THE STRUCTURE AND PROPERTIES OF DICOUMAROL AND RELATED COMPOUNDS

A thesis submitted to the University of Warwick in part fulfilment of the requirements for the degree of Doctor of Philosophy

by

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-1968-

PREFACE

The work described in this thesis was carried out in the School of Molecular Sciences, the University of Warwick, during the period of October 1965 to April 1968. Except where otherwise stated, the author's original work is described.

The author wishes to thank Dr. D.W. Hutchinson, who directed this work, and also Professor V.M. Clark for his helpful suggestions.

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INTRODUCTION

Coumarin derivatives as Anticoagulants.

Dicoumarol was first prepared in 1903, by the reaction of formaldehyde and 4-hydroxycoumarin[1]. Little interest was taken in the compound, however, until 1940, when Link[2] identified it as the haemorrhagic agent in the spoiled sweet clover disease of cattle. Link[3] investigated the properties of the anticoagulant in rabbits, along with a great many other derivatives, in an attempt to find a relationship between structure and activity for this type of compound. Although he had little success in his researches as far as structural relationships were concerned, he discovered other useful anticoagulants, such as warfarin, pelantan and macoumar (3-(α-phenyl-β-acetylethyl)-4-hydroxycoumarin, ethyl glyoxylate dicoumarol and 3-(1'-phenylpropyl)-4-hydroxycoumarin respectively).

According to the accepted theory of blood coagulation[4,5] prothrombin is converted to thrombin under the influence of calcium ions and thromboplastin (effectively an enzyme for the process). The thrombin thus produced reacts with fibrinogen to produce the fibrin of coagulated blood. This process is inhibited by dicoumarol. The study of this phenomenon has relied on the analysis of blood and blood serum obtained from animals fed on diets containing these anticoagulants. Examples of the types of analysis involved are found in refs. 6-8.

The factors produced (or lacking) in the blood of the treated animals are given names (such as Factor VII and the Christmas factor) as the chemical constitution of the factors is unknown[5].

A subject that has received considerable attention is the relationship of coumarin anticoagulants to the natural and synthetic forms of vitamin K[9]. It is well known that vitamin K deficiency in mammals gives rise to hypothrombinaemia, and the resulting haemorrhagic condition bears a very close resemblance to that which is produced by the administration of coumarin anticoagulants. Furthermore, the haemorrhagia caused by coumarins is rapidly cured by treatment of the animal with vitamin K_1, (2-methyl-3-phytyl-1,4-naphthaquinone), suggesting that there exists between the coumarin and the vitamin an antagonism resembling that observed by Woods [10,11] between sulphonamides and p-aminobenzoic acid, and having a similar origin, namely a structural similarity between the coumarin and the essential body metabolite vitamin K. Since vitamin K is required for the synthesis of prothrombin by the liver, the coumarin anticoagulants may act as competitive inhibitors which prevent the utilization of the vitamin by one of the enzymes involved in this synthesis [12,13].

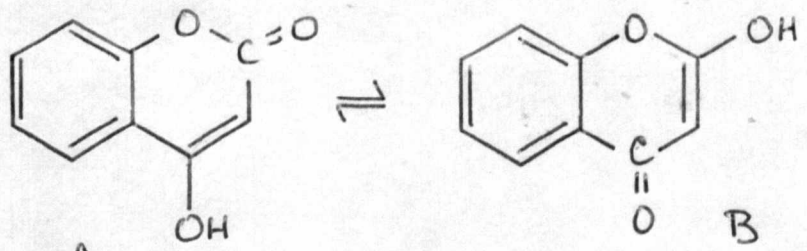
In addition to papers dealing with the clinical use of coumarin anticoagulants, many papers have been published as the result of preparations of new coumarin derivatives and their testing for anticoagulant activity. (See for instance refs. 14-17)

A number of suggestions as to structure reactivity relationships have been made, but no satisfactory formulation has ever been presented. It seems that much more will have to be known about the intimate mechanism of the blood clotting process before any advances can be made along these lines. (For a general review of the field see ref. 5.)

Structure of 4-hydroxycoumarin derivatives.

4-hydroxycoumarin

The structure of 4-hydroxycoumarin has been well established. Arndt [18] has shown that in solutions of 4-hydroxycoumarin, the 4-hydroxycoumarin form (diagram IA) is in equilibrium with the 2-hydroxychromone form (diagram IB)



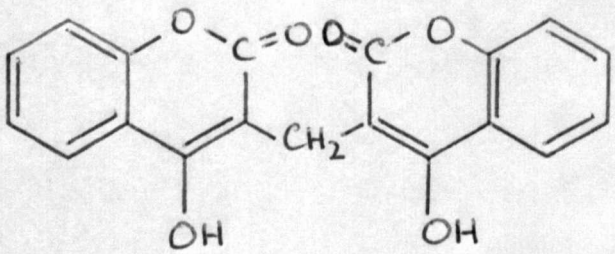
I

Rapid methylation with diazomethane produces predominately 4-methoxycoumarin, whereas in the case of a slow addition of diazomethane a mixture is formed in which the content of 2-methoxychromone is higher than in the former case. The authors conclude that in the equilibrium state the 4-hydroxycoumarin structure predominates. The formation of the chromone derivative during slow methylation is explained by the fact that

2-hydroxychromone is a more acid tautomer, and consequently it is methylated more easily. Although the concentration of the chromone tautomer in the mixture is low, the equilibrium upset by the removal of one component is restored only slowly. The possibility of the contribution of the diketo structure (2,4-diketochromone) has been ruled out by the work of Link[19], who showed by titration that an alcoholic solution of 4-hydroxycoumarin is almost 100% enol. (Assuming a possible rate of enol-keto tautomerisation similar to that shown by ethyl acetoacetate[20].)

Structure of dicoumarols and related work.

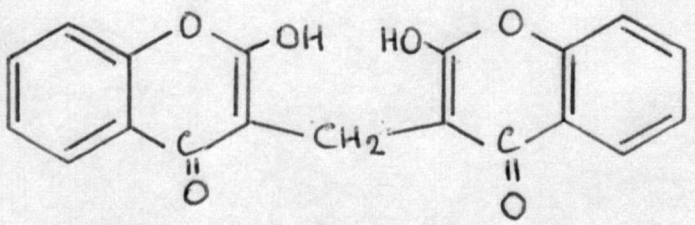
Link did not investigate the tautomerism of dicoumarol, and wrote the structure in the logical coumarin form below[21]. (diagram II)



II

Following on from Link's work Knobloch and co-workers [22,23] investigated the various tautomeric possibilities by comparing the i.r. and u.v. spectra of dicoumarol derivatives and related compounds. In the paper by Knobloch Kakac and Macha[22] they put forward the hypothesis that dicoumarols exist very

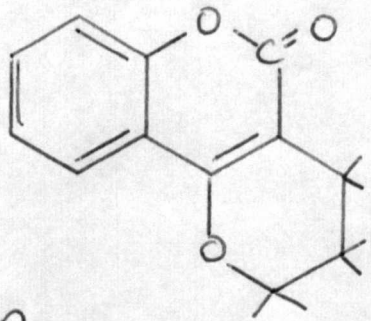
largely in the chromone form. This conclusion is drawn from the observation that peaks in the u.v. spectra of dicoumarols may be ascribed to absorptions characteristic of both chromone and coumarin, and that the pKa_1 value of pelantan (ethyl glyoxalate dicoumarol) is approximately 2.7 in 20% ethanol. The latter point, they say, proves the existence of a hydroxychromone group, which is more acidic than a 4-hydroxycoumarin group (they give the pKa value of 4-hydroxycoumarin in the same solvent as 4.4.) The later paper by Knobloch and Prochazka[23] takes into account the i.r. spectra of these derivatives. They show that the maximum absorption in the carbonyl region of the i.r. (νmax C=0) for dicoumarol is 1660 cm^{-1}, and 1653 cm^{-1} for chromone (spectra in ethylene dichloride). On the other hand νmax C=0 for simple coumarin derivatives is between 1700 and 1720 cm^{-1}. They come to the conclusion that dicoumarol exists in the dichromone structure below. (Diagram III)



III

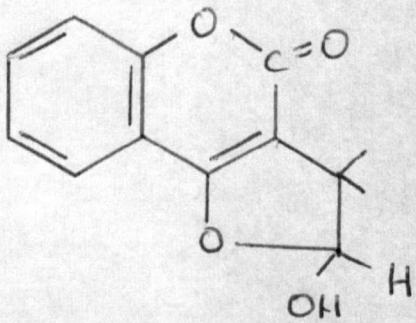
They also suggest from the O-H stretch area of i.r. spectrum that the chromonyl hydroxyl groups are hydrogen bonded intramolecularly to the chromonyl carbonyl groups of the other ring.

Chmielewska[24] has discussed the tautomerism of dicoumarol, and puts forward the suggestion that it exists as half coumarin and half chromone. The basis for this suggestion was the view that anticoagulant activity could be correlated with vitamin K antagonism and the active form of a coumarin anticoagulant must be a cyclic ketal as in diagram IV, A or B



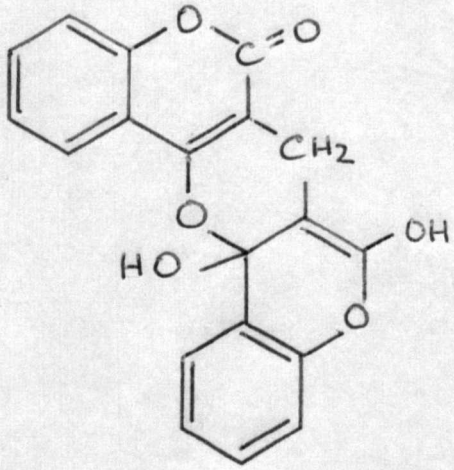
A

IV



B

These can arise only from 3-substituted 4-hydroxycoumarins in which the side chain has a carbonyl or a potential carbonyl in position 2' or 3'. The half chromone half coumarin structure of dicoumarol would lead to the derived ketal in diagram V.



V

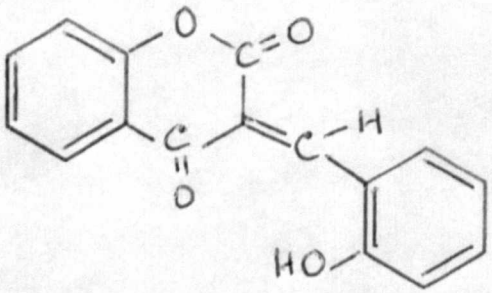
Chmielewska investigates the methylation reactions of dicoumarols, and the methylation of the 4-hydroxycoumarin and dicoumarol analogues 4-hydroxy-6-methyl-α-pyrone and 3,3-methylene-bis-4-hydroxy-6-methyl-α-pyrone. The i.r. spectra of the 4,4'- and 2,4'-dimethyl ethers of dicoumarols in carbon tetrachloride are given, along with the spectrum of 2-methoxychromone in this solvent. Whereas 2-methoxychromone has a band attributable to the carbonyl group at 1659 cm^{-1}, the 4,2'-dimethyl ether does not have a band in this area, and Chmielewska says that no explanation can be offered for this behaviour. Following this work some of the properties of 4-hydroxy-6-methyl-α-pyrones have been investigated (4-hydroxy-6-methyl-α-pyrone is readily available from dehydracetic acid, an ethyl aceto-acetate derivative.) The absence of the 1659 cm^{-1} band from the spectra of the 4,2'-dimethyl ethers is also investigated.

The above work on the structure of dicoumarol is unconvincing. The authors conclusions from limited data seem highly questionable, and in consequence this investigation was re-opened with the additional advantage readily available from n.m.r. spectroscopy.

Structure of other 4-hydroxycoumarin derivatives.

Link[21] prepared a whole range of bridge substituted dicoumarol derivatives, by treating 4-hydroxycoumarin with a variety of aldehydes, including salicylaldehyde. He discovered that if salicylaldehyde and 4-hydroxycoumarin were boiled under reflux

for a short time, (about 10 minutes), then, on cooling, pale yellow needles were precipitated from solution. If boiling was continued for a period of hours, then a colourless product was precipitated. On the basis of elemental analysis, he identified the yellow compound as 3-o-hydroxybenzylidene-2,4-diketochroman (diagram VI)



VI

The colourless derivative he identified as the product of the addition of 4-hydroxycoumarin to the benzylidene carbon atom of the above compound. This Michael addition to the double bond is followed by loss of water between the hydroxyl group of one of the 4-hydroxycoumarin residues, and the hydroxyl group of the salicylaldehyde residue. Link concluded that all additions of 4-hydroxycoumarin to aldehydes proceed by this route, first addition of 4-hydroxycoumarin to the carbonyl group of the aldehyde through the carbanion, followed by loss of water to give a diketochroman, and subsequent Michael addition of another molecule of 4-hydroxycoumarin.

An investigation was carried out into the unusual class

of compounds characterised by the diketochroman above, and many other such derivatives were prepared. Their reactions were studied and the spectra of these compounds along with those of their derivatives have led to a rationalisation of their structure. Similar derivatives were prepared using the 4-hydroxycoumarin analogue 4-hydroxy-6-methyl-α-pyrone.

Another route for the preparation of 4-hydroxycoumarin derivatives investigated by Link was the Michael addition of 4-hydroxycoumarin to the double bond of an αβ unsaturated ketone[25]. (The first preparation of warfarin was in fact by the addition of 4-hydroxycoumarin to benzylacetone.) One such reaction investigated by Link was the addition of 4-hydroxycoumarin to mesityl oxide[26]. Two products were obtained from this reaction, one of which, an acidic product, seemed to be the normal Michael addition product, and the other, a comparatively low melting point solid soluble in heptane, had an analysis which showed it to be a condensation product of 4-hydroxycoumarin and mesityl oxide. The structures of these two products were re-investigated, both by spectroscopy on the compounds themselves, and in the case of the heptane soluble compound, spectroscopy of the reduction product as well.

Hydrogen bonding and I.R. spectra.

In molecules containing hydroxyl groups hydrogen bonded to carbonyl groups, two features may be observed in the i.r. spectra attributable to the hydrogen bonding. The frequency of the

O-H stretch will be lower and the absorption band broader, and the stretching frequency of the carbonyl group to which the hydroxyl proton is hydrogen bonded will also be reduced. These points are well illustrated in the i.r. spectra of the carboxylic acid dimers, which have been extensively studied by Bratoz, Hadzi and Sheppard.[27] Farmer[28] has made a thorough study of the i.r. spectra of substituted deuterated and non-deuterated 4-hydroxycoumarin derivatives, from which he concludes that all 4-hydroxycoumarin derivatives are predominately in the coumarin form. The results in this thesis are in agreement with his findings (see Discussion Section) Analysis of i.r. spectra are made with reference to the work by Bellamy.[29]

Hydrogen Bonding and n.m.r. spectra.

One of the first applications of n.m.r. spectroscopy was to hydrogen bonding, in a study of the hydroxyl proton chemical shifts of alcohols (30-32) and phenols [33] in carbon tetrachloride solution. The results showed that the greater the concentration of alcohol or phenol the lower the chemical shifts of the hydroxyl protons. The amount of hydrogen bonding will increase with the concentration, and will result in a sharing of the electron of the hydrogen-bonded proton with another oxygen atom (apart from the one to which it is bonded already), with a resulting deshielding of the nucleus. Analysis of n.m.r. spectra are based on the works by Roberts;[34] and Pople, Schneider and Bernstein.[35]

Tautomerism and n.m.r. spectra.

When a compound is a liquid, or dissolves in a suitable solvent to give an equilibrium mixture of two or more tautomers, n.m.r. spectroscopy may provide an excellent method for studying this tautomerism. The most widely studied compound of this type has been acetylacetone. In the spectrum of pure liquid acetylacetone it is possible to distinguish between peaks of both enol and keto tautomers, and obtain an estimation of the percentages of these forms present at equilibrium from the integrated areas of the peaks. The chemical shift of the hydrogen bonded hydroxyl proton of the enol form (-3.6τ) was at the time the lowest recorded proton chemical shift.[36]

The spectrum of dimedone has been recorded by Cortes,[37] and the spectrum of 1,3 cyclohexanedione extensively studied by Cyr and Reeves.[38] From a study of the n.m.r. spectrum of 1,3 cyclohexanedione over a range of concentrations in chloroform solution Cyr and Reeves have formulated an equilibrium for this compound between 3 species, the keto form, the enol form and an enol dimer. (Unlike acetylacetone, this compound is sterically unable to form intramolecular hydrogen bonds). They found that as the concentration of 1,3 cyclohexanedione increased, the chemical shift of the hydroxyl proton decreased, this decrease being a measure of the increase in dimer concentration. By extrapolating the graph of the reciprocal of the square root of the 1,3 cyclohexanedione concentration against the chemical

shift of the hydroxyl proton (which was found empirically to give a straight line) they determined the chemical shift of the hydroxyl proton at infinite concentration. The value they obtained (-2.32τ) was presumed to be the chemical shift of the hydroxyl protons of the dimer. Study of the n.m.r. spectrum of this compound at different temperatures has also allowed these workers to estimate the equilibrium constants for the interconversion of the species present in chloroform solution.

Other Studies on the Tautomerism of Dimedone.

The i.r. and u.v. spectra of dimedone and many of its simple derivatives (such as enol ethers) have been studied by numerous authors.[39-42] Their researches have shown that in hydroxylic solvents, such as water and alcohol, dimedone exists as almost 100% enol. In less polar solvents (such as dioxan and chloroform) appreciable amounts of keto form are in equilibrium with the enol. In chloroform solution, for example, a doublet of bands in the i.r. at 1710 cm^{-1} is attributable to the carbonyl groups of the keto form, and a broad band at 1600 cm^{-1} is attributable to the carbonyl group and double bond of the enol form.

Bellamy[43] has investigated the i.r. spectrum of formaldehyde dimethone, and deduces an intramolecularly hydrogen bonded structure analogous to the one propounded in the Discussion Section on the basis of n.m.r. spectra.

Hydrogen bonding and pKa values.

a) Monobasic Acids.

The presence of a hydrogen bond between a dissociating proton and another group in a molecule has been put forward on several occasions to explain anomalously high pKa values. Sprengling[44] has accurately determined the pKa values of o- m- and p- substituted hydroxymethyl phenols, and has shown that the pKa values of the o- substituted derivatives are some 0.12 pKa units higher than would be expected on the grounds of inductive effects. Arnold[45] has studied the pKa values of various 2- substituted 1- naphthols, as compared with the comparable 4- substituted compounds, and concludes that chelation results in acid weakening in the case of 2- substitution. The difficulty involved in this kind of study is that changing a parameter to include possible hydrogen bonding necessitates change in other parameters (such as inductive, mesomeric and steric effects, and solvation energies of ions) and it is usually extremely difficult to differentiate between the effects. The effects produced by hydrogen bonding in mono-anions, described below, are more clearly defined, however.

b) Dibasic Acids.

The ratio of the first to the second dissociation constant of a symmetrical dicarboxylic acid is always greater than 4 (the statistical factor) and approaches 4 as the distance between the carboxyl groups increases[46] e.g. in the acids $(CH_2)_n(COOH)_2$, where n=1 the ratio of the dissociation constants (r) = 800;

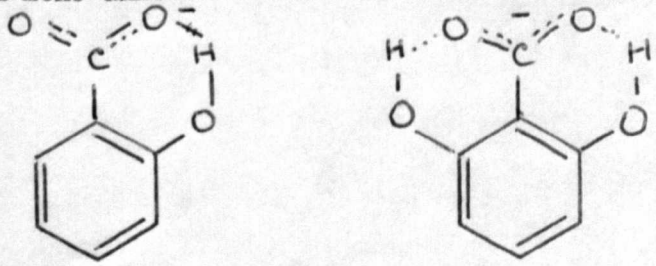
n = 2, r = 25; n = 4, r = 13; n = 7, r = 11.[47]

Kirkwood and Westheimer [48,49] have developed an electrostatic theory to give pKa_1 to pKa_2 ratios of these acids in terms of the interprotonic distance, and an effective dielectric constant which is a function of the size and shape of the molecule. Eberson[50] has concluded from calculations on some simple short chain dicarboxylic acids (including succinic acids and maleic acid) that the Kirkwood and Westheimer treatment alone is insufficient to explain the high ratios of the first and second dissociation constants, but that together with the assumption of an intramolecularly hydrogen bonded mono-anion the theory is in satisfactory agreement with experimental data.

The pKa values of maleic acid are 2.22 and 8.82, as compared with fumaric acid (4.37 and 6.19). From Shahat's crystallographic work[51] it has been shown that the O-H-O distance in crystalline maleic acid is rather short, 2.46A°. This distance is fairly close to that which would be expected for a symmetrical hydrogen bond,[52] but nevertheless the bond distances in the two carboxyl groups show quite conclusively that the hydrogen atom is more firmly associated with one than the other. Orgel[53] concludes from the i.r. spectra of maleic acid and its mono-potassium salt that hydrogen bonding in the mono-anion is probably symmetrical. A parallel conclusion is reached for phthalic acid and its mono-anion. Similar work by Eberson[54] on the i.r. spectra of some substituted succinic acids has shown that hydrogen bonding occurs both in the neutral molecule and in

the mono-anion. Further evidence for such a structure is found on comparing the strengths of the half esters of dicarboxylic acids with the strengths of the corresponding free acids. There is considerable evidence that the polar effect of a carbalkoxy group must be very similar to that of a carboxyl group.[55] Consequently, in the absence of specific interactions between the two functional groups, it would be expected that the ratio of the first ionization constants of the dicarboxylic acid to that for the monoester of that acid should have the value 2, corresponding to the statistical value for the dicarboxylic acid.[46] A number of acids show this behaviour, e.g. fumaric acid (2.0), succinic acid (2.12), and malonic acid (3.6). Other acids for which hydrogen bonding has been proposed exhibit considerably larger ratios, maleic acid (10.6), tetramethyl succinic acid (27), and diethyl malonic acid (32).

Hydrogen bonding of carboxylate anions to the protons of phenolic hydroxyl groups in substituted benzoic acids may also have a dramatic effect on pKa values. The pKa values of salicylic acid are 3.0 and 13.3,[56] the related 2,6-dihydroxybenzoic acid is a stronger acid (pKa 2.30) comparable to phosphoric acid.[57] The hydrogen bonded mono-anions of these acids are shown in diagram VII



VII

Dicoumarols in Oxidative Phosphorylation.

The first investigation on this class of compounds was conducted by Martius and Nitz-Litzow,[58,59] who were interested in the influence of vitamin K in oxidative phosphorylation, and knew of the antivitamin K properties of the coumarin anticoagulants. They found significant reductions in the P:O ratios of respiring rat liver mitochondria in the presence of small quantities of the anticoagulants (in the order of 10^{-5} molar). A rough correspondence was found between the known effectiveness of the anticoagulant, and its activity in terms of the concentration necessary to have a significant effect on the P:O ratios. Dicoumarol (the most active anticoagulant) was effective at the lowest concentration; at a concentration of 5×10^{-6} molar the P:O ratio was found to be 0.89, as compared with a control experiment of 2.36 (62% uncoupling on this basis). The inhibitory effect of dicoumarols was also noted, in terms of a slowing down of the respiration rate, but no quantitative assessment was made of this property. No mechanism of action was put forward by these workers.

Mechanism of Uncoupling.

Although the actual mechanism of coupling of mitochondrial electron transport to the energy conservation reactions leading to the synthesis of ATP is not known, a generally accepted concept is available in terms of chemical reactions. It is generally believed that during the oxidation and reduction of a respiratory

chain carrier (A), the carrier interacts with an unknown component (I) to form a high energy compound ($A \sim I$). This high energy compound reacts, by an unknown sequence of reaction, with ADP and P_i to form ATP. This sequence may be represented by the equations 1-3 below, where B is the electron carrier which accepts electrons from AH_2. The uncoupler (U) is then supposed to act by breaking down $A \sim I$ as in equation 4, by binding with I (reversibly, as in equation 5).

$$AH_2 + B + I = A \sim I + BH_2 \qquad (1)$$

$$A \sim I + P_i = A + I \sim P \qquad (2)$$

$$I \sim P + ADP = I + ATP \qquad (3)$$

$$A \sim I + U = A + U - I \qquad (4)$$

$$U - I = U + I \qquad (5)$$

Evidence for this type of sequence may be found in the work of Chance [60,61], who studied the state of oxidation of respiratory chain carriers spectrophotometrically in the presence of ADP and uncouplers.

Mechanism of Inhibition.

