A THESIS

entitled

STUDIES OF ALKYLCOBALOXIMES AS MODELS FOR A B_{12}-DEPENDENT REARRANGEMENT

by

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ABBREVIATIONS

Å	Angstrom
Ac	acetyl
AdoCbl	adenosylcobalamin
Aq.	aqueous
B	base
b.p.	boiling point
^{o}C	degree centigrade
C_{α},C_{β},C-1,C-2,...	carbon atom relative to a defined functional group
Conc.	concentrated
Co-R	chelated cobalt σ-bonded to an alkyl group R
D	deuterium
δ	chemical shift in parts per million
5,6-DMB	5,6-dimethylbenzimidazole
DMG, dmgH	monoanion of dimethylglyoxime
DMS	dimethanesulphonate
DMSO	dimethyl sulphoxide
DNA	deoxyribonucleic acid
EDA	ethyl diazoacetate
e.i.m.s.	electron impact mass spectroscopy
E.S.R., e.s.r.	electron spin resonance
Et	Ethyl radical
EtOAc	ethyl acetate

g	gram
GC	gas-liquid chromatography
h	hour
HMPA	hexamethylphosphoramide
i.r.	infrared
λ	wavelength
L_α	ligand in the α position
LDA	lithium diisopropylamide
M	molar
Me	methyl radical
MeIT	methyl itaconate
α-MG	α-methyleneglutarate
MHz	megahertz
min	minute
(m)mol	(milli)mole
Ms	mesyl (methanesulphonyl)
m.p.	melting point
m/z	mass/charge
nm	nanometer
n.m.r.	nuclear magnetic resonance
nOe	nuclear Overhauser effect
Ph	phenyl
p.p.m.	parts per million
py,Py	pyridine

r.b.	round bottom flask
R_f	relative flow
r.t.	room temperature
RX	alkyl halide
TFA	trifluoroacetic acid
THF	tetrahydrofuran
t.l.c.	thin layer chromatography
TMS	tetramethylsilane
TSS	3-(trimethylsilyl)propionic acid, sodium salt
U.V.,u.v.	ultra violet

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I also acknowledge all the secretarial and technical services provided by the Department of Chemistry and Molecular Sciences, which were usually dispensed with a smile and good humour.

***

DECLARATION

The work herein described was performed in the Department of Chemistry and Molecular Sciences at the University of Warwick, England, during the period commencing November, 1980 to November 1983.

This work is thought to be original, and where other work is quoted, this is made clear and due acknowledgements are accorded. The work herein described has not been submitted for any other degree previously.

***

PUBLICATIONS

Synthesis and rearrangements of carboxylate-substituted but-3-enyl- and cyclopropylcarbinylcobaloximes
B. T. Golding and S. M. Kibende,
J. Chem. Soc., Chem. Comm., 1983, 1103

Rearrangements and dioxygen insertion into the Co-C σ bond of but-3-enylcobaloximes; X-ray structure of $EtO_2CCH{=}CHCHOOCoMe$
N W Alcock, B. T. Golding, and S. M. Kibende
Manuscript in preparation

And whatever you do, in word or deed, do everything in the name of the Lord Jesus, giving thanks to God the father through Him.

Whatever your task, work heartily as serving the Lord and not men, knowing that from the Lord you will receive inheritance as your reward; you are serving the Lord Christ.

The Bible, Colossians, 3:17, 23, 24 (R.S.V.)

To my dear Mother,
whose love and support
made all this work possible

ABSTRACT

The necessity for vitamin B_{12} in animals and the consequences of lack of it are well-documented in the literature. Its unique and indispensable role, and its active form as the coenzyme for at least eleven different enzymic rearrangements *in vivo*, are also known. The enzymic conversion of α-MG to MeIT, which is the centre of interest in the present work, is one of these rearrangements.

However, the mechanism by which most of these rearrangements proceed is still a matter of speculation, based on logic and inconclusive experimental observations. Organocobalt model systems, which resulted from the suggestion that substrate-Co species are intermediates in these rearrangements, have been synthesised and studied in their thousands by various workers, and have in some instances shed light on this intriguing mechanistic problem.

This thesis is a description of the synthesis, characterisation and rearrangement of monocarboxylate-substituted but-3-enyl- and cyclopropylmethyl(pyridine)-cobaloximes, in a model study of the B_{12}-dependent enzymic rearrangement of α-MG. The syntheses of the alkylating agents are described, and novel compounds, which are fully characterised, are reported.

The findings from this study suggest that alkyl-cobalamins postulated as intermediates in the reaction catalysed by α-MG mutase would be insufficiently reactive towards rearrangement. However, rapidly interconverting carboxy-substituted but-3-enyl and cyclopropylmethyl radicals are suggested to be plausible intermediates. An addenda to this thesis contains synthetic proposals for the continuation of this work.

A separate chapter (Chapter 5) describes the preparation and testing of the cyclic analogues of the antitumour drug busulphan, viz., *cis*- and *trans*-di(hydroxymethyl)cyclopropane dimethanesulphonate. Both compounds showed little antitumour activity near their toxic dose. The implications of these observations for the mode of action of busulphan are discussed.

***

CHAPTER 1

INTRODUCTION

1.0 VITAMIN B_{12}

1.0.0 General Considerations

Vitamin B_{12}, an indispensable factor in the metabolic reactions and the largest of the coenzymes, belongs to the family of substances known as corrinoids which are derivatives of a macrocycle called corrin. Vitamin B_{12} cannot be synthesised in the mammalian body and therefore it has to be taken in pure form or as an ingredient in food.

As far as is known to date, vitamin B_{12} is synthesised exclusively by microorganisms. In this respect it is unique among the vitamins. Wherever it is found in nature its origin can be traced back to bacteria or microorganisms, growing in soil or water or in the rumen or intestine of some animal.

All animals require vitamin B_{12} in their metabolism, whereas all or most plants do not. On the other hand, some microorganisms require B_{12} and synthesise it, whereas others need it but cannot synthesise it. The latter types have to take it in from their environment.

Vitamin B_{12} is now called cyanocobalamin (CN-Cbl). Human beings require an average daily dose of 3 to 7 µg of this vitamin, which is normally

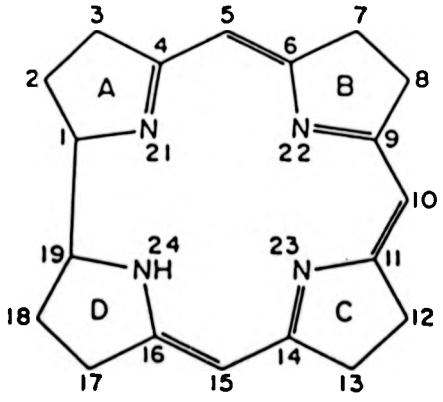
available from dairy products. Cereals, nuts, vegetables and fruits do not contain B_{12} unless it is artificially added. Human plasma contains several other analogues of vitamin B_{12}, the major components being methylcobalamin (MeCbl), adenosylcobalamin (AdoCbl) and hydroxocobalamin (OH-Cbl). Methylcobalamin is the dominant cobalamin in blood. It has been found in various human tissues and fluids including the milk and plasma of nursing mothers, where it occurs in about equal amounts.

Methylcobalamin and adenosylcobalamin are coenzymes. They catalyse enzymic transmethylation and isomerisation reactions, respectively. It is now known that these coenzymes are the active forms of vitamin B_{12}, rather than cyanocobalamin as was erroneously believed for a long time. OH-Cbl, which can be converted into the active form of vitamin B_{12} more easily than CN-Cbl, is nowadays administered in the treatment of various diseases instead of CN-Cbl. The latter is sometimes found in the blood of vitamin B_{12}-deficient patients because of failure in their metabolic system to convert it into adenosylcobalamin. The recognition of these coenzyme forms of vitamin B_{12} opened up a vast new field of research for investigation and although progress has been made in this area, there is still a lot more to be done.

Vitamin B_{12}, being a member of the corrinoid group, is made up of reduced pyrrole rings with side-chains and joined into a macrocyclic ring by links between their α carbons; three of these links are formed by a

one-carbon unit and the other by a direct C_α-C_α bond. In CN-Cbl, there is a cyanide group above the ring (denoted as β-position for convenience) and a 5,6-dimethylbenzimidazole (5,6-DMB) group below the ring (α position). All these groups are directly attached to the central cobalt(III) atom *via* the nitrogen atoms except for the substituent at the β-position. The latter is attached to cobalt by a unique Co-C σ bond. The numbering of the carbon and nitrogen atoms of the corrin ring corresponds to that in porphyrins except that corrin lacks a C-20 atom. The four tetrapyrrole rings are labelled A, B, C and D in a clockwise direction.

Figure 1



Corrin

1.0.1 Historical

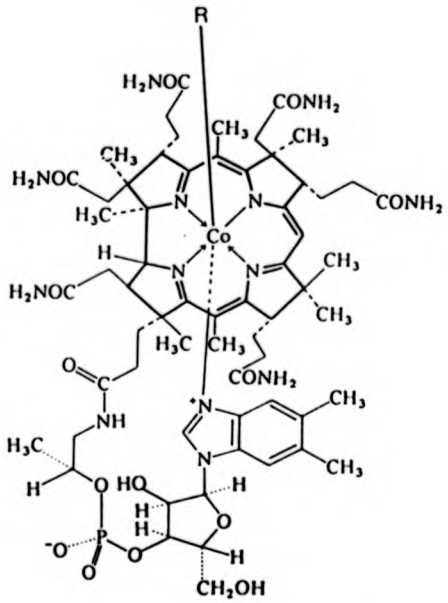
A disease by the name of pernicious anaemia was first clearly described in the 19th Century. It is characterised by neurological disorders and diminution of the red blood cells, many of which lose their disc-like shape. A sore tongue and absence of hydrochloric acid in the stomach are some of the many symptoms that beset a patient with this disease. Before 1926 pernicious anaemia was invariably fatal. Minot and Murphy, during that year, published[1] their landmark treatment of pernicious anaemia by a special diet of raw liver administered to forty-five patients with the disease. From their clinical observations they concluded that raw liver contains an anti-pernicious-anaemia (APA) factor, and if eaten in large quantities would be of tremendous benefit to the patient. However, the concentration of this factor in the liver is very small ($\sim$ 1 ppm). This meant that the patient had to eat rather too much raw liver to exhibit diagnosable improvement. In the ensuing work vitamin B_{12} was isolated[2,3] as red crystals from liver extracts and later on from bacterial fermentation processes. Finally, this compound was successfully synthesised in the laboratory, although bacterial sources are still cheaper by far for commercial purposes. Its chemistry and structure have been targets of research attention for many years.

1.0.2 Structure of Vitamin B_{12}

Much work was undertaken to find out the chemical nature of vitamin B_{12} and after long and tedious efforts, the correct structure was established[4] by Hodgkin in 1956 using X-rays. From the structure it was clear that the molecule contains a hexaco-ordinate diamagnetic cobalt atom, surrounded by four nitrogen atoms and a cyanide group in the β-position. The Co-C bond in vitamin B_{12} (1.92 Å) is shorter than that in AdoCbl (2.03 Å) and other cobalamins, perhaps solely due to steric and electronic properties of the β-ligands. The corrin nucleus is well protected by the surrounding ligands from chemical attack. The four corrinoid rings are buckled to project the nitrogen atoms alternately 0.05 Å below and above the equatorial plane.

The central cobalt atom is suited for its position and role because its size allows it to fit in the middle of the corrin ring without undue distortion and concomitant stress in the ring. Furthermore, its ability to change oxidation states from Co(III) to Co(II) and Co(I) is an indispensable factor in the activity of coenzyme B_{12} in reactions that depend on it. The bond angles and bond lengths (bond strength) of the axial ligands (L_β-Co-L_α) vary from ligand to ligand in organocobalt complexes depending on the steric hindrance and electron withdrawing/donating capabilities of these ligands, e.g. for isopropyl(pyridine)cobaloxime the Co-C bond is 2.085 Å and CO-N (pyridine) is 2.099 Å. If Ph_3P, which is a more tightly bound and hindered ligand than pyridine

Figure 2

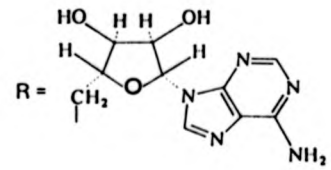


R = OH, (OH-Cbl)

R = Me, (Me-Cbl)

R = CN, (CN-Cbl)

R = adenosyl, (AdoCbl)



Adenosyl

replaces the latter, the Co-C bond stretches to 2.20 Å, the longest Co-C bond known to date. However, if pyridine is replaced by H_2O, the bond should contract because water is a poor base and is less hindered. For this reason secondary cobalamins, which are unstable and hard to prepare, can be prepared much more easily if L_α (5,6-DMB) is prevented from co-ordinating to cobalt by quarternising it first. L_α and L_β seem to be engaged in a perpetual competition for the Co central atom which in turn may protrude out of the equatorial plane to the side of the "stronger" ligand. It can therefore be seen that if L_α in coenzyme B_{12} is replaced by a hindered but strongly bound part of the transport protein or the enzyme, the nature of this group could control the making and breaking of the Co-C of the coenzyme. This argument gives support to hypothetical radical mechanisms put forward to explain the B_{12}-dependent enzymic rearrangements.

The sequence of side chains in naturally occurring corrins, e.g. vitamin B_{12}, is the same as that found in the naturally occurring porphyrins such as haemoglobin and chlorophyll, due to their origin by a common biosynthetic pathway. These alternating acetamide and propionamide side chains make the vitamin more hydrophilic in character. It is not known which one of these amide groups is employed as a point of attachment on to the enzyme or transport protein during the enzymic rearrangements. Nevertheless, it is now known that the amide groups are indispensable in coenzyme B_{12} and if

they are hydrolysed to the corresponding carboxylates, the B_{12}-dependent enzyme is inhibited. A large number of coenzyme B_{12} analogues with L_β modified in the sugar and/or the base moiety of the nucleoside have been prepared[5] in an effort to locate which functional groups of the adenosyl ligand take part in the rearrangements. Most of these analogues could function as cofactors for ribonucleotide reductase[5] whereas others could not. It was also noticed during those experiments that the primary amino group of the adenine moiety did not affect the activity of the coenzyme if it were modified or left out. In a similar way Hogenkamp[5] has shown that a coenzyme B_{12} analogue which is active with a particular enzyme may be totally inactive or an inhibitor of another enzyme.

1.1 MECHANISM OF THE B_{12}-DEPENDENT ENZYMIC REACTIONS

1.1.0 Background Aspects

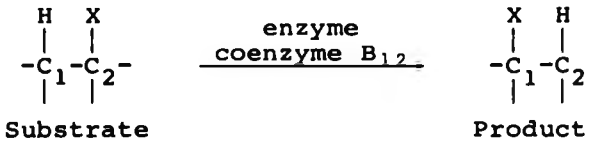
A total of eleven enzymic reactions (Table 1) are now known to be dependent on coenzyme B_{12}. The respective enzymes are almost totally inactive in the absence of coenzyme B_{12}. In nature, when B_{12}-dependent enzymes are synthesised by bacteria, usually some analogue of the coenzyme is also made in the system in order to enable the enzyme to carry out its function. Although these unique enzymic rearrangements have been known for a long time, it is only recently, about 15 years ago, that

TABLE 1 Enzymatic Reactions Catalysed by Coenzyme B_{12} (AdoCbl)

Enzyme	a	b	c	d	X
Dioldehydrase	OH	H	H	e.g. H or CH_3	OH
Glycerol dehydrase	OH	H	H	CH_2OH	OH
Ethanolamine Ammonia-Lyase	OH	H	H	H or CH_3	NH_2
(*R*)-Methylmalonyl-CoA Mutase	H or CH_3	H	CO_2H	H	COSCoA
(*S*)-Glutamate Mutase	H	H	H	CO_2H	$CH\overset{+}{N}H_3CO_2^-$
α-Methyleneglutarate	H	H	H	CO_2H	$C(=CH_2)CO_2H$
Aminomutase utilising either					
(i) (*S*)-3,6-Diaminohexanoate	$CH_2CH\overset{+}{N}H_3CH_2CO_2^-$	H	H	H	NH_2
(ii) (*R*)-2,6-Diaminohexanoate	$(CH_2)_2CH\overset{+}{N}H_3CO_2^-$	H	H	H	NH_2
(iii) (*R*)-2,5-Diaminopentanoate	$CH_2CH\overset{+}{N}H_3CO_2^-$	H	H	H	NH_2
(iv) α- or β-Leucine	$(CH_3)_2CH$	H	H	CO_2H	NH_2

tremendous interest has arisen aimed at finding out the actual role and mode of action of coenzyme B_{12} in these rearrangements. Interests among researchers has varied from studying model compounds that closely simulate the coenzyme (e.g. alkylcobaloximes) through to studying actual enzyme systems[6]. The integral value of all this research is that it has yielded a lot of insight towards solving the mechanistic problem of the B_{12}-dependent rearrangements. To this end, suggestions based on sound experimental observations have been made in an effort to explain the intriguing mechanisms of these rearrangements[7,8].

Essentially, these reactions consist of a group X on C-1 swopping positions with a hydrogen atom on C-2, in an unprecedented manner, quite unknown to the organic or inorganic chemist.



(1)

Group X could be acrylyl as in α-methyleneglutarate (αMG), an $-NH_2$ as in ethanolamine ammonia-lyase, -COS-CoA as in methylmalonyl-CoA etc.

Although the suggestions that have been made to explain the mechanisms are varied, most researchers seem to agree that:

1. the first step in these rearrangements is the breaking of Co-C bond of coenzyme B_{12};

2. the resulting adenosyl moiety abstracts a hydrogen atom from C-1 which it "later on" returns to C-2 of the same substrate molecule.

This trend of thought has been supported experimentally in the case of ethanolamine ammonia-lyase[9,10]. Studies[11] with coenzyme and substrate specifically labelled with deuterium and tritium have revealed:

(a) that C-5′ hydrogens of the coenzyme do take part in these enzymic rearrangements;

(b) that there is no transfer of hydrogen from solvent to either substrate or AdoCbl in these reactions except in the case of ribonucleotide reductase.

The Dowd model[7] fails in this respect because he observed hydrogen transfer between solvent and substrate.

Despite all these studies, up to now there is no impeccable evidence available to confirm the mechanistic suggestions. One general trend in these studies is the implication of the nature of Co-C bond as one of the major factors behind these rearrangements.

1.1.1 The Co-C σ-bond[12]

(i) Alkylcobalt complexes are diamagnetic and hence give good ^{1}H n.m.r. spectra. Contrary to the classical expectations of a highly polarised M-C bond in an organometallic compound, the Co-C σ bond is more covalent than it is ionic and the cobalt central atom is considered to be in the oxidation state of +3

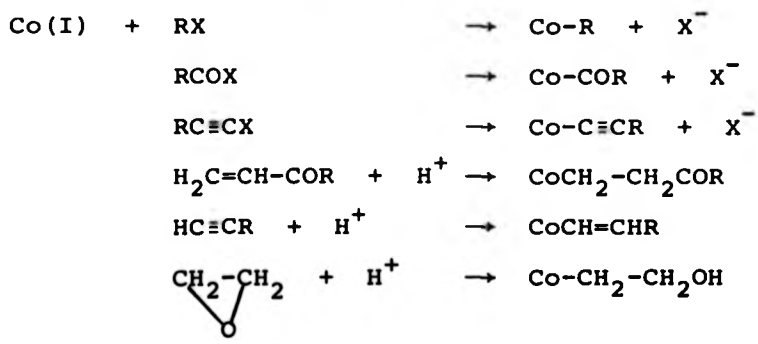
in all uncharged hexacoordinate cobalt complexes. In the infrared region it usually absorbs between 315-335 cm^{-1}, subject to the nature of the alkyl ligand and the other ligation around the cobalt atom[12a,b]. A peak at about 450 nm in the u.v. region also signifies its presence. The latter peak has been ascribed to d-d transitions[12c] whereas on other occasions it has been attributed to charge-transfer absorption[19] involving the promotion of an electron from the highest filled σ-bonding orbital to the corresponding σ-antibonding orbital. The charge-transfer assignment is justified on the grounds that the extinction coefficients pertinent to such absorptions are of the order of 10^3.

The Co-C bond is usually stable to water and air, but it is dissociated by light. Alkylcobalamins are more light-sensitive than model complexes, e.g. alkylcobaloximes, especially in solution. These complexes seem to stand up well to heat as compared to light, although the Co-C bond will break (homolytically, presumably) at elevated temperatures. In both cases, however, stability is strongly influenced by the thermodynamic state of the molecule, e.g. steric hindrance as in secondary alkylcobaloximes and to a good extent by the α-substituent (L_α). In this work pyridine was exclusively used as the α-ligand.

(ii) <u>Formation of Co-C bond</u>: The Co-C σ bond can be formed by three different routes (Scheme 1): Co(I) reacting with electrophilic reagents (Co(I) is still

the most nucleophilic reagent known), Co(II) with organic radicals and Co(III) with nucleophilic reagents. The reaction involving Co(I) is the most commonly used one and was employed to synthesise almost all the alkylcobaloximes in this project. Electrophiles in this case may vary from organohalides and acylhalides, acetylenes, through to epoxides and active double bonds.

Scheme 1



The thermodynamic state of the resulting organocobalt complex determines whether any of these reactions in Scheme 1 is going to work or not, just as much as the same principle governs their stability under varying conditions of light, heat, etc. Some organocobalt compounds are too hindered to form (e.g. there is no reaction between cob(I)alamin and neopentyl chloride) while others do form, but are too unstable to be isolated. Hexacoordinate secondary alkylcobalamins are a typical example of the latter case, detectable only in reaction mixtures. Co(I) needed in these reactions

has been generated in various ways, usually in alcohols like EtOH, MeOH, i-PrOH, although other solvents like THF, DMF, etc. have been used. Most reducing agents used in the laboratory are capable of reducing Co(III) to Co(II) and then to Co(I). However, sodium borohydride is usually the reagent of choice for generation of Co(I). This is because of its easy handling and subsequent work-up, besides being cheap and readily available. It reduces Co(III) to Co(I) rapidly and efficiently. It was used in this work almost exclusively, occasionally with a pellet of sodium hydroxide, to generate Co(I). Although Co(I) is stable at high pH, it decomposes in aqueous acid[13]. Nevertheless, the protonated species (Co-H) can be prepared in anhydrous acid according to G. N. Schrauzer and R. J. Holland's method[14]. Another method[15] of generating Co-H under milder conditions was used in this study. It entails warming β-hydroxy-α-phenethylcobaloxime solution at 40°. The reaction proceeds by β-elimination of hydrogen to give an enol and Co-H. The cobalt hydride thus generated may then react *in situ* with the appropriate activated double bond or acetylene, etc.

(iii) Cleavage of Co-C σ Bond and Rearrangement: As was pointed out earlier, most researchers are agreed that the cleavage of the Co-C σ bond precedes rearrangement in all the B_{12}-dependent enzymic reactions. However, divergent opinions are expressed in discussions of some of the rearrangements at least, regarding the

nature of the bond cleavage, the role of cobalt (if any) after the cleavage and the nature of the intermediates. Carboxylate substituted cyclopropylcarbinylcobalt species which have been suggested as possible intermediates in the B_{12}-dependent αMG rearrangement have been synthesised and studied in this project. The results obtained point to the fact that these alkylcobaloximes rearrange only slowly when treated with TFA in $CDCl_3$ or heated up to 45^{o} for an hour. This observation casts doubts on their intermediary role in actual enzymic rearrangements. Again this observation is in sharp contrast with earlier experimental results[16,17] employing the related, methyl-substituted cyclopropylcarbinyl cobaloximes. The latter cobaloximes were shown to rearrange readily (with or without catalysis by TFA in $CDCl_3$) to a mixture of the corresponding but-3-enylcobaloximes.

The Co-C bond can be cleaved in various ways:

(a) homolytically with formation of Co(II) and an organic radical $R^{\cdot}$;

(b) heterolytically with formation of Co(I) and $R^{\oplus}$ or Co(III) and $R^{\ominus}$;

(c) β-elimination with formation of Co-H and an olefin (or an alkenol, e.g. in the case of β-hydroxy-α-phenethylcobaloxime).

Although the mode of cleavage of the Co-C σ bond in the B_{12}-dependent enzymic systems is still a subject of much study, experimental evidence accumulated from non-enzymic photolysis and thermolysis of organocobalt

compounds indicates that homolytic fission[18,19] competes with β-elimination[20] in these reactions, e.g. ethylcobalamin photolysed under nitrogen, gave ethylene as the main product. This can be explained by the β-elimination mechanism. However, the increase of the rate of this thermolysis if oxygen or i-PrOH is present and the preponderance of ethane in the products if mercaptoethanol is added, indicate that homolysis takes precedence under these conditions.

Cleavage of the Co-C σ bond has been studied in a few B_{12}-dependent enzymes by e.s.r. spectroscopy and there is ample evidence that cleavage of this bond is the first step in these enzymic reactions. If homolysis of the Co-C bond occurs, it is expected that an R$^{\cdot}$ and a Co(II) e.s.r. signals should appear during the enzyme catalysis, and it has been observed[21,22-26]. However, the fact that Co(II) and free radicals can be detected during enzymatic reactions by their e.s.r. and u.v.-visible spectra strongly suggests, but does not prove, that the Co-C bond undergoes homolytic fission. The Co(II) species could arise in such circumstances from some other side reaction like oxidation of Co(I) by aerial O_2 as was shown by kinetic studies with ribonucleotide reductase[27]. Alternatively, the e.s.r. observations could be explained[28] by invoking a direct atomic orbital overlap of Co(II) + radical pair with a Co---C distance of ≥ 10 Å. Nevertheless, e.s.r. observations are still meaningful in as much as bond distances of ≥ 10 Å seem incompatible with structures of organocobalt complexes (Co-substrate) in which Co-C bond lengths

lie between 2.0 and 2.2 Å. Similar peaks in the e.s.r. spectra have been observed for the non-enzymic photolysis of organocobalt complexes[29,30].

During this work it has been observed that heating alkylcobaloximes up to 40° in $CDCl_3$/TFA solution broadens their ^1H n.m.r. peaks, whereas no such broadening is observed during aerobic photolysis experiments. There was not enough evidence however, to implicate the participation of Co(II) in these experiments.

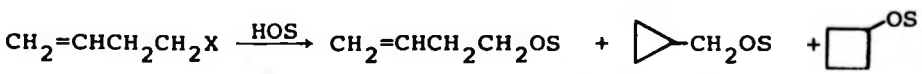
As is well-known, higher alkylcobalamins containing the structural fragment Co-C-CH decompose photochemically under anaerobic conditions to give hydridocobalamin (Co-H) and unrearranged olefins[18,19]. This phenomenon was also observed for cobaloximes even under aerobic conditions[31] and has been confirmed during this project. However, in addition to β-elimination to give Co-H, we have observed olefin rearrangements as well.

1.1.2 Further Mechanistic Considerations

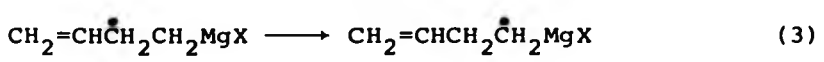
(i) The similarity in structural changes in the B_{12}-dependent enzymatic rearrangements provokes one to generalise the latter under one distinct mechanism. This hypothetical suggestion however, will hold only as long as none of these rearrangements is thoroughly understood. This suggestion is augmented by the fact that all the rearrangements depend on the nature of one bond *viz.* the Co-C bond of AdoCbl although there are several possible routes by which this bond could

cleave, as has been pointed out earlier on. Breaking of this bond would generate a 5'-deoxyadenosyl radical or a carbonium ion or a carbanion, depending on the mode of cleavage, and whatever happens after this, is still a matter of postulation and research. The nature of the adenosyl species will govern the initial nature of the species produced from a substrate molecule by abstraction of a hydrogen atom (substrate-derived radical), hydride ion (substrate-derived carbonium ion) or proton (substrate-derived carbanion).

Several questions remain unanswered, e.g. does the substrate drift away from the coordination sphere of cobalt and hence, subsequently, rearrange with no help from cobalt; does the substrate rearrange as a carbonium ion, a carbanion or as a radical, (all the three possibilities being plausible because of the facile nature of electron transfer reactions which could occur even after an initial homolytic fission). Yet another possibility is the formation of a substrate-cobalt bond which then breaks after rearrangement. Experiments have been done in order to answer some of these questions. It is well known that appropriately substituted allylcarbinyl derivatives solvolyse to give carbonium ions which rearrange to a mixture of cyclopropylcarbinyl and cyclobutyl products. On the other hand, allycarbinyl Grignard reagents, which can be envisaged as carbanions, do rearrange by way of 1,2-vinyl group migration to give products with C_α and C_β swapped.

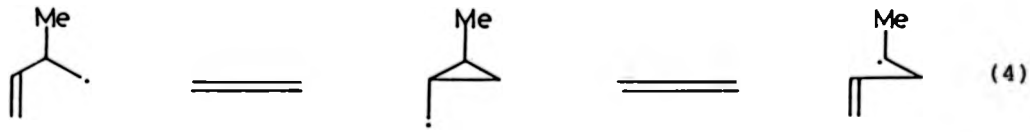


(2)

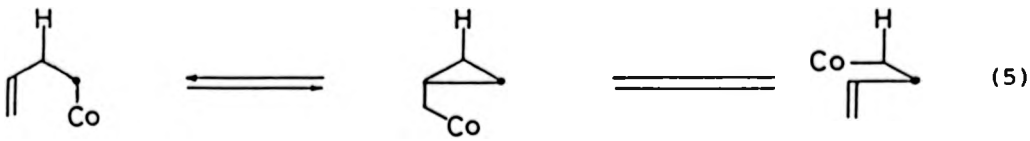


(3)

By the strength of these *in vitro* observations it would not be unreasonable to suggest the involvement of carbonium ions or carbanions in the B_{12}-dependent rearrangements. To complete the argument, L. K. Montgomery and J. W. Matt[32] have generated and studied homoallylic free radicals (equation 4) with a view to ascertaining whether or not allylcarbinyl radicals undergo rearrangements similar to those encountered with either cationic or anionic allylcarbinyl species. From their observations they concluded that not only do allylcarbinyl radicals give products related to those from either cationic or anionic rearrangements, but also that cyclopropylcarbinyl radicals are important intermediates and lie along the reaction coordinate for 1,2-vinyl group migration. When this observation is extrapolated to its logical conclusion, it is easy to see why cyclopropylcarbinyl radicals have been suggested as intermediates in some of the B_{12}-dependent rearrangements. Such intermediates would easily explain the $C_\alpha \neq C_\beta$ swopping observed for both radical and cation rearrangements in allylcarbinyls. Incidentally, such swopping of carbon positions has been demonstrated by B. T. Golding *et al.*[16] for allylcarbinylcobaloximes using $^{13}C_\alpha$-labelled compounds (equation 5).



(4)

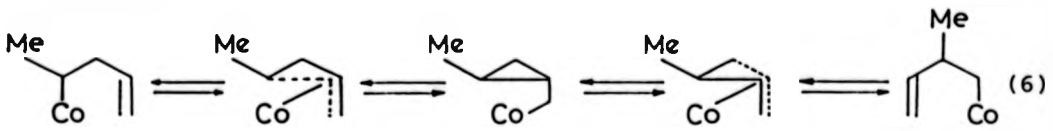


(5)

Work with ^{2}H-labelled materials has also been carried out reaching a similar conclusion. Although these *in vitro* rearrangements are non-enzymic and in no way confirmatory, they strongly suggest that cyclopropylcarbinyl species might be intermediates in the enzymic B_{12}-dependent rearrangement *in vivo*.

