether (10 cm^3) was added, and the mixture was stirred. During the addition of benzoyl chloride, a precipitate formed. The mixture was then stirred at room temperature overnight. The precipitate, which is $Et_3\overset{+}{N}HCl$ (4.5 g, 33 mmol) was removed. The excess ether was evaporated. Distillation afforded a yellow liquid (4.96 g, 63 %), b.p. 88 ^oC at 0.001 mmHg.

^{1}H NMR (CCl_4, 60 °C) : 0.88 [3H, t, $Me(CH_2)_2$], 1.2 [3H, t, $MeCH_2$], 1.35 [2H, sextet, $MeCH_2CH_2$], 1.92 [2H, q, $CH_2CH=CH$], 3.7 [2H, q, CH_2N], 4.92 [1H, pair of triplets, $CH=CHN$], 6.4 [1H, d, $CH=CHN$, J = 18 Hz, trans coupling], and 7.32 [5H, s, Ar H].

Found (CHN) : C, 76.34 ; H, 8.87 ; N, 6.30 %. $C_{14}H_{19}NO$ (217) requires : C, 77.38 ; H, 8.81 ; N, 6.44 %.

2.13.2 N-Cyclopentenyl-N-ethylbenzamide

The title compound was prepared similarly. The compound was a clear liquid, yield 49 %, b.p. 86-88 °C at 0.04 mmHg. TLC : alumina F-254 (Merck); ether; Rf 0.75.

^{1}H NMR (CCl_4, 60 °C) : 1.14 [3H, t, Me], 1.76 [2H, qt, $CH_2CH_2CH_2$], 2.17 [4H, m, $CH_2CH=CCH_2$], 3.62 [2H, q, CH_2N], 5.07 [1H, perturbed t, $-CH=CN$] and 7.2 - 7.4 [5H, m, Ar H].

2.13.3 N-Ethyl-N-vinylbenzamide [CH_2=CHN(COPh)Et]

The title compound was prepared similarly. The compound was a colourless oil, yield 49 %, b.p. 66-7 °C at 0.04 mmHg.

^{1}H NMR (CCl_4, 60 °C) : 1.21 [3H, t, Me], 3.76 [2H, q, NCH_2], 4.14 [1H, d, J 10 Hz, $H_{gem/cis}$], 4.38

[1H, d, J 15 Hz, $H_{gem/trans}$], 6.68 [1H, q, J 10 and 15 Hz, $\underline{H}C=CH_2$] and 7.34 [5H, s, Ph H].

Found (CHN) : C, 74.83 ; H, 7.58 ; N, 7.89 %. $C_{11}H_{13}NO$ (175) requires : C, 75.40 ; H, 7.48 ; N, 7.99 %.

m/z : 175 (12), 147 (16), 105 (100).

2.13.4 N-Ethyl-N-Camphorenylacetamide

N-Ethylcamphorimine (1.264 g, 7.10 mmol) and acetyl chloride (0.5 cm^3, 7.1 mmol) in $CHCl_3$ (5 cm^3) were heated to reflux with stirring for 6 h. Triethylamine (1 cm^3, 7.17 mmol) was added. Some precipitate was observed which went into solution when more $CHCl_3$ (4 cm^3) was added. The red coloured solution was heated to reflux for 4 days. Petroleum ether (40-60) was added to precipitate the $Et_3\overset{+}{N}HCl$ salt. The supernatant was concentrated, giving a clear viscous liquid (1.76 g). Distillation gave a pale yellow liquid, b.p 113 °C at 0.1 mmHg, which was further distilled, b.p. 75-80 °C at 0.1 mmHg (1.1066 g). TLC : Alumina F-254 (Merck); ether - petroleum ether (60-80), 3 : 7; Rf 0.56 and Rf 0.24 (the major fraction). Preparative chromatography was carried out on Grade 3 alumina: 50 g; column packing, 2.5 cm x 12.5 cm; eluting solvent, ether - pet. ether (60-80) in the proportion of 3 : 7. A minor fraction (295 mg), which moved with the solvent front, was found to be the unreacted imine. The slower moving fraction was

the title compound (745 mg, 47 %), a colourless liquid. ^{1}H NMR ($CDCl_3$): 0.8, 0.9, and 0.96 [each 3H, s, 3 x C-Me], 1.05 [3H, t, J 7 Hz, NCH_2Me], 1.1 - 1.3, 1.5 - 1.7 [4H, m, H-5 and H-6], 2.0 [3H, s, MeCO], 2.38 [1H, t, J 2 Hz, H-4], 3.45 [2H, m, NCH_2Me], and 5.68 [1H, d, J 2 Hz, CH=C].

2.14 METHODS FOR LABELLING CARBONYL COMPOUNDS

2.14.1 [^{17}O]/[^{18}O]pentanal via hydrolysis of PrCH=CHNEt(COPh)

To N-pent-1-enyl-N-ethylbenzamide (2.1 g, 9.8 mmol) in dry ether (15 cm^3) was added 25 atom-% [^{18}O]water (0.18 cm^3, 9.75 mmol), followed by the addition of a catalytic quantity of HCl in ether (0.05 m. equiv., titrated as 1 molar). Dry HCl was prepared by the addition of conc. HCl (15 cm^3) to conc. H_2SO_4 (20 cm^3) [86] and the HCl gas released was bubbled into dry ether (100 cm^3). An aliquot (1 cm^3) of this stock solution was diluted 100 x with water, and 25 cm^3 removed and titrated with 0.1M NaOH, bromothymol blue being used as an indicator. The hydrolysis experiment was conducted in a N_2 flushed glove-box. The mixture was stirred and the hydrolysis monitored by TLC and ^{1}H NMR spectroscopy of an aliquot. TLC : alumina F-254 (Merck); ether; Rf 0.73 (starting material) and Rf 0.41 (N-ethylbenzamide). Hydrolysis was judged to be complete when the starting material was replaced by the product N-ethylbenzamide (ca 2 h). To this mixture was added a polymeric base,

diisopropylaminopolystyrene (1.00 g, 1.2 mequiv./g), which neutralized the acid. After 30 min stirring, the solid was filtered off. The filtrate was evaporated to low volume by partial vacuum distillation using a water-pump at room temperature. Some N-ethylbenzamide began to crystallize from the concentrated ether solution. The ether was removed by filtration and cooled in a dry-ice and acetone bath. A trap-to-trap distillation at low pressure using an oil-pump gave a clean ethereal solution of the labelled pentanal, free from amide contaminant. Further purification by preparative GLC : 20 % DEGS on chromosorb WHP, column temperature 130 ^{0}C, flow rate 42 cm^{3}/min, He carrier gas, gave 25 atom % [^{18}O]pentanal (432.1 mg, 51.5 %). Similarly, the hydrolysis of this enamide in 31 atom-% [^{17}O]water afforded 31 atom-% [^{17}O]pentanal whose IR spectrum is shown in Fig. 5.

IR (CH_2Cl_2) : C=^{16}O (1725 cm-^{1}) and C=^{18}O (1690 cm-^{1}) in the ratio of 4 : 1. The [^{18}O]pentanal from one experiment was reduced by $LiAlH_4$ to an alcohol, which was then derivatized with 1-naphthyl isocyanate. The atom-% enrichment was determined from the mass spectrum of the labelled urethane. Lit. [87], $C_{18}H_{19}O_2N$, m.p. 68 ^{0}C. [^{18}O]urethane, m.p. 65 ^{0}C. To show that the amide was devoid of ^{18}O-enrichment, the mass spectrum of the by-product NH(COPh)Et was compared with an authentic sample [88]. The authentic NH(COPh)Et was recrystallised from ethanol at -20 ^{0}C , m.p. 68 ^{0}C, Lit. [88], 68.5 ^{0}C. The m.p. of NHCOPhEt from one ^{18}O experiment was 68-69 ^{0}C.

^1H NMR ($CDCl_3$) for $C_{10}H_7NH-CO-[^{18}O]-(CH_2)_4Me$: 0.9 [3H, t, Me], 1.35 and 1.68 [6H, m, $(CH_2)_3Me$], 4.18 [2H, t, OCH_2], 6.88 [1H, broad s, NH], and 7.3 - 7.9 [7H, m, Ar H].

m/z of the 25 atom-% [^{18}O]urethane: 260 (1.7), 259 (10.9), 258 (9.8), 257 (33.1), 187 (17.2), 169 (91.2), 143 (100.0), 115 (49.3), 87 (19.6), 69 (20.8), 55 (35.1), 43 (94.2), and 29 (32.9). The same sample kept for a year gave m/z : 260 (0.6), 259 (4.2), 258 (2.8), 257 (17.2), 170 (15.8), 169 (100.0), 143 (17.7), 141 (22.3), 140 (20.4), 44 (23.6), and 28 (37.2). An authentic unlabelled urethane give m/z : 259 (0.3), 258 (2.9), 257 (16.4), 170 (14.4), 169 (100.0), 143 (11.3), 141 (18.7) and 140 (19.7).

^1H NMR (CCl_4) for NH(COPh)Et : 1.2 [3H, t, Me], 3.45 [2H, qt, CH_2], 6.62 [1H, broad s, NH] and 7.2 - 7.8 [5H, m, Ar H].

m/z of the authentic NH(COPh)Et: 150 (5.0), 149 (45.4), 106 (8.6), 105 (100), 77 (45.1), and 51 (12.9). m/z of the by-product NH(COPh)Et from the preparation of 25 atom-% [^{18}O]pentanal: 150 (4.2), 149 (41.9), 106 (7.6), 105 (94.1), 77 (39.6), and 51 (11.7).

2.14.2 Hydrolysis of N-cyclopent-1-enyl-N-ethylbenzamide

Similarly the title compound was hydrolysed with

one equiv. of 23 atom-% [^{18}O]water. The hydrolysis was completed in 10 min. Distillation (Kugelrohr) afforded 23 atom-% [^{18}O]cyclopentanone, yield 47 %, b.p. 70-90 ^{0}C at 98 mmHg.

IR (CCl_4) : C=^{16}O (1750 cm^{-1}) and C=^{18}O (1710 cm^{-1}) in the ratio of ca 4 : 1.

2.14.3 Hydrolysis of [PhCH(Cl)-N(COPh)Et]

A solution of benzoyl chloride (1.13 g, 8 mmol) in dry ether (20 cm^{3}) was stirred, and PhCH=NEt (1.33 g, 10 mmol) added. A clear homogeneous solution was observed initially which gradually turned cloudy. After stirring for 0.5 h, 23 atom % [^{18}O]water (0.18 cm^{3}, 9.74 mmol) was added. Effervescence occurred as HCl gas formed. Two layers appeared after stirring for 15 min. The ether layer was removed and the viscous layer, which is mainly NH(COPh)Et and some PhCH=NH(Cl)Et was washed with ether (3 x, 5 cm^{3}). The ether solvent was removed and distillation (Kugelrohr) afforded 23 atom % [^{18}O]benzaldehyde (728.6 mg, 86 %), b.p. 60-70 ^{0}C at 9 mmHg. The fraction which did not distil over, was extracted into MeOH and crystallized from this solvent. ^{1}H NMR spectroscopy showed this crystalline material to be N-ethylbenzamide (645 mg, 4.4 mmol).

IR (CCl_4) : C=^{16}O (1710 cm^{-1}) and C=^{18}O (1682 cm^{-1}).

23 atom-% [^{18}O]Benzaldehyde, m/z: 109 (1.6), 108 (30.4), 107 (38.1), 106 (100.0), 105 (98.1), 78 (16.0), 77 (98.0), and 51 (27.6).

2.14.4 Hydrolysis of N-cyclohex-1-enyl-N-benzylbenzamide

The title compound was prepared on a small scale by reacting N-cyclohexylidene-N-benzylamine (101 mg, 0.534 mmol) with one equiv. of benzoyl chloride and triethylamine in $CDCl_3$ (0.5 cm^3). The enamide was formed within 10 min and the $CDCl_3$ was evaporated. Petroleum-ether (40-60) was added to extract the enamide. The insoluble $Et_3\overset{+}{N}HCl$ salt was discarded. A yield of 106 mg (69 %) of enamide was obtained. This enamide was directly hydrolysed with one equiv. of unlabelled water and 0.05 mequiv. of HCl. ^{1}H NMR spectrosopy after several hours revealed complete hydrolysis to cyclohexanone and N-benzylbenzamide.

^{1}H NMR ($CDCl_3$) for title enamide : 1.2 - 1.5 [4H, m, $-CH_2CH_2-$], 1.7 - 1.9 [4H, m, $-CH_2-CH=C-CH_2-$], 4.72 [2H, s, NCH_2Ph], 5.17 [1H, t, -CH=C-], and 7.1 - 7.5 [5H, m, Ar H].

2.14.5 Hydrolysis of EtCH=C(Me)N(COPh)CH_2Ph and PrC(=CH_2)N(COPh)CH_2Ph

To PrC(Me)=NCH_2Ph (122 mg, 0.7 mmol) in CH_2Cl_2 (0.5 cm^3) was added one equiv. of triethylamine and benzoyl chloride . ^{1}H NMR spectroscopy showed that the enamide appeared as two isomers in the ratio of 3 : 1. TLC : (silica-gel F 254, ether) gave two spots

with Rf 0.61 and Rf 0.48. Visualisation of the plate under a u.v. lamp also indicated a product ratio of ca 3 : 1. The enamide was also formed in these proportions when Hünig's base was used. Work-up of the triethylamine reaction gave a 78 % yield of enamide. When this enamide was hydrolysed with one equivalent of unlabelled water and 0.05 mequiv. of HCl in $CDCl_3$ (0.5 cm^3), ^1H NMR spectroscopy showed only two products, pentan-2-one and the by-product N-benzylbenzamide, which crystallised from the solution.

For $EtCH{=}C(Me)N(COPh)CH_2Ph$, ^1H NMR ($CDCl_3$) : 0.52 [3H, t, $MeCH_2CH{=}CMe$], 1.62 [3H, s, $CH{=}CMe$], 1.60 [2H, qt, $MeCH_2CH{=}C$], 4.67 [2H, s, CH_2Ph], 4.84 [1H, t masked by the isomer protons, $CH{=}CMe$] and 7.1 - 7.54 [10H, m, isomers of Ar H].

For $PrC(=CH_2)N(COPh)CH_2Ph$, ^1H NMR ($CDCl_3$) : 0.74 [3H, t, $MeCH_2CH_2$], 1.30 [2H, qt, $MeCH_2CH_2$], 1.90 [2H, t, $CH_2(C{=}CH_2)N$], 4.77 [2H, d, CH_2Ar], and 7.1 - 7.54 [10 H, m, protons from the two isomers Ar H].

2.15 N-ETHYLCAMPHORIMINE HYDROCHLORIDE

2.15.1 Preparation

The title compound was prepared by titrating N-ethylcamphorimine (1.57 g, 8.8 mmol) in ether (50 cm^3) with one equiv. of dry HCl. A white precipitate

formed immediately and was removed by filtration. The ^1H NMR spectrum of this iminium salt was recorded (see below and cf Fig. 2). The structure of this iminium salt was confirmed by reversible hydrolysis to the starting imine. Thus, after dissolving the iminium salt (192 mg, 0.8 mmol) in water (2 cm^3) followed by addition of 12.5 M NaOH (0.2 cm^3, 2.5 mmol) and stirring for 5 min, work-up by extraction into ether gave the imine material identified by its ^1H NMR spectrum.

^1H NMR ($CDCl_3$): 0.88, 1.02, and 1.48 [each 3H, s, 3 x C-Me], 1.46 [3H, t, NCH_2Me], 1.3 - 2.5 [6H, m, $-CH_2CH_2CH$ and H-3 endo], 2.95 [1H, dd, J 20 Hz, H_{gem}; J 5 Hz, H_{vic}, H-3 exo], and 3.78 [2H, q, NCH_2].

2.15.2 Hydrolysis

N-Ethylcamphorimine hydrochloride (68.7 mg, 0.3195 mmol) and one equiv. of water (0.006 cm^3) were dissolved in deuterated DMSO (0.5 cm^3) in a NMR tube. The solution was kept in an oil bath heated to 150 °C for 24 h. The hydrolysis was monitored by ^1H NMR. The sample was cooled, and was then diluted with water (10 cm^3). Petroleum ether (40-60) (10 cm^3) was added to extract the camphor. The petroleum ether was washed with saturated NaCl and then dried (Na_2SO_4). Sublimation onto a cold finger afforded pure camphor (40 %).

2.16 RESULTS AND DISCUSSION

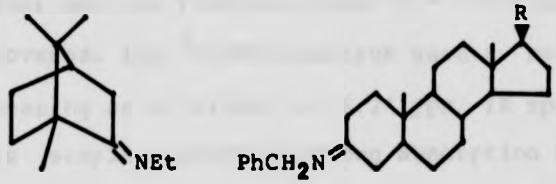
2.16.1 Imines

Table 1 lists a number of aldimines and ketimines that were prepared. The aldimines were prepared by direct addition of an aldehyde to ethylamine using KOH as a dehydrating agent. These reactions were conveniently monitored by either ^{1}H NMR or IR spectroscopy. Ketones, especially cyclohexanone and pentan-2-one, would only form imines if they were reacted with the primary amine in the presence of molecular sieves (type 3A) as a dehydrating agent. Attempts to prepare a ketimine by azeotropic distillation of a mixture of cyclopentanone and benzylamine in toluene gave after 7 h a poor recovery of the imine. Pentan-2-one was found to condense with benzylamine in the presence of KOH, if the mixture was heated at 60 °C. However, with molecular sieves 3A at room temperature these reactants gave after 13 h complete imine formation (with KOH only ca 50 % imine was formed). A sterically hindered ketimine (61) was prepared by refluxing d-camphor and ethylamine in toluene with a Lewis acid catalyst ($TiCl_4$), a procedure described in the literature [9]. Steroidal ketimines of structure (62) and (63) were prepared in CH_2Cl_2 solvent containing molecular sieves 3A. These condensations took 5 days at r.t. to complete, compared to the reaction of androsterone with n-butylamine, which has been reported to form a

ketimine in 3 weeks at r.t. in the presence of molecular sieves 4A. The aldimines and ketimines in Table 1 were obtained by distillation under reduced pressure of the crude imines. The freshly distilled imines were colourless liquids, and were best stored at 4 ^{o}C or below to avoid deterioration. The physical properties of the imines are shown in Table 1, together with data for those imines that were found in the literature. The imino-compounds were characterised by a sharp -C=N- absorption in the IR region [89, 90]. The ^{1}H NMR spectra of the aldimines showed a characteristic methine (CH=N) resonance at ca 7.5 - 8.0 ppm. We did not record the mass spectra of the imines, because it had been shown [91], by mass spectrometry, that samples of aldimines kept at 4 ^{o}C for several days contained a large amount of impurities due to the self-condensation of the aldimines.

Table 1: Aldimines and Ketimines (RR'C=NR")

R	R'	R"	Yield (%)	B.p. (^{o}C)
H	Me	Et	54	47, Lit. [91], 46
H	Bu	Et	42	67-68/95 mmHg, Lit.[92], 125-127
H	Me$(CH_2)_6$	Et	58	82/15 mmHg, Lit.[93], 89/21 mmHg
H	Ph	Et	60	85/14 mmHg, Lit.[94], 98-99/22 mmHg
-$(CH_2)_4$-		Et	64	74-78/74 mmHg
-$(CH_2)_5$-		CH_2Ph	46	76-80/0.04 mmHg, Lit. [95], 130/5 mmHg
Me	Pr	CH_2Ph	50	46/13 mmHg



(61) R = $MeCH(CH_2)_3CH(Me)_2$ (62)

Yield (58 %) R = OH (63)

b.p. 88 ^0C/9 mmHg

2.16.2 Hydrolysis of iminium salts

Initially, a method we examined for preparing [^{17}O]/[^{18}O]-carbonyl compounds involved the hydrolysis of protonated aldimines or ketimines. Thus, pentylideneethylamine in excess ether solvent containing 1 mol equiv. of water was titrated with an equiv. of HCl. However, this procedure resulted in the precipitation of a white insoluble iminium salt. The ^1H NMR spectrum of this salt is shown in Fig. 1. This salt decomposed on prolonged contact with moisture giving a characteristic odour of pentanal. A solution of $BuCH{=}\overset{+}{N}Et(HCl)$ in D_2O, monitored by ^1H NMR spectroscopy, gave in 3 h signals for pentanal and $Et\overset{+}{N}H_2Cl$. Complete hydrolysis was observed after 24 h at room temperature. We found that refluxing octylideneethylamine (10 mmol) in ether (100 cm^3) with 1 mol equiv. of water, followed by the slow addition of one equiv. of HCl over a 2 h period, then removal of ether solvent, and distillation from a Kugelrohr apparatus, afforded 21 % octanal. In addition, a

higher boiling fraction from 70 - 110 °C at 17 mmHg was recovered. Its ^{1}H NMR spectrum gave a methine signal appearing as a triplet at 6.24 ppm. IR spectroscopy of this sample showed a strong absorption at 1690 cm^{-1}. This spectroscopic data showed that the by-product had the structure R'(CHO)C=CHR", where R' = C_6H_{13} and R" = C_7H_{13}. This structure was the result of an acid catalysed aldol-type condensation of octanal. BuCH=NEt underwent a similar aldol-type condensation with CF_3COOH in $CDCl_3$, to give R'(CH=NEt)C=CHR", where R' = C_3H_7 and R" = C_4H_9 , as shown by ^{1}H NMR spectroscopy. Protonated imines, e.g RCH=$\overset{+}{N}$H(Cl)Et where R is C_4H_9 and C_7H_{15}, were thus found to be unsuitable as derivatives for the preparation of isotopically enriched carbonyl compounds. These aldimine salts were not soluble in many organic solvents and were readily decomposed by moisture.

Although the alkylidene iminium salts were found to be generally unsatisfactory for preparing [^{17}O]/[^{18}O]-carbonyl compounds, a sterically hindered ketimine salt, prepared by reacting together N-ethylcamphorimine and HCl, was suitable for preparing e.g. [^{18}O]camphor. This ketimine salt was stable for 1 week in air at r.t. after which the characteristic smell of camphor could be detected. Heating N-ethylcamphorimine hydrochloride in deuterated DMSO with one equiv. of water at 150 °C for > 24 h gave a mixture whose ^{1}H NMR spectrum indicated that camphor and ethylamine hydrochloride had been formed. The camphor was obtained by direct sublimation

after dilution of the reaction mixture with water, extraction with petroleum ether and evaporation of the solvent.

2.16.3 Hydrolysis of N-1-haloalkylamides

We have shown that protonated aldimines were not suitable for preparing ^{18}O-labelled aldehydes. We therefore studied another imine derivative. Thus, addition of one equiv. of PhCOCl to BuCH=NEt in $CDCl_3$ gave BuCH(Cl)-N(COPh)Et. Addition of one equiv. of water to this derivative and monitoring by ^1H NMR spectroscopy indicated that some hydrolysed products were formed (pentanal and N-ethylbenzamide). However, as hydrolysis was slow and incomplete, addition of a tertiary base (1/10 equiv triethylamine) gave the two hydrolysis products immediately and some $Et_3\overset{+}{N}HCl$. When this mixture was kept for 24 h, aldol condensation occurred, as judged by the weakening of the formyl signals. Similarly, octylideneethylamine reacted with acetyl chloride to give the N-1-haloalkylamide adduct, which was also hydrolysed readily by water (with catalytic Et_3N) to octanal, MeCONHEt and $Et_3\overset{+}{N}HCl$. The ^1H NMR spectrum of this solution after 24 h gave a characteristic triplet resonance at 6.28 ppm, indicative of aldol condensation of the octanal. However, the N-1-haloalkylamide adduct of PhCH=NEt and benzoyl chloride was readily hydrolysed to benzaldehyde with one equiv. of water without any added base. Besides, benzaldehyde, lacking a C-2 proton, cannot form an aldol condensation product.

Using [^{18}O]water in this hydrolysis, we obtained 23 atom-% [^{18}O]benzaldehyde. An IR spectrum of this compound is shown in Fig. 4. We also observed that addition of aldimine to an acylating agent followed by triethylamine, before addition of water, did not give hydrolysis products. Some enamide formation was observed in the ^{1}H NMR spectrum. The enamides were stable to aqueous base but not aqueous acid. Their suitability for preparing [^{17}O]/[^{18}O]-carbonyl compounds is now described.

2.16.4 Enamides

Table 2 lists a number of enamides prepared as described by the method of Breederveld [41]. Only enamide (67) was found in the literature [95]. The enamides (64), (65) and (66) were readily prepared by reacting the corresponding imines with benzoyl chloride in ether, followed by the addition of triethylamine and stirring the mixture at r.t. for 24 h. The addition of the base before benzoyl chloride, does not affect the yield of the enamide obtained. Either procedure resulted in the precipitation of a trialkylammonium salt. The enamide obtained was purified by quick filtration through grade 3 alumina or by direct distillation. The enamide was obtained as a clear or pale yellow liquid which was best stored at -20 °C. Elemental analyses of enamide (65) and (66) were not satisfactorily obtained.

Enamides (67) and (68) were prepared on a

small scale by reacting the ketimines with benzoyl chloride and triethylamine in $CDCl_3$ or CH_2Cl_2. The reaction was followed in a NMR tube. The haloalkane solvent was removed and the enamide was extracted with petroleum ether (40-60) or pentane, leaving behind insoluble $Et_3\overset{+}{N}HCl$.

Enamide (68) was shown by ^1H NMR spectroscopy to be a mixture of isomers (Scheme 62). When Hünig's base was used instead of triethylamine, the same proportion of isomers was observed. The intermediate (70) has available both C-1 methyl and C-2 methylene protons, which can be readily removed by a base. ^1H NMR spectroscopy showed preference for removal of C-1 methyl protons and hence isomer (71) predominated over the other isomer (72) in the ratio of 3 : 1. A sterically hindered ketimine prepared from d-camphor did not give an enamide with benzoyl chloride or acetyl chloride after treatment with triethylamine in ether or haloalkane solvents at r.t. However, we found that camphor enamide (69) was obtained after refluxing the camphor ketimine with acetyl chloride and triethylamine in $CHCl_3$ for 4 days. Even then, some starting ketimine was recovered. Reacting this ketimine with benzoyl chloride and triethylamine under similar conditions did not produce the corresponding enamide.

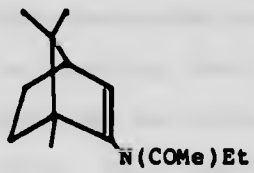
We attempted alternative procedures for preparing enamides from e.g oximes (Barton et al) and amides

(Ben-Ishai *et al*). However, the ^1H NMR spectra of the enamides prepared from a cyclohexanone- and a steroidal-oxime showed weak alkene signals, which appeared at different chemical shifts compared to the enamide prepared from imine, acetyl chloride and a base. The method described by Ben-Ishai *et al*, i.e. direct condensation of an aldehyde with an amide to give an enamide, under acid-catalysed conditions, appeared to give the enamide contaminated with amidals.

Table 2: Enamides [RR'C=CR"N(COPh)R"']

	R	R'	R"	R"'	Yield (%)	B.p. (^oC)
(64)	H	H	H	Et	49	66-67/0.04 mmHg
(65)	Pr	H	H	Et	63	88/0.01 mmHg
(66)	H	$-(CH_2)_3-$		Et	49	86-88/0.04 mmHg
(67)	H	$-(CH_2)_4-$		CH_2Ph	69	... Lit. [95], (m.p. 73-74 ^oC)
(68)	Et	H	Me	CH_2Ph	78*	
	H	H	Pr	CH_2Ph		

*obtained as a mixture (cf Scheme 62)



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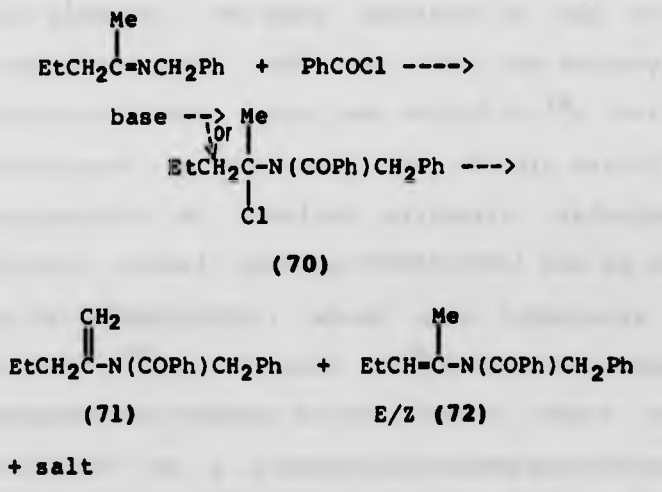


Scheme 62

2.16.5 Hydrolysis of Enamides

N-Pent-1-enyl-N-ethylbenzamide in dry ether was hydrolysed with one equiv. of 31 atom-% [^{17}O]water and 25 atom-% [^{18}O]water. HCl (1/20 equiv.) was added as a catalyst to the reaction, which was stirred at r.t. Hydrolysis gave a mixture of [^{17}O]/[^{18}O]pentanal and N-ethylbenzamide, (Scheme 63). Although the hydrolysis was mild, it was essential to add a polymeric base to neutralise the acid, to prevent further acid-catalysed aldol condensation of the aldehyde. N-Ethylbenzamide readily crystallised from the ether by cooling the solution. A sample of 25 atom-% [^{18}O]pentanal was reduced with $LiAlH_4$ to the alcohol, which was derivatised with 1-naphthyl isocyanate. The mass spectrum of this urethane derivative together with the IR spectrum of the [^{17}]/[^{18}O]pentanal indicated that most of the ^{17}O/^{18}O isotopes from the labelled water were transferred to

the aldehyde. The mass spectrum of the by-product N-ethylbenzamide indicated that the carbonyl of the amide functional group, was devoid of ^{18}O enrichment. Encouraged by the simplicity of this method for the preparation of labelled aliphatic aldehydes, we further showed that H_2C=CHNEt(COPh) can be converted to BrCH=CHNEt(COPh), which upon hydrolysis with 7 atom-% [^{17}O]water gave [^{17}O]bromoacetaldehyde. The compound was reduced to the alcohol which was then converted to a 1-naphthylurethane derivative (73), from which the ^{17}O content can be determined from a mass spectrum taken of this sample (cf Chapter 3). Furthermore, the hydrolysis of enamide (66), with 23 atom-% [^{18}O]water, gave 23 atom-% [^{18}O]cyclopentanone. The versatility of enamides for the preparation of $^{17}O/^{18}O$ aldehydes and ketones was further demonstrated with enamide (t) and enamide (68). Hydrolysis of enamides (67) and (68) with one equivalent of water and catalytic HCl followed by ^1H NMR in $CH_2Cl_2/CDCl_3$ gave spectra indicative of the corresponding ketones together with N-benzylbenzamide. A limitation in the application of enamide hydrolysis for the preparation of labelled aldehydes or ketones appeared with enamide (69), which was expected to give a labelled camphor. Instead, attempted hydrolysis produced a complicated ^1H NMR spectrum in the alkyl region. This may have arisen from an acid-catalysed rearrangement of an intermediate cation (cf rearrangements of norbornyl cations) [96].

$$RCH{=}CHN(COR')Et \xrightarrow[---------]{H^+/H_2^{\bullet}O} RCH^{\bullet}O + NH(COR')Et$$

R = Pr, R' = Ph R = Bu

Scheme 63

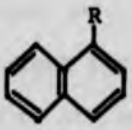
2.16.6 Reactions of enamides

Literature reports indicate that enamides are reactive towards powerful electrophiles such as bromine [58], ozone, peracids, and lead (IV) acetate [49]. We decided to explore some reactions of our enamides that were known for the analogous enamines. For example, we monitored by ^1H NMR spectroscopy possible reactions of the enamides (64) and (66) with methyl iodide and acrylonitrile, respectively, in $CDCl_3$ or deuterated acetone. However, under these conditions. alkylation and Michael-type addition did not occur.