Although it has been known for some time that uncouplers of oxidative phosphorylation (including dicoumarol) inhibit mitochondrial respiration, little attention has been paid to this process until recently (within the last two years). This is at least partly attributable to the complexity of the uncoupler effect on mitochondrial respiration and other mitochondrial enzyme activities, such as ATPase. Hemker,[62] working on nitrophenols,

has proposed that in high concentrations the uncouplers bind I (equation 5) to such an extent that it is no longer available for reaction in equation 1. However, it has been shown that the inhibitory effect of uncouplers may be relieved by greater substrate concentration,[63] which Wilson has shown to be opposed to the above mechanism.[64] (According to this mechanism, at low substrate concentrations in state 3, where ADP and P_i are in excess, there would be a larger steady-state concentration of I and the addition of uncoupler would have little effect on the respiration. At high substrate concentrations the greater electron flux would increase AH_2 concentration, and the binding of I by the uncoupler would have a more pronounced effect. This is contrary to the above observation). Wilson[64] has studied the effect of succinate concentrations on the inhibition of succinate oxidation at a fixed concentration of uncoupler, and shown from Lineweaver-Burk plots that competitive inhibition occurs between substrate and uncoupler, probably at the primary dehydrogenase stage. The Lineweaver-Burk plots, though straight lines, do not have the correct slope for simple competitive inhibition, and Wilson concludes that the competitive inhibition is of a more complex nature, possibly occurring at more than one site. Support for the proposed interaction of uncoupler and dehydrogenase is found in the work of Jurtshuk,[65] who observed an inhibition of α-hydroxybutyric dehydrogenase by dicoumarol and 2,4-dinitrophenol.

Wilson has compared the uncoupling and inhibiting strengths of a series of uncouplers in terms of concentration, and has found that there is no relationship between the two effects on this basis.

Van Dam[66] has investigated the effect of dicoumarol on the respiration of rat liver mitochondria, both in the presence and absence of ADP (State 3 and State 4 respiration respectively). The response to dicoumarol in State 4 is analogous to the type of response found in this work, the rate of respiration passes through a maximum at 5×10^{-6} molar dicoumarol, and the concentration required for half inhibition is 1.5×10^{-5} molar. Van Dam studies the state of oxidation of the respiratory chain carriers in State 3, and finds that in the presence of sufficient dicoumarol to cause inhibition, complete oxidation of the chain has occurred. This is further evidence for the proposed influence of the uncoupler at the level of the interaction of the substrate with the primary dehydrogenase. Any binding that occurs is reversible, as has been shown by the addition of bovine serum albumin to inhibited mitochondria, which leeches out the dicoumarol by binding on to the albumin and restores the respiratory control of the mitochondria.[67]

In this work a whole range of coumarin derivatives was taken, and their activity studied at a fixed substrate concentration, in order to obtain detailed information on reactivity relationships among this class of uncouplers.

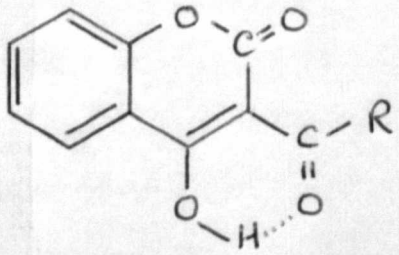
DISCUSSION SECTION

Hydrogen Bonding and Spectra.

Chemical Shifts of Hydroxyl Protons.

As mentioned in the introduction, the chemical shift of protons has been used as a criterion of whether or not they are hydrogen bonded. A simple comparison between the chemical shift of a hydrogen bonded and a non hydrogen bonded proton is found on comparison of the n.m.r. spectra of o and p-vanillin. The chemical shift of the hydroxyl proton in o-vanillin is -0.77τ, and does not vary on dilution of the solution. The chemical shift of the hydroxyl proton in p-vanillin is 2.78τ in a 1.3 molar solution, and changes to 3.45τ on ten-fold dilution. In the case of the o-vanillin, the hydrogen bonding is intramolecular, between the proton of the hydroxyl group, and the adjacent carbonyl group, and consequently dilution does not affect the degree of hydrogen bonding. In the case of p-vanillin, on the other hand, the hydrogen bonding is intermolecular, and dilution reduces the amount of hydrogen bonding as seen by an increase in the chemical shift of the proton of the hydroxyl group. Other carbonyl compounds of this sort in which intramolecular hydrogen bonds are formed as 6-membered rings are as follows, methyl salicylate (τ_{OH}, - 1.24), salicylaldehyde (τ_{OH}, - 1.19), methyl acetyl-5-bromosalicylate (τ_{OH}, - 0.41), 1-formyl-2-hydroxynaphthalene (τ_{OH}, - 3.20). 2, 3-dihydroxybenzaldehyde has both an intramolecularly hydrogen bonded proton (τ, - 0.3) and a hydroxyl proton for which no such bonding is possible (τ, 3.6).

Two compounds of the acetylacetone type which have been prepared are 3-formyl-4-hydroxycoumarin (where R=H in diagram VIII)



VIII

and 3-acetyl-4-hydroxycoumarin, (where R = CH_3.) The chemical shifts of the hydroxyl protons are - 2.00 and - 8.29τ respectively. The difference in chemical shifts may be explained, in part at least, by the +I effect of the methyl group increasing the negative charge of the acetyl carbonyl group (and hence the strength of the hydrogen bond) in the 3-acetyl as compared with the 3-formyl-4-hydroxycoumarin. Similar structures and low chemical shifts have been encountered in 2-formyldimedone (τ_{OH}, - 5.60) and dehydracetic acid (τ_{OH}, - 7.01). The n.m.r. spectra of tricarbonyl compounds of this type are discussed in detail by Merenyi and Nilsson[68].

Effect of hydrogen bonding on i.r. spectra.

The effect of hydrogen bonding discussed most fully here is its influence on the i.r. frequencies of carbonyl groups. The i.r. frequency of the carbonyl group of o-vanillin in a molar solution in chloroform is 1658 cm^{-1}, that of p-vanillin under the same condition is 1686 cm^{-1}, some 28 cm^{-1} higher. Similar changes in frequency have been observed for many of the compounds discussed here. The influence of hydrogen bonding on the O-H stretching

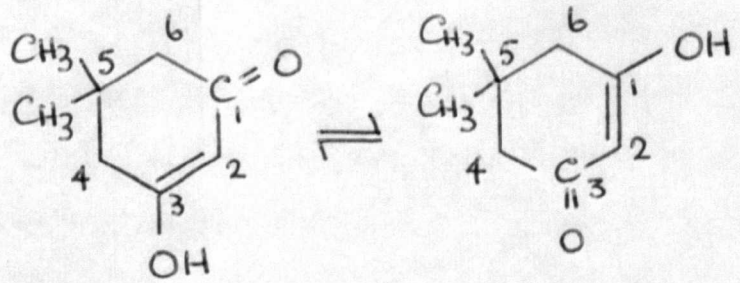
frequency has not been found of great value in structural elucidation, (accordingly only frequencies of strong bands in the area associated with carbonyl stretching frequencies, 1550 - 1800 cm^{-1}, have been recorded in detail in the experimental section). Bis-derivatives (e.g. dicoumarols, dimethones, etc.) all show a medium band at ca. 3100 cm^{-1}, with subsidiary maxima at ca. 2760 and 2630 cm^{-1}, indicative of strong hydrogen bonding.[27] The simple 3-substituted 4-hydroxycoumarins also show these bands, but under conditions in which their i.r. spectra can be recorded (i.e. in the solid state, as a Nujol mull, or in dioxan or ethanol solution) they may form strong intermolecular hydrogen bonds, either with themselves or with the solvent.

N.m.r. Spectra of Dimedone Derivatives.

a) Dimedone

The i.r. spectrum of dimedone in chloroform shows the presence of both enol and keto tautomers, though there is no way of measuring accurately the percentage of these forms present in the solution. The n.m.r. spectrum of a solution of dimedone in chloroform is, however, quite simple, (see experimental section) and allows an estimate of the percentages of the tautomers by comparison of the areas of the signals. The most suitable

signals for this purpose were found to be those of the 4- and 6-methylene groups of the enol and keto forms. (Although the 4- and 6-methylene groups of the enol form are formally different, one being adjacent to a carbonyl group, and the other being adjacent to an enol hydroxyl group, the chemical shifts of the 4- and 6-methylene protons are identical. This is on account of the rapid exchange, which may be written diagramatically as below. (IX)



IX

Several values of the percentages of keto and enol present are given in the experimental section. It is seen that even in a saturated solution in chloroform there is a predominance of the keto form.

The changes in the n.m.r. spectrum of dimedone in deuterochloroform solution over a range of concentrations may (as in Cyr and Reeves work with 1,3 cyclohexanedione) be interpreted in terms of an equilibrium between keto, enol and enol dimers. It was found empirically that a straight line plot was obtained if the reciprocal of the concentration was plotted against the chemical shift of the hydroxyl proton (diagram XL)

The value of the chemical shift at infinite concentration is -2.24τ, presumably the chemical shift of the protons of the enol dimer. This value compares with the value of -1.31τ for the hydroxyl protons of formaldehyde dimethone given below. (Cyr and Reeves value for the dimer of 1,3 cyclohexanedione (-2.32τ) compares with the value of -1.64τ found in this work for the chemical shifts of the hydroxyl protons of 2,2'-methylene-bis-1,3-cyclohexanedione.) The analysis of the n.m.r. spectrum of dimedone by the Mexican workers is at variance with the analysis presented here. They ascribe the signal of the 4- and 6-methylene protons of the enol form to the hydroxyl proton, and do not find any signal corresponding with the one found here for the hydroxyl proton. How they arrived at their conclusions is not clear.

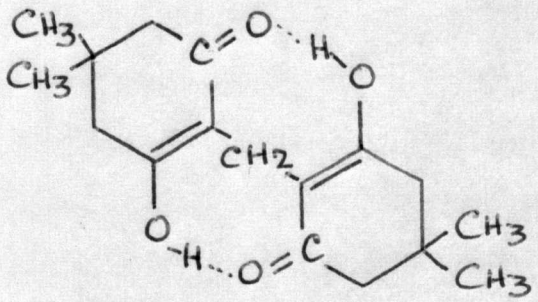
N.m.r. spectrum of dimedone in other solvents.

The solvents used for this investigation were not deuterated. In consequence it was impossible to observe the signals of the methylene groups of the enol and keto forms of dimedone, and estimations of the percentage of tautomers were made by integrations of the signals of the 5-methyl groups of the enol and keto forms. The results showed that in acetonitrile the percentage of enol is a little lower than at the same concentration in chloroform, and in dioxan very much higher. According to studies on the i.r. spectrum of dimedone in dioxan[69], dimedone forms good hydrogen bonds with dioxan, a result that is borne out by this investigation. In sodium deuteroxide solution it is

possible to follow the slow exchange of the vinyl proton, by integration of the spectrum after a known time. The exchange was found to be first order, $K_{30^\circ} = 1.15 \times 10^{-4}$ sec^{-1}.

N.m.r. spectra of dimethones.

The spectra of formaldehyde dimethone shows that in this compound both dimedone rings are in the enol form. There is a unique chemical shift for the 4- and 6-methylene protons at 7.74τ, corresponding with the 7.70τ value for the 4- and 6-methylene groups of dimedone enol. The hydroxyl protons have a chemical shift of -1.31τ, which does not vary as the solution is diluted. The conclusion drawn from this is that formaldehyde dimethone exists in chloroform solution as the intramolecularly hydrogen bonded structure in diagram X



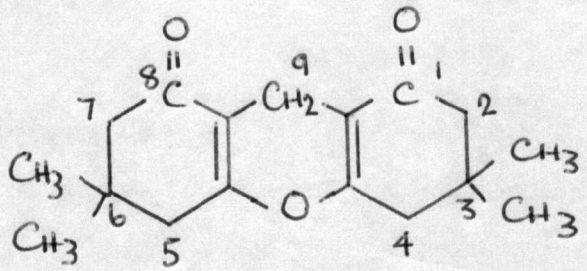
X

The substitution of one of the protons in the methylene bridge by a phenyl group is seen to affect the hydroxyl proton chemical shifts as now two signals are found in this area, one at -1.76τ, and the other at -0.57τ. Substitution of one of the methylene protons by a methyl group has an even more dramatic effect, the signals being found at -2.76τ and a very broad signal centred

roughly on 0.4τ . The effect of the substituent is presumably to interfere sterically with the hydrogen bonding, and the symmetry of the molecule.

N.m.r. spectra of dimethone anhydrides.

In dimethone anhydrides the chemical shifts of the protons of the methylene groups corresponding with the 4- and 6-methylene groups of dimedone are no longer identical. In formaldehyde dimethone anhydride, (1,8 (2H, 5H,)-dione-3,4,6,7-tetrahydro-3,3,6,6-tetramethylxanthen, see diagram XI), the 4- and 5-methylene protons have a chemical shift of 7.80τ , and the 2- and 7-methylene protons have a chemical shift of 7.66τ . Similar chemical shifts are found in acetaldehyde and cinnamaldehyde dimethone anhydrides.



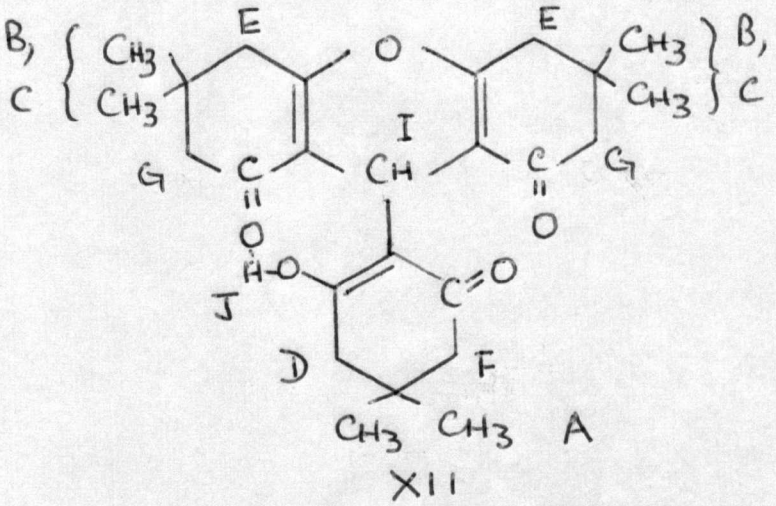
XI

Substitution of one of the 9-methylene protons with a cinnamyl residue is accompanied by a splitting of the signals of the 3- and 6-methyl groups in a similar manner to that described for 2-formyldimedone dimethone below. A similar splitting is not seen in acetaldehyde dimethone anhydride. Presumably the 9-methyl group is too small to give any resolvable splitting.

N.m.r. Spectra of more complex dimedone derivatives.

a) 2-formyldimedone dimethone.

The dimethone of 2-formyldimedone consists of 3 dimedone residues linked by the 2-position to a central carbon atom, with loss of a molecule of water between 2 of the residues, as in diagram XII



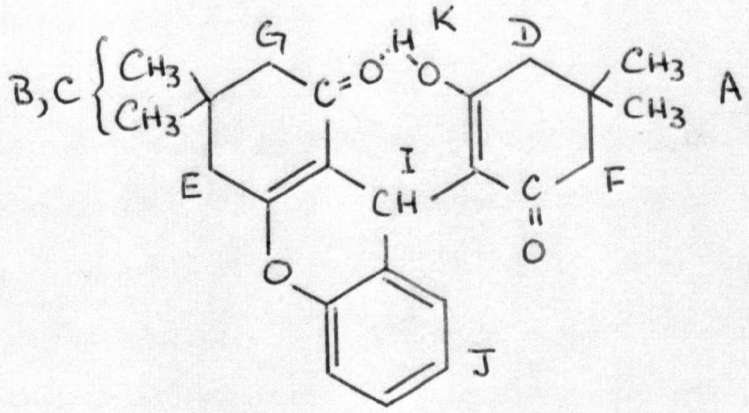
XII

The analysis of the chemical shifts of the methylene protons was based upon the integrated areas of the peaks (as there are 2 residues of one type and one of another). The analysis of the methyl proton chemical shifts is based on the n.m.r. spectrum of salicylaldehyde dimethone given below.

A, 9.01 (6H); B, C, 8.99 (6H) and 8.88 (6H); D, 7.97 (2H); E, 7.72 (4H); F, 7.64 (2H); G, 7.52 (4H); I, 5.58 (1H); J, -0.03 (1H). The spectrum shows that the methyl groups of the octahydroxanthen moiety appear as a doublet. They are held in a rigid conformation, and the presence of the third dimedone residue is sufficient to render them non equivalent. The hydroxyl proton has a negative chemical shift, suggestive of a small amount

of hydrogen bonding. Molecular models of this type of compound suggest that the steric requirements of hydrogen bonding are not met as well as with the simple dimethones with two hydrogen bonds.

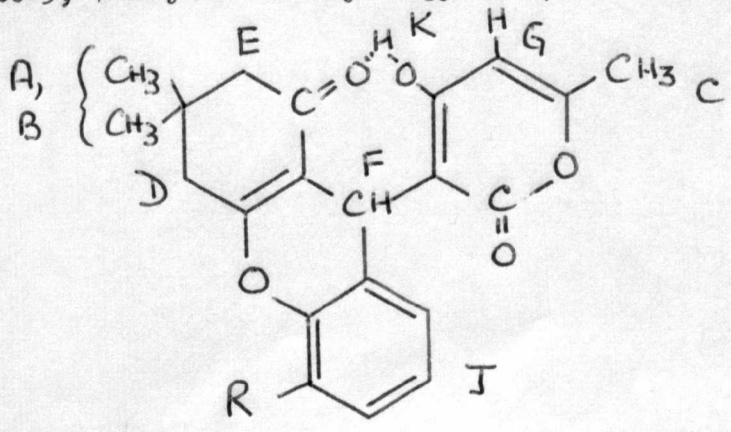
b) Salicylaldehyde dimethone (diagram XIII)



XIII

A, 9.24 (6H); B,C, 9.20 (3H), 9.11 (3H); D, 8.05 (2H); E, F, 7.69 (2H), 7.61 (2H); G, 7.49 (2H); I, 5.33 (1H), J, 2.99 (3H, tight multiplet); K, -0.42 (1H). (The individual identity of E and F is in doubt, due to the proximity of these peaks and their identical size).

b) Dimedone addition product of 3-o-hydroxybenzylidene-2, 4-diketo-3, 4-dihydro-6-methyl-α-pyrone (R = H in diagram XIV)



XIV

A, B, 8.94 (3H), 8.87 (3H); C, 7.94 (3H); D, 7.74 (2H); E, 7.67 (2H); F, 5.06 (1H); G, 4.14 (1H); J, 2.96 (4H, tight multiplet); K, -0.42 (1H). (The spectrum of the dimedone addition product where R = OCH_3 may be seen to correspond extremely closely with this spectrum - see Experimental Section).

The i.r. and u.v. spectra of Dimedone Derivatives.

The absorptions in the area of the i.r. associated with carbonyl stretching frequencies is characterised for simple dimethones by a broad band centred on 1600 cm^{-1} (Spectrum in chloroform or as Nujol mull). The lack of absorption at 1700 cm^{-1} indicates that these compounds exist as almost 100% enol, in keeping with the hydrogen bonded structure above. The i.r. spectra of dimethone anhydrides show bands at 1660 and 1630 cm^{-1}; the former may be attributed to the carbonyl groups (which are unsaturated) and the latter to the double bonds. Dimethone anhydrides have also distinctive peaks in the u.v., at ca. 233 and 292 m.μ. These characteristic peaks may be seen to have an influence on the absorption of other more complex compounds containing this grouping (2-formyldimedone dimethone, for example).

The influence of hydrogen bonding may be seen on the i.r. spectra of more complex dimethones. Salicylaldehyde dimethone (diagram XII) shows a band at 1648 cm^{-1} for the hydrogen bonded carbonyl group, and 2-formyldimedone dimethone shows a similar band (at 1646 cm^{-1}) for the hydrogen bonded carbonyl group of the octahydroxanthen residue.

Structure of dicoumarol and other 4 hydroxycoumarin derivatives.

Structure of 4-hydroxycoumarin

The data collected here is entirely consistent with the predominance of the 4-hydroxycoumarin structure for all simple 3- substituted 4-hydroxycoumarins, both in solution and the solid state. Simple coumarin derivatives have distinctive u.v. spectra, with peaks at 303 - 314 mμ, and 279 - 285 mμ, and another peak or shoulder at 269 - 276 mμ. (See diagram XVI) They show this type of spectrum whether they are O-alkylated or not. Unfortunately 4-hydroxycoumarin is extremely insoluble in chloroform, and no assessment of the hydrogen bonding of this compound was possible as was possible in the case of dimedone.

The structure of dicoumarols.

Knobloch's dichromone structure of dicoumarol is dismissed on the following grounds:-

a) The frequency attributable to the chromone carbonyl group in the i.r. spectra in chloroform solution of 2-methoxychromone, 3-methyl-2-methoxychromone, and the 2,2'-dimethyl ether of dicoumarol is ca. 1630 cm^{-1}. (That of chromone itself is a little higher, at 1647 cm^{-1}). This value is 30 cm^{-1} lower than ν_{max} C = O dicoumarol, and any hydrogen bonding, either inter- or

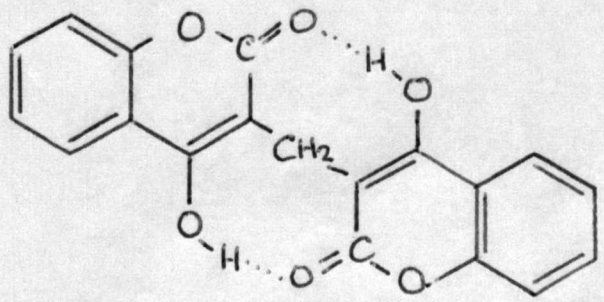
intramolecular, of the chromone carbonyl group would decrease, rather than increase, the carbonyl frequency.

b) If dicoumarol were a dihydroxychromone, then on account of the greater acidity of hydroxychromones as compared with hydroxycoumarins, diazomethane would react with dicoumarol to give largely the 2,2'-dimethyl ether. It has been found that dicoumarol reacts to give mostly the 4, 4'-dimethyl ether. Only a small quantity of the 2,2'-dimethyl ether could be obtained on chromatographic separation of the products of this reaction.

c) The simple non hydrogen bonded chromone structure would presumably have a u.v. spectrum like that of 2-methoxychromone. The u.v. spectrum of dicoumarol is unlike that of 2-methoxychromone or 4-hydroxycoumarin (see diagram XVI) This fact also precludes the simple dicoumarin structure drawn by Link (diagram II).

d) Knobloch makes no attempt to explain why the chromone structure is energetically more favourable for dicoumarols than it is for simple 4-hydroxycoumarins.