The equilibration of 1- and 2-methylbut-3-enylcobaloximes has been established independently by A. Bury *et al.*[33] and B. T. Golding *et al.*[16] and two possible mechanisms have been suggested (equations 6 and 7).

unimolecular



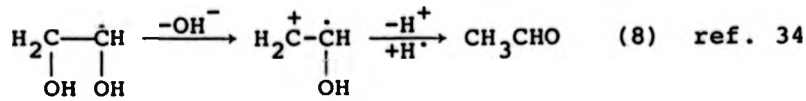
bimolecular

Both research groups were agreed that cyclopropylcarbinyl-cobaloximes are intermediates and once formed they can rearrange into the but-3-enylcobaloximes either (1) *via* a $^{3}\eta$-homoallylic cobalt species (unimolecular mechanism) or (2) by Co(II) catalysis (bimolecular mechanism). The bimolecular mechanism in which the fortuitous presence of catalytic Co(II) is essential, was favoured by A. Bury *et al*. However, it has also been shown[17] that the acid catalysed equilibration of optically active 1- and 2-methylbut-3-enylcobaloximes is stereospecific and intramolecular. These results exclude a

dissociative mechanism involving homolysis or heterolysis of the Co-C bond due to the fact that the interconversion is stereospecific. In addition, the intramolecular nature of these rearrangements implies that the bimolecular mechanism is not as plausible as originally thought, at least for acid-catalysed rearrangements. A pentacoordinate species derived from the removal of L_α (pyridine) by acid was suggested as the precursor of the $^3\eta$-homoallylic alkylcobalt intermediate in the intramolecular mechanism. Again from these results it seems that the presence of cobalt during rearrangement is indispendable.

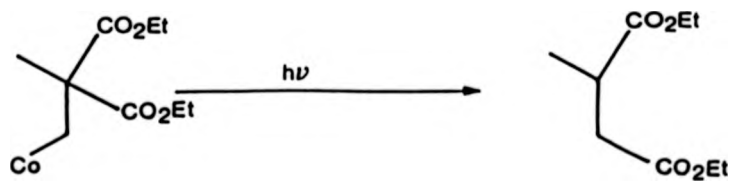
(ii) Cobalt Involvement: The role of cobalt in the B_{12}-dependent rearrangements and their model systems is still unclear and experiments are under way to try and elucidate it. In some of the experiments it seems that cobalt is indispensable in the rearrangements, whereas in other experiments the contrary seems to be true. Alkyl radicals of various "substrates" have been generated in the absence of cobalt and their subsequent reactions studied. The feasibility of direct rearrangement without further interaction with cobalt was demonstrated for the conversion of ethylene glycol to acetaldehyde in the absence of the B_{12}-dependent dioldehydrase which normally catalyses this reaction. The ethylene glycol radical was generated using Fenton's reagent and it was observed to rearrange (equation 8). One can never be sure however, that in the absence of cobalt, iron did not do the catalysis in this experiment (Fenton's reagent =

Fe^{2+}/H_2O_2). Nevertheless, a mechanism which does not involve either metal was suggested to explain the observed interconversion[34].

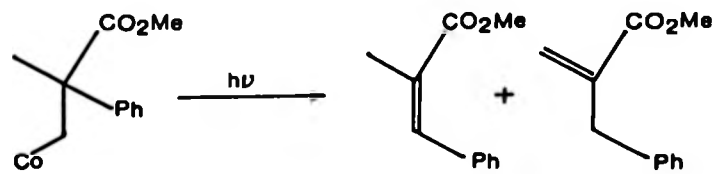


(8) ref. 34

This finding seems to indicate that cobalt might also be dispensable after the formation of substrate-derived radical in the enzymic rearrangement. It has also been shown that various alkyl radicals obtained from the corresponding alkylcobaloximes by photolysis, do rearrange although in small and variable yields, e.g.

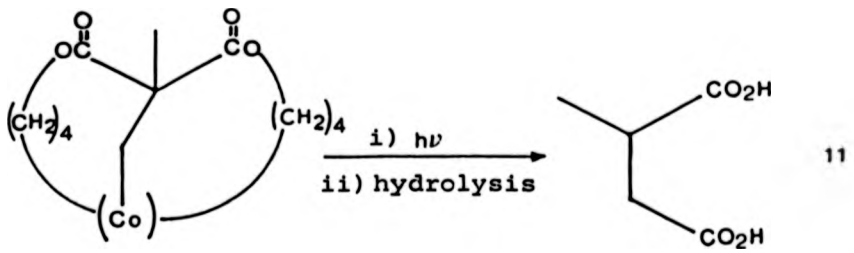


9 (ref. 8)



10 (ref. 35)

The poor yields obtained in these experiments could be explained by suggesting that soon after the formation of the alkyl radical, the latter drifts away from Co(II) and is deprived of the cobalt's catalytic effect. Therefore a model alkylcobaloxime with the alkyl ligand held by the equatorial ligands in such a way that it cannot drift away, even after cleavage, would help to clarify this matter. Indeed, such an ingenious model (equation 11) was proposed and made by J. Rétey *et al.*[36] and on photolysis one rearranged product was obtained exclusively and in high yield (*cf.* refs. 8, 35).

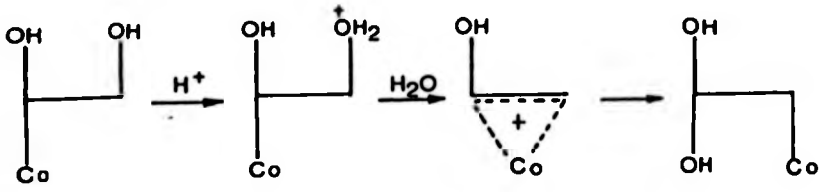


11

These observations seem to indicate that the catalytic effect of cobalt is indispensable in this type of rearrangement. However, the above experimental evidence is in no way conclusive because it has also been shown[37] that an alkylcobalamin with an alkyl moiety related

to that of the Rétey model, but unbridged to the equatorial ligands, gives one rearranged product exclusively and in yields of up to 70%. With such conflicting experimental evidence, the role of cobalt in these rearrangements of model compounds remains as obscure as it has been in the related enzymic systems.

Another mechanistic pathway for B_{12}-catalysed rearrangements, which has been investigated by Dolphin *et al.*, involves the rearrangement of the substrate while the latter is still σ-bonded to cobalt. After the transalkylation of the substrate for 5'-deoxyadenosyl *via* radical intermediates it is envisaged that the bound substrate then rearranges to the product. Such a mechanism however, would only be plausible in the presence of an enzyme which would then act as the "driving factor" for the reaction; otherwise without breaking the Co-C bond it is hard to see, on thermodynamic grounds, how such group migrations would be induced. Such a pathway, for at least some of the B_{12}-catalysed reactions, involving an olefinic π-complex has been suggested[38] (*cf.* equation 12). It is particularly designed to explain the changes and products observed during both enzymic and non-enzymic studies on dioldehydrase substrate-like compounds.



12

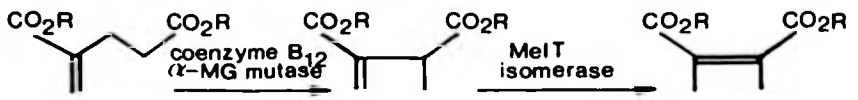
During these studies alkylcobalt compounds with good leaving groups (-OCOR) on the C_α and C_β were synthesised and it was observed that the group at C_β was further labilised by the well-documented "organometallic β-effect", e.g. when 2-acetoxyethylcobaloxime, specifically labelled with ^{13}C at C-1 was solvolysed in methanol it gave 2-methoxyethylcobaloxime which was a 1:1 mixture of two isomers containing ^{13}C at C-1 or C-2. This implies that at some time during the solvolysis C-1 and C-2 become equivalent and this is well explained by invoking the intermediacy of a π-complex.

1.2 COENZYME B_{12}-DEPENDENT α-METHYLENEGLUTARATE (α-MG) MUTASE:

1.2.0 Background Aspects

Out of the eleven B_{12}-dependent enzymic rearrangements known to date, three involve unique carbon-skeleton rearrangements; carbon bonds are broken and new ones are formed in a typically specific enzymic manner. In this work our interest has been focused on the α-MG mutase rearrangement, using cobaloximes as models to simulate the role of coenzyme B_{12}. α-MG mutase is known to catalyse the reversible interconversion between α-MG and methylitaconate (MeIT). MeIT isomerase which does not need coenzyme B_{12} for activity and is found together with α-MG mutase in bacterial cultures, is responsible for taking the interconversion reaction a step further from MeIT to dimethyl-

maleate (equation 13).



13

R ≡ H

1.2.1 Induction and Isolation of α-MG Mutase[6]

(i) α-Methyleneglutarate mutase was first isolated from cell extracts of a bacterium species *Clostridium barkeri*, growing anaerobically on nicotinic acid. During those studies it was also noticed that bacteria growing on glucose instead of nicotinate did not give any α-MG mutase in detectable amounts; addition of glucose to the culture inhibited the induction of the mutase. Among the contaminants of α-MG mutase obtained in this way were MeIT isomerase and a red compound of much lower molecular weight which turned out to be coenzyme B_{12}. The original studies on the induction of the mutase[39] indicate that the bacteria (and hence the mutase) multiply best at 30° as opposed to 25° or 37° and at an optimum pH of 8.2. Subsequent experiments on the rearrangement of α-MG have been done at temperatures ranging from 30° to 40° and at a pH of 7.7.

(ii) Purification of α-MG Mutase: The enzyme was purified at 0-4° *via* a series of tedious processes, during which most of the coenzyme B_{12} was also removed. In the "absence" of this coenzyme, the mutase still exhibited some activity. This rather unprecedented enzymic activity could be attributed to the residual cobamide analogues which were not completely removed by the separation techniques. The accompanying enzyme, MeIT isomerase, was enriched as well in this process of purification and the two enzymes were partially resolved on the basis of their size difference using agarose chromatography. Further resolution was achieved by fractional recrystallisation with ammonium sulphate. The fact that its activity rapidly diminishes in air, in low protein medium and when warmed, makes it very hard to isolate in pure form. Although the enzyme (α-MG mutase) has not yet been isolated in pure form, various experimental observations elucidate its nature and activity.

1.2.2 Nature and Activity of α-MG Mutase

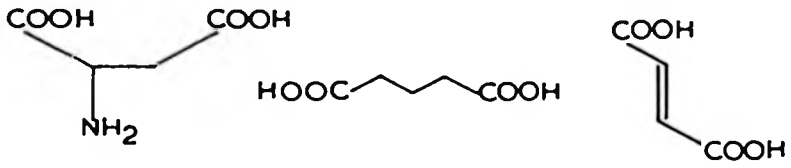
Chromatography on agarose indicated that the enzyme was relatively small; its approximate molecular weight of about 170,000 was determined by sucrose density gradient centrifugation and is comparable with that of methylmalonyl-CoA mutase. α-MG mutase is very unstable in low protein concentrations even if it is stored at -80°. Its stability is not changed by addition of

substrate or reducing agents or a combination of the two. Addition of monovalent or divalent cation like Na^+, NH_4^+ or Ca^{2+}, Mn^{2+} to the enzyme has no effect at all. Similarly, adding chelating agents like EDTA or α,α'-dipyridyl does not alter the enzymic activity. This suggests that no monovalent or divalent cations are involved in the interconversion catalysed by this enzyme, in contrast to AdoCbl-dependent diol dehydratase. There are only two substrates known for this enzyme: α-MG and MeIT. Many other related dicarboxylates were tested[6] and found either to inhibit the enzyme or not to be substrates at all. Those carboxylates that were neither inhibitors nor substrates of α-MG mutase include: glutarate, glutamate, fumarate, β-methylaspartate, 1,2-cyclobutanedicarboxylates, etc. On the contrary *cis*- and *trans*-1-methyl-1,2-cyclopropyldicarboxylates which were originally suggested as intermediates in the enzymic α-MG ⇌ MeIT interconversion were found to inhibit the enzyme. Other inhibitors include: succinate, itaconate, etc.

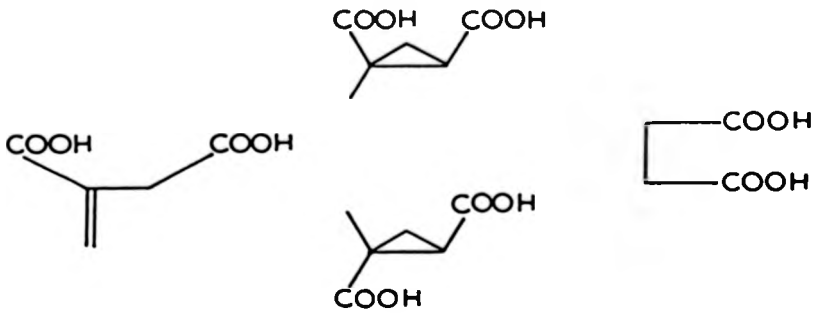
α-MG mutase is not heat stable, e.g. it will lose 50% of its activity if heated at 58° for 5 minutes. This is in sharp contrast with the stability of other enzymes like MeIT isomerase (which does not depend on coenzyme B_{12} for activity) whose activity is almost unchanged under these conditions. The dependence of α-MG mutase on coenzyme B_{12} was illustrated[6] by the fact that its activity was greatly diminished by exposing it to visible light or rigorous purification or treating

Figure 3

Non-Substrates:



Inhibitors:



it with charcoal. All these three processes are known either to destroy or remove the coenzyme from the mutase. The enzyme contains an essential sulphydryl group because it was inhibited by reacting it with iodoacetate. However, it is not known yet what part this group plays in the rearrangement.

1.3 ORGANOCOBALOXIMES AS MODELS FOR COENZYME B_{12}

1.3.0 Cobaloximes *versus* Cobalamins

(i) Organocobaloximes is a name given to a class of organocobalt complexes which were devised and made essentially to simulate coenzyme B_{12} (an organocobalamin), because of their close resemblance to the latter. Although many other models have been made and studied[40], cobaloximes have been shown to be among the closest non-corrin or porphyrin models to adenosylcobalamin, and their name is meant to emphasise this fact. Typically, four nitrogen atoms from two molecules of dimethylglyoxime (DMG) surround a low-spin cobalt atom in the equatorial position; an electron-donating base and an organic ligand occupy the α and the β axial positions, respectively (Figure 4). These ligands can and have been modified, sometimes with added advantage. In this work alkyl(pyridine)cobaloximes have been made, characterised and studied under varying conditions in order to shed some light on the coenzyme B_{12}-catalysed enzymic rearrangement of α-MG. Besides the advantages offered by these model systems, it is as well to know the similarity and differences between the model (cobaloximes) and the system of interest (coenzyme B_{12}).

(ii) Similarities: The most conspicuous relationship between organocobaloximes and adenosylcobalamin

(coenzyme B_{12}) is the presence of Co-C σ bond in both classes of compound besides the usually octahedral coordination chemistry around the cobalt central atom. Their synthesis is similar, usually from Co(I) and alkyl-halides (see Scheme 1). In both types of complexes the ligands are capable of stabilising all the three oxidation states of cobalt (i.e. +1, +2 and +3) which is essential during the coenzyme B_{12}-catalysed rearrangements. Both systems can form the remarkably stable Co-H species which, in this work, has been shown to take part in some of the carbon-skeleton rearrangements caused by light.

Thermolysis and photolysis of both cobalamins and cobaloximes are similar processes, usually giving rise to identical products in the two systems. It seems, at least in some of the thermolytic and photolytic processes, that cobalt acts as a good protecting group for the alkyl radical, which explains the similarity in the products. Their chemical reactions are also related.

X-ray diffraction has been a powerful tool in the studies of vitamin B_{12} since Hodgkin used it to determine the structure of this vitamin (CN-Cbl). It has in particular revealed the variability of the Co-C bond length (and hence strength) in both cobalamins and cobaloximes, depending on the ligation around the cobalt atom. It has also emphasised the close structural similarity, especially in the vicinity of the cobalt atom[41]. The folding of the equatorial ligand towards the less-hindered axial ligand and the concomitant

changes in bond angles and bond lengths are common to both cobalamins and cobaloximes. These physical or structural similarities are often reflected in the chemical behaviour of the two classes of compounds, e.g. the *trans* effect controls the stability of Co-C bond during reactions of both cobalamins and cobaloximes according to the degree of strain originally inherent in the bond.

(iii) Differences: Several studies have been conducted on both cobalamins and cobaloximes in order to highlight their differences, which should be taken into account when interpreting experimental observations where cobaloximes are used as models for the coenzyme B_{12}. An ideal model should have thermodynamic, electrochemical and kinetic properties identical to those of the coenzyme it is simulating, but indeed that is an ideal and it is the coenzyme itself. Hence, there are a few (though not trivial) differences between the model and the coenzyme, especially because of the difference in ligation around the cobalt central atom. These differences include: the axial Co-N bond is shorter in cobaloximes than in cobalamins, although their Co-C bonds are of comparable length. The consequence of this is a slightly more positive charge on cobalt in cobaloximes. This difference in charge is in turn reflected in the electrochemistry of these compounds. Steric factors cause other differences because cobalamins generally have more bulky side chains on the corrin rings and

their L_α is much bigger than, e.g. pyridine in cobaloximes. The effect of the bulkiness of L_α in conjunction with its base-donor properties is to stretch and hence labilise the Co-C bond by pulling the cobalt atom slightly out of the equatorial plane. This factor is demonstrated by the Co-C bond length in *iso*-propylcobaloxime which is 2.085 Å if the base (L_α) is pyridine and 2.20 Å if the base is Ph_3P. These differences are again reflected in the electrochemistry, bond dissociation energies and general reactivity of these complexes. A typical example of this is observed in the one-electron oxidation of alkylcobaloximes, which is virtually unknown for the corresponding alkylcobalamins[42]. On the other hand Cob(II)alamins react with alkyl iodides in aqueous solutions, whereas Cob(II)aloximes do not[43] (equations 14-17).

$$\text{Alkylcob(III)aloxime} \xrightarrow{-\,e} \text{Co(IV)} \qquad (14)$$

$$\text{Alkylcob(III)alamin} \xrightarrow{-\,e} \times \qquad (15)$$

$$\text{Cob(II)alamin} + \text{RI} \xrightarrow{\text{aq. solution}} \text{R-cob(III)alamin} \qquad (16)$$

$$\text{Cob(II)aloxime} + \text{RI} \xrightarrow{\text{aq. solution}} \times \qquad (17)$$

Benzylcobaloxime is a known, stable and isolable compound whereas benzylcobalamin has never been detected even in reaction mixtures[35]. Cobaloximes which are known to have *cis* coordination sites should be capable of catalysing or undergoing reactions which are beyond the reach of cobalamins by the strength of this property.

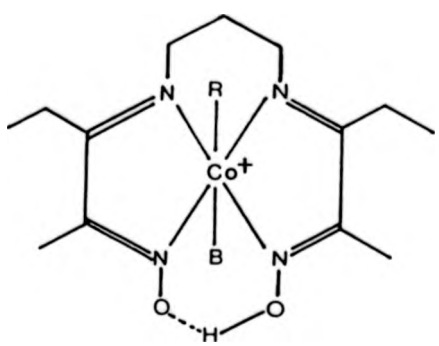
In their communication in 1981, C. M. Elliot *et al.*[44] compared electrochemical properties of cobaloximes and those of cobalamins and seem to have emerged with a rather

pessimistic view of cobaloximes as models. Their results and conclusion can be summarised: cobaloximes have too strong a Co-C bond, a wrong overall charge and hence wrong electrochemical properties. Their axial base constants are too high and generally cobaloximes have a different symmetry from that of the coenzyme they are meant to model. The authors recommend the Costa model, $(Co[C_2(DO)(DOH)_{pn}])$, as a closer substitute for the coenzyme B_{12}. However, they noticed that "lack of an appended axial benzimidazole, the different apparent Co-R bond stabilities, the *ca.* 0.4 V too positive $E_{\frac{1}{2}}$ values of the cobalt alkyls and the inability of any simple model to reproduce possible conformational changes and steric effects in the more complicated coenzyme are, however, limitations of the Costa model".

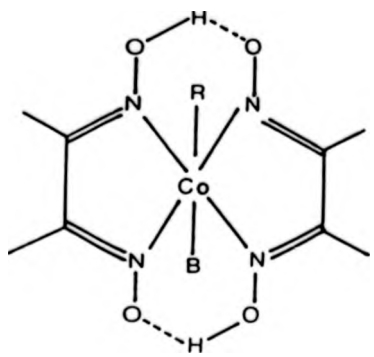
(iv) Advantages of Cobaloximes as Models: The differences between cobaloximes and cobalamins, e.g. the easier methods of preparing cobaloximes, the simplicity of their ^1H n.m.r. and other spectroscopic data compared to those of cobalamins could be seen as advantages. If cobalt and the ligation around it in cobalamins is just a protecting group for the reactive organic free radical, then cobaloximes can provide this protection cheaply and effectively.

The Co-C bond being strong in cobaloximes is a definite advantage in that it has enabled the preparation of secondary cobaloximes and studies[45] on them have revealed that the Co-C bond length (and

Figure 4



$R\text{-}Co[C_2(DO)(DOH)_{pn}]B$



R-Co(dmgH)B

R = alkyl
B = base

hence bond strength) is more dependent on steric hindrance and less on the electronic properties of the cobalt atom. Secondary or other hindered cobalamins would be too unstable for such studies. Alkylcobaloximes are not as photosensitive as cobalamins and this property means that cobaloximes are easier to handle, usually in dim light. Most alkylcobaloximes are much easier to purify than the corresponding cobalamins; the usual methods include silica gel column chromatography or fractional recrystallisation from CH_2Cl_2/30-40^o petroleum spirit or acetone/H_2O. Cobaloximes are stoichiometric complexes

usually with no water of crystallisation, whereas cobalamin crystals usually contain varying amounts of water which makes it impossible to do any microanalysis on them.

All these advantages offered by cobaloximes make it a lot easier and faster for the researcher to probe and hopefully solve the still intriguing mechanisms of the B_{12}-dependent enzymic rearrangements. However, notwithstanding these similarities and advantages, any conclusion drawn from model studies must eventually be authenticated by studying actual enzymic systems because of the differences mentioned above. As the search for the ideal model rages on, more differences continue to emerge and hopefully a better model will be found.

1.4 α-MG-RELATED ALKYLATING AGENTS AND THEIR COBALOXIMES

It has been postulated that during B_{12}-dependent enzymic rearrangement of α-MG, after the cleavage of the AdoCbl Co-C σ bond, the adenosyl radical abstracts a hydrogen atom from C-4 of α-MG and forms a substrate-derived organocobalt intermediate (I, Scheme 2). It has also been suggested[16,33,46] that this intermediate further rearranges *via* a cyclopropylcarbinylcobalt intermediate (IIA/B, Scheme 2) in equilibrium with a π-homoallylcobalt species, to give the product after replacing cobalt with a hydrogem atom. This pathway has some precedent in the chemistry of cyclopropylcarbinyl and but-3-enyl radicals in solution and in the gas phase[47].

Scheme 2

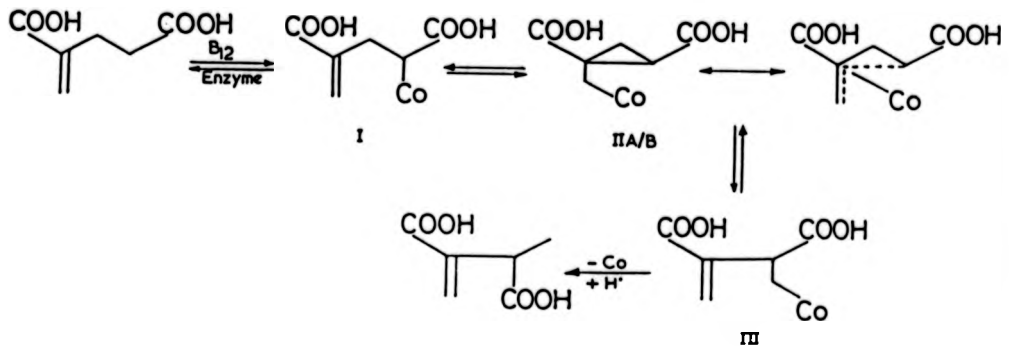
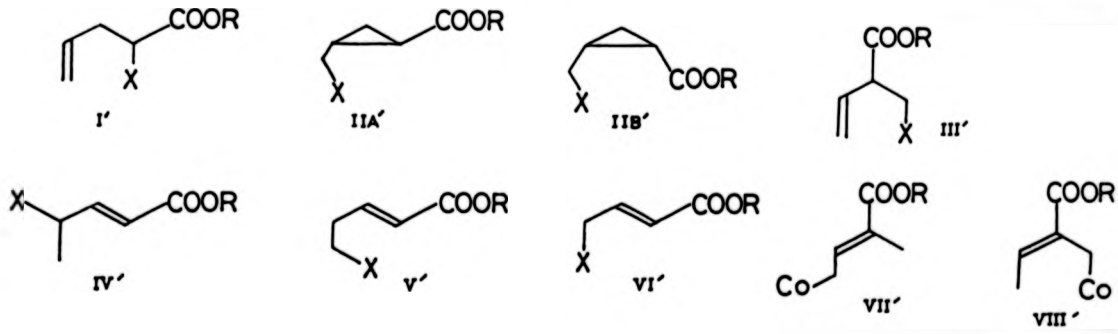


Figure 5



X = Cl, Br, I, $Co(dmgH)_2Py$

R = Et, Me

During this project interest has been focused on the preparation of alkylating agents and their corresponding cobaloximes (I'-V') which are related to I, II and III (Scheme 2), but having only one carboxylate group. By studying such model compounds one should be able to learn whether, e.g. starting with IIA' it is possible to obtain I' or III'. The rate of such rearrangement would indicate whether the suggestion of cyclopropylcarbinylcobalt intermediacy is viable or not. Indeed, thermolysis and acid-catalysed rearrangement of IIA' or III' into I' are so slow that this suggestion should be questioned. Since the carboxyl groups do not seem to take part in α-MG rearrangement, the ester derivatives were employed in this study. This variation would also show whether the carboxyl groups are actually essential in the rearrangement. Such a connection between rearrangement and presence of carboxyl groups was not observed at all during this work.

Previous work has been done on related systems, e.g. methyl-substituted cyclopropylcarbinyl and but-3-enylcobaloximes[46], but these were further away from the actual α-MG which contains two carboxyl groups. The results obtained in this work do augment this point in that the most stable alkylcobaloxime in their system was III' (methyl instead of COOEt), whereas this study has revealed that I' is the most stable and hence the dominant species in acid or heat equilibrated mixtures. The two systems are fundamentally and inherently different in that the methyl groups are electron-donating,

whereas the carboxylate groups are electron-withdrawing. Some preliminary attemps to synthesise the series of but-3-enyl and cyclopropylcarbinyl alkylating agents possessing two carboxylate groups, along with the corresponding cobaloximes have been made. These compounds would give an even truer picture of the α-MG system.

CHAPTER 2

METHODS AND INSTRUMENTATION

Conventional methods were used throughout this work, and where special techniques were used, they are described. Methods suggested in the "Purification of Laboratory Chemicals" by D. D. Perrin, W. L. F. Armarego, and D. R. Perrin, Second Edition, were found most useful and some of them were employed to obtain pure and dry solvents and reagents.

2.1 METHODS

(i) As they are potential carcinogens, special care was taken to avoid skin-contact or inhalation of the various kinds of alkylating agents which were synthesised in this work. Efficient fume cupboards and hand gloves were used routinely.

(ii) Air-sensitive compounds like butyl lithium and Co(I) were handled under an atmosphere of argon or nitrogen gas. Butyl lithium was transferred by needle and syringe after letting in dry nitrogen into the stock bottle *via* another needle. In cases where BuLi was required as a solution in another solvent, the original solvent was pumped off under nitrogen, at the Schlenk line. Its concentration was determined by the ^{1}H n.m.r. spectroscopy method*.

(iii) Moisture-sensitive compounds like $LiAlH_4$, MeONa, were kept in the dry box, whereas hygroscopic compounds like HMPA were kept over appropriate drying agents, in tightly closed bottles, sealed with "Parafilm".

(iv) Photosensitive compounds like organocobalt compounds and alkyl halides (particularly iodides) were handled in dim light. The alkyl iodides were normally kept at -20° in aluminium-foil-wrapped containers.

(v) Solutions of compounds (products) in ether or CH_2Cl_2 or hydrocarbons, were usually dried over anhydrous $MgSO_4$ or Na_2SO_4. Throughout the experimental section, "... solvent was removed at the pump ..." refers to the use of a Büchi rotary evaporator, which was equipped with a water pump capable of pressures down to 12 mmHg.

(vi) An assortment of home-made and aluminium-backed commercial t.l.c. plates was used: silica gel (60-120 mesh, 0.125-0.25 mm) was used for ordinary chromatographic columns, silica gel 60 (GF_{254}) for home-made t.l.c. plates and silica gel 60 (230-400 mesh, 0.040-0.063 mm) for flash chromatographic columns. Eluting solvents were freshly made-up, and where ratios are quoted they refer to volume:volume.

(vii) Where the presence of any extraneous dissolved metal was undesirable, e.g., in the case of acid-catalysed rearrangements of alkylcobaloximes, TFA/$CDCl_3$ solutions

were measured, transferred and dispensed by an "Agla" (all-glass) syringe. The TFA/$CDCl_3$ solution was originally standardised against a standard aqueous solution of NaOH, in the usual way.

(viii) When very dry glassware was required, the flame-drying method was preferred. The flask or reaction vessel was warmed with a flame while purging it with a stream of dry nitrogen. This method was found quicker and more effective than the oven-drying method.

2.2 INSTRUMENTATION

(i) An annular teflon spinning-band distillation column with theoretical plates in excess of 125 at atmospheric pressure was used. The annular still was 5 mm x 61 cm and is surrounded by a thermostated glass jacket. Liquids with boiling point difference of only $3\text{-}6^{\circ}$ were completely separated on this column. A minimum rate of 4 drops per minute at the still head gave the best separation.

(ii) G.l.c. analyses were done on a Perkin-Elmer (model F-11) instrument, with nitrogen as the carrier gas. Preparative g.l.c. was carried out on a Carlo-Erba (model, Fractovap 2450) instrument.

(iii) ^1H n.m.r. spectra were recorded on a 220 MHz Perkin-Elmer (model R34), or a 400 MHz Bruker (model WH400)

instruments. The spectra were calibrated in p.p.m. and all the chemical shifts are relative to a particular internal standard, TMS (or TSS if D_2O is the solvent). Peaks are described in the experimental section by their chemical shift δ in p.p.m. The figure and letters in brackets refer to the nature of the peak: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, p = quintet, dd = double doublet, ddd = double-double doublet, dt = double triplet. J refers to the coupling constant as measured from these peaks. An italicised subscript (e.g. J_{trans}) refers to the nature of the coupling constant. Coupling constants were either measured directly from the peaks of interest or after proton-spin decoupling at a related resonance, by the usual homonuclear decoupling method.