But the enamides may be useful intermediates for preparing deuterated aldehydes. Thus, enamides RCH=CHN(COPh)Et where R is H or a butyl group, on hydrolysis with one equiv. of D_2O and 1/20 equiv. DCl for 3 h, gave deuterated aldehydes and NHCOPhEt. The ^1H NMR spectrum of a deuterated pentanal gave the formyl proton resonance as a singlet at 9.64 ppm.

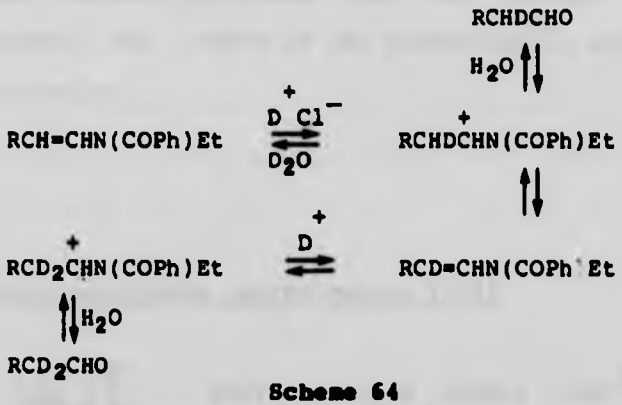
The deuterium content was determined by reducing the deuterated pentanal to the corresponding alcohol with $LiAlH_4$. The ^1H NMR spectrum of this product in CCl_4 showed resonances indicative of $PrCD_2CH_2OH$ (the

hydroxymethyl resonance at 3.5 ppm appeared as a doublet). The mass spectrum of the 1-naphthylurethane derivative (74) of this deuterated pentanol gave m/z : 260 (2.5), 259 (15.5) 258 (24.8) and 257 (9.3). The mass spectrum of undeuterated 1-naphthylcarbamic acid, pentyl ester showed m/z : 259 (0.3), 258 (2.9) and 257 (16.4). Hence, there was 21 % undeuterated, 53 monodeuterated and 26 % dideuterated pentanal with atom-% d = 53 %. The m.s. data indicated mono- and dideuteration during hydrolysis of PrCH=CHN(COPh)Et. A mechanism invoking an equilibrium protonation would account for this observation (Scheme 64).



R = NH-CO-^{17}O-CH_2-CH_2-Br (73)

R = NH-CO-O-CH_2-CD_2-Pr (74)



Scheme 64

2.17 IR spectra of $^{17}O/^{18}O$-carbonyl compounds

Halmann and Pinchas [97] showed in 1958 that the

C=^{18}O band of benzophenone appears at 1635 cm^{-1}, whereas the C=^{16}O band is at 1664 cm^{-1}. This 29 cm^{-1} shift is similar to those obtained by numerous observers studying esters, ketones, and aldehydes [98]. Fig. 5 shows the IR spectrum for 31 atom-% [^{17}O]pentanal. The replacement of a light isotope by a heavy one in a molecule leads to a shift of the lines in the Raman and IR spectra. This isotope shift, always taking a course toward lower frequencies, can be calculated to a first approximation by means of the harmonic oscillator approximation (see below for an example on a 31 atom-% [^{17}O]pentanal). Using this infrared technique, it is impossible to determine the exact extent of ^{17}O incorporation. The molar extinction coefficient for the heavy isotope band has been shown not to be the same as for the ^{16}O band, so the extinction coefficients are not yet predictable. A rough comparison of the peak sizes of the ^{17}O/^{18}O aldehydes and ketones prepared indicates that the isotope C=O band approaches the atom-% of the theoretically possible ^{18}O incorporation.

Harmonic oscillator approximation [135]

$\nu = \frac{1}{2\pi c}\sqrt{\frac{k}{\mu}}$, where ν = wave number (cm^{-1}), c = velocity of light, k = force constant and μ = reduced mass (assuming k to be the same in both cases); the following expression is obtained for the ratio of the absorption frequencies:

$$\mu = \frac{ma \times mb}{ma + mb}$$

Thus, for example, 31 atom-% [^{17}O]pentanal

$$\frac{\nu(C{=}{}^{17}O)}{\nu(C{=}{}^{16}O)} = \sqrt{\frac{\mu(C{=}{}^{17}O)}{\mu(C{=}{}^{16}O)}}$$

$$= \sqrt{\frac{12 \times 16}{12 \times 17}\,\frac{(12 + 17)}{(12 + 16)}} = 0.9873$$

Similarly, for ^{18}O-nucleus, the ratio = 0.9759

The isotopic shift, $\Delta\nu$ = 1712 (1 - 0.9873)

= 21 cm^{-1} (^{17}O)

The isotopic shift, $\Delta\nu$ = 1697 (1 - 0.9759)

= 40 cm^{-1} (^{18}O)

These calculated values (21 cm^{-1} and 40 cm^{-1}) are in fair agreement with the observed values of 19 cm^{-1} and 33 cm^{-1} respectively for the ^{17}O and ^{18}O nuclei (cf. Fig. 5).

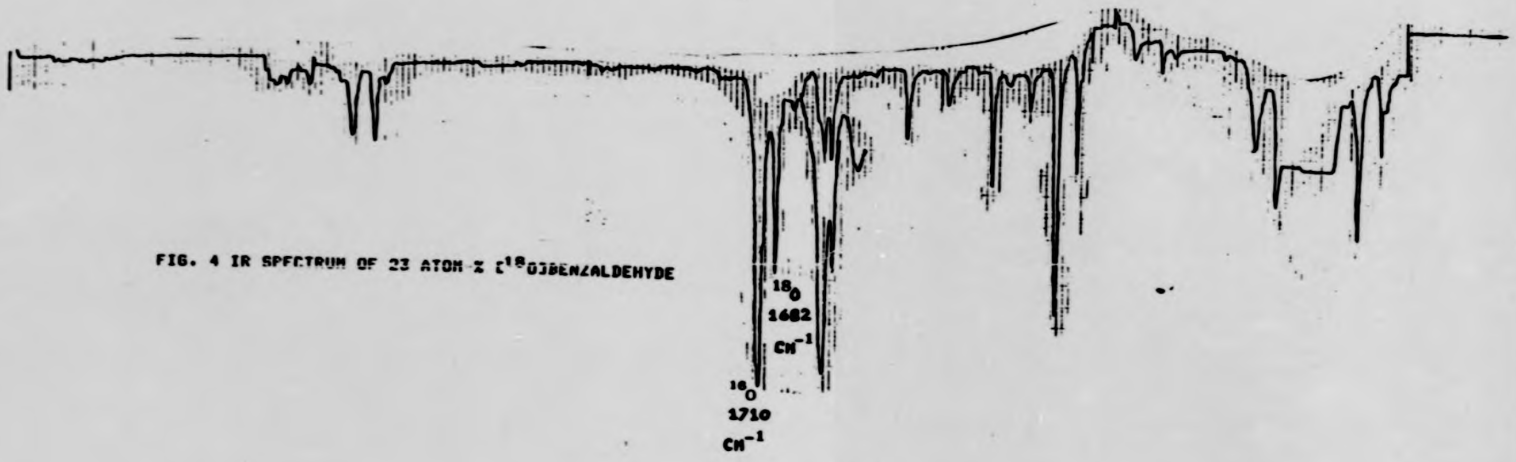


FIG. 4 IR SPECTRUM OF 23 ATOM % [^{18}O]BENZALDEHYDE

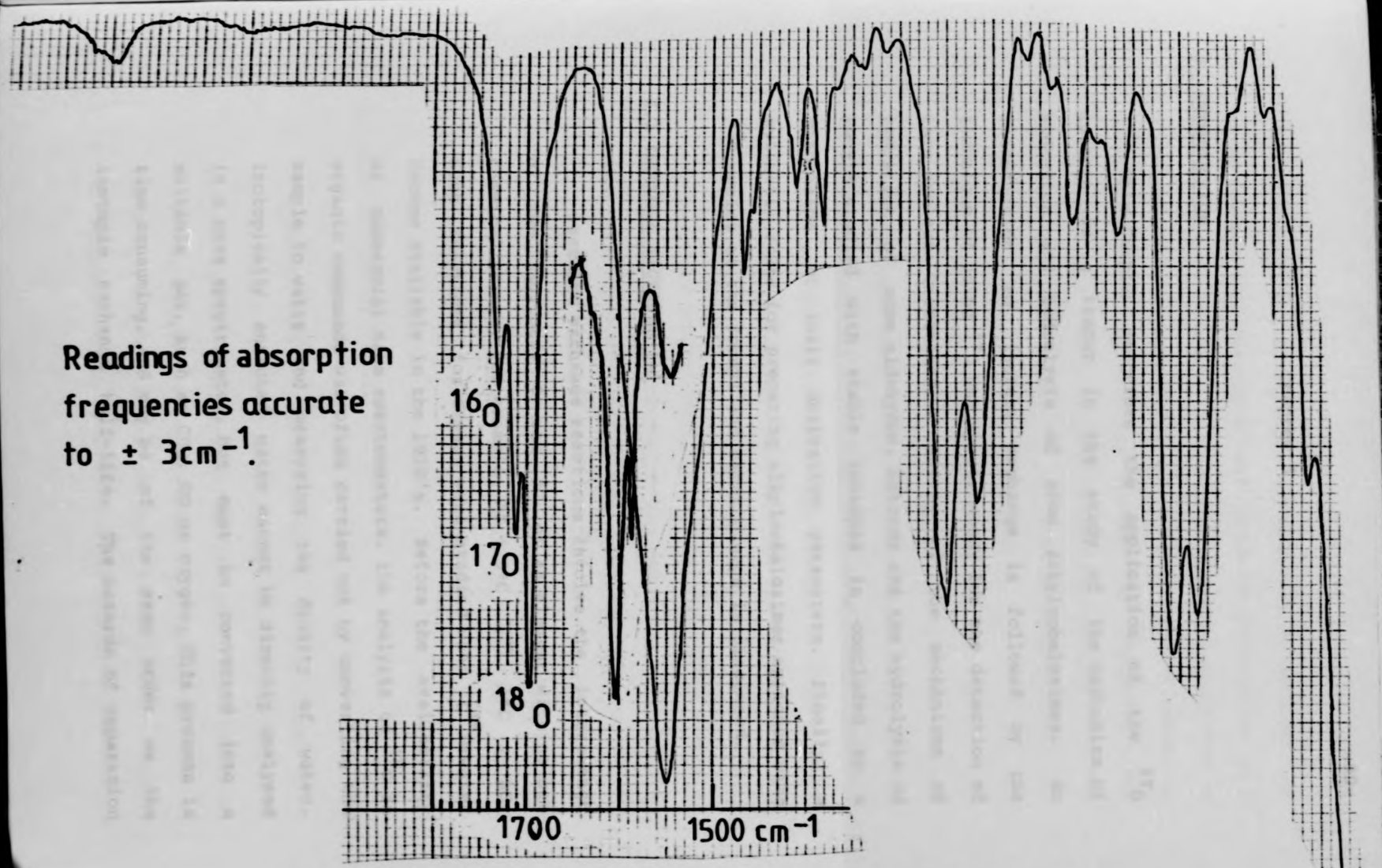


Fig.5 IR SPECTRUM OF 31 ATOM-% ^{17}O 1PENTANAL

Chapter 3

3. INTRODUCTION

This Chapter describes the application of the ^{17}O isotope as a tracer in the study of the mechanism of hydration and hydrolysis of some alkylcobaloximes. An introduction to isotope exchange is followed by the technique of using ^{17}O NMR spectroscopy for the detection of this nucleus. Thereafter, a survey of the mechanisms of hydration of some aldehydes, ketones and the hydrolysis of esters studied with stable isotopes is concluded by a discussion of their activation parameters. Finally, a synthetic route for preparing alkylcobaloximes enriched with ^{17}O nuclei at the formyl and ester groups is discussed.

3.1 ISOTOPIC EXCHANGE [1]

Isotopic exchange reactions involve the interchange of two isotopes, C and *C of an element with no net chemical change, for example, AC + B*C $\rightleftharpoons$ A*C + BC. Such reactions for oxygen were studied when [^{18}O]water became available in the 1930's. Before the availability of commercial mass spectrometers, the analysis of ^{18}O in organic compounds was often carried out by converting the sample to water and measuring the density of water. Isotopically enriched water cannot be directly analysed in a mass spectrometer, but must be converted into a suitable gas, such as CO_2, CO or oxygen. This process is time consuming, and may be of the same order as the isotopic exchange half-life. The hazards of separation

and purification of organic compounds may be avoided by analysing the reactants and products in solution by a spectroscopic method. ^{17}O NMR spectroscopy provides a means of overcoming these drawbacks. Oxygen exchange *in situ* may be conveniently studied by following the changes with time of the intensity of the relevant absorption peaks during the exchange reaction.

3.2 NMR OF SENSITIVE NUCLEI [2, 3]

Among the three stable oxygen isotopes, only ^{17}O has a nuclear spin, I = 5/2, and thus shows magnetic properties. The sensitivity of a nucleus to investigation by an NMR experiment depends on the magnitude of its magnetic moment, μ. At constant field, the signal strength is proportional to $(I + 1)/I^2 \times \mu^3 B_0^2$, where B_0 = applied field strength. In addition, the natural abundance is a critical factor. The NMR spectroscopy of ^{17}O was thus restricted in early years by the low concentration of its nuclei in molecules with natural isotopic distributions. The so-called receptivity of ^{17}O relative to hydrogen (^1H) is $1.08 \times 10^{-5} : 1$.

3.3 SPECTRAL LINEWIDTH [3]

Nuclei with $I > 1/2$ possess a nuclear quadrupole moment Q as a result of the non-spherical distribution of nuclear charge. These nuclei can interact with electrical field gradients in the environment, especially those of the electron shell in the molecule in which the nucleus is situated. These interactions contribute to relaxation

phenomena. The nuclear magnetic relaxation time (T_1) is a function of molecular motion and can be expressed as $1/T_1 = f\tau_c$, where τ_c is the so-called rotational correlation time of a given nucleus. For a spherical molecule in a continuous medium, τ_c is related to temperature T, solution viscosity η and the molecular radius a: $\tau_c = 4\pi\eta a^3/3KT$ (Eq. 1). Eq. 1 can be used qualitatively to relate sample conditions to observed line-widths. Line narrowing may be achieved either by increasing temperature, or using solvent of low viscosity, or a diluted sample. Eq. 1 also implies that smaller molecules will give better resolved spectra. For relatively small molecules line-widths are typically from several tens to several hundred Hz, when observing ^{17}O nuclei (cf < 1 Hz for ^{1}H or ^{13}C nuclei). If the molecular diameter is large, the molecular tumbling time becomes relatively slow and line-widths of 1 kHz or over may be obtained.

3.4 ^{17}O NMR SPECTROSCOPY [3, 4]

Advances in NMR instrumentation, particulary pulse FT and increased availability of ^{17}O-enriched materials, have overcome some of the difficulties with ^{17}O nuclei. The precise balance between degree of enrichment and amount of signal accumulation will depend on the relative time and cost factors involved in each alternative. These limitations are compensated for by the large chemical shifts often observed for ^{17}O-compounds, which aid in the resolution of quadrupole broadened resonances. Thus, chemical shifts of ^{17}O resonance lines extend over a

range of about 1000 ppm. The time required to obtain a ^{17}O-spectrum ranges from a few minutes to an hour or more. This is for relatively small molecules studied as neat liquids or concentrated solutions in a sample tube of 10 mm diameter using a few grams or cm^3 of sample. However, diffulties accumulate for larger molecules because of their weak signals caused by limited solubility and/or broad line-widths.

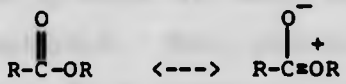
3.4.1 Chemical shifts of ^{17}O-compounds [4]

Chemical shifts for ^{17}O resonances of oxygen compounds are shown in **Table 3**. There is a clear distinction between the ^{17}O resonance of the ether-like linkage (-O-) and those of the carbonyl groups. For example, in methyl acetate, the carbonyl oxygen resonates at lower field (355 ppm) compared to the methoxyl at 137 ppm (relative to water as internal standard). Primary alcohols and ethers absorb close to the ^{17}O resonance of water, but branching of the attached hydrocarbon groups causes substantial chemical shifts downfield. The ^{17}O resonance of the bridging oxygen in an ether-like site (R-O-R) is shifted downfield, if one of the R groups is replaced by a π-accepting substituent such as an acyl group, which introduces π-character into the bridging oxygen (**Scheme 65**). A carboxylic acid gives a single chemical shift value about half-way between the -O- and $-\overset{|}{C}=O$ resonances of esters, because of averaging caused by rapid proton transfer between the intermolecular hydrogen bonded species (**Scheme 66**).

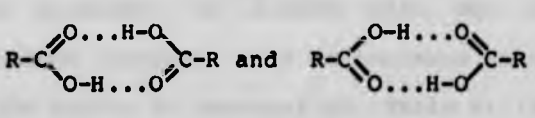
The $-\overset{|}{C}=O$ resonances of aldehydes and ketones around 600 ppm are shifted upfield by substituents which are electron-releasing, such as an amino group. This effect weakens the carbonyl π-bond (**Scheme 67**). Recently, ^{17}O NMR spectroscopy has been used to study the ^{17}O chemical shift in a number of substituted benzaldehydes, acetophenones [5], cyclohexanones [6], and alcohols [7]. The configurational differences in the diastereomers of cyclic 2'-deoxyadenosine 3',5'-[^{17}O,^{18}O]monophosphate were determined by ^{17}O NMR spectroscopy, from which the stereochemical course of the hydrolysis of this nucleotide was distinguished [8]. The magnetic non-equivalence of diastereotopic oxygens in sulphones has been reported [9].

Table 3. ^{17}O chemical shifts and linewidths for selected organic compounds in the liquid state [cf 3]

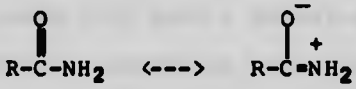
Compound	δ/ppm	$W_{1/2}$ Hz
MeOH	37	60
EtOH	7.4	110
Bu^tOH	66.2	600
Et_2O	12	90
MeCOOMe	137	120
MeCOOH	250	110
$(MeCO)_2O$	265	130
$MeCONH_2$	286	200
Me_2CO	572	45



Scheme 65



Scheme 66



Scheme 67

3.5 HYDRATION OF ALDEHYDES AND KETONES

The hydration of an aldehyde or ketone [10 a-d] is described by Scheme 68. The equilibrium for hydration depends on the nature of the groups attached to the carbonyl functional group and to the nature of the solvents. Formaldehyde is almost completely hydrated in aqueous solution, acetaldehyde is about 60 % hydrated, whereas acetone is scarcely hydrated at all. In strong donor solvents, e.g. pyridine and DMSO, formation of the hydrate is far more favourable than in water or other poor donor solvents [11]. The factors influencing the equilibrium are partly electronic, partly steric. Electron donors stabilise the partial positive charge on the carbonyl carbon atom; bulky groups destabilise the tetrahedral diol; both effects discourage addition. The equilibrium constants for the hydration of aldehydes and ketones via the gem-diol have been determined from u.v. [12] , ^{17}O NMR [10 c], and ^{1}H NMR spectroscopy [10 c].

The hydration is both general acid- and general base-catalysed. The general acid-base catalysis is related by Eq. 2, where k_{Obsd} is the pseudo-first order rate constant. In accord with Eq. 2, the rate of hydration increases with an increase in the concentration of the buffer at constant pH (Table 4) [12].

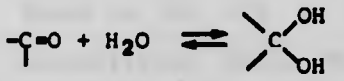
Jencks [15] gave a detailed account of the mechanism of addition reactions to carbonyl compounds. Jencks proposed a concerted mechanism for the general acid- and general base-catalysed hydration of carbonyl compounds (Scheme 69). General acid-catalysis involves the concerted addition of HX (H_2-[^{18}O]) and transfer of a proton. General base-catalysis involves the concerted removal of a proton from the attacking reagent, to facilitate attack at the carbonyl group. In the back reactions, the roles of the acid and base catalysts are reversed, thus completing the ^{18}O exchange. Bell et al [16] have shown that the kinetic order with respect to water, for the uncatalysed hydration of $CH_2ClCOCH_2Cl$ in aqueous dioxan and aqueous MeCN, is close to 3. They suggested that 3 molecules of water are present in the transition state, as in structure (75). From the energies of activation and solvent isotope effects, they proposed a stepwise mechanism [17].

Rates of ^{18}O-exchange for a range of aromatic aldehydes and ketones in acidified THF [18] and acidified acetonitrile [19] have been measured by IR spectroscopy. Oxygen exchange in acetone, isopropyl methyl ketone and pinacolone has been studied by following the decay of the

^{17}O-NMR signal of the ^{17}O-labelled ketone in aqueous buffers [20 a-b]. The carbonyl and gem-diol resonances are separated by 500 ppm, so attainment of equilibrium can be conveniently monitored ($T_{1/2}$ varies from 6 min to 2 h).

Table 4. Comparison of the rate constants for hydration of some simple aldehydes

Aldehyde	Medium	pH	Temp	k_{obsd}	$T_{1/2}$	Ref
Propanal	0.01M acetic acid	4.28	0 °C	$12.28 \times 10^{-3}\ s^{-1}$	-	[12]
Propanal	0.1M acetic acid	4.17	0 °C	$24.7 \times 10^{-3}\ s^{-1}$	-	[12]
Propanal	0.005M phosphate	7.16	0 °C	$3.12 \times 10^{-3}\ s^{-1}$		[12]
HCHO	water	-	20 °C	$9.8\ s^{-1}$	70 ms	[13]
MeCHO	0.002M phosphate	7.00	15 °C	-	ca 2 min	[14]
MeCHO	0.034M phosphate	7.00	15 °C	-	ca 15 s	[14]

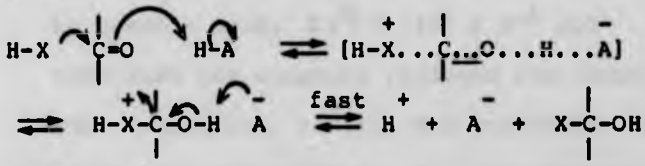


Scheme 68

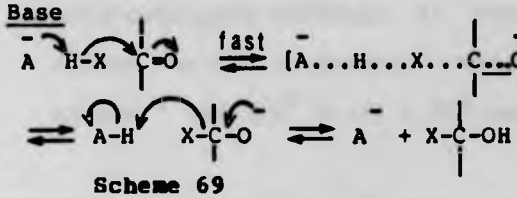
$$k_{obsd} = k_o + k_{H_3O^+}[H_3O]^+ + k_{OH^-}[OH^-] + k_{HA}[HA] + k_{A^-}[A^-]$$

Eq. 2

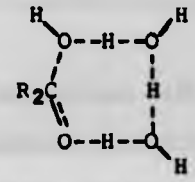
Acid



Base



Scheme 69



(75)

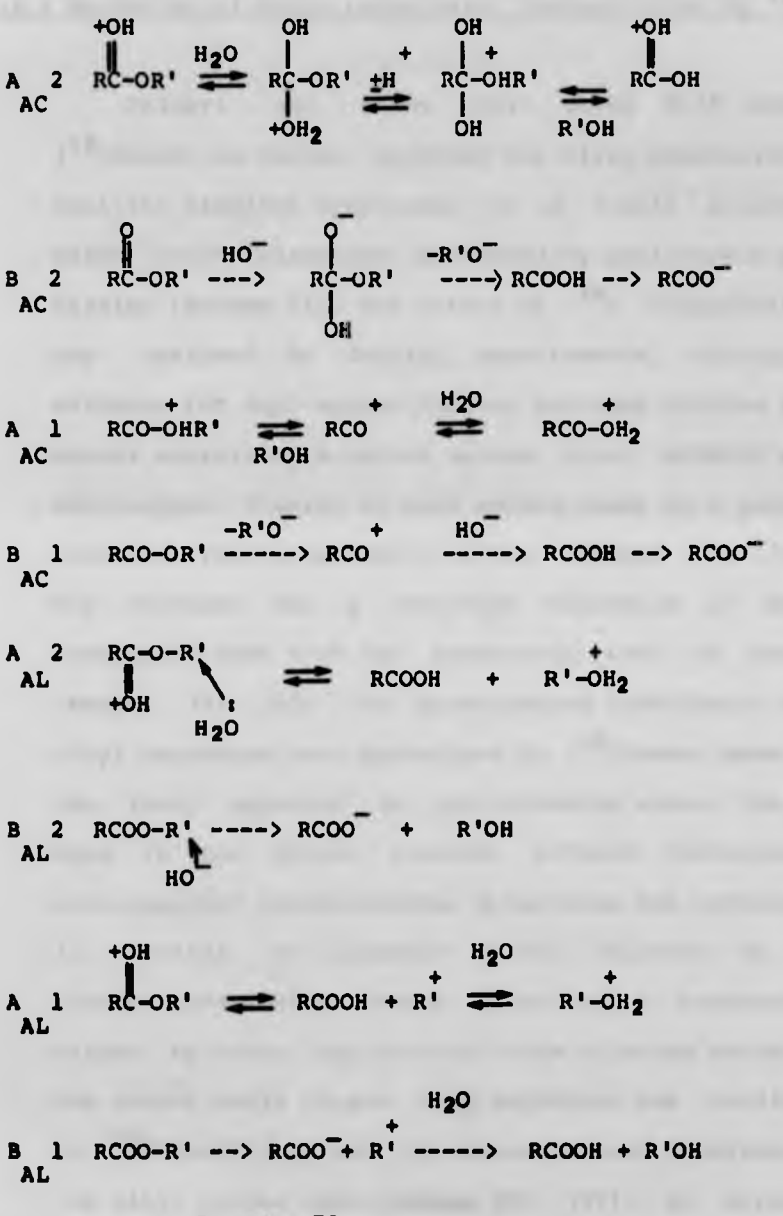
3.5.1 Thermodynamic data for hydration of $-\overset{|}{C}=O$ compounds

From the rates and equilibrium constants for the reversible acid- and base-catalysed hydration of isobutyraldehyde, the standard enthalpy ($\Delta H^{\ominus}$) and entropy ($\Delta S^{\ominus}$) for isobutyraldehyde hydration was found to be -23 kJ mol^{-1} and -83 J K^{-1} mol^{-1}, respectively [21]. These values are comparable to those obtained by Pocker and Dickerson [12] for propanal ($\Delta H^{\ominus}$, -23 kJ mol^{-1}; $\Delta S^{\ominus}$, -77 J K^{-1} mol^{-1}), as well as data for 2,2-dimethylpropanal and isobutyraldehyde. Bell and Sorensen [16] have shown that the entropy of activation, $\Delta S^{\ddagger}$ for the hydration of $ClCH_2COCH_2Cl$ in aqueous dioxan is -271 J K^{-1} mol^{-1}, a large and negative value, which is consistent with a cyclic transition state containing two extra

water molecules. The less negative value for catalysis by benzoic acid, $\Delta S^{\neq} = -197$ J K^{-1} mol^{-1}, supports the view that the catalyst replaces one water molecule in the transition state. The activation parameters for acid-catalysed exchange of benzophenone in aqueous dioxan for the temperature range 40-75 ^{0}C are $\Delta H^{\neq} = 78$ kJ mol^{-1} and $\Delta S^{\neq} = -81$ J K^{-1} mol^{-1} [16].