The conclusion reached is that dicoumarol is a dicoumarin structure with hydrogen bonds between the hydroxyl groups of one 4-hydroxycoumarin residue and the carbonyl groups of the other, as in diagram XV



XV

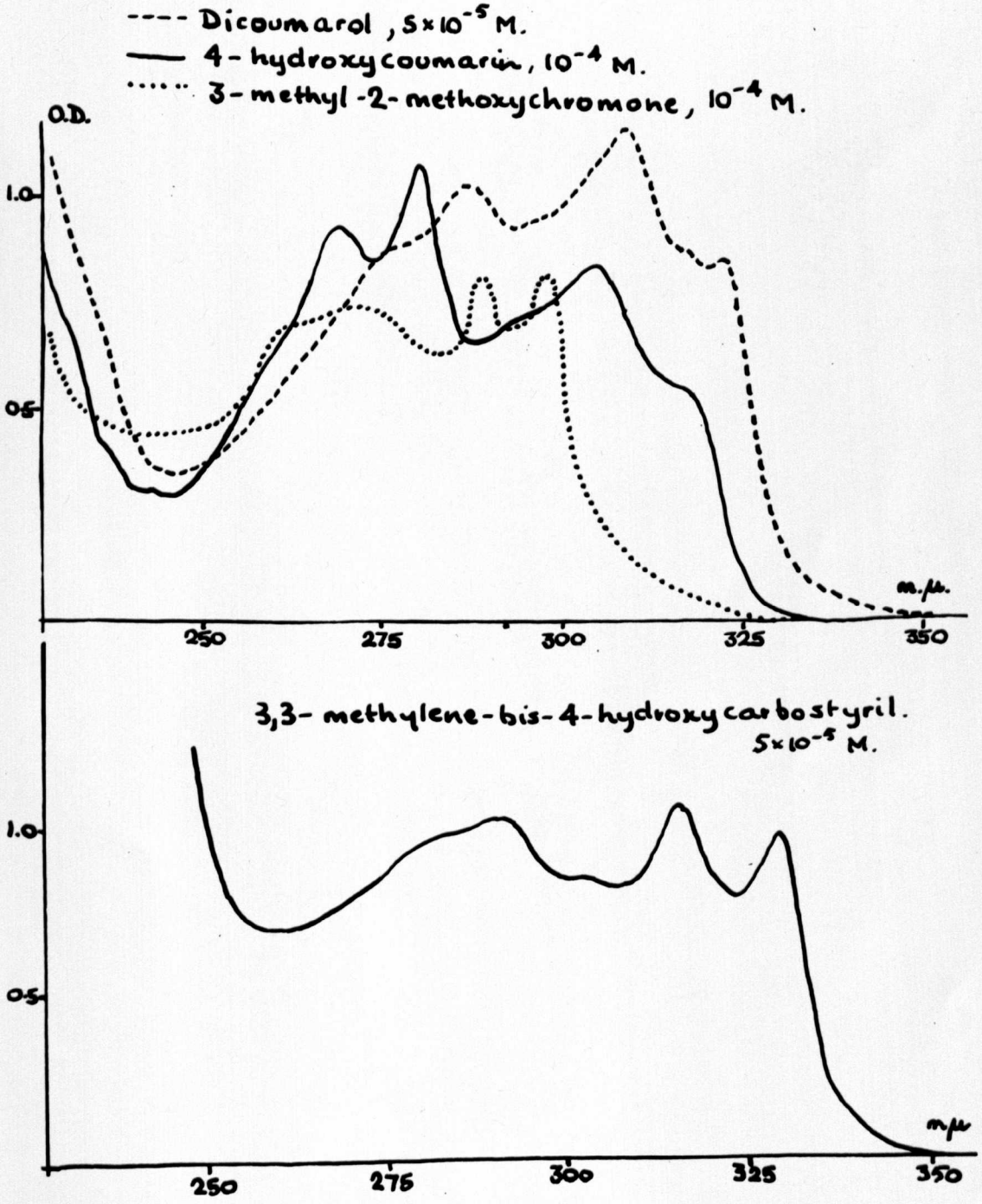

---- Dicoumarol, 5×10^{-5} M.
—— 4-hydroxycoumarin, 10^{-4} M.
..... 3-methyl-2-methoxychromone, 10^{-4} M.
O.D.
1.0
0.5
m.μ.
250
275
300
325
350
3,3-methylene-bis-4-hydroxycarbostyril.
5×10^{-5} M.
1.0
0.5
mμ
250
275
300
325
350

Diagram XVI

This model will explain the above points, and is supported by the following facts.

a) The spectrum of dicoumarol is essentially the same whether taken with dicoumarol in the solid state (as Nujol mull or KCl disc) or in solution (chloroform or dioxan). 4-hydroxycoumarin has a νmax C = O which varies from solvent to solvent. (νmax C = O in dioxan, 1730; νmax C = O: chloroform containing 2% ethanol, 1695; νmax C = O as Nujol mull, 1700.)

b) 4-hydroxycoumarin is insoluble in any solvent with which it cannot form strong intermolecular hydrogen bonds, but dicoumarol is soluble in chloroform, and higher homologues of dicoumarol are appreciably soluble in carbon tetrachloride.

c) The n.m.r. spectra of dicoumarols show protons with low chemical shifts (τ_{OH} dicoumarol, - 1.70). In a similar way to that described for dimethones, substitution of one of the protons of the methylene group by a larger unit may interfere with the hydrogen bonding. In this case however, the planar phenyl group does not cause the chemical shifts of the two hydroxyl protons to be different from one another, but the smaller spherical methyl group does interfere with the hydrogen bonding in this way. Substitution in the para-position of the phenyl group by the fairly bulky nitro-group, does cause a small modification of the hydrogen bonding as seen in the n.m.r. spectra. Another feature of the n.m.r. spectra of dicoumarols, is that the 5-protons of the 4-hydroxycoumarin residues have a lower chemical shift than any of the other aromatic protons. This may be

attributed to the increase in the electron density (or double bond character) of the C - O bond of the 4-hydroxyl group, caused by the hydrogen bonding. This feature is seen in the n.m.r. spectra of chromones, where there is a carbonyl group α - to the aromatic ring. As would be expected none of the 4-methyl ethers of coumarins show this property. It seems that all 4-hydroxycoumarin residues in compounds where there is hydrogen bonding of the hydroxyl group intramolecularly to the carbonyl group of another residue show this feature (see later for other examples).

d) The low pKa value of dicoumarols as compared with 4-hydroxycoumarin is not explained in terms of the more acidic hydroxychromone groups, but in terms of the stability of the mono-anion. See later for discussion of these values.

e) A molecular model of this structure shows the steric feasibility of the hydrogen bonds.

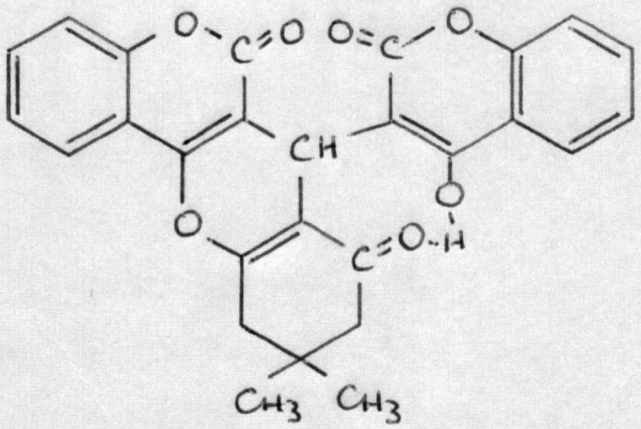
(Note: Chmielewska failed to find a "chromone band" in the i.r. spectrum of the 2,4'-dimethyl ether of dicoumarol because she expected a band at 1659, by analogy with the i.r. spectrum of 2-methoxychromone as a Nujol mull. The 2,4'-dimethyl ether, however, resembles 3-methyl-2-methoxychromone in that the i.r. frequency of the chromone carbonyl group appears at 1630 cm^{-1} either in chloroform solution or as a Nujol mull).

Other features of the i.r. spectra of 4 hydroxycoumarin derivatives.

As well as the features discussed above, some other reproducible characteristics have been observed in these spectra. All compounds containing 4-hydroxycoumarin residues show a band at ca. 1569 cm^{-1}.

However when the 4-hydroxycoumarin residue condenses with the hydroxyl group of another residue, as in the case in dicoumarol epoxide, there is always a band in the spectrum at 1610 ± 1 cm^{-1}. Both these bands are associated with the double bonds of the benzene rings of the coumarin residues.

The i.r. frequency of the carbonyl group of a coumarin residue not involved in hydrogen bonding is greater than 1710 cm^{-1}. When the residue is involved in hydrogen bonding, either through the hydroxyl group or the carbonyl group, the frequency is less than 1700 cm^{-1}. Accordingly, it can be assumed that any intramolecular hydrogen bonding in 2-formyldimedone dicoumarol occurs between the hydroxyl group of the 4-hydroxycoumarin residue and the carbonyl group of the dimethone anhydride residue as shown in diagram XVII



XVII

I.r. frequencies: 1728, 1669.

Similar analyses may be made of the carbonyl stretching frequencies of other coumarin derivatives prepared, the spectra of which are given in the experimental section.

Structure of other dicoumarols and related compounds

p-dimethylaminobenzaldehyde dicoumarol

This compound is unusual in being highly coloured, with a band in the u.v. at 497 mμ. (ε, 7000). Recrystallisation and chromatography show the colour not to be due to any highly coloured impurity. On addition of either alkali or acid to a solution of the compound in ethanol, the colour disappears. The i.r. spectrum of a saturated solution of the compound in chloroform shows that the absorptions are in line with those of other dicoumarols, but the solubility in chloroform is insufficient to allow an n.m.r. spectrum to be taken in this solvent.

The corresponding dimethone (a pale orange compound) has been prepared, and its n.m.r. spectrum does not indicate any divergence in structure from that of any other dimethone prepared.

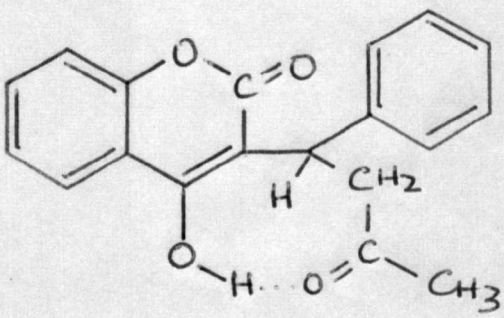
(The chemical shift of the hydroxyl protons are similar to those observed for benzaldehyde dimethone). In a neutral alcohol solution of the dicoumarol, or in the solid state, it seems likely that some protonation of the N-dimethyl group occurs by the proton of one of the 4-hydroxycoumarin residues, and the colour may be due to some type of charge transfer complex between ionic forms present. The addition of acid or alkali will disturb the proportion of any charged forms present, the former by protonation of the N-dimethyl group, and the latter by removal of the hydroxyl protons of the 4-hydroxycoumarin residues.

Pelantan.

In this compound there is the possibility of hydrogen bonding between the hydroxyl groups of the 4-hydroxycoumarin residues, and the carbonyl of the carbethoxy group. However, there is no evidence in the spectra of pelantan that this type of bonding occurs.

Warfarin.

The u.v. spectrum of this compound shows it to be essentially a simple coumarin derivative. The νmax C=O (i.r. spectrum as Nujol mull) is 1688 cm^{-1}, and on account of this rather low value for the stretching frequency of the carbonyl group of the α-acetonyl residue, the following hydrogen bonded structure seems likely. (Diagram XVIII)



XVIII

The pKa value of warfarin (see later) provides further evidence for this postulate.

4-hydroxy-6-methyl-α-pyrone derivatives.

The spectra of these derivatives are generally speaking rather less complex than the spectra of their 4-hydroxycoumarin analogues. The u.v. spectra of 4-hydroxy-6-methyl-α-pyrone and the bis

derivatives studied all show a single maximum above 230 mμ. The i.r. spectrum of the simple α-pyrone shows a νmax C=O of 1700 cm^{-1}, and the i.r. spectra of the bis-derivatives show a νmax C=O of 1680 cm^{-1}. This relationship is similar to that encountered with 4-hydroxycoumarin and dicoumarols. Unfortunately, as has been found for 4-hydroxycoumarin, the α-pyrone is highly insoluble in chloroform, and so again no examination of hydrogen bonding in this solvent is possible. The n.m.r. spectra of the bis-α-pyrones shows bonding similar to that observed in dicoumarols, with hydroxyl proton chemical shifts of around -1τ. In the case of these derivatives the substitution of a methyl group for a proton of the methylene bridge of the formaldehyde bis-α-pyrone does not interfere with the hydrogen bonding sufficiently to make the hydrogen bonds non-identical (as evidenced by the n.m.r. spectra). The following table shows the relationship between the type of bis-derivative, the substituent on the bridge, and the chemical shift of the hydroxyl protons.

Group	Dimethone	Dicoumarol	Bis-pyrone
H	-1.31	-1.70	-0.80
C_6H_5	-1.76, -0.57	-1.43	-0.91
CH_3	-2.74, -0.40	-2.24, -1.44	-1.22

Hydrogen bonding does not seem to be as effective in bis-pyrones as in dicoumarols or dimethones (using the criterion of negative chemical shift as a guide to the strength of the bonding).

This is reflected in the νmax C=O for bis-pyrones being 20 cm^{-1} higher than the νmax C=O for dicoumarols. The aromatic ring in 4-hydroxycoumarins evidently causes some steric restraint on the α-pyrone ring which leads to more efficient hydrogen bonding in dicoumarols as compared with bis-pyrones.

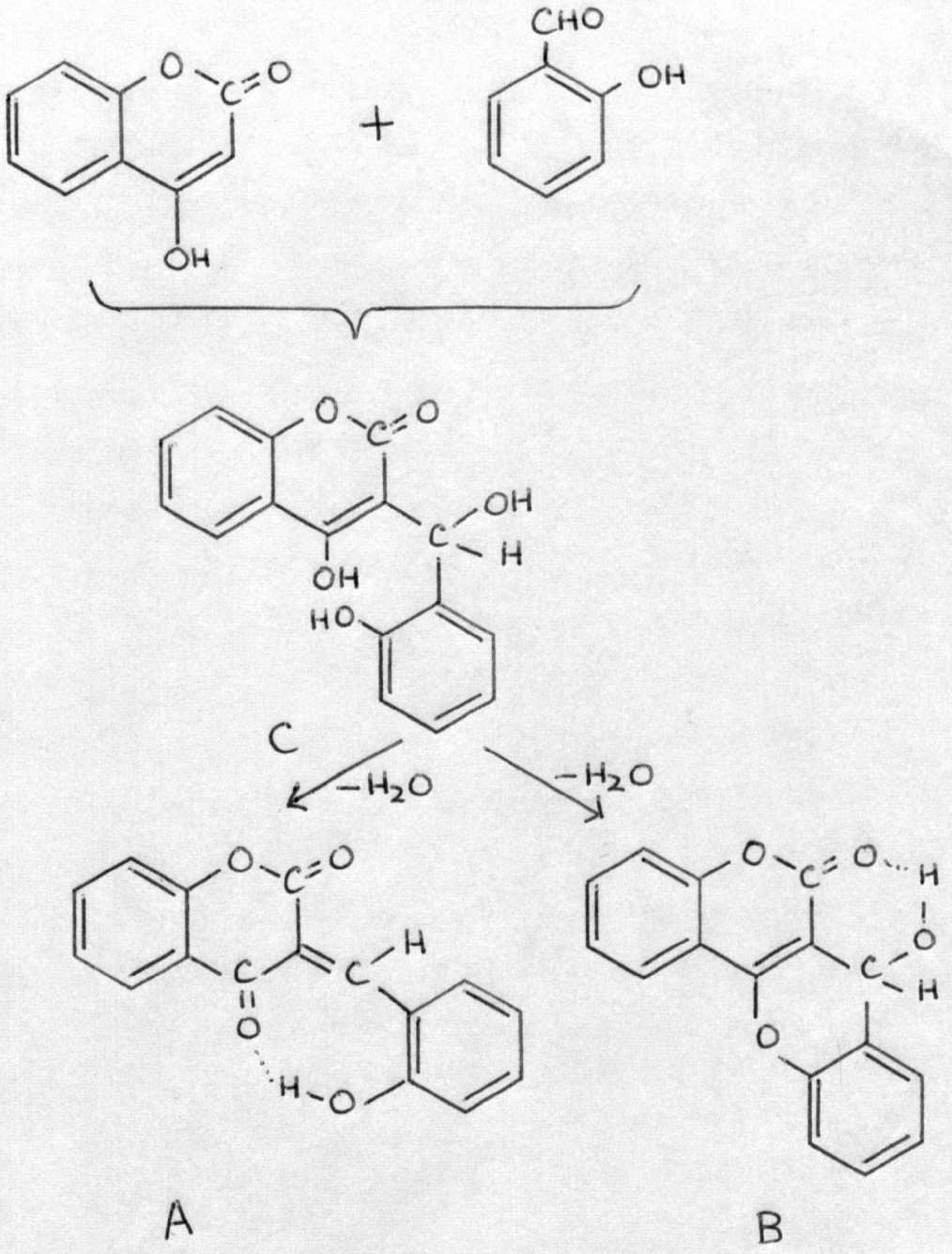
Other dicoumarol analogues.

The two 3,3'-methylene-bis-carbostyril derivatives prepared ~~both~~ show u.v. spectra very similar to dicoumarol. (The u.v. spectrum of 3,3'-methylene-bis-4-hydroxycarbostyril is shown in diagram XVI). The absorption in the carbonyl region of the i.r. is also very similar to dicoumarol, again suggesting a dicoumarol type structure for these compounds. The limited data on 3,3'-thio-bis-4-hydroxycoumarin indicatives a similar structure for this compound.

The Structure and Properties of the Mono-addition products of o-hydroxybenzaldehydes and 4-hydroxy-α-pyrones.

a) 3-o-hydroxybenzylidene-2, 4,-diketochroman.

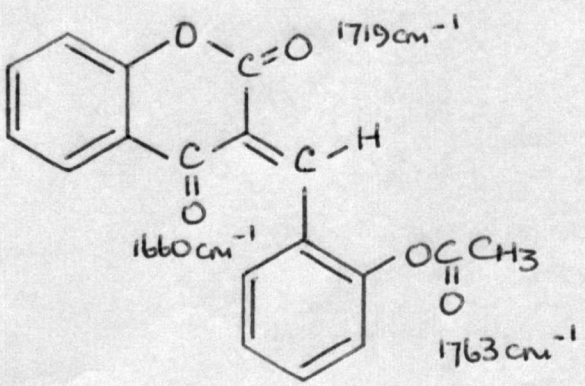
Link discussed the reaction of 4-hydroxycoumarin and salicylaldehyde in terms of the reaction sequence below to give a product of structure XIX(A)[21]. His formulation was supported by analy~~sis~~tical data, and the yellow colour of the compound, which he attributed to its highly conjugated structure. (The hydrogen bond has been added after study of the n.m.r. spectrum of this compound, τ_{OH}, - 1.70. The drawing of the 8-membered ring is schematic, the benzylidene phenyl group is not coplanar with the diketochroman residue).



XIX

Another mode of dehydration of the first formed addition product XIX(C) would be to give structure XIX(B), which would have the same elemental analysis as XIX(A). However, structure XIX(B), despite its more orthodox hydrogen bond as part of a 6-membered rather than an 8-membered ring, seems unlikely on consideration of the spectra. The n.m.r. spectrum shows a proton with a chemical shift of 2.00τ. By analogy with the n.m.r. spectra of other secondary carbinols, the secondary carbinol proton of structure XIX(B) would be expected to show a chemical shift of around 6τ. The value of 2.00τ is, however, in the region expected for the benzylidene proton of structure XIX(A). The i.r. spectrum shows a band at 1720 cm^{-1}, a higher value than would be expected for the hydrogen bonded carbonyl group in structure XIX(B), but not an unreasonable value for the non-hydrogen bonded carbonyl group in structure XIX(A).

Acetyl derivative. The above compound forms a stable mono-acetyl derivative, the i.r. spectrum of which is rather informative, showing bands at 1763, 1719 and 1660 cm^{-1}. The 1763 cm^{-1} band is typical of the carbonyl stretching frequency of an aromatic acetoxy group. (see below).

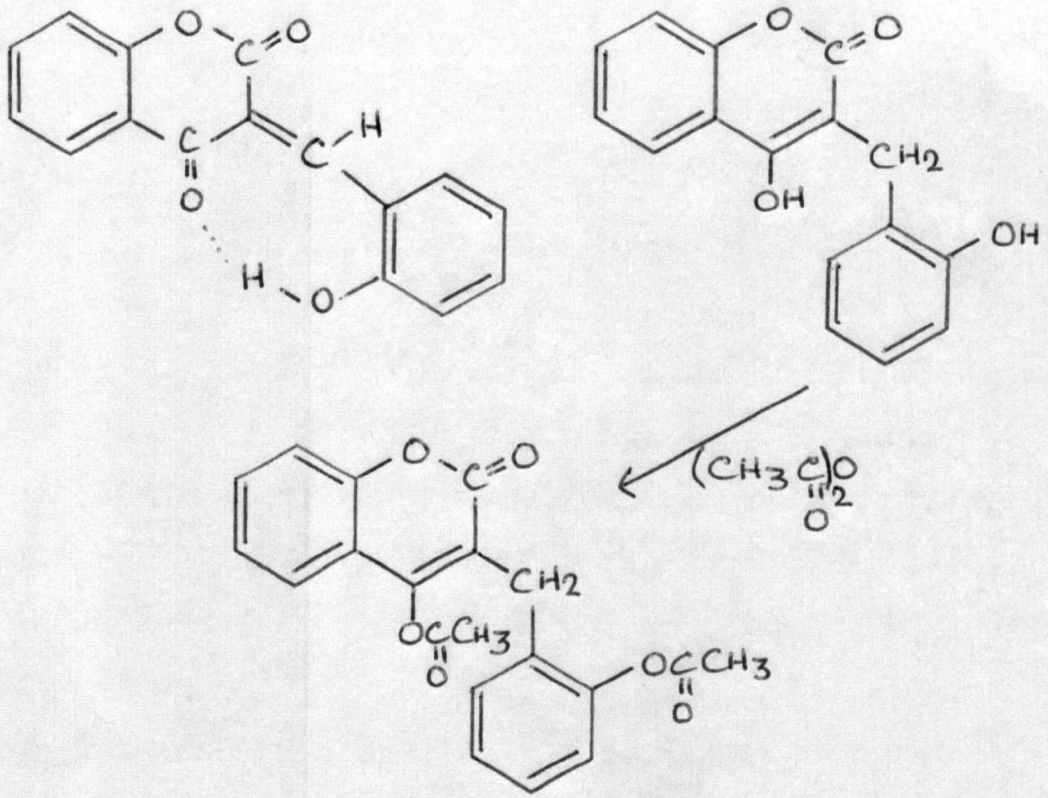


XX

The diketochroman structure (XX) will explain this band and the two other bands adequately. The band at 1719 cm^{-1} corresponds with the band at 1720 cm^{-1} for the carbonyl group of structure XIX(A). The 4-carbonyl group of structure XIX(A) is hydrogen bonded, and does not show an absorption in the i.r. of above 1630 cm^{-1}. However, the 4-carbonyl group in the above structure (XX) is non-hydrogen bonded, and the appearance of the new band at 1660 cm^{-1} on formation of the acetyl compound is indicative of this change. The n.m.r. spectrum of the acetyl compound shows a single proton at 1.72τ, corresponding with the benzylidene proton of structure XX, and the u.v. spectrum does not show any influence of coumarin chromaphores.

Reduction On reduction, one mole of hydrogen is taken up to give a coumarin derivative (as indicated by the u.v. spectrum), which forms in turn a diacetyl derivative. The n.m.r. spectrum of the diacetyl derivative shows there to be a methylene group, and two methyl groups. The i.r. spectrum shows bands at 1777 and 1750 cm^{-1}. By analogy with 4-acetoxycoumarin, with a band at 1790 cm^{-1}, the 1777 cm^{-1} band could be due to the carbonyl of a similar 4-acetoxy group, and by analogy with methyl *o*-phenacylsalicylate (1760 cm^{-1}), and *p*-acetoxybenzaldehyde dicoumarol epoxide (1760 cm^{-1}), the 1750 cm^{-1} band could be due to the carbonyl group of an aromatic acetyl group. The evidence as presented here suggests the following reaction scheme,

(diagram XXI) starting from structure XIX(A)



XXI

The compound formed on reduction is 3-o-hydroxybenzyl-4-hydroxycoumarin.

Conclusion The reactions of the mono- addition product of 4-hydroxycoumarin and salicylaldehyde, including its addition reactions described below, show conclusively that it reacts as if it were structure XIX(A). The spectra of the molecule itself also strongly indicate structure XIX(A). A molecular model of this compound shows the steric feasibility of the hydrogen bond, the geometry of which is similar to that of the

hydrogen bonds in dimethones and dicoumarols. It may be noted that the alternative structure involving hydrogen bonding with the 2-carbonyl group is ruled out, on the ground that if this were the case then there would be no carbonyl with as high a frequency as 1720 cm^{-1} in the i.r. The changes in i.r. spectrum involved on formation of the acetyl derivative also preclude this structure.

Other diketochromans.

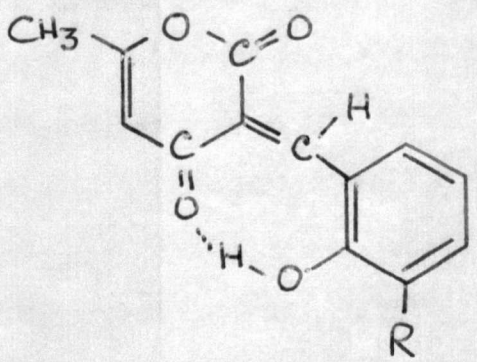
The reactions of the mono-addition product of o-vanillin and 4-hydroxycoumarin (addition of dimedone and acylation) indicate a similar structure for this compound. Spectroscopic changes on formation of the acetyl derivative are analogous to those observed with the salicylaldehyde compound. The 1-formyl-2-hydroxynaphthalene-4-hydroxycoumarin mono-addition compound was found to be inert to addition of dimedone or 4-hydroxycoumarin in boiling ethanol. It did, however, form an acetyl compound analogous to those described above.

4-hydroxy-6-methyl-α-pyrone analogues.

4-hydroxy-6-methyl-α-pyrone and salicylaldehyde undergo exactly analogous reactions to those described above for 4-hydroxycoumarin and salicylaldehyde.

On boiling under reflux a solution of equimolar quantities of salicylaldehyde and 4-hydroxy-6-methyl-α-pyrone for a short time (about 10 minutes) and cooling, orange-yellow crystals of the mono-addition compound separate. The n.m.r.

spectrum of this compound shows the hydroxyl proton (τ, - 5.69) to be more tightly hydrogen bonded than the hydroxyl proton of the 4-hydroxycoumarin analogue. The i.r. spectrum shows a band at 1728 cm^{-1} for the 4-carbonyl group. The structure is XXII, where R=H.