^{13}C n.m.r. spectra were recorded at 22.63 MHz on a 90 MHz Bruker (model WH90) instrument, or at 100.62 MHz on a 400 MHz Bruker (model WH400) instrument. All peaks were broad-band proton decoupled and their chemical shifts were measured relative to TMS as an internal standard. They all appear as singlets. A new n.m.r. technique by the name of "Distortionless Enhancement by Polarisation Transfer" (DEPT), capable of inverting all the peaks due to $-CH_2-$ carbon atoms, and at the same time eliminate all the peaks due to protonless carbon atoms, while leaving CH_3- and -CH- peaks intact, was found very useful for interpreting ^{13}C spectra**.

(iv) Infrared (ν_{max}) spectra were recorded on a Perkin-Elmer (model 580B) instrument. The samples were run as liquid films or Nujol mulls on NaCl plates or as solids in KBr pellets. The peaks are designated by their wavenumbers (cm^{-1}), whereas the nature of the peaks is depicted by s = strong or m = medium or w = weak or just broad. The scan range was normally 4000 cm^{-1} to 300 cm^{-1}.

(v) Ultra violet (λ_{max}) spectra were recorded on a Shimadzu (model UV-365) instrument. Compounds were run as standard solutions in absolute ethanol, and peaks are recorded in nanometres (nm), followed by the extinction coefficient in brackets. The latter were calculated in the usual way from the measured absorbances and solution concentrations.

(vi) Mass spectra were recorded on a Kratos MS80 instrument. Peaks are quoted as m/z, and the ion giving rise to the peak is shown in brackets. The intensity of the ion of interest is shown as a % immediately after the brackets. Ammonia was used exclusively for c.i.m.s. Accurate mass measurements are expressed in number of p.p.m. of deviation from the calculated mass of the molecular ion. A negative or positive sign before the quoted figure signifies whether the measured mass was lower or higher than the calculated mass, respectively.

(vii) Microanalysis was carried out for carbon, hydrogen sulphur and nitrogen by C.H.N. and E.M.A.L. Laboratories.

(viii) Electron spin resonance (e.s.r.) spectra were run for alkyl radicals generated from some of the alkylating agents, at Leicester University.

(ix) *In vivo* testing of the cyclic analogues of busulphan for antitumour activity was done at the Patterson Laboratories of Christie Hospital and Holt Radium Institute, Manchester.

*A. Silveira, Jr., and D. Bretherick, Jr., *J. Chem. Ed.*, 1979, 56, 560

**M. R. Bendall, D. M. Doddrell, and D. T. Pegg, *J. Magn. Reson.*, 1981, 44, 238

CHAPTER 3

SYNTHESIS OF ALKYLATING AGENTS AND THEIR COBALOXIMES

3.0 INTRODUCTION

Alkylcobalamins derived from enzyme substrates and AdoCbl have been postulated to be among the active intermediates during the eleven B_{12}-dependent enzymic rearrangements, their formation being aided *in situ* by the respective enzymes. However, unlike these very specific enzymic systems in which side reactions are minimal or altogether non-existent, during non-enzymic organocobalt complex syntheses, there are side reactions which give rise to by-products, e.g. desalkylcobalt species. Therefore, during model studies the organocobalt complex of interest not only has to be synthesised from a suitable alkylating agent and a pentacoordinate cobalt species, but has to be isolated and characterised as well, before proper studies on it can proceed. Usually the alkylating agent is also synthesised and characterised beforehand. Part of the present work has entailed comprehensive organic syntheses and characterisation of monocarboxylate-substituted cyclopropylcarbinyl and but-3-enyl alkylating agents and their corresponding cobaloximes (I'-V', Fig. 5).

All the alkylating agents were prepared by known literature methods and/or routes devised in this laboratory. Various synthetic routes were followed in an

effort to obtain gram quantities of each of these alkylating agents. In the case of substituted cyclopropanes at least three routes were tried; one failed, the second one gave the desired products in a yield of about 1% after 10 reaction steps, and the third one which employed ethyl diazoacetate and a rhodium carboxylate catalyst gave good yields of *cis*- and *trans*-cyclopropane derivatives.

Purification methods like spinning-band distillation, preparative GC and flash column chromatography were employed to give alkylating agents of high purity, before their characterisation and subsequent conversion into alkylcobaloximes. 220 MHz ^{1}H n.m.r. spectroscopy was the routine method for checking purity and characterisation of both alkylating agents and alkylcobaloximes, besides the conventional t.l.c. method. Alkylcobaloximes usually show a strong singlet at about 2.1 δ due to ^{4}Me of the dimethylglyoximes (DMG's), whereas the corresponding feature appears further downfield at *ca.* 2.4 δ in desalkylcobaloximes, which makes ^{1}H n.m.r. an effective tool for analysing these compounds. Desalkylcobaloximes which may arise from the unwanted oxidation of Co(I) by, e.g. aerial oxygen during the preparation of Co-R, have a smaller R_f on silica gel than the latter and normally stay at the origin during chromatography unless polar solvents like methanol are used. An alkylperoxycobaloxime (Co-OO-R) which was observed during this work, had an intermediate R_f on silica gel and therefore it was quite easy to separate the three cobaloximes chromatographically,

i.e. desalkyl, peroxy and alkylcobaloximes. Reaction of Co(I) with alkyl halides or mesylates was the method of choice for preparing alkylcobaloximes. Except in the case of the halide being α to an ester group (*cf.* Scheme 1), it was found most appropriate to convert chlorides to iodides (better-leaving group) by the classical Finkelstein method before attempting reaction with cobalt(I). New compounds were fully characterised.

3.1 SYNTHESIS OF ALKYLATING AGENTS

3.1.1 Cyclopropanation

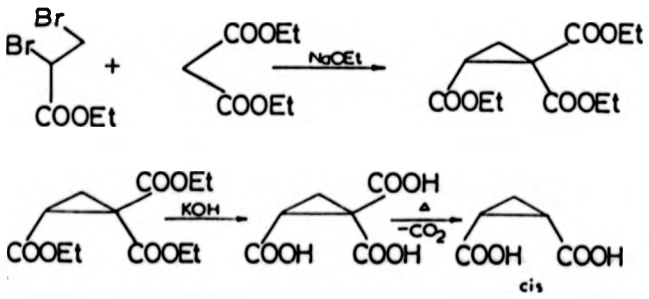
(i) The synthesis and chemistry of substituted cyclopropanes are well documented in the literature because some of these compounds have biological activity and some are good starting materials in a variety of organic syntheses; they have been synthesised by various methods. Three-membered ring formation is kinetically favourable but thermodynamically unfavourable so that three-membered rings may be destroyed under the conditions of their formation. Therefore, during cyclopropanation, lowering reaction temperature favours cyclisation and hence increases the yield of the cyclopropane product, whereas higher temperatures favour formation of acyclic by-products.

Substituted cyclopropanes are accessible *via* a variety of synthetic routes including the addition of a carbene/carbenoid species to the π-bond of an unsaturated molecule or by base-promoted carbonyl condensation followed

by 1,3-intramolecular cyclisation. The carbene/carbenoid species can be derived from haloforms (HCX_3/base, X = halide), diazoacetates (N_2CHCO_2R/Δ or $h\nu$ or catalyst), Simmons-Smith reaction (Zn-Cu couple/CH_2I_2) etc. During this work *cis*- and *trans*-cyclopropane dicarboxylates and tricarboxylates were synthesised by some of these methods in an effort to obtain *cis*- and *trans*-1-alkoxycarbonyl-2-(halomethyl)-cyclopropanes which were needed for the B_{12} studies. The latter were prepared pure and characterised before using them to make cobaloximes.

(ii) Cyclopropanation by Carbonyl Condensation:
Malonate diester is known[48] to react with 2,3-dihalopropionates in the presence of a base to give 1,1,2-cyclopropane tricarboxylates in good yield (> 60%). In this work diethylmalonate was deprotonated using sodium ethoxide in ethanol and then ethyl 2,3-dibromopropionate was added dropwise to the cooled mixture. After the ensuing condensation, the base abstracts another proton from diethyl malonate methine generating another carbanion, which in turn cyclises by an intramolecular S_N2 reaction to give the cyclopropane tricarboxylate.

Scheme 3



The triester was hydrolysed with potassium hydroxide with a view to obtaining the *cis*- and *trans*- dicarboxylic acids after decarboxylation according to M. Cutz and M. Conrad's procedure[49]. Although this procedure is ostensibly simple, we could obtain only ~ 4% yield of the *cis*-isomer (m.p. 139°) in spite of several attempts. Some of the tricarboxylic acid was recovered unchanged and was removed from the *cis*-isomer product by fractional crystallisation (ether/40-50° petroleum ether).

The structure of the *cis*-dicarboxylic acid isomer was confirmed by ^{1}H n.m.r. (D_2O): δ 1.90 (m, 1H), 2.00 (m, 1H), 2.25 (m, 2H). The two protons on the unsubstituted carbon atom of the ring are experiencing different shielding/deshielding by the carboxylate groups because of their dissimilar disposition in space and therefore they resonate at different chemical shifts. The other two ring protons have identical chemical environment and hence resonate together. However, their peak appears more downfield because of the shielding and electron-withdrawing effects afforded by the carboxylate groups.

During decarboxylation most of the required dicarboxylic acid was lost perhaps due to ring-opening because of the high temperature (185°) required for the process. Due to low yields, this route was abandoned as a way of eventually obtaining 1-alkoxycarbonyl-2-(halomethyl)cyclopropanes.

A related reaction between acrylate and haloacetate in the presence of a base, e.g. MeO^-Na^+ gives very good yields of cyclopropane dicarboxylates (*cis* and *trans*).

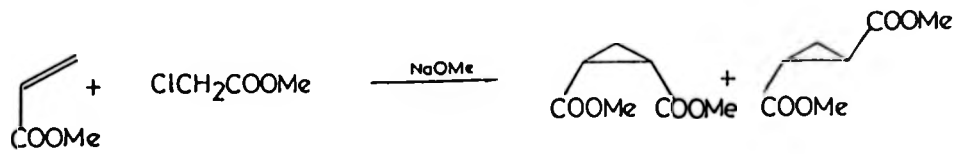
The best procedure is that by McCoy[50] and its modification by Baldwin *et al.*[51]. This is the only method so far which gives very good yields (> 80%) of these compounds and the *cis*-isomer forms in preponderance to the *trans*-isomer. The reagents are cheap and readily available; methyl-acrylate, methylchloroacetate and $\overset{+}{N}aO\bar{M}e$ were used in this work. The base ($\overset{+}{N}aO\bar{M}e$) abstracts a proton from haloacetate methylene to give a carbanion. The carbanion, which is stabilised by the carbonyl of the ester group, attacks the acrylate by Michael addition to give another carbanion. The latter cyclises by S_N2 attack on the halide-bearing carbon. At low temperatures (-78^{o}) this reaction is still quite fast, whereas the thermodynamically-favoured de-cyclisation is almost totally eliminated .

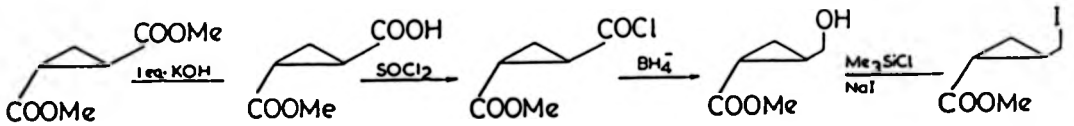
Cis- and *trans*-cyclopropane dicarboxylates were separated using a spinning-band column by exploiting the slight difference in their polarity, which causes a little difference in their boiling points ($\sim$ 3 C^{o}).

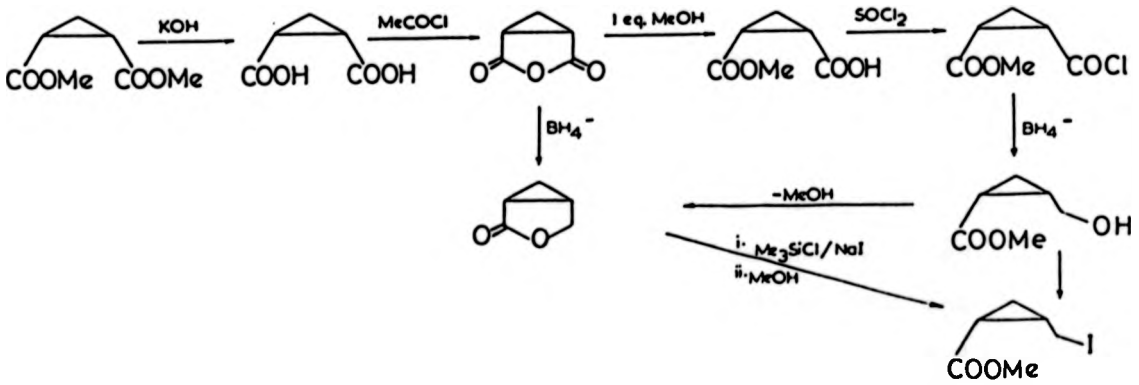
Selective hydrolysis of one of the ester groups of the *trans*-isomer gave *ca.* 50% yield of *trans*-methoxycarbonyl-cyclopropane carboxylic acid (*cf.* ref. 52). The conversion of the monoacid into the corresponding acid chloride with $SOCl_2$ and reduction to alcohol by $NaBH_4$ in THF went smoothly to give pure *trans*-1-methoxycarbonyl-2-(hydroxy-methyl)cyclopropane. An impurity (the diester) was easily separated from the more polar alcohol by eluting the product through a short silica gel column with dichloromethane. The alcohol which remained on the column was washed off with CH_2Cl_2:MeOH (90:10) and

Scheme 4

Preparation of *cis*- and *trans*-1-(Iodomethyl)-2-methoxycarbonylcyclopropane







collected. A modification of the procedure developed by G. A. Olah *et al.*[53] employing trimethylsilyliodide generated *in situ* from Me_3SiCl/NaI was used to convert this alcohol into the corresponding iodide. However, a yield of only 24% was procured from this step of the reaction. This was in spite of arduous attempts to maximise the yield by re-esterifying the carboxylate group, just in case it was cleaved by Me_3SiI, by the end of the reaction time (1½ hours) at room temperature; re-esterification was attempted as follows: dry methanol with 3 drops of conc. HCl was added to the reaction mixture and stirring was continued for another 12 hours before work-up and chromatography on a silica gel column. It is not clear whether this modification of the Olah *et al.* procedure affected the yield of the iodide at all. It seems other reagents (e.g. Ph_3P/I_2, Ph_3P/CCl_4 followed by Finkelstein reaction) could have given better yields as they do not react with ester groups.

The diacid obtained by hydrolysis of *cis*-1,2-dimethoxycarbonylcyclopropane was pure by ^1H n.m.r. spectroscopy and its spectrum was identical to that of the compound previously obtained by decarboxylation of 1,1,2-cyclopropanetricarbocylic acid. The formation of the acid anhydride was quantitative but the reduction of one of the carbonyl groups gave only a fair yield (29%). The resulting lactone was also pure by ^1H n.m.r. ($CDCl_3$): δ 0.80 (m, 1H) and 1.24 (m, 1H) due to the two apex protons of the cyclopropane ring; 2.00 (m, 1H) and 2.22 (m, 1H) due to the bridge carbon protons; 4.17

(d, 1H) and 4.30 (m, 1H) due to the two protons on the five-membered ring of the lactone. The formation of the monoacid and the corresponding acid chloride was fast and efficient. However, the formation of *cis*-1-(hydroxymethyl)-2-methoxycarbonylcyclopropane by reducing *cis*-1-methoxycarbonylcyclopropane-2-carboxyl chloride presented a particular problem because the alcohol thus obtained easily cyclised giving the lactone. This lactonisation takes place spontaneously and gradually if the alcohol is left at room temperature for a long time, or in the presence of an acid catalyst, e.g. a Lewis acid ($AlCl_3$), mineral acid (HCl), for a short while. This property of the *cis*-alcohol was utilised during this work to separate it from the *trans*-isomer in other preparations; the *trans*-isomer alcohol does not cyclise because of its stereochemistry. The lactone was cleaved by Me_3SiI[53] and re-esterified with methanol to give the final product, *cis*-1-(iodomethyl)-2-methoxycarbonylcyclopropane. This reagent seems quite suitable for the conversion because it does not only react with free alcohol, but also with the lactone to give the same end product after re-esterification (*cf.* Scheme 4). However, in practice only a poor yield (∿ 7%) from the pure lactone was obtained by following this procedure.

The alkyl iodides (IIA' and IIB', Fig. 5) from these reactions were fully characterised by i.r., ^1H n.m.r., accurate m.s., and then stored at -20° for subsequent reactions. They were unchanged even after 2 years under these conditions, except for the brownish

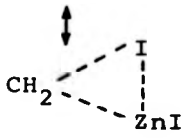
tinge of iodine which was more apparent in the *cis*- than the *trans*-isomer. Because these compounds were required in gram quantities, other cyclopropanation methods were sought and tried out during this project. The routes described so far did not provide enough of these very important alkylating agents.

(iii) Cyclopropanation by the Simmons-Smith Reaction[54]: The reaction of an organozinc reagent, prepared from zinc-copper-couple and diiodomethane, with substituted unsaturated compounds has over the years proved versatile and convenient for the synthesis of cyclopropanes since it was discovered by H. E. Simmons and R. D. Smith in 1958[55]. Their original procedure has long since been modified in most instances, to give superior yields. The Simmons-Smith cyclopropanation reaction is stereospecific with regard to the olefin, is usually free from serious side reactions, is normally conducted under mild conditions and often gives good yields. It has been used widely in the synthesis of substituted cyclopropanes.

It has been shown that the process of cyclopropanation in the Simmons-Smith reaction does not involve a very reactive species like a free carbene; the intervention of free radicals formed on the zinc surface is, however, not totally excluded. The generally accepted mechanism is that of an organozinc intermediate leading to a methylene transfer[56]. It was proposed that this organometallic species might be best represented as a methylene and zinc iodide complex or as a structure

with some electron donation from the carbon-bound iodine to the zinc atom. Although it attacks double bonds, this zinc reagent does not give products expected of a free carbene,

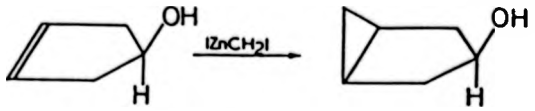
$$CH_2I_2 + Zn(Cu) \longrightarrow ICH_2ZnI + (Cu)$$



e.g. it does not insert in C-H or O-H bonds as carbenes do. It has also been shown[55b] that during Simmons-Smith reactions copper plays no other role than activating the zinc surface, because zinc on its own was found to be effective in some cyclopropanation reactions.

Experiments[56,57] with olefin mixtures have revealed that the methylene iodide/zinc reagent behaves as a weak electrophile and hence olefin reactivity increases with increased alkyl substitution around the double bond; this effect is offset by concurrently increasing steric hindrance. A neighbouring functional group, e.g. OH, CO_2R, NH_2 on the olefin, capable of coordinating to the zinc reagent was not only found to accelerate the Simmons-Smith reaction, but also to direct the cyclopropanation process stereospecifically. This phenomenon is particularly apparent in rings which contain double bonds and an -OH group nearby (*cf.* equation 20). This property has been utilised in many synthetic methods to obtain selective cyclopropanation,

e.g. in the case of cyclopent-3-enol only one isomer was obtained.



(20)

The olefin of interest for the present work, *viz.* (E)-4-hydroxybut-2-enoate, contains two functional groups (OH and CO_2R) capable of directing the stereochemistry of the cyclopropanation reaction. It was hoped that the coordinating effect of these functional groups would make up for the reaction-retardation effect due to the electron-withdrawing nature of the ester carbonyl group. Starting with the methyl ester of this olefin, M. P. Atkins attempted[48] to prepare pure samples of *trans*-1-(hydroxymethyl)-2-methoxycarbonylcyclopropane by employing the Simmons-Smith reaction, but was not successful. When a similar experiment was repeated during the present work, *trans*-1-(hydroxymethyl)-2-methoxycarbonylcyclopropane was obtained contaminated by a number of unsaturated acyclic impurities (Scheme 5). Distillation alone could not remove the impurities and only when the product was run on an Apiezon L Chromosorb gas-liquid chromatographic column (120^{o}), was a good separation obtained.

There are a few requirements for the Simmons-Smith reaction to take place readily: the reagents must be dry because any water present will foul the Zn/Cu couple surface. The solvent must be able to stabilise the zinc reagent thus formed. The zinc reagent must not be too reactive towards the solvent at the usual reaction

temperature range of 25-60°. The solvent on the other hand, must not promote the destruction of the zinc reagent. As solvents, ethers go a long way in fulfilling these requirements. However, 1,2-dimethoxyethane in which the Simmons-Smith cyclopropanation goes most rapidly, also reacts with the zinc reagent fastest of all the ethers. Tetrahydrofuran has the opposite effect, whereas diethyl ether has been shown to be a good compromise and therefore it is generally used. In some reactions it has been found advantageous to use preformed zinc reagents, especially if the olefin is decomposed or polymerised by zinc iodide; by-products in these reactions include polymeric material among other compounds. α,β-Unsaturated esters undergo cyclopropanation by this method, but the yields are dependent on the specific ester. This expresses the delicate balance between electronic and steric factors regarding the olefin in Simmons-Smith reactions.

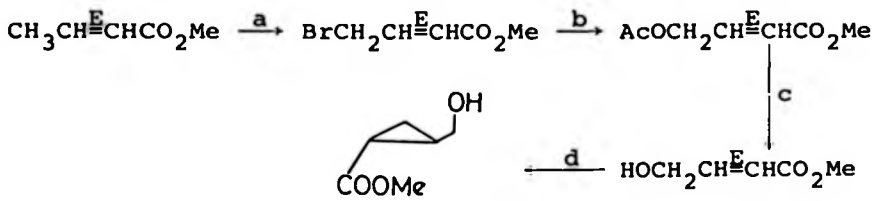
The Zinc-Copper Couple: The Simmons-Smith reaction depends heavily on the quality of this couple; the method by which it is prepared influences the rate of reaction and the ultimate yield. The role of copper in the couple is just to activate the zinc surface, because experiments conducted with zinc alone, activated in other ways, have given decent yields of cyclopropanes. The original couple used by the discoverers of the reaction was made by reduction of zinc powder and cupric oxide with H_2 by heating them at 500°. The main advantage of a couple prepared in this way is that it does not lose activity on storage even in

the presence of aerial moisture. However, its cumbersome preparation stops it from being used regularly. The most widely used couple is that prepared by precipitation of copper from $CuSO_4$ onto freshly acid-washed zinc dust surface. It was used in this project. Other types of Zn/Cu couple have been prepared either by adding zinc dust to a hot solution of $Cu(OAc)_2$ in HOAc or to CuCl in ether and heating under nitrogen. Other metals have been used besides copper (e.g. silver) with some advantages. The Zn/Ag couple requires shorter reaction times and gives higher cyclopropanation yields compared to its Zn/Cu counterpart. However, what is gained in shorter reaction times and higher yields does not seem to make up for what is lost in buying the metal - silver being much more expensive than copper. Therefore, the Zn/Ag couple is not as widely used.

Since this reaction is stereospecific, it was hoped that during the present work, starting with the (*E*)- and (*Z*)-4-hydroxybut-2-enoate isomers it would be possible to obtain both *trans*- and *cis*-1-(hydroxymethyl)-2-methoxycarbonylcyclopropane, respectively. (*E*)-Methyl 4-hydroxybut-2-enoate was originally obtained by allylic bromination of (*E*)-methylbut-2-enoate and subsequent reaction with silver acetate followed by $MeOH/H^+$ (*cf.* Scheme 5). After obtaining a mixture of products from a Simmons-Smith reaction between CH_2I_2 and $HOCH_2CH^{E}{=}CHCO_2Me$, it was clear that this method was not going to yield the desired gram quantities of alkylating agents. The best method for preparing these compounds turned out to be the

Scheme 5

Preparation of *trans*-1-(hydroxymethyl)-2-methoxycarbonylcyclopropane



trans

[a] N-bromosuccinimide, CCl_4, Δ

[b] Silver acetate, CCl_4, Δ

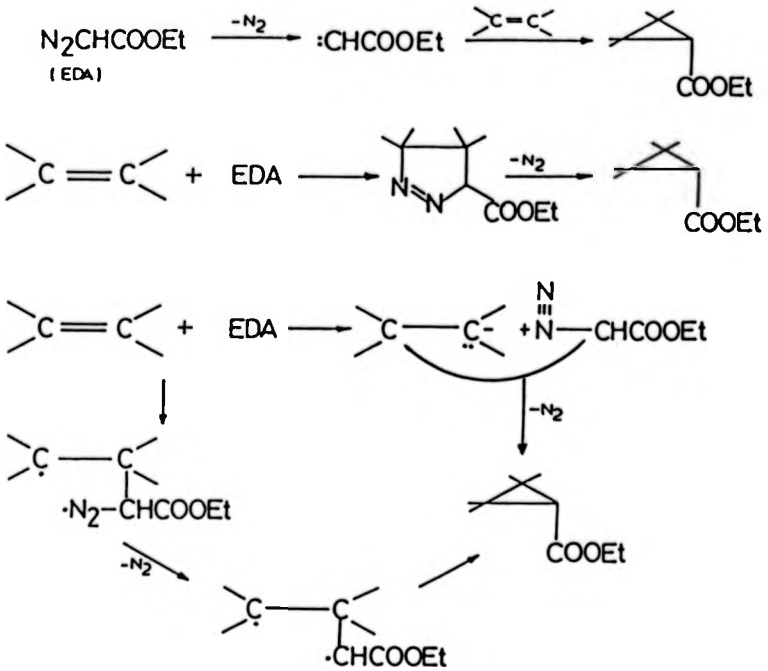
[c] $MeOH/H^+$

[d] Zn/Cu couple, ether Δ

reaction of ethyl diazoacetate and allylic compounds.

(iv) Cyclopropanation with Ethyl Diazoacetate (EDA)[58]: For a long time ethyl diazoacetate, among other carboalkoxy-diazo compounds, has been known to react with olefins when heated or photolysed or in the presence of a metal catalyst, to give substituted cyclopropanes in good yields. The catalysts used to decompose EDA have varied from metals, e.g. Cu, Pd, metal salts like $CuSO_4$, through to chelated metal complexes with chiral ligands. The nature of the ligand or counterion has been shown to affect the nature of the products in such addition reactions. In this project rhodium acetate dimer was used as recommended by Hubert *et al.*[59] and good yields of *cis*- and *trans*-substituted cyclopropanes were obtained (*cf.* Scheme 6).

Addition Mechanism: Although the mechanism of the cyclopropanation reaction with EDA is not known precisely, it has been established[60] that like other diazoacetate esters, EDA reacts with olefins under the influence of heat or light, by three distinct pathways. In the first pathway EDA may lose nitrogen and react as a carbene. In the second pathway, EDA itself may add to the olefin double bond to give a pyrazoline which then gives the corresponding cyclopropane on heating ($T > 150^{o}$, if no catalyst present). Alternatively, the C_{α} of EDA might form a bond with one carbon of the olefin, hence generating a diradical or a zwitterionic intermediate. Loss of nitrogen due to ring closure would then complete the process (equation 23).



Pyrazoline intermediates are most likely to form by 1,3-dipolar addition when activated olefins (e.g. crotonate, acrylate) react with EDA under thermolytic conditions. Contrary to the general view that pyrazolines do not decompose catalytically with copper salts, it has been demonstrated[61] that pyrazolines can, in some instances, decompose if heated in the presence of a catalyst: a preformed pyrazoline was decomposed 100% in 1/4 hour by boiling it in benzene in the presence of $Cu(BF_4)_2$ catalyst. The same compound was unchanged when it was boiled in benzene for 4 hours in the absence of any catalyst. This implies that EDA could be the active species in catalysed reactions, giving rise to pyrazolines which decompose subsequently, to give cyclopropanes and nitrogen.

It has been demonstrated[62] that using bulky

chiral ligands attached to a metal atom, e.g. Cu,it is possible to synthesise optically active substituted cyclopropanes, although in low optical yields (~ 6%). This observation strongly supports the view that the reactive species in such systems is a carbene-metal complex. Furthermore, the fact that the bulkiness of the catalyst ligand was found[63] to affect the *endo/exo* ratio of the products from cyclic olefins and EDA implies that the role of the catalyst is more than just to liberate a carbene from the diazoacetate ester.

In the presence of metal catalysts the reaction pathway is most likely different from, and much more complex than the one discussed above. In their comprehensive study, D. S. Wulfman *et al.* pointed to the complexity of almost all reactions between diazoalkanes and olefins if they are catalysed by copper salts, e.g. in one case 57 products were formed although only 30 were identified[64]. The yields from such reactions were demonstrated to depend on variables like the rate at which the reagents are mixed, catalyst concentration, reaction temperature, etc. The graph of % yield of various products *versus* catalyst concentration showed two maxima, which indicates that there were at least two processes taking place in such reactions. It was also shown in that study that the ratio of maleate to fumarate in the products depends chiefly on the catalyst concentration besides other factors. When these observations are extrapolated to the present study with EDA, they are very informative and help explain some of the results.

Addition; Stereospecificity: Addition of carboalkoxycarbenes (hν) to *cis*- and *trans*-but-2-enes has revealed that the carbene produced by direct photolysis adds to olefins in a highly stereospecific manner. The sensitised photolysis afforded products due to addition but with substantial loss in stereospecificity. This observation gives credence to the suggestion that $:CHCO_2Et(h\nu)$ from direct photolysis is in the singlet state, whereas the carbene from sensitised photolysis has less energy and is in the triplet state. It is known that carbenoids generated in the presence of catalysts react with olefins stereospecifically as well. During this work $:CHCO_2Et$ carbenoid has been generated catalytically and reacted with allyl halides and acetate. The question of stereospecificity did not arise here because the olefins are not disubstituted (*cf.* Scheme 6). However, the stereospecificity factor would be of tremendous significance during the synthesis of dialkoxycarbonyl-2-(hydroxymethyl)cyclopropanes starting with (*E*)- or (*Z*)-4-hydroxybut-2-enoates as outlined in the synthesis proposals (page 155).