3.6 INGOLD'S CLASSIFICATION OF ESTER HYDROLYSES [22 a-b]

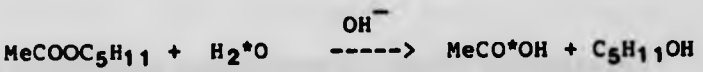
Mechanisms of ester hydrolysis, for which there are 8 possible combinations (Scheme 70), are given the following designations; $A_{AC}2$, $B_{AC}2$, $A_{AC}1$, $B_{AC}1$, $A_{AL}2$, $B_{AL}2$, $A_{AL}1$, and $B_{AL}1$. The letter A denotes an acid-catalysed reaction. B means an uncatalysed or base-induced reaction. The subscripts AC (Acyl) or AL (Alkyl) denote which carbon-oxygen bond is cleaved. The number 1 or 2 is the molecularity of the reaction, namely unimolecular or bimolecular. The acid-catalysed reactions are reversible, whereas base-induced reactions are irreversible due to the formation of carboxylate ions from the carboxylic acid. The $A_{AC}2$ and $B_{AC}2$ routes are common among primary and secondary alkyl esters. The $A_{AC}1$ route is observed in strongly acidic solution for esters of hindered aromatic acids. The $B_{AC}1$ route is unknown. The $B_{AL}2$ route occurs only in exceptionally severely hindered acyl groups and in the neutral hydrolysis of β-lactones. The $A_{AL}2$ route is unknown. Tertiary alkyl (aryl) esters (e.g. Me.CO.OCPh$_3$) in neutral (or acidic) solution react by the $B_{AL}1$ ($A_{AL}1$) mechanism [23]. In alkaline solution a competitive $B_{AC}2$ process may occur.



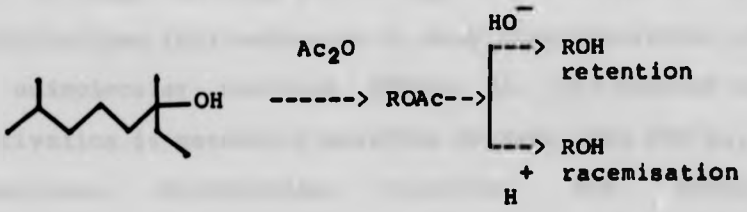
Scheme 70

3.6.1 Mechanism of ester hydrolysis: investigation by ^{18}O-labelling

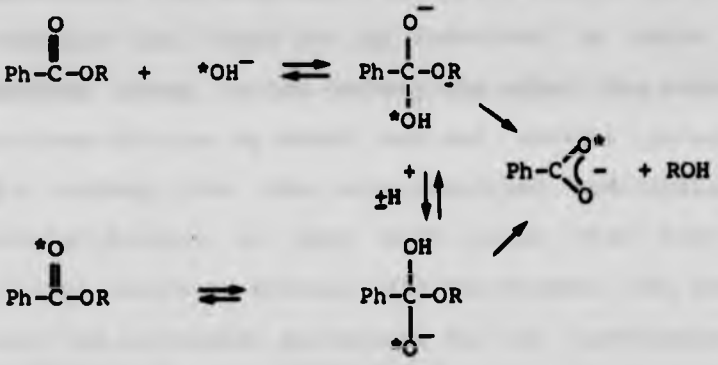
Polanyi and Szabo [24] using 0.35 atom-% [^{18}O]water as tracer, provided the first demonstration that the alkaline hydrolysis of a simple aliphatic ester (n-pentylacetate) proceeded by acyl-oxygen bond fission (Scheme 71). The extent of ^{18}O incorporation was analysed by density measurements. Structural evidence for acyl-oxygen fission includes studies with esters containing a chiral centre (i.e. RCOOCHR'R"). Acyl-oxygen fission of such esters leads to a product alcohol which is optically active (Scheme 72) [25]. The evidence for a two-stage mechanism in ester hydrolysis came from the pioneering work of Bender (Scheme 73) [26] for base-induced hydrolysis. When alkyl benzoates were hydrolysed in [^{18}O]water some of the label appeared in the unreacted ester. The key step is the proton transfer between isotopically distinguished intermediates. β-Lactones are hydrolysed in neutral or slightly acidic solution by the alkyl-oxygen fission route involving a bimolecular attack by water, due to this route offering relief of the severe angle strain. This mechanism was confirmed by ^{18}O-labelling and by stereochemical inversion at the alkyl carbon atom (Scheme 74) [27]. In strongly acidic solution the alkoxyl oxygen can be protonated in a rapid pre-equilibrium and the ring can then be opened by unimolecular acyl-oxygen cleavage.



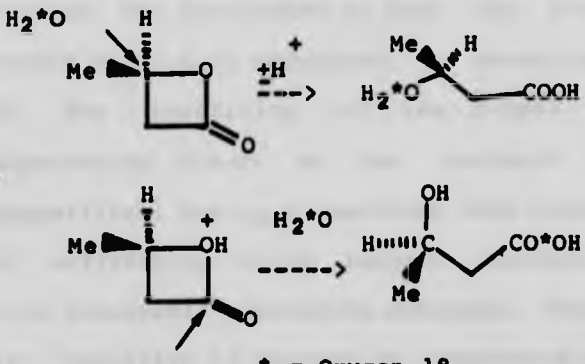
Scheme 71



Scheme 72



Scheme 73



* = Oxygen-18

Scheme 74

3.6.2 Activation parameters for ester hydrolysis

A high enthalpy and entropy of activation for an alkyl-oxygen (AL) mechanism is very characteristic of a unimolecular reaction (Table 5). The entropy of activation is generally positive or near zero for $A_{AL}1$ reactions. Unimolecular reactions are mostly encountered with tertiary alkyl esters and similar substrates that dissociate to form a relatively stable carbonium ion. These are S_N1 reactions, in which the leaving group is the carboxylate anion. The reaction is thus affected by added ions and solvent polarity. The entropy for the acid-catalysed hydrolysis of t-butyl formate is very much lower than that of t-butyl acetate. Stimson [28] and Salomaa [29] showed that the activation parameters for the hydrolysis of t-butyl formate are comparable to those of ethyl formate. The conclusion is that the t-butyl formate reacts by an $A_{AC}2$ mechanism. The exception here is due to the reactivity of the formyl group making bimolecular attack at the carbonyl group more competitive. The $A_{AL}1$ reactions have higher enthalpies of activation, which largely compensates for their more favourable activation entropies. These reactions are sensitive to changes in temperatures, and become important at higher temperatures, even for t-butyl formate [29].

The $B_{AL}1$ uncatalysed mechanism is less common. An example appears to be the hydrolysis of t-butyl

trifluoroacetate in aqueous acetone for which a high value of enthalpy comparable with other unimolecular dissociations is observed, but for which the entropy is -18 J K^{-1} mol^{-1} [30]. The small negative entropy value is compatible with neither the $A_{AL}1$ nor $A_{AC}2$ mechanism (part of the $\Delta S^{\neq}$ term for $A_{AL}1$ comes from the pre-equilibrium protonation, for which $\Delta S^{\ominus}$ is probably slightly positive).

Table 5. Activation parameters for the hydrolysis of esters

Esters	Temp ^{o}C	Medium	$\Delta H^{\neq}$(kJ mol^{-1})	$\Delta S^{\neq}$(J K^{-1} mol^{-1})	Mechanism	
a t-Butylacetate	15-35	aq HCl	109	54.8	$A_{AL}1$	[31]
b t-Butylformate	15-45	aq HCl	59.4	-88.7	$A_{AC}2$	[29]
c Ethylformate	15-45	aq HCl	62	-86	$A_{AC}2$	[29]
d Methylmesitoate	25-45	9.8M H_2SO_4	120	70	$A_{AC}1$	[32]
e t-Butyltrifluoroacetate	25-45	70 % acetone	102	-18	$B_{AL}1$	[30]

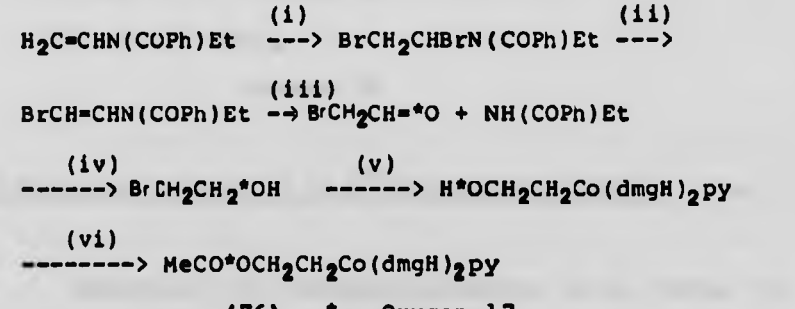
3.7 SYNTHETIC ROUTE TO [^{17}OAc]-2-ACETOXYETHYL(PYRIDINE)COBALOXIME

The title cobaloxime (76) was prepared as outlined in **Scheme 75.** The enamide H_2C=CHN(COPh)Et undergoes rapid bromine addition in CCl_4. An attempt to brominate with N-bromosuccinimide gave unidentified products. The compound $BrCH_2CHBrN(COPh)Et$ was not isolated (N.B. compound (77) has been obtained as a crystalline material by Bohme *et al* [33].) Crude BrCH=CHN(COPh)Et was purified by filtering through alumina and eluting with dry CCl_4. Hydrolysis of this brominated enamide with 7 atom-% [^{17}O]water gave [^{17}O]$BrCH_2CHO$ and a precipitate of N-ethylbenzamide. $BrCH_2CHO$ is a powerful lacrymator and readily forms the trimer [34]. The reduction of $BrCH_2CHO$ to the corresponding halohydrin was effected by a hydride-transfer reagent (**Section 3.7.2.**).

3.7.1 α-Halocarbonyl compounds

As shown above, enamides can be used to prepare α-haloaldehydes or α-haloketones. The classic preparation of such compounds involves treatment of the ketone or aldehyde with molecular bromine, chlorine or occasionally iodine in alkaline solution (**Scheme 76**) [35]. This method depends on the ease of enolisation of the carbonyl compound. The saturated aldehydes have been brominated at the α-position with bromine in DMF, containing p-toluene-sulphonic acid as catalyst [36], or in ether with a small quantity of dioxan [37]. In some cases, α-bromoaldehydes may be

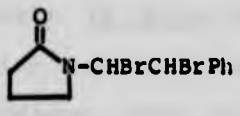
prepared from trimethylsilyl enol ethers [38]. Reactions of a trialkylborane with α-bromoacrolein in aqueous THF gave an α-bromoaldehyde (Scheme 77). If the reaction mixture contained triethylorthoformate, stable α-bromoacetals could be obtained directly [39]. The described procedures are invalid for preparing [^{17}O]haloaldehydes. The enamines [40], when treated with bromine/water, gave haloaldehydes and this method could be suitable for making labelled haloaldehydes (Scheme 78). Bromodiethylacetal, a commercially available acetal, when exposed to water/catalytic HCl, failed to hydrolyse under the conditions which are satisfactory for 2-bromovinyl-N-ethylbenzamide.



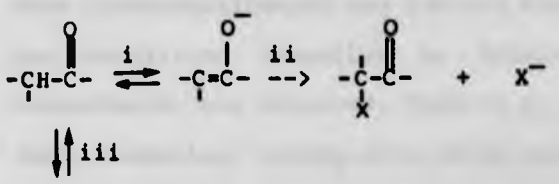
(76) * = Oxygen-17

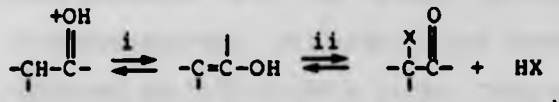
(i) Br_2/CCl_4 (ii) Et_3N (iii) cat. $H^+/H_2{}^*O$
(iv) $Zn(BH_4)_2$/ether (v) $BrCo(dmgH)_2py/NaBH_4$/ethanol
(vi) acetic anhydride/pyridine

Scheme 75



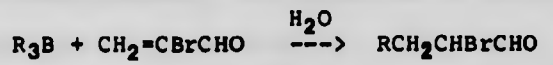
(77)



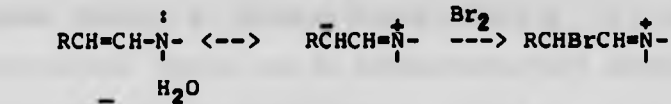


i, base; ii, Br_2, fast step; iii, H^+

Scheme 76



Scheme 77



Scheme 78

3.7.2 Selective reduction of [^{17}O]bromoacetaldehyde

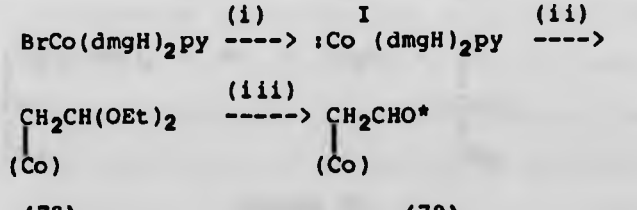
Reduction of bromoacetaldehyde with $NaBH_4$ or $LiAlH_4$ gave poor results. $NaBH_4$ in ethanol solvent can form traces of ethoxide ion, causing the product [^{17}O]bromoethanol to undergo a base-catalysed cyclisation to form ethylene oxide. The crude reaction mixture from reducing bromoacetaldehyde with $NaBH_4$ was reacted directly *in situ* with cobaloxime(I) in ethanol, which gave an unsatisfactory yield of $HOCH_2CH_2Co(dmgH)_2py$ (note that cobaloxime(I) can displace epoxide). Sakrikar [41] showed that the reduction of $BrCH_2COBr$ with $LiAlD_4$ in excess of ether solvent for 2 h at -5^{0}C afforded 52 % of $BrCH_2CD_2OH$.

When bromoacetaldehyde was reduced with $LiAlH_4$ under the conditions described by Sakrikar, only 10 % of bromoethanol was obtained. Nystrom et al [42] found that combining $LiAlH_4$ with $AlCl_3$ rendered the $LiAlH_4$ less alkaline and the mixed hydride reduction of 3-bromopropionyl chloride to the bromohydrin could be achieved at 35°C in 80 % yield. They also showed that $LiAlH_4$ in ether at -15 °C can give an 80 % yield of 3-bromopropanol. The reduction of halogen was shown to be slow or prevented by using the mixed hydride. $Zn(BH_4)_2$ has similar reducing powers to $NaBH_4$ but is less basic. A prostaglandin-bearing a carbonyl functional group and an alkali-sensitive lactone-ring was selectively reduced with $Zn(BH_4)_2$, leaving the lactone-ring intact [43].

We found that $BrCH_2CHO$ was satisfactorily reduced with $Zn(BH_4)_2$ in ether. The four-step synthesis (Scheme 75) leading to [^{17}O]bromoethanol, including the reduction of [^{17}O]$BrCH_2CHO$ with $Zn(BH_4)_2$, afforded a distilled fraction of this alcohol in an overall yield of 27 %. Also, we showed that $Zn(BH_4)_2$ could reduce bromoacetylbromide to the halohydrin in 72 % yield at r.t. within 10 min. The [^{17}O]bromoethanol was derivatised with 1-naphthyl isocyanate to the 1-napthylurethane. The extent of the [^{17}O]-label in the [^{17}O]bromoethanol was determined from the electron impact mass spectrum of its derivative.

3.8 [^{17}O-FORMYL]FORMYLMETHYL(PYRIDINE)COBALOXIME (79)

[^{17}O-Formyl]Formylmethyl(pyridine)cobaloxime (79) was prepared by hydrolysing the cobaloxime (78) with one equiv. of 31 atom-% [^{17}O]water and 1/20 equiv. HCl, (Scheme 79). The amount of ^{17}O isotope in (79) was estimated from the intensity of the isotopic carbonyl absorption in the IR spectrum. A mechanism for the hydrolysis of the cobaloxime (78) is shown in Scheme 80 [44]. In addition, acid decomposition can cause cobalt-carbon bond cleavage (cf Scheme 81) [45]. Schrauzer showed that acidic decompostion of the cobaloxime (78) gave MeCHO, together with ethanol. Dolphin et al were able to detect another intermediate, ethyl vinyl ether. The $NaBH_4$/ethanol method for preparing the cobaloxime (78) from $BrCo(dmgH)_2py$ and $BrCH_2CH(OEt)_2$ gave a yield of 48 %. Schrauzer was able to prepare $(MeO)_2CHCH_2Co(dmgH)_2py$ (Scheme 82), but the yield was low (14 %). Subsequent acid hydrolysis of this cobaloxime gave $OCHCH_2Co(dmgH)_2HOH$ - the yield was not reported [46]. Dolphin et al [47] showed that an electron-rich olefin such as ethyl vinyl ether could interact with the electron deficient cobaloxime(III) via a π-complex, which then reacted with ethanol to form the compound (78). Dolphin et al was unable to prepare this cobaloxime pure, and so they chromatographed the material on silica-gel, which caused hydrolysis of (78) to cobaloxime (79), which was then crystallised (Scheme 83).

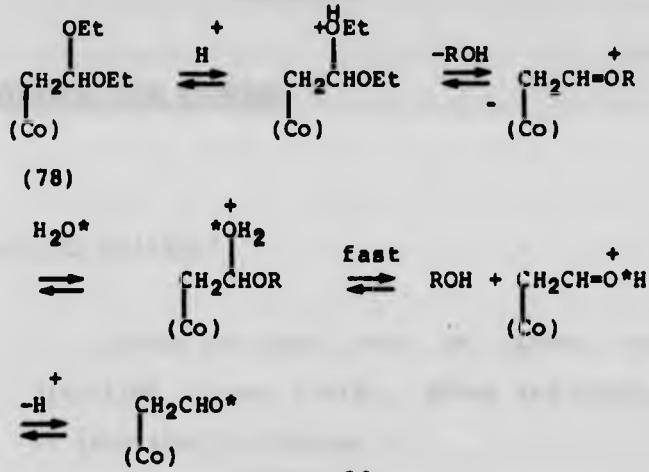


(78) (79)

(i) $NaBH_4$/EtOH (ii) $BrCH_2CH(OEt)_2$

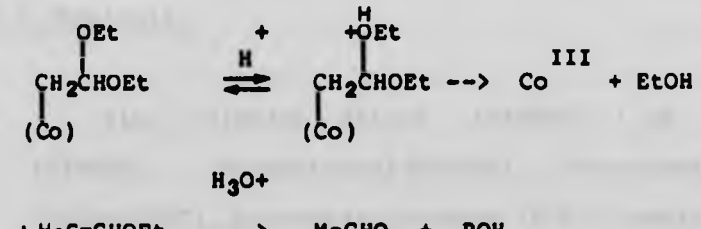
(iii) *[^{17}O]water/cat. HCl/CH_2Cl_2

Scheme 79

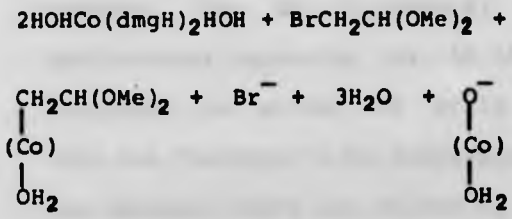


(78)

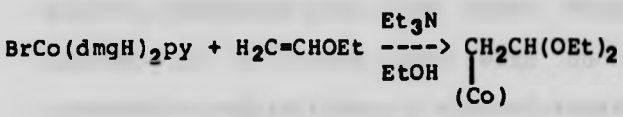
Scheme 80



Scheme 81



Scheme 82



Scheme 83

3.9 MATERIALS AND METHODS

3.9.1 Dry solvents

Dioxan was kept over 3A sieves overnight and distilled from $LiAlH_4$. Ether and CH_2Cl_2 were dried as described in Chapter 2.

3.9.2 Chemicals

Zinc chloride sticks (FISONS). AR Bromine (FISONS). Bromoethanol (FISONS). Bromoacetaldehyde (KOCH-LIGHT). Bromoacetylbromide (BDH). Cobalt bromide (FISONS). Dimethyglyoxime (May and Baker). Silica-gel MN Kieselgel (Merck).

3.9.3 ^{17}O NMR measurements

The ^{17}O NMR spectra were obtained from enriched

samples (up to 31 atom-%) on a Bruker WH400 spectrometer operating at 54.24 MHz. Samples were contained in either 10 or 15 mm diameter tubes and were run "non-spin". No field-frequency lock was used. The spectral width was 40,000 Hz and free induction decays were stored as either 512, 1024 or 4096 data points, depending upon the exact experiment. Pulse angles of 90^0 were used with no delay between successive acquisitions; a typical repetition time was 0.05 seconds. Low power ^1H decoupling was employed. Temperatures were monitored and held constant ($\pm 1^0$) with a standard Bruker control unit. All free induction decays less than 4 K data points were zero filled to either 4 K or 8 K and an exponential multiplication function was used to enhance the signal to noise ratio. Chemical shifts are reported in p.p.m. relative to internal water at $\delta = 0$. Integrals were estimated from peak heights as the width at half height remained constant for each run. Kinetic runs were controlled by a standard microprogramme from the spectrometer's computer. Sufficient scans were obtained to allow reasonable signal to noise enhancement and repeated every 30 minutes or 1 h for the ^{17}O isotopically enriched cobaloximes, and every 18 seconds for the [^{17}O]pentanal.

3.10 EXPERIMENTAL

3.11 2,2'-DIETHOXYETHYL(PYRIDINE)COBALOXIME (78)

To bromo(pyridine)cobaloxime (4.49 g, 20 mmol) in ethanol (70 cm^3), deaerated by bubbling argon through the suspension, was added $NaBH_4$ (757 mg, 20 mmol), followed by bromoacetaldehyde diethylacetal (1.5 cm^3, 10 mmol). The reaction mixture was stirred for 1 h, and then air was bubbled through the mixture (0.5 h). Cold water was added to the solution giving a yellow precipitate of the title compound. The product (2.3 g, 48%) was dried in a vacuum desiccator containing a mixture of silica-gel and $CaCl_2$. The material was pure by TLC: silica-gel, F-254 (Merck); MeOH (1cm^3) : $CHCl_3$ (19 cm^3) : pyridine (0.1 cm^3); Rf 0.47. This cobaloxime was insoluble in ether or dioxan, but was soluble in CH_2Cl_2.

Found (CHN): C,46.99; H, 6.60; N, 14.46 %. $C_{19}H_{13}CoN_5O_6$ (MW 485) requires: C, 47.01; H, 6.64; N, 14.42 %.

^1H NMR ($CDCl_3$): 1.1 [6H, t, $(Me)_2$], 1.57 [2H, d, $CoCH_2$], 2.13 [12H, s, 4 x dmgH Me], 3.28 - 3.6 [4H, m, $(OCH_2)_2$], 4.29 [1H, t, CH], 7.3 [2H, t, meta py H], 7.71 [1H, t, para py H] and 8.85 [2H, d, ortho py H].

3.12 [^{17}O-FORMYL]FORMYLMETHYL(PYRIDINE)COBALOXIME (79)

2,2-Diethoxyethyl(pyridine)cobaloxime (400 mg, 0.82 mmol) was dissolved in CH_2Cl_2 (4 cm^3) and 31 atom-% [^{17}O]water was added, followed by a catalytic quantity of HCl (0.05 mequiv., 0.04 mmol) dissolved in ether. The hydrolysis was monitored by ^1H NMR spectroscopy - by observing the appearance of the formyl proton. TLC was

inadequate because the Rf's of the starting material and product were close to one another. The solution was stirred for 3 h, the solvent was removed, together with the by-product ethanol, and the solid dissolved in CH_2Cl_2 and filtered under suction through MN Kieselgel. The filtrate was evaporated to dryness giving dark red crystals of the title compound (340 mg, 100 %) . The IR absorption spectrum of the ^{17}O-enriched title compound indicated three carbonyl absorption peaks due to ^{16}O : ^{17}O : ^{18}O and these absorption intensities were in the ratio of 2 :1 : 2. A repeated experiment whereby, instead of purifying the material by chromatography, evaporation of the solvent and recrystallisation from(1 : 1)CH_2Cl_2 : cyclohexane at 4 ^0C was used; affording the compound in 82 % yield. Its IR carbonyl absorptions indicated that ^{16}O : ^{17}O : ^{18}O were in the ratio of 2 : 3 : 5. The carbonyl isotopic peaks were well resolved in a KBr disc, but in solution (CH_2Cl_2) a broad band was observed. ^1H NMR spectroscopy of the labelled compound, obtained by both methods of purification, indicated 6-7 % of desalkylated cobaloxime - 2.4 [12H, s, 4 x dmgH, Me] to be present. Hydrolysis of 2,2-diethoxyethyl(pyridine)cobaloxime with 100 equiv. of HCl or 1/20 equivalent of tosic acid was too extreme, leading to the precipitation of a large quantity of desalkylcobaloxime. ^1H NMR ($CDCl_3$): 1.8 [2H, d, J 5 Hz $CoCH_2$], 2.2 [12H, s, 4 x dmgH Me], 9.26 [1H, t, J 5 Hz, CHO], and 5 py H.

3.13 ZINC BOROHYDRIDE [48]

Anhydrous zinc chloride (4.0 g, 29 mmol) was suspended in dry ether (50 cm^3) and heated to reflux for 75 min. After cooling, insoluble particles were removed. The resultant ethereal solution of $ZnCl_2$ was added dropwise to a suspension of $NaBH_4$ (2.7 g, 71 mmol) in 150 cm^3 of dry ether within 10 min, and was allowed to stir for 33 h. The ethereal zinc borohydride thus prepared was filtered through a filter paper to remove suspended particles and was used immediately for the reduction.

3.14 2-BROMOVINYL-N-ETHYLBENZAMIDE

A solution of bromine (1.8 cm^3, 34.8 mmol) in CCl_4 (20 cm^3) was added gradually to $H_2C{=}CHN(COPh)Et$ (6.0 g, 34.3 mmol) diluted with CCl_4 (20 cm^3), over a period of 5 min. When the bromine colour had been discharged, an aliquot observed by ^1H NMR spectroscopy indicated the formation of $BrCH_2CHBrN(COPh)Et$. The solution was then treated with triethylamine (48 cm^3, 34.4 mmol) and left to stir for 2 h, during which time a heavy precipitate of the trialkylammonium salt appeared (identity confirmed by ^1H NMR). The suspension was filtered under suction either through a glass sinter or through grade 3 alumina (this removed the salt) and was eluted with more dry CCl_4 solvent giving the title compound (9.647 g, 80 %); pure by ^1H NMR spectroscopy. TLC: alumina F-254 (Merck); ether; Rf 0.54, gave a single blue spot visualized under u.v. The yield of $Et_3\overset{+}{N}HCl$ was 79 %.

$BrCH_2CHBrN(COPh)Et$, ^1H NMR (CCl_4): 1.35 [3H, t, Me], 3.53 [2H, m, NCH_2], 3.85 [1H, dd, J 5 Hz H-2 gauche to H-1], 4.07 [1H, t, J 12 Hz H-2 trans to H-1], 6.31 [1H, dd, J 5 Hz BrCHN], and 7.45 [5H, m, Ph H].

$BrCH{=}CHN(COPh)Et$, ^1H NMR ($CDCl_3$): 1.22 [3H, t, Me], 3.70 [2H, q, CH_2], 5.56 [1H, d, J 12.5 Hz BrCH], 7.06 [1H, d, J 12.5 Hz, BrCH=CH], and 7.39 [5H, s, Ph H]. $Et_3\overset{+}{N}HBr$ ($CDCl_3$): 1.45 [9H, t, $N(CH_2Me)_3$] and 3.2 [6H, m, $N(CH_2Me)_3$].

m/z: 255 (5.4), 253 (5.6), 174 (44), 105 (100), 77 (80), and 51 (20).

3.15 HYDROLYSIS OF BrCH=CHN(COPh)Et

To the above compound (9.6 g, 27.8 mmol) in dry ether (60 cm^3) was added 7.26 atom-% [^{17}O]water (0.5 cm^3, 27.8 mmol) followed by a catalytic quantity of ethereal HCl, 1/20 equivalent. The solution was stirred and the hydrolysis monitored by ^1H NMR spectroscopy and TLC. ^1H NMR spectroscopy showed signals for the [^{17}O]$BrCH_2CHO$ and EtCONHPh. TLC: alumina F-254 (Merck); ether; Rf 0.26 for EtCONHPh. The hydrolysis was completed after 29.5 h. A viscous brown syrup of NH(COPh)Et was obtained. The ether layer was decanted into another flask containing diisopropylaminopolystyrene (a polymeric base) (2.0 g, 1.2 mequiv/g) and the suspension stirred for 45 min. The polymeric base was removed and the ether supernatant was shown by ^1H NMR spectroscopy to contain mainly [^{17}O]$BrCH_2CHO$ and N-ethylbenzamide. It was used

directly for the next stage (reduction).