XXII

In contrast to the 4-hydroxycoumarin analogues, this compound did not form an acetyl derivative under any of the conditions tried (see Experimental Section). Presumably this is due to steric hindrance by the 2-carbonyl group in the transition state, coupled with the loss of the energy of the hydrogen bond, being a prohibitive factor in this case. The compound in which R=OH in diagram XXII reacts readily with acetic anhydride to give a mono-acetyl compound, which is clearly formed by acylation of the 3-hydroxyl group. (The n.m.r. spectrum shows a hydrogen-bonded hydroxyl proton at -5.78τ, and a signal for the benzylidene proton at 1.40τ. The u.v. spectrum is very similar to those of the other compounds prepared of structure XXII, where R=H, OH, or OCH_3.) The dimedone addition products of

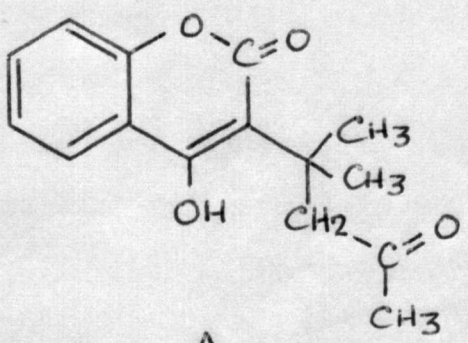
XXII, where R=H and OCH_3, have been prepared, an analysis of the n.m.r. spectra of these compounds is given on page 30.

The reduction of XXII (R=H) follows the same route as that of its 4-hydroxycoumarin analogue, one mole of hydrogen being ~~is~~ taken up to give 3-o-hydroxybenzyl-4-hydroxy-6-methyl-α-pyrone, which forms a diacetyl derivative. The n.m.r. spectrum of the diacetyl compound shows, inter alia, 3 methyl groups (though owing to the proximity of these peaks and their equal areas it is not possible to ascribe them individually) and a methylene group.

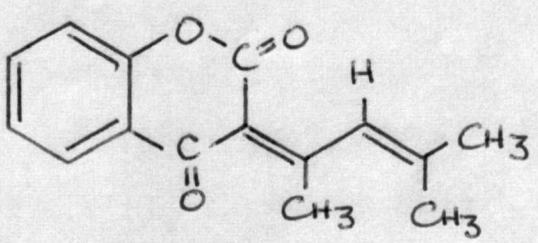
On boiling under reflux a solution of salicylaldehyde and 4-hydroxy-6-methyl-α-pyrone for a long period (as described in the Experimental Section) colourless crystals separated. The n.m.r. spectrum of this compound shows it to have an analogous structure to the bis-derivative of 4-hydroxycoumarin and salicylaldehyde. (See Experimental Section).

The reaction of 4-hydroxycoumarin and mesityl oxide.

Link identified the two products of this reaction as an acidic product (XXIIIA) and a heptane soluble product (XXIIIB)[26]



A

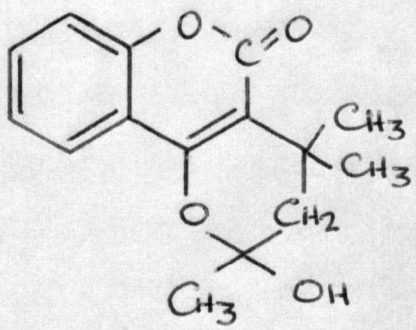


B

XXIII

A is formed by the Michael addition of a molecule of 4-hydroxycoumarin to the double bond, and B by condensation of 4-hydroxycoumarin with the carbonyl group of mesityl oxide.

a) Acidic product. All the properties of this compound studied, indicate that Link's structure is correct. The i.r. spectrum shows a band at 1668 cm^{-1}, the highest wavelength for a band in the carbonyl area. This corresponds with the carbonyl group of the side chain, intramolecularly hydrogen bonded with the hydroxyl group in a similar way to that proposed for warfarin. The u.v. spectrum shows the compound to be a coumarin derivative. The compound is insoluble in chloroform, but the n.m.r. spectrum in trifluoro-acetic acid corresponds with the compound the structure of which is shown below. (XXIV) The methylene group appears as an AB pattern. The two 2'-methyl groups have chemical shifts of 8.95 and 8.75τ, and the 4'-methyl group gives a signal at 8.27τ.

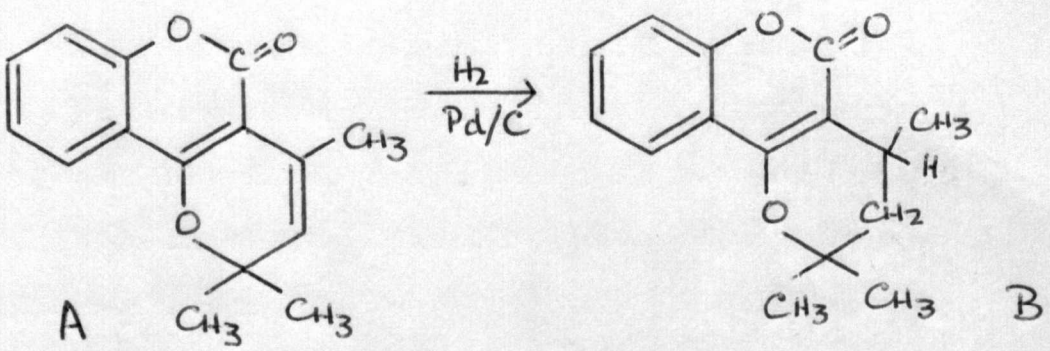


XXIV

Link has shown that compounds of the general structure of XXIII(A) form acetals when reacted with a solution of hydrogen chloride in methanol[70]. Presumably the compound XXIII(A) has formed the hemi-acetal above on solution in trifluoro-acetic acid.

b) Heptane soluble product. The properties of this compound show Link's structure to be incorrect. On the basis of structure XXIII(B) there should be long range coupling in the n.m.r. spectrum between the β-hydrogen atom and the two γ-methyl groups. However, long range coupling is observed between a proton and one methyl group. The chemical shift of the γ-methyl groups would be expected to be around 7.8τ, but it is rather higher than this at 8.5τ. The i.r. and u.v. spectra do not give a useful guide to the structure, except to show that it is not a simple coumarin.

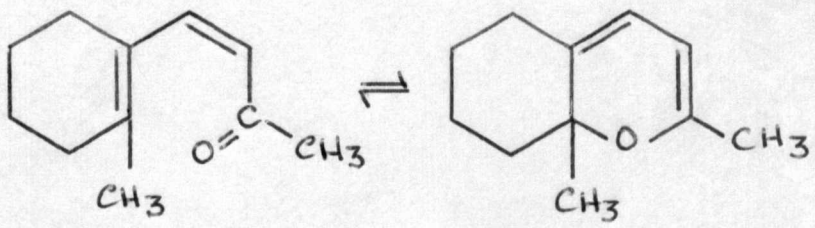
On reduction of this compound with palladium on charcoal only one mole of hydrogen is taken up, whereas a compound of structure XXIII(B) would be expected to take up 2 moles. The product has the u.v. spectrum of a simple coumarin derivative. These facts, coupled with the n.m.r. data above, has lead to the conclusion that Link's formula XXIII(B) represents the first formed product, which then undergoes an electrocyclic reaction to give structure XXV(A)



XXV

Reduction of XXV(A) then gives the coumarin XXV(B) (3,4-(2',2',4'-trimethyl)-dihydropyranocoumarin). The n.m.r. spectrum of this derivative is analysed as follows: 8.63 (3H), 8.45 (3H), (2'-methyl groups); 8.60 (3H, doublet, J=6.5 c./sec. for 4'-methyl group). The 3' methylene group is part of an A_{BX} system, where X is the 4' proton. H_A, 7.92; H_B, 8.35; J_{AB}, 13.9 c./sec. J_{AX}, 6.7 c./sec. J_{BX}, 10.0 c./sec. X is a complex multiplet, (7.15τ). Splittings are attributable to the 4'-methyl group as well as the 2'-methylene group. (The possibility that this compound is 3,4-(2',4',4'-trimethyl)-dihydropyranocoumarin is ruled out, on the ground that the 2'-proton in this structure would have a chemical shift of around 6τ, as compared with the 7.15τ of the 4' proton of the above structure. This in turn precludes the possibility that the original 4-hydroxycoumarin-mesityl oxide addition product is the 2',4',4'-trimethyl pyranocoumarin, rather than the 2', 2',4'-trimethyl pyranocoumarin

derivative). Cyclisations of structures such as XXIII(B) have been observed before. β-ionone, for example, is in equilibrium with the α-pyran structure below[71] (Diagram XXVI)



XXVI

The equilibrium constant in ethylenetetrachloride solution at 18° has been found to be 4. In the case of the mesityl oxide adduct above no indication of any equilibrium could be found, either in its spectra or in its reactions.

Preparation and properties of "mixed" dicoumarols.

By a "mixed" dicoumarol is meant a compound containing a 4-hydroxycoumarin nucleus attached through the 3-position to a carbon atom attached in turn to the 3-position of one of the other enolic residues considered, (or in the case of dimedone attached to the 2-position). Water may be lost between either residue and the o-hydroxy group of an o-hydroxybenzaldehyde residue. Three methods have been employed in the preparation of these compounds.

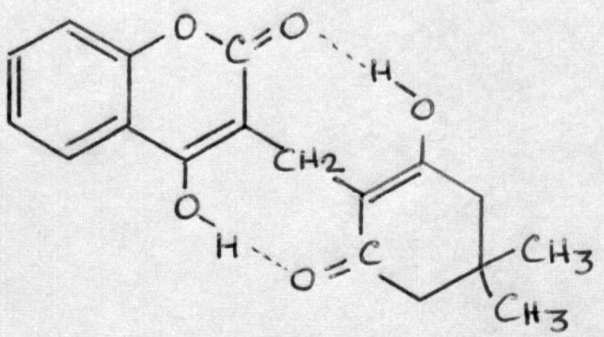
(1) The reaction of an aldehyde with a mixture of nucleophiles, followed by separation of the products.

(2) The addition of a suitable nucleophile to products formed by the addition of one mole of 4-hydroxycoumarin to one mole of an o-hydroxybenzaldehyde (diketochromans).

(3) The use of 3-N-piperidinomethyl-4-hydroxycoumarin.

Route 1

An example of this route is the preparation of a series of compounds by Hellmann and Shroder,[72] in which a substituted 1,3-cyclohexanedione derivative is linked through a methylene bridge to the 3-position of 4-hydroxycoumarin. The dimedone compound was prepared via this route. (Diagram XXVII)



XXVII

This compound shows some interesting properties, which may be summarised by saying that it is half a dicoumarol and half a dimethone. The i.r. spectrum shows a band at 1660 cm^{-1}, typical of the 4-hydroxycoumarin residues in dicoumarols. The u.v. spectrum shows chromophores typical of both dicoumarol and formaldehyde dimethone. (The extinction coefficients at

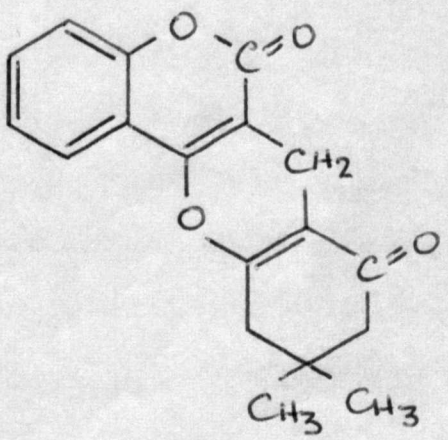
a given wavelength are approximately equal to the sum of half the extinction co-efficients of dicoumarol and formaldehyde dimethone at that wavelength). The n.m.r. spectrum shows the following points.

a) Two non-equivalent hydrogen bonded protons at -1.10 and -2.25τ.

b) The 5-proton of the 4-hydroxycoumarin residue again has a lower chemical shift than the other 3 aromatic protons.

c) Unlike the simple dimethones, in which the 4- and 6-methylene groups show a unique chemical shift, there are two separate signals for the methylene groups. Presumably, as the two tautomeric forms of the 4-hydroxycoumarin (the coumarin and the chromone) are non equivalent, a certain amount of "bond fixing" occurs in the dimedone residue to which it is hydrogen bonded.

Another interesting feature of this compound is its dehydration. Under the mild conditions in which formaldehyde dimethone will undergo dehydration, the compound remains unreactive. However, using a 12.5% solution of concentrated sulphuric acid in methanol, under which conditions dicoumarol would not dehydrate,[73] dehydration does occur to give the anhydride XXVIII



XXVIII

Two features of the n.m.r. spectrum of this compound might be commented on here.

a) Unlike the compound above, the 5-proton of the coumarin residue does not have a chemical shift distinguishable from those of the other 3 protons.

b) The asymmetry of the molecule is insufficient to cause the two 5-methyl groups to show distinguishable chemical shifts.

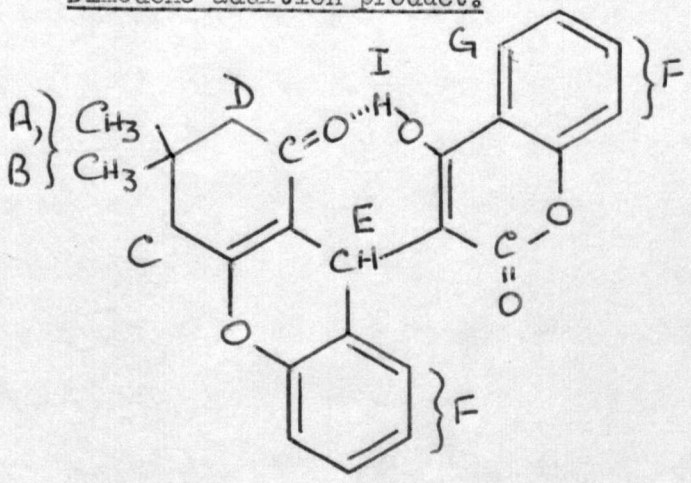
The i.r. spectrum shows a band at 1720 cm^{-1} for the carbonyl group of the coumarin residue, a band at 1660 cm^{-1} for the carbonyl group of the dimedone residue, and a band at 1611 cm^{-1} for the aromatic ring of the cyclised coumarinyl.

Route 2

Link treated 6-methyl-4-hydroxycoumarin with 3-o-hydroxybenzylidene-2,4-diketochroman and obtained a product analogous to salicylaldehyde dicoumarol[21]. The product had lost a molecule of water between one of the 4-hydroxycoumarin residues and the hydroxyl proton of the salicylaldehyde residue.

Link did not distinguish between the possibilities. Presumably, owing to the similarity of the two residues, it would be extremely difficult to solve the problem without recourse to mass spectrometry. Two of these addition derivatives were prepared in this work.

a) Dimedone addition product.



XXIX

The structure of this compound is shown very clearly to be as above (XXIX), on consideration of its spectra. N.m.r. spectrum analysis. A, B, 8.97 (3H), 8.87 (3H); C, 7.65 (2H); D, 7.42 (2H); E, 4.95 (1H); F, 2.6 - 3.2 (6H, complex multiplet); G, 1.7 - 1.9 (1H, complex multiplet); I, -0.10 (1H).

The asymmetry of the molecule is sufficient to cause the rigidly fixed methyl groups of the dimedone residue to be in different chemical environments, and the chemical shift of the 5-proton of the 4-hydroxycoumarinyl residue is distinguishable from the shifts of the other aromatic protons. The u.v. spectrum is dominated by the 4-hydroxycoumarin chromaphores in acidic and alkaline solution. It will be seen later that the pKa of this compound is 4.38, a little higher than the pKa values of the simple 3-substituted 4-hydroxycoumarins studied.

b) The 4-hydroxycarbostyril addition product.

The compound is a colourless microcrystalline solid with a high melting-point, which is extremely insoluble in all the common solvents at room temperature. Analysis again confirms that loss of water occurs after addition of the 4-hydroxycarbostyril in the normal manner, but unfortunately the spectroscopic data is too confused to allow the mode of dehydration to be established.

c) The 4-hydroxy-6-methyl-α-pyrone addition product.

This compound could not be prepared either by the addition of 4-hydroxy-6-methyl-α-pyrone to 3-benzylidene-2,4-diketochroman, or by addition of 4-hydroxycoumarin to the salicylaldehyde-4-hydroxy-6-methyl-α-pyrone addition product. In view of the stability of both salicylaldehyde dicoumarol and the corresponding 4-hydroxy-6-methyl-α-pyrone compounds, this is rather inexplicable.

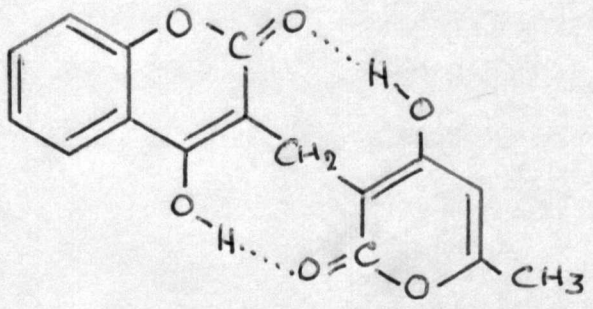
Reactions of other diketochromans.

The dimedone adduct of the mono-addition product of 4-hydroxycoumarin and o-vanillin has also been characterised.

Route 3

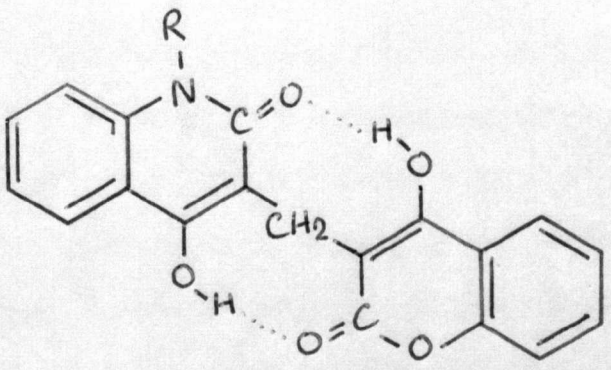
3-N-piperidinomethyl-4-hydroxycoumarin was first prepared by Link [74] by the Mannich reaction of 4-hydroxycoumarin, piperidine and formaldehyde. The properties and structure of this compound were studied by Abramovich and Gear[75], who found that quaternisation of the piperidine residue with methyl iodide

gave a compound that would react with another 4-hydroxycoumarin derivative to give a dicoumarol. Accordingly they prepared dicoumarols in which one only of the two 4-hydroxycoumarin residues was substituted in the aromatic ring. Mohlo and Mentzner[76] have used this compound to prepare the 4-hydroxy-6-methyl-α-pyrone compound XXX, which has also been prepared in this work by this method.



XXX

In a similar way to the corresponding dimedone derivative (see above) the spectra show this molecule to have properties of both dicoumarol and bis-4-hydroxy-α-pyrone. The hydroxyl protons have identical shifts (-1.09τ), showing the similar geometry of the coumarin and α-pyrone ring systems.
The i.r. spectrum shows bands at 1679 and 1660 cm^{-1} for the hydrogen bonded carbonyl groups of the α-pyronyl and coumarinyl residues respectively.
Two new compounds have been prepared by this route, where R=H and R=CH_3 in diagram XXXI



XXXI

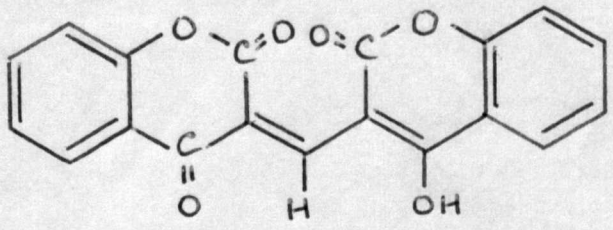
These compounds are insoluble in any suitable n.m.r. solvent for the observation of hydrogen bonding, but the i.r. and u.v. spectra again indicate properties associated with both hydrogen-bonded residues.

Discussion of Preparative Methods.

Reaction of Dicoumarols

Dicoumarol is a very inert substance, and compatible with its high melting point (288-289°), is comparatively insoluble in organic solvents. Most of the reactions of dicoumarols which have been studied involve the hydroxyl groups (e.g. acylation[77], phosphorylation[77], epoxidisation[73], methylation, with diazomethane[21] and dimethyl sulphate[78].) It is soluble in alkali, and on boiling under reflux in alkali will undergo ring opening and decarboxylation, these reactions occurring more or less selectively with one 4-hydroxycoumarin residue initially, but with both residues on prolonged periods of boiling[79].

One reaction of dicoumarol that has not been reported is its oxidation. It is possible to conceive of a scheme in which the methylene bridge is oxidised to give the diketochroman structure XXXII. (Removal of a hydride anion from the bridge methylene group by an oxidising agent, followed by the loss of a proton from one of the 4-hydroxycoumarin residues). This compound would probably be reactive in terms of Michael addition at the benzylidene carbon atom in the same way as is 3-o-hydroxybenzylidene-2,4-diketochroman. (XXIIIA).



XXXII

Dicoumarol was found however, to be inert to most oxidising agents, with the exception of ceric ammonium nitrate, which caused disruption of the coumarin rings to give a variety of salicoyl degradation products. The benzylidene proton in benzaldehyde is unreactive to N-bromosuccinimide. This may well be on account of the steric hindrance by the hydrogen bonded system between the 4-hydroxycoumarin residues to attack by bromine atoms. Another factor in this case may be the energetically unfavourable disruption of the hydrogen bonds that would be caused in the product by the bulky bromine atom.

Acetone and 4-hydroxycoumarin.

Under conditions in which 4-hydroxycoumarin will react with aldehydes it is completely unreactive towards acetone. Attempts to prepare the dicoumarol derivative of acetone by heating 4-hydroxycoumarin and acetone together in sealed tubes at elevated temperatures, yielded only condensation products of 4-hydroxycoumarin and mesityl oxide.

Methyl ethers.

Arndt's method of separation of 2-methoxychromone from 4-methoxycoumarin utilises the solubility of the former in concentrated hydrochloric acid[18]. However, one extraction with acid was found insufficient for complete separation, and accordingly, the sample obtained from the extraction (enriched in 2-methoxychromone) was separated by thin layer chromatography. Using silica GF_{254} (which contains phosphor) and ether as developing solvent, two clear and widely separated bands could be seen under u.v. light for the two methyl ethers. The more polar 2-methoxychromone has the lower R_F value, and the extraction of the silica corresponding to this band with chloroform yielded pure 2-methoxychromone on evaporation of the solvent.

Chmielewska[80] separated 3-methyl-2-methoxychromone from an ether solution of a mixture of this compound and 3-methyl-4-methoxycoumarin by adding perchloric acid, which forms the insoluble perchloric acid salt of the 3-methyl-2-methoxychromone.

A similar method of separation of these two methyl ethers to the one described above was found convenient. This method has the additional advantage of precluding any rearrangement that might occur in the presence of the perchloric acid.

From the reaction of dicoumarol with diazomethane, Chmielewska obtained two dimethyl ethers, the 4,4'- and the 4,2'. The possibility of there being any 2,2'-dimethyl ether was not discussed[24]. However, careful chromatographic separation of the products of this reaction show that a small amount of 2,2'-ether is produced. The i.r. and u.v. spectra of this compound are, as expected, very similar to those of 3-methyl-2-methoxychromone.

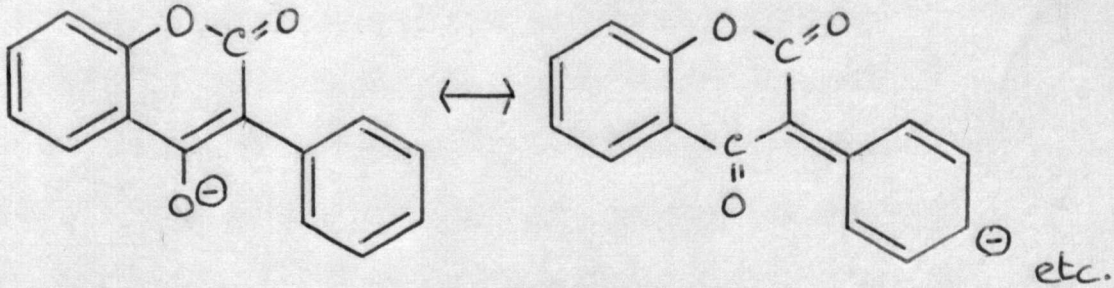
3-formyl-4-hydroxycoumarin.

Two methods are available in the literature for the preparation of this compound. Ziegler uses the formylation of 4-hydroxycoumarin by N-methyl-N-phenylformamide in the presence of phosphorus oxychloride[81]. The use of the more readily available N, N-dimethylformamide as formylating agent reduces the yield considerably; (although a small quantity of product was obtained here, some workers have failed to obtain any product with this reaction).[82] The method of Cecchi,[83] in which 4-hydroxycoumarin is condensed with formamide and the resulting imine hydrolysed, was found to be satisfactory.

pKa values.

a) Monobasic acids.