Insertion Versus Addition: In 1951 D'yakonov and Vinogradova demonstrated[65] that EDA reacts with refluxing allyl bromide in the presence of copper or copper sulphate to give ethyl 2-bromopent-4-enoate in 70% yield by $:CH_2CO_2Et$ carbenoid insertion into the C-Br bond; neither carbene dimers nor cyclopropane-containing products were observed. This reaction was later extended to allyl acetate[66] and allyl chloride[67]. Allyl acetate gave *cis*-

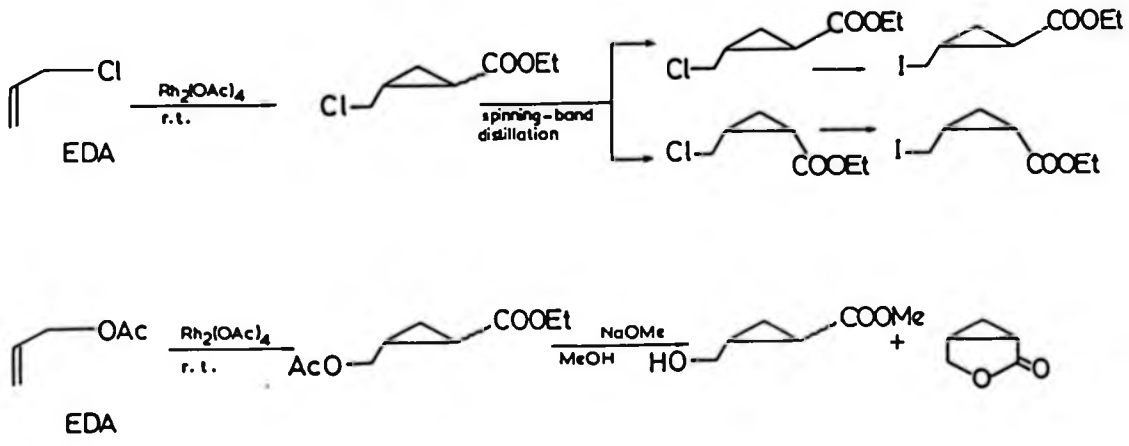
and *trans*-2-(acetoxymethyl)cyclopropane carboxylates in 33% yield and no insertion products, whereas using Cu(I)Cl catalyst allyl chloride also gave the corresponding *cis*- and *trans*-cyclopropanes, but in 4% yield and the insertion product, ethyl 2-chloropent-4-enoate, in 6% yield. During the present work, it has been shown that using rhodium acetate catalyst, EDA reacts with allyl chloride at room temperature to give the *cis*- and *trans*-cyclopropane esters predominantly, in > 60% crude yield. Alternatively, refluxing allyl bromide reacted with EDA in the presence of copper bronze to give the "insertion" product in 55% yield after purification; cyclopropane esters constituted < 5% of the total yield as judged from ^{1}H n.m.r. spectroscopy. No maleate or fumarate was detected in the products. However, these carbene dimers were found among the products of this reaction when $Rh_2(OAc)_4$ was used as catalyst instead of Cu bronze.

EDA was prepared according to a literature procedure[68] from glycine ethyl ester and HNO_2. $Rh_2(OAc)_4$ was prepared from $Rh(OH)_3.H_2O$ and acetic acid also according to a known[69] procedure. Starting with allyl chloride and $Rh_2(OAc)_4$ catalyst at room temperature, EDA was added gradually with stirring, over a period of 10 hours, to avoid carbenoid dimerisation. The resulting chlorides were converted into the iodides according to the Finkelstein method, after separation by spinning-band distillation.

In the case of allyl acetate, the resulting *cis*- and *trans*-cyclopropanes were transesterified with

Scheme 6

Preparation of *cis*- and *trans*-2-alkoxy-carbonylcyclopropanecarbinyls

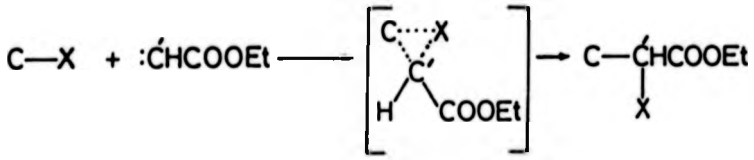


Scheme 7

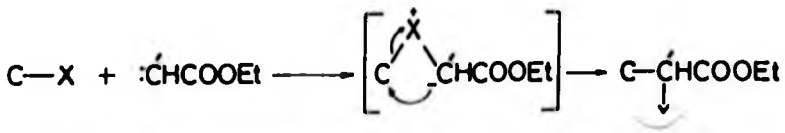
Preparation of ethyl 2-bromopent-4-enoate

$MeO^{-}Na^{+}$/MeOH and the acetate group was removed concurrently to give *cis*- and *trans*-1-(hydroxymethyl)-2-methoxycarbonyl-cyclopropane in 78% yield. The *cis*-isomer lactonised gradually on standing, hence aiding separation of the two isomers on a silica gel flash column through which the lactone eluted first, far ahead of the *trans*-isomer.

Insertion Mechanism: Like other reactions involving carboalkoxycarbenes and an olefin, there are at least two competing reactions when EDA reacts with allyl halides *viz.* "insertion" in the C-X bond to give ethyl 2-halopent-4-enoates and addition across the C=C bond of the olefin to give *cis*- and *trans*-1-ethoxycarbonyl-2-(halomethyl)cyclopropane The ratio of insertion to addition products depends on the mode of decomposition of EDA, e.g. singlet carbethoxycarbene produced by direct photolysis of EDA preferentially attacks the halogen atom in allylic halides to afford predominantly C-X "insertion" products, whereas the corresponding triplet carbene generated by benzophenone sensitised photolysis adds preferentially to the C=C bond of the olefin. The word "insertion" is usually applied to reactions (e.g. equation 24) in which the breaking of C-X bond and the formation of the new C-C' and C'-X bonds is a concerted process.



(24)



(25)

Equation 25 above shows a possible pathway for the "insertion" process, although one can never be sure that the active species is $:CHCO_2Et$, because of the reasons mentioned earlier, especially in the presence of a catalyst like copper. In the case of allyl halides several mechanisms have been suggested[70] to explain the occurrence of isomeric "insertion" products; all of them implicate the involvement of the C=C bond in the insertion process. For allyl bromide the two isomers arising from (S_N2') 1,2-shift of the C=C bond during insertion are practically indistinguishable.

3.1.2 Deconjugative Hydroxymethylation of But-2-enoate-(crotonate)

Ethyl 2-(iodomethyl)but-3-enoate (III', Fig. 5) is another alkylating agent that was needed during this work to prepare the corresponding alkylcobaloxime and study its rearrangement under various conditions; this alkylating agent is a but-3-enyl acyclic analogue of the cyclopropanes discussed above and it has been obtained in pure form from the alkylation of the anion derived from 4-bromobut-2-enoate.

Although numerous examples of compounds in this class can be found in literature[71,72], there is no single example where ethyl 2-(iodomethyl) or 2-(hydroxymethyl)but-3-enoate has been successfully prepared, isolated and characterised, let alone its cobaloxime. This could be attributed to the facile isomerisation of this compound by double bond migration or by dehydrohalogenation in the case of the iodide. These

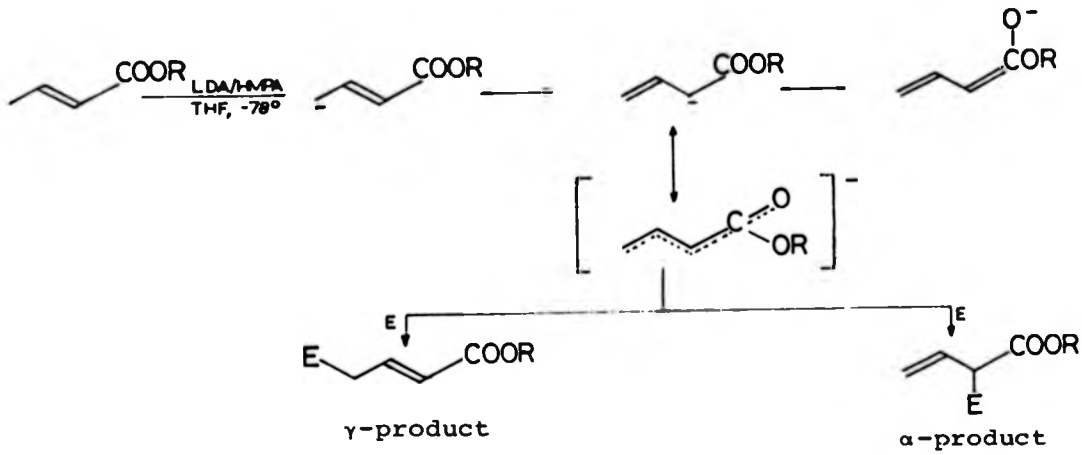
processes are enhanced by the relatively low pKa of H_α which permits the thermodynamically favourable restoration of conjugation to take place very easily.

It is well established[71] that crotonate anions react with electrophiles predominantly at the C_α due to the polarisation of the negative charge by the ester carbonyl centre. There are many examples in the literature where crotonate anions have been generated both from the acids and the esters, using a variety of strong bases (e.g. LDA/HMPA in THF[71], lithium isopropyl cyclohexylamide in THF[73] etc., usually at -78°) or from 4-bromocrotonates using Zn metal[72] in the typical Reformatsky manner. Electrophiles used in these reactions range from alkyl halides and sulphonates through to ketones and aldehydes. Varying ratios of α- to γ-alkylation products have also been obtained with these electrophiles (Scheme 8). This ratio has been shown to depend on a number of factors including reaction temperature[72,74], the metal counterion and the solvent system[75] used, whereas the stereochemistry of the γ-alkylation product does not depend on these factors as much as it does on the nature of the anion and the alkylating agent. It was observed[74] that during the reaction of either crotonic or 3-methylcrotonic dilithiated anion and carbonyl compounds (aldehydes or ketones), there exists an equilibrium between the α- and γ-alkylation products. The former is formed first at low temperature and is subsequently transformed by prolonged heating into the γ-product.

A similar result was obtained by treatment of

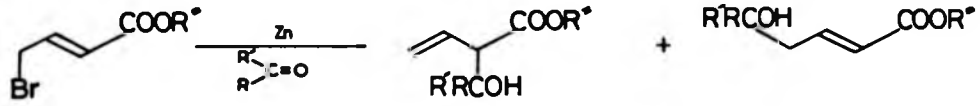
Scheme 8

Alkylation of but-2-enoate

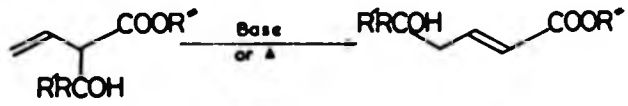


(26)

E = Electrophiles, e.g. alkyl halide and sulphonates, ketones and aldehydes



(27)



(28)

the initially formed β-hydroxy acid with two equivalents of base (*cf.* Scheme 8, equation 28). The previously observed (*Z*)-stereoselectivity for the γ-attack product was confirmed for 3-methylcrotonic acid, whereas crotonic acid leads to the (*E*)-isomer of its γ-alkylation product[74].

R. H. Schlessinger *et al.*[71] have studied the deconjugative alkylation of ethyl crotonate using various alkylating agents; LDA/HMPA in THF at -78° was employed as base. During the present work it was hoped that by following their procedure it would be possible to obtain compounds like CH_2=CHCH(CH_2OR)CO_2R which, according to their communication, they obtained in 92% yield. Their results could not be reproduced even after several attempts, particularly for chloromethyl methyl ether. When diiodomethane was reacted with crotonate anion produced according to their procedure, only crotonate dimers and polymers were detected. It is envisaged though that CH_2=CHCH(CH_2I)CO_2Et might have initially formed but was quickly dehydrohalogenated in the presence of base and the resulting but-1,3-diene carboxylate polymerised. Alternatively, the alkyl iodide initially formed could have been scavenged by the unreacted crotonate anions.

However, quenching the crotonate anion with methyl iodide afforded a good yield of γ- and α-alkylation products, in another experiment conducted under similar conditions. When the crotonate anion was again quenched with chloromethyl methyl ether according to the procedure in ref. 71, after work-up the products were separated by preparative GC (SE30 column, 150°). Two major

components in the reaction mixture were identified as ethyl 2-(methoxymethyl)but-2-enoate:

^{1}H n.m.r. (CCl_4) δ, 1.30 (t, 3H), 1.90 (d, 3H), 3.25 (s, 3H), 4.08 (s, 2H), 4.15 (q, 2H), and 6.95 (q, 1H)

e.i.m.s., m/z: 158(M^+), 143, 128, 113, 97, 85

and ethyl 2,2-bis(methoxymethyl)but-3-enoate:

^{1}H n.m.r. (CCl_4) δ, 1.25 (t, 3H), 3.30 (s, 6H), 3.54 (dd, 4H, J = 21 Hz), 4.11 (q, 2H), 5.09 (d, 1H, J_{cis} = 10.8 Hz), 5.10 (d, 1H, J_{trans} = 17.6 Hz) and 5.74 (dd, 1H, J_{cis}= 10.8 Hz, J_{trans}= 17.6 Hz)

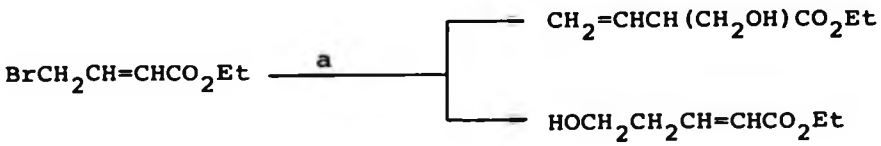
e.i.m.s., m/z: 203($M + H)^+$, 170, 157, 126, 98, 45

None of the desired compound was obtained although it is obvious it did actually form initially and then isomerised by double bond migration, back into conjugation to give $CH_3CH=C(CH_2OCH_3)CO_2Et$. Japanese scientists[76] generated crotonate anion from 4-bromocrotonate using diethylaluminium chloride and Zn/CuBr and reacted it with benzaldehyde to give the corresponding β-hydroxy ester (*threo*- and *erythro*-isomers) in an excellent yield (100%), although they did it on a microscale. When their method was followed using paraformaldehyde instead of benzaldehyde on a bigger scale, a mixture of products was obtained with no definite evidence for the presence of the wanted compound, 2-(hydroxymethyl)but-3-enoate. The method was therefore abandoned. At this time a related procedure employing Cr(II)/THF to reduce crotyl iodide appeared in the

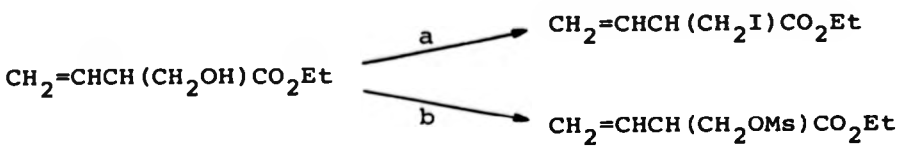
literature[77]. In that procedure Cr(II) is obtained by reducing Cr(III) with $NaBH_4$. However, aware of the possible reaction between $NaBH_4$ and conjugated double bonds in the presence of a transition metal-like chromium, this method had to be modified to suit the prevailing need. During the present work it was observed that activated zinc metal is also capable of cleanly reducing Cr(III) in THF, at room temperature, within less than an hour. The purple colour of Cr(III) gradually turns green indicating the formation of Cr(II). When chloromethyl methyl ether was added to this reaction mixture and the temperature lowered to -50^{o}, methyl 4-bromocrotonate solution in THF was added dropwise over a period of one hour to avoid the accumulation of unionised crotonate which would react with the ionised crotonate to give dimers and polymers, and hence lower the yield. After work-up, the ^{1}H n.m.r. spectrum of the product showed the presence of both γ- and α-alkylation products, in a ratio of about 1:4, respectively. Conducting this experiment at -60^{o} increased the yield of the α-product over the γ-product appreciably. Although a similar change in this ratio was observed[74] for the β-hydroxybut-3-enoates and was attributed to retroaddition of the α-product which is initially formed, such an explanation is unsatisfactory for molecules like CH_2=CHCH(CH_2OCH_3)CO_2Me which do not possess β-hydroxy moieties. Another mechanistic pathway must be found to explain this observation.

Scheme 9

Formylation of Crotonate Anion



(29)



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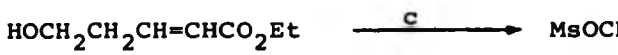
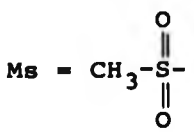


(31)

[a]Activated zinc dust/dry ether/room temperature, 3 hours (ref. 72)

[b]Ph_3P/I_2/MeCN/room temperatue, 12 hours (ref. 78)

[c]$MeSO_2Cl$ /CH_2Cl_2/Et_3N/0° to -10°, 15 minutes (ref. 79)



The advantage of zinc over $NaBH_4$ in the reduction of Cr(III) to Cr(II) is worth noting, especially in reactions involving easily-reduced groups like -CHO, -COCl, e.t.c. The activity of the zinc dust used in this reaction is vital, e.g. freshly activated zinc dust will reduce Cr(III)/THF within 30 minutes, whereas unactivated zinc will not reduce it at all, at room temperature.

The generation of the crotonate anion by the classical Reformatsky reaction, using activated zinc dust and 4-bromocrotonate, and subsequent quenching with gaseous formaldehyde in ether, gave the best yield of 2-(hydroxymethyl)but-3-enoate. Zinc dust was activated by washing it several times with 0.5 M HCl, water, absolute ethanol and dry ether. It was then heated at $\sim 150^{o}$ for 10 minutes under an atmosphere of nitrogen gas, in a 50 cm^3 r.b. flask. By pumping out the heated gas from the flask and letting in drier nitrogen alternately, all the moisture in the flask was removed to give dry zinc dust. This method of drying zinc dust is not only convenient but fast as well; the whole procedure is over within less than one hour from the time of washing to drying. Zinc dust prepared in this way was found to react with Reformatsky bromides (bromoacetates or bromocrotonates) quite exothermically and the reaction was over within a couple of hours. There was no need to reflux the reaction mixture contrary to the Reformatsky procedure and the yield (40%) of pure ethyl 2-(hydroxymethyl)but-3-enoate was good considering that a gaseous aldehyde was one of the starting materials. The two isomers thus

obtained were carefully separated on a flash silica gel column which afforded pure samples of each. Ethyl 2-(iodomethyl)but-3-enoate was prepared from the corresponding alcohol (i.e. the α-product) which was obtained as above, using Ph_3P/I_2 in acetonitrile[78]. This alcohol was also found to react more quickly and more efficiently with Ph_3P/CCl_4: when the alcohol was simmered with a slight excess of Ph_3P dissolved in CCl_4 it was totally converted to the chloride within 3 hours. However, notwithstanding such an efficient reaction that involves no acid at any stage, it meant that the chloride thus produced had to be taken through yet another reaction step (Finkelstein reaction), to convert it into the iodide. Therefore the reaction with Ph_3P/I_2 was preferred. The alcohol was found to form a methyl-sulphonate derivative readily. The latter compound is quite reactive as expected: when it was added to preformed cob(I)aloxime the only alkylcobaloximes obtained and identified by their ^1H n.m.r. spectra did not include the expected alkyl cobaloxime. One of the cobaloximes from this reaction was $CH_3CH{=}C\ (CH_2Co)CO_2Et$ and the other, $CoCH_2CH{=}CCH_3CO_2Et$. Aware that the corresponding alkyl iodide does react with cob(I)aloxime quite smoothly to give the expected S_N2 product, and that iodide is almost as good a leaving group as methyl-sulphonate, the explanation for the above observation remains obscure. However, a secondary reaction involving buta-1,3-dienylcarboxylate and Co(I) can be envisaged.

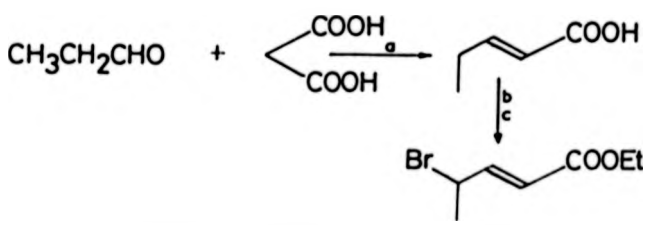
(*E*)-Ethyl 5-hydroxypent-2-enoate (the γ-product)

was obtained pure from a flash column and was fully characterised. It was then reacted with methanesulphonyl chloride according to a literature procedure[79], to make the corresponding methanesulphonate, which was then used to make the alkyl cobaloxime. This methanesulphonate is yet another isomer of the but-3-enylcarboxylate series of alkylating agents, whose cobaloximes have been studied during the present work. The corresponding alkyl-cobaloxime was made starting with Co(I) and this mesylate.

3.1.3 Synthesis of (*E*)-Ethyl 4-bromopent-2-enoate (IV', Fig. 5)

Another compound in the series of but-3-enyl carboxylate isomers needed in this study (the title compound), was made according to literature procedure[95]: the acid obtained by the reaction of malonic acid with propionaldehyde was esterified with $EtOH/H^+$ and the resulting ester was brominated at C-4 using N-bromosuccinimide/hν and Δ to give ethyl (*E*)-4-bromopent-2-enoate. Pure samples at each stage were obtained by fractional distillation and were characterised by ^1H n.m.r. spectroscopy and c.i.m.s. in the case of the bromide, (Scheme 10).

Scheme 10



[a] Pyridine, reflux
[b] $EtOH/H^+$
[c] N-bromosuccinimide/CCl_4, hν and reflux

3.1.4 Synthesis of (*E*)-Ethyl penta-2,4-dienoate

During this study the above compound was also required for the synthesis of the corresponding alkylcobaloxime using cobalt hydride (Co-H). It was synthesised in a very good yield (82%) by dehydrohalogenating ethyl 2-bromopent-4-enoate with triethylamine in acetone. The starting material, ethyl 2-bromopent-4-enoate is readily available from the carbenoid reaction described earlier on, involving allyl bromide and EDA. This method was found most convenient and efficient for the preparation of this buta-1,3-diene carboxylate and even more so, as polymeric material, which would be expected to accompany such a synthesis, could not be detected after filtering the ethereal solution of the product through a short column of silica gel. The resulting compound, which was pure by ^1H n.m.r. spectroscopy, was kept at -20° in the dark to avoid its polymerisation. As expected, the (*E*)-isomer was the only detectable product from the elimination reaction:

(32)

3.2 SYNTHESIS OF ALKYL(PYRIDINE)COBALOXIMES

3.2.0 Introduction

Throughout this study alkyl(pyridine)cobaloximes have been prepared according to standard literature procedures, either from Co(I) reacting with alkyl halides and alkyl methanesulphonates or Co-H reacting with activated double bonds, e.g. penta-2,4-dienoate. Co(I) was obtained by reducing a halo(pyridine)cobaloxime with $NaBH_4$ in either ethanol or methanol. Although well over 2,000 cobalt complexes in this class were known[80] and had been made as early as 1973, it is still worth noting a few observations, precautions and difficulties encountered during the present work, particularly as some of the compounds here described are novel.

It is now accepted that Co(I) reacts with simple alkyl halides by the well-known S_N2 mechanism[81] and not by an electron transfer (equations 33 and 34) mechanism.

$$Co(I) + RX \longrightarrow Co(II) + R^{\cdot} + X^{-} \qquad (33)$$

$$Co(II) + R^{\cdot} \longrightarrow Co\text{-}R \qquad (34)$$

This means that highly optically pure isomers of Co-R can be obtained starting with optically pure alkylating agents. In the present work, however, all chiral alkylating agents were used as racemates. The suggestion that Co(I) reacts with alkyl halides by an S_N2 mechanism has since been supported by other workers[82] when they demonstrated

the inversion of configuration at the displacement centre of substituted cyclohexyl bromides and tosylates during the reaction of the latter with Cob(I)aloxime. Many other examples have also appeared in literature[83] in support of the S_N2 mechanism. Notwithstanding all these convincing observations, there are some examples whereby alkylcobaloximes are formed with retention, e.g. 1-adamantyl- and 1-norbornyl(pyridine)cobaloximes. In this case, the electron transfer mechanism (equations 33 and 34) seems most likely. The alkylating agents used in this project are not as sterically hindered as either 1-adamantyl or 1-norbornyl halides, and so the S_N2 mechanism is generally assumed during their reaction with Co(I). Because of this, alkyl iodides were expected to give better yields than the corresponding chlorides. Thus, alkyl chlorides were converted into alkyliodides by the Finkelstein method.

In an effort to find optimum conditions for the synthesis of the alkylcobaloximes, reaction conditions e.g., the reaction time and temperature, the amount of $NaBH_4$ used, and concentration of reagents were varied successively. It was noticed that whereas excess of $NaBH_4$ ensures total reduction of Co(III) to Co(I) before the alkylating agent is added, it also reacts with and destroys the alkylcobaloxime that is initially formed. To counteract this yield-attenuating process, a slight excess of $NaBH_4$ and several-fold equivalents of the alkylating agent were usually used, expecially when the latter is readily available. In practice, however, one finds that a slight excess of $NaBH_4$ is usually

insufficient, particularly because $NaBH_4$ reacts with the solvent. Hence, the reduction of Co(III) to Co(I) as judged from the colour of the reaction mixture takes a long time to go to completion and sometimes a mixture of Co(I) and Co(II) is obtained. In the light of this, some researchers have recommended the use of excess of $NaBH_4$ and then either adding an acetone solution of the alkylating agent or just adding acetone to the reaction mixture when the reduction is complete. Acetone reacts with the excess of $NaBH_4$ to give isopropylalcohol which does not affect the reaction. However, during this study, adding acetone to the reaction mixture did not improve the yields of alkylcobaloximes at all. On the other hand, adding free DMG to the reaction mixture before $NaBH_4$ is added was found to help the reduction of halo(pyridine)-cobaloxime. The reaction mixture turned from dark brown to dark blue and became homogeneous much faster, compared to other runs where free DMG was not added. Less $NaBH_4$ was required to effect complete reduction and the best yields ($\simeq$ 100%) of alkylcobaloximes were obtained when free DMG was used. This observation seems to suggest that at least one of the DMG-containing intermediate species, e.g. cob(I)aloxime, has the tendency to dissociate and therefore adding free DMG to the reaction mixture suppresses the dissociation according to the Le Chatelier's principle.

By taking and immediately working-up equal aliquots from one of the reaction mixtures at periodical intervals of 5 minutes, 10 minutes, 20 minutes and

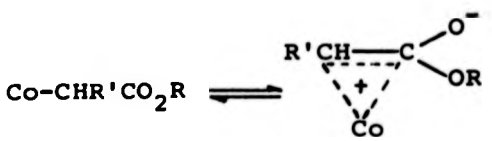
30 minutes, measured from the time the alkylating agent was added, it was possible to establish that at least the reaction between alkyliodides and Co(I) is very fast, because there was very little difference in the alkylcobaloxime yields obtained from these aliquots. This observation was augmented by t.l.c. analyses of the reaction mixtures at various times. Generally, the reaction between Co(I) and alkyl iodides is over within ½ hour to 1 hour. Although some of the reaction mixtures were left stirring for up to 3 hours, there was no obvious increase in the yield at the end of this time. Conducting these reactions at 0° did not make any detectable difference, at least in the cases where cyclopropylcarbinyliodides were used as alkylating agents.

Pure samples of alkylcobaloximes were obtained by either fractional crystallisation or/and column chromatography on silica gel; [eluant, CH_2Cl_2:Py:MeOH (95:1:5)]. Both free DMG and desalkylcobaloximes were easy to remove because they remained at the top of the column under these conditions, while alkylcobaloximes eluted as yellow/orange bands ($R_f \approx 0.45$ on t.l.c. using the above solvent system) and excess alkylating agents moved with the solvent front. When M. P. Atkins[48] chromatographed the related series of alkyl cobaloximes with methyl-substituted alkyl ligands as opposed to carboxylate-substituted alkyl ligands which have been studied in the present work, he found it necessary to add at least 1% pyridine to the eluting solvent in order to suppress the facile rearrangements of the alkyl ligands.

This could have been necessary because the methyl-substituted but-3-enylcobaloximes which he studied do rearrange rapidly in the presence of acid, unlike the carboxylate but-3-enylcobaloximes. Omitting pyridine in the eluant made no difference either to the separation or stability of the alkyl cobaloximes during the present work, if light was excluded as much as possible. The alkylcobaloximes were isolated as reddish-orange crystalline compounds, pumped dry at the freeze-drier and fully characterised by ^{1}H n.m.r., ^{13}C n.m.r., i.r., u.v. and microanalysis.

3.2.1 Discussion

(i) 1-Ethoxycarbonylbut-3-enyl(pyridine)cobaloxime (I', Fig. 5): Although ethyl 2-bromopent-4-enoate might be expected to give a low yield of this secondary alkylcobaloxime on account of its steric hindrance, a yield of 55% was obtained. There was even no need to convert the bromide into the iodide because the former is well labilised by its proximity to the carboxylate group and the S_N2 attack by Co(I) is smooth and fast. The resulting alkylcobaloxime is also stabilised by what is generally known as the β-effect[84] (equation 35).



(35)

This phenomenon is reflected in several physical constants of such organocobalt carboxylic esters:

if R = H, the pKa of the compound is unusually high and the ν_{max} for the carbonyl group is much lower compared with other compounds, e.g. β-organocobalt carboxylic acids and esters; in the case of 1-ethoxycarbonylbut-3-enyl(pyridine)cobaloxime ν_{max} (KBr), 1680 cm^{-1}, whereas in the corresponding bromoester ν_{max}, 1738 cm^{-1}. The chemical behaviour of the complex is also altered by the β-effect to a good extent; e.g. it is known[85] that α-organocobalt carboxylic ester groups (Co-C-CO_2R) are not easily saponified. They have to be treated with warm concentrated H_2SO_4 followed by water in order to hydrolyse them (equation 36). Schrauzer and Windgassen attributed this observation to the steric hindrance afforded by the equatorial ligands of the cobaloxime moiety. Although protonation of the ester alkoxy group is possible, subsequent displacement of the alcohol from the acylium cation by water, is apparently severely inhibited for steric reasons. The related β-organocobalt carboxylic esters on the contrary, were readily hydrolysed[85] in the presence of cold 1 M HCl. During the present work however, adding NaOH (40 mmol) pellets to a reaction mixture containing ethyl 2-bromopent-4-enoate and chloro(pyridine)cob(I)aloxime (∿ 20 mmol) in AR methanol (120 cm^3), and stirring for 3 hours at room temperature, 1-methoxycarbonylbut-3-enyl(pyridine)-cobaloxime was isolated in 14% yield, containing a trace of the ethyl ester cobaloxime (equation 37).

$$Co\text{-}CH_2CO_2Et \xrightarrow[\Delta]{\text{conc. } H_2SO_4} Co\text{-}CH_2CO_2H \qquad (36)$$

$$CH_2{=}CHCH_2CHCoCO_2Et \xrightarrow[RT]{dil.NaOH/MeOH} CH_2{=}CHCH_2CHCoCO_2Me \quad (37)$$

It is realised, however, that according to the procedure described above, the observed transesterification might have taken place either before or after the cobaloxime formation. If the transesterification took place after the α-organocobalt carboxylic ester was formed, then this would be in sharp contrast to the steric hindrance explanation given above concerning the saponification of $Co\text{-}CH_2CO_2R$ which invokes steric hindrance.

The proton n.m.r. of 1-ethoxycarbonylbut-3-enyl-(pyridine)cobaloxime shows typical peaks expected of an α-organocobalt ester: the four DMG methyl groups are inequivalent and resonate at 2.2 δ as a doublet, whereas all the other resonances due to the alkyl ligand were appreciably shifted upfield, in some cases by as much as 2 p.p.m. (Co-CH) and 0.6 p.p.m. ($Co\text{-}C\text{-}CH_2\text{-}$), compared to the precursor bromide ester. The coupling constants of the olefinic protons of the alkyl ligand were obtained by spin-decoupling experiments, e.g. irradiating at 1.87 p.p.m. (Co-CH- and $Co\text{-}C\text{-}CH_2\text{-}$) rendered the multiplet at 5.61 p.p.m. (C=CH-C-) a double doublet with J_{cis} = 10.2 Hz and J_{trans} = 17.2 Hz. The multiplet at 5.61 p.p.m. is so wide that it could not be irradiated totally, and this would complicate the multiplicity pattern of related resonances.