3.15.1 Preparation of [^{17}O]bromoethanol

The homogeneous ethereal solution containing the crude [^{17}O]$BrCH_2CHO$ and NH(COPh)Et was added to a freshly prepared $Zn(BH_4)_2$ in ether (100 cm^3, 13.9 mmol), over a period of 10 min at r.t.. Effervescence was observed. The reduction was rapid, ^{1}H NMR spectroscopy of an aliquot showing the absence of a signal due to the formyl proton, whereas the proton signals for EtCONHPh were intact. An insoluble white precipitate was observed. The mixture was cooled in dry-ice, and cold water (25 cm^3) was added cautiously. When effervescence had ceased, conc. H_2SO_4 (4 cm^3) diluted with water (25 cm^3) was added. The white particles dissolved and clear aqueous and ether phases were observed. The organic phase was removed and the aqueous phase further extracted with ether (25 cm^3, 3 x). The ether extract was combined and dried ($MgSO_4$). The ether was removed, and ^{1}H NMR spectroscopy of the crude product showed [^{17}O]$BrCH_2CH_2OH$ and NH(COPh)Et. Distillation (Kugelrohr apparatus) afforded [^{17}O]bromoethanol (936 mg, 27 %), b.p. 70-90 ^{0}C at 7-16 mmHg. The ^{1}H NMR spectrum agreed with that of authentic bromoethanol (Aldrich).

^{1}H NMR ($CDCl_3$): 3.55 [2H, t, CH_2OH] and 3.94 [2H, t, $BrCH_2$].

^{17}O NMR ($CDCl_3$): 1.35 (external ref. dioxan),

width at half-height = 100 Hz.

3.15.2 Reaction of Br-CH_2-CH_2-[^{17}O]-H with 1-naphthyl isocyanate

To 7 atom-% [^{17}O]bromoethanol was added a 10 % excess of 1-naphthyl isocyanate (0.049 cm^3, 0.34 mmol). The mixture was kept for 3 days in a glove box flushed with nitrogen. The mixture solidified and hot petroleum-ether (100-120) was added to the urethane. Filtration removed some insoluble urea by-product. The filtrate was cooled to 4 °C, whereupon white crystals of $C_{10}H_7NHCO$-[^{17}O]CH_2CH_2Br (24 mg, 27 %, m.p. 85 °C) were obtained. TLC: silica-gel, F-254 (Merck); ether; Rf 0.52. Lit. [49], m.p. 86-87 °C.

^{1}H NMR ($CDCl_3$): 3.6 [2H, t, CH_2Br], 4.52 [2H, t, $COOCH_2$], 7.02 [1H, s, NH broad], and 7.4 - 7.9 [7H, m, $C_{10}H_7$]. [^{17}O]-labelled urethane, m/z: 293 (5.7), 294 (1.4), 295 (6.6), 296 (1.5), 297 (1.1) and 169 (100). Unlabelled urethane, m/z: 293 (6.7), 294 (1.0), 295 (6.6), 296 (0.9), 169 (90.5), and 140 (100).

3.15.3 Preparation of [^{17}O]$HOCH_2$-CH_2-Co(dmgH)$_2$py

Argon was bubbled into ethanol (50 cm^3) for 10 min, followed by the addition of $BrCo(dmgH)_2py$ (3.2 g, 7.2 mmol). The suspension was stirred and kept under Argon. After 5 min $NaBH_4$ (541 mg, 14.3 mmol) was added to the suspension which became warm and turned dark blue. After 5 min, the mixture turned dark red when [^{17}O]bromoethanol (894 mg, 7.2 mmol) in 1 cm^3 of

ethanol was added. The suspension was stirred for 2 h and then air was blown into the suspension for 1/2 h. Water (100 cm^3) and pyridine (0.5 cm^3) were added, but did not precipitate the product, which was extracted into CH_2Cl_2 (50 cm^3, 5 x). The CH_2Cl_2 solution was concentrated and cyclohexane added until cloudiness appeared. When the mixed solvent was kept at -20 ^0C overnight, the title compound appeared as orange-brown crystals (934.2 mg, 31.6 %), pure by TLC. TLC: silica-gel, F-254 (Merck); MeOH (1 cm^3) : $CHCl_3$ (19 cm^3) : pyridine (0.1 cm^3); Rf 0.25. When the solvent from the mother liquor was removed, the crude title compound (921 mg, 31 %), which was then converted in the next stage to Ac-[^{17}O]-CH_2-CH_2-Co(dmgH)$_2$py.

^1H NMR ($CDCl_3$): 1.67 [2H, t, J 7 Hz, $CoCH_2$], 2.13 [12H, s, 4 x dmgH, Me], 3.0 [2H, t, 7 Hz, CH_2OH], and 5 py H.

^{17}O NMR ($CDCl_3$, 21 ^0C): 15.64 ppm; width at half-height = 800 Hz.

3.15.4 [^{17}OAc]-2-Acetoxyethyl(pyridine)cobaloxime

To crude [^{17}O]$HOCH_2CH_2$Co(dmgH)$_2$py (921 mg, 2.23 mmol) was added pyridine (5.0 cm^3), followed by acetic anhydride (1.0 cm^3, 10 mmol). The mixture was kept at r.t. overnight, whereupon the title compound crystallised out of the solution. The excess reagent and solvent were evaporated on a Büchi to a low volume. The crystals were filtered off and washed

with cold water affording an orange-red crystalline product (336 mg, 33 %). TLC: silica-gel, F-254 (Merck); MeOH (1 cm^3), $CHCl_3$ (19 cm^3), pyridine (0.1 cm^3); Rf 0.47.

^1H NMR ($CDCl_3$): 1.52 [2H, t, J 7 Hz, $CoCH_2$], 1.95 [3H, s, MeCo], 2.15 [12H, s, 4 x dmgH Me], 3.75 [2H, t, CH_2OAc] and 5 py H.

^{17}O NMR ($CDCl_3$, 21 °C, ext. ref. dioxan): 174 ppm; width at half-height = 1100 Hz.

3.16 [^{17}O]PENTANAL & [^{17}O-FORMYL]FORMYLMETHYL(PYRIDINE)COBALOXIME

3.16.1 Hydration of [^{17}O]pentanal

The hydration of [^{17}O]pentanal (0.026 cm^3, 0.24 mmol) was followed by ^{17}O NMR spectroscopy in 0.025 M citric - 0.05 M sodium phosphate buffer (3.0 cm^3), pH 4.5 and another run was carried out in 0.1 M sodium phosphate (3.0 cm^3), pH 6.8. The buffer was pre-equilibrated to 5 °C in the NMR probe, before the [^{17}O]pentanal was introduced. The progress of the hydration was followed by measuring the increase in intensity of the [^{17}O]water signal.

3.16.2 [^{17}O-Formyl]formylmethyl(pyridine)cobaloxime

The hydration of (79) (45 mg, 0.109 mmol) in dioxan (3 cm^3) : water (2 cm^3) was followed by

measuring the increase in intensity of the [^{17}O]water signal. Experiments were carried out at 40, 50 and 60 ^{o}C. After an overnight run, the aqueous solvent was removed and the solid dissolved in $CDCl_3$ to confirm that the unlabelled $OCHCH_2Co(dmgH)_2py$ was still intact (< 10 % of an aquated cobaloxime was obtained). The IR spectrum of the solid confirmed that the isotopic carbonyl bands (C=^{17}O and C=^{18}O) were replaced by the C=^{16}O band. A similar amount of (79) was dissolved in dioxan (3 cm^3) : 0.1M sodium phosphate (2 cm^3), pH 6.8, and the hydration was monitored at 50 ^{o}C.

3.17 HYDROLYSIS OF [^{17}OAC]-2-ACETOXYETHYL(PYRIDINE)COBALOXIME

This cobaloxime (60 mg, 0.132 mmol) was dissolved in dioxan (4 cm^3) by warming the solution. The solution was equilibrated in the NMR probe to 50 ^{o}C. Doubly distilled water (2.0 cm^3) was added and the rate of the hydrolysis followed by measuring the increase in intensity of the [$^{17}O_2$]acetate signals which appeared at ca 273 ppm. The intensity of the [$^{17}O_2$]acetate signal reached its maximum after 10 h and it remained constant. The solution was removed, frozen in dry-ice and attached to a freeze-drier unit. When the aqueous solvent had been removed (24 h), the solid $HOCH_2CH_2Co(dmgH)_2py$ was dissolved in $CDCl_3$/TMS. A ^{1}H NMR of this sample was recorded and showed > 90 % of 2-hydroxyethyl(pyridine)cobaloxime. The ^{17}O NMR of this sample was scanned for 1 h but no ^{17}O signal was detected from 0 - 500 ppm. A control experiment in which [^{17}O]H-CH_2-CH_2-Co(dmgH)$_2$py (50 mg, 0.1204 mmol) in aqueous dioxan (4 cm^3) : water (2 cm^3) containing one

equiv. of acetic acid was kept for 24 h at 50 °C gave, applying a similar work-up procedure to the above, no detectable loss of the ^{17}O-label. A duplicate experiment with the title cobaloxime was conducted and showed reproducibility of the above results.

3.18 RESULTS AND DISCUSSION

3.18.1 [^{17}O-Formyl]formylmethyl(pyridine)cobaloxime (79)

An IR spectrum of (79) is shown in Fig. 6. The two additional isotopically substituted carbonyl bands had absorptions at 1640 cm^{-1} ($C=^{17}O$) and 1630 cm^{-1} ($C=^{18}O$). The IR spectrum showed that the relative intensities of ^{16}O : ^{17}O : ^{18}O carbonyl absorptions were in the ratio of 2 : 3 : 5. This compound was prepared by the hydrolysis of $(EtO)_2CHCH_2Co(dmgH)_2py$ with 1 mol equiv. of 31 atom-% [^{17}O]water. The relative intensities of the IR carbonyl absorptions suggested that most of the isotopic label from the 31 atom-% [^{17}O]water was incorporated into the cobaloxime (79). When this cobaloxime was purified through a silica-gel column, its IR carbonyl absorptions showed ^{16}O : ^{17}O : ^{18}O in the ratio of 2 : 1 : 2. The decrease in the intensities of the isotopic C=O absorptions were a result of the acidicity of the silica-gel, which caused isotopic exchange between the labile aldehyde functional group and water absorbed on the silica-gel.

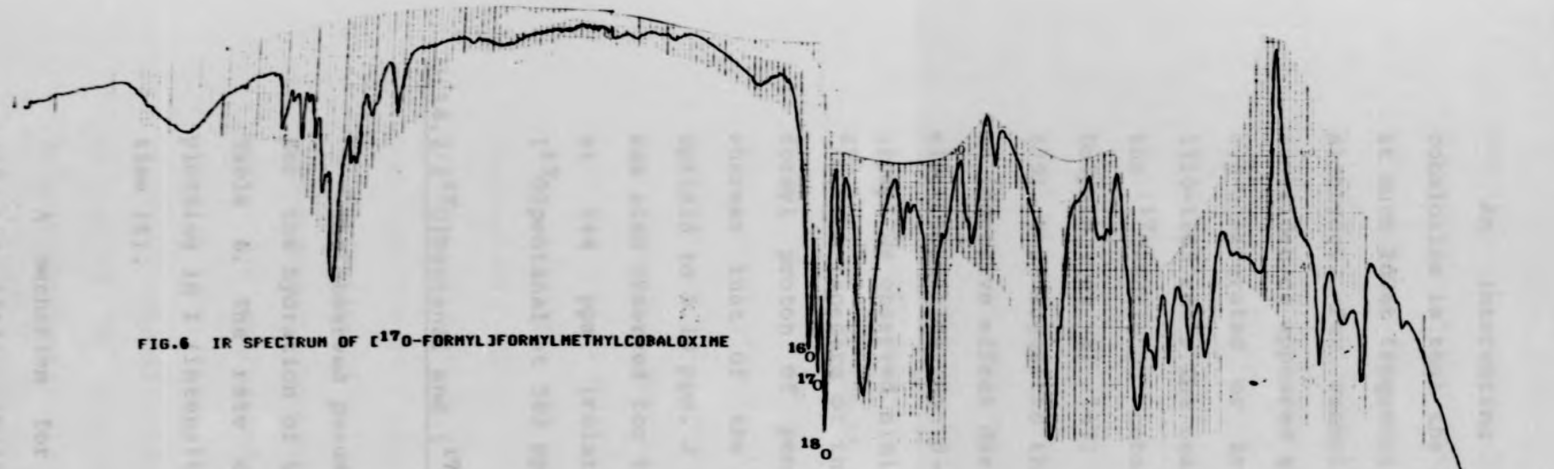


FIG.6 IR SPECTRUM OF [^{17}O-FORMYL]FORMYLMETHYLCOBALOXIME

^{16}O : ^{17}O : ^{18}O

1658 : 1640 : 1630 CM^{-1}

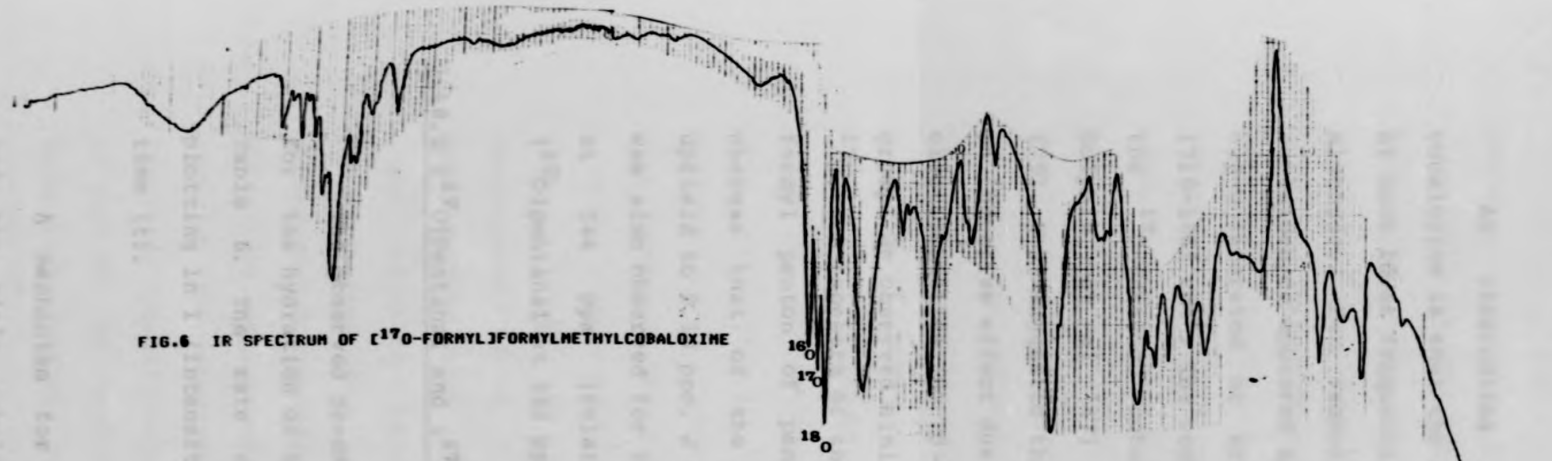


FIG.6 IR SPECTRUM OF [^{17}O-FORMYL]FORMYLMETHYLCOBALOXIME

^{16}O : ^{17}O : ^{18}O

1658 : 1640 : 1630 CM^{-1}

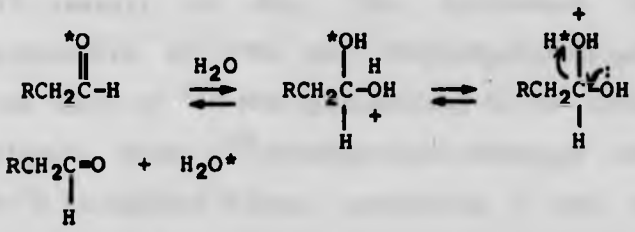
An interesting feature of this labelled cobaloxime is that the carbonyl stretching values were at much lower frequencies compared to typical organic aldehydes. For example, the carbonyl absorption of acetaldehyde appeared at 1730 cm^{-1} and that of an α,β-unsaturated or aromatic aldehyde in the region 1710-1685 cm^{-1}. The weakening of the carbonyl bands in the ^{17}O-enriched cobaloxime had been observed by Dolphin et al [47] on the unlabelled compound (79). They suggested that this phenomenon arose from an inductive effect due to the interaction of the more electropositive Co d-orbitals with the β-carbonyl group. We observed similar effects in the ^{1}H NMR and ^{17}O NMR spectra of this compound. The characteristic formyl proton of pentanal appeared at 9.66 ppm, whereas that of the labelled cobaloxime was shifted upfield to 9.10 ppm, J = 3.5 Hz. This upfield shift was also observed for the ^{17}O resonance which appeared at 544 ppm (relative to water) compared to [^{17}O]pentanal at 583 ppm.

3.18.2 [^{17}O]Pentanal and [^{17}O-formyl]formyl(pyridine)cobaloxime

The observed pseudo-first order rate constants for the hydration of the title compounds are shown in Table 6. The rate constants were obtained by plotting ln I (intensity of ^{17}O[water] signal) against time (t).

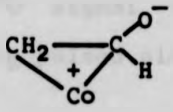
A mechanism for the hydration of [^{17}O]pentanal and the title cobaloxime, thought to go via a

gem-diol, is shown in Scheme 84. An interesting observation is that the kinetics of hydration of the cobaloxime (79) in aqueous dioxan, showed a half-life $T_{1/2}$= 4 h at 50 ^{0}C, which is extremely slow relative to aromatic aldehydes or even ketonic compounds. In accord with Eq. 2, the rate of hydration of a similar amount of (79), but in dioxan containing phosphate buffer (pH 6.8) at 50 ^{0}C, has a half life of 2 h. The lack of reactivity of the formyl group in $OCHCH_2Co(dmgH)_2py$ was evident when attempts to reduce the compound to $HOCH_2CH_2Co(dmgH)_2py$ with $NaBH_4$ and $LiAlH_4$ failed, although conversion can be achieved with diborane [50]. This lack of reactivity may be attributed to the occurrence of a significant sigma to π-hyperconjugation as in complex (80), thus deactivating the carbonyl groups towards nucleophilic addition by the water molecule. Brown [51] observed that the pKa of $HOOCCH_2Co(dmgH)_2py$ was 7.14 and that of $HO_2CCH_2CH_2Co(dmgH)_2py$ was 5.70. The weak acidity of the former complex was a consequence of σ - π hyperconjugation. σ-π Hyperconconjugation was described [52] as the stabilization of a neighbouring cationic centre by the delocalisation of a σ bond. The length or angles around such a σ bond are not changed as the transition state is approached. Such delocalisation without changing the reactant geometry is also known as 'vertical stabilisation'. This effect is in contrast to that of the bridged-ion theory of neighbouring group participation.



R = Pr or $-Co(dmgH)_2py$, * = Oxygen-17

Scheme 84



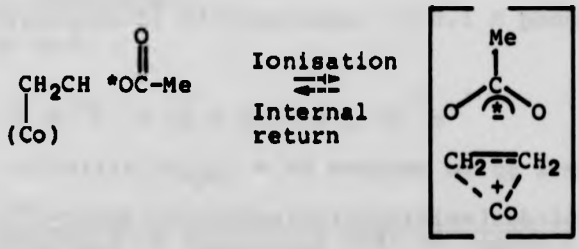
(80)

3.18.3 [^{17}OAc]-2-Acetoxyethyl(pyridine)cobaloxime (76)

The rate constants for the hydrolysis of cobaloxime (76) are shown in Table 6. Fig. 7 shows a ^{17}O NMR spectrum taken during the hydrolysis of cobaloxime (76) at 50 ^{o}C in aqueous dioxan. The intensity of the [$^{17}O_2$]acetate signal increased with time, whilst that of the water and the dioxan remained constant throughout the experiment; no other signals were detected. The appearance of ^{17}O in the acetate ion indicated that the hydrolysis involved breakage of the alkyl-oxygen bond. From Ingold's classification of the mechanism of ester hydrolysis, the neutral hydrolysis (76) is a $B_{Al}1$ type, a relatively uncommon reaction, the one example known being $CF_3CO.OC(Me)_3$ (Section 3.6.2). The hydrolysis of (76) in aq. dioxan at 50^{o}C has a half-life of 3 h. N.B., the neutral hydrolysis of ethyl acetate is exceptionally slow (the estimated half-life at 25^{o}C is

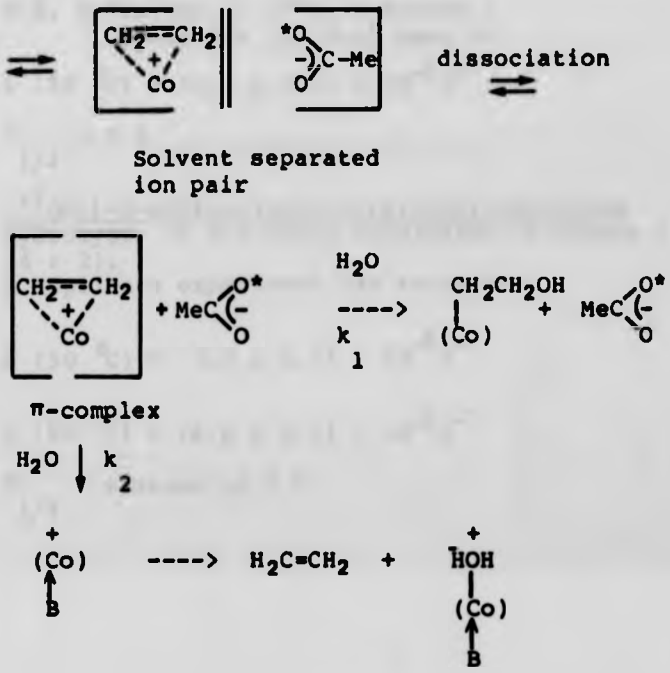
75 years), [cf 22]. The by-product from the hydrolysis of (76) was $HOCH_2CH_2Co(dmgH)_2py$, which was shown by ^{17}O NMR spectroscopy to be free of ^{17}O signal. When $[^{17}O]HOCH_2-CH_2-Co(dmgH)_2py$ was held at 50 °C in aqueous dioxan containing 1 mol equiv. of acetic acid, for 24 h, on removal of the solvent and redissolving the cobaloxime in $CDCl_3$ the sample showed an ^{17}O signal comparable to an untreated sample in $CDCl_3$ pulsed similarly for 1 h.

A mechanism which is consistent with these results (Scheme 85), proposes a π-ethylene intermediate of cobaloxime(III), which undergoes rapid capture of H_2O to give $HOCH_2CH_2Co(dmgH)_2py$ or loses ethene to give $HOCo(dmgH)_2py$. The rates of these reactions are such that the relative amount of the former compared to the latter is in the ratio of 3 : 1 (from ^1H NMR spectroscopy).



Intimate ion pair

cotd.



Scheme 85

Table 6. Rate constants for the hydration and hydrolysis of ^{17}O-enriched compounds

[^{17}O]Pentanal

Hydration of [^{17}O]pentanal in 0.1 M phosphate buffer, pH 6.8.

k (5 °C) = (1.6 ± 0.1) x 10^{-2} s^{-1}

Half-life ($T_{1/2}$) = 43 seconds or ca 1 min.

[^{17}O-Formyl]Formylmethyl(pyridine)cobaloxime

Hydration of cobaloxime (79) in dioxan : water (3 : 2)

k (40 °C) = (1.6 ± 0.4) x 10^{-5} s^{-1}

k (50 °C) = (4.5 ± 0.4) x 10^{-5} s^{-1}

k (60 °C) = (1.6 ± 0.2) x 10^{-4} s^{-1}

$T_{1/2}$ = 4 h (50 °C)

Cotd...

N.B. Hydration of (79) in dioxan : phosphate (pH 6.8) gave :-

k (50 ^{0}C) = (1.0 ± 0.1) x 10^{-5} s^{-1}

$T_{1/2}$ = 2 h

[^{17}OAc]-2-Acetoxylethyl(pyridine)cobaloxime

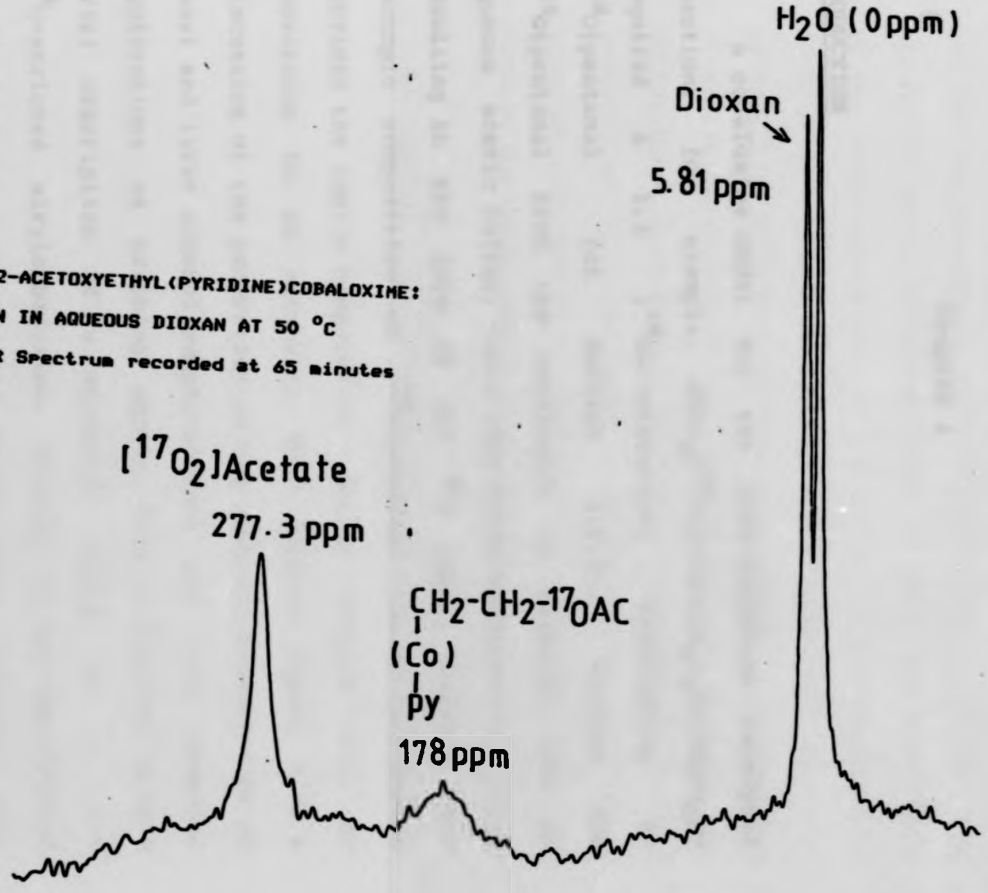
Hydrolysis of the title cobaloxime in dioxan : water (4 : 2).
A duplicate experiment was recorded.

k (50 ^{0}C) = (6.5 ± 0.1) x 10^{-5} s^{-1}

k (50 ^{0}C) = (6.6 ± 0.4) x 10^{-5} s^{-1}

$T_{1/2}$ = average ca 3 h

FIG. 7 [^{17}OAC]-2-ACETOXYETHYL(PYRIDINE)COBALOXIME:
HYDRATION IN AQUEOUS DIOXAN AT 50 °C
A ^{17}O NMR Spectrum recorded at 65 minutes



Chapter 4

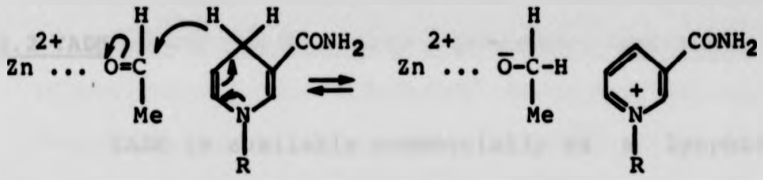
4. INTRODUCTION

A cobaloxime model for the diol-dehydrase catalysed reaction, for example, $HOCH_2[^{18}O]H\text{-}CH\text{-}(CH_2)_3Co(dmgH)_2py$ required a 1,2 (^{18}OH)-migration, terminating at [^{18}O]pentanal (cf Section 1.6.3). Because the [^{18}O]pentanal from the cobaloxime is released into an aqueous acetic buffer, rapid reversible hydration may occur resulting in the loss of the ^{18}O label. However, the isotopic composition of [^{18}O]pentanal can be ascertained provided the labile compound is rapidly trapped (e.g. by conversion to an alcohol). This Chapter begins with a discussion of the properties of the reducing enzymes such as yeast and liver alcohol dehydrogenases and their possible applications as trapping agents. This is followed up by a brief description of a synthetic route to a C-4 ^{18}O-enriched alkylcobaloxime. Finally, in the Experimental Sections, there are presented results which indicated that the hydration of 23 atom-% [^{18}O]benzaldehyde was relatively slow compared to a 25 atom-% [^{18}O]BuCHO in aqueous-organic solvent. These results implied that an organic solvent may be used as trapping agent, and hence an alternative cobaloxime model is proposed in the Discussion Section.

4.1 ALCOHOL DEHYDROGENASES [1 a-b]

These are zinc metalloenzymes of broad specificity, oxidising a wide range of aliphatic and aromatic alcohols to their corresponding aldehydes and ketones using NADH

and NAD^+ as coenzymes. The two most studied enzymes are those from yeast and horse liver. At pH 7, the equilibria of the redox reactions lie heavily in favour of carbonyl reduction. Scheme 86 shows a mechanism for the oxidation of alcohols. The basic mechanism is essentially electrophilic catalysis mediated by the zinc atom at the active site. Polarization of the alcoholate anion by Zn^{2+} acting as a Lewis acid facilitates the subsequent hydrogen transfer step. This process involves only one side of the nicotinamide ring of NADH.