The pKa value of 4-hydroxycoumarin is 4.20 ± 0.02 at 21°. Substitution of the 3-proton by a methyl group has little effect on the pKa value, now 4.17± 0.04. Substitution by a phenyl group, however, reduces the pKa value to 3.76, presumably due to the stabilisation of the mono-anion by the sharing of the charge on the oxygen atom on to the phenyl group as shown in diagram XXXIII



XXXIII

3-α-phenyl-β-acetylethyl-4-hydroxycoumarin (warfarin) has a pKa value of 5.05, rather higher than those of the other 3- substituted 4-hydroxycoumarins investigated. This value may be explained in terms of a hydrogen bond between the 4-hydroxy group and the carbonyl group of the acetonyl residue stabilising the neutral molecule with respect to the anion in the same way as was indicated for the hydrogen bonded monobasic acids in the introduction. (See diagram XVIII). The comparatively hydrophobic side chain would also be expected to have an acid weakening effect as compared with 4-hydroxycoumarin. The pKa value of salicylaldehyde semidimethone-dicoumarol (the dimedone addition product of 3-o-hydroxybenzylidene-2,4-diketochroman,

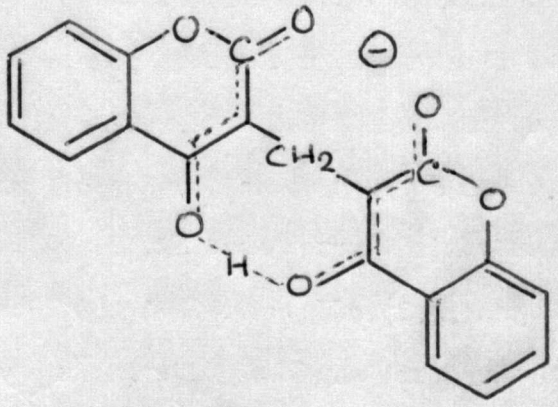
(diagram XXIX) in which the n.m.r. spectrum indicates hydrogen bonding for the hydroxyl group, is 4.36, only slightly above the value for 4-hydroxycoumarin; that of 3-formyl-4-hydroxycoumarin dimethone is 4.98. These increases in pKa value may be due to hydrogen bonding in the neutral molecule, but in any event the values are useful in as much as they provide further evidence that both compounds contain a 4-hydroxycoumarin rather than a dimedone residue.

Both salicylaldehyde dimethone (diagram XIII) and 2-formyldimedone dimethone (diagram XII) contain dimedone residues, the hydroxyl groups of which appear to be hydrogen bonded. In both these cases a large enhancement of pKa value over dimedone is shown, the former having a pKa of 6.10, and the latter a pKa of 6.95, as compared with a pKa of 5.05 for dimedone.

b) Dibasic acids.

Dicoumarols and dimethones present a rather more complex picture. Superficially the presence of two hydrogen bonds might be expected to give two pKa values higher than that of the corresponding "monomer" (4-hydroxycoumarin or dimedone). This is found to be the case for formaldehyde dimethone and acetaldehyde dimethone, but for all the other bis derivatives examined, one pKa value was found to be lower, and one very much higher than that of the "monomer". All these results indicate that the mono-anion is very stable (i.e. easy dissociation to give the mono-anion, followed by a difficult dissociation

to give the di-anion:- pKa_2 for these compounds is generally 5 pKa units or more greater than the pKa of the corresponding "monomer"). This state of affairs may be compared with the dissociation of maleic acid; pKa_1, 2.22, pKa_2, 8.82 described in the Introduction as compared with, say, acetic acid (pKa 4.76) or formic acid (pKa 3.75). The neutral maleic acid molecule may form an intramolecular hydrogen bond, but hydrogen bonding in the mono-anion appears to be much more ideal in terms of O-H-O bond lengths. A similar phenomenon would explain the pKa values of dicoumarols and dimethones. The mono-anion of dicoumarol is shown is diagram XXXIV. Various canonical forms of this anion may be drawn and the negative charge may be shared over both rings. Knobloch has pointed out that the u.v. spectra of anions of 4-hydroxycoumarin residues indicate a chromone rather than a coumarin structure for the anions (i.e. the negative charge resides mostly on the 2-carbonyl group) so it would seem that much of the charge may be on the 2-carbonyl groups in this case too.



XXXIV

Presumably, if it were assumed that no hydrogen bonding was involved, then calculations on the basis of the Kirkwood-Wesheimer treatment (in which the ratio of the two pKa values is inversely proportional to the interprotonic distance) would only predict a comparatively small difference in the pKa values of dicoumarols, in which the interprotonic distance is much larger than in acids such as maleic and succinic.

Mitochondrial Studies.

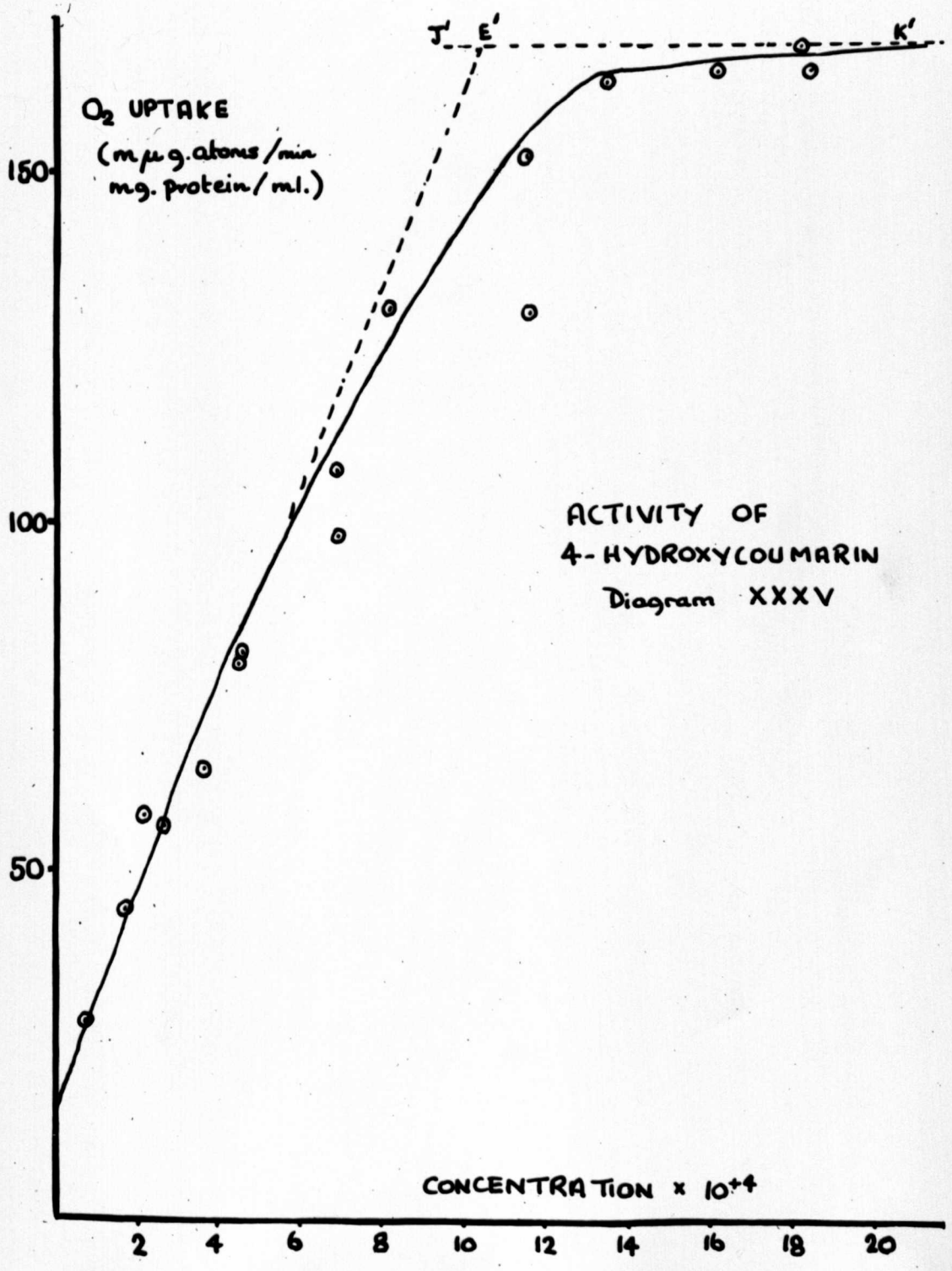
The compounds tried in this investigation may be divided into three classes on the basis of their effect on mitochondrial respiration.

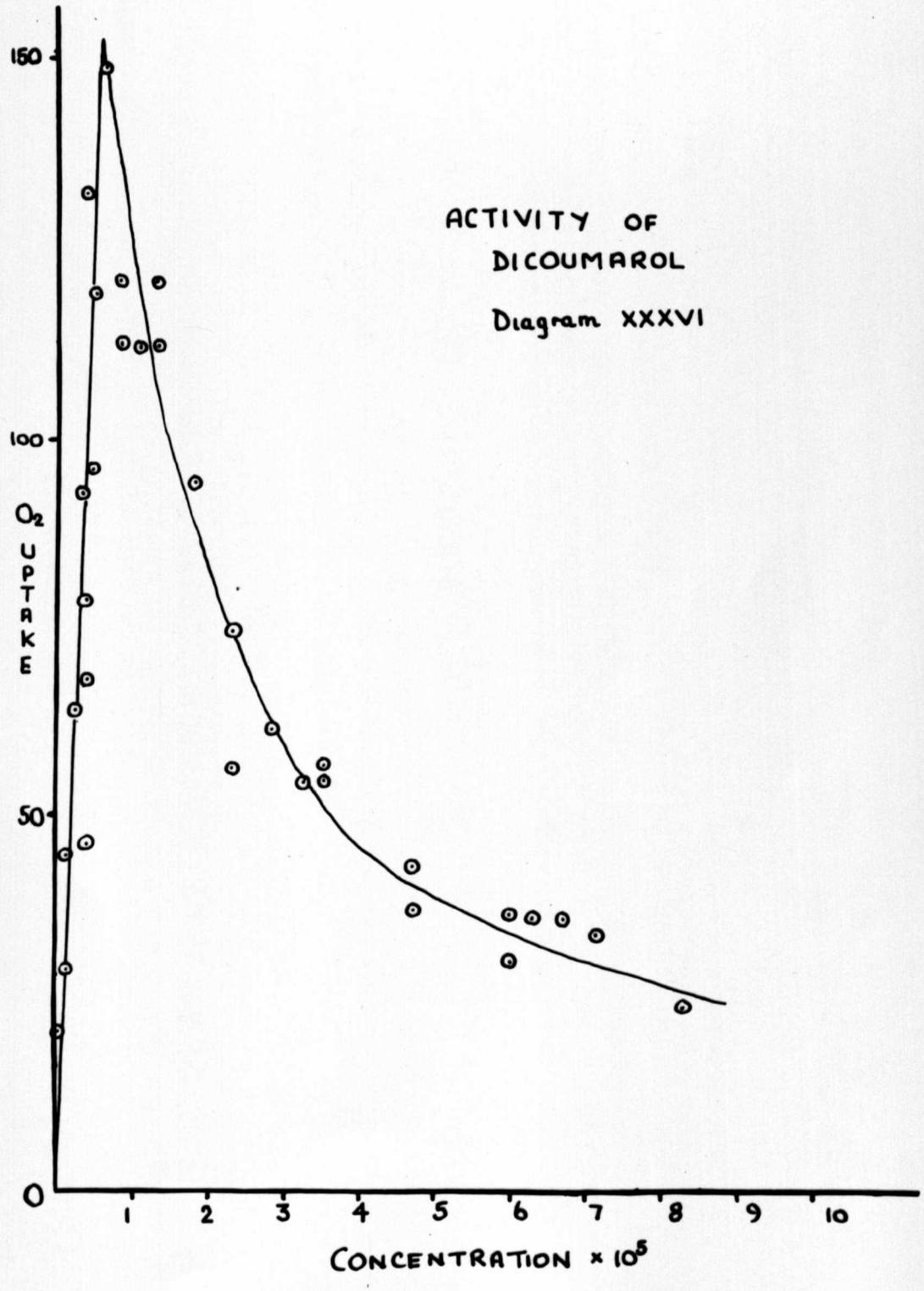
a) Those that uncouple, but do not inhibit, exemplified by 4-hydroxycoumarin, the concentration versus rate of oxygen uptake plot of which is given in diagram XXXV.

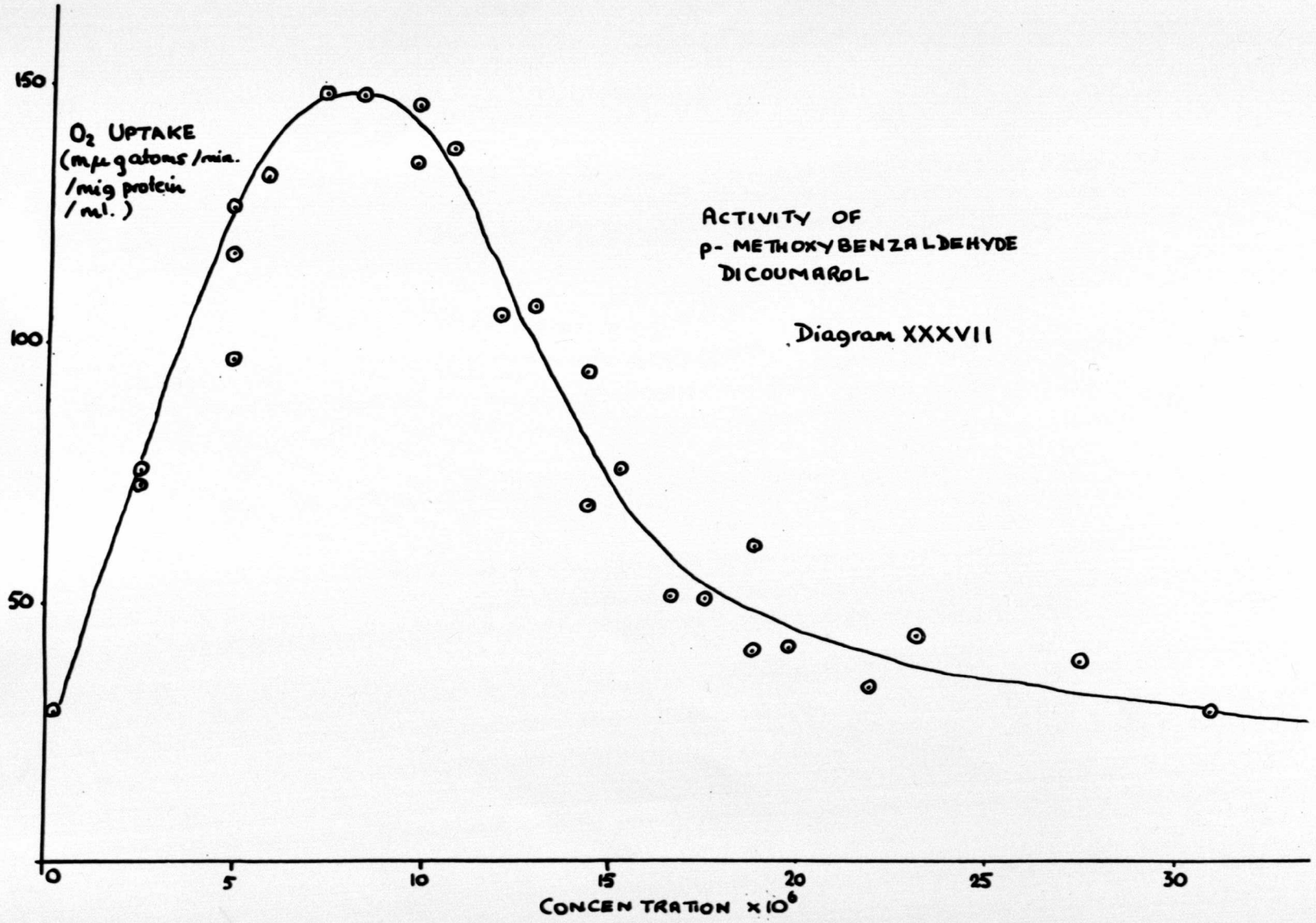
b) Those that uncouple at low concentrations, but inhibit at comparatively higher concentrations (See XXXVI and XXXVII for dicoumarol and p-methoxybenzaldehyde dicoumarol).

c) Inactive compounds.

A distinct structural difference is noted between compounds of type a) and compounds of type b). All compounds in group b) may form intramolecular hydrogen bonds involving 8-membered rings, none of those in group a) may do so. An idealised rate plot for a compound of group b) is given in diagram XXXVIII.

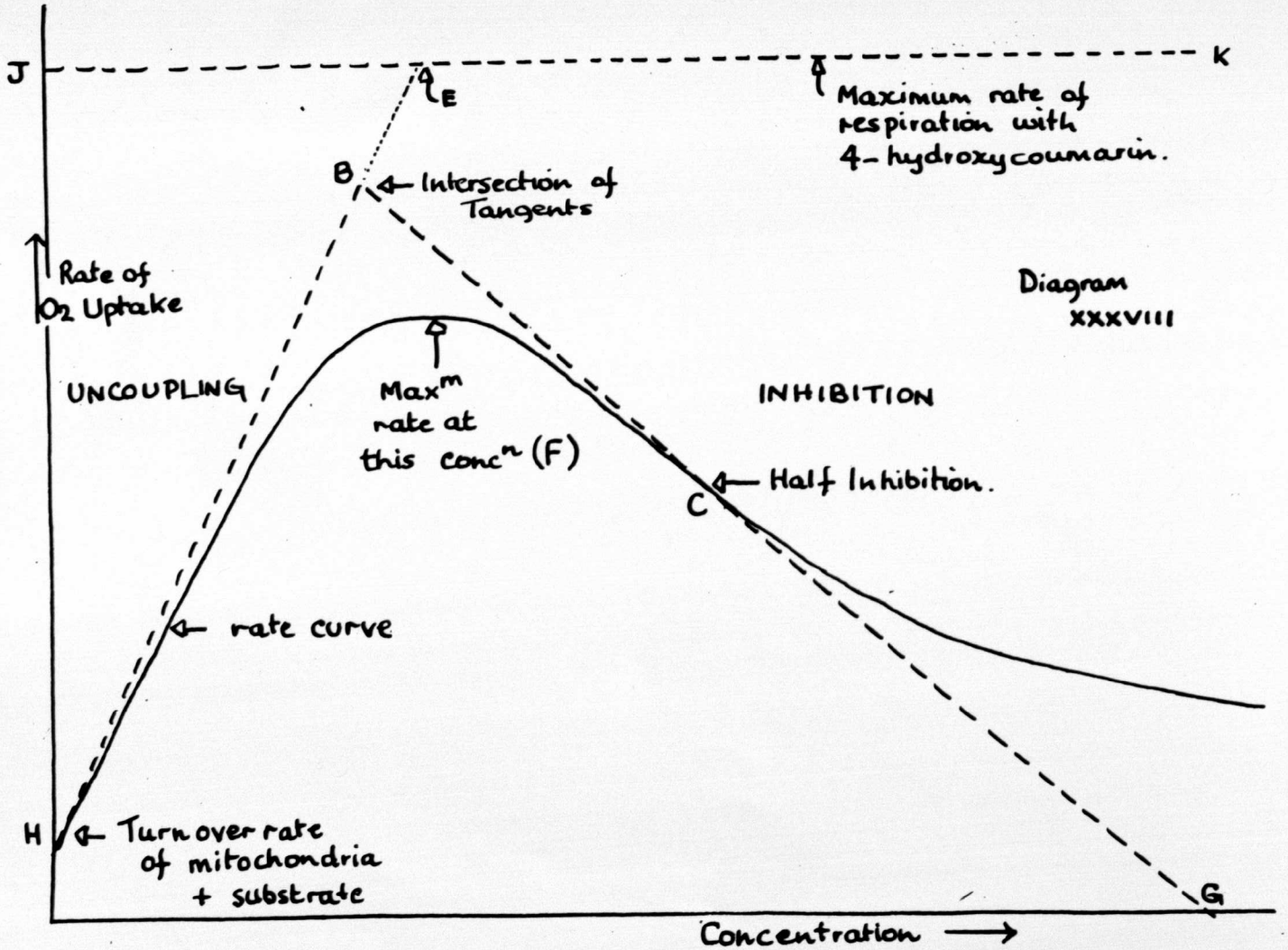

J'
E'
K'
O_2 UPTAKE
(mμg.atoms/min
mg. protein/ml.)
150
100
50
ACTIVITY OF
4-HYDROXYCOUMARIN
Diagram XXXV
CONCENTRATION x 10^{+4}
2
4
6
8
10
12
14
16
18
20


ACTIVITY OF
DICOUMAROL
Diagram XXXVI
150
100
50
0
O_2 UPTAKE
1
2
3
4
5
6
7
8
9
10
CONCENTRATION × 10^5



ACTIVITY OF
p-METHOXYBENZALDEHYDE
DICOUMAROL

Diagram XXXVII


J
K
E
Maximum rate of respiration with 4-hydroxycoumarin.
B
Intersection of Tangents
Rate of O2 Uptake
Diagram XXXVIII
UNCOUPLING
Max^m rate at this conc^n (F)
INHIBITION
Half Inhibition.
C
rate curve
H
Turnover rate of mitochondria + substrate
G
Concentration

HE is the initial slope of the rate curve, and intersects the line JK, which is an asymptote to the rate curve of a compound of group a). (See line J'K' in 4-hydroxycoumarin graph) BG is a tanget to the rate curve at point C, defined by the condition BC = CG. B is the point of intersection of the two tangents. The concentrations equivalent to points B,C, and E are recorded under those headings for a range of derivatives in the tables on p 122-3 . Column A is defined as maximum observed rate with uncoupler (see (F)) divided by the maximum rate obtained with dicoumarol. Column D is the ratio of the slope of BG to the slope of HB. It is a measurement of the effectiveness of uncoupling and inhibition, as evidenced by small changes of rate produced by vanishingly small additions of compound at points H and C. The data in tables allows a rough reconstruction of the rate plots for all the compounds cited.

Some of the points which may be deduced from the results are inherent in the above description of the graph. The maximum rates produced by compounds of group a) are equal to within experimental error, and are all greater than those observed for compounds of group b) (see column A) Presumably, if compounds of group b) did not inhibit, their graphs would resemble those of group a) compounds, and all give the same maximum rate. Accordingly, the concentration E should give a direct comparison with 4-hydroxycoumarin (concentration E' in diagram XXXVIII) of their efficiency as uncouplers in terms of concentration.

If the uncoupling is caused by binding with an enzyme, the value E will be inversely proportioned to K_s, the binding constant (see below).

It may be seen, then, that the results may be interpreted in terms of a common mechanism for all these derivatives. The results are in accord with the findings of Wilson and Merz, that dicoumarol acts at two independent sites, possibly the phosphorylation and substrate binding to dehydrogenase sites that they suggest. On this interpretation, compounds of group a) are presumably unable to bind to the enzyme(s) that bind substrate. It has been shown, as has been noted in the Introduction, that the respiration response to dicoumarol may be eradicated by adding bovine serum albumin to the dicoumarol treated mitochondria. It thus seems that interaction of dicoumarol with the mitochondria must involve reversible bindings with relevant enzymes, rather than a reaction with some functional group of importance in the phosphorylation sequence. The results here are in agreement with this theory, the large range of concentrations needed for maximal activity over the number of uncouplers and inhibitors examined is suggestive of binding phenomena, rather than mole for mole reactions. This suggestion is also consistent with the stability of dicoumarol referred to earlier, the possibility of easy oxidation of the protons of the methylene bridge, for instance, has been shown to be unlikely.

Further support for an enzyme hypothesis may be seen in diagram XLI, a graph of the rate of oxygen uptake at a fixed concentration of dicoumarol (7.1×10^{-1} molar, the concentration giving the maximum rate of respiration) against the concentration of mitochrondrial protein. This linear plot is to be expected if binding occurs to an enzyme, (E), by uncoupler (S) and $K_s \gg [E]$ Results shows that $K_s > 10^{-6}$ molar, and the concentration of inhibitors (such as Piericidin A) which are thought to act in mole for mole reactions with specific "sites" in the phosphorylation sequence act at concentrations in the order of 10^{-7} molar[103].

The substrate used in the above experiments was a mixture of glutamate and malate, which is known to give three molecules of ATP per mole equivalent of oxidisable compound in coupled electron transport. Investigations using 0.0027 molar succinate (which on the same basis as above gives two molecules of ATP) indicate that dicoumarol has a similar action in this case. Using equivalent values to those quoted in the tables, B = 0.35 , C = 0.49 , D = 0.67. These figures show that with succinate as substrate dicoumarol is effective in smaller concentrations than it is with glutamate and malate, and that inhibition is comparatively more effective in this case; (D for glutamate and malate is 2.88).

Structure-activity relationships.