The proton-spin-decoupled ^{13}C spectrum was typical of this class of compounds with a broad peak at 33.15 δ

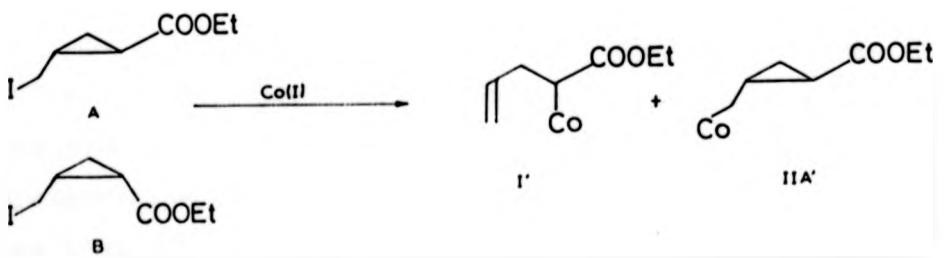
attributable to Co-C_α. This feature is common to most organocobalt compounds which contain a Co-C σ bond, and was observed with other cobaloximes. In the u.v. region, a peak at 430 nm was ascribed to charge transfer absorptions of the Co-C bond, although its extinction coefficient of 450 is less than expected[19] ($\sim 10^3$), perhaps due to hyperconjugation.

(ii) 2-Ethoxycarbonylbut-3-enyl(pyridine)cobaloxime: The alkylating agent used to make this compound was an alkyl iodide [although the chloride was easier to make], in order to maximise the yield . The ester group is separated from the leaving group by two carbons and cannot assist the S_N2 displacement process by labilising the halide. There is no hyperconjugation in this cobaloxime and the slight decrease in ν_{max} (KBr), 1728 cm^{-1} compared to the original alkyl iodide ν_{max}, 1737 cm^{-1} can only be attributed to inductive effects. The molecule is less stable than 1-ethoxycarbonylbut-3-enyl(pyridine)cobaloxime in the presence of light, acids or bases, perhaps due to the absence of the β-effect. It rearranges into the latter under acidic conditions. The presence of a Co-C σ bond was confirmed by a band in the u.v. region with a peak at 447 nm. Its extinction coefficient of 950 is within the expected range ($\sim 10^3$) (ref. 19). An upfield shift of all the ^1H n.m.r. peaks of the cobaloxime alkyl ligand compared to the original iodo ester, was also observed. The non-equivalence of the Co-$C_\alpha H_2$ protons was demonstrated by a chemical shift difference of 0.41 p.p.m. between

their resonances. This cobaloxime could not be isolated starting with the corresponding mesylate and Co(I); other cobaloximes were identified instead, resulting from the reaction of Co(I) and buta-1,3-diene-2-carboxylate.

(iii) *Cis*- and *trans*-2-ethoxycarbonylcyclopropylmethyl(pyridine)cobaloxime: Attempts to prepare pure samples of either isomer were frustrated by concomitant partial de-cyclisation of the cyclopropane ring giving rise to 1-ethoxycarbonylbut-3-enyl(pyridine)cobaloxime in varying ratio . The latter was identified by comparison with an authentic sample prepared from the corresponding bromo ester (see above). Reaction conditions were changed successively in each case but to no avail; the product always contained some but-3-enylcobaloxime. Starting with either A or B (Scheme 11), the only cyclopropylcobaloxime that could be identified (^{1}H n.m.r.) was IIA', and from one of the runs starting with A, almost pure IIA' was obtained, contaminated with < 5% of I'.

Scheme 11



The product from this experiment was purified, characterised by ^{1}H n.m.r. and its stability under various conditions, e.g. heat and acidic medium was studied. However, subsequent attempts to make more of IIA' of the same purity or better (i.e. free from the but-3-enylcobaloxime) were unsuccessful. Factors that influence this decyclisation are discussed under the "Rearrangements" section in Chapter 4. ^{1}H n.m.r. peaks in the region 0.5-1.5 δ, typical of the *trans*-cyclopropyl ligand, were observed and appropriately assigned. None of the *cis*-isomer was detected using either the 220 MHz or the 400 MHz instrument. The decrease of only $\sim$ 6 cm^{-1} in the ester carbonyl stretch frequency compared to the original alkyl iodide is pertinent to the structure because the ester group is γ to the cobalt atom. This cobaloxime was found on the whole to be more stable under various conditions than the methyl-substituted cyclopropylmethyl(pyridine)cobaloxime studied by M. P. Atkins[48].

(iv) <u>(*E*)-4-Ethoxycarbonylbut-3-enyl(pyridine)cobaloxime</u>: This was prepared from the alkyl methanesulphonate in low yield. This is rather surprising as the methanesulphonate moiety is an excellent leaving group, even towards weak nucleophiles like H_2O. It is possible that Co(I) reacted with the sulphonate centre as well to form cobaloximes of the type $BCoSO_2Me$, hence lowering the yield of the alkylcobaloxime. Although (*E*)-4-ethoxycarbonylbut-3-enyl(pyridine)cobaloxime is an orange/yellow solid, it is less crystalline than the related but-3-enyl cobaloximes

described above. This property was reflected in its reluctance to come out of solution during the recrystallisation process. The ester carbonyl stretch frequency is lowered from ν_{max} 1720 cm^{-1} in the precursor methanesulphonate to 1708 cm^{-1} (KBr) in the alkyl cobaloxime, perhaps because of an inductive effect. The β-effect cannot be operative here because the double bond and the cobalt centre are separated by two sp^3 carbons; $Co\text{-}CH_2CH_2CH{=}CHCO_2Et$.

(v) (*E*)Ethyl 4-[dioxy(pyridine)cobaloxime]pent-2-enoate: During an attempt to purify 1-ethoxycarbonylbut-3-enyl(pyridine)cobaloxime from CH_2Cl_2/30-40° petroleum spirit, another alkyl cobaloxime was formed photolytically (bright sunlight). When it was later isolated by chromatography and characterised, its ^1H n.m.r. spectrum was similar to that expected of (*E*)-ethyl 4-[(pyridine)-cobaloxime]pent-2-enoate. However, this proved not to be the compound: elemental analysis together with an X-ray structure showed that the new cobaloxime was the title alkylcobaloxime having dioxygen inserted between cobalt and a carbon atom.

This alkylperoxycobaloxime was also obtained from the photolysis of $CH_2{=}CHCH(CH_2Co)CO_2Et$ (III', Fig. 5) and $CoCH_2CH_2CH\overset{E}{=}CHCO_2Et$ (V', Fig. 5) in $CDCl_3$ and in d_4-MeOH. When Co(I) was reacted with $CH_3CHBrCH\overset{E}{=}CHCO_2Et$, the same alkylperoxycobaloxime was again obtained. (*E*)-Ethyl 4-[dioxy(pyridine)cobaloxime]pent-2-enoate is different from alkylcobaloximes in many respects: it is dark brown

Fig. 6 Single Crystal X-Ray Structure of (*E*)-Ethyl 4-[dioxy(pyridine)cobaloxime]pent-2-enoate

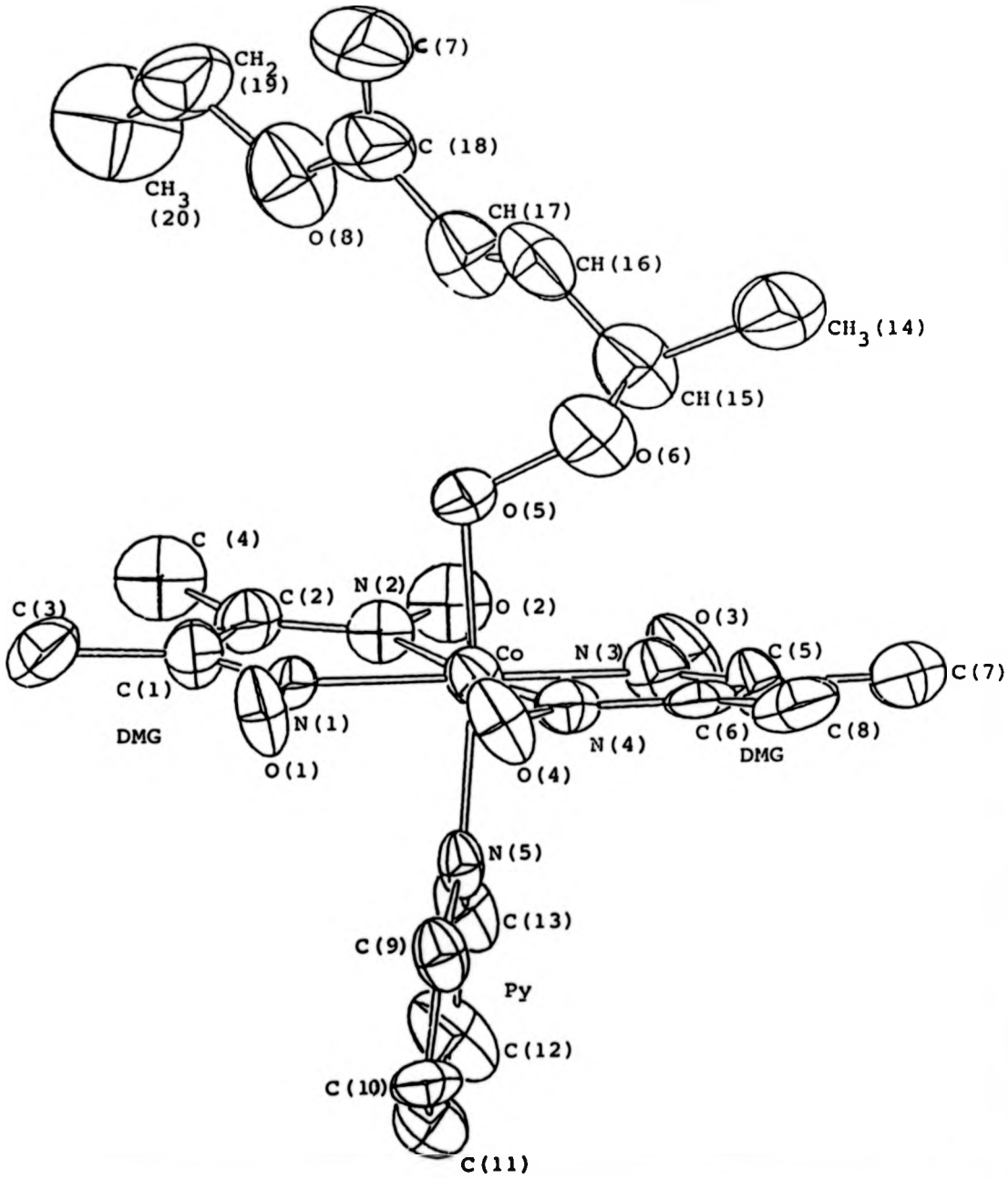


Table 2

Selected Bond Lengths and Bond Angles (see Fig. 6)

Bond Lengths (Å)		Bond Angles (°)	
Co-N(1-4) (oxime)	1.896(3)*	N(1,3)-Co-N(2,4)	81.7(1)*
		N(1,2)-Co-N(3,4)	98.4(1)*
Co-N(5)	2.007(5)	Co-N(1,4)-C(1,2,5,6)	116.1(3)*
Co-O(5)	1.923(4)	N(1)-Co-O(5)	84.0(2)
N(1-4)-O(1-4) (oxime)	1.347(4)*	N(2)-Co-O(5)	86.0(2)
		N(3)-Co-O(5)	96.2(2)
N(1-4)-C(1,2,5,6) (oxime)	1.289(5)*	N(4)-Co-O(5)	89.3(2)
		Co-O(5)-O(6)	115.3(3)
C(1,2)-C(5,6) (oxime)	1.471(7)*	O(5)-O(6)-C(15)	108.2(5)
O(5)-O(6)	1.415(7)		
O(6)-C(15)	1.428(8)		
C(14)-C(15)	1.500(12)		
C(15)-C(16)	1.480(10)		
C(16)-C(17)	1.322(10)		
C(17)-C(18)	1.457(11)		
O(1)---O(4)	2.493(7)		
O(2)---O(3)	2.495(8)		
O(1)-H(O-1,3)	1.51(9)		
O(4)-H(O-1,4)	1.03(9)		
O(2)-H(O-2,3)	1.30(8)		
O(3)-H(O-2,3)	1.22(8)		

*Averaged over equivalent values

in colour, whereas alkylcobaloximes are orange/yellow; it has a much smaller R_f value on silica gel than alkylcobaloximes, and the absence of a Co-C σ bond in this compound is betrayed by the absence of any peak around 450 ± 30 nm in the u.v. region. Its structure, which was determined by single crystal X-ray method, is shown in Fig. 6.

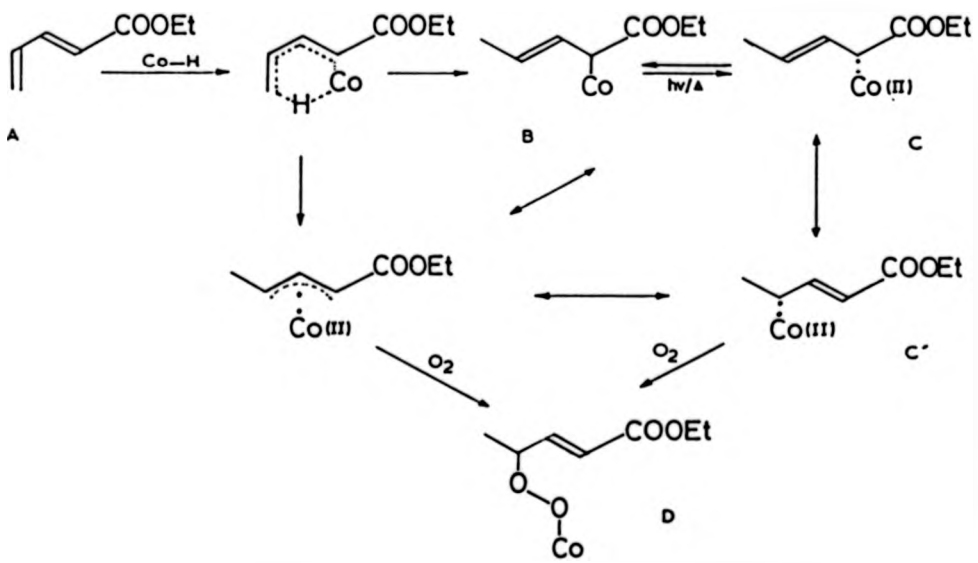
Mechanism: A possible mechanism for the photolytic formation of the alkylperoxycobaloxime from I' and V' (Fig. 5) involves β-elimination giving rise to Co-H and CH_2=CHCH$\overset{E}{=}$CHCO_2Et. Subsequent recombination followed by dioxygen insertion completes the process.

To test this hypothesis, two experiments were done: (1) Incubating CH_2=CHCH=CHCO_2Et and Co-H at 40° in $CDCl_3$ gave the alkylperoxycobaloxime in 17% yield; Co-H was generated[15] *in situ* from β-hydroxy-α-phenethyl-(pyridine)cobaloxime. (2) Photolysing CH_2=CHCH_2CHCoCO_2Me in the presence of an equimolar quantity of the corresponding diene ethyl ester *viz.* (*E*)-ethyl penta-2,4-dienoate, gave a 1:1 mixture of (*E*)-ethyl and (*E*)-methyl 4-[dioxy(pyridine)-cobaloxime]penta-2,4-dienoate. These two results support the mechanistic hypothesis above as far as β-elimination and recombination are concerned. However, the mechanism of both the recombination of Co-H and CH_2=CHCH=CHCO_2R (R = Me or Et) and dioxygen insertion cannot be deduced from these observations.

The reaction of Co-H and a variety of activated olefins has been shown[91] to proceed through free radical or free-radical-like transition states resulting from

H· transfer from cobalt to the olefin. The involvement of free radicals does explain the double bond isomerisation after recombination of Co-H and CH_2=CHCH=$CHCO_2R$ (Scheme 12). After the formation alkylcobaloxime B (Scheme 12) the Co-C bond is cleaved again by hν/Δ to give a well-stabilised radical and Co(II). The alkyl radical thus produced is delocalised into the adjacent α,β-unsaturated ester moiety; dioxygen insertion completes the reaction.

Scheme 12



Compound (B) could not be identified in the reaction perhaps because of its transient nature.

In the literature[86-90] it is well known that allylic cobaloximes insert dioxygen quite readily, even

at 20° if they are in solution. It is highly likely that in all the reactions studied during this work, in which the alkylperoxycobaloxime is formed, the corresponding allylic alkylcobaloxime (B) is formed first and then when the reaction is exposed to air, e.g. during the work-up, the latter cobaloxime inserts oxygen as depicted in Scheme 12. For comparison, (*E*)-3-ethoxycarbonylprop-2-enyl-(pyridine)cobaloxime (VI', Fig. 5) which is an allylic alkylcobaloxime but with no methyl group to cause steric hindrance, was prepared during this work from (*E*)-ethyl 4-bromobut-2-enoate and Co(I). It was isolated and purified by chromatography on silica gel. It is very stable at room temperature, both in solution and in solid state and shows no tendency to insert dioxygen under these conditions. This observation implies that steric destabilisation caused by the extra CH_3 group of [$CH_3CHCoCH{=}CHCO_2Et$] plays a big part in the labilisation of the Co-C bond leading to dioxygen insertion. It also shows that it is not just the allylic nature of Co-C bond that helps the cobaloxime to insert oxygen, but the weakness of the bond. If the Co-C σ bond is suitably labilised by steric strain coupled with its allylic nature, then even dim light and/or heat from the surroundings, at room temperature, is enough to homolyse it. This is particularly so if the resulting alkyl radical can be stabilised by delocalisation *via* an ester group or any other electron-withdrawing group capable of stabilising the free radical.

Although unstrained and non-allylic alkylcobaloximes have been shown[86-90] to insert dioxygen as well, they do

so only under more stringent conditions, e.g. at high temperatures or when photolysed. This observation backs up the suggestion that dioxygen insertion involves homolysis of the Co-C σ-bond of the alkylcobaloxime and trapping of the resulting alkyl radical by dioxygen (equation 38).

$$\text{Co-R} \xrightarrow{h\nu/\Delta} \text{Co(II)} + \text{R}^{\cdot} \xrightarrow{O_2} \text{Co(II)} + \text{RO}_2^{\cdot} \longrightarrow \text{Co-OO-R} \qquad (38)$$

Further support for this radical mechanism is afforded by the findings of Jensen and Kiskis[90]. Working with optically active 2-butyl(pyridine)cobaloxime, they demonstrated that dioxygen insertion occurs with racemisation at the cobalt-bearing carbon of the alkyl ligand.

3.3 EXPERIMENTAL

3.3.1 Synthetic Intermediates *en route* to *cis*- and *trans*-1-Alkoxycarbonyl-2-(iodomethyl)-cyclopropanes

1,1,2-Triethoxycarbonylcyclopropane: To a one litre r.b. flask fitted with a reflux condenser and a pressure-equalising dropping funnel was added absolute ethanol (250 cm^3) and clean sodium metal (15.18 g, 0.66 mol). Diethyl malonate (52.8 g, 0.33 mol) was then added with stirring, followed by ethyl 2,3-dibromopropionate (86.2 g, 0.33 mol). A white precipitate of NaBr was seen. The reaction mixture was stirred until it was neutral to

litmus paper (~ 4 hours). Most of the ethanol was removed at the pump and water (500 cm^3) was added to the resulting slurry. The dark upper layer was separated off and the aqueous layer extracted with ether (2 x 100 cm^3). The combined organic layers were dried ($MgSO_4$), and distilled to yield 1,1,2-triethoxycarbonylcyclopropane, 54 g (62%), b.p. 100°/0.5 mmHg. (Lit. b.p. 123.5°/2 mmHg, 276°/760 mmHg)[49].
^1H n.m.r. (CCl_4) δ, 1.27 (m, 9H), 1.65 (m, 1H), 1.95 (m, 1H), 2.56 (m, 1H) and 4.19 (m, 6H).

Cyclopropane-1,1,2-tricarboxylic Acid: This was made by refluxing the triester with excess aqueous NaOH for 15 hours. The resulting reaction mixture was acidified with concentrated HCl to pH 1 and was extracted continuously for 2 days with ether. The solid tricarboxylic acid was recrystallised from EtOAc/CH_2Cl_2 to afford a yield of 67% of pure material. (Lit. m.p. 189°) C.A., 53:17922.
^1H n.m.r. (D_2O) δ 2.70 (t, 1H), 2.95 (t, 1H), and 2.85 (m, 1H)

1,2-Cyclopropane dicarboxylic Acid: Cyclopropane-1,1,2-tricarboxylic acid (1 g, 5.75 mmol) was pyrolysed in a Kugelröhr apparatus at 180-190° under reduced pressure (15 mmHg) until effervescence ceased. The resulting liquid was distilled from the same apparatus to give an oily yellowish product which on recrystallising from ether/40-50° petroleum ether, gave white crystals of *cis*-1,2-cyclopropane dicarboxylic acid: 0.03 g (~ 4%). (Lit. m.p. 139-142°)[50].
^1H n.m.r. (D_2O) δ 2.90 (m, 1H), 2.00 (m, 1H), and 2.25 (m, 2H).

Cis- and trans-1,2-dimethoxycarbonylcyclopropane: To a three-necked two litre flask equipped with gas inlet and solid delivery adaptors and a mechanical stirrer, was added dry methyl chloroacetate (132.5 g, 107 cm^3, 1.22 mol) and methyl acrylate (334.6 g, 350 cm^3, 3.9 mol) under an inert atmosphere of nitrogen gas. The mixture was cooled (- 78°) and solid sodium methoxide (77 g, 1.43 mol) was added gradually over 1½ hours. The reaction mixture was then stirred at - 78° for another 5 hours. Water (500 cm^3) was added to dissolve the precipitated NaCl and the reaction mixture concentrated at the pump to remove most of the excess of methyl acrylate, before extracting it with ether (6 x 100 cm^3). The organic layers were combined and dried ($MgSO_4$). The solvent was removed at the pump and the product was distilled using a spinning-band column apparatus to give the *cis*-diester, 45 g (23%) b.p. 90°/12 mmHg.

^1H n.m.r. (CCl_4) δ 1.20 (m, 1H), 1.52 (q, 1H), 2.01 (t, 2H), and 3.64 (s, 6H);

and *trans*-diester, 43 g (22%) b.p. 86-88°/12 mmHg.

^1H n.m.r. (CCl_4) δ, 1.36 (t, 2H), 2.06 (t, 2H) and 3.66 (s, 6H).

Trans-1-methoxycarbonylcyclopropane-2-carboxylic Acid: This was obtained by adding half equivalent of aqueous KOH (19 mmol) gradually over 2 hours to a refluxing solution of *trans*-1,2-dimethoxycarbonylcyclopropane (18 mmol) in methanol (5 cm^3). The reaction mixture was cooled and acidified with concentrated HCl to

pH 3 before extracting it with ether (4 x 5 cm^3). Removal of solvent followed by distillation gave the required product, 1.37 g (58%) ≈ b.p. 145°/2 mmHg (distillation done with a Kugelröhr apparatus).

^1H n.m.r. ($CDCl_3$) δ 1.44 (m, 2H), 2.15 (m, 2H), 3.66 (s, 3H). A trace of the diester was the only contaminant in the product.

Trans-1-(hydroxymethyl)-2-methoxycarbonylcyclopropane[92]: was obtained *via* three independent procedures.

(1) By reducing the corresponding acid chloride cyclopropane ester with $NaBH_4$ or Bu_4NBH_4[93] in THF. The acid chloride ester was originally obtained by treating *trans*-1-methoxycarbonylcyclopropane-2-carboxylic acid with $SOCl_2$ at room temperature for 12 hours (see Scheme 4).

(2) By the reaction of allyl acetate and EDA, catalysed by $Rh_2(OAc)_4$ and then treating the product with NaOMe in dry MeOH: To a 250 cm^3 r.b. flask equipped with a dropping funnel and a drying tube was added allyl acetate (98.0 g, 105 cm^3, 0.974 mol) and $Rh_2(OAc)_4$ (0.287 g, 0.65 mmol) and the mixture was stirred for 5 minutes at room temperature. EDA (34.7 g, 32 cm^3, 0.304 mol) was then added dropwise over a period of 3 hours, with stirring. The catalyst was filtered off and the product distilled to afford a mixture of *cis*- and *trans*-1-(acetoxymethyl)-2-ethoxycarbonyl-

cyclopropane (44 g, 78%). This mixture was then stirred with a 10% solution of NaOMe in dry methanol for 12 hours at room temperature. Subsequent work-up afforded a mixture of *cis*- and *trans*-1-(hydroxymethyl)-2-methoxycarbonyl-cyclopropanes and the lactone, 3-oxabicyclo[3,1,0]-hexan-2-one which were separated by flash column chromatography on a silica gel column (eluent: dichloromethane); *trans*-1-(hydroxymethyl)-2-methoxycarbonylcyclopropane,

^{1}H n.m.r. (CCl_4) δ 0.84 (m, 1H), 1.13 (m, 1H), 1.52 (m, 1H), 1.60 (m, 1H), 3.28 (broad, 1H), 3.35 (dd, 1H, J_1 = 6.8 Hz, J_2 = 12.2 Hz), 3.58 (dd, 1H, J_1 = 6.8 Hz, J_2 = 12.2 Hz) and 3.65 (s, 3H).

(3) By the Simmons-Smith reaction between diiodo-methane and (*E*)-methyl 4-hydroxybut-2-enoate. To a 250 cm^3 r.b. flask equipped with a pressure equalising funnel, a reflux condenser and a gas inlet adaptor, was added zinc/copper couple[48] type II (4.7 g) in dry ether (70 cm^3) under an atmosphere of nitrogen gas. Diiodomethane (13 g, 48.5 mmol) was added and followed by dropwise addition of (*E*)-methyl 4-hydroxybut-2-enoate (2.2 g, 20 mmol) solution in ether (15 cm^3) over a 30 minute period, before refluxing the reaction mixture for 24 hours. Saturated ammonium chloride (50 cm^3) was

added to the cooled reaction mixture, the ethereal layer was separated off and washed with water (50 cm^3) and dried ($MgSO_4$). The solvent was removed at the pump and the ^1H n.m.r. spectrum of the distilled product (0.4 g) showed a substantial yield (> 50%) of *trans*-1-(hydroxymethyl)-2-methoxycarbonyl-cyclopropane besides many other by-products, by comparing its spectrum with that of the same but purer compound prepared otherwise. Good separation was achieved by using an Apiezon L Chromosorb GC column at 150°.

3-Oxabicyclo(3,1,0)hexan-2-one: This was made and isolated as described above. It was also made by partial reduction[92] of *cis*-1,2-cyclopropane dicarboxylic anhydride with $NaBH_4$ in THF.

A mixture of sodium borohydride (0.14 g, 3.7 mmol) in dry THF (6 cm^3) was stirred and cooled in an ice bath while *cis*-1,2-cyclopropane dicarboxylic anhydride (0.4 g, 3.6 mmol) in THF (21 cm^3) was added over a period of 5 minutes. The ice bath was removed and stirring continued for another $1\,^3/_4$ hours. 6 M Hydrochloric acid was added cautiously and the mixture concentrated. Water (50 cm^3) was added and the mixture extracted with CH_2Cl_2 (2 x 25 cm^3). The organic layers were combined and dried (Na_2SO_4). Removing the solvent afforded a pure sample of the lactone (29%).

^1H n.m.r. (CCl_4) δ, 0.80 (m, 1H), 1.22 (m, 1H), 1.94 (m,

1H), 2.20 (m, 1H), 4.13 (d, 1H, H_1 = 9.5 Hz) and 4.28 (dd, 1H, J_1 = 4.9 Hz, J_2 = 9.5 Hz).

Trans-1-(iodomethyl)-2-methoxycarbonylcyclopropane: To a 50 cm^3 three-necked flask, equipped with a reflux condenser and a dropping funnel, was added acetonitrile (6 cm^3), sodium iodide (0.8 g, 5.2 mmol) and *trans*-1-(hydroxymethyl)-2-methoxycarbonylcyclopropane (0.34 g, 6.2 mmol). Trimethylsilyl chloride (0.56 g, 0.66 cm^3, 5.2 mmol) in acetonitrile (4 cm^3) was added gradually to the stirring reaction mixture and stirring continued for another 1½ hours before dry methanol (10 cm^3) and three drops of concentrated HCl were added. Stirring was then continued for 12 hours before the reaction mixture was extracted with ether (5 x 10 cm^3), the extracts were combined and treated with saturated $Na_2S_2O_3$ (aq.) before drying ($MgSO_4$). Removing the solvent gave a pure sample of the desired iodide, 0.15 g (24%).

^1H n.m.r. (CCl_4) δ 0.85 (m, 1H), 1.43 (m, 1H), 1.54 (m, 1H), 1.85 (m, 1H), 3.03 (dd, 1H, J_1 = 8.3 Hz, J_2 = 9.8 Hz), 3.20 (dd, 1H, J_1 = 7.3 Hz, J_2 = 9.8 Hz), and 3.65 (s, 3H).

Cis-1-(iodomethyl)-2-methoxycarbonylcyclopropane: To a 50 cm^3 three-necked flask equipped with a condenser and flushed with N_2 gas, was added NaI (3.6 g, 24 mmol), acetonitrile (10 cm^3) and 3-oxabicyclo[3.1.0]hexan-2-one (0.8 g, 8 mmol) followed by trimethylsilyl chloride

(2.6 g, 24 mmol) while stirring. The reaction mixture was refluxed for 24 hours, cooled to room temperature and then dry methanol (20 cm^3) and acetyl chloride (1 cm^3) added. The resulting reaction mixture was then stirred at room temperature for 12 hours before adding water (50 cm^3) to it and extracting the product with ether (5 x 10 cm^3). The combined organic layers were washed with a saturated solution of $Na_2S_2O_3$, dried (Na_2SO_4) and solvent removed at the pump. The product was chromatographed on a silica gel column (eluted with CH_2Cl_2) to give pure *cis*-iodide, 0.14 g (7%).

^1H n.m.r. (CCl_4) δ 1.11 (dt, 1H, J_{gem} = 4.9 Hz, J_{trans} = 5.9 Hz), 1.27 (dt, 1H, J_{gem} = 4.9 Hz, J_{cis} = 8.3 Hz), 1.72-1.97 (m, 2H), 3.32 (t, 1H, J = 9.8 Hz), 3.50 (dd, 1H, J_1 = 7.3 Hz, J_{gem} = 9.8 Hz) and 3.69 (s, 3H) p.p.m.

ν_{max}^{film} 1726 (>C=O), 1005 (ring C-C), 575 (CH_2-I) cm^{-1};

e.i.m.s., m/z 240 $(M)^+$ 16.9%, 209 $(M - 31)^+$ 23.9%, 181 $(M - 59)^+$ 3.8%, 127 $(M - 113)^+$ 16.8%, 113 $(M - 127)^+$ 100%.