Scheme 86

4.2 HLADH AND YADH [2]

4.2.1 HLADH

The alcohol dehydrogenase from horse liver, HLADH, is available commercially in crystalline form, and is stable for months at 4 °C. The enzyme catalyses the oxidoreductions of a broad spectrum of substrates ranging from ethanol and acetaldehyde up to steroidal alcohols and ketones [2]. The commercial preparations and those isolated directly from horse liver can be separated into a complex series of isozymes [1]. Each isozyme is a dimeric species and the monomeric units

that constitute the isozyme can be classified as the ethanol type (E) or steroid type (S) [2]. EE-Dimers are catalytically active if ethanol is the substrate but are inactive against steroid alcohols, whereas SS-isozymes are fully active with steroid alcohols. The largest fraction (60 to 90 %) of the commercial enzyme seems to be composed of EE-dimer which has a molecular weight of 84,000 and contains two identical monomers with a known sequence of 374 amino acid residues each [3].

4.2.2 YADH

YADH is available commercially as a lyophilized powder or as a buffered crystalline suspension. In these forms it is stable for 4 to 12 months at 5 ^{o}C. The usual sources for YADH are baker's or brewer's yeasts. YADH catalyses the oxidoreductions of a very limited range of acyclic alcohols and their corresponding aldehydes or ketones. Cyclic alcohols or ketones are not substrates.

4.3 FACTORS AFFECTING THE ACTIVITY AND STABILITY OF HLADH AND YADH

An understanding of such factors as changes in content and composition of the reaction solution is useful when applying HLADH or YADH as a chiral catalyst for preparative scale asymmetric synthesis [2] or as a trapping agent.

4.3.1 Effects of temperature

HLADH catalysed reactions are usually assayed at 4 ^{0}C to 25 ^{0}C. Within this temperature range, HLADH remains catalytically active when kept for a week or more. HLADH does not tolerate temperatures in excess of 30 ^{0}C well, and at 75 ^{0}C rapid denaturation of the enzyme occurs [4]. The rate of heat denaturation is prevented slightly by the presence of stabilizers such as cysteine or bovine serum albumin, but is accelerated by NAD^{+} [5]. Addition of an inhibitor such as isobutyramide also protects against heat inactivation; the HLADH-NAD^{+}-isobutyramide ternary complex is completely stable at 75 ^{0}C [4]. Ternary complexes with a good substrate may also prevent inactivation at high temperatures to a limited extent [6].

YADH is relatively unstable compared to HLADH. YADH activity is very sensitive to changes in temperature. Most assays are performed at ca 25 ^{0}C but the enzyme is progressively inactivated even at this temperature [7]. YADH loses half its activity in ca 20 min at 43 ^{0}C, but addition of stabilisers such as glutathione [7], cysteine, or bovine serum albumin [5] gave protection against higher temperature inactivation. Whether or not the prior addition of NAD^{+} provides partial protection against heat denaturation is not clear [5].

4.3.2 Effects of pH

HLADH is active in the pH range 5 to 10. Because oxidoreductions are equilibria in which the concentration of H^+ is an important factor, the pH of a reaction solution is often selected to provide the optimum displacement of the reaction in the desired direction. The pH optimum for catalytic reduction of a carbonyl substrate is ca 7. Acidic solutions have adverse effects on the catalytic process. Exposure of NADH to solutions of low pH leads to a rapid loss of its coenzyme property because the dihydropyridine moiety is susceptible to acid catalyed reactions [8 9]. HLADH by itself is almost completely inactivated in 2 h at pH 4 and 23.5°C, but addition of this enzyme to coenzyme-isobutyramide ternary complex has a considerable stabilising effect. Complexing HLADH in this manner protects it from the effects of pH 4 solutions [4]. We found that after premixing NADH and HLADH in 0.01M sodium phosphate buffer (pH 6.8) and adding this binary complex to a pH 4.2 solution followed by the aldehyde, the enzyme was catalytically active. It is conceivable that (HLADH-NADH) complexes are similary stabilised to the ternary complex, and that the normal pH 5 to 9 operating range of the enzyme can be extended in some cases using this procedure.

YADH becomes increasingly unstable outside the pH range 6 to 8.5 and the pH optimum is reported to be

ca 8 [10]. The rate of acid denaturation is slowed in the presence of NAD^+ [2]. We found that after prior addition of NADH and BSA to YADH, followed by the aldehyde in pH 4.2 solution, the enzyme was catalytically inactive.

4.3.3 Effects of electrolytes

Oxidoreductions catalysed by HLADH can be performed in a variety of buffers. These include phosphate [1a], citrate [11], acetate [4], etc. The ionic srength of the medium is normally controlled by the concentration of the buffer used; 0.05 to 0.1M is the usual concentration range but more dilute solutions can be employed [1]. We found that the concentration of the citrate-phosphate buffer at pH 4.2 appeared to be critical for HLADH activity. Higher buffer concentrations appeared to retard the rate of conversion of aldehyde to alcohol. The nature of the buffer or its concentration does not seem to be critical for YADH in its normal pH range.

4.3.4 Effects of organic solvents

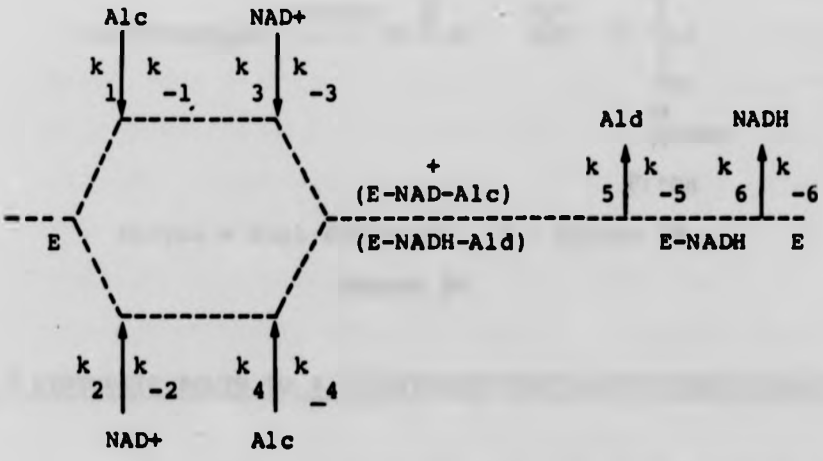
For HLADH, the addition of 3 % methanol lowers the rate of cyclohexanone reduction by a factor of 4 [2] and the SS-dimer is totally inactive in 40 % ethanol [2]. The reduction of ketone by HLADH is inhibited in the presence of dioxan [12].

In contrast, 1.7 % of diethyleneglycol dimethyl ether has little effect on the reduction of cyclohexanone by HLADH, and small amounts of ethyleneglycol monomethyl ether acetate actually accelerate the reduction process [2]. YADH is relatively stable in the presence of 20 % acetone [7] and remains catalytically active in aqueous solutions containing 30 % ethylene glycol [13].

4.4 ADH AS A TRAPPING AGENT

A [^{17}O]-aldehyde exchanges its label rapidly via a geminal diol in aqueous solution (cf Chapter 3). To minimise the extent of loss of the isotopic tracer, the labile carbonyl must be trapped, for example, by converting the compound into an inert group, such as an alcohol. Trapping of this isotopic tracer may be achieved chemically or biologically with a hydride-transfer agent. Reduction of the carbonyl function in acidic solvents by $LiBH_3CN$ is slow and inefficient [14]. Enzymes are remarkable catalysts since they can bring about reactions with great speed and high yield. Of these enzymes, the YADH and HLADH are suitable reducing agents. Scheme 87 shows a basic rate mechanism for the oxidation of ethanol [15]. A stopped flow rapid mixing kinetic technique found the conversion of an aldehyde to an alcohol to have a rate constant between 200 to 400 s^{-1} (i.e. $T_{1/2}$ 2-3 msec) [16]. The hydration of [^{17}O]pentanal at pH 7 (5^{0}C) has a $T_{1/2}$ of ca 1 min. In Scheme 87, K_4, the rate of diffusion of the substrate to the E-NAD^+ complex is 10^4 M^{-1} s^{-1}. The bimolecular rate constants for favourable

proton transfers to electronegative atoms such as O, N or S are extremely fast, being diffusion controlled in the order of 10^{10} - 10^{11} s^{-1} M^{-1}. [17]. These data provide an estimation that some label may be retained as [^{18}O]-alcohol when trapped with ADH enzymes. Retey et al [18] were able to trap [^{18}O]propanal at neutral pH with an excess of YADH (Scheme 88). The trapped [^{18}O]propanol product was derivatised with 1-naphthyl isocyanate and the extent of [^{18}O]-label retained determined by mass spectrometry. They reported that almost half of the ^{18}O isotope appeared in the buffer as [^{18}O]water. Biellmann et al [19] also reported that for HLADH, the hydrated form of acetaldehyde does not inhibit the rate of the enzyme-catalysed reduction.

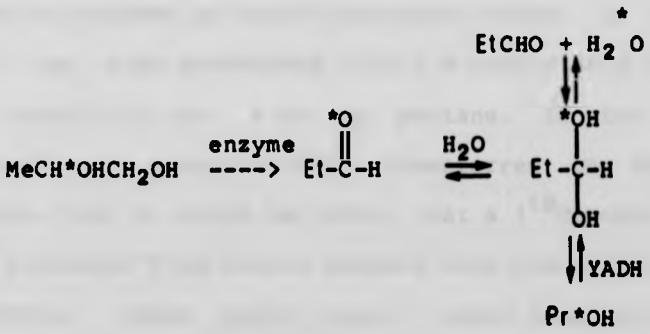


cotd.

k_1	$1.04 \times 10^4\ M^{-1}\ s^{-1}$	k_{-1}	$3.96\ s^{-1}$
k_2	$7.34 \times 10^4\ M^{-1}\ s^{-1}$	k_{-2}	$0.338\ s^{-1}$
k_3	$7.16 \times 10^5\ M^{-1}\ s^{-1}$	k_{-3}	$32\ s^{-1}$
k_4	$2.86 \times 10^4\ M^{-1}\ s^{-1}$	k_{-4}	$104\ s^{-1}$
k_5	$176\ s^{-1}$		
k_6	$9.5\ s^{-1}$		

Rate constants for the oxidation of ethanol at 27 ^{0}C catalysed by HLADH. Data taken from the work by Wong and Hanes [cf ref. 15].

Scheme 87



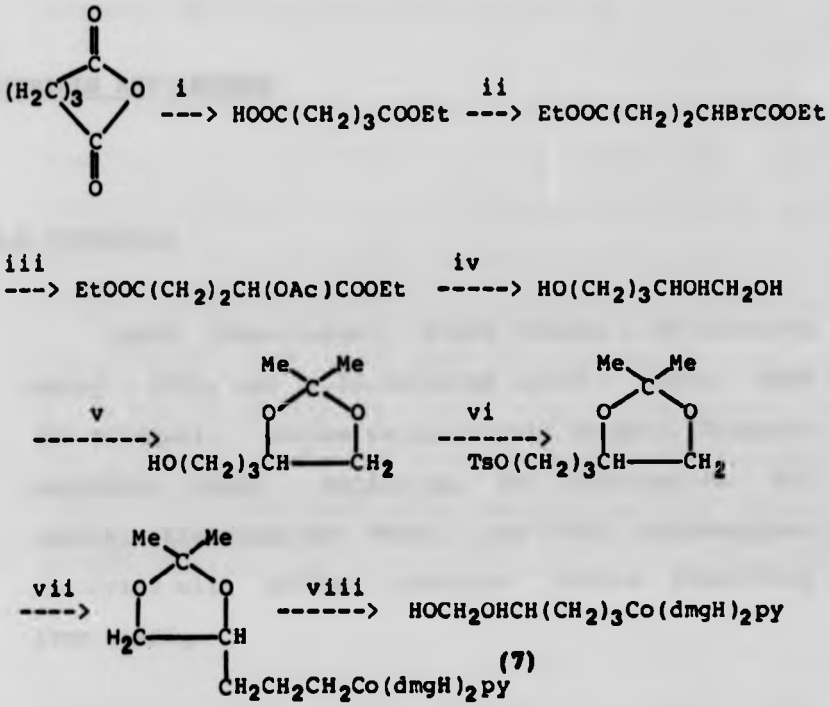
Enzyme = diol dehydrase * = Oxygen 18

Scheme 88

4.5 SYNTHETIC ROUTE TO 4,5-DIHYDROXYPENTYL(PYRIDINE)COBALOXIME

The title cobaloxime (7) has been prepared from pentane-1,2,5-triol (**Scheme 89**) [20]. We have used a different route to this triol because one of its precursors ($EtOOCCH_2CH_2CHBrCOOEt$) can be reacted with one equivalent of acetate ion. By labelling this acetate ion (**Scheme 90**), with one equivalent of [^{18}O]water, a

method for preparing cobaloxime (7) enriched at the C-2 oxygen position can be carried out. Cobaloxime (7) was synthesised because, as was described in Chapter 1, photolysis of (7) in aqueous acetic acid gave a 10 % yield of pentanal as one of the rearrangement products. Golding et al also showed that variable yields of pentanal were obtained depending on the acidities and the temperature of the buffers used in the photolysis. In particular, we needed to confirm that photolysis of (7) in citrate - phosphate buffer (pH 4.2) gave ca 8 % yield of pentanal. It was at pH 4.2 that HLADH was required to trap the pentanal. Although photolysis in 0.1 M acetic acid (pH 3) gave a maximum yield of pentanal, such an extreme pH would inactivate HLADH. In addition, (7) was also photolysed in 0.1 M acetic acid containing an organic solvent such as pentane. If the pentanal formed was quantitatively transferred to the organic phase, then it would be likely that a [^{18}O]pentanal could be protected from direct contact with the acidic aqueous solvent, which would cause rapid isotopic exchange, thus avoiding the addition of HLADH as a trapping agent. Finally, the cobaloxime (7) was photolysed in the presence of HLADH, and any pentanol formed could be identified by derivatisation with 1-naphthyl isocyanate.



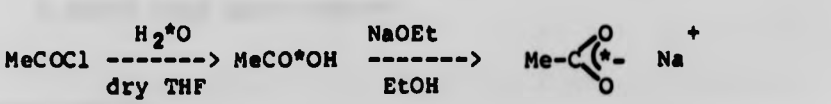
i, ethanol/reflux ii, $SOCl_2/Br_2/H_2O$/ethanol

iii, KOAc/ethanol/heat iv, $LiAlH_4$/ether v, acetone/H^+

vi, TsCl/py vii, $BrCo(dmgH)_2py/NaBH_4$/ethanol

vii, aqueous HCl

Scheme 89



+ diethyl 2-bromoglutarate --> $EtOOC(CH_2)_2CH(*OAc)COOEt$

* = Oxygen 18

Scheme 90

4.6 MATERIALS AND METHODS

4.6.1 Chemicals

NADH (Boehringer), HLADH (Sigma, 1.35 units/mg solid - 1972, and 1.85 units/mg solid - 1980). YADH (Boehringer). Bovine serum albumin (Sigma). Glutaric anhydride (BDH). Silica-gel for preparative TLC (Merck, Kieselgel-60 PF254, Art 7747). Dihydropyran was dried with sodium carbonate before distilling from $LiAlH_4$.

4.6.2 Instruments

U.v./visible spectra were recorded on a Unicam SP 500 or a Perkin-Elmer Model 552 spectrophotometer. The enzymic assays were recorded on a Unicam SP 1800 u.v. spectrophotometer. Mass spectra were recorded on a MS902 mass spectrometer.

4.7 EXPERIMENTAL

4.7.1 ENZYMIC ASSAY

Stock buffers prepared for the alcohol dehydrogenase assays were 0.1M sodium phosphate (pH 6.8) and 0.1M citrate - 0.2M sodium phosphate (pH 3.8) [21]. In addition the following stock reagents were

prepared for an enzymic assay at pH 6.8.

Stock reagents: HLADH - 0.7 mg of HLADH was dissolved in 1 cm^3 of 0.1M sodium phosphate (pH 6.8), YADH (30 mg/cm^3) - 0.05 cm^3 was removed and diluted with 1.5 cm^3 of 0.1M sodium phosphate (pH 6.8) containing 2.7 mg of BSA, giving a final concentration of 1 mg/cm^3; NADH - was prepared as 10 mg in 1 cm^3 of 0.1M sodium phosphate (pH 6.8); Substrates - acetaldehyde (0.010 cm^3, 0.179 mmol) and pentanal (0.010 cm^3, 0.094 mmol) were each diluted with dioxan (0.090 cm^3).

The enzymic assays were as follows: 3 cm^3 of 0.1M sodium phosphate buffer (pH 6.8); 0.1 cm^3 of NADH; 0.05 cm^3 of enzyme, were mixed in cuvettes in both the sample and reference beams. The enzymic reaction was initiated by the addition of the substrates (0.005 cm^3) to the sample cuvette. The decrease in optical density at 340 nm with time enabled the enzymic activities for HLADH and YADH to be compared for the substrates MeCHO and pentanal at pH 6.8. The specific activities of ADH were calculated from the absorbance maximum of NADH at 340 nm (ϵ 6.22 cm^3/micromole). The specific activity = Δ O.D. x volume of buffer/6.22 x amount of enzyme. The results for the HLADH and YADH activities at pH 6.8 are shown in Table 7

Table 7. Specific activities of HLADH and YADH in 0.1M sodium phosphate (pH 6.8)

Enzymes	volume (cm^3) of enzymes in assays	substrates	O.D./min	specific activities micromole/ min/mg
HLADH	0.05	pentanal	0.65	ca 9
HLADH	0.02	pentanal	0.26	ca 9
HLADH	0.02	MeCHO	0.53	ca 18
YADH	0.02	pentanal	0.40	ca 10
YADH	0.02	MeCHO	rapid	-

4.7.2 Enzymic assay at pH 4.2

A modification of the stock reagents was necessary for the comparison of YADH and HLADH acting on MeCHO and pentanal in 0.025M citric - 0.05M sodium phosphate (pH 4.2). Stock reagents: HLADH (1 mg) and NADH (10 mg), were premixed in 1 cm^3 of 0.01M sodium phosphate buffer (pH 7.0); YADH (30 mg/cm^3) - 0.05 cm^3 was removed and diluted with 1.35 cm^3 of 0.01M sodium phosphate buffer (pH 7.0), to which had previously been added NADH (15 mg) and BSA (2.7 mg). The final concentration of YADH was 1 mg/cm^3; Substrates - prepared as described earlier.

In the enzymic assays, the sample cuvette and the reference cuvette each contained 3.0 cm^3 of the buffer (pH 4.2) and 0.10 cm^3 of the NADH - Enzyme - BSA premix. The enzymic reactions were initiated by adding 0.005 cm^3 of the substrates to the sample cuvette. YADH was found to be inactivated in citrate-phosphate buffer (pH 4.2) and was thus

unsuitable for use as a trapping agent for pentanal at this pH. Table 8 shows the specific activities of HLADH at three different concentrations of citrate-phosphate buffer. Control expts. showed that NADH does not decay rapidly when kept at pH 4.2 over a 1 min period.

Table 8. Specific activities of HLADH

Citric-phosphate	pH	O.D.	specific activity micromole/ min/mg.
0.01M - 0.125M	4.5	0.67	ca 2
*0.025M - 0.05M	4.2	0.23	ca 1
0.05M - 0.10M	3.9	0.11	ca 0.3

* A repeat experiment using the more recent HLADH (1980) gave Δ O.D./min = 0.74, from which the specific activity was calculated to be ca 4 micromole/min/mg

4.7.3 Reaction of pentanol with 1-naphthyl isocyanate

We expected that the photolysis of 0.1 mmol of (7) in citrate-phosphate buffer (pH 4.2) would give ca 8 % yield of pentanal. Assuming a quantitative reduction of pentanal to pentanol by HLADH at this pH, the efficiency of recovery of pentanol as its urethane derivative can thus be determined from this experiment. A stock solution of pentanol (0.010 cm^3, 0.092 mmol) in 10 cm^3 of 0.025M citric - 0.05M sodium phosphate (pH 4.2) was prepared. 1 cm^3 of this stock buffer was removed (0.0092 mmol of pentanol) and diluted with 50 cm^3 of citric-phosphate buffer. The

pentanol was extracted into ether (10 cm^3, 3x) and the ether extract dried with Na_2SO_4. To determine the amount of pentanol recovered, 1-naphthyl isocyanate (0.014 cm^3) was added to the ether solvent. The ether was evaporated and the mixture was kept in a nitrogen flushed glove-box overnight whereupon the urethane derivative was formed. The derivative was extracted with hot petroleum ether (100-120 ^0C). The solvent was removed and CH_2Cl_2 was added to the derivative. Preparative TLC: silica-gel (Merck); 20 x 20 cm, thickness = 0.5 mm, was used to remove the unwanted excess reagent and urea, which had a lower Rf value compared to 1-naphthylcarbamic acid, pentyl ester, Rf = 0.64. The weight of the crude derivative was 12.6 mg. The exact amount was determined by u.v. spectroscopy. The molar extinction coefficient (ε_{max}) determined for this urethane derivative was 7.15 x 10^3. From this value, a 30 % recovery of pentanol as its urethane was estimated.

4.7.4 Reduction of [^{18}O]pentanal with HLADH at pH 4.2

To 50 cm^3 of 0.025M citric - 0.05M phosphate buffer (pH 4.2) was added 1.6 cm^3 of a solution of 0.1M sodium - phosphate buffer (pH 6.8) containing NADH (100 mg) and HLADH (10 mg). The solution was stirred and 25 atom-% [^{18}O]pentanal (1 mg in 0.01 cm^3 dioxan) added immediately. After 45 min, the product (expected [^{18}O]pentanol) was extracted with ether (10 cm^3, 4x) and the resultant emulsion broken up by addition of NaCl. The ether extract was filtered

through glass wool and dried (Na_2SO_4) for 3/4 h. The drying agent was removed and 1-naphthyl isocyanate (0.0143 cm^3) was added to the ether. The rest of the procedure was similar to that described in the last Section. In addition, the extent of ^{18}O retained in the labelled urethane derivative was determined from the mass spectrum of this sample.

4.7.5 Ethyl hydrogen glutarate - $EtOOC(CH_2)_3COOH$ [22]

The title compound was prepared by refluxing glutaric anhydride (22.6 g, 0.20 mol) in ethanol (12 cm^3) for 2 h. Direct distillation gave a small fraction, b.p. 50 - 90 °C at 0.15 mmHg, which was discarded. The main fraction (64 %), b.p. 90 - 96 °C at 0.15 mmHg was collected. Lit. [22], b.p. 159-165 °C at 17 mmHg.

^1H NMR (CCl_4): 1.24 [3H, t, Me], 1.91 [2H, m, CH_2COOEt], 2.36 [4H, m, CH_2CH_2COOH], 4.08 [2H, q, OCH_2], and 11.46 [1H, s, COOH].

4.7.6 Diethyl 2-bromoglutarate - $EtOOCCH_2CH_2CHBrCOOEt$ [23]

Ethyl hydrogen glutarate (20.5 g, 0.125 mmol) and thionyl chloride (36.4 cm^3, 0.5 mol) were heated to reflux with stirring for 2 h. To this solution was added AR bromine (21 g, 0.132 mol) over 1 h. The mixture was heated to reflux for 1/2 h before leaving at r.t. overnight. Ethanol (62.5 cm^3, 1.09 mol) was added cautiously, resulting in the formation of 2

layers. Ether was added to extract the products. The ether extract was washed with water, 5 % $NaHCO_3$, and then water, until neutral. The ether was dried (Na_2SO_4). Distillation afforded a main fraction, b.p. 74 - 80 °C at 0.04 mmHg. A main fraction (47 %), b.p. 94 °C at 0.2 mmHg was obtained after redistillation. Lit.[23], b.p. 122-124 °C at 2 mmHg.

^1H NMR (CCl_4): 1.4 [6H, m, $(COOCH_2Me)_2$], 2.2 - 2.6 [4H, m, CH_2CH_2], and 4.1 - 4.42 [5H, m, $(COOCH_2Me)_2$ and CHBr].

4.7.7 Diethyl 2-acetoxyglutarate

To diethyl 2-bromoglutarate (4 g, 15 mmol) in ethanol (40 cm^3) was added solid KOAc (3 g, 31 mmol). The mixture was heated to reflux with stirring overnight. The precipitated KBr was removed and the solvent evaporated. The residue was extracted with ether and washed with water, then dried with $MgSO_4$. Distillation afforded a fraction, b.p. 83 °C at 0.2 mmHg and a purer fraction, b.p. 90 °C at 0.2 mmHg. The two fractions (2.8 g, 58 %) were combined. Lit. [24], b.p. 138 - 140 °C at 4 mmHg.

^1H NMR (CCl_4): 1.28 [6H, m, $(COOCH_2Me)_2$], 2.08 [3H, s, MeCO], 2.32 [4H, m, CH_2CH_2], 4.15 [4H, m, $(COOCH_2Me)_2$, and 4.86 [1H, t, CHOAc].

4.7.8 A method for preparing diethyl-[^{18}OAc]-2-acetoxyglutarate

Acetyl chloride (0.690 cm^3, 10 mmol) was added to dry THF (3.0 cm^3), followed by distilled water (0.180 cm^3, 10 mmol). To this solution was added 0.457M NaOEt (44 cm^3). The mixture was stirred for 1 h and diethyl 2-bromoglutarate (2.4 g, 9 mmol) was added. The reaction was heated to reflux for 72 h whereupon an aliquot was shown by ^1H NMR spectroscopy to be diethyl 2-acetoxyglutarate.

4.7.9 Reduction of diethyl 2-acetoxyglutarate with $LiAlH_4$

Diethyl 2-acetoxyglutarate (1 g, 4.1 mmol) in dry ether (5 cm^3) was added slowly to a suspension of $LiAlH_4$ (419 mg, 8.12 mmol) in dry ether (20 cm^3). The mixture was kept at r.t. overnight. The work-up procedure required the addition of water (0.4 cm^3), 15 % NaOH (0.4 cm^3), again water, and stirring for 1/2 h. The white slurry was filtered through Celite, eluting the product with propan-2-ol. No products were obtained when ethanol or ether was used. The excess solvent was removed on a Büchi kept at 60 ^0C. Distillation (Kugelrohr) afforded product (132 mg, 27 %), b.p. 145 - 146 ^0C at 0.001 mmHg.

^1H NMR (D_2O/H_2O): 1.70 [4H, m, CH_2CH_2) and 3.7 [5H, m, ($CH_2OH)_2$ & $CHOH$]. The preparation of 4,5-dihydroxypentyl(pyridine)cobaloxime is well described [25]. Only a small description of the quantity of the reagents used is reported.

4.7.10 4-(3-Hydroxypropyl)-2,2-dimethyl-1,3-dioxan

Pentane-1,2,5-diol (980 mg, 8 mmol) and p-toluenesulphonic acid (31 mg) in acetone-petroleum ether (40-60) (1 : 1; 5 cm^3) was heated to reflux. The ketal was obtained as an oil (378 mg, 29 %), and used in the next stage. Lit. [25], b.p. 64 - 65 °C at 0.06 mmHg.

^1H NMR (CCl_4): 1.32 [6H, 2 x s, 2 x Me], 1.58 [4H, m, $-(CH_2)_2-$], 3.4 - 4.8 [5H, m, $OHCH_2$ & $CHCH_2$].

4.7.11 2,2-Dimethyl-4-(3-p-tolylsulphonyloxy)-1,3-dioxolan

To the alcohol (378 mg, 2.34 mmol) and dry pyridine (1.1 cm^3) at -15 °C was added tosyl chloride (490 mg, 2.58 mol) in dry pyridine (1.2 cm^3). The product was an oil (55 mg, 94%).

^1H NMR (CCl_4): 1.25 [6H, s, $(Me)_2$], 1.47 - 1.77 [4H, m, CH_2CH_2], 2.45 [3H, s, MeAr], 3.37 - 4.07 [5H, m, $OCHCH_2O$ & CH_2OSO_2], and 7.29 - 7.72 [4H, ABq, 4 x Ar H].

4.7.12 4,5-Dihydroxypentyl(pyridine)cobaloxime

Bromo(pyridine)cobaloxime (990 mg, 2.2 mmol) in ethanol (10 cm^3), $NaBH_4$ (250 mg, 6.6 mmol), and the tosylate (551 mg, 2.2 mmol) in ethanol (5 cm^3), were reacted together for 24 h. The product was precipitated from cold water as a yellow crystalline

solid (530 mg, 45 %). TLC: silica-gel, F-254 (Merck); $CHCl_3$: MeOH : py (19 : 1 : 0.1); Rf 0.61.

^1H NMR ($CDCl_3$): 1.35 [6H, s, $(Me)_2$], 0.95 - 1.7 [6H, m, $CoCH_2CH_2CH_2$], 2.14 [12H, s, 4 x dmgH, Me], 3.3 - 4.1 [3H, m, CH_2CH_2] and 5 pyH.

The cobaloxime (400 mg, 0.78 mmol) was hydrolysed in 0.1M HCl (12 cm^3), for 4 h. The product was purified by elution through silica-gel (55 g), with $CHCl_3$: MeOH : py (190 : 10 : 1). TLC: silica-gel, F-254 (Merck); $CHCl_3$: MeOH : py (19 : 1 : 0.1); Rf 0.12.

^1H NMR ($CDCl_3$): 0.9 - 1.8 (6H, m, $CoCH_2CH_2CH_2$), 2.18 (12H, s, 4 x dmgH, Me], 3.3 - 3.8 [3H, m, $CHCH_2$ and 5 py H].