Apart from the division made earlier on the grounds of hydrogen bonding, several other relationships may be seen from the tables on p 122-3. Comparison of the figures for methylene, ethylene,

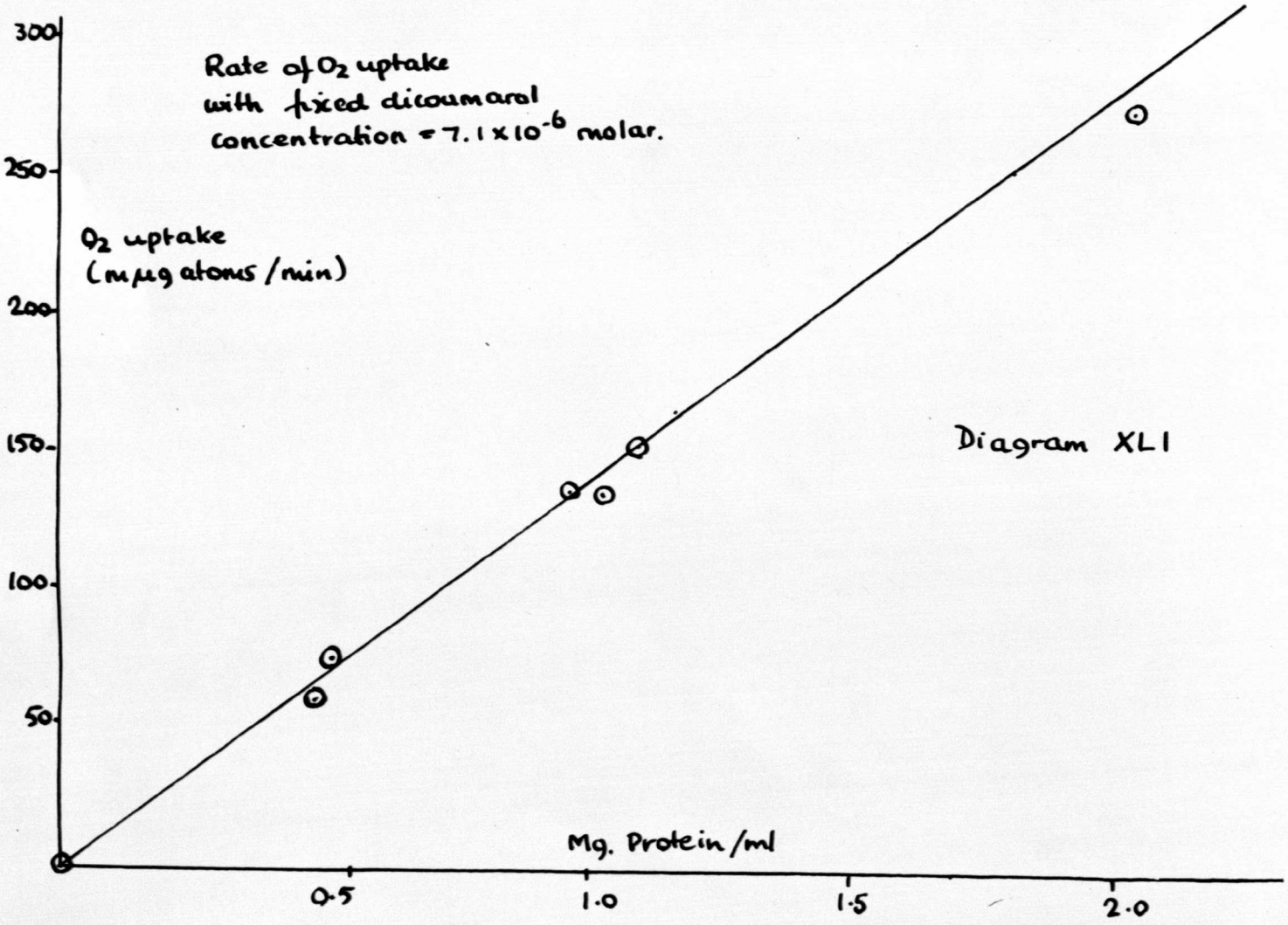

Rate of O_2 uptake
with fixed dicoumarol
concentration = 7.1×10^{-6} molar.
O_2 uptake
(mμg atoms / min)
300
250
200
150
100
50
Mg. Protein / ml
0·5
1·0
1·5
2·0

Diagram XL I

isopropylidene and benzylidene bis-4-hydroxycoumarins shows that in this series increase in the size of the substituent on the bridge carbon atom lowers the effectiveness of the compound in terms of the concentration necessary to give the maximum observed rate of respiration. Figures in column D indicate that this increase in size has a greater effect on binding at the site of uncoupling than at the site of inhibition. Substitution in the para-position of the phenyl group of benzaldehyde dicoumarol may give compounds that are more or less active than the parent compound. Substitution by methyl, methoxy or chloro groups (all of which groups mesomerically donate electrons to the π system of the phenyl group) give compounds more active than benzaldehyde dicoumarol, and in the case of p-tolualdehyde dicoumarol appreciably more active than dicoumarol itself. Substitution by the electron withdrawing nitro group, the phenolic hydroxyl group, or the basic dimethylamino group reduces the activity of the dicoumarol. A rough correlation between maximum observed rate (column A) and uncoupling efficiency (inversely proportional to the figures in column E) may also be seen.

It would be interesting to investigate the activity of dicoumarols in which both the protons of the methylene bridge of dicoumarol were replaced by other groups. Attempts to prepare acetone dicoumarol failed (see Experimental Section) and the only compound of this sort investigated was pyruvic acid dicoumarol. This compound was found to be inactive, though this

may be due to the carboxyl group rather than the di-substitution.

Replacement of one of the 4-hydroxycoumarin residues in dicoumarol by the analogous 4-hydroxy-6-methyl-α-pyrone residue gives a compound which retains much of the activity of dicoumarol. Replacement of both residues (as in ethylidene-bis-4-hydroxy-6-methyl-α-pyrone) gives a compound that is inactive. In a similar way, 4-hydroxy-6-methyl-α-pyrone itself in inactive.

The activity of 3-(o-hydroxybenzylidene)-2,4-diketochroman may possibly be due to reaction to give salicylaldehyde dicoumarol.

Replacement of the pyran oxygen atom of 4-hydroxycoumarin by an N-H group to give 4-hydroxycarbostyril does not greatly affect the activity, whereas analogous replacement by an $N-CH_3$ group to give N-methyl-4-hydroxycarbostyril produces an inactive compound.

Relationship with Activity as Anticoagulants.

Nitz - Litzow's discovery of the activity as uncouplers in oxidative phosphorylation of the well known anticoagulants of the coumarin series has provided the only appraisal of any possibility of a link between the two processes. (See Introduction). Dicoumarol, the most active anticoagulant he tried, was also the most effective uncoupler, in terms of concentration. Other similar relationships have been noted here. Arora et. al.[82] have investigated the anticoagulant activity of a series of p-substituted benzaldehyde dicoumarols in terms of their coagulation valency (an arbitrary scale set to the value 100 for an inactive compound and 0 for dicoumarol). Their results are given in the table on p122.

The close correspondence to the uncoupling activity is seen by comparison with the figures in column E. The only compound in this series which is out of order when comparing the activities of anticoagulation and uncoupling is p-hydroxybenzaldehyde dicoumarol, which was found to be inactive as an uncoupler at the concentration shown. A similar investigation on a series of bridge substituted dicoumarols by Guminska and Eckstein[84] has shown similar results in the case of p-nitro and p-chloro substituted benzaldehyde dicoumarols. The greater anticoagulant activity of methoxy as compared with hydroxy substituted derivatives of 4-hydroxycoumarins and dicoumarols, found by Arora and Mathur[85], is also consistent with the uncoupling activity found in this work. Although there is no known reason to tie up the chemical processes involved in blood coagulation and oxidative phosphorylation (mostly through lack of information on the intimate mechanism of the former process) these types of structure activity relationships do indicate that there may well be some connection.

EXPERIMENTAL SECTION

Starting materials

Starting materials were normally commercially available.

Chloroform, for spectroscopy, was purified by the method of Vogel.[86]

Spectra

N.m.r. spectra were run on a Perkin-Elmer R.10. spectrometer, and were run in deuterochloroform against tetramethylsilane as standard, unless stated to the contrary. Chemical shifts are recorded in τ values.

I.r. spectra were run on a Perkin-Elmer P.E. 237 spectrometer. Frequencies are recorded in cm^{-1}

U.v. spectra were run on a Unicam S.P.800 recording spectrophotometer, and were run using absolute ethanol as solvent unless stated to the contrary. Wavelengths are recorded in $m\mu$.

Spectra of Starting Materials

The following spectra are referred to in the text

4-hydroxycoumarin. N.m.r. spectrum in trifluoro-acetic acid, 4.01 (1H), 2.2 - 3.0 (4H, complex multiplet)

I.r. spectrum as Nujol mull, 1700, 1636, 1614, 1570.

I.r. spectrum in chloroform containing 2% ethanol, 1695, 1675, 1627, 1569. I.r. spectrum in dioxan, 1730, 1633, 1611, 1572. U.v. spectrum. λmax; 305 (8,500), 208 (10,900), 269 (9,400), 243 (3,200); sh; 318, 293, 257, 237, 232. In alkali, λmax; 297, 288; sh; 276, 242, 235.

Dicoumarol. N.m.r. spectrum, 6.46 (2H), 2.2 - 3.0 (6H, complex multiplet), 1.8 - 2.0 (2H, complex multiplet), - 1.7 (2H). I.r. spectrum in chloroform, 1659, 1630, 1603, 1573. I.r. spectrum in dioxan, 1659, 1627, 1599, 1569. I.r. spectrum as Nujol mull, 1655, 1630, 1602, 1569. I.r. spectrum as KCl disc. 1655, 1630, 1600, 1570. U.v. spectrum, λmax; 323 (16,900), 309 (23,100), 287 (20,500); sh; 317, 297, 276. In alkali, λmax; 315; sh; 292, 255, 242.

Pelantan. (Ethyl glyoxylate dicoumarol). N.m.r. spectrum, 8.70 (3H, triplet, J = 6.6 c./sec.), 5.61 (2H, quartet, J = 6.6 c./sec.), 4.44 (1H), 2.0 - 2.6 (6H, complex multiplet), 1.7 - 1.9 (2H, complex multiplet), - 1.48 (2H). I.r. spectrum in chloroform, 1735, 1658, 1618, 1600, 1568. U.v. spectrum, λmax; 310 (21,700), 283 (19,800); sh; 323, 277. In alkali; λmax; 313; sh; 294, 242.

Warfarin. (3-(α-acetonylbenzyl)-4-hydroxycoumarin) N.m.r. spectrum taken in sodium deuteroxide solution in deuterium oxide, 7.74 (3H), 7.14 (2H, doublet, J = 7.2 c./sec.), 4.92 (1H, triplet, J = 7.2 c./sec.), 2.3 - 2.9 (7H, complex multiplet), 1.8 - 2.0 (2H, complex multiplet). I.r. spectrum as Nujol mull, 1688, 1621, 1579. U.v. spectrum, λmax; 307 (11,200), 282 (12,900), 271 (11,500); sh; 320, 296, 260. In alkali, λmax; 313; sh; 292, 280.

Dehydracetic acid. N.m.r. spectrum, 7.73 (3H, doublet, J = 0.7 c./sec.), 7.35 (3H), 4.05 (1H, quartet, J = 0.7 c./sec.), - 7.01 (1H). I.r. spectrum in chloroform, 1740, 1721, 1644, 1611, 1560.

Methyl salicylate. N.m.r. spectrum, 6.03 (3H), 2.0 - 3.3 (4H, complex multiplet), - 1.24 (1H).

Salicylaldehyde. N.m.r. spectrum, 2.75 - 3.05 (2H, complex multiplet), 2.2 - 2.5 (2H, complex multiplet), - 0.10 (1H), - 1.19 (1H).

o-vanillin. N.m.r. spectrum, 6.20 (3H), 2.7 - 3.2 (3H, complex multiplet), 0.20 (1H), - 0.77 (1H).

I.r. spectrum in chloroform, 1658, 1587.

p-vanillin. N.m.r. spectrum, concentration 1.3 mole/litre, 6.02 (3H), 2.78 (1H), 2.7 - 2.9 (1H, ortho to hydroxyl group, complex multiplet), 2.3 - 2.5 (2H, ortho to formyl group, complex multiplet), 0.01 (1H). On tenfold dilution the chemical shift of the hydroxyl proton changes from 2.78 to 3.45.

I.r. spectrum in chloroform, 1686, 1600.

1-formyl-2-hydroxynaphthalene. N.m.r. spectrum, 1.2 - 3.0 (6H, complex multiplet), - 0.79 (1H), - 3.20 (1H).

2, 3-dihydroxybenzaldehyde. N.m.r. spectrum, 3.6 (1H), 2.6 - 3.1 (3H, complex multiplet), 0.07 (1H), - 0.3 (1H) (concentration, 1 mole/litre).

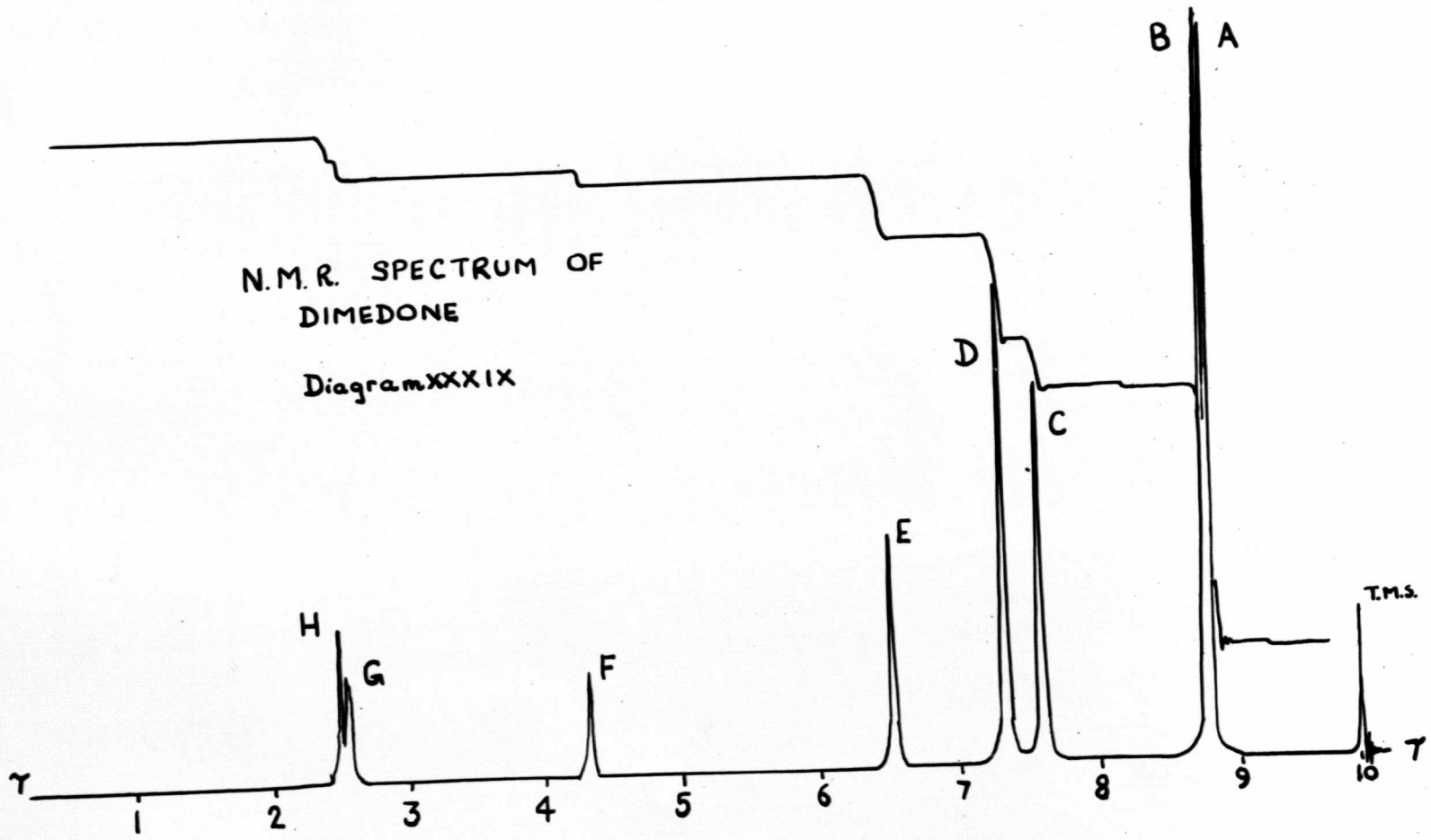
Dimedone. The N.m.r. spectrum in deuterochloroform is shown in Diagram XXIX. The spectrum shows peaks for both diketo and enol tautomers, and is analysed as follows.

A, 8.96, 5-methyl groups of keto form.

B, 8.90, 5-methyl groups of enol form.

C, 7.70, 4- and 6-methylene groups of enol form.

D, 7.42, 4- and 6-methylene groups of keto form.


N.M.R. SPECTRUM OF
DIMEDONE
Diagram XXXIX
B
A
D
C
E
H
G
F
T.M.S.
τ
1
2
3
4
5
6
7
8
9
10
τ

E, 6.60, 2-methylene group of keto form.

F, 4.41, 2-vinyl proton of enol form.

G, 2.62, hydroxyl group of enol form.

H, 2.54, H atom of chloroform impurity in deuterochloroform.

The assignments were made on the basis of the integration of the signals in the spectrum, and on the change in intensity of the signals with changing concentration as in the experiment described below.

Effect of concentration on the n.m.r. spectrum of Dimedone.

On dilution of a solution of dimedone in deuterochloroform (saturated at 30°) two changes were observed. The value of the chemical shift of the hydroxyl proton varied uniformly over the range of concentrations, being greater at high concentration, and the percentage of keto and enol forms in the solution, as measured by the integrated areas of the signals, changed towards a higher percentage of enol with increase in concentration of dimedone. At a concentration of dimedone of 0.39 moles/litre the percentage of enol was 40%, at 0.24 moles/litre the percentage of enol was 27%, and at 0.14 moles/litre the percentage of enol was 16%. Diagram XL is a plot of the chemical shift of the hydroxyl proton against the reciprocal of the concentration. At infinite concentration the value of the chemical shift of the hydroxyl proton is - 2.24.

Percentage of tautomers in other solutions.

The percentage of tautomers was estimated by comparison of integration of the 5-methyl peaks of the enol and keto forms

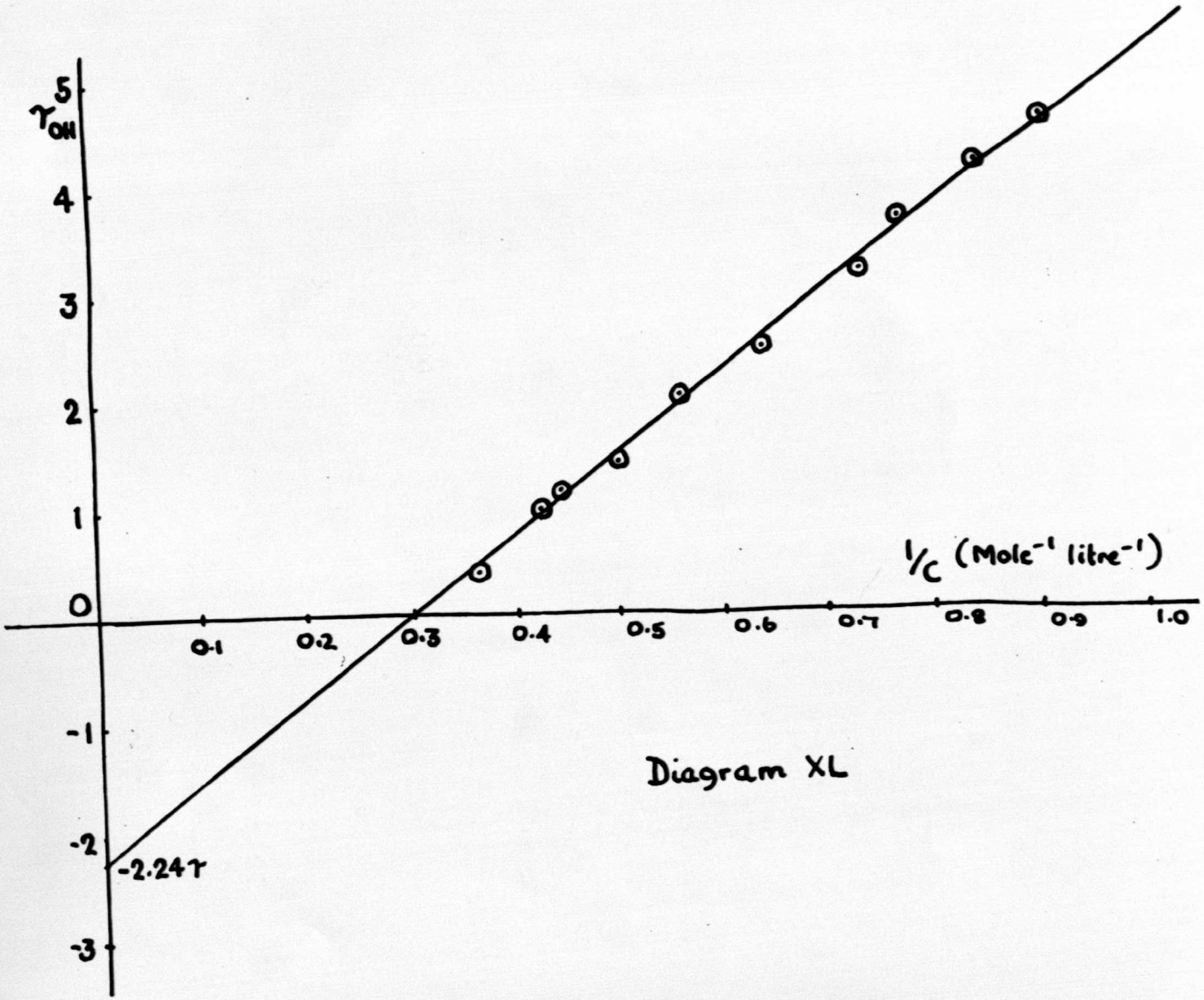

γ_{OH}
5
4
3
2
1
0
-1
-2
-3
-2.24τ
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
1/C (Mole^{-1} litre^{-1})

Diagram XL

in 0.25 molar solutions of dimedone in the following solvents. Methylene dichloride, 17% enol. Dioxan, 80% enol. Acetonitrile, 56% enol. (Dimedone was found to be too insoluble in carbon tetrachloride to give a satisfactory spectrum). The solvents used in this experiment were redistilled and dried over molecular sieve, grade 3A.

Spectrum in 2N sodium deuteroxide. Only peaks due to the enol form were detected in this solvent. 9.11 (6H), 7.87 (4H), 4.84 (1H). Tertiary butyl alcohol (CH_3 = 8.78) was used as standard. The vinyl proton, with chemical shift 4.84, was found to exchange. A rate plot showed the reaction to be first order with respect to dimedone, $k_{30^\circ} = 1.15 \times 10^{-4}$ sec. $^{-1}$.

Other spectra.

I.r. spectrum in chloroform, 1733, 1706, 1608, 1580.

I.r. spectrum as Nujol mull, 1610, 1580.

U.v. spectrum, λmax; 255 (17,700). In alkali, λmax; 278 (28,600).

Preparation of compounds.

3-methyl-4-hydroxycoumarin.

This compound was prepared from methyl salicylate according to the method of Link.[87] It was recrystallised from ethanol as colourless needles, m.p. 229-230°, after treatment with animal charcoal. Calc. for $C_{10}H_8O_3$; C: 68.18%; H: 4.58% Found, C: 68.37%, H: 4.68%. N.m.r. spectrum in trifluoro-acetic acid, 8.20 (3H), 2.2 - 3.0 (4H, complex multiplet). I.r. spectrum as Nujol mull, 1668, 1630, 1613, 1566.

I.r. spectrum in dioxan, 1700, 1626, 1609, 1570.
U.V. spectrum, λmax; 306 (13,200), 279 (13,600), 268 (12,300); sh; 320, 296, 258. In alkali, λmax; 311: sh: 292.

3-phenyl-4-hydroxycoumarin.

This compound was also prepared by the method of Link.[87] The methyl O-phenacylsalicylate was obtained from the crude reaction product by distillation at 1mm. b.p. 202°, m.p. 55°. Yield 43% theoretical. N.m.r. spectrum, 6.23 (3H), 6.11 (2H), 1.8 - 2.0 (1H, complex multiplet), 2.4 - 3.0 (8H, complex multiplet). I.r. spectrum in chloroform, 1760, 1722, 1680, 1610, 1587. The 3-phenyl-4-hydroxycoumarin was recrystallised from ethanol as colourless prisms, m.p. 264 - 266°, after treatment with animal charcoal. Yield, 57% theoretical. Calc. for $C_{15}H_{10}O_3$; C. 75.62%; H: 4.23%. Found: C: 75.29%; H: 4.49%. N.m.r. spectrum in trifluoro-acetic acid, 2.3 - 3.1 (complex multiplet). I.r. spectrum as Nujol mull, 1672, 1622, 1611, 1600. U.v. spectrum, λmax; 312, 280, 270. In alkali, λmax; 308; sh; 287, 243.