(E)-Methyl 4-acetoxybut-2-enoate: Carbon tetrachloride (400 cm^3) was added to a one litre r.b. flask containing silver acetate (39.63 g, 0.24 mol) and (*E*)-methyl 4-bromobut-2-enoate (46.5 g, 0.26 mol) and the reaction mixture refluxed while stirring, for 20 hours. The solvent was then removed at the pump and the product was distilled using a spinning-band column apparatus to

give very pure (*E*)-methyl 4-acetoxybut-2-enoate, 13.2 g (32%), b.p. 100°/12 mmHg which solidified on standing to give a low-melting white solid, m.p. 26°.

^{1}H n.m.r. (CCl_4) δ 2.08 (s, 3H), 3.71 (s, 3H), 4.68 (d, 2H, J = 4.4 Hz), 5.95 (d, 1H, J = 16.1 Hz), and 6.85 (dt, 1H, J_1 = 4.4 Hz, J_2 = 16.1 Hz).

(E)-Methyl 4-hydroxybut-2-enoate: To a 50 cm^3 r.b. flask was added crystals of (*E*)-methyl acetoxybut-2-enoate (13.2 g, 83 mmol), dry methanol (35 cm^3) and concentrated H_2SO_4 (1.1 cm^3) and the reaction mixture was stirred at room temperature for 12 hours. Solid sodium bicarbonate was added followed by water (60 cm^3), the mixture extracted with ether (3 x 60 cm^3) and the combined organic layers were dried ($MgSO_4$). Removing the solvent followed by distillation afforded pure (*E*)-methyl 4-hydroxybut-2-enoate, 5 g, (51%), b.p. 112°/12 mmHg.

^{1}H n.m.r. (CCl_4) δ 3.69 (s, 3H), 4.23 (d, 2H, J = 3.2 Hz), 5.99 (d, 1H, J = 14.5 Hz), and 6.94 (dt, 1H, J_1 = 3.2 Hz, J_2 = 14.5 Hz).

Ethyl diazoacetate (EDA): This was prepared according to literature procedure[68] in 85% yield. It was pure enough to use for the cyclopropanation and "insertion" reactions without having to distil it[94].

^{1}H n.m.r. ($CDCl_3$) δ 1.28 (t, 3H), 4.23 (q, 2H) and 4.77 (s, 1H).

Ethyl chloroacetate, which is the commonest contaminant,

constituted ∿ 3% of this product.

Cis- and trans-1-(chloromethyl)-2-ethoxycarbonyl-cyclopropane: A 500 cm^3 r.b. flask equipped with a pressure-equalising funnel, a stirring bar and a drying tube, held by a two-necked adaptor, was charged with distilled allyl chloride (137 g, 1.8 mol) and rhodium acetate dimer (0.3835 g, 0.8676 mmol). The mixture was stirred for about 3 minutes at room temperature before EDA (58 g, 0.51 mol) was added dropwise at this temperature over a period of about 10 hours. The effervescence that was quite evident at the beginning decreased gradually towards the end; vigorous stirring was maintained all the time. Excess of allyl chloride was removed at the pump and the crude product was distilled using a spinning-band column apparatus to give pure samples of the two isomers:
cis-1-(chloromethyl)-2-ethoxycarbonylcyclopropane, 21 g, (26%), b.p. 83°/16 mmHg,
^{1}H n.m.r. (CCl_4) δ 1.15 (m, 2H), 1.29 (t, 3H), 1.68 (m, 1H), 1.82 (m, 1H), 3.63 (q, 1H), 3.81 (q, 1H), and 4.12 (m, 2H), and
trans-(chloromethyl)-2-ethoxycarbonylcyclopropane, 25 g, (30%), b.p. 89-90°/16 mmHg.
^{1}H n.m.r. (CCl_4) δ 0.92 (m, 1H), 1.27 (m, 4H), 1.57 (m, 1H), 1.77 (m, 1H), 3.46 (m, 2H), and 4.08 (q, 2H).

Cis-1-ethoxycarbonyl-2-(iodomethyl)cyclopropane: This was obtained by refluxing the corresponding chloride with 2 equivalents of NaI in acetone (600 cm^3 for every mole of NaI used) for 12 hours. It was then left stirring at room temperature for two days. Acetone was removed at the pump and the product was extracted with ether. Removing the solvent gave a slightly yellow liquid, which was fully characterised and shown to be the *cis*-1-ethoxycarbonyl-2-(iodomethyl)cyclopropane, in 96% yield.

R_f = 0.74 (silica gel; CH_2Cl_2/40-60° petroleum ether, 1:1).

^1H n.m.r. (CCl_4) δ 1.09 (q, 1H, J_{gem} = 4.9 Hz, J_2 = 11.2 Hz), 1.25 (m, 1H), 1.29 (t, 3H), 1.85 (m, 2H), 3.31 (t, 1H, J = 9.3 Hz), 3.49 (dd, 1H, J_1 = 7.3 Hz, J_2 = 9.3 Hz), and 4.10 (m, 2H).

ν_{max}^{film} 1723 (s, ester C=O), 1027 (m, ring C-C), 575 (s, CH_2-I) cm^{-1}.

e.i.m.s., m/z 254 $(M)^+$ 7.4%, 209 $(M - 45)^+$ 27%, 127 $(M - 127)^+$ 100%, 99 $(M - 155)^+$ 87%, 69 $(M - 185)^+$ 34%. Accurate mass deviation, -0.79 p.p.m.

Trans-1-ethoxycarbonyl-2-(iodomethyl)cyclopropane: This was obtained by the Finkelstein reaction on the corresponding chloride as above, in 93% yield.

R_f = 0.65 (silica gel; CH_2Cl_2/40-60° petroleum ether, 1:1)

^1H n.m.r. (CCl_4) δ 0.84 (m, 1H), 1.26 (t, 3H), 1.40 (m, 1H), 1.53 (m, 1H), 1.84 (m, 1H), 3.01 (dd, 1H, J_1 = 8.8 Hz, J_2 = 9.8 Hz),

3.23 (dd, 1H, J_1 = 7.3 Hz, J_2 = 9.8 Hz), and 4.08 (q, 2H).

ν_{max}, 1726 (s, ester C=O), 992 (m, ring C-C) cm^{-1}

e.i.m.s., m/z 254 $(M)^+$ 1.6%, 209 $(M - 45)^+$ 22.5%, 127 $(m - 127)^+$ 100%, 99 $(M - 155)^+$ 77.2%. 85 $(M - 169)^+$ 16.9%.

Accurate mass deviation, +3.94 p.p.m.

3.3.2 Preparation of Carboxylate-substituted But-3-enyl Alkylating Agents

Ethyl 2-bromopent-4-enoate: Redistilled allyl bromide (60 g, 43 cm^3, 0.5 mol) and copper bronze (0.1 g) were put in a 250 cm^3 two-necked flask which was equipped with a condenser and a dropping funnel containing ethyl diazoacetate (14.15 g, 13 cm^3, 0.124 mol). The temperature of the reaction mixture was raised until it started to reflux gently. EDA was then added dropwise over a period of 4 hours with stirring. Excess allyl bromide was removed at the pump at room temperature and the resulting liquid distilled to give pure ethyl 2-bromopent-4-enoate, 14 g (55%), b.p. 78-80°/12 mmHg, lit. b.p. 78-79°/12 mmHg.

^1H n.m.r. (CCl_4) δ 1.30 (t, 3H), 2.75 (m, 2H, J_1 = 7.3 Hz, J_2 = 14.7 Hz), 4.09 (t, 1H, J = 7.3 Hz) 4.18 (q, 2H), 5.11 (d, 1H, J_{cis} = 9.7 Hz), 5.13 (d, 1H, J_{trans} = 18.5 Hz), and 5.71 (m, 1H).

R_f = 0.80 (siliga gel; CH_2Cl_2/40-60° petroleum ether, 1:1).

ν_{max}, 3084 (m, =C-H), 1740 (s, ester C=O), 1643 (m, C=C) cm^{-1}.

e.i.m.s., m/z: 208 (M, $^{81}Br)^+$ 0.9%, 206 (M, $^{79}Br)^+$ 1%, 163 (M, ^{81}Br-45) 5.5%, 161 (M, ^{79}Br-45)$^+$ 5.7%, 135 (M, ^{81}Br-73)$^+$ 15.4%, 133 (M, ^{79}Br-73) 15.9%, 127 (M-Br)$^+$ 100%, 99 (M-Br-28)$^+$ 86.2%.

Analysis by c.i.m.s. showed peaks, m/z: 226 (M, $^{81}Br + NH_4)^+$, 224 (M, $^{79}Br + NH_4)^+$, 209 (M, $^{81}Br + H)^+$, 207 (M, $^{79}Br + H)^+$.

Ethyl 2-(hydroxymethyl)but-3-enoate and (E)-Ethyl-5-hydroxypent-2-enoate: A 500 cm^3 three-necked r.b. flask was equipped with a reflux condenser and a drying tube, and was flame-dried while purging it with a stream of dry N_2 gas (the gas was originally passing over paraformaldehyde in a 50 cm^3 r.b. flask, attached to one of the side arms). The apparatus was left to cool with N_2 still flowing. The reaction flask was then charged with sodium-dried ether (200 cm^3), activated zinc metal dust (13 g, 0.2 mol) and (*E*)-ethyl 4-bromobut-2-enoate (9.65 g, 0.05 mol) in ether (10 cm^3) while stirring. One crystal of iodine which was then dried turned the reaction mixture reddish-brown, but the colour was quickly discharged within one minute, hence indicating that the reaction had started. The reaction mixture was then saturated with formaldehyde gas (∿ 30 minutes), which was carried over by a slow stream of dry N_2 gas flowing over heated paraformaldehyde. The ensueing slightly exothermic reaction generated enough heat to reflux the ether for about 10 minutes. Stirring was continued for another 3 hours before 1 M HCl (30 cm^3) was added. The

aqueous layer was continuously extracted with ether, the organic layers combined and dried ($MgSO_4$). On removing the solvent a crude product was obtained. It was then chromatographed on a flash silica gel column (eluted with ethyl-acetate/30-40° petroleum ether, 1:3; R_f = 0.45), to give pure samples of:

(1) Ethyl 2-(hydromethyl)but-3-enoate, 2.6 g (36%).

^{1}H n.m.r. (CCl_4) δ 1.28 (t, 3H), 3.16 (m, 1H), 3.60 (dd, 1H, J_{vic} = 4.8 Hz, J_{gem}= 10.8 Hz), 3.75 (dd, 1H, J_{vic} = 8.3 Hz, J_{gem} = 10.8 Hz), 4.14 (q, 2H), 5.16 (d, 1H, J_{cis} = 9.7 Hz), 5.18 (d, 1H, J_{trans}= 18.1 Hz), and 5.75 (ddd, 1H, J = 8.3 Hz, J_{cis} = 9.7 Hz, J_{trans} = 18.1 Hz).

ν_{max}, 3340 (broad, O-H), 3085 (w, =C-H), 1730 (s, ester C=O), 1640 (m, $C=CH_2$) cm^{-1}.

Analysis with c.i.m.s. gave peaks, m/z: 162 $(M + NH_4)^+$, 145 $(M + H)^+$, 127 $(M + H - H_2O)^+$

When e.i.m.s. was used, a protonated molecular ion, 145 $(M + H)^+$, was invariably observed despite several attempts.

(2) (*E*)-Ethyl 5-hydroxypent-2-enoate, 1.0 g (14%) resulting from γ-alkylation.

^{1}H n.m.r. (CCl_4) δ 1.28 (t, 3H), 2.40 (q, 2H), 3.66 (t, 2H), 4.12 (q, 2H), 5.82 (d, 1H, J_{trans} = 16.1 Hz) and 6.90 (dt, 1H, J = 7.3 Hz, J_{trans}= 16.1 Hz).

Ethyl 2-(iodomethyl)but-3-enoate: A 250 cm^3 r.b. flask equipped with a drying tube and a stirring bar was charged with CaH_2-dried acetonitrile (60 cm^3), triphenyl phosphine (6.55 g, 25 mmol) (originally recrystallised from methanol and dried at 0.05 mmHg for several hours), and commercial resublimed iodine (5.08 g, 20 mmol). The mixture was stirred at room temperature for 5 minutes during which time it changed colour from dark red to light yellow, hence indicating the formation of Ph_3PI_2. (The colour change can be speeded by warming the reaction mixture.) Ethyl 2-(hydroxymethyl)but-3-enoate (2.7 g, 18.75 mmol) in dry acetonitrile (5 cm^3) was then added and the reaction mixture stirred at room temperature for 12 hours. At the end of this time the alcohol had been totally converted into the iodide; the reaction progress was monitored at regular intervals by ^1H n.m.r. spectroscopy. Acetonitrile was pumped off and the resulting mixture was chromatographed on a flash silica gel column, (CH_2Cl_2/ 40-60° petroleum ether, 1:3). After removing the solvent at the pump, the product was then distilled using a Kugelröhr apparatus to give pure ethyl 2-(iodomethyl)but-3-enoate, 3.7 g (69%). R_f = 0.79 (silica gel, CH_2Cl_2/40-60° petroleum ether, 1:1).

^1H n.m.r. (CCl_4) δ 1.29 (t, 3H), 3.17 (m, 1H), 3.36 (m, 2H), 4.15 (q, 2H), 5.22 (d, 1H, J_{trans} = 17.6 Hz), 5.23 (d, 1H, J_{cis} = 10.3 Hz), 5.74 (ddd, 1H, J = 7.3 Hz, J_{cis} = 10.3 Hz, J_{trans} = 17.6 Hz).

ν_{max}, 3085 (w, =C-H), 1737 (s, ester C=O), 1638 (m, C=C) cm^{-1}

Ethyl 2-(iodomethyl)but-3-enoate: A 250 cm^3 r.b. flask equipped with a drying tube and a stirring bar was charged with CaH_2-dried acetonitrile (60 cm^3), triphenyl phosphine (6.55 g, 25 mmol) (originally recrystallised from methanol and dried at 0.05 mmHg for several hours), and commercial resublimed iodine (5.08 g, 20 mmol). The mixture was stirred at room temperature for 5 minutes during which time it changed colour from dark red to light yellow, hence indicating the formation of Ph_3PI_2. (The colour change can be speeded by warming the reaction mixture.) Ethyl 2-(hydroxymethyl)but-3-enoate (2.7 g, 18.75 mmol) in dry acetonitrile (5 cm^3) was then added and the reaction mixture stirred at room temperature for 12 hours. At the end of this time the alcohol had been totally converted into the iodide; the reaction progress was monitored at regular intervals by ^1H n.m.r. spectroscopy. Acetonitrile was pumped off and the resulting mixture was chromatographed on a flash silica gel column, (CH_2Cl_2/ 40-60° petroleum ether, 1:3). After removing the solvent at the pump, the product was then distilled using a Kugelröhr apparatus to give pure ethyl 2-(iodomethyl)but-3-enoate, 3.7 g (69%). R_f = 0.79 (silica gel, CH_2Cl_2/40-60° petroleum ether, 1:1).

^1H n.m.r. (CCl_4) δ 1.29 (t, 3H), 3.17 (m, 1H), 3.36 (m, 2H), 4.15 (q, 2H), 5.22 (d, 1H, J_{trans} = 17.6 Hz), 5.23 (d, 1H, J_{cis} = 10.3 Hz), 5.74 (ddd, 1H, J = 7.3 Hz, J_{cis} = 10.3 Hz, J_{trans} = 17.6 Hz).

ν_{max}, 3085 (w, =C-H), 1737 (s, ester C=O), 1638 (m, C=C) cm^{-1}

e.i.m.s., m/z: 254 $(M)^+$ 5.3%, 209 $(M - 45)^+$ 5.7%, 181 $(M - 73)^+$ 76.6%, 127 $(M - 127)^+$ 100%, 99 $(M - 155)^+$ 65.9%

Accurate mass deviation, - 4.72 p.p.m.

(E)-Ethyl 5-(methanesulphonate)pent-2-enoate: This was prepared according to the literature procedure[79] from the corresponding alcohol, $MeSO_2Cl/Et_3N$ in CH_2Cl_2, and was isolated as an oil in 58% yield.

^1H n.m.r. (CCl_4) δ 1.29 (t, 3H), 2.65 (q, 2H, J = 6.4 Hz), 2.96 (s, 3H), 4.15 (q, 2H), 4.29 (t, 2H, J = 6.4 Hz), 5.89 (d, 1H, J_{trans} = 16.1 Hz), and 6.82 (dt, 1H, J = 6.4 Hz, J_{trans} = 16.1 Hz).

ν_{max}, 3020 (w, =C-H), 1719 (s, ester C=O), 1660 (m, C=O), 1355, 1175 (s, S=O)cm^{-1}

(E)-Ethyl 4-bromopent-2-enoate: This was prepared by allylic bromination of (*E*)-ethyl pent-2-enoate. (The latter was originally prepared by the Doebner condensation of malonic acid and propionaldehyde followed by esterification[95].)

To 100 cm^3 r.b. flask equipped with a condenser, drying tube and a stirring bar was added analytical grade N-bromosuccinimide (NBS) (6.25 g, 0.035 mol), dry carbon tetrachloride (25 cm^3), and (*E*)-ethyl pent-2-enoate (4.5 g, 0.035 mol). The reaction mixture was stirred and refluxed while radiating it with a 150 W bulb for 16 hours. At the end of this time all the NBS had been

converted into succinimide which was now floating on top of the dense CCl_4. The reaction mixture was cooled and filtered through a glass sintered funnel before removing the solvent at the pump. Distillation through a short fractionating column afforded (*E*)-ethyl 4-bromopent-2-enoate, 6.40 g (88%), b.p. 980/15 mmHg.

^1H n.m.r. (CCl_4) δ 1.30 (t, 3H), 1.83 (d, 3H, J = 6.8 Hz), 4.16 (q, 2H), 5.66 (dq, 1H, J_1 = 6.8 Hz, J_2 = 7.8 Hz), 5.85 (d, 1H, J_{trans} = 15.6 Hz), and 6.95 (dd, 1H, J_1 = 7.8 Hz, J_{trans} = 15.6 Hz).

ν_{max}, 1722 (s, ester C=O), 1653 (m, C=C), 725 (m, C-Br) cm^{-1}.

c.i.m.s., m/z: 226 $(M\ ^{81}Br + NH_4)^+$, 224 $(M\ ^{79}Br + NH_4)^+$, 209 $(M\ ^{81}Br + H)^+$, 207 $(M\ ^{79}Br + H)^+$, 163 $(M\ ^{81}Br\text{-ethanol})^+$, 161 $(M\ ^{79}Br\text{-ethanol})^+$, 127 $(M\text{-}Br)^+$.

(E)-Ethyl penta-2,4-dienoate: To a 100 cm^3 r.b. flask was added ethyl 2-bromopent-4-enoate (1.65 g, 7.97 mmol), acetone (15 cm^3) and triethylamine (5 cm^3). The reaction mixture was stirred at 55° for 5½ hours during which time $Et_3\overset{+}{N}HBr^-$ precipitated out of solution as white solid. The reaction mixture was cooled to room temperature and filtered thorough a sintered-glass funnel before removing the solvent at the pump. In order to remove all the remaining triethylammonium hydrobromide, the product was dissolved in ether (10 cm^3), and filtered through a short column (~ 10 cm) of silica gel. The solvent was removed at the pump (temp. < 30°), to give (*E*)-ethyl penta-2,4-dienoate,

0.82 g (82%).

^{1}H n.m.r. (CCl_4) δ 1.29 (t, 3H), 4.18 (q, 2H), 5.46 (d, 1H, J = 10.7 Hz), 5.59 (d, 1H, J = 17 Hz), 5.85 (d, 1H, J = 15.5 Hz), 6.44 (dt, 1H, J_1 = 10.7 Hz, J_2 = 17 Hz) and 7.19 (dd, 1H, J_1 = 10.7 Hz, J_2 = 17 Hz).

ν_{max}^{film} 3100-3000 (w, =C-H), 1715 (s, ester C=O), 1647 (m, $-C=CH_2$), 1603 (m, $-CH=CCO_2Et$) cm^{-1}

e.i.m.s., m/z 126 $(M)^+$ 33.3%, 98 $(M - 28)^+$ 36.8%, 81 $(M - 45)^+$ 100%, 70 $(M - 56)^+$ 13.7%, 53 $(M - 73)^+$ 88.9%, and 27 $(M - 99)^+$ 70%.

3.3.3 Preparation of Alkyl(pyridine)cobaloximes

1-Ethoxycarbonylbut-3-enyl(pyridine)cobaloxime (I', Fig. 5): Absolute ethanol (10 cm^3) and bromo(pyridine)cobaloxime (0.448 g, 1 mmol) contained in a 100 cm^3 Schlenk tube, were purged by stirring them for 1 hour with a gentle stream of nitrogen gas passing over the top of the suspension. Sodium borohydride (0.038 g, 1 mmol) in absolute ethanol (20 cm^3) was added and the mixture was stirred for 10 minutes at room temperature. During this time the reaction mixture changed from dark brown to dark blue and became homogeneous. Ethyl 2-bromopent-4-enoate (0.311 g, 1.5 mmol) in absolute ethanol (10 cm^3) was then added and the reaction mixture was stirred at room temperature for another 1 hour. The solvent was removed at the pump (< 30°) and the resulting solid was chromatographed

on a flash silica gel column, (CH_2Cl_2:MeOH:Pyridine, 96:3:1) to give pure 1-ethoxycarbonylbut-3-enyl(pyridine)cobaloxime, 0.27 g (55%).

R_f = 0.44 (silica gel; CH_2Cl_2:MeOH:Pyridine, 94:1:5)

^1H n.m.r. ($CDCl_3$) δ 1.20 (t, 3H), 1.87 (m, 3H), 2.22 (d, 12 H), 3.92 (q, 2H), 4.75 (dd, 1H, J_{gem} = 2.1 Hz, J_{cis} = 10.2 Hz), 4.86 (dd, 1H J_{gem} = 2.1 Hz, J_{trans} = 17.2 Hz), 7.30 (t, 2H) 7.70 (t, 1H) and 8.51 (d, 2H).

ν_{max} (KBr) 3450 (broad, NO-H), 3100 (w, =C-H), 1680 (s, ester C=O), 1636 (m, $C=CH_2$), 1600 (w, Py. C=N), 1557 (s, C=NOH). 1240, 1090 (s, N-O), 515 (s, Co-N) cm^{-1}. (The Co-C bond at ∿ 320 was not observed perhaps because of the β-effect.)

λ_{max}^{EtOH} 305 nm (10,600) and 430 nm (450)

Calculated: C, 48.49; H, 6.10; N, 14.14

Found: C, 48.30; H, 6.04; N, 14.17

$\{^1H\}^{13}C$ n.m.r. ($CDCl_3$) δ 12.41 (4 Me DMG), 14.17 (H_2C_β), 33.15 (Co-C), 34.97 (CH_3 ester), 59.47 (CH_2 ester), 114.33 (CH_2=), 125.3 (C *meta*-pyridine), 136.95 (HC_γ-), 137.86 (C *para*-pyridine), 150.01 (C *ortho*-pyridine), 150.99 (4C=N DMG), 178.93 (-CO_2-ester).

2-Ethoxycarbonylbut-3-enyl(pyridine)cobaloxime (III', Fig. 5): Bromo(pyridine)cobaloxime (0.448 g, 1 mmol) and absolute ethanol (40 cm^3), contained in a 100 cm^3 Schlenk tube

were flushed with argon gas while stirring for 2 hours. When $NaBH_4$ (0.066 g, 1.7 mmol) was added there was vigorous effervescence and the reaction became dark blue and homogeneous. Ethyl 2-(iodomethyl)but-3-enoate (0.23 g, 0.906 mmol) in absolute ethanol (10 cm^3) was added and stirring continued at room temperature for 1 hour. The solvent was removed at the pump and the resulting solid was recrystallised from CH_2Cl_2/30-40° petroleum ether to give a yellow/orange solid which was chromatographed on silica gel column, (CH_2Cl_2:MeOH:pyridine, 94:1:5). Removing the solvent afforded the desired cobaloxime, 2-ethoxycarbonylbut-3-enyl(pyridine)cobaloxime, 0.11 g(25%).

R_f = 0.45 (silica gel; CH_2Cl_2:MeOH:pyridine, 94:1:5).

^1H n.m.r. ($CDCl_3$) δ 1.24 (t, 3H), 1.55 (dd, 1H, J_{vic} = 3.9 Hz, J_{gem} 8.8 Hz), 1.96 (t, 1H, J = 8.8 Hz), 2.10 (d, 12H), 2.51 (dt, 1H, J_1 = 3.9 Hz, J_2 = 8.8 Hz), 4.07 (q, 2H), 4.89 (d, 1H, J_{cis} = 9.7 Hz), 4.99 (d, 1H, J_{trans}= 17.1 Hz), 5.65 (ddd, 1H, J_{vic} = 8.8 Hz, J_{cis} = 9.7 Hz, J_{trans} 17.1 Hz), 7.31 (t, 2H), 7.73 (t, 1H), and 8.56 (d, 2H).

ν_{max} (KBr) 3450 (broad, NO-H), 3200 (w, =C-H), 1728 (s, ester C=O), 1630 (w, CH_2=C), 1600 (m, pyridine C=N), 1560 (s, C=NOH), 1238, 1089 (s, N-O), 518 (m, Co-N), 320 (w, Co-C) cm^{-1}.

λ_{max}^{EtOH} 447 nm (950), 380 nm (1,800), 290 nm (7,100)

Calculated: C, 48.49; H, 6.10; N, 14.14

Found: C, 48.36; H, 6.11; N, 13.95

$\{^1H\}^{13}C$ n.m.r. ($CDCl_3$) δ, 12.03 (4Me DMG), 14.12 (CH_3 ester), 50.31 (HC-Co), 60.18 (CH_2 ester), 114.16 ($H_2C=$), 125.12 (C *meta*-pyridine), 137.45 (C *para*-pyridine), 137.87 (HC_γ), 149.73 (C=N DMG), 149.87 (C *ortho*-pyridine), and 173.49 (CO_2 ester).

Trans-1-ethoxycarbonyl-2-cyclopropanecarbinyl(pyridine)-cobaloxime (IIA', Fig. 5): Although attempts to prepare the above compound were repeatedly frustrated by the attendant formation of the but-3-enyl(pyridine)cobaloxime, resulting from the decyclisation of the cyclopropyl moiety, one of the trial runs gave almost pure IIA' (~ 96%). During this successful attempt the reaction was also done in the usual way.

A suspension of bromo(pyridine)cobaloxime (0.996 g, 2.2 mmol) in pyridine (1 cm^3) and ethanol (30 cm^3) contained in a 200 cm^3 Schlenk tube, was stirred and purged with a stream of nitrogen for 45 minutes. $NaBH_4$ (0.152 g, 4 mmol) was added cautiously and the mixture stirred for 10 minutes, during which time it turned dark blue and became homogeneous. *Trans*-1-ethoxycarbonyl-2-(iodomethyl)cyclopropane (0.762 g, 3 mmol) was then added and stirring was continued for only 30 minutes, before ethanol was removed at the pump. The resulting solid was recrystallised from CH_2Cl_2/30-40° petroleum ether overnight. The orange/red crystals thus

obtained were pumped dry at the freeze-drier; yield, 0.1 g (10%).

^{1}H n.m.r. ($CDCl_3$) δ 0.56 (m, 1H), 1.00 (m, 1H), 1.23 (m, 5H), 1.49 (m, 2H), 2.12 (s, 12H), 4.07 (q, 2H), 7.32 (t, 2H), 7.76 (t, 1H), and 8.58 (d, 2H)

ν_{max}(KBr) 3420 (broad, NO-H), 3100-300 (w, ring C-H), 1720 (s, ester C=O), 1608 (m, pyridine C=N), 1566 (s, C=NOH), 1010, 980 (w, ring C-C), 520 (m, Co-N) cm^{-1}

Calculated: C, 48.49; H, 6.10; N, 14.14

Found: C, 47.60; H, 6.02; N, 14.02

(E)-4-Ethoxycarbonylbut-3-enyl(pyridine)cobaloxime (V', Fig. 5): This was also prepared in the usual way using the following quantities of reagents: absolute ethanol (50 cm^3), bromo(pyridine)cobaloxime (0.448 g, 1 mmol), $NaBH_4$ (0.08 g, 2.1 mmol) and (*E*)-ethyl 5-(methanesulphonate)pent-2-enoate (2.5 g, 11.3 mmol). Chromatography and recrystallisation gave the pure product as orange/red crystals.

^{1}H n.m.r. ($CDCl_3$) δ 1.25 (t, 3H), 1.50 (t, 2H, J = 8 Hz), 1.27 (q, 2H), 2.12 (s, 12H), 4.13 (q, 2H), 5.72 (d, 1H, J = 16.4 Hz), 6.87 (dt, 1H, J_{vic} = 6.4 Hz, J_{trans} = 16.4 Hz), 7.32 (t, 2H), 7.73 (t, 1H), and 8.57 (d, 2H)

ν_{max}(KBr) 3420 (broad, NO-H), 3100-3000 (w, =C-H), 1708 (s, ester C=O), 1649 (m, C=CCO_2Et), 1605 (w, pyridine C=N), 1565 (s, C=NOH),

1235, 1092 (s, N-OH), 519 (m, Co-N) cm^{-1}.

Calculated: C, 48.49; H, 6.10; N, 14.14

Found: C, 47.07; H, 5.95; N, 13.74 (in spite of several attempts)

(E)-Ethyl 4-[(pyridine)cobaloxime]but-2-enoate (VI', Fig. 5): This was prepared from (*E*)-ethyl 4-bromobut-2-enoate and Co(I) in the usual way. Chromatography of the product gave the desired cobaloxime in 26% yield.

^1H n.m.r. ($CDCl_3$) δ 1.25 (t, 3H), 2.17 (s, 12H), 2.25 (d, 2H, J = 9.7 Hz), 4.10 (q, 2H), 5.56 (d, 1H, J_{trans} = 15.6 Hz), 6.70 (dt, 1H, J_1 = 9.70 Hz, J_{trans} = 15.6 Hz), 7.32 (t, 2H), 7.75 (t, 1H) and 8.49 (d, 2H).

ν_{max}(KBr) 3450 (broad, NO-H), 3100-3000 (w, =C-H), 1700 (s, ester C=O) 1640 (w, pyridine C=C), 1612 (w, $C=CCO_2Et$, 1602 (w, pyridine C=N), 1560 (s, C=NOH), 1236 and 1092 (s, N-OH), 518 (m, Co-N) cm^{-1}.

(E)-Ethyl 4-[dioxy(pyridine)cobaloxime]pent-2-enoate (D, Scheme 12): Besides being the product from various photolysis experiments, the above alkylperoxycobaloxime was prepared from the corresponding alkyl bromide in the usual way using the following quantities of reagents: absolute ethanol (40 cm^3), bromo(pyridine)cobaloxime (0.448 g, 1 mmol), pyridine (1 cm^3), $NaBH_4$ (0.076 g, 2 mmol), (*E*)-ethyl 4-bromopent-2-enoate (0.207 g, 1 mmol). Subsequent work-up gave a dark brown solid, 0.053 g (11%),

which was shown to be the title compound.

^{1}H n.m.r. ($CDCl_3$) δ 1.00 (d, 3H, J = 7.0 Hz), 1.26 (t, 3H), 2.30 (s, 12H), 3.86 (dq, 1H, J = 7.0 Hz), 5.23 (d, 1H, J_{trans} = 16.0 Hz), 6.73 (dd, 1H, J_1 = 7.0 Hz, J_{trans} = 16.0 Hz), 7.27 (t, 2H), 7.71 (t, 1H), and 8.37 (d, 2H).