4.7.13 Photolysis of 4,5-dihydroxypentyl(pyridine)cobaloxime

4,5-Dihydroxypentyl(pyridine)cobaloxime (50 mg, 0.106 mmol) was photolysed in 0.1M acetic acid (pH 3.0) (50 cm^3). The procedure for isolating pentanal as its dinitrophenylhydrazone has been described by P.J. Sellars [25]. The pentanal-DNP was dissolved in AR MeOH (100 cm^3). The absorbance of this solution was measured at the 358 nm maximum (ϵ 2.2 x 10^4 l mol^{-1} cm^{-1}) on a calibrated Unicam SP 500 spectrophotometer. Three experiments are described below. In the third experiment, HLADH was added to the cobaloxime before photolysis. Such photolysis caused the reduction of

the pentanal formed to pentanol, which was then derivatised with 1-naphthyl isocyanate. In addition, a fourth experiment was conducted, whereby pentanal (0.010 cm^3, 0.1 mmol) was injected into equal proportions of aqueous 0.1M acetic acid : pentane (10 cm^3 : 10 cm^3). The distribution of pentanal in the two phases may be determined by separating the two layers and recovery of the pentanal as its DNP.

Photolysis of the cobaloxime (7): (i) in 0.025M citrate - 0.05M phosphate (pH 4.4), 50 cm^3; (ii) in 0.1M acetic acid (pH 3) and pentane (50 cm^3 : 10 cm^3); and (iii) in 0.025M citrate - 0.05M phosphate containing HLADH (25 mg) plus NADH (98 mg) dissolved in 0.1M sodium phosphate (pH 6.8), 1 cm^3. The results of the above experiments were as follows:

4.7.14 Experiment 1

The stock solution of pentanal-DNP in AR MeOH (100 cm^3) was diluted 5 x. O.D. (358 nm) = 0.3695 (10 mm cell). O.D. = ϵ x c x l. Thus amount of pentanal-DNP = 8.397 micromole. % yield of pentanal-DNP = 8.397/106 x 100 = 7.9 %.

4.7.15 Experiment 2

The stock solution of pentanal-DNP in AR MeOH (100 cm^3) was read without dilution. Pentanal DNP in aqueous acetic-acid, O.D. (358 nm) = 0.680 (10 mm cell). Pentanal DNP in pentane phase, O.D. (358 nm) =

1.102 (10 mm cell). Thus, distribution of pentanal-DNP in pentane phase/aq. MeCOOH = 5 micromole/3micromole = 4.7 % /2.9 %.

4.7.16 Experiment 3

A 1-naphthylurethane was identified by mass spectrometry. The mass spectrum showed that 1-naphthylcarbamic acid, pentyl ester was not recovered as expected. Instead, the molecular ion corresponded to that of 1-napthylcarbamic acid, ethyl ester.

4.7.17 Experiment 4

The stock solution was diluted 10 x. Pentanal-DNP from pentane phase, O.D. (358 nm) = 0.270 (2 mm cell). Distribution of pentanal-DNP in pentane/0.1M acetic acid = 61.36 micromole/7.72 micromole = 8/1.

4.7.18 Hydration of 23 atom-% [^{18}O]benzaldehyde monitored by IR

[^{18}O]Benzaldehyde (23 atom-%, 0.010 cm^3, 98 micromole) was injected into an aqueous acetic buffer in 3 ways: (i) directly injected into 0.1M acetic acid, pH 3 (10 cm^3); the solution was vigorously stirred for 5 min, and [^{18}O]PhCHO re-extracted into CH_2Cl_2; (ii) in CH_2Cl_2 (3 cm^3), added to 0.1M acetic acid (10 cm^3); this solution was vigorously stirred for 5 min, and the CH_2Cl_2 layer was removed for IR monitoring; (iii) injected into a vigorously stirred

mixture made up of 0.1M acetic acid : CH_2Cl_2 (50 cm^3 : 15 cm^3); 1 cm^3 of the CH_2Cl_2 solution was removed after 5 min and 10 min periods for inspection in an IR spectrophotometer. The hydration of [^{18}O]PhCHO was quantitatively estimated from the ratio of ^{16}O : ^{18}O carbonyl absorptions at 1710 cm^{-1} and 1682 cm^{-1} respectively. The [^{18}O]benzaldehyde in the CH_2Cl_2 extract was dried with $MgSO_4$ before introducing the sample into the NaCl solution cell. A control experiment made up of 23 atom-% [^{18}O]benzaldehyde (0.010 cm^3) in CH_2Cl_2 (3 cm^3) showed that the ratio of ^{16}O : ^{18}O carbonyl absorptions were 2.1 : 1. This was determined by multiplying the width at half-height of the respective carbonyl peak by the height of the peak. The results indicated that in: (i) only one major C=^{18}O band was observed; (ii) ^{16}O : ^{18}O were in the ratio of 4 : 1; (iii) a sample after 5 min, contained ^{16}O : ^{18}O carbonyl absorptions in the ratio of 6 . 5 : 1, which increased to 8 : 1 in 10 min, indicating that more of the ^{18}O isotope had exchanged with the ^{16}O from the water (cf Fig. 8).

4.7.19 Hydration of [^{18}O]pentanal monitored by IR

25 Atom-% [^{18}O]pentanal (29 mg) in dioxan (0.3 cm^3) and CH_2Cl_2 (1 cm^3) was shaken with 10 cm^3 of 0.025M citric - 0.05M phosphate (pH 4.2) for 1 min. The CH_2Cl_2 layer was removed and dried (Na_2SO_4). IR spectroscopy of this solution gave evidence of C=^{18}O absorption. However, when 25 atom-% [^{18}O]pentanal in CH_2Cl_2 (2 cm^3) was shaken with 50 cm^3 of 0.1M acetic

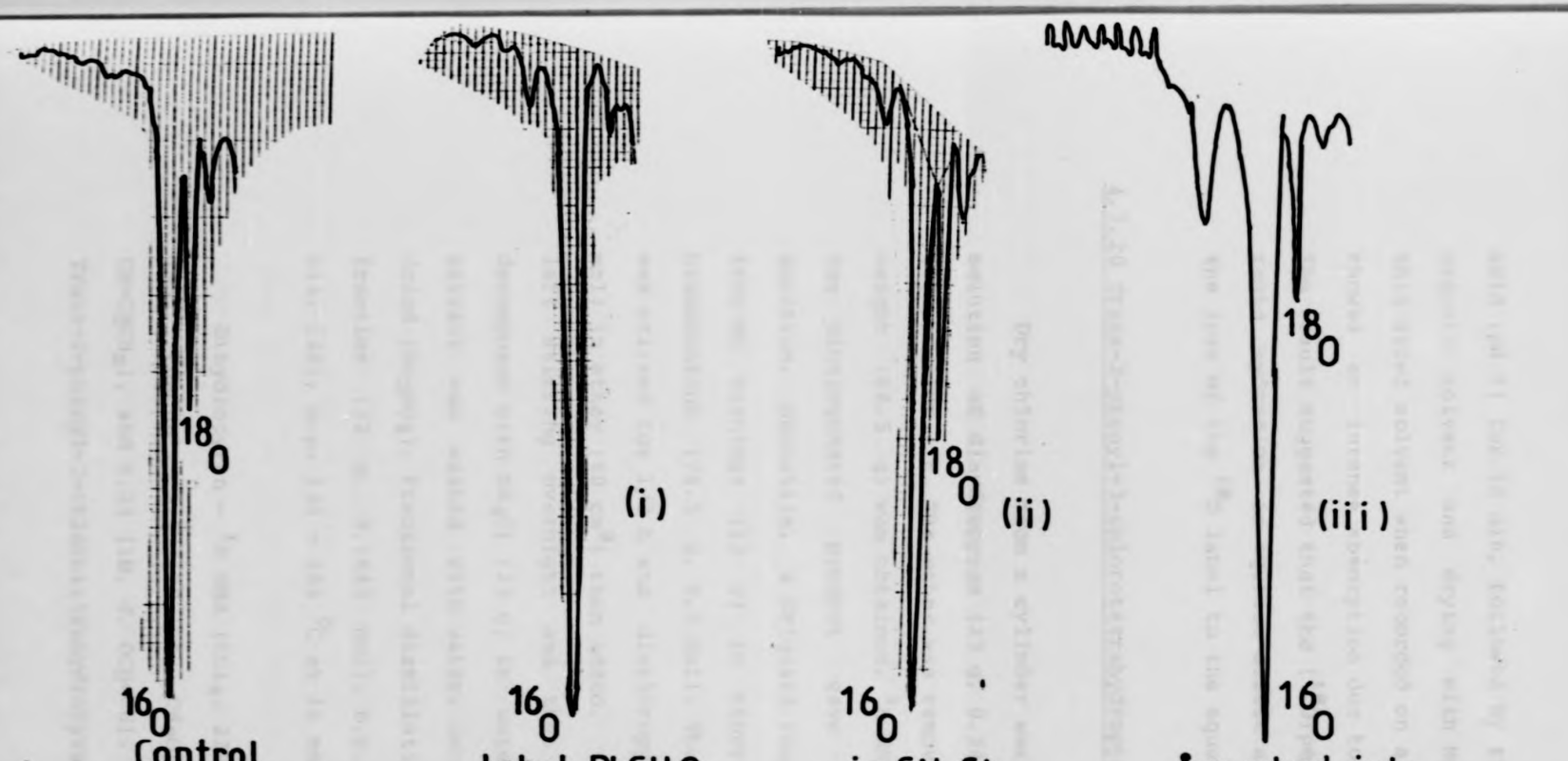


Fig. 8 Hydration of 23 atom % [^{18}O]benzaldehyde

acid (pH 3) for 15 min, followed by the removal of the organic solvent and drying with $MgSO_4$, a sample of this dried solvent when recorded on a IR instrument, showed an intense absorption due to the $-C=^{16}O$ band. The result suggested that the [^{18}O]pentanal underwent rapid hydration in aqueous acidic acid, resulting in the loss of the ^{18}O label to the aqueous solvent.

4.7.20 Trans-2-phenyl-3-chlorotetrahydropyran [26]

Dry chlorine from a cylinder was bubbled into a solution of dihydropyran (29 g, 0.34 mol) in AR ether (60 cm^3) for 1 h. The ether was removed and a crude weight (68.5 g) was obtained. ^1H NMR spectroscopy of the dihalogenated product gave a complicated spectrum. Meanwhile, a Grignard reagent was prepared from Mg turnings (12 g) in ether (100 cm^3) and bromobenzene (78.5 g, 0.5 mol). The Grignard reagent was stirred for 1/2 h and dichloropyran (68.5 g, 0.44 mol) in ether (60 cm^3) then added. The mixture was left stirring overnight and then the mixture was decomposed with NH_4Cl (33 g) in water. The ethereal extract was washed with water, saturated NaCl, then dried (Na_2SO_4). Fractional distillation gave a main fraction (32 g, 0.1645 mol), b.p. 114 ^oC at 9 mmHg. Lit. [26], b.p. 153 - 154 ^oC at 16 mmHg.

Dihydropyran - ^1H NMR (CCl_4, 220 MHz): 1.7 - 2.1 [4H, CH_2CH_2], 3.9 [2H, t, CH_2O], 4.55 [1H, m, $CH=CHCH_2$], and 6.24 [1H, d, $OCH=CH$].

Trans-2-phenyl-3-chlorotetrahydropyran (CCl_4, 220

MHz): 1.6 - 2.0, 2.3 - 2.5 [4H, m, CH_2CH_2]; 3.3 - 3.5, 3.5 - 3.8, 3.9 - 4.1 [4H, m, $CH_2OCHCHCl$] and 7.23 [5H, s, Ar H].

2,3-Dichlorotetrahydropyran (CCl_4): 1.3 - 2.8 [4H, m, CH_2CH_2], 3.5 - 4.3 [3H, m, $ClCHCH_2CH_2CH_2O$], and 6.0 [1H, d, CHClO].

4.7.21 5-Hydroxy-1-phenylpent-1-ene ($PhCH=CH(CH_2)_3OH$)

Sodium (1.5 g) was placed in xylene (25 cm^3) and the suspension was heated to 120 °C, whereupon the sodium melted to give an oval mass with a silvery appearance. The flask was held with a thick cloth and vigorously shaken to give a fine suspension of sodium with a sandy appearance. In a nitrogen-flushed glove-box, the xylene was decanted off and the residue washed several times with dry ether. The sodium sand was diluted with ether (25 cm^3), followed by addition of trans-2-phenyl-3-chlorotetrahydropyran (5 g, 26 mmol). The suspension became yellow and an effervescence and cloudiness resulted, followed by the dissolution of the sodium metal. After 24 h, ethanol-water, was carefully added until excess sodium had been destroyed. The product was extracted into ether and the ether washed with sat. NaCl, water, then dried (Na_2SO_4). The product (2g) was a clear liquid, b.p. 114 - 115 °C at 0.6 mmHg. Lit. [27], b.p. 102 °C at 0.1 mmHg. TLC: silica-gel, F-254 (Merck); ether; Rf 0.47 (intense) and Rf 0.82 (weak). ^1H NMR spectroscopy showed two components. The

by-product was a saturated compound, which arose from the further reduction of the title alkene. The ratio of alkene to alkane was 1 : 1 from the ^1H NMR spectrum.

^1H NMR (CCl_4, 220 MHz): $PhCH{=}CH(CH_2)_3OH$ gave signals at 2.22 [2H, q, $CHPh{=}CHC\underline{H}_2$] and 6 - 6.4 [2H, m, CH=CH], whereas $Ph(CH_2)_4CH_2OH$ had a signal at 2.52 [2H, t, CH_2Ph].

4.8 RESULTS AND DISCUSSION

4.8.1 Photolysis of 4,5-dihydroxypentyl(pyridine)cobaloxime (7)

Photolysis of (7) in citrate-phosphate buffer (pH 4.2) yielded 8 % pentanal. The pentanal was recovered as its DNP derivative. The yield obtained was in agreement with P.J Sellars' result [25]. An 8 % yield was equivalent to 8 micromole of pentanal based on the 0.106 mmol of the cobaloxime (7) that was photolysed. It was further shown that photolysis of 0.1 mmol of the cobaloxime (7) in 0.1M acetic acid - pentane (50 cm^3 : 10 cm^3), followed by separation of the two phases, and derivatization with DNP gave 2.9 % pentanal-DNP in the aqueous phase and 4.7 % pentanal-DNP in the organic phase. When 0.1 mmol of pentanal was introduced into equal proportions of 0.1M acetic acid : pentane (10 cm^3 : 10 cm^3), recovery as pentanal-DNP showed predominant transfer to the organic phase.

4.8.2 Enzymic assay on HLADH and YADH

Tables comparing the specific activities of HLADH and YADH acting on acetaldehyde and pentanal at pH 6.8 and pH 4.2 are tabulated in the Experimental Section. The reduction of pentanal to pentanol in 0.025M citrate-0.05M phosphate (pH 4.2) showed that HLADH is a better reducing enzyme compared to YADH, which completely lost its activity in this buffer. The specific activity of HLADH at pH 4.2 was ca 4 micromole/min/mg.

4.8.3 Reduction of [^{18}O]pentanal with HLADH

When 25 atom-% [^{18}O]pentanal was injected into citrate-phosphate buffer (pH 4.2) containing an excess of HLADH and NADH, the resulting [^{18}O]pentanol was recovered as its urethane derivative. Control experiments showed that the % efficiency of recovery of pentanol as its urethane derivative to be 30 %. The mass spectrum for [^{18}O]urethane showed evidence of the (M + 2) ion, approaching the expected ratio of 20 atom-% enrichment. However, to rule out the possibility that impurities may be causing inaccuracies, a further experiment would be desirable, preferably with a more highly enriched, e.g. (^{18}O)pentanal. The photolysis of the cobaloxime (7) in citrate-phosphate buffer (pH 4.2) containing an excess of NADH and HLADH did not result in the isolation of the urethane derivative of pentanol. Instead, a mass spectrum of the urethane derivative

indicated the presence of 1-napthylcarbamic acid, ethyl ester. This was traced to the fact that the commercial NADH (Sigma) was contaminated with ca 10 % of ethanol [28].

4.8.4 Efficiency of HLADH as a trapping agent

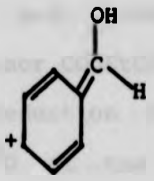
HLADH thus showed the capability of reducing [^{18}O]pentanal to [^{18}O]pentanol at pH 4.2, as evidenced by a mass spectrum of the [^{18}O]-labelled urethane derivative. The enzymic activity of HLADH in citrate-phosphate buffer (pH 4.2) was found to be 4 micromole/min/mg for the substrate pentanal. Thus, a ten-fold excess of HLADH would convert ca 40 micromole of pentanal to pentanol in 1 min. The photolysis of cobaloxime (7) in this buffer was expected to give 8 micromole of pentanal. Thus the time required for HLADH to convert 8 micromole of pentanal to pentanol would be about 5 s. At this rate, most of the isotopic label in pentanal was expected to be retained. The half-life for propanal in 0.1M acetic acid (pH 4.2) at 0 ^{0}C is 28 s.

The observation that photolysis of (7) in citrate-phosphate buffer (pH 4.2) containing an excess of NADH and HLADH gave no indication of pentanol recovered as its naphthylurethane needs further investigation. The effect of u.v. light on NADH and thus the activity of HLADH on its substrate have to be considered.

4.8.5 Alternative approach

An alternative approach without the need for HLADH is to photolyse cobaloxime (7) in 0.1M aqueous acetic acid together with an organic solvent. Such a system would enable the pentanal formed to diffuse into the organic phase, thus preventing direct contact of the labile [^{18}O]pentanal with the acidic aqueous phase. However, it was demonstrated that not all the pentanal appeared in the organic phase containing acetic acid : pentane in the ratio of 5 : 1. A predominant transfer of pentanal to the organic phase occurred when a 1 : 1 proportion of aqueous acetic acid : pentane was used. Such an increase in organic solvent was undesirable and likely to decrease the yield of pentanal upon photolysis of the cobaloxime (7) (N.B. in neutral solvent alk-1-ene product dominates, cf Section 1.5.3.). Besides, 25 atom-% [^{18}O] pentanal in CH_2Cl_2 when introduced into a citrate-phosphate buffer (pH 4.2) was shown by IR spectroscopy to lose its ^{18}O label rapidly in 1 min. However, when 23 atom-% [^{18}O]PhCHO in CH_2Cl_2 (3 cm^3) was introduced into 0.1M acetic acid (pH 3) (10 cm^3) and vigorously stirred for 5 min, an IR spectrum of this solution in CH_2Cl_2 showed only half of the isotope was lost to the water. With direct introduction of [^{18}O]PhCHO into a vigorously stirred solvent, made up of acetic acid and CH_2Cl_2 in the proportion of 10 : 3, more than half of the ^{18}O label was lost after 5 min. The effect of the aromatic system in PhCHO was to act as an electron donor to

delocalise the positive charge on the carbonyl carbon and, hence, reduce the reactivity of the group in addition reactions, as shown by the following resonance form (81). This ability of an aromatic system to delocalise charge retards the rate of isotopic exchange with water relative to [^{18}O]BuCHO.



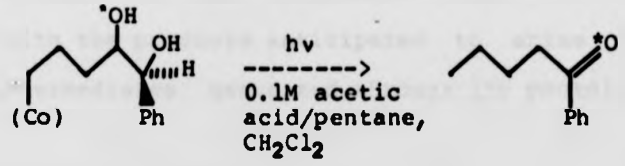
(81)

4.8.6 Conclusions

Investigations of the trapping of [^{18}O]pentanal with HLADH and the diffusion of [^{18}O]BuCHO and [^{18}O]PhCHO into an organic solvent pointed to the advantages of synthesising an [^{18}O]-labelled cobaloxime such as (82). Photolysis of the cobaloxime (82) in a mixed aqueous acetic acid and organic solvent - assuming the possibility of a [1,2] radical rearrangement, would produce [^{18}O]BuCOPh (Scheme 91). This barely hydrated arylketone would be trapped in the organic solvent and should retain most of its ^{18}O label. Initial steps have been taken to synthesize an unlabelled cobaloxime (82) (Scheme 92) to show that photolysis would generate BuCOPh.

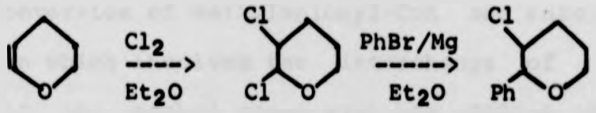
A method for labelling the cobaloxime (7) at

the C-2 oxygen position required the initial preparation of an [$^{18}O_2$]acetate ion, which could then dispace bromide ion from the precursor diethyl 2-bromoglutarate (**Scheme 90**). In the [$^{18}O_2$]acetate ion the [^{18}O]-label became diluted two-fold. However, from observations made (cf **Chapter 2**) whilst labelling ketones and aldehydes with $^{17}O/^{18}O$ isotopes, the precursor $COOEtCH_2CH_2COCOOEt$ would be an ideal reagent. Reduction of this ketonic ester labelled with $^{17}O/^{18}O$ at the ketone position with $LiAlH_4$ would then give $HO-(CH_2)_3-[^{18}OH]CH-CH_2OH$. Applying the procedure of **Scheme 89**, the path to a highly enriched $OH-CH_2-[^{18}OH]CH-(CH_2)_3Co(dmgH)_2py$ can be envisaged.



(82) * = Oxygen-18

Scheme 91



Na, Et_2O → $PhCH{=}CH(CH_2)_3OH$ → $PhCH(OH)CHOH(CH_2)_3OH$

Scheme 92

Chapter 5

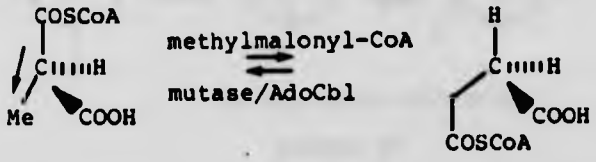
5. INTRODUCTION

This Chapter describes a preliminary study of the cobaloxime $(EtOOC)_2CMe(CH_2)_3Co(dmgH)_2py$ (**101**). This was synthesised in the hope of finding a model system for the 1,2-skeletal rearrangement catalysed by AdoCbl-dependent methylmalonyl-CoA mutase. An introductory discussion on chemical models deals with cobalamin and cobaloxime models reported in the literature that are known to undergo a facile 1,2-carbon shift, possibly via a carbanion intermediate. Thereafter, various cobaloxime models demonstrated to undergo a 1,2-carbon skeletal rearrangement via radical intermediates are presented. Finally, a synthetic route to the cobaloxime (**101**) is discussed together with the products anticipated to arise from the radical intermediates generated through its photolysis.

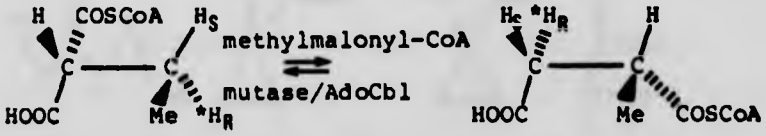
5.1 METHYLMALONYL-CoA MUTASE [1]

Methylmalonyl-CoA mutase catalyses the interconversion of methylmalonyl-CoA and succinyl-CoA, a reaction which involves the interchange of a hydrogen atom at the methyl group with the -COSCoA (**Scheme 93**). Only the (R)-isomer of methylmalonyl-CoA is a substrate for AdoCbl-dependent methylmalonyl-CoA mutase. Experiments with carbon-13 and carbon-14 [2, 3] labelled substrates showed that the -COSCoA group shifts from C-2 on to the Me carbon atom of methylmalonyl-CoA. This shift proceeds intramolecularly. The migration of the hydrogen

atom in the opposite direction occurs intermolecularly, the cobalt-bound methylene group of AdoCbl serving as the acceptor-donor site for the migrating H atom. Retey and Zagalak [4] further extended the studies of the stereochemistry of the methylmalonyl-CoA mutase reaction with a deuterated ethylmalonyl-CoA. Their results are summarised in **Scheme 94.** The enzyme removes the pro-R hydrogen from the methylene group of ethylmalonyl-CoA and the interchange of -COSCoA with H took place with retention of configuration. In diol dehydrase, the interchange of the migrating groups leads to inversion of configuration at the carbon terminus.



Scheme 93

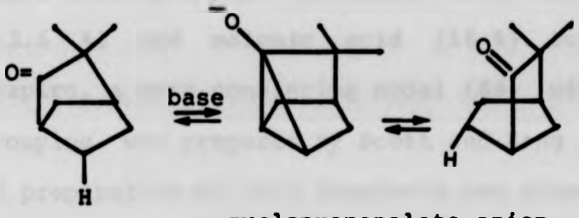


Scheme 94

5.2 CHEMICAL MODELS FOR CARBANION REARRANGEMENT

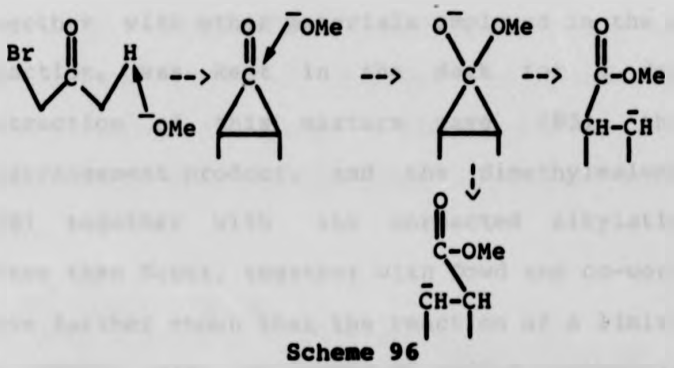
Ingraham [5] was the first to propose that the homoenolate ion of methylmalonyl-CoA could equilibrate (via a cyclopropanolate anion) with the carbanion of succinyl-CoA (cf **Section 1.6.2.3**). Experimental evidence for the existence of a homoenolate anion was provided by Nickon and Lambert [6] from their experiment on the racemisation of camphenilone. The camphenilone

racemisation was postulated to occur by way of enantiomeric homoenolate ion which could equilibrate via a cyclopropanolate anion intermediate (Scheme 95). One other model which shows the existence of a cyclopropanolate anion is the Favorskii rearrangement. In the Favorskii rearrangement (Scheme 96) [7], the products obtained will depend on the manner in which the cyclopropanolate anion equilibrates with its homoenolate anions.



cyclopropanolate anion

Scheme 95



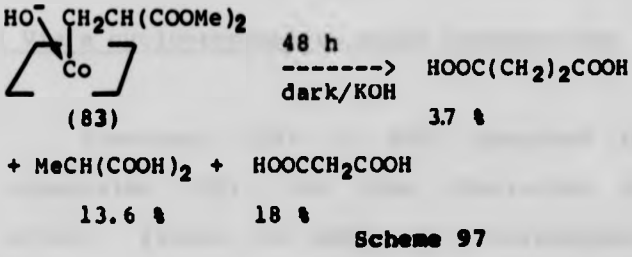
Scheme 96

5.3 COBALALAMIN MODELS FOR -COSCoA MIGRATION

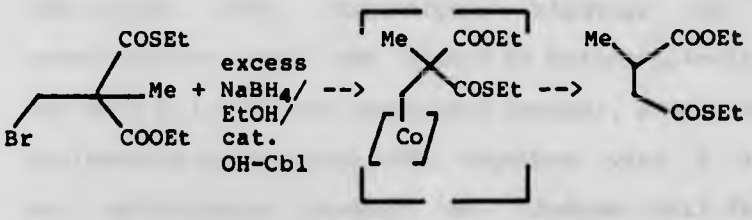
Dowd and Shapiro [8] in 1976 attempted to prepare a cobalamin model (83) to simulate the rearrangement that occurs in the enzymic system. The cobalamin (83) was too unstable to be isolated as a pure material.

Therefore, cob(I)alamin, prepared from the reduction of OH-Cbl with excess $NaBH_4$ in water, at pH 8.9, was reacted with the alkylating agent $BrCH_2CH(COOMe)_2$. After 6 min, the aqueous alkaline solution of (83) had u.v. and visible spectral characteristics of an alkylcobalamin (Scheme 97), suggesting that a Co-C bond had been formed. The alkaline cobalamin solution was kept for 48 h. The products after extraction and saponification gave 3.7 % of the expected rearrangement product succinic acid. Other products isolated were methylmalonic acid (13.6 %) and malonic acid (18 %). Following Dowd and Shapiro, a more convincing model (84) with a thioester grouping was prepared by Scott and Kang [9a]. The method of preparation of this cobalamin was almost identical to that of Dowd and co-workers. A crude mixture containing the model cobalamin, again identified spectroscopically, together with other materials employed in the alkylation reaction, was kept in the dark for a day. Ether extraction of this mixture gave (85), the expected rearrangement product, and the dimethylmalonate ester (86) together with the unreacted alkylating agent. Since then Scott, together with Dowd and co-workers [9b], have further shown that the reaction of a limited amount of HO-Cbl with an excess of sodium borohydride and the thioethyl ester of bromomethyl methylmalonate in ethanol, rapidly yielded the carbon skeletal rearranged thioethyl ester of methylsuccinate (85) in 1 h, together with the unrearranged thioethyl ester of dimethylmalonate (86) (Scheme 98). Scott and co-workers [10] also repeated their thio ester model reaction in EtOD solvent whereby the excess amount of sodium borohydride used in the

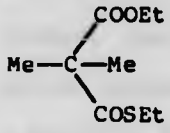
preparation of the alkylcobalamin intermediates was destroyed by the addition of MeCHO following the formation of the alkyl-cobalt bond. The deuterium contents of (85) and (86) were analysed and it was concluded from the extent of deuteration of these products that (i), the C-Co bond in the alkylcobalamin intermediate was capable of both carbanionic and homolytic cleavage; (ii), the skeletal rearrangement most probably occured at the radical stage. The products obtained indicated that the alkyl group on the model cobalamin underwent rearrangement. The position of the methyl group in the methylsuccinate ester was proof that the rearrangement occurred by migration of the thioester group rather than the ester group, as observed in the methylmalonyl-CoA mutase reaction. That the migration was in favour of the thio ester grouping has been suggested by Merkelbach and Buck [11] using CNDO-2 calculations. They based their conclusions on several proposed cyclopropanolate anion intermediates, of which I (Scheme 99) was the favoured intermediate that controlled the exclusive formation of (85).