4-hydroxycarbostyril.

This compound was prepared by the method of Ziegler[88] N.m.r. spectrum in trifluoro-acetic acid, 3.20 (1H), 1.4 - 2.6 (3H complex multiplet). I.r. spectrum as Nujol mull, 1660, 1632, 1608, 1595, 1560. U.v. spectrum, λmax; 315, 279, 268; sh; 327, 300, 260. In alkali, λmax; 300, 238.

N-methyl-4-hydroxycarbostyril.

This compound was also prepared by the method of Ziegler.[88]

It was recrystallised from glacial acetic acid as yellow needles, m.p. 256-260°, after treatment with animal charcoal. Calc. for $C_{10}H_9O_2N$; C: 68.56; H: 5.18; N: 8.00. Found, C: 68.61; H: 5.49; N: 8.49. N.m.r. in trifluoro-acetic acid, 6.30 (3H), 3.40 (1H), 1.8 - 2.9 (4H, complex multiplet). I.r. spectrum as Nujol mull, 1643, 1611, 1578, 1555. U.v. spectrum in dioxan, λmax; 331 (16,600), 317 (18,400), 293 (16,000); sh; 280.

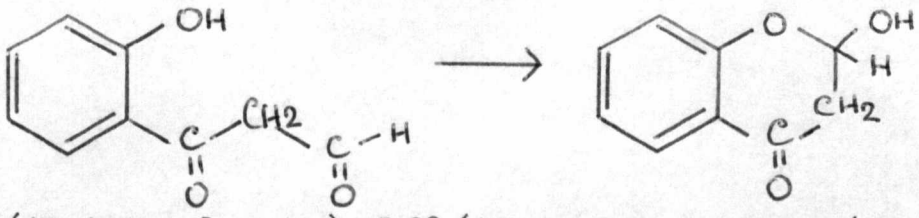
6-methyl-4-hydroxy-α-pyrone.

This compound was prepared by the method of Collie.[89] The crude product was washed with chloroform to remove any unreacted dehydracetic acid, and recrystallised from water as colourless needles, m.p. 178-189°. N.m.r. spectrum in trifluoro-acetic acid, 7.47 (3H), 3.74 (1H, in 5-position), 3.44 (1H, in 3-position). The signal at 3.74 was designated to the 5-proton by reference to the chemical shifts of the 5-protons of other 6-methyl-4-hydroxy--pyrones prepared. I.r. spectrum as Nujol mull, 1711, 1655, 1625, 1587. U.v. spectrum, λmax; 283 (6,600). In alkali, λmax; 278, 237.

Chromone.

Chromone was prepared by the method of Schonberg and Sina.[90] The ω-formylacetophenone did not crystallise from the reaction mixture on acidification, and was extracted from the reaction mixture with ether. It was obtained on evaporation of the ether after washing with sodium hydrogen carbonate solution. It was recrystallised from a mixture of benzene and light petroleum, b.p. 40°-60°. The n.m.r. spectrum of ω-formylacetophenone in chloroform

shows that it exists in the cyclic structure below in this solvent.



5.18 (1H, hydroxyl proton), 3.98 (1H, triplet, J = 4.3 c./sec.), 1.8 - 2.9 (4H, complex multiplet). 7.10 (2H, complex multiplet). The last peak is a doublet (J = 4.3 c./sec.) with further small splittings. It is part of an ABX system where J_{AB} is much smaller than J_{AX} which is almost equal to J_{BX}.

Chromone was recrystallised from 40° - 60° petroleum ether as pale yellow needles, m.p. 52-53°. N.m.r. spectrum in carbon tetrachloride, 3.62 (1H, α to carbonyl group, doublet, J = 6.2 c./sec.), 1.99 (1H, doublet, J = 6.2 c./sec.), 2.1 - 2.5 (3H, complex multiplet) 1.7 - 1.9 (1H, complex multiplet). I.r. spectrum in chloroform, 1647, 1619, 1601, 1569. U.v. spectrum, λmax; 303 (9,400), 298 (7,800); sh; 240.

Coumarin.

Coumarin was prepared by the general method for the Perkin reaction described in Organic Reactions[91], and purified by the method of Buckles.[92] It was obtained on recrystallisation from aqueous methanol as colourless needles, m.p. 65-68°. N.m.r. spectrum, 3.47 (1H, α to carbonyl group, doublet, J = 9.7 c./sec.), 2.12 (1H, doublet, J = 9.7 c./sec.), 2.3 - 2.8 (4H, complex multiplet). I.r. spectrum in chloroform, 1714, 1622, 1608, 1565. U.v. spectrum, λmax; 312 (8,200), 274 (16,200); sh; 283

Preparation of Dicoumarols

Dicoumarols were prepared by boiling under reflux a one and a half fold excess of the aldehyde in a 20% alcoholic solution of 4-hydroxycoumarin in ethanol, after the method of Link.[21] In the case of o-vanillin dicoumarol the half product of the reaction is precipitated initially, but reacts further to give the bis-product. For details, see Table p.82.

N.m.r. spectra of Dicoumarols.

Acetaldehyde dicoumarol, 7.97 (3H, doublet, J = 7.2 c./sec.) 5.04 (1H, quartet, J = 7.2 c./sec.) 2.0 - 2.6 (6H, complex multiplet, 1.6 - 1.8 (2H, complex multiplet), - 1.44 (1H), - 2.24 (1H).

Propionaldehyde dicoumarol, 9.00 (3H, triplet, J = 7.5 c./sec.), 7.51 (2H, complex multiplet), 5.19 (1H, triplet, J = 8.4 c./sec.), 2.0 - 2.7 (6H, complex mutliplet), 1.7 - 1.9 (2H, complex multiplet), - 1.84 (1H), - 2.66 (1H).

Benzaldehyde dicoumarol, 3.94 (1H), 1.9 - 2.9 (13H, complex multiplet), - 1.43 (2H).

Salicyaldehyde dicoumarol. Spectrum in trifluoro-acetic acid, 5.17 (1H), 2.3 - 3.4 (12H, complex multiplet).

o-vanillin dicoumarol. Spectrum in trifluoro-acetic acid, 6.36 (3H), 4.98 (1H), 2.2 - 3.8 (11H, complex multiplet)

p-hydroxybenzaldehyde dicoumarol mono-ethanolate, 8.76 (3H, triplet, J = 7.2 c./sec.), 7.26 (2H, quartet, J = 7.2 c./sec.) 3.93 (1H), 2.2 - 3.3 (10H, complex multiplet) 1.8 - 2.0 (2H, complex multiplet), - 1.48 (2H)

Preparation of Dicoumarols

Parent Aldehyde	% Yield	m.p. °C	Molecular Formula	H.calc.	H.found	C.calc.	C.found	Solvent for recrystallisation
Acetaldehyde	71	172-174	$C_{20}H_{14}O_6$	4.03	4.15	68.57	68.67	EtOH/$CHCl_3$
Propionaldehyde	60	142-144	$C_{21}H_{16}O_6$	4.40	4.68	69.23	69.58	EtOH/Dioxan
Benzaldehyde	73	226-230	$C_{25}H_{16}O_6$	3.91	3.87	72.81	72.80	EtOH/$CHCl_3$
Salicylaldehyde	85	245-247	$C_{25}H_{14}O_6$	3.41	3.40	73.16	73.29	EtOH
o-vanillin	83	281-283	$C_{26}H_{16}O_7$	3.66	3.98	70.91	70.88	Cyclohexanone
p-hydroxybenzaldehyde [a]	77	217-220	$C_{27}H_{22}O_8$	4.64	4.65	68.33	68.13	EtOH/$CHCl_3$
p-vanillin	75	245-248	$C_{26}H_{18}O_8$	3.96	3.94	68.12	68.59	EtOH
p-methoxybenzaldehyde	88	248-252	$C_{26}H_{18}O_7$	4.10	4.40	70.58	69.95	EtOH/$CHCl_3$
p-dimethylamino-benzaldehyde [b]	91	215-217	$C_{27}H_{21}O_6N$	4.65	4.79	71.20	70.98	EtOH/$CHCl_3$
p-nitrobenzaldehyde [c]	48	248-250	$C_{25}H_{15}O_8N$	3.30	3.48	65.65	65.50	EtOH/$CHCl_3$
p-tolualdehyde	79	268-271	$C_{26}H_{18}O_6$	4.26	4.30	73.23	72.87	EtOH/$CHCl_3$
p-chlorobenzaldehyde [d]	75	237-240	$C_{25}H_{15}O_6Cl$	3.36	3.54	67.19	66.99	EtOH/$CHCl_3$

a Recrystallised as mono-alcoholate
b Nitrogen analysis Calc; 3.08. Found, 3.22
c Nitrogen analysis Calc; 3.06. Found, 3.32
d Chlorine analysis Calc; 7.95. Found, 7.89

p-vanillin dicoumarol, 6.14 (3H), 3.93 (1H), 3.1 - 3.3 (4H, complex multiplet), 2.2 - 2.8 (6H, complex multiplet), 1.8 - 2.0 (2H, complex multiplet), - 1.44 (2H). Addition of deuterum oxide and reintegration of the signals showed that the hydroxyl proton of the p-vanillin residue has a chemical shift of between 2.2 and 2.8. (The deuterium exchange of all 3 hydroxyl protons is rapid on shaking the chloroform solution with deuterium oxide).

p-methoxybenzaldehyde dicoumarol, 6.22 (3H), 3.98 (1H), 2.2 - 3.3 (10H, complex multiplet), 1.9 - 2.1 (2H, complex multiplet), - 1.35 (2H).

p-dimethylaminobenzaldehyde dicoumarol. Spectrum in trifluoro-acetic acid, 6.56 (6H, doublet, J = 10.2 c./sec.), 3.65 (1H), 1.7 - 2.6 (12H, complex multiplet). (The splitting of the signal of the methyl groups is due to protonation on nitrogen by the trifluoro-acetic acid).

p-nitrobenzaldehyde dicoumarol, 3.90 (1H), 1.7 - 2.8 (12H, complex multiplet), - 1.35 (1H), - 1.53 (1H)

p-tolualdehyde dicoumarol, 7.67 (3H), 3.93 (1H), 2.90 (5H), 2.2 - 2.8 (6H, complex multiplet), 1.8 - 2.1 (2H, complex multiplet) - 1.42 (2H). The signal at 2.90 is due to the protons on the aromatic ring of the tolualdehyde residue. It is an unresolved tight multiplet.

p-chlorobenzaldehyde dicoumarol, 3.92 (1H), 2.2 - 3.0 (10H, complex multiplet), 1.8 - 2.0 (2H, complex multiplet), - 1.58 (1H), signal for other hydroxyl proton very broad, centred on 0.2.

U.v. and I.r. spectra of dicoumarols.

The U.v. and I.r. spectra of dicoumarols are given in Tables p. 85-6.

3, 3-thio-bis-4-hydroxycoumarin.

Prepared by the method of Klosa,[93] and recrystallised from cyclohexanone as colourless needles m.p. 311 (d).

Calc. for $C_{18}H_{10}O_4S$; C: 61.01; H: 2.82; S: 9.04.

Found; C: 60.95; H: 2.93; S: 8.92. I.r. spectrum as Nujol mull, 1672, 1610, 1599, 1547. U.v. spectrum λmax; 329, 317, 292; sh; 280. In alkali, λmax; 299, 277; sh; 287.

Pyruvic acid dicoumarol

4-hydroxycoumarin (3.26 g., 0.02 mole) was boiled under reflux in glacial-acetic acid (50 ml.) with pyruvic acid (0.88 g., 0.1 mole) for 45 minutes. After this time a mass of pink crystals had separated, which were filtered at the pump, dried, and recrystallised from cyclohexanone as colourless microcrystalline material, m.p. 220°(d). Yield, 59% theoretical. $C_{21}H_{14}O_8$ Requires C: 63.96; H: 3.58. Found, C: 63.96; H; 3.95. N.m.r. spectrum in sodium deuteroxide solution in deuterium oxide, 8.33 (3H), 1.9 - 2.9 (8H, complex multiplet). I.r. spectrum as Nujol mull, 1722, 1661, 1629, 1603, 1556. (This spectrum is very similar to the spectrum of pelantanic acid as described by Knobloch[23]). U.v. spectrum, λmax; 310, 285, 274; sh; 322.

3, 3'-methylene-bis-6-bromo-4-hydroxycoumarin.

This compound was prepared by the method of Link.[94] The methyl 5-bromosalicylate was prepared by the following method. Bromine (80 g., 1 mole) and methyl salicylate (77 g., 0.5 mole) were boiled under reflux for 5 hours carbon disulphide (800 ml.).

I.R. and U.V. spectra of Dicoumarols (1)

Parent aldehyde	U.v. spectrum in alcohol	U.v. spectrum in alkali	I.r. spectrum
Acetaldehyde	λmax; 310 (23,100) 288 (19,700); sh; 323, 319, 278	λmax; 316; sh; 293, 257, 242	1659, 1622, 1607, 1569.
Propionaldehyde	λmax; 311 (23,500) 289 (19,800); sh; 324, 319, 278	λmax; 315; sh; 294, 257, 242	1660, 1622, 1609, 1571
Benzaldehyde	λmax; 310 (21,600) 287 (19,800); sh; 324, 299, 277, 258	λmax; 310; sh; 293, 258, 240	1660, 1620, 1604, 1579.
Salicylaldehyde	λmax; 313 (21,700) 287 (19,100), 273 (20,900); sh; 328, 267	λmax; 303, 267; sh; 328, 317, 295, 290, 282, 256	1700, 1672, 1648, a 1615, 1608, 1570
o-vanillin	λmax; 310, 284, 278, sh; 328, 267	λmax; 305, 267, sh; 293, 255, 245	1720, 1662, 1641, a 1620, 1610, 1570.
p-hydroxybenzaldehyde	λmax; 310 (18,200) 288 (16,200); sh; 324, 277	λmax; 310, 240; sh; 293	1662, 1620, 1606 1571

a. spectrum as Nujol mull, compound insoluble in chloroform.

I.R. and U.V. spectra of Dicoumarols (2)

Parent Aldehyde	U.v. spectrum in alcohol	U.v. spectrum in alkali	I.r. spectrum
p-vanillin	λmax; 310 (22,400), 288 (21,100); sh; 325, 278	λmax; 308; sh; 295, 343	1660, 1628, 1610 [a] 1568
p-methoxybenzaldehyde	λmax; 311, (22,000) 288 (19,000), sh; 325, 278	λmax; 313; sh; 292	1660, 1620, 1604, 1580
p-dimethylaminobenzaldehyde [b]	λmax; 497 (7,000), 306 (22,200) 290 (18,700) 268 (19,300) sh; 326	λmax; 312, 244; sh; 295, 256	1661, 1620, 1609, 1580
p-nitrobenzaldehyde	λmax; 305 (22,400) 288 (22,800) 279 (22,600); sh; 325	λmax; 312; sh; 288, 255, 243	1660, 1620, 1603, 1578
p-tolualdehyde	λmax; 311 (20,300), 290 (19,200); sh; 325, 277	λmax; 313; sh; 294, 255, 242	1660, 1620, 1604 1579
p-chlorobenzaldehyde	λmax; 307 (19,900) 290 (17,900), 280 (17,000); sh; 313, 327	λmax; 313; sh; 294, 255, 243	1660, 1620, 1603 1589

[a] 3560 band for phenolic hydroxyl group.

[b] U.v. spectrum in acid, λmax; 312, 289.

On evaporation of the carbon disulphide and recrystallisation of the crude product from methanol, 100 gms of methyl 5-bromosalicylate were obtained as colourless needles, m.p. 61-62°. Yield, 70% theoretical. N.m.r. spectrum in carbon tetrachloride, 6.13 (3H), 3.27 (1H, doublet, J = 8.4 c./sec. for proton o- to hydroxyl group), 2.59 (1H, doublet of doublets, J = 8.4 c./sec. and J = 1.5 c./sec.), for proton p- to ~~acetyl~~ methoxycarbonyl group), 2.21 (1H, doublet J = 1.5 c./sec., for proton o- to ~~acetyl~~ methoxy-carbonyl. group), - 0.41 (1H). Methyl acetyl-5-bromosalicylate was recrystallised from methanol with the aid of a carbon dioxide acetone bath as colourless needles, m.p. 31.5 - 32°. N.m.r. spectrum, 7.81 (3H), 6.23 (3H), 3.13 (1H, doublet, J = 8.6 c./sec. for proton o- to acetyl group), 2.42 (1H, doublet of doublets, J = 8.6 c./sec. and 2.6 c./sec. for proton p to acetoxy group), 1.96 (1H, doublet, J = 2.6 c./sec., for proton o to acetoxy group).

The 3, 3'-methylene-bis-5-bromo-4-hydroxycoumarin was recrystallised twice from cyclohexanone and twice from ethanon-chloroform mixtures as colourless prisms, m.p. 310°(d). I.r. spectrum as Nujol mull, 1655, 1611, 1597, 1562.

3, 3'-methylene-bis-4-hydroxycarbostyril.

This compound was prepared by the method of Ziegler [95], and recrystallised from benzyl alcohol as colourless needles, m.p. 360 (d). N.m.r. spectrum in sodium deuteroxide solution in deuterium oxide, 6.06 (2H), 2.2 - 2.8 (6H, complex multiplet), 1.7 - 2.0 (2H, complex multiplet). I.r. spectrum as Nujol mull,

1660, 1609, 1560. U.v. spectrum, λmax; 330, 316, 303, 292; sh; 282. In alkali, λmax; 316; sh; 303.

3, 3'-methylene-bis-N-methyl-4-hydroxycarbostyril.

This compound was also prepared by the method of Ziegler,[95] and recrystallised from dimethylformamide as pale yellow needles, m.p. 344°(d). N.m.r. spectrum in trifluoro-acetic acid, 6.49 (6H), 6.22 (2H), 2.0 - 3.0 (8H, complex multiplet). I.r. spectrum as Nujol mull, 1643, 1611, 1578, 1555. U.v. spectrum in dioxan, λmax; 331 (16,600), 317 (18,400), 293 (16,000); sh; 280.

2-methylene-(3',4'-hydroxycoumarinyl)-dimedone.

This compound was prepared by the method of Hellmann and Shroder[72]. N.m.r. spectrum, 8.90 (6H), 7.63 (2H, protons of 6-methylene group), 7.56 (2H, protons of 4-methylene group), 6.44 (2H, protons of methylene bridge), 2.2 - 2.7 (3H, complex multiplet), 1.8 - 2.0 (1H, complex multiplet), - 1.10 (1H), - 2.25 (1H). I.r. spectrum in chloroform, 1657, 1628, 1603, 1572. U.v. spectrum, λmax; 322 (8,600), 308 (12,000), 269 (22,800); sh; 285. In alkali, λmax; 289.

Anhydride:- The above compound (0.100 g.) was dissolved in a mixture of methanol (7 ml.) and concentrated sulphuric acid (1 ml.) The whole was boiled under reflux for 1 hour, and on cooling a white crystalline product separated. The product was filtered off, washed with water, and recrystallised from methanol as colourless needles, m.p. 235 - 237°. Yield, 73% theoretical. $C_{18}H_{16}O_4$ requires C: 72.96, H: 5.44. Found, C: 72.50;

H: 5.58. N.m.r. spectrum, 8.82 (6H), 7.62 (2H, protons of 6-methylene group), 7.45 (2H, protons of 4-methylene group), 6.79 (2H, protons of methylene bridge), 2.1 - 3.0 (4H, complex multiplet). I.r. spectrum as Nujol mull, 1707, 1666, 1630, 1609. U.v. spectrum, λmax; 303 (7,000), 256 (20,400); sh; 337, 320, 292, 267, 248.

3-N-piperidinomethyl-4-hydroxycoumarin

This compound was prepared by the method of Link[74]. It separated from the alcohol solution after addition of ether as fine colourless plates, m.p. 182(d). Yield 70% theoretical. N.m.r. spectrum in trifluoro-acetic acid, 8.3 - 9.1 (6H, complex multiplet, protons of 3,-4- and 5-methylene groups of piperidino residue), 6.7 - 7.9 (4H, protons of 2- and 6-methylene groups of piperidino residue) 6.25 (2H, protons of methylene bridge), 2.3 - 3.5 (3H, complex multiplet). The chemical shifts of the 2- and 6-methylene groups are downfield with respect to the chemical shifts of the 3-, 4- and 5-methylene groups due to protonation of the nitrogen atom by trifluoro-acetic acid. I.r. spectrum as Nujol mull, 1671, 1610, 1580. U.v. spectrum, λmax; 288 (12,700); sh; 294, 274, 242, 234. In alkali, λmax; 308; sh; 291, 241. In acid, λmax; 310, 284, 273; sh; 323.

3-methylene-(3',4'-hydroxycoumarinyl)-4-hydroxycarbostyril.

3-N-piperidinomethyl-4-hydroxycoumarin (0.259 g., 0.001 mole) was added to a mixture of ethanol (15 ml.) and methyl iodide (1ml.). The resulting solution was boiled under reflux for

2 minutes, and finely ground 4-hydroxycarbostyril (0.161 g., 0.001 mole) added. The mixture was boiled under reflux for a further 3 hours, and on cooling, the product filtered at the pump. The product was recrystallised from benzyl alcohol as colourless microcrystalline material, m.p. 294 - 297°. Yield, 59% theoretical. $C_{25}H_{15}O_5N$ requires C: 73.34; H: 3.69, N: 3.42. Found, C: 73.05; H: 3.85; N: 3.12.

I.r. spectrum as Nujol mull, 1660, 1629, 1605, 1573.

U.v. spectrum, λmax; 309, 287; sh; 322, 277. In alkali, λmax; 313; sh; 290, 260.

3-methylene-(3', 4' -hydroxycoumarinyl)-N-methyl-4-hydroxycarbostyril

This compound was prepared by the method above, using N-methyl-4-hydroxycarbostyril (0.175 g., 0.001 mole) in place of the 4-hydroxycarbostyril. The product was recrystallised from glacial acetic acid as colourless needles, m.p. 229 - 232°. Yield, 58% theoretical. $C_{20}H_{15}O_5N$ requires C: 68.06; H: 3.91; N: 4.18. Found, C: 67.90, H: 4.23; N: 4.46.

N.m.r. spectrum in sodium deuteroxide solution in deuterium oxide, 6.78 (3H), 6.30 (2H), 1.9 - 3.2 (8H, complex multiplet).

I.r. spectrum as Nujol mull, 1660, 1631, 1608, 1572, 1550.

U.v. spectrum, λmax; 308 (17,300), 289 (16,300); sh; 331, 323, 316, 275. In alkali, λmax; 315, sh; 292, 257.

3-methylene-(3', 4' -hydroxycoumarinyl)-4-hydroxy-6-methyl- α -pyrone

This compound was prepared by the method of Molho and Mentzer.[76] It was recrystallised from ethanol as colourless needles, m.p. 188-190°.

Yield, 71% theoretical. N.m.r. spectrum, 7.73 (3H), 6.31 (2H), 3.94 (1H), 2.2 - 2.7 (3H, complex multiplet), 1.9 - 2.1 (1H, complex multiplet). - 2.09 (2H). I.r. spectrum in chloroform, 1679, 1660, 1633, 1612, 1603, 1576. U.v. spectrum, λmax; 307 (15,900), 286 (16,100); sh; 322, 274. In alkali, λmax; 307; sh; 292, 280, 241.

Addition products of 4-hydroxycoumarin and mesityl oxide.

Two products are formed when 4-hydroxycoumarin is boiled under reflux with mesityl oxide in ethanol solution. These products were prepared and separated by the method of Link.[26]

a) Acidic product. N.m.r. spectrum in trifluoro-acetic acid, 8.95 (3H), 8.75 (3H), 8.27 (3H), 2.2 - 2.9 (4H, complex multiplet). Also on AB pattern, with H_A = 7.78, H_B = 7.16, J_{AB} = 15.0 c./sec. I.r. spectrum as Nujol mull, 1668, 1617, 1572. U.v. spectrum, λmax; 318 (7,500), 304 (11,300), 281 (13,300), 269 (12,100); sh; 322, 308.

b) Heptane soluble product. N.m.r. spectrum, 8.51 (6H), 7.82 (3H, doublet, J = 1.6 c./sec.), 4.80 (1H, quartet, J = 1.6 c./sec.), 2.2 - 3.0 (4H, complex multiplet). I.r. spectrum in chloroform, 1703, 1651, 1611, 1605, 1552. U.v. spectrum, λmax; 347 (7,600), 246 (10,650).

Reduction of heptane soluble product:- This compound (1 g.) was shaken at room temperature (21°) with 10% palladium on charcoal (0.100 g.) in ethanol (20 ml.) under an atmosphere of hydrogen. Within 1 hour one molar equivalent of hydrogen was taken up.