ν_{max}(KBr) 3450 (broad, NO-H), 3120-3000 (w, =C-H), 1722 (s, ester C=O), 1658 (m, $C=CCO_2Et$), 1640, 1620 (w, pyridine C=C), 1605 (w, pyridine C=N), 1568 (s, C=NOH), 1242, 1092 (S, N-O), 514 (m, Co-N) cm^{-1}.

λ_{max}^{EtOH} 310 nm (7,500) no peak at ∿ 450 nm ± 30 nm.

$\{^1H\}^{13}C$ n.m.r. ($CDCl_3$) δ 12.30 (4Me DMG), 14.08 (CH_3 ester), 18.19 (H_3C-5), 59.83 (CH_2 ester), 78.05 (Co-OO-CH), 120-80 (=HC_β), 125.05 (C *meta*-pyridine), 138.08 (C *para*-pyridine), 149.78 (HC-3), 150.78 (C *ortho*-pyridine), 151.40 and 151.52 (C=NOH), and 166.30 (CO_2Et).

The above alkylperoxycobaloxime was also obtained from the individual photolyses of cobaloxime I', III' and V' (Fig. 5), and the reaction between Co-H and (*E*)-ethyl penta-2,4-dienoate.

(i) Photolysis Experiments: Typically, a measured quantity of the appropriate alkylcobaloxime was dissolved in either $CDCl_3$ (∿ 0.5 cm^3) or CD_3OD (∿ 0.5 cm^3) and the solution put in a well-stoppered 5 mm n.m.r.

tube. Photolysis was achieved by either exposing the solution to sunlight or to a 150 W electric bulb for varying lengths of time. The reaction progress was monitored at intervals by ^{1}H n.m.r. spectroscopy. At the end of each photolysis experiment, the contents of the tube were chromatographed on a flash silica gel column, (CH_2Cl_2/pyridine/MeOH, 95:1:5). Depending on the reaction time and light intensity some of the starting cobaloxime was, in some instances, isolated unchanged, whereas the alkylperoxycobaloxime was the major product that was identified. In the case where photolysis of the respective alkylcobaloxime was done in $CDCl_3$, a mixture of Cl_2DC-cobaloxime and Cl_2HC-cobaloxime was also isolated as a yellow by-product.

^{1}H n.m.r. ($CDCl_3$) δ 2.22 (s, 12H), 5.79 (s, 1H), 7.33 (t, 2H), 7.76 (t, 1H) and 8.52 (d, 2H).

(ii) The reaction between Co-H and (*E*)-ethyl pent-2,4-dienoate: β-Hydroxy-α-phenethyl(pyridine)cobaloxime (0.16 g, 0.33 mmol), originally prepared from Co(I) and styrene oxide, was weighed into a 5 mm n.m.r. tube together with $CDCl_3$ (∿ 0.6 cm^3). (*E*)-Ethyl penta-2,4-dienoate (0.025 g, 0.198 mmol) was then added to the mixture in the tube and the contents were mixed thoroughly by shaking the tube before running a ^{1}H n.m.r. spectrum. With the tube still in the n.m.r. spectrometer, the probe was then heated to 40° and kept at this temperature for 1 hour while taking ^{1}H n.m.r. spectra of the reaction mixture at regular intervals of 10 minutes. During this

time the peaks of the original diene were seen to disappear while new ones were coming up. Although there was no further substantial change after 1 hour, the temperature of the probe was raised to 45° and kept at this for another hour. The contents of the tube were chromatographed in the usual way on a flash silica gel column. Removing the solvent afforded the alkylperoxycobaloxime, 0.018 g (17%).

(iii) Photolysis of 1-Methoxycarbonylbut-3-enyl(pyridine)-Cobaloxime in the Presence of (*E*)-ethyl Penta-2,4-dienoate: 1-Methoxycarbonylbut-3-enyl(pyridine)cobaloxime (0.121 g, 0.24 mmol) and (*E*)-ethyl penta-2,4-dienoate (0.031 g, 0.24 mmol) were dissolved in $CDCl_3$ (∿ 0.6 cm^3) and put in a stoppered 5 mm n.m.r. tube. The tube was stood in a beaker of water and irradiated with a 150 W electric bulb for 4 hours. The reaction mixture was then emptied on to a flash silica gel column and eluted with CH_2Cl_2/MeOH/pyridine (95:5:1). After removing the solvent the ethyl and methyl ester peroxycobaloxime D (Scheme 12) were obtained unresolved in ∿ 1:1 ratio, in a total yield of 21%. Using a 220 MHz ^1H n.m.r. spectrometer most of the related protons of the two peroxycobaloximes seem to resonate together except for the two olefinic protons; even then the latter are separated by only 3.5 Hz, with those of the methyl ester peroxycobaloxime at higher field.

CHAPTER 4

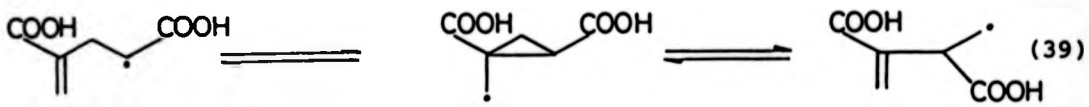
REARRANGEMENTS OF ALKYLCOBALOXIMES

4.0 INTRODUCTION

4.0.1 Mechanisms

Two extreme mechanisms may be postulated for the B_{12}-dependent α-MG rearrangement to MeIT. One involves alkyl radical intermediates[96] rearranging in the absence of cobalt. After the cleavage of the AdoCbl C-Co σ-bond, the resulting adenosyl radical abstracts a hydrogen atom from the substrate (α-MG) producing an α-MG radical. It is this radical that is thought to rearrange *via* a cyclopropylmethyl radical to a MeIT radical. The abstraction of a hydrogen atom from Ad-H by the MeIT radical completes the process (*cf.* Scheme 13).

Scheme 13

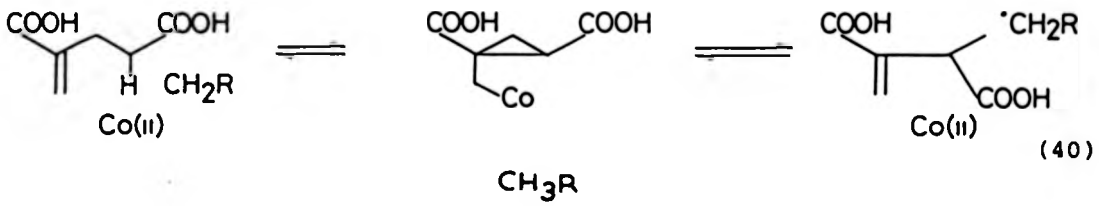


(39)

A second mechanism[97] invokes the involvement of cobalt in the rearrangement process. After the formation of the α-MG radical as above, the radical combines with Cob(II)alamin which is formed in the first step, to give an α-MG-derived organocobalt species. The organocobalt thus formed rearranges in a concerted manner to give MeIT-Cob(III)alamin, (*cf.* Scheme 2).

In contrast, Johnson *et al.*[33] have suggested an ingenious mechanism in which the key step involves the dual action of adenosyl radical and Cob(II)alamin on the substrate (α-MG), giving rise to an intermediate cyclopropylcarbinylcobalamin species (Scheme 14).

Scheme 14



(40)

In this mechanism Cob(II)alamin is responsible for the opening and the closing of the cyclopropyl ring.

Evidence that rearrangement can occur *via* organocobalt intermediates has been furnished by Chemaly and Pratt[98]. They found that when cyclopropylcarbinyl(pyridine)cobaloxime is heated at 60° in

various solvents, it rearranges to but-3-enyl(pyridine)-cobaloxime. The rate of this reaction is independent of both solvent polarity and the presence of oxygen. They inferred that the rearrangement perhaps does not involve charged species. Secondly, the mediation of Co(II) seemed unlikely because if it were involved, it would be oxidised rapidly to Co(III) in the presence of oxygen, hence attenuating the rate of the rearrangement.

4.0.2 Methyl- *versus* Carboxylate-substituted Ligands

The fact that 2-methylcyclopropylcarbinyl(pyridine)-cobaloxime rearranges[48] quite readily to an equilibrium mixture of 1- and 2-methylcyclopropylcarbinyl(pyridine)-cobaloxime if heated or in the presence of acid, could be taken to suggest that carboxylate-substituted cyclopropyl-carbinylcobalt species might be intermediates in the α-MG rearrangement. It should be borne in mind, however, that the observed rearrangement could be a unique property of the methyl- and not the carboxylate-substituted organocobalt complexes. In this respect, the methyl-substituted but-3-enyl- and cyclopropylcarbinylcobaloximes are poorer models for the α-MG-cobalt system, compared to the alkylcobaloximes which have been studied in the present work. This is because the course and products of the rearrangement of organocobalt compounds depend largely on the nature of the alkyl ligand in the α-position; the substitution around the ligand with the attendent electronic effects and steric hindrance do influence

the speed and the process by which it rearranges. The methyl group exhibits an electronic effect (hyperconjugative electron release) opposite to that afforded by -COOR (electron withdrawal *via* inductive or mesomeric effects). α-MG contains two electron-withdrawing carboxylate groups and should be better modelled by the organocobalt compounds which contain at least one carboxylate group, like those studied here.

However, a profound difference may be realised between α-MG acid and model alkyl ligands which contain esterified carboxyl groups. At physiological pH most of the α-MG is ionised, and the ionised $-COO^-$ groups have a different electronic effect from that afforded by the esterified carboxylate groups. This difference is expected to affect the stability of substrate-derived radicals, and to influence the nature of the products. Nevertheless, esters have the advantage of being easy to handle and if necessary, they can be hydrolysed after the formation of the organocobalt complex (*cf.* ref. 85) to the corresponding acid. The organo-ester cobaloximes prepared during this work were studied under various conditions of heat, light, acid and base as described below.

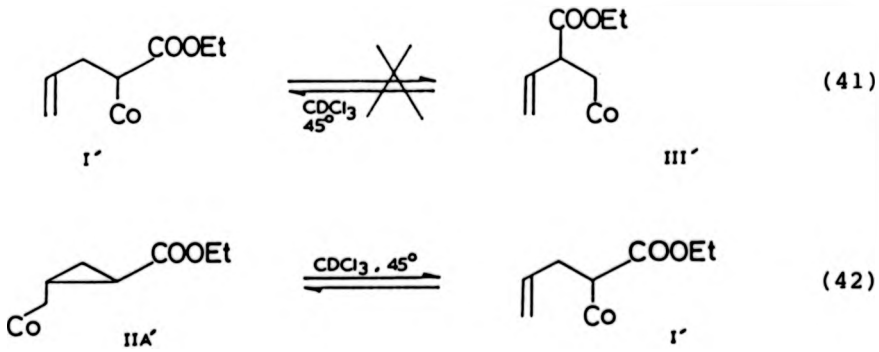
4.1 REARRANGEMENTS

4.1.1 Thermal Rearrangement

Contrary to the previous studies[48] with 1- and 2-methyl-substituted but-3-enylcobaloximes, the

related 1- and 2-carboxylate-substituted but-3-enylcobaloximes neither equilibrated nor rearranged in any way when each was incubated as a solution in $CDCl_3$ at 50° for over 20 hours, in the dark. The only change that was apparent in each case, according to ^1H n.m.r. spectroscopy, was slight decomposition to desalkylcobaloximes and a mixture of organic products.

Scheme 15



When a $CDCl_3$ solution of the cyclopropyl isomer (IIA', eq. 42) was left at room temperature ($\sim 22^{\circ}$) for 3 hours, there was no change in its ^1H n.m.r. spectrum. This was not surprising because unchanged crystals of this compound had been previously obtained by overnight recrystallisation from a mixture of CH_2Cl_2/30-40° petroleum ether. However, when a solution of the same cobaloxime IIA'

in $CDCl_3$ was left in the dark, at room temperature (~ 22°) for 30 hours, there was some conversion into I' according to analysis by ^1H n.m.r. spectroscopy; none of III' was detected. Warming the solution of IIA' at 45° for another hour caused little, but detectable further conversion into I'. Cobaloxime III' was not observed at all in the rearrangement mixture.

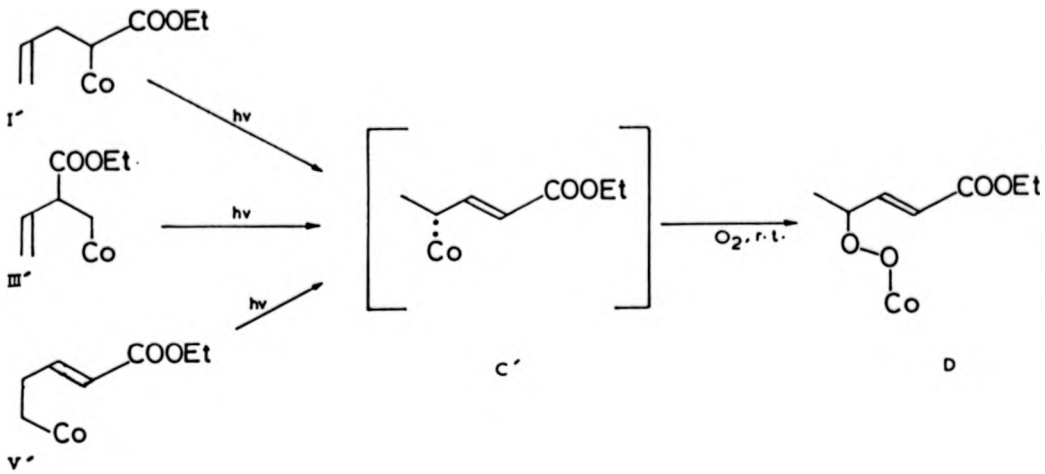
4.1.2 Photolytic Rearrangements

The most conspicuous result from the aerobic photolyses of cobaloximes I', III' and V' was that they all yielded the same alkylperoxycobaloxime D (Scheme 16), besides desalkylcobaloximes and organic by-products. The cobaloximes were photolysed as solutions in either $CDCl_3$ or CD_3OD, using direct bright sunlight or a 150 W electric bulb as described in Chapter 3. The rearrangement progress was monitored by ^1H n.m.r. spectroscopy. No broadening of peaks was observed. The formation of D from III' is slightly different because it involves carbon-skeleton rearrangement before dioxygen insertion, unlike the formation of D from cobaloximes I' or V'.

4.1.3 Base-induced Reactions of Cobaloximes

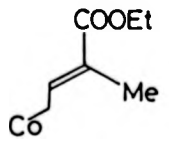
During the preparation of cobaloxime III' from Co(I) and the corresponding alkylmethanesulphonate, it was realised that if the reaction mixture was very basic then very little of the desired compound was isolated.

Scheme 16

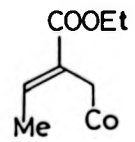


Two other cobaloximes (VII' and VIII') were isolated instead, in varying ratios; from one of the preparations none of III' was identified. One of the two cobaloximes was characterised by ^{1}H n.m.r. spectroscopy as (*E*)-3-ethoxycarbonylbut-2-enyl(pyridine)cobaloxime, (compound VII'). The most unique resonance for this cobaloxime was a singlet at δ 1.33 due to the methyl group and a triplet at δ 6.55 due to the olefinic proton. The second cobaloxime, (compound VIII') was 2-ethoxycarbonylbut-2-enyl(pyridine)cobaloxime. It

had a doublet at δ 1.31 due to the CH_3 group, and a quartet at δ 6.86 due to the olefinic proton. These resonances were most diagnostic regarding the structures of these two cobaloximes.



VII'



VIII'

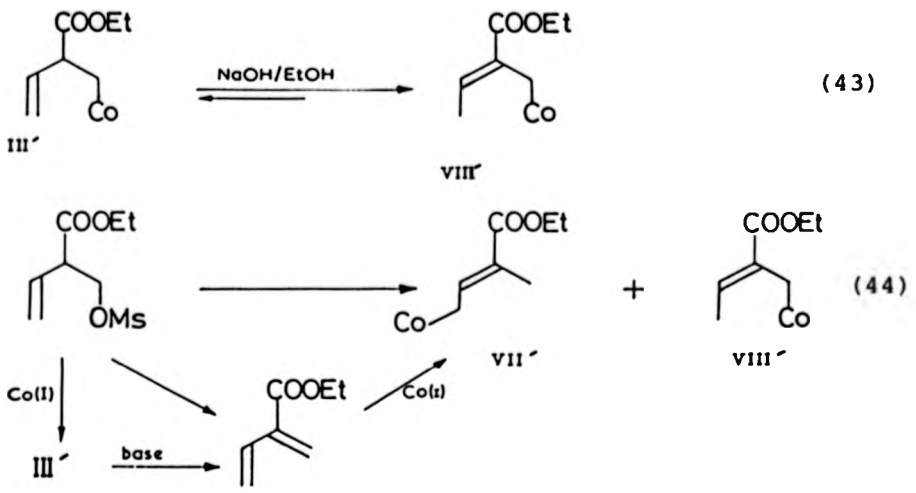
Compound VII' did not show any nuclear Overhauser effect (nOe) between the protons of the $CH_3\dot{C}$=CH- moiety, and therefore it was assigned the (*E*)-configuration.

Adding a pellet of sodium hydroxide to a reaction mixture containing 2-ethoxycarbonylbut-3-enyl-(pyridine)cobaloxime (compound III') (~ 1 mmol), gave a mixture of III' and VIII'. In another experiment, an attempt to make III' from the corresponding alkylmethane-sulphonate and Co(I) was frustrated by the formation of VII' and VIII' instead (Scheme 17).

4.1.4 Acid Rearrangements

There was no observable change in the ^1H n.m.r. spectrum of cobaloxime III' when a solution of it in $CDCl_3$, containing 0.992 M TFA, was left at room temperature for several hours. Fast rearrangement to I' was observed when this solution was warmed up to 40°. After 2½ hours at this temperature the peaks became too broad for any

Scheme 17

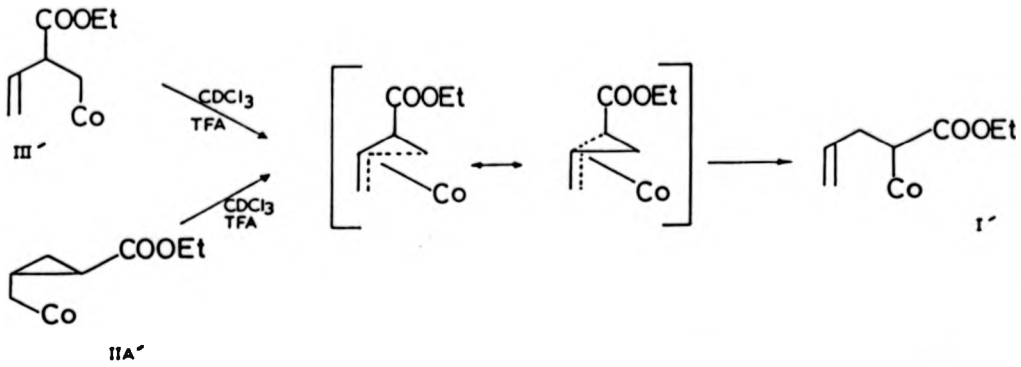


reasonable interpretation to be made. The reaction was stopped and worked up. From this reaction, a mixture of I' and III' (∿ 3:2) was obtained. In another experiment with III'/TFA in $CDCl_3$, which was conducted at room temperature, none of III' was observed in the reaction mixture after 36 hours. Cobaloxime I' did not rearrange at all when it was treated with TFA under similar conditions. However, it did decompose slightly giving rise to desalkylcobalt compounds and organic products. The major organic product was (*E*)-ethyl penta-2,4-dienoate, which was identified by comparing its ^1H n.m.r. spectrum

with that of an authentic sample.

Incubating a 1:1 mixture of I' and IIA' in $CDCl_3$, containing 0.992 M TFA for 36 hours (22^{o}), followed by 6 hours at 45^{o}, totally removed the peaks due to IIA' from the ^{1}H n.m.r. spectrum of the reaction mixture. Cobaloxime I' was isolated from this rearrangement mixture in 58% yield.

Scheme 18



4.1.5 De-cyclisation of the Cyclopropyl Ring in the Preparation of II'

During the preparation of II' (A or B) from Co(I) and the corresponding cyclopropylcarbinyl iodide, cobaloxime I' was inevitably formed as well, in varying ratios. It was clear that I' was originating from the unwanted de-cyclisation of the cyclopropyl ring,

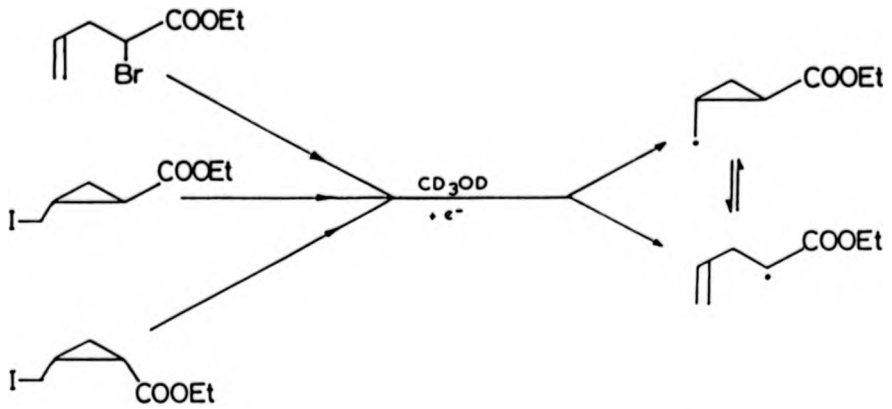
either before or after the formation of cobaloxime II'. This was in spite of carrying out the preparation at various temperatures (e.g., 0^{o} and -80^{o}). The possibility of the alkyl iodide de-cyclising before reacting with Co(I) was ruled out because these cyclopropylcarbinyl iodides are known[99] to stand a variety of conditions without de-cyclising, including bases like aqueous $NaHCO_3$ and KOH, and acids like PCl_3 and 25% H_2SO_4 (aq.).

4.1.6 Equilibration of (2-Ethoxycarbonylcyclopropyl)-methyl and 1-Ethoxycarbonylbut-3-enyl Radicals: E.S.R. Studies

In experiments carried out at the University of Leicester by Professor M. C. R. Symons and Dr. D. N. R. Rao, ethyl 2-bromopent-4-enoate, *cis*- and *trans*-1-ethoxycarbonyl-2-(iodomethyl)cyclopropane were used to generate the corresponding alkyl radicals in CD_3OD by removing the halide atom with a beam of electrons. The three compounds gave the same or very similar e.s.r. spectra. All the spectra showed rapid interconversion between cyclic and acyclic radicals (Scheme 19). (*E*)-Ethyl 4-bromopent-2-enoate gave an allylic radical different from the other three, under the same conditions.

$$CH_3CHBrCH{=}CHCO_2Et \xrightarrow[CD_3OD]{+e^-} CH_3\dot{C}H{-}CH{=}CHCO_2Et + Br^-$$

Scheme 19



4.2 DISCUSSION

4.2.1 Mechanism

It is highly likely that in the thermally-induced rearrangement of IIA' to I', the radical mechanism was followed exclusively. This would be possible, especially after the cleavage of the Co-C σ-bond. The resulting (2-ethoxycarbonylcyclopropyl)methyl radical then partly de-cyclises as was observed in an e.s.r. experiment, to a better-stabilised 1-ethoxycarbonylbut-

3-enyl radical, (*cf.* Scheme 19). The electron-beam method of generating the free radicals seems very attractive because the energy of the resulting alkyl radical can be controlled quite easily, and hence the products. Usually thermally produced alkyl radicals are generated at high temperatures and therefore possess an unknown and substantial amount of internal energy, and the course and products of the rearrangements that may ensue are dependent on that state of the molecule.

It is well established[19,100,101] that the aerobic photolysis of alkylcobalt compounds in acidic or neutral media ultimately gives rise to Co(II) species and organic products. The organic products are derived from alkyl radicals, which are known[100-103] to be intermediates during these photolyses. The nature of the organic products has been used *a posteriori* as proof for the formation of the organic radicals besides the evidence afforded by e.s.r. studies discussed in Chapter 1.

The fact that the proton n.m.r. peaks of the compounds which were photolysed in the present work did not broaden during the experiments, was attributed to the participation of secondary processes in the rearrangements. Co(II) might have been removed by these processes so fast that its concentration at any given time was not sufficient to cause the broadening of ^{1}H n.m.r. spectra peaks. These secondary processes include the recombination of Co(II) and R$^{\bullet}$ and the insertion of dioxygen in the CoII/R$^{\bullet}$ pair. Dioxygen insertion into methyl(pyridine)cobaloxime during photolysis is known[104,105]

to be about 75 times faster than the recombination process. In the case of cobaloximes I' and V' the mechanism is mostly probably similar to that depicted in Scheme 12: β-elimination gives (*E*)-ethyl penta-2,4-dienoate and Co-H, which recombine to give cobaloxime B (Scheme 12). Cobaloxime B then rearranges and inserts dioxygen as outlined in that Scheme to give alkylperoxycobaloxime D. In the case of cobaloxime III' it is thought that the initial process involves homolysis of Co-C bond, followed by cyclisation to give (2-ethoxycarbonylcyclopropyl)-methyl radical and Co(II). The alkyl radical then de-cyclises as was described in the thermal rearrangement experiments, to give 1-ethoxycarbonylbut-3-enyl radical (CH_2=$CHCH_2\dot{C}HCO_2Et$). Elimination of a hydrogen atom from C_β of this radical gives ethyl penta-2,4-dienoate. The latter, together with Co(II), then insert dioxygen by the route of Scheme 12. During the photolysis of III', the corresponding alkyl-peroxycobaloxime resulting from mere oxygen insertion, was not observed at all according to ^1H n.m.r. spectroscopy.

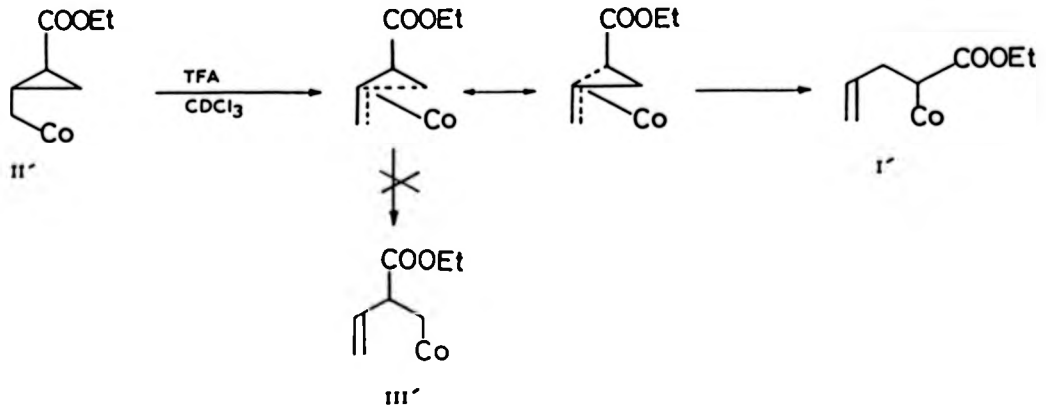
In the base-induced reactions of cobaloxime III', cobaloxime VIII' arose *via* the abstraction of the C_α-H proton by the base, and hence restored conjugation. Alternatively, in a basic medium, the alkylmethanesulphonate underwent elimination to give 2-ethoxycarbonylbuta-1,3-diene. The diene then reacted with Co(I) to give VII' and/or VIII' depending on the double bond that was attacked (Scheme 17).

It has been suggested[16,17] that the TFA-catalysed rearrangement of 1- and 2-methylbut-3-enyl(pyridine)-

cobaloxime proceed *via* a protonated or pentacoordinate and homoallylic transition state or intermediate. This suggestion in effect excludes a free radical mechanism in such a rearrangement, and employs the well-known property of transition metals to coordinate to homoallylic species. It is also an attractive suggestion as far as α-MG enzymic rearrangement is concerned. It is possible to envisage a group on the enzyme acidic enough to remove the 5,6-DMB from the α-position of an α-MG-cobalamin, followed by rearrangement similar to that of the but-3-enylcobaloximes described above. This is the most attractive mechanism that can account for the acid-catalysed rearrangements described earlier for the conversion of III' to I'. Compound II' is possibly an intermediate in this conversion (see Fig. 7).
In the experiments which employed the methyl-substituted analogues of I', II', and III', the equilibrium mixture of those analogues contained more of the analogue of III' (primary cobaloxime), and this was attributed to a steric factor. However, it appears that the stabilisation of I' by hyperconjugation (*cf.* equation 35), more than compensates for its destabilisation by steric hindrance, and hence it preponderates over III' in the rearrangements studied here. This is borne out by the rearrangement (thermal and acid-induced) of IIA', which gave I' and none of III'.

Starting with II' (i.e., the *cis* or the *trans*-isomer), the cyclopropane ring can de-cyclise in two ways in theory, with formation of a terminal double bond and migration of cobalt.

Scheme 20



In practice, however, only one way is possible as shown in Scheme 20. An energy diagram for the acid-catalysed rearrangement of IIA' and III' to I' shown in Figure 7, is proposed on the basis of the observations obtained in this work.

The formation of (*E*)-ethyl penta-2,4-dienoate from I' in the presence of CF_3COOH can be accounted for as shown in equation 45. After the protonation of one of the DMG ligands and removal of pyridine as a pyridinium ion, cobalt remains with a partial positive charge. Abstraction of a proton from C_β-H of the alkyl ligand and elimination of Co(I) species, i.e., $(CF_3COO^-)_2Co(dmgH)(dmgH_2)$, gives the diene.

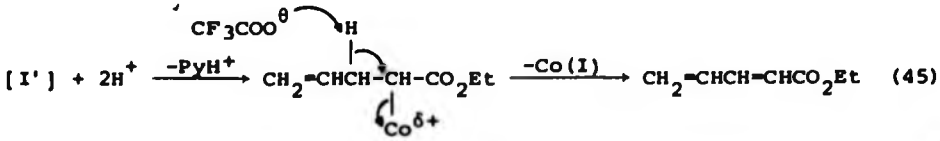
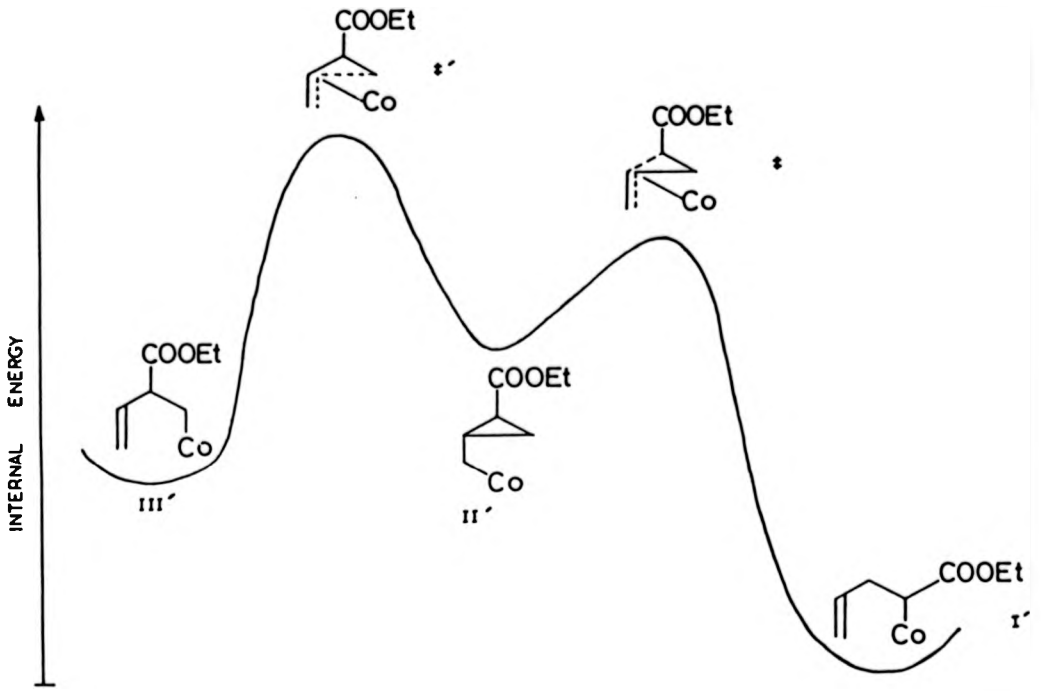


Figure 7

Acid-Catalysed Rearrangement of III' and II'



The cobalt(II) species generated in this way is responsible for the broadening of peaks in the ^{1}H n.m.r. spectrum of the reaction mixture, which was observed.