Scheme 97

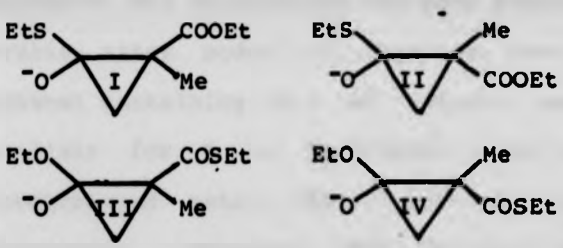


(84) (85)



(86)

Scheme 98



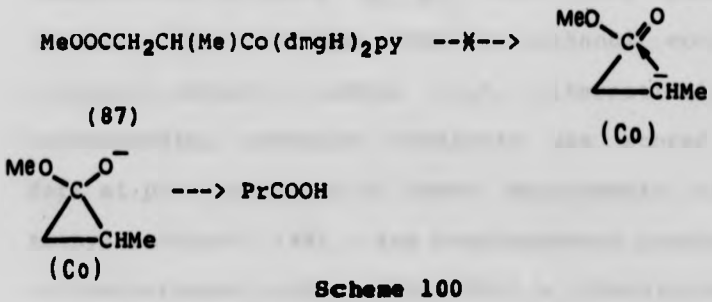
Scheme 99

5.4 COBALOXIME MODELS FOR -COSCoA MIGRATION

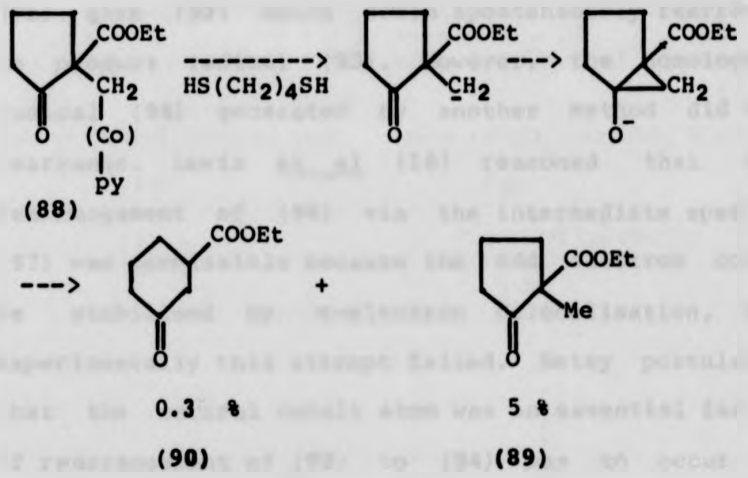
5.4.1 Via a cyclopropanolate anion intermediate

Schrauzer [12] in 1967 prepared the acyclic cobaloxime (87), but this cobaloxime in aqueous alkali failed to show any rearrangement product (isobutyric acid), although the cobalt-carbon bond was cleaved heterolytically. Instead, butyric acid was obtained after saponification, an indication that the formation of a cyclopropanolate anion did not occur

(Scheme 100). Ingraham [13] prepared the cyclic cobaloxime (88). Heterolytic cleavage of the cobalt-carbon bond was caused by butane-1,4-dithiol and gave 0.3 % of the rearranged product, ethyl-3-keto cyclohexane carboxylate (89), together with 5 % of an unrearranged product (90) (Scheme 101). That the rearrangement of the acyclic cobaloxime of Schrauzer failed, may be because the carbanion generated adds a proton before the appropriate groups have rotated into a position for the carbanion to attack the carbonyl group and so cause rearrangement as suggested by Ingraham. The orientation was more favourable in the cyclic ester model of Ingraham. Cobaloxime (88) in ethanol containing 10 % of toluene was exposed to sunlight for 6 h. Photolysis gave 0.2 % of the unrearranged ester (89), but no (< 0.01 %) rearranged products (90) by gas chromatographic analysis. The photolysis results thus support an ionic mechanism for the methylmalonyl-CoA mutase reaction.



Scheme 100



Scheme 101

5.4.2 Via a radical intermediate

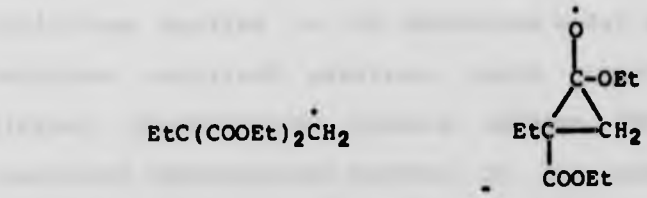
A mechanism for -COSCoA migration via radical intermediates was first proposed by Eggerer *et al* in 1960 [14]. Their proposal was based on the then known Urry and Kharasch rearrangement of 2-phenylethyl radicals. A cobaloxime and cobalamin model, which could rearrange by a free radical mechanism, was demonstrated by Retey *et al* [15]. They photolysed compound (91) (**Scheme 102**) in ethanol containing catalytic amount of acetic acid. Alternatively, the corresponding unstable cobalamin was stored in the dark at pH 7 for a few h. These experiments afforded methylsuccinate (94), the rearrangement product, and an unrearranged product (95). Such a photolysis gave variable products, with no yields given, except that catalytic acetic acid was essential for consistent results [N.B. experiments of Golding *et al* with the model cobaloxime (7)]. Photolysis of the cobaloxime

(91) gave (92) which could spontaneously rearrange to produce radical (93). However, the homologous radical (96) generated by another method did not rearrange. Lewis et al [16] reasoned that the rearrangement of (96) via the intermediate species (97) was permissible because the odd electron could be stabilised by π-electron delocalisation, but experimentally this attempt failed. Retey postulated that the central cobalt atom was an essential factor if rearrangement of (92) to (94) was to occur in reasonable yield. Retey et al [17] then prepared a capped cobaloxime (98) (Scheme 103), in which the "substrate" was anchored covalently by two methylene bridges to a planar part of the complex. Photolysis of (98) in methanol indeed gave a higher yield of the rearranged product because this caged structure prevented the radical formed by photolysis from diffusing away from the catalytic cobalt (II) species. The ester group migrated via the postulated cobalt-stabilised transient intermediate (99). Alkaline hydrolysis of the photolysed ester gave methylsuccinic acid as the only rearrangement product. Interestingly, the cobaloxime model (88) prepared by Ingraham has been reinvestigated. Photolysis of (88) in several solvents, e.g. benzene, chloroform, acetonitrile, all appeared to give > 30 % yield of the rearrangement product (100). The present study showed that the radical rearrangement of an acyl group was a favoured process even without the assistance of cobalt [18].

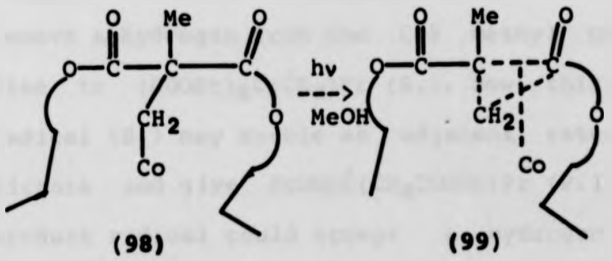
$(EtOOC)_2CMeCH_2Co(dmgH)_2py$ (91) $\xrightarrow{h\nu/EtOH,\ cat.\ MeCOOH}$ $(EtOOC)_2C\dot{M}eCH_2$ (92)

$\longrightarrow$ $(EtOOC)\dot{C}MeCH_2COOEt$ (93) $\longrightarrow$ $(EtOOC)CHMeCH_2COOEt$ (94) + $(EtOOC)_2C(Me)Me$ (95)

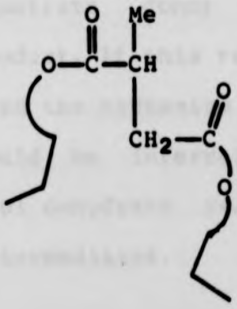
Scheme 102



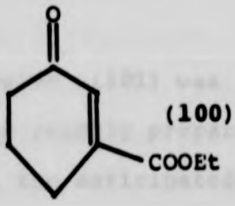
(96) (97)



(98) (99)



Scheme 103



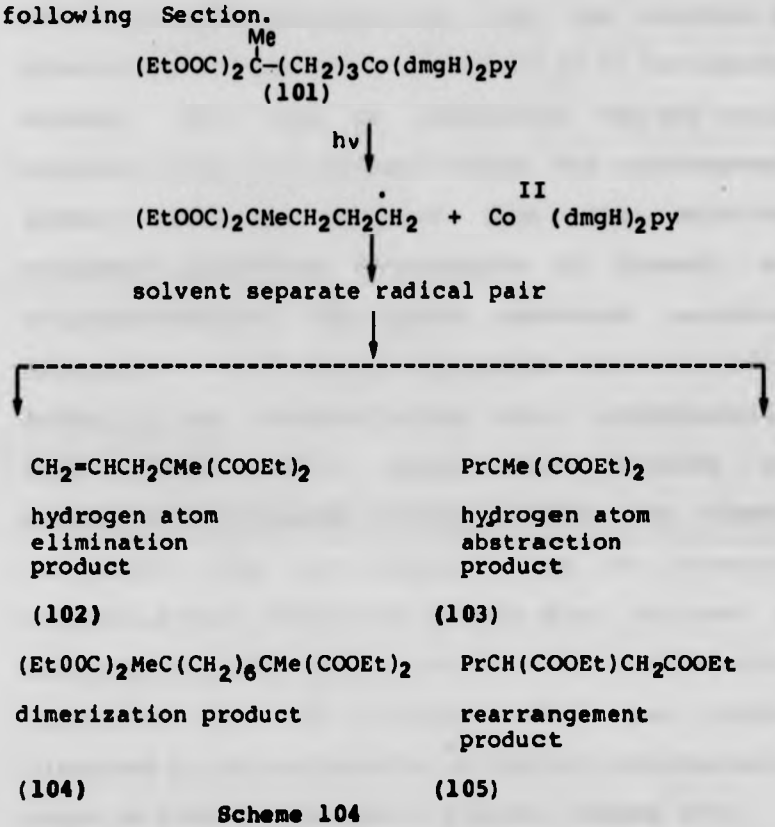
(100)

5.5 A COBALOXIME MODEL FOR METHYLMALONYLCoA MUTASE REACTION

Studies on the model compound (7) showed that the photolysis of this compound in aqueous acetic acid generated radical intermediates, one of which allowed migration of a C-2 hydroxy group, thus giving rise to a rearrangement product (cf **Section 1.6.3**). An analogous cobaloxime model, (**101**) upon photolysis under the conditions applied to the cobaloxime model for the diol dehydrase catalysed reaction, could proceed via a similar photophysical process (**Scheme 104**). Such a postulated photophysical process in the acid mediated pathway initially gives a caged radical pair. The organic radical $[(COOEt)_2CMeCH_2CH_2\dot{C}H_2]$ of this pair could then remove a hydrogen from the C-5 methyl position giving rise to $(COOEt)_2C(\dot{C}H_2)Pr$ (S.). Now, this substrate-like radical (S.) may enable an adjacent ester grouping to migrate and give $EtOOC\dot{C}(CH_2COOEt)Pr$ (P.). Finally, this product radical could accept a hydrogen atom from a substrate donor and terminate to the rearrangement product. If this rearrangement product were identified, then the mechanism of action of methylmalonyl-CoA mutase could be inferred to be like that postulated for the diol dehydrase reaction that is via organic radical intermediates.

The model cobaloxime (**101**) was initially studied because this compound was readily prepared from available precursors. Besides, all the anticipated products which arose from a radical elimination (**102**), termination (**103**), dimerisation (**104**), and rearrangement (**105**),

for example, are readily synthesised as described in the following Section.



Scheme 104

5.5.1 Preparation of model products (101) and (102-105)

The model cobaloxime together with two of the expected products, (102) and (103) were prepared from the precursor $MeCH(COOEt)_2$ (Scheme 105). The α-proton of the methylmalonic ester was readily removed under basic conditions. The carbanion generated then reacted with the respective alkylating agents to form the corresponding products. The model cobaloxime was prepared by the alkylation of the cobaloxime(I) anion with $Cl(CH_2)_3C(COOEt)_2Me$ using the procedure described by Schrauzer. N.B. Widdowson

and Roussi [19] have described the preparation of alkoxycarbonylalkylcobaloximes from the reaction of cobaloxime(II) with α-halogenoesters in a non-aqueous solvent. This type of cobaloximes was not easily prepared using the Schrauzer method. The rearrangement product (105) was prepared from the acid-base catalysed aldol-type condensation of butanal and ethylcyanoacetate. The olefin generated underwent nucleophilic addition by the cyanide ion. Eventually, hydrolysis and decarboxylation gave propylsuccinic acid (Scheme 106), which was isolated and esterified with ethanol to give (105). The dimeric tetraester (104) was prepared from the precursor hexane-1,6-diol. Tosylation of this diol followed by nucleophilic displacement of the alkyl tosylate with iodide ion gave the di-iodide, which was further displaced by two equivalents of diethyl methylmalonate anion to give the tetraester product (Scheme 107).

$$MeCH(COOEt)_2 \xrightarrow[EtOH]{NaOEt} Me\bar{C}(COOEt)_2 + RBr \longrightarrow RC(COOEt)_2Me$$

$$R = Pr,\ CH_2{=}CH{-}\dot{C}H_2,\ Cl(CH_2)_3-$$

Scheme 105

$$PrCHO + CH_2CNCOOEt \xrightarrow[\text{ii) EtOH/KCN}\ \text{iii) HCl/H}_2\text{O}\ \text{iv) EtOH/HCl}]{\text{i) py/MeCOOH}} PrCH(COOEt)(CH_2COOEt) \quad (105)$$

Scheme 106

$$HOCH_2(CH_2)_4CH_2OH \xrightarrow{i} TsOCH_2(CH_2)_4CH_2OTs \xrightarrow{ii}$$
$$ICH_2(CH_2)_4CH_2I \xrightarrow{iii} (EtOOC)_2MeC(CH_2)_6CMe(COOEt)_2$$

(104)

i, TsCl/py ii, NaI/acetone iii, $NaOEt/MeCH(COOEt)_2$

Scheme 107

5.6 MATERIALS AND METHODS

5.6.1 Instruments

NMR spectra were recorded on: (i) Perkin Elmer (model R-12) 60 MHz for ^1H, and (ii) Bruker (model WH90) at 22.63 mHz for ^{13}C. We thank Paul Benson for recording the ^{13}C spectra.

Irradiation technique - Fig. 9 shows the irradiation apparatus used for photochemical experiments described in this thesis. This simple photochemical reactor consists of a medium pressure mercury arc lamp encased in a synthetic quartz envelope. The lamp generates ozone and oxides of nitrogen in air, hence the surrounding area was flushed with nitrogen. The medium-pressure mercury arc operates at internal pressures from 1 to 10 atmospheres and emits radiation over the region 200-1400 nm, with particularly intense emissions at 313 nm, 366 nm, 435.8 nm and 546.1 nm. The light source unit was separated from the solution in the reaction vessel by a water-cooled jacket made of

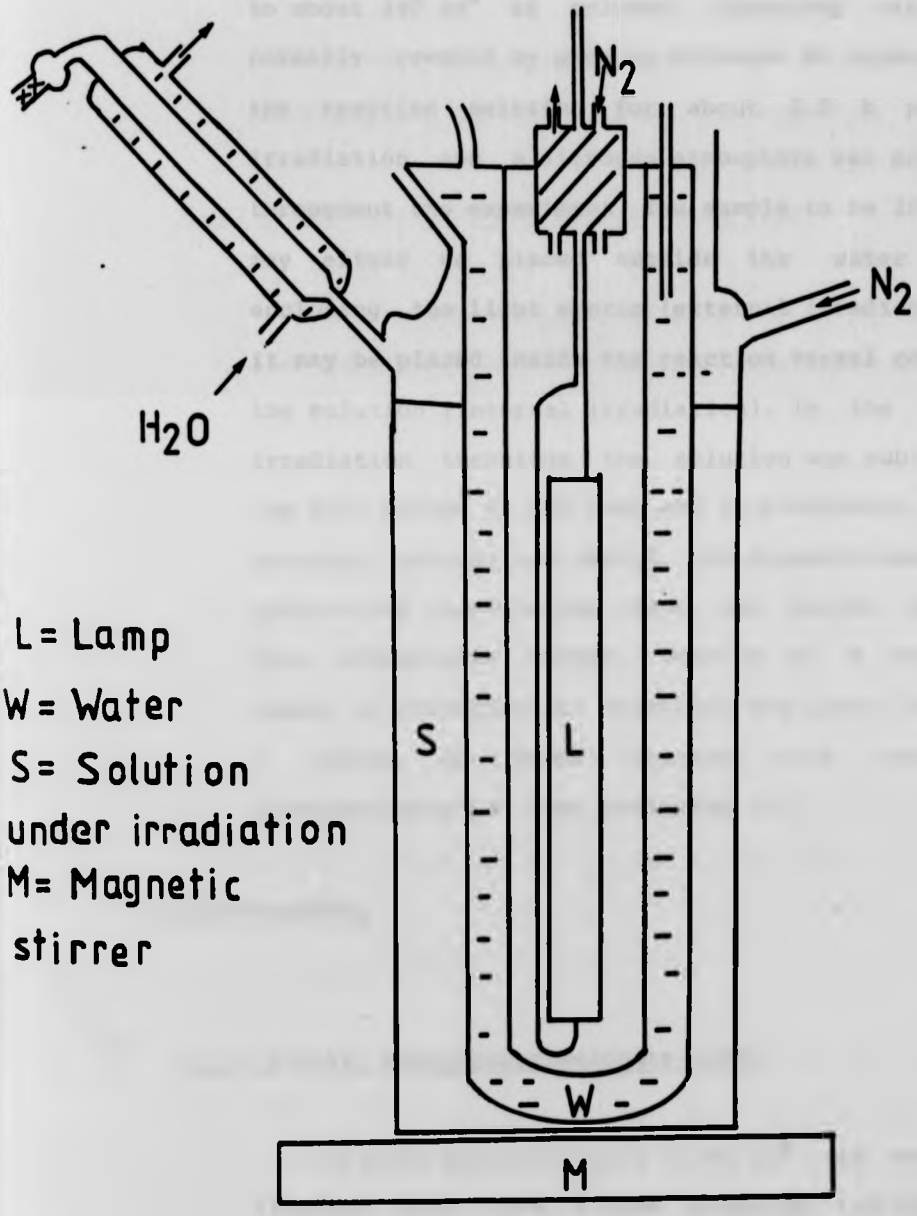


Fig.9 A photochemical reactor

Pyrex. The water-cooled jacket, besides protecting the lamp from the reaction solvent, also prevents the heat generated by the lamp from overheating the solution in the reaction vessel. The reaction vessel can hold up to about 300 cm^3 of solvent. Dissolved oxygen was normally removed by passing nitrogen or argon through the reaction solution for about 0.5 h prior to irradiation and a nitrogen atmosphere was maintained throughout the experiment. The sample to be irradiated may either be placed outside the water jacket enclosing the light source (external irradiation), or it may be placed inside the reaction vessel containing the solution (internal irradiation). In the internal irradiation technique the solution was subjected to the full output of the lamp and is preferable. In the external irradiation method, the degassed sample in a suba-sealed round-bottom flask was better protected from atmospheric oxygen. Details of a very large number of photochemical reactions are given [20], and a series of books dealing with preparative photochemistry has been published [21].

5.7 EXPERIMENTAL

5.7.1 Diethyl methylpropylmalonate (103)

Diethyl methylmalonate (1.86 cm^3, 15 mmol) was titrated with 1.1M sodium ethoxide (13.60 cm^3, 15 mmol), followed by the addition of 3-bromopropane (1.48 cm^3, 15 mmol). The solution was heated to reflux

for a few h. The NaBr precipitate was removed and the filtrate was distilled to give a product (1.3 g, 41 %), b.p. 88 °C at 0.2 mmHg. Lit. [22], b.p. 114-120 °C at 19 mmHg.

^{1}H NMR (CCl_4 60 MHz): 0.7 - 2.1 [16H, m, PrCMe & $(COOCH_2\underline{Me})_2$], and 4.09 [4H, q, $(COOC\underline{H}_2Me)_2$].

5.7.2 Diethyl 2-allyl-2-methylmalonate (102)

To diethyl methylmalonate (1.21 cm^3, 9.9 mmol) was added 1.1M sodium ethoxide (9 cm^3, 9.9 mmol). The solution was kept at reflux for 1 h, cooled and then allylbromide (0.86 cm^3, 9.9 mmol) was added. The precipitate was removed using Celite. The filtrate was distilled (Kugelrohr) giving the product (1 g, 47 %), b.p. 132 °C at 1.1 mmHg. Lit. [23], b.p. 84-90 °C at 2.5 mmHg.

^{1}H NMR (CCl_4, 60 MHz): 1 - 1.6 [9H, m, CMe & $(CH_2\underline{Me})_2$], 2.52 [2H, d, $C\underline{H}_2CH$], 4.15 [4H, q, $(C\underline{H}_2Me)_2$], and 4.8 - 5.9 [3H, m, CH_2=CH].

5.7.3 Hexane-1,6-diol ditosylate

Hexane-1,6-diol (10 g, 85 mmol) in dry pyridine (150 cm^3) was cooled in an ice-salt bath. Tosyl chloride (48.4 g, 255 mmol) was added to this solution. The solution was kept at 4 °C when it changed colour from yellow to brown. At this stage long needles of pyridinium hydrochloride precipitated.

When no more salt appeared to form, the mixture was poured into 400 g of ice. This caused precipitation of the title compound, which was then recrystallised from hot methanol (24 g, 66 %). Lit. [24], m.p. 70 °C.

^{1}H NMR (CCl_4, 60 MHz): 1.16 - 1.86 [8H, m, $-(CH_2)_4-$], 2.42 [6H, s, $(MeAr)_2$], 3.26 - 4.16 [4H, t, $(-CH_2O-)_2$], and 7.16 - 7.86 [8H, ABq, $(4 \times HAr)_2$].

5.7.4 1,6-Diiodohexane

Hexane-1,6-diol ditosylate (6 g, 14 mmol) and NaI (6 g, 40 mmol) in AR acetone (50 cm^3) was kept at reflux overnight. The mixture was cooled and the excess NaI filtered off. The acetone was evaporated, and the product redissolved in CH_2Cl_2. The CH_2Cl_2 extract was washed with water, and dried ($MgSO_4$). The product was distilled (3.6 g, 71 %), b.p. 66 °C at 0.05 mmHg. Lit. [25], b.p. 123 - 128 °C at 4 mmHg.

^{1}H NMR (CCl_4, 60 MHz): 1.2 - 2.2 [8H, m, $-(CH_2)_4-$] and 3.2 [4H, t, $(CH_2I)_2$].

5.7.5 2,9-Dimethyl-2,9-di(ethoxycarbonyl)decan-1,10-dioate (104)

Prepared from the following reactants: super-dry ethanol (15 cm^3); sodium (0.5 g, 22.8 mmol); Me(COOEt)CH(COOEt) (5.6 g, 31.8 mmol), and 1,6-diiodohexane (3.6g, 10.6 mmol). The solution was kept at reflux for 24 h. The product (1.39 g, 31 %), b.p. 134 °C - 138 °C at 0.005 mmHg, solidified on

leaving at r.t.. ^{1}H NMR (CCl_4, 60 MHz): 1 - 1.5 [30H, m, $-(CH_2)_8-$, $(COOCH_2\underline{Me})_4$, $(C\underline{Me})_2$], 3.45 and 4.25 [8H, q, $(COOC\underline{H}_2Me)_4$].

5.7.6 Propylsuccinic acid [26]

Butanal (7 g, 98 mmol), pyridine (6 g, 80 mmol), ethylcyanoacetate (9 g, 80 mmol), and glacial acetic acid (6.4 cm^3, 112 mmol) was kept at reflux at 100 ^{0}C for 1.5 h. Ethanol (10 cm^3) was added, followed by KCN (80 mmol) in small portions, to the hot solution. After refluxing for another hour, the brown solution was cooled, and decomposed with HCl (100 cm^3, 1 : 3 dilution). An oil was separated from the aqueous layer The aqueous layer was extracted with ether and the ether extract and the oily layer combined. The ether was removed, and the oily residue was heated to reflux with conc. HCl (50 cm^3) for 12 h. The hydrolysate was evaporated to dryness on a oil bath, and the dried mass was extracted with ether. The ether was removed giving an oil which solidified. The solid was recrystallised twice from ether and petroleum ether (40 - 60) affording the product (1 g, 7.5 %), m.p. 92 - 93 ^{0}C. Lit. [26], m.p. 98 - 99 ^{0}C.

^{1}H NMR (CCl_4, 60 MHz): 0.7 - 2 [7H, m, $MeCH_2CH_2$], 2.3 - 3.2 [3H, m, $CHCH_2$] and 11.74 [2H, s, $(COOH)_2$].

5.7.7 Diethyl propylsuccinate (105)

To 2-propylsuccinic acid (1 g, 7.35 mmol) in

ethanol (8 cm^3) was added acetyl chloride (0.472 cm^3) to generate HCl (ca 3 % w/v). The solution was kept at reflux for 3 h and left to stand overnight. The ethanol was removed, and the residue diluted with CH_2Cl_2, which was then washed with aqueous saturated NaCl and water. The CH_2Cl_2 extract was dried ($MgSO_4$). Distillation (Kugelrohr) gave a colourless liquid (812 mg, 48 %), b.p. 91 °C at 0.01 mmHg. Lit. [27], b.p. 112 - 117 °C at 7 - 9 mmHg.

^1H NMR (CCl_4, 60 MHz): 0.8 - 1.7 [13H, m, $(COOCH_2\underline{Me})_2$ & Pr], 2.1 - 2.75 [3H, m, $CHCH_2$], and 4.15 [4H, q, $(COOCH_2Me)_2$]. IR (neat): C=O, 1735 cm^{-1}. ^{13}C ($CDCl_3$, TMS): 175.1, 172.1, 78.5, 77.1, 75.7, 60.5, 41.1, 36.2, 34.2, 20.2, 14.2, and 13.9.

5.7.8 2,2-Diethoxycarbonyl-5-chloropentane - $Cl(CH_2)_3C(COOEt)_2Me$

To super-dry ethanol (100 cm^3) under a nitrogen atmosphere, was added sodium (3.5 g, 152 mmol). When the sodium had reacted, diethyl methylmalonate (28 g, 160 mmol) was added. The reaction mixture was stirred, followed by the addition of $Br(CH_2)_3Cl$ (14.8 cm^3, 150 mmol). After stirring for 20 h, the precipitate was filtered and the residue on distillation gave a fraction b.p. 60 - 64 °C at 0.65 mmHg, which was mainly the unreacted diethyl methylmalonate and was discarded. Further distillation afforded the product (16.7 g, 41 %), b.p. 80 - 116 °C at 0.65 mmHg. Lit. [28], b.p. 134 - 136 °C at 10 mmHg.

^{1}H NMR (CCl_4, 60 MHz), 1.25 [6H, t, $(CH_2Me)_2$], 1.36 [3H, s, CMe], 1.6 - 2.0 [4H, m, CH_2CH_2], 3.5 [2H, t, CH_2Cl], and 4.16 [4H, q, $(CH_2Me)_2$].

5.7.9 4,4-Diethoxycarbonylpentyl(pyridine)cobaloxime (101)

To dimethylglyoxime (8.6 g, 75 mmol) and cobalt (II) chloride hexa-hydrate (8.9g, 37.5 mmol) was added AR methanol (100 cm^3). The suspension was stirred for 10 min under a stream of argon. To the suspension 50 % aqueous NaOH (3 g, 6 cm^3, 75 mmol) was added, followed by pyridine (3 g, 37.5 mmol). The solution turned dark on addition of NaOH. The suspension was cooled to -10 ^{0}C and stirred for 20 min with argon bubbling into the mixture. To this suspension was again added 50 % NaOH (3 g, 75 mmol), followed by $NaBH_4$ (200 mg, 5.28 mmol) dissolved in water (2.5 cm^3). The reaction mixture became dark green and $Cl(CH_2)_3CMe(COOEt)_2$ (10 g, 40 mmol) was added to complete the reaction. The reaction mixture was stirred for 10 h, and air was bubbled in to reduce the solvent to half its volume. The suspension was poured into ice-cold water (200 cm^3), whereupon the product precipitated from the solution. The orange-yellow product was filtered and recrystallised from aqueous ethanol, affording the title cobaloxime (7.6140 g, 32 %).

^{1}H NMR (CCl_4, 60 MHz): 1 - 2 [15H, m, $(Me)_2$ & $(CH_2)_3CMe$], 2.10 [12H, s, 4 x dmgH, Me], 4.15 [4H, q, $(COOCH_2Me)_2$], and 5 py H. Found (CHN): C, 49.2, H, 6.48; N, 11.86 %. $C_{24}H_{38}CoN_5O_8$ (584) requires: C,

49.4; H, 6.5; N, 12.00 %.

5.7.10 Photolysis of 4,4-diethoxycarbonylpentyl(pyridine)cobaloxime

The cobaloxime (100 mg, 0.1713 mmol) in 250 cm^3 of 0.1M acetic acid (pH 3.0) was stirred, until it dissolved, in the dark (24 h). This solution may be irradiated internally or stoppered with a serum cap for external photolysis. In either case , the solution was deoxygenated by bubbling argon (pre-washed with Cr^{II}, NaOH and acetic acid) for 30 min. For external photolysis, the serum-capped flask was transfered to a water bath kept at r.t. and the solution photolysed with constant stirring. The photolysis took ca 7 - 10 min, when the original yellow solution turned colourless. The products were extracted into ether, which was washed with $NaHCO_3$ and then dried ($NaSO_4$). The ether was removed and the solid was diluted with a mixed solvent consisting of ether (3 cm^3) : pet-ether (40-60) (10 cm^3). The mixed solvent was filtered under suction through a pad of silica-gel (60-120 mesh) to remove inorganic by-product. GLC: model F & M; 20 % DEGS on Chromosorb WHP; 180 ^0C, carrier gas He; flow rate, 1.25 cm^3/min; was used to identify the photolysis products - by comparison of their retention times (Rt) with those of authentic samples and also by co-injections.

5.8 RESULTS AND DISCUSSION

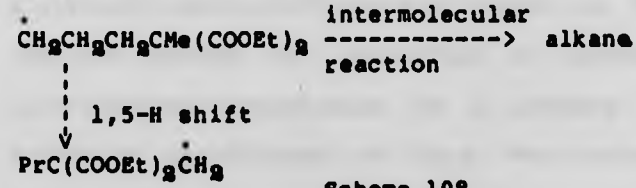
Photolysis of 4,4-diethoxycarbonylpentylcobaloxime

in deaerated aqueous acetic acid at pH 3, did not give the rearrangement product (diethyl 2-propylsuccinate). TLC: silica-gel; 5 x 20 cm; ether - petroleum ether (40 - 60), (3 : 10); iodine stain; showed a single intense spot at Rf 0.49. Only two major products were eluted from GLC. These were identified as $PrCMe(COOEt)_2$, Rt of 4 min 35 s, and $H_2C{=}CHCH_2CMe(COOEt)_2$, Rt of 5 min 29 s, formed in ca equal proportions. Under similar GLC conditions, authentic $PrCH(COOEt)CH_2COOEt$ has a Rt of 12 min, whereas the dimer tetraester (102) failed to elute because of its non-volatility. Thermolysis under aereated and deaerated conditions in aqueous acetic acid gave products identical to those from the photolysis in deaerated solvent. Thermolysis (deaerated) in a non-polar solvent, e.g. benzene, also gave similar types of products. Thus, in all the above reactions, there was not a trace of the rearrangement product, as detected by GLC analysis.

The failure to detect a rearrangement product could be explained by the reactions outlined in **Scheme 108.** The crucial step leading to a 1,2-intramolecular rearrangement of an ethoxycarbonyl group is the initial 1,5-intramolecular hydrogen shift to generate a 2,2-diethoxycarbonylpentyl radical, which is not demonstrable by this model. Golding et al [29] had shown that (7) deuterated at the 1,1,5,5-pentyl positions, when photolysed in aqueous acetic acid, gave deuterated pentanal containing three deuterated atoms in the terminal methyl group. This is expected if a favoured intramolecular 1,5-hydrogen transfer takes precedent

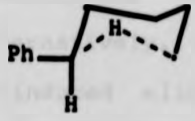
(Scheme 109). That a 1,5-intramolecular (H) transfer is kinetically favourable may be attributed to the transition state of this intermediate, which resembles the geometry of a cyclohexane ring, for example, the species (106) proposed by Tedder [30].

Thus, the only reasonable conclusion to be drawn from the experiments described on the photolysis of 4,4-diethoxycarbonyl(pyridine)cobaloxime is that soon after homolytic cleavage of its cobalt-carbon bond, the rates of intermolecular reaction, giving rise to radical abstraction and disproportionation products, are far faster than a 1,5-intramolecular hydrogen shift in the derived radical. There is no net gain in stabilisation energy from an initial primary radical going to another primary radical via a 1,5 radical migration, whereas with the 4,5-dihydroxypentylcobaloxime model the initial radical upon intramolecular 1,5-hydrogen shift generates a secondary radical which is additionally stabilised by the adjacent oxygen atom.



Scheme 108

Scheme 109



(106)

Chapter 6

6. INTRODUCTION

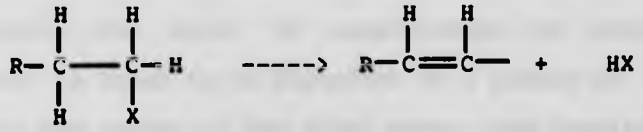
This Chapter describes methods for the preparation of alk-1-enes and alkan-2-ones by the neutral photolysis and thermolysis of alkylcobaloximes. An introductory survey on the scope and limitations of existing literature methods for alk-1-ene preparation from primary alkyl halides or their derived primary substrates is followed by a discussion on alkylcobaloximes as alternative intermediates for olefin synthesis.

6.1 FORMATION OF THE $-\overset{|}{C}=\overset{|}{C}-$ DOUBLE BOND

Various methods for constructing the $-\overset{|}{C}=\overset{|}{C}-$ double bonds have been reviewed [1, 2, 3]. In this thesis we will consider only β-elimination of the type shown in Scheme 110, where X = e.g. OH, OCOR, Br, OSO_2R, $\overset{+}{N}R_3$ etc. Some examples of reactions that are known to produce a straight chain alk-1-ene are shown in Table 9. The simplest method for preparing an alkene would be the acid-catalysed dehydration of a primary alcohol. The synthetic uselfulness of this dehydration process is limited, because under acidic conditions, the alk-1-ene could be isomerised into the more stable alk-2-ene or alk-3-ene [4]. However, a high yield of alk-1-ene can be obtained by dehydrating the vaporised alcohol over hot alumina [5]. Alternatively, alk-1-ene can be obtained from the base-induced elimination of a primary alkyl halide or tosylate. The side-products in this case often

arise from a competitive substitution reaction, especially when oxygenated bases [6] are used to induce elimination. Conditions which favour elimination over substitution may be achieved by heating the primary alkyl halides with a sterically hindered base (e.g. Hünig's base) [2] or with a tertiary alkoxide base in benzene [6]. Schlosser and Tarchini [6] have shown that a primary alkyl ammonium salt (formed by a reaction of octyl bromide with trimethylamine) suspended in tetraglyme when heated with an excess of powdered KOH, gave exclusively oct-1-ene. Monson [7] found that alkyl bromides readily undergo elimination to form an alk-1-ene when heated directly in HMPA solvent.

So far we have discussed the direct method for obtaining an alk-1-ene from a readily available primary alcohol or primary alkyl halide. Many other workers had converted these accessible compounds into the primary alkyl acetate, xanthate (ROCSSMe), amine oxide, sulphoxide or selenoxide etc., and they found that heating these derived compounds alone could provide sufficient energy for the leaving group X to function as an intramolecular base. The temperature required to induce this pyrolytic elimination to give an alk-1-ene appeared to vary with the nature of the group X, such that the selenoxides are more suitable in view of the mild conditions required to effect their elimination.



Scheme 110

Table 9. Preparation of alk-1-ene from primary alkyl substrates

R	X	Reaction conditions	Alk-1-ene	Yield (%)	Ref.
Hexyl	-OH	Al_2O_3, 350 °C	oct-1-ene	98	[5]
Hexyl	-Br	Hünig's base, 180 °C, 12 h	oct-1-ene	99	[2]
Hexyl	$-\overset{+}{N}Me_3$	tetraglyme, KOH, 100 °C, 20 h	oct-1-ene	98	[6]
Hexyl	-Br	HMPA, 1 h, 180 - 210 °C	oct-1-ene	68	[7]
Ethyl	-OCOMe	N_2, 500 °C	but-1-ene	100	[8]
Propyl	-OCSSMe	reflux	pent-1-ene	15	[9]
Octyl	$-NOMe_2$	160 °C	dec-1-ene	80	[10]
Pentadecyl	-SOMe	reflux in DMSO	heptadec-1-ene	85	[11]
Decyl	-TeOAr (Ar = p-$MeOC_6H_4$)	toluene, 110 °C, 12 h	dodec-1-ene	58	[12]
Decyl	-SeOAr (Ar = 2-$NO_2C_6H_4$)	25 °C	dodec-1-ene	62	[13]

6.2 COMMENTS

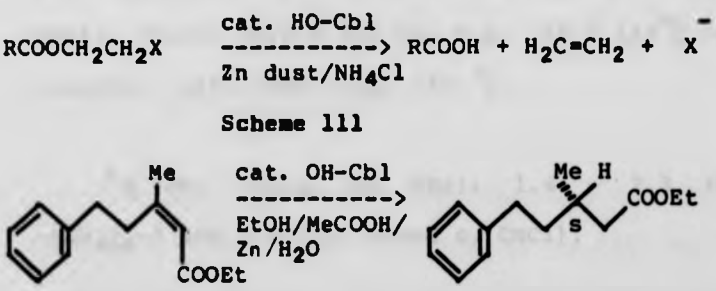
Although there are several methods described in the literature for the direct conversion of a primary alkyl halide or a derived primary alkyl substrate into the corresponding alk-1-ene, some of the procedures suffer from competitive side reactions or require extreme conditions. For instance, simple alkyl halides or

tosylates can undergo elimination as well as substitution reactions. The ratio of substitution to elimination products is known to be dependent on a number of factors such as the nature of the alkyl group, the leaving group, the base, solvent and the temperature [3]. Schlosser's method gave a good yield of oct-1-ene, but required strongly basic conditions [6]. Pyrolysis of an acetate [14] to give an alkene is usually effected at a temperature of ca 300 - 500 ^{0}C. The high temperature required for this elimination is a disadvantage because the alkene that is being formed may not be stable under the reaction conditions. Pyrolysis of amine oxides is relatively mild compared to the acetate, but the manipulations required to construct the desired amine oxide functionality reduce the efficiency in terms of the yield and time required for the procedure. The work-up procedure after the pyrolysis of a xanthate [15] is frequently difficult because of sulphur-containing impurities, which often have to be removed by distillation from sodium. In this thesis, we describe a method for preparing terminal alkenes, applicable to base-labile substrates, from the thermolysis and photolysis of alkylcobaloximes under neutral conditions. The alkylcobaloximes were readily prepared from available precursors such as alkyl halides or tosylates.

6.3 SYNTHETIC APPLICATIONS OF ALKYLCOBALOXIMES

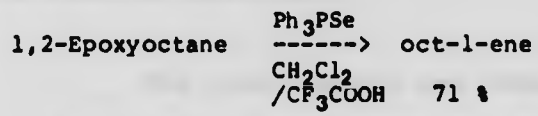
Although alkylcobaloximes have been widely studied as models of the AdoCbl-dependent enzymic reactions, recently, a few publications have appeared indicating

that alkylcobaloximes (or even cobalamins) are useful in synthetic applications. For example, the alkylation of an orthoester halide with cobaloxime(I) gave an orthoester alkylcobaloxime, which was then hydrolysed to an alkylcobaloxime containing a carboxyl function attached to a cobalt-alkyl group [16]. Such cobaloximes were difficult to prepare directly from halogenoacids, by the frequently used technique, involving the in situ generation of cobaloxime(I) anion in an aqueous methanolic solution at high pH (risk of lactonisation of the halogenoacid under the basic conditions). Cob(I)alamin has been found to catalyse the removal of a β-haloethyl protecting group (**Scheme** 111) [17], the reduction of an α,β - unsaturated ethyl ester (**Scheme** 112) [18], and the reduction of 1-naphthylnitrile to its amine [19]. These reactions used catalytic amounts of cob(I)alamin and an excess of metallic zinc as the source of electrons. The finding of Golding et al [20] that photolyses of alkylcobaloximes under neutral conditions gave exclusively alk-1-enes, is the basis of the mild and convenient synthetic method using alkylcobaloxime intermediates, which is described here. Initially, octyl(pyridine)cobaloxime was studied for comparison with Schlosser's findings [6]. We have also studied the conversion of 2-hydroxyalkylcobaloximes (readily prepared from an epoxide), to alk-1-enes and alkan-2-ones, respectively. The reaction of **Scheme** 113 [21] had been reported, but required a toxic selenium reagent. Cobaloximes provide an alternative method for such conversions.



Scheme 111

Scheme 112



Scheme 113

6.4 EXPERIMENTAL

6.4.1 Cyclopentanol

Cyclopentanol was prepared from the following reactants: cyclopentanone (66 cm^3, 750 mmol) in ethanol (75 cm^3), $NaBH_4$ (7.2 g, 189 mmol) in water (75 cm^3), 2M NaOH (9 cm^3) and ethanol (45 cm^3). Yield: 49 g (76 %), b.p. 86 - 88 ^{0}C at 104 mmHg (a clear liquid). Lit.[22], b.p. 141 ^{0}C.

^{1}H NMR (CCl_4, 60 MHz): 1.1 - 2 [8H, m, $-(CH_2)_4-$], 3.6 [1H, s, CHOH] and 4.2 [1H, broad s, OH].

6.4.2 Cyclopentylchloride

The title compound was prepared by heating the following reactants: cyclopentanol (22.4 g, 260 mmol);

conc. HCl (66 cm^3, 660 mmol); and $CaCl_2$ (26 g, 234 mmol). Yield: 15.2 g (55 %), b.p. 110 - 114 ^oC (a clear liquid). Lit. [22], b.p. 114 ^oC.

^1H NMR (CCl_4, 60 MHz): 1.4 - 2.3 [8H, m, $-(CH_2)_4-$] and 4.4 [1H, broad s, CHCl].

6.4.3 Cyclopentylcarbinol

The title compound was prepared by refluxing the following reactants together: cyclopentylchloride (37 g, 0.358 mmol) in ether (150 cm^3); Mg turnings (9.2 g, 0.386 mmol) and then addition of paraformylaldehyde (17.85 g, 595 mmol). Yield: 9.2 g (26 %), b.p. 65 - 70 ^oC at 18 mmHg (a clear liquid). Lit. [23], b.p. 162 ^oC.

^1H NMR (CCl_4, 60 MHz): 1 - 2.4 [9H, m, cyclic protons C_5H_9], 3.17 [1H, s, OH, dissappear on shaking in D_2O], and 3.4 [2H, d, CH_2OH].

6.4.4 Cyclopentylcarbinyltosylate

The title compound was prepared from the following reactants at r.t.: cyclopentylcarbinol (6.6 g, 66 mmol) in pyridine (6 cm^3) and tosyl chloride (14.49 g) in pyridine (35 cm^3). Yield: 16 g (90 %). An oily liquid. Lit. [24], m.p. 8 - 11 ^oC.

^1H NMR (CCl_4, 60 MHz): 1.31 - 1.71 [9H, m, cyclic protons - C_5H_9], 2.41 [3H, s, MeAr], 3.81 [2H, d,

OCH_2], 7.26 & 7.71 [4H, ABq, 4 x Ar H]

6.4.5 Cyclopentylcarbinyliodide

Cyclopentylcarbinyl tosylate (15 g, 59 mmol), acetone (150 cm^3) and NaI (69.6 g, 0.464 mol) were heated to reflux. Work-up gave the title compound, yield (6.4 g, 43 %), b.p. 63 ^0C - 65 ^0C at 12 mmHg (a clear light pink liquid). Lit. [25], b.p. 75 ^0C at 19 mmHg.

^1H NMR (CCl_4, 60 MHz): 1 - 2.5 [9H, broad m, cyclic protons - C_5H_9] and 3.14 [2H, d, CH_2I].

6.4.6 Cyclopentylcarbinyl(pyridine)cobaloxime

Schrauzer's method: $DMGH_2$ (6.6 g, 57 mmol), AR $CoCl_2 6H_2O$ (6.8 g, 28.6 mmol), MeOH (76 cm^3), 50 % NaOH (2.3 g, 4.6 cm^3, 57 mmol), pyridine (2.3 g, 29 mmol), 50 % NaOH (57 mmol), $NaBH_4$ (153 mg, 5 mmol) and finally cyclopentylcarbinyl iodide (6.4 g, 25 mmol). The product was precipitated by addition of cold water. Yield: 6.8 g (59 %).

^1H NMR ($CDCl_3$, 220 MHz): 0.8 - 1.7 [9H, m, cyclic protons], 1.77 [2H, d, $CoCH_2$], 2.1 [12H, s, 4 x dmgH, Me], and 5 py H.

6.4.7 1-Methylcyclopentanol

The title compound was prepared as described in

Vogel [26]: Mg turnings (9.8 g, 0.4 mol) in dried ether (60 cm^3), and cyclopentanone (17 g, 0.2 mol) in ether (20 cm^3). Reflux for 1 h. Yield: 11.86 g (59%), b.p. 57.5 ^{0}C at 25 mmHg (low m.p. white solid). Lit. [27], b.p. 43 ^{0}C at 9 mmHg, m.p. 36 - 37 ^{0}C.

^{1}H NMR (CCl_4, 60 MHz): 1.3 [3H, s, Me], 1.4 - 2 [8H, m, $-(CH_2)_4-$], and 2.7 [1H, s, OH, disappeared in D_2O].

6.4.8 1-Methylcyclopentene [27]

Methylcyclopentanol (12 g, 0.12 mol) was heated with iodine (50 mg) at 100 ^{0}C until the product distilled over. The olefin-water mixture was dried (Na_2SO_4), and the crude product was carefully fractionated with a column of helices (1 ft): Yield: 5.9 g , b.p. 74 - 74.2 ^{0}C. A clear liquid, lit. [27], b.p. 75 ^{0}C.

^{1}H NMR (CCl_4, 60 MHz): 1.7 [3H, s, Me], 1.8 - 2.5 [6H, m, cyclic $(CH_2)_3$ protons], and 5.28 [1H, m, -C=CH]. Lit. [27], reported that an IR spectrum showed contamination by methylenecylopentane (10 %). GLC: model F-11, 15 % squalane on Chromosorb WHP) gave the title product Rt 5 min containing < 10 % contaminant.

6.4.9 Methylenecyclopentane

Methylenecyclopentane was prepared from methyltriphenylphosphonium iodide (9 g, 22 mmol) in

DMSO (50 cm^3), sublimed potassium t-butoxide (2.38 g, 21 mmol), and cyclopentanone (1.7 g, 20 mmol). Yield: 214 mg (13 %), b.p. 75 - 76 ^oC (a clear liquid). Lit. [28], b.p. 75 - 76 ^oC.

6.4.10 Octyl(pyridine)cobaloxime

Octyl(pyridine)cobaloxime was prepared by a procedure described by Schrauzer from octylbromide which afforded 54 % of the alkylcobaloxime. The procedure from bromo(pyridine)cobaloxime, 2 equiv. KBH_4, DMSO, octylbromide, gave after purification through a silica gel column (pet ether : acetone; 1 : 1), and crystallisation of the product from aqueous ethanol (4 ^oC), 31 % of a yellow-orange crystalline material. This cobaloxime was insoluble in pentane. ^1H NMR ($CDCl_3$, 60 MHz): 0.5 - 2.0 [17H, m, octyl protons] 2.12 [12H, s, 4 x dmgH, Me], and 5 py H.

Found (CHN): C, 54.42; H, 7.57; N, 14.80 %. $C_{21}H_{36}CoN_5O_4$ (MW 481) requires: C, 52.39; H, 7.54; N, 14.55 %.

6.4.11 1,2-Epoxyhexane

1,2-Epoxyhexane was prepared by reacting hex-1-ene (12.48 cm^3, 0.10 M) in CH_2Cl_2 (150 cm^3) with meta-chloroperbenzoic acid (22 g, 0.108 mol) in CH_2Cl_2 (240 cm^3). The reaction was stirred for 5 h at r.t.. The method of extraction of the product was as described in the literature [29]. Yield: 46 %, b.p.

114 - 116 °C (a clear liquid). Lit. [30], b.p. 113.5 - 114 °C.

^{1}H NMR (CCl_4, 60 MHz): 0.85 - 1.25 [3H, broad shouldered, Me], 1.35 - 1.75 [6H, broad $(CH_2)_3$], and 2.55 [3H, m, cyclic protons].

6.4.12 2-Hydroxyhexyl(pyridine)cobaloxime

2-Hydroxyhexylcobaloxime was prepared from 1,2-epoxyhexane (4 g, 40 mmol), dimethylglyoxime (8.6 g, 75 mmol), cobalt (II) chloride 6-hydrate (8.9 g, 75 mmol), MeOH (100 cm^3), NaOH and $NaBH_4$. The product obtained was crystallised from aqueous methanol. Yield: 1.9 g, (11 %).

Found (CHN): C, 49.74; H, 6.97, N, 15.66 %. $C_{18}H_{31}CoN_5O_5$ (MW 468) requires: C, 48.61, H, 6.87; N, 14.91 %.

^{1}H NMR ($CDCl_3$, 60 MHz): 0.7 - 1.6 [12H, broad band, alkyl protons], 2.18 [12H, s, 4 x dmgH, Me], and 5 py H.

6.4.13 Photolysis of octyl(pyridine)cobaloxime)

The irradiation technique was as described in Chapter 5. Octylcobaloxime (481 mg, 1 mmol) was photolysed in a non-polar solvent (benzene, 250 cm^3, 8 h 20 min) and a protic solvent (ethanol, 250 cm^3, 1 h 25 min). External irradiation was applied in each

case. Photolysis was monitored by TLC (disappearance of starting material). The benzene solvent was distilled to low volume, pentane was added and the solution filtered through silica gel to remove inorganic by-products. The pentane was concentrated and the sample was injected into a GLC column and was found to contain mainly oct-1-ene. To determine the yield of oct-1-ene in ethanol, 25 cm^3 of ethanol was removed and a known quantity of toluene (0.005 cm^3) was added as an internal standard. A control expt. with authentic toluene (2 mmol) and oct-1-ene (2 mmol) in 25 cm^3 n-pentane (25 cm^3) gave peaks of equal proportions by GLC analysis. An aliquot from the photolysis in ethanol was filtered through a small alumina column before injecting into a GLC column. GLC: Model F-11, F.I.D; 15 % squalane in Chromosorb WHP, 100 ^0C; carrier gas nitrogen, 20 psi; Rt 9 min 50 s (toluene) and Rt 12 min (oct-1-ene). N.B. No octane was detected. Authentic octane appeared at Rt 13 min 45 s. Control experiments e.g. (i) photolysis of octylbromide (10 mmol) and $NaBH_4$ (20 mmol) in deaerated ethanol (250 cm^3) for 3 h gave mainly octane; (ii) photolysis of the same quantities of materials but containing a catalytic amount of $BrCo(dmgH)_2py$ (1 mmol) in ethanol for 2 h gave a high proportion of oct-1-ene together with octane.

6.4.14 Thermolysis of octyl(pyridine)cobaloxime

Octylcobaloxime (50 mg, 0.10 mmol) was heated at reflux in various solvents (50 cm^3): benzene,

chlorobenzene, ethanol, and AR ethane-1,2-diol. In each thermolysis, nitrogen was bubbled through the solution. Work up for the thermolysis in the hydroxylic solvent required addition of water before extraction of the products into pentane. The pentane was evaporated to low volume and filtered through alumina. An internal standard (toluene) was then added. Analysis of oct-1-ene in the non-polar solvents involved adding the internal standard directly to a known volume of the solvent. The yield of oct-1-ene was determined from GLC: F-11, 15 % squalane, 100 °C.

6.4.15 Photolysis of 2-hydroxyhexyl(pyridine)cobaloxime (111)

2-Hydroxyhexylcobaloxime (55 mg, 0.1 mmol) in doubly distilled water (55 cm^3) was degassed for 3.5 h with argon before photolysis at r.t. (external irradiation). The reaction was monitored by removing an aliquot, extracting the product into CH_2Cl_2, and observing the disappearance of starting material (12 h) by TLC. The reaction mixture was poured into 0.4 % acidic 2,4-dinitrophenylhydrazine. The rest of the procedure included extracting into carbonyl-free solvent, followed by chromatography on specially activated silica gel to remove contaminants such as propanone DNP and the by-product biacetylmonooxime DNP [31]. 2-Hexanone DNP (50 mg, 84 %) was obtained. TLC: silica gel, F-254 (Merck), $CHCl_3$, Rf 0.8 was similar to authentic 2-hexanone-DNP. The material was dissolved in AR MeOH (100 cm^3), the sample was diluted 50 x and an O.D. reading of 0.38 at 358 nm in a 1 cm

cell was found (Unicam SP 500 u.v. spectrometer). The % yield estimated from u.v. spectroscopy was 81 % (Molar extinction coefficient at 358 nm = 2.2 x 10^4 mol^{-1} 1 cm^{-1}). This suggested an actual yield of hexan-2-one of nearly 100 %, because isolation of DNP derivative is known to be ca 80 % efficient [31].

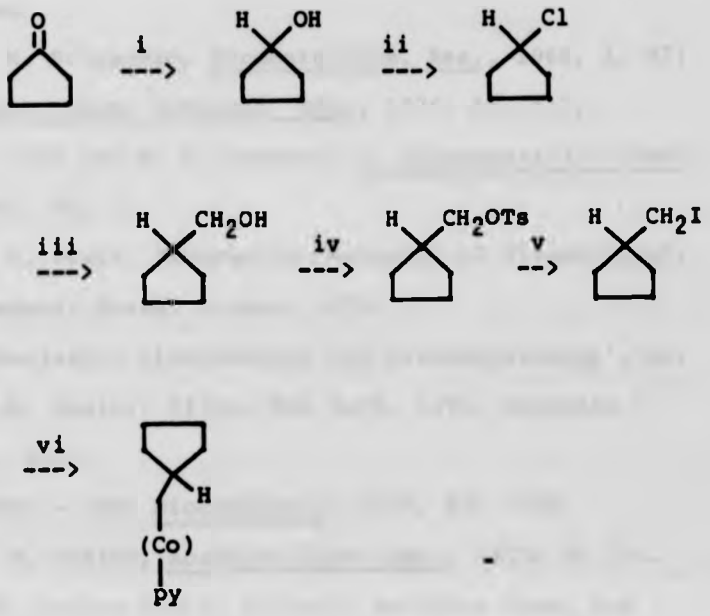
6.5 RESULTS AND DISCUSSION

Photolysis of octylcobaloxime in deaerated benzene was slow (8 h). The product was identified by GLC (comparison with authentic material), which showed exclusively oct-1-ene. However, photolysis of this alkylcobaloxime in a polar solvent e.g. ethanol required only 1 h 20 min for completion, giving 98 % of oct-1-ene. Thermolysis of octylcobaloxime in several solvent systems (benzene, chlorobenzene, ethanol, ethane-diol) was monitored by TLC for the disappearance of the starting material. However, TLC showed many unidentified degradation products in contrast to the clean disappearance of the starting material from the photolysis experiment. The best solvent system in the thermolysis expt. was ethanol which gave 33 % of oct-1-ene after a 4 day reflux. When the octylcobaloxime was heated at reflux in a higher solvent (e.g. ethanediol), a very poor yield of oct-1-ene was obtained.

Besides generating an alk-1-ene from the photolysis of octylcobaloxime, the terminal alkene can be readily produced from 2-hydroxyalkyl(pyridine)cobaloxime. We showed that 2-hydroxyhexyl(pyridine)cobaloxime (100 mg,

0.213 mmol) with trifluoroacetic acid (0.181 cm^3, 0.234 mmol) in $CDCl_3$ (0.5 cm^3) in a NMR tube, underwent acid-catalysed fragmentation within 5 min, to give signals which indicated the presence of hex-1-ene. Quick distillation (Kugelrohr) and collection of this material gave a ^1H NMR spectrum identical to that of authentic hex-1-ene. Photolysis of cobaloxime (111) in a neutral buffer (water) gave after a 12 h irradiation, exclusively hexan-2-one. The ketone was isolated as its DNP derivative in ca quantitative yield. The cobaloxime (111) was prepared from 1,2-epoxyhexane. Hence, a terminal epoxide can be converted to a terminal olefin or ketone depending on the reactions applied to the derived cobaloxime (111).

The generation of a terminal olefin from an alkylcobaloxime requires the expulsion of one of the β-hydrogens in the alkyl portion. The mechanism of production of a terminal olefin would either be a concerted β-elimination from an excited state of the alkylcobaloxime or a stepwise process via a caged cobaloxime(II) - radical pair. However, to rule out the possibility of contamination by an internal alkene, cyclopentylcarbinylcobaloxime (112) (Scheme 114) was investigated. In this case, the product of direct elimination, methylenecyclopentane, is easily converted (e.g. mild acid catalysis) to the more stable 1-methylcyclopentene and so the method is subjected to a strigent test. Although the relevant compounds had been prepared, there was insufficient time to complete the analysis of this system.



(112)

Scheme 114

(i) $NaBH_4$/ethanol (ii) $H^+/CaCl_2$ (iii) Mg/$(HCHO)_n$ (iv) py/TosCl (v) NaI/acetone (vi) Schrauzer procedure

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