The real enzymic system may not be as acidic as the conditions in the above experiment, and side-reactions like elimination may be unknown *in vivo*. However, the removal of the base ligand (L_α) (pyridine in the model system and 5,6-DMB in the enzymic system), provides a chance to probe the behaviour of the alkyl ligand in the absence of the base (L_α), and this has been achieved.

The isolation of varying ratios of I'/II' from

the attempted synthesis of II' (*cis* and *trans*) is understood to have arisen from partial de-cyclisation of the cyclopropane ring. This can happen either during or after the formation of II' by:

(1) Co(I) reacting with RX by an electron transfer mechanism (equation 46)[106,107];

(2) Co(II) reacting with RX by an electron transfer mechanism (equation 47)[108];

(3) the catalytic action of Co(II) on II' (equation 48)[33].

Scheme 21

$$Co(I) + RX \rightarrow Co(II) + RX^{\cdot -} \rightarrow Co(II) + R^{\cdot} + X^{-} \rightarrow Co(III)R + X^{-} \quad (46)$$

$$Co(II) + RX \rightarrow X\text{-}Co(III) + R^{\cdot} \xrightarrow{Co(II)} R\text{-}Co(III) + X\text{-}Co(III) \quad (47)$$

(48)

X = iodide

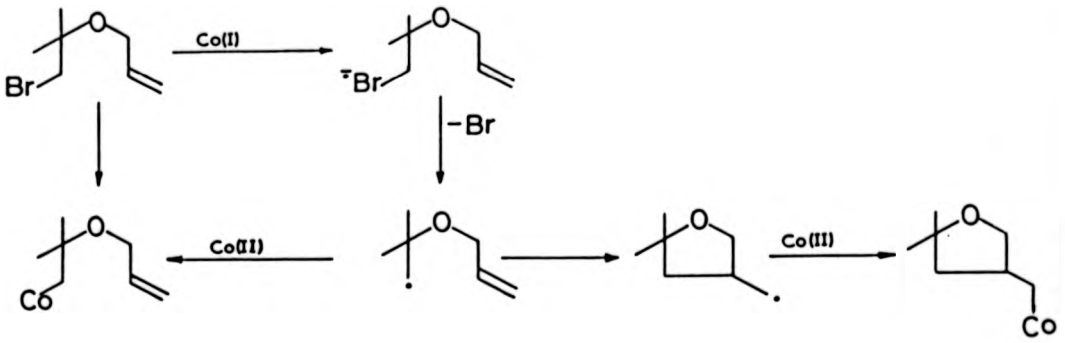
In equations 46 and 47 the alkyl radical R$^{\cdot}$ is the (2-ethoxycarbonylcyclopropyl)methyl radical which can ring-open as already described, to the 1-ethoxycarbonyl-but-3-enyl radical.

The above observations support the results of Schaffler and Rétey[106], and of Tada *et al.*[107] that electron transfer reactions are very important during

the reaction of Co(I) and alkyl bromides or iodides, and this is in sharp contrast to the generally accepted S_N2 mechanism[81].

Tada and Okabe[107] employed the cyclisation of the allyloxyethyl radicals as proof for the intermediary role of radicals in the reactions between Co(I) and RX (X = Br, I) (*cf.* Scheme 22).

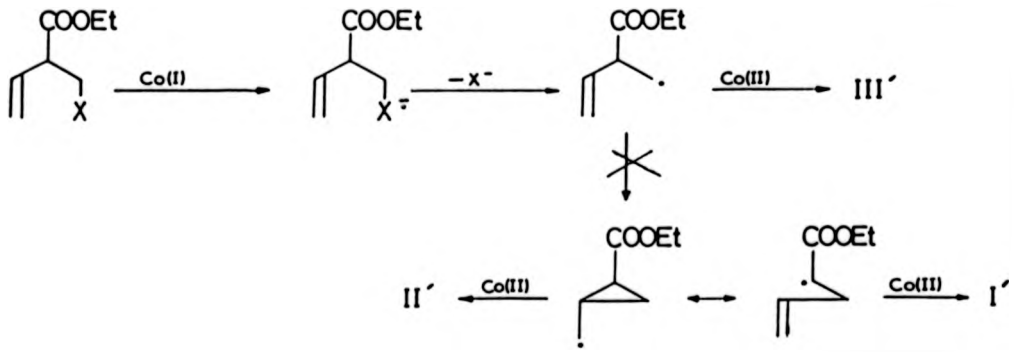
Scheme 22



This electron transfer mechanism is exceptional because during the present work cobaloxime I' (or II') was not observed in the preparation of cobaloxime III', using Co(I) and the corresponding alkyl iodide, (*cf.* Scheme 23). However, it is realised that the combination of the preformed alkyl radical, 2-ethoxy-carbonylbut-3-enyl with Co(II), could be much faster

than its cyclisation to the (2-ethoxycarbonylcyclopropyl)-methyl radical, hence the subsequent formation of I' and II' is negligible (Scheme 23). In the light of this, the reaction of Co(I) with RX by the conventional S_N2 mechanism, and the subsequent de-cyclisation by the catalytic action of Co(II) (see Scheme 21) appears to be the most attractive mechanism for the formation of cobaloxime I' in the attempted synthesis of II'.

Scheme 23



During the attempted synthesis of II', Co(II) could arise from partial reduction of the halo(pyridine)-cobaloxime [i.e. Co(III)]: Co(III) → Co(II) → Co(I). The first step is fast, but the second one can be as long as 10 minutes, to go to completion. Co(II) could also

arise from the oxidation of preformed Co(I) by traces of aerial oxygen in the inert gas atmosphere in the reaction vessel. Therefore, its concentration [Co(II)], and hence the I'/II' ratio, in the reaction mixture would vary with the amount of $NaBH_4$ used, the time allowed for the reduction and the purity of the inert gas in the reaction vessel; the variation in the I'/II' ratio was observed from various trial runs.

4.3 CONCLUSION

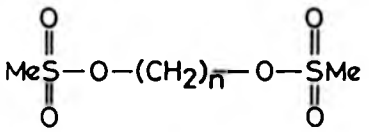
From the observations above it appears that alkylcobalamins which were postulated[12,7] as intermediates in the reaction catalysed by α-methyleneglutarate mutase (Scheme 2) would be insufficiently reactive towards rearrangement. However, rapidly interconverting ethoxycarbonyl-substituted but-3-enyl and cyclopropyl radicals are plausible intermediates in the formation of I' from II'. This supports a mechanism for α-methyleneglutarate mutase *via* intermediate carboxy-substituted but-3-enyl and cyclopropylmethyl radicals[12,109].

CHAPTER 5

CYCLIC ANALOGUES OF BUSULPHAN

5.0 INTRODUCTION

Busulphan is the common name given to butane-1,4-diol dimethanesulphonate, which is used clinically in the treatment of chronic myleroid leukemia. Busulphan is also known[110] as 1,4-dimethanesulphonyloxybutane, myleran or GT-41. It belongs to a group of bifunctional alkylating agents which exhibit antitumour activity[111] to varying extents.



n = 4, busulfan

These diesters are known to exert a markedly more powerful cytotoxic action than the corresponding monofunctional alkylating agents. Their usefulness as drugs depends on their specific cytotoxicity and this property varies dramatically on going from n = 1 to n = 10. Busulphan (n = 4) is of particular interest because of its specific activity against the myleroid leukemia cells and its ether/water solubility[112], which is well-balanced to allow efficient distribution of this drug in the body. The other members of the homologous series have also been used chemotherapeutically, when and where

they are suited for the purpose.

These dimethanesulphonate (DMS) esters are easily synthesised from the corresponding 1,ω-alkanediols, using $MeSO_2Cl/Et_3N$, as already described. Because they possess two good leaving groups per molecule, they are all effective alkylating agents, able to employ one or both of these groups in their interaction with nucleophiles including biological macromolecules.

5.1 MODE OF ACTION OF ALKYL-DMS ESTERS *in vivo*

5.1.1 Cross-linking

Many alkylating agents that are used as anticancer drugs owe their curative power to their ability to alkylate macromolecules at nucleophilic sites with a degree of specificity. By so doing (e.g. in the case where the macromolecule is DNA) busulphan is believed to kill off the malignant cells of myleroid leukemia. Alkylation of the DNA strand(s) causes breaks in these strands and interferes with replication. The powerful cytotoxic action of these difunctional alkylating agents was attributed to their ability to cross-link fibrous macromolecules, particularly those involved in the production of chromosomes[113,114]. A reaction of this type had originally been suggested[115] by Elmore, Gulland, Jordan and Taylor for the mustard gas ($ClCH_2CH_2SCH_2CH_2Cl$) and DNA interaction. There are numerous other examples of this kind in literature.

In their recent work[112], Fox and Bedford have tried to correlate cross-linking and cytotoxicity both *in vivo* and *in vitro*, for the busulphan homologous series. They first demonstrated that all dimethane-sulphonic acid esters with n = 1-10 are capable of forming interstrand cross-links with DNA, excepting n = 2. Then they investigated cross-linking *versus* cytotoxicity and cross-linking *versus* specific cytotoxicity (antitumour activity). In both cases they found a rather random relationship between the variables, throughout the homologous series.

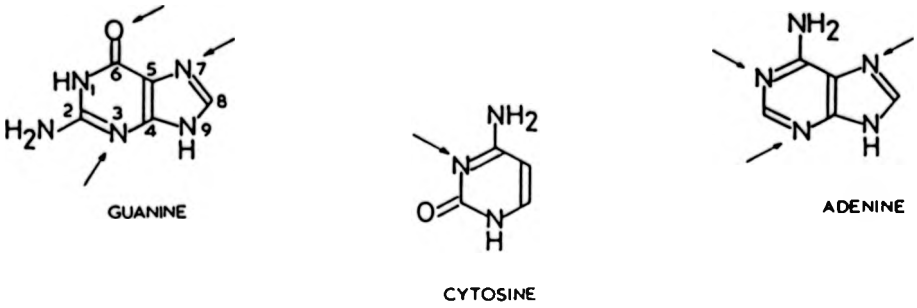
It can be inferred from these results that the number of cross-links is not solely responsible for the detrimental effect on the cells (normal or cancerous), but that other factors such as the nature of cross-links (e.g. the way the alkylating agent binds and possibly the position at which it binds) may play a significant part. This may be illustrated by referring to the case of hexane-DMS which yielded six times more cross-links than busulphan, yet it is over twenty times as cytotoxic as the latter[112].

There is evidence that the primary target of busulphan in the body is DNA, particularly at the N-7 of guanine. Experimental results in support of this have been obtained[116-119] by various research groups. Although it is less likely, the nucleophilic centres of pyrimidines, purines, proteins, etc., can also be alkylated. Ludlum and Tong[120] have demonstrated this alkylation of DNA by busulphan. They isolated and characterised a compound which they have shown

to contain a 1,ω-di(7-guanine)butane moiety.

Figure 7

Sites of Alkylation in DNA

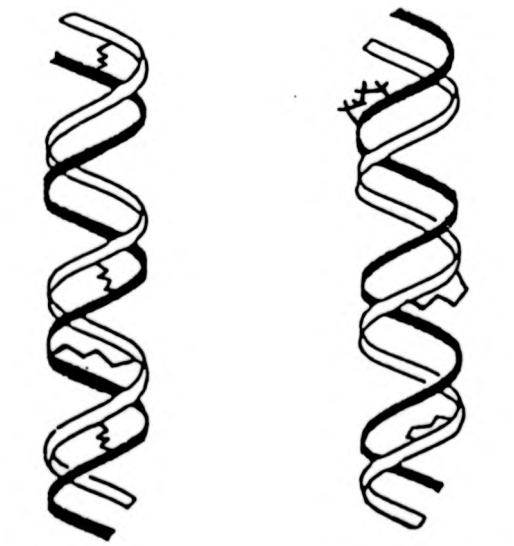


Ross[121] classified alkylating agents according to whether they react with nucleophiles by an S_N1 or S_N2 mechanism; busulphan falls in the latter group. Reacting by this mechanism, alkylating agents (difunctional) are capable of alkylating DNA to form either interstrand or intrastrand cross-links. Originally, only intrastrand cross-links were known[122,123] for busulphan, but in their recent communication, Fox and Bedford[112] have reported interstrand cross-linking for busulphan as well.

These recent results seem to dispense with the original explanation as to why busulphan could not cross-link the two DNA strands. It was thought that the distance between the nucleophilic centres in the two strands was too wide for the butane chain (~ 4.62 Å, stretched conformation) to span. However, to account for the newly observed interstrand cross-linking, it was suggested that one end of busulphan chain reacts with O-6 guanine on one strand and the other end reacts with

N-4 cytosine of the second strand. This looks plausible as the distances involved are only 5.6 Å (wide groove) or 4.3 Å (narrow groove). Intrastrand cross-linking of guanine, whereby busulphan employs both of its functional groups to alkylate one strand, cannot be overlooked on the account of steric hindrance. However, this would require the butane chain to assume a less probable non-extended conformation.

Figure 8



Cross-linking of DNA

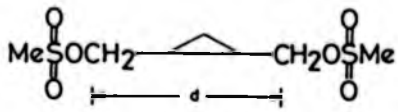
An extra carbon atom in a homologue of these alkyl-DMS esters can mean a concomitant change in several of its properties like steric hindrance, oil/water solubility, the distance (d) the alkyl chain can span, etc. Therefore, it becomes difficult to attribute variation in antitumour activity to one particular variable. The best comparison can be made by altering only one variable, while keeping constant as many of the other variables as possible. This was attempted during the present work by using the *cis*- and the *trans*-isomers of one compound that is an analogue of busulphan.

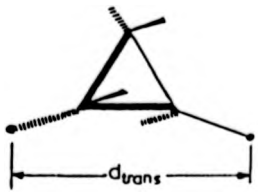
5.2 *Cis*- and *trans*-1,2-Di(hydroxymethyl)cyclopropane Dimethanesulphonate

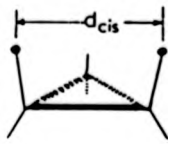
These two isomers are cyclic analogues of busulphan. They have a four-carbon chain similar to that of busulphan, except that this time it is held in a rigid conformation by a cyclopropane ring. The rigidity in turn limits the distance (d) the alkyl moiety can span during its interaction with DNA. This distance is shorter for the *cis*-isomer than the *trans*-isomer.

It was hoped that by studying the interaction of these isomers with macromolecules *in vivo*, it would be possible to learn something about the relationship between antitumour activity and chain length, while keeping the rest of the variables constant. However, by changing from the *trans*- to the *cis*-isomer, it was noticed that besides the change in the distance (d),

Figure 9







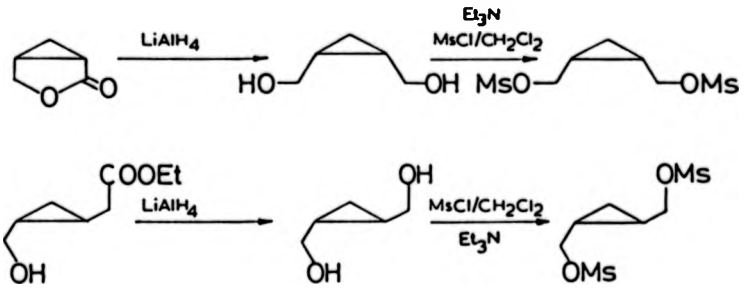
the steric hindrance against nucleophilic attack was altered as well.

These cyclic analogues of busulphan were prepared in the usual way by the reaction of $MeSO_2Cl/Et_3N$ and the corresponding diols. The diols had been prepared from the reduction of 3-oxabicyclo(3,1,0)hexan-2-one and *trans*-1-(hydroxymethyl)-2-methoxycarbonylcyclopropane, as described in Chapter 3.

5.3 RESULTS AND DISCUSSION

Surprisingly, *in vivo* tests, which were done by B. W. Fox in Manchester, showed that these cyclic analogues of busulphan were not as active against Yoshida tumour cells as was expected on the account of their close

Scheme 24



resemblance to busulphan and its homologues. They showed excessive gastrointestinal toxicity - the *trans*-isomer more than the *cis*-isomer, but both showed a little antitumour activity near the toxic dose. In an earlier *in vitro* experiment carried out by the same researchers, it could not be established whether these cyclic analogues formed any links with DNA, even when the sensitive alkaline elution technique was used.

These results indicate that the distance (d) is not the only limiting factor that determines antitumour activity (specific cytotoxicity) or cytotoxicity in general. If (d) were the limiting factor then the toxicity of these cyclic analogues would be closely related to that of n = 3-5 because they have about the same chain length. The differential toxicity observed between the two cyclic analogues themselves, was attributed

to difference in reactivity as dictated by steric hindrance.

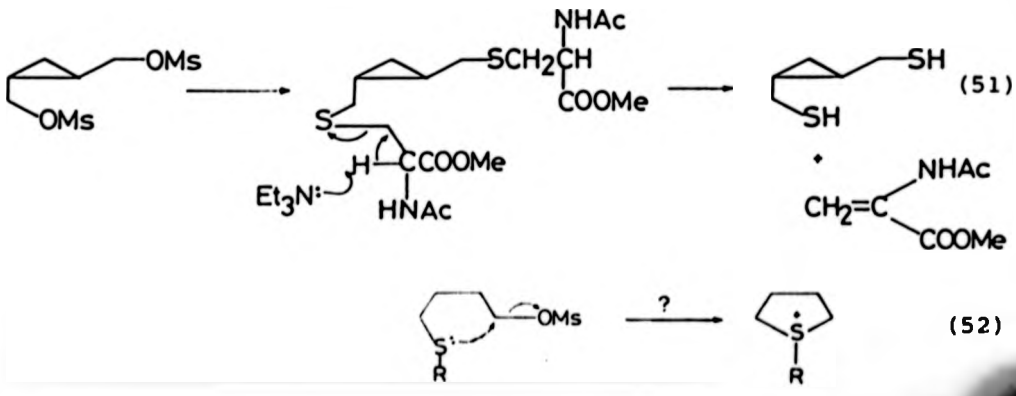
5.3.1 Mechanism of Alkylation

As was mentioned earlier, busulphan most likely reacts with nucleophilic centres *in vivo* by the S_N2 mechanism. Some of these nucleophilic centres include sulphydryl groups of amino acids like cystein.

In another experiment, this mechanism was probed by reacting N-acetyl-l-cysteine methyl ester with busulphan and the cyclic analogues, in the presence of Et_3N and in $DMSO/D_2O$. There was no evidence for the formation of the expected sulphonium salt from either busulphan or the cyclic analogues. There was, however, satisfactory evidence for the formation of tetrahydrothiophene, when the experiment was done in $DMSO/D_2O$ (analysis by ^1H n.m.r. spectroscopy). 2-(N-Acetylamino)acrylate was the product in all the cases where Et_3N was used as base.

A possible mechanism for the formation of 2-(N-acetylamino)acrylate is shown below in equation 51.

Scheme 25



(51)

(52)

On the other hand, an S_N1 mechanism would entail the solvolysis of the DMS compounds to carbonium ions. The existence of the carbonium ions (and hence the S_N1 mechanism) would be betrayed by the concomitant de-cyclisation of the cyclopropyl ring to give either a four- or five-membered ring and/or an olefin[124,125]. None of such compounds was observed using ^1H n.m.r. spectroscopy. This was taken to augment the view that in the presence of nucleophiles, busulphan and its analogues will react by the S_N2 mechanism.

5.4 EXPERIMENTAL

Cis-1,2-di(hydroxymethyl)cyclopropane: 3-Oxabicyclo(3,1,0)-hexan-2-one (0.99 g, 10 mmol) and $LiAlH_4$ (0.74 g, 19.5 mmol), in dry ether (50 cm^3), were stirred together for 33 hours at room temperature. A ground mixture of $Na_2SO_4.10H_2O$ and Celite ($\sim$ 1:1) was added carefully to the reaction mixture with stirring, before the reaction mixture was filtered. The resulting Na_2SO_4/Celite cake was washed several times with ether, before further extraction in a Soxhlet continuous extractor apparatus, for 6 hours. Removing the solvent gave the pure diol, 0.79 g (77 %).

^1H n.m.r. (CCl_4): δ 0.15 (q, 1H, J = 6.0 Hz), 0.74 (m, 1H), 1.24 (m, 2H), 3.16 (t, 2H, J = 11 Hz), 3.94 (dd, 2H, J_1 = 5.0 Hz, J_2 = 11.0 Hz), and 4.57 (s, 2H)

Trans-1,2-di(hydroxymethyl)cyclopropane: was made by reducing *trans*-1-hydroxymethyl-2-methoxycarbonylcyclopropane

with a six-fold excess of $LiAlH_4$ in ether. The work-up was carried out as above. The diol was isolated in 88% yield. (Lit. b.p. 112-113°/3 mmHg)[126-128].

^1H n.m.r. ($CDCl_3$) δ 0.42 (t, 2H, J = 6.0 Hz), 0.99 (m, 2H), 3.50 (t, 2H, J = 12.0 Hz) 3.78 (dd, 2H, J_1= 6.0Hz, J_2 = 12.0 Hz), 4.05 (broad, 2H), and 4.95 (broad, 1H).

Cis-1,2-di(hydroxymethyl)cyclopropane dimethanesulphonate: was prepared according to the literature method in reference 79, from the *cis*-diol. It was then recrystallised from CH_2Cl_2/30-40° petroleum ether to give white crystals. m.p. 71°, decomposes to a black mass at 125°.

^1H n.m.r. ($CDCl_3$) δ 0.55 (dt, 1H, J_{gem} = 6.1 Hz, J_{trans} = 5.4 Hz), 1.10 (dt, 1H, J_{gem} = 6.1 Hz, J_{cis} = 8.6 Hz), 1.55 (m, 2H), 3.05 (s, 6H), 4.12 (dd, 2H, J_{gem} = 5.9 Hz, J_{vic} = 8.8 Hz), and 4.46 (dd, 2H, J_{gem} = 5.9 Hz, J_{vic}= 10.8 Hz)

$\{^1H\}^{13}C$ n.m.r. ($CDCl_3$) δ 9.34 (ring $-CH_2-$), 15.61 (ring, 2CH-), 37.82 ($2CH_2O-$), and 69.76 ($2CH_3S-$)[128].

Calculated for $C_7H_4O_6S_2$ C, 32.55; H, 5.46; S, 24.83

Found C, 32.57; H, 5.52; S, 23.29

Trans-1,2-di(hydroxymethyl)cyclopropane dimethanesulphonate: was also prepared as in reference 79, and was recrystallised twice from CH_2Cl_2/30-40° petroleum ether for analysis.

m.p. 68-69°, decomposes to a black mass at 125°.

Lit. m.p. 60-62° with decomposition[126].

^{1}H n.m.r. ($CDCl_3$) δ 0.82 (t, 2H, J = 6.8 Hz), 1.36 (m, 2H), 3.04 (s, 6H), 4.07 (dd, 2H, J_{vic} = 7.8 Hz, J_{gem} = 9.7 Hz), 4.19 (dd, 2H, J_{vic} = 6.4 Hz, J_{gem} = 9.7 Hz).

$\{^1H\}^{13}C$ n.m.r. ($CDCl_3$) δ 9.60 (ring $-CH_2-$), 16.91 (ring 2CH-), 37.89 ($2CH_2O-$), and 72.63 ($2CH_3S-$)[129].

C.i.m.s., m/z 276 $(M + NH_4)^+$ 100%, 180 $(M + NH_4 - MeSO_3H)^+$ 10%, 163 $(M - MeSO_3^-)$ 29%, 84 $(M - 2MeSO_3H + NH_4)^+$ 9%, 67 $(M - 2MeSO_3H + H)^+$.

Calculated for $C_7H_4O_6S_2$	C, 32.55;	H, 5.46;	S, 24.83
Found	C, 32.50;	H, 5.56;	S, 22.38

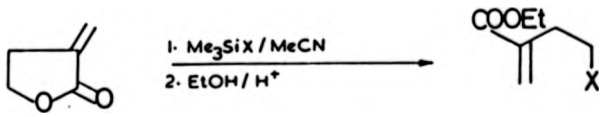
FUTURE WORK

ALKYLATING AGENTS SYNTHESIS PROPOSALS

(i) Monocarboxylate-Substituted Series

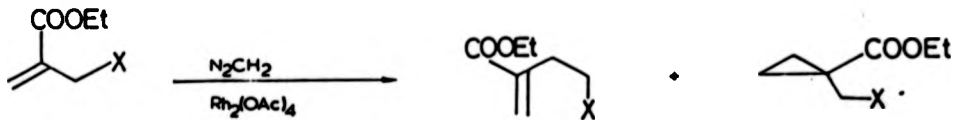
The alkylating agents which have been synthesised and used to make alkylcobaloximes in the present work are but part of a group of compounds related to α-MG and the intermediates involved in its enzymic rearrangement. Two other members of this series of alkylating agents are 3-ethoxycarbonylbut-3-enyl halide (A, Scheme 26), and 1-ethoxycarbonyl-1-(halomethyl)cyclopropane (B, Scheme 26). Possible routes to these compounds are suggested here below.

Scheme 26



A (53)

(*cf.* refs. 53, 130, 131)

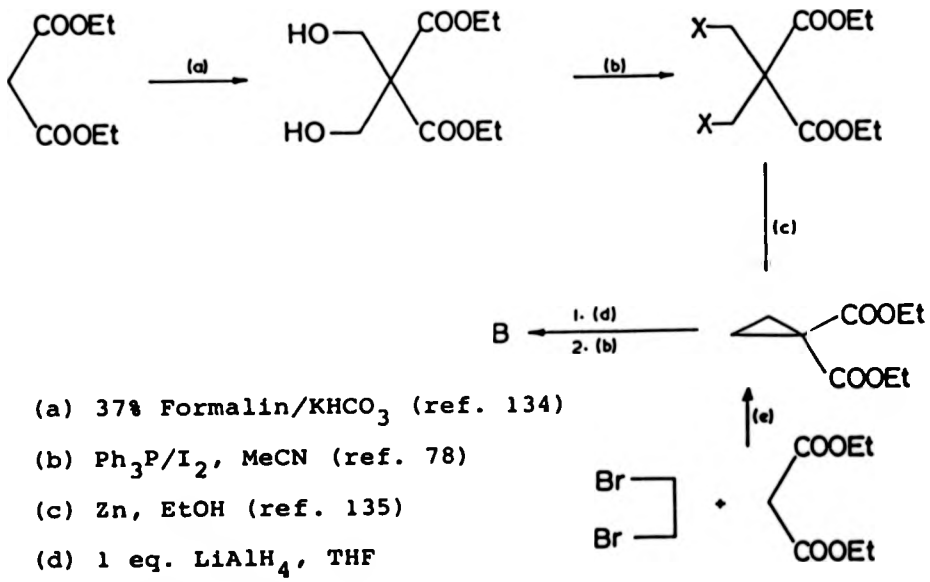


A B (54)

(*cf.* ref. 132)

The cleavage of esters and lactones by Me_3SiX is well-documented in the literature[53]. The starting material, α-methylene-γ-butyrolactone is commercially available. The reaction of diazomethane with bromomethacrylate has precedent in the reactions of EDA with allyl halides and acetate described in Chapter 3, whereas 2-(bromomethyl)prop-2-enoate is obtainable in good yield from a recent procedure[133]. Compounds A and B thus prepared would be separated by distillation on a spinning-band column. Another possible method for preparing B is *via* a series of well-known reactions as shown in Scheme 27.

Scheme 27



(a) 37% Formalin/$KHCO_3$ (ref. 134)

(b) Ph_3P/I_2, MeCN (ref. 78)

(c) Zn, EtOH (ref. 135)

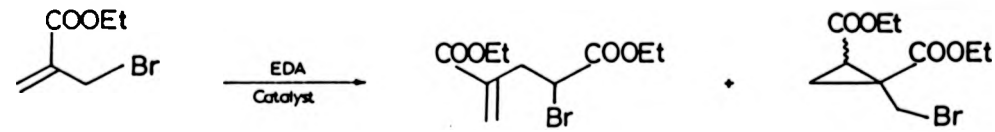
(d) 1 eq. $LiAlH_4$, THF

(e) Na/EtOH (ref. 136).

(ii) Dicarboxylate-Substituted Series

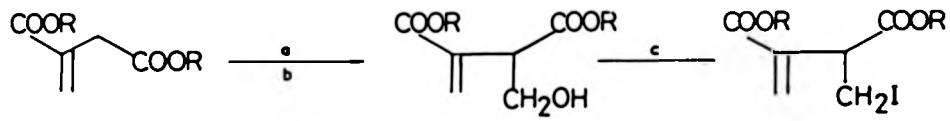
Besides α-MG-Cbl and MeIT-Cbl, 1,2-dihydroxycarbonyl cyclopropylmethylcobalamin has been postulated as an intermediate in the actual α-MG enzymic rearrangement. The alkyl ligands needed to synthesise these intermediates *in vitro* can be prepared as shown in the Scheme below. The starting materials in each case, i.e.

Scheme 28



(55)

(*cf.* ref. 137)



(56)

(a) N-bromosuccinimide/CCl_4
(b) Zn dust/Et_2O, H_2CO(g)
(c) Me_3SiX/MeCN, EtOH/H^+

] or LDA/HMPA, H_2CO

2-(Bromomethyl)prop-2-enoate and itaconic acid, are readily obtainable; the latter is cheaply available commercially. There is a choice between whether to use the diester of itaconic acid or the acid itself. If the diester is employed in the reaction of Scheme 28, then hydroxymethylation will be followed by spontaneous lactonisation to give β-ethoxycarbonyl-α-methylene-γ-butyrolactone. This would mean using Me_3SiX to cleave the lactone (and the ester) afterwards, prior to final re-esterification. On the other hand, using the acid as in the Scheme means that 2/3 of all the LDA used will be wasted making the dilithium salt of the acid.

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