During a further 3 hours no further hydrogen was taken up. The reaction mixture was filtered through Kieselguhr, and the alcohol evaporated under vacuo leaving an oil. The oil, which could not be crystallised from any of the solvent systems tried, did not form a picrate when boiled under reflux with a saturated solution of picric acid in ethanol. N.m.r. spectrum in carbon tetrachloride, 8.63 (3H), 8.45 (3H), 8.60 (3H, doublet, J = 6.5 c./sec.). Methylene group and vicinal proton as ABX system, H_A at 7.92, H_B at 8.35, J_{AB} = 13.9 c./sec., J_{AX} = 6.7 c./sec. J_{BX} = 10.0 c./sec. 7.15 (1H, complex multiplet), 2.2 - 3.0 (3H, complex multiplet). I.r. spectrum as liquid film, 1712, 1622, 1575. U.v. spectrum, λmax; 317, 304, 281, 270; sh; 312, 293, 258, 243.

Diazomethane

Diazomethane was prepared from p-tolylsulphonylmethylnitrosamide, as described in Organic Syntheses.[96]

Methyl ethers of 4-hydroxycoumarin

An excess of diazomethane was added to a suspension of 4-hydroxycoumarin (3 g.) in ether (200 ml.). After standing for 2 hours, 4-methoxycoumarin (1.5 g.) was precipitated. The remaining solution was evaporated to dryness, and extracted 3 times with ice cold 20% hydrochloric acid (10 ml.) The combined hydrochloric acid extracts were treated with sodium carbonate, extracted with ether, and washed with 2N sodium hydroxide solution. On evaporation of the ether, the remaining solid (0.250 g.) was separated by thin layer chromatography on silica, GF 254, using dry

ether as developing solvent, to give two fractions, one with R_F, 0.6 (identified on extraction as 4-methoxycoumarin) and the other with R_F 0.25 (identified on extraction as 2-methoxychromone). 0.200 g. of pure 2-methoxychromone (m.p. 103-4) were obtained by this method.

Spectra of 4-methoxycoumarin, n.m.r. spectrum in carbon disulphide, 6.00 (3H), 4.46 (1H), 2.2 - 3.0 (4H, complex multiplet).

I.r. spectrum in chloroform, 1710, 1619, 1603, 1562.

I.r. spectrum as Nujol mull, 1713, 1618, 1602, 1562.

U.v. spectrum, λmax; 309 (8,000), 282 (10,900), 272 (11,600); sh; 325, 300.

Spectra of 2-methoxychromone, n.m.r. spectrum, 6.05 (3H), 4.41 (1H), 2.2 - 2.8 (3H, complex multiplet), 1.7 - 1.9 (1H, complex multiplet). I.r. spectrum in chloroform, 1633, 1629, 1573. I.r. spectrum in dioxan, 1651, 1627, 1573. I.r. spectrum in ethanol, 1622, 1568. I.r. spectrum as Nujol mull, 1659, 1623, 1609, 1571. U.v. spectrum, λmax; 293 (5,500), 282 (5,900), 261 (8,300); sh; 252.

Methyl ethers of 3-methyl-4-hydroxycoumarin

The method of separation of the isomers was conducted as described above for the methyl ethers of 4-hydroxycoumarin. The 3-methyl-4-methoxycoumarin did not crystallise from the ether on standing, and was obtained on recrystallisation of the residue remaining after the 20% hydrochloric acid extraction. Colourless needles, (m.p. 43-44°) were obtained on recrystallisation from light petroleum, b.p. 40°-60°. Using dry ether as developing solvent

under the same condition as above, the mean R_F value of the 3-methyl-2-methoxychromone was 0.33. It was obtained as colourless needles, m.p. 82 - 84°, on extraction from the chromatography plate.

Spectra of 3-methyl-4-methoxycoumarin, n.m.r. spectrum in carbon tetrachloride, 7.87 (3H), 5.97 (3H), 2.2 - 2.9 (4H, complex multiplet). I.r. spectrum in chloroform, 1710, 1632, 1618, 1578. U.v. spectrum, λmax; 310 (7,800), 282 (10,500), 272 (11,400).

Spectra of 3-methyl-2-methoxychromone, n.m.r. spectrum in carbon tetrachloride, 8.12 (3H), 5.87 (3H), 2.4 - 2.8 (3H, complex multiplet), 1.7 - 1.9 (1H, complex multiplet). I.r. spectrum in chloroform, 1630, 1565. I.r. spectrum as Nujol mull, 1630, 1570. U.v. spectrum, 297 (8,400), 289 (8,400), 272 (7,500); sh; 263.

Dimethyl ethers of dicoumarol.

Dicoumarol (1.200 g.) was treated with excess diazomethane (solution in ether), and allowed to stand for 2 hours. On evaporation of the ether, 1.274 g. of material were given. Separation on a silica column with ether-heptane mixtures allowed the separation of the 4, 4' -dimethyl ether(0.606 g.), and the 4, 2' -dimethyl ether (0.314 g.). On extraction of the silica in the column with methanol a further 0.070 g. of material was obtained, which was separated on thin layer chromatography with silica, using ether as developing solvent, into 3 bands. The band of lowest R_F (0.025)

was extracted, and on evaporation gave 0.006 g. of a colourless crystalline material, identified as the 2, 2' -dimethyl ether by means of the spectra recorded below. Spectra of 4, 4' - dimethyl ether, n.m.r. spectrum, 5.87 (6H), 5.95 (2H), 2.2 - 2.9 (8H, complex multiplet). I.r. spectrum in chloroform, 1725, 1630, 1579. U.v. spectrum, λmax; 314 (23,600), 285 (21,300), 275 (19,400); sh; 323. Spectra of 4, 2' -dimethyl ether, n.m.r. spectrum, 5.83 (6H), 6.08 (2H), 2.2 - 2.8 (7H complex multiplet), 1.7 - 1.9 (1H, complex multiplet). (The chemical shifts of the 2' -methoxy and 4-methoxy groups are not separable using deuterochloroform as solvent.) Spectrum in trifluoro-acetic acid, 6.40 (2H), 6.17 (3H), 5.90 (3H), 2.2 - 2.8 (7H, complex multiplet) 1.7 - 1.9 (1H, complex multiplet). I.r. spectrum in chloroform, 1725, 1630, 1581, 1572. U.v. spectrum λmax; 310 (21,000), 285 (21,300), 275 (19,400); sh; 323. 2, 2' -dimethyl ether, i.r. spectrum in chloroform, 1627, 1565. U.v. spectrum, λmax; 289, 267.

Dicoumarol epoxide

This compound was prepared by the method of Link.[73] It was twice recrystallised from cyclohexanone, and gave colourless needles. m.p. 324 - 326°. N.m.r. spectrum in trifluoro-acetic acid, 7.14 (2H), 2.2 - 2.8 (8H, complex multiplet). I.r. spectrum as Nujol mull, 1711, 1679, 1633, 1611. U.v. spectrum in dioxan, λmax; 310, 294, 264, 256; sh; 275.

4-Monomethyl ether of Dicoumarol.

This compound was prepared from dicoumarol epoxide in the manner

described by Link.[73] It was recrystallised from methanol as colourless needles, m.p. 172 - 174°. N.m.r. spectrum in trifluoro-acetic acid, 6.39 (2H), 6.11 (3H), 2.2 - 2.8 (8H, complex multiplet). I.r. spectrum as Nujol mull, 1694, 1667, 1617, 1570. U.v. spectrum, λmax; 310 (17,900), 285 (19,400), 275 (17,100); sh; 322. In alkali, λmax; 312, 285; sh; 274, 242.

Benzaldehyde dicoumarol epoxide.

Prepared by the method of Link.[73] N.m.r. spectrum in trifluoro-acetic acid, 4.95 (1H), 1.7 - 3.0 (13H, complex multiplet). I.r. spectrum as Nujol mull, 1730, 1719, 1669, 1610. U.v. spectrum, λmax; 289, 264, 248, 209; sh; 332, 317.

4-monomethyl ether of benzaldehyde dicoumarol.

This compound was prepared from the epoxide by the method described by Link for the 4-monomethyl ether of dicoumarol.[73] On heating, or attempting recrystallisation from alcohols, or standing over a period of days, the compound reverted to the epoxide. N.m.r. spectrum in trifluoro-acetic acid 6.25 (3H), 3.83 (1H), 2.3 - 3.2 (13H, complex multiplet).

p-acetoxybenzaldehyde dicoumarol epoxide.

p-hydroxybenzaldehyde dicoumarol mono-ethanolate (0.498 g., 0.001 mole), was dissovled in a 50% solution of acetic anhydride in pyridine (5 ml.). On standing for 2 hours at room temperature a colourless solid had separated, which was collected by filtration, and recrystallised from a mixture of ethanol and chloroform as a colourless prisms, m.p. 350°. Yield, 74%

theoretical. $C_{27}H_{26}O_7$ requires C: 71.68; H: 3.56. Found, C: 71.55; H: 3.70. N.m.r. spectrum, 7.77 (3H), 4.81 (1H), 1.7 - 3.4 (12H, complex multiplet). I.r. spectrum in chloroform, 1760, 1735, 1669, 1611, 1583. U.v. spectrum, 305, (11,500), 291 (12,500), 263.5 (20,300), 256 (20,000), 249 (21,900); sh; 334, 317.

p-acetoxy-o-methoxybenzaldehyde dicoumarol epoxide

p-vanillin dicoumarol (0.229 g., 0.0005 mole) was dissolved in a 40% solution of acetic anhydride in pyridine (5 ml.). On standing for two days the solution was poured into water. A white solid separated, which was filtered off, washed with water, and recrystallised from chloroform as colourless prisms, m.p. 281 - 284°. $C_{28}H_{18}O_8$ requires C: 69.71; H: 3.76. Found, C: 69.64; H: 4.21. N.m.r. spectrum, 7.79 (3H), 6.19 (3H), 4.89 (1H), 3.2 - 3.3 (2H, complex multiplet), 2.4 - 2.8 (7H, complex multiplet), 1.8 - 2.1 (2H, complex multiplet). I.r. spectrum in chloroform, 1762, 1733, 1726, 1669, 1611. U.v. spectrum, λmax; 315 (15,500), 262 (24,700), 247 (26,00); sh; 333, 307.

Dimethones.

Dimethones were prepared by boiling dimedone under reflux in 50% aqueous ethanol with a one and a half fold excess of aldehyde. They were recrystallised from aqueous ethanol. Yields were between 70% and 80% theoretical.

Spectra of Dimethones.

Formaldehyde dimethone. N.m.r. spectrum, 8.92 (12H), 7.74 (8H),

6.92 (2H), - 1.31 (2H). I.r. spectrum in chloroform, 1605 - 1585 (broad band). I.r. spectrum as Nujol mull, 1605 - 1585 (broad band). U.v. spectrum, λmax; 254 (30,000). In alkali, λmax; 285 (47,200).

Acetaldehyde dimethone. N.m.r. spectrum, 8.92 (12H), 7.79 (8H), 8.48 (3H, doublet, J = 7.4 c./sec.) 5.77 (1H, quartet, J = 7.4 c./sec.), - 2.74 (1H). Other hydroxyl proton, very broad, centred around - 0.4.

Benzaldehyde dimethone. N.m.r. spectrum, 8.85 (12H), 7.63 (8H), 4.56 (1H), 2.83 (5H, centre of tight multiplet for protons on phenyl group), - 0.57 (1H), - 1.76 (1H).

Salicylaldehyde dimethone. N.m.r. spectrum, 9.24 (6H), 9.20 (3H), 9.11 (3H), 8.05 (2H), 7.69 (2H), 7.61 (2H), 7.49 (2H), 5.33 (1H), 2.99 (4H, tight multiplet), - 0.42 (1H). I.r. spectrum in chloroform, 1650, 1597, 1583. U.v. spectrum, λmax; 267 (15,900), 223 (14, 900); sh; 295. In alkali, λmax; 285 (23,600); sh; 233.

p- dimethylaminobenzaldehyde dimethone. N.m.r. spectrum, 8.85 (12H), 7.64 (6H, methyl protons of dimethylamino group), 7.16 (8H), 4.60 (1H), - 0.76 (1H), - 1.71 (1H).

Dimethone Anhydrides.

When dimethones are dissolved in hot ethanol to which a small quantity of concentrated hydrochloric acid has been added, loss of water occurs between the two dimedone residues to give the anhydride. On cooling, the anhydride separates from the solution.[97] The following anhydrides, the n.m.r. spectra of which are given below,

were prepared in this way.

Formaldehyde dimethone anhydride. N.m.r. spectrum in carbon tetrachloride, 8.87 (12H), 7.80 (4H), 7.66 (4H), 6.50 (2H). I.r. spectrum in chloroform, 1660, 1621.

Acetaldehyde dimethone anhydride. N.m.r. spectrum, 8.91 (3H, doublet, J = 6.9 c./sec.), 8.89 (12H), 7.73 (4H), 7.63 (4H), 6.32 (1H, quartet, J = 6.9 c./sec.).

Cinnamaldehyde dimethone anhydride. N.m.r. spectrum in trifluoro-acetic acid, 8.90 (6H), 8.79 (6H), 7.51 (4H), 7.35 (4H), 5.7 (1H, doublet, J = 5.0 c./sec.), 4.3 - 4.6 (1H, complex multiplet), 2.5 - 3.1 (6H, complex multiplet).

2, 2' - methylene-bis-1, 3-cylohexanedione.

This compound was prepared by the method of King and Felton.[98] N.m.r. spectrum, 8.04 (4H, quintet, J = 6.0 c./sec.), 7.62 (8H, triplet, J = 6.0 c./sec.) 6.88 (2H), - 1.64 (2H). I.r. spectrum in chloroform, 1600 (broad). I.r. spectrum as Nujol mull, 1603 - 1573 (broad band). U.v. spectrum, λ max; 263 (30,600); sh; 292. In alkali, λmax; 282, sh; 303.

3, 3 - methylene-bis-4-hydroxy-6-methyl-α-pyrone

4-hydroxy-6-methyl-α-pyrone (0.504 g., 0.004 mole) was dissolved in methanol (5 ml.) and formaldehyde added (as 45% w/v formalin, 1 ml.) On standing for 7 days and dilution with water, colourless needles were precipitated, which were filtered off and recrystallised from aqueous methanol as colourless needles, m.p. 244-245°. Yield, 67% theoretical. N.m.r. spectrum, 7.76 (6H), 6.50 (2H), 4.01 (2H), - 0.80 (2H).

I.r. spectrum in chloroform, 1680, 1617, 1583. I.r. spectrum as Nujol mull, 1679, 1615, 1580. U.v. spectrum, λmax; 290 (15,200). In alkali, λmax; 288; sh; 245.

3,3-ethylidene-bis-4-hydroxy-6-methyl-α-pyrone.

4-hydroxy-6-methyl-α-pyrone (0.504 g., 0.004 mole) was dissolved in 33% aqueous ethanol (15 ml.) and a two fold excess of acetaldehyde added. The mixture was boiled under reflux for 30 minutes, and the solvent removed under vacuo. The residue was recrystallised from aqueous methanol as colourless needles, m.p. 147-148°. Yield 41% theoretical. $C_{14}H_{14}O_6$ requires C; 60.43; H: 5.07. Found, C: 60.19; H: 5.02.

N.m.r. spectrum, 8.30 (3H, doublet, J = 7.2 c./sec.), 7.75 (6H), 5.59 (1H, quartet, J = 7.2 c./sec.), 4.00 (2H), - 1.22 (2H).

I.r. spectrum in chloroform, 1681, 1517, 1573. U.v. spectrum, λmax; 290 (15,900). In alkali, λmax; 288.

3,3-benzylidene-bis-4-hydroxy-6-methyl-α-pyrone

This compound was prepared by the method above for the 3,3-ethylidene-bis-4-hydroxy-6-methyl-α-pyrone, only using a two fold excess of benzaldehyde in place of the acetaldehyde. The product was recrystallised from aqueous methanol as colourless prisms, m.p. 208 - 210°. Yield, 30% theoretical. $C_{19}H_{16}O_6$ requires C: 67.05; H: 4.75. Found, C: 67.15; H: 4.81.

N.m.r. spectrum, 7.71 (3H), 4.19 (1H), 3.91 (2H), 2.72 (5H, tight multiplet), - 0.91 (2H). I.r. spectrum in chloroform, 1683, 1616, 1572. I.r. spectrum as Nujol mull, 1680, 1623, 1575.

U.v. spectrum, λmax; 290 (16,200). In alkali, λmax; 287.

3-acetyl-4-hydroxycoumarin and 4-acetoxycoumarin.

These compounds were prepared from 4-hydroxycoumarin and acetyl chloride by the method of Arakawa.[99]

3-acetyl-4-hydroxycoumarin was recrystallised from ethanol as yellow needles, m.p. 134-135°. (The crystals sintered at 115°). N.m.r. spectrum, 7.18 (3H), 1.7 - 2.7 (4H, complex multiplet), - 8.29 (1H). I.r. spectrum in chloroform, 1720, 1615 1549. I.r. spectrum as Nujol mull, 1720, 1615, 1549. U.v. spectrum, λmax; 325 (13,700), 302 (19,200); sh; 290. In alkali, λmax; 300. U.v. spectrum in light petroleum, b.p. 40-60° λmax; 337 (13,700), 326 (15,600), 302 (18,700), 289 (15,800). 4-acetoxycoumarin was recrystallised from a mixture of benzene and light petroleum, b.p. 60-80° as fine colourless needles, m.p. 105-106°. N.m.r. spectrum, 7.50 (3H), 3.26 (1H), 2.1 - 2.6 (4H, complex multiplet). I.r. spectrum in chloroform, 1790, 1728, 1628, 1610, 1572. U.v. spectrum, λmax; 310 (3,200), 270 (5,600); sh; 280.

3-formyl-4-hydroxycoumarin.

This compound was prepared by two methods.

a) The method of Ziegler and Maier[81] was adapted, N, N-dimethylformamide dried over molecular sieve, (grade 3A) was used in place of the N-methyl-N-phenylformamide, in equimolar quantities. The reaction mixture was warmed and stirred for 2 hours, when 4.5 g. of 4-hydroxycoumarin separated, and were removed by filtration. On dilution of the filtrate with water,

0.500 g. of 3-formyl-4-hydroxycoumarin dicoumarol were obtained as microcrystalline material, m.p. $>330^{\circ}$.

On separating the 3-formyl-4-hydroxycoumarin by extraction of the remaining solution with chloroform, and recrystallisation the residue remaining after the evaporation of the chloroform from cyclohexane, 3-formyl-4-hydroxycoumarin (0.138 g.) was obtained as yellow needles, m.p. 134-136°. Calc. for $C_{10}H_6O_4$, C: 63.16; H: 3.18. Found, C: 63.49; H: 3.38.

b) The method of Checchi.[83] The 3-formyl-4-hydroxycoumarin was recrystallised from cyclohexane as above. Calc. for $C_{10}H_6O_4$, C: 63.16; H: 3.18. Found, C: 63.41; H: 3.36. N.m.r. spectrum, 1.7 - 2.7 (4H, complex multiplet), - 0.21 (1H), - 2.00 (1H). I.r. spectrum in chloroform, 1733, 1641, 1628, 1568. U.v. spectrum, λmax; 304 (13,700); sh; 345, 283, 270. In alkali, λmax; 301. U.v. spectrum in light petroleum, b.p. 40°-60°, λmax; 348 (11,300), 334 (12,400), 304 (16,100), 293 (12,600); sh; 320.

3-formyl-4-hydroxycoumarin dicoumarol.

This compound was obtained as above as a biproduct in the preparation of 3-formyl-4-hydroxycoumarin. N.m.r. spectrum in trifluoro-acetic acid, 4.96 (1H), 2.0 - 3.3 (12H, complex multiplet). I.r. spectrum as Nujol mull, 1728, 1694, 1669, 1610, 1570. U.v. spectrum, λmax; 312, 268, 248; sh; 335, 240. In alkali, λmax; 297, 269, 238; sh; 355, 247.

2-formyldimedone.

This compound was prepared by the method of Akehurst and Bartels-Keith[100]

N.m.r. spectrum, 8.88 (6H), 7.61 (2H), 7.40 (2H), 0.20 (1H), - 5.60 (1H). I.r. spectrum in chloroform, 1671, 1630, 1589. U.v. spectrum, λmax; 274, 233. In alkali, λmax; 272, 258; sh; 243.

2-formyldimedone dimethone.

This compound was obtained as a biproduct from the reaction above as described by Akehurst and Bartels-Keith.[100] N.m.r. spectrum, 9.03 (6H), 8.99 (6H), 8.88 (6H), 7.97 (2H), 7.72 (4H), 7.64 (2H), 7.52 (4H), 5.58 (1H), - 0.03 (1H). I.r. spectrum in chloroform, 1660, 1643, 1619. U.v. spectrum, λmax; 236 (20,800); sh; 330, 310. In alkali, λmax; 282 (16,500), 238 (21,500); sh; 320, 256.

3-formyl-4-hydroxycoumarin dimethone.

3-formyl-4-hydroxycoumarin (0.190 g., 0.001 mole) was boiled under reflux in 40% aqueous ethanol (10 ml.) with dimedone (a one and a half molar excess) for 10 minutes. The product, which separated on cooling, was recrystallised from ethanol as a colourless microcrystalline material, m.p. 227 (d). Yield, 60% theoretical. $C_{26}H_{26}O_6$ requires C: 71.87; H: 6.03. Found, C: 71.51; H: 5.89. N.m.r. spectrum, 8.93 (6H), 8.87 (6H), 7.67 (4H), 7.43 (4H), 5.14 (1H), 2.2 - 2.8 (3H, complex multiplet), 1.7 - 1.9 (1H, complex multiplet), - 0.58 (1H). I.r. spectrum in chloroform, 1700, 1660, 1641, 1623, 1573. U.v. spectrum, λmax; 312 (14,800), 286 (13,000), 236 (19,700); sh; 270, 260. In alkali, λmax; 308; sh; 290, 233.

2-formyldimedone dicoumarol.

2-formyldimedone (0.168 g., 0.001 mole) was boiled under reflux in ethanol (10 ml.) with 4-hydroxycoumarin (0.324 g., 0.002 mole) for 2 hours. On cooling a solid separated, which was obtained by filtration and recrystallised from ethanol as colourless needles, m.p. 257 (d). Yield, 45% theoretical. $C_{27}H_{20}O_7$ requires C: 71.04; H: 4.42. Found C: 71.00; H: 439.

N.m.r. spectrum, 8.87 (3H), 8.82 (3H), 7.57 (2H), 7.24 (2H), 4.94 (1H), 1.7 - 2.7 (8H, complex multiplet), - 0.23 (1H).

I.r. spectrum, 1728, 1669, 1657, 1622, 1610, 1570.

U.v. spectrum, λmax; 307 (16,300), 260 (23,400); sh; 328, 292, 269, 252. In alkali, λmax; 293, 259, 238; sh; 360, 305.

3-o-hydroxybenzylidine- 2,4-diketochroman.

This compound was prepared by the method of Link.[21] Calc. for $C_{16}H_{10}O_4$, C: 72.18; H: 3.79. Found, C: 72.05; H: 3.77.

N.m.r. spectrum, 2.2 - 3.2 (8H, complex multiplet), 2.00 (1H), - 1.70 (1H). I.r. spectrum as Nujol mull, 1720, 1630, 1610, 1592, 1567. U.v. spectrum, λmax; 326 (10,300), 289 (13,800), 241 (8,300); sh; 258. In alkali, λmax; 307, sh; 290, 240.

Acetyl derivative. 3-o-hydroxybenzylidene-2,4-diketochroman (0.266 g., 0.001 mole) was boiled under reflux in acetic anhydride(5 ml.) for 2 hours. The resulting solution was then poured into water (100 ml.) and allowed to stand. The precipitate which formed was filtered off, and recrystallised from aqueous ethanol as colourless prisms, m.p. 125-126°. Yield, 80% theoretical. $C_{18}H_{12}O_5$ requires C: 70.13;

H: 3.92. Found, C: 70.12; H: 3.83. N.m.r. spectrum, 7.99 (3H), 2.2 - 2.8 (8H, complex multiplet), 1.72 (1H). I.r. spectrum as Nujol mull, 1763, 1719, 1660, 1616, 1602, 1562. U.v. spectrum, λmax; 297 (16,600); sh; 337, 255, 243.

Dimedone addition product. 3-o-hydroxybenzylidene-2, 4-diketochroman (0.266 g., 0.001 mole) was boiled under reflux in ethanol (15 ml.) with dimedone (0.140 g., 0.001 mole) for 20 hours. On cooling, colourless needles separated, m.p. 252 (d). Yield, 68% theoretical. $C_{24}H_{20}O_5$ requires C: 74.21; H: 5.19. Found, C: 74.10; H: 5.17. N.m.r. spectrum, 8.97 (3H), 8.87 (3H), 7.65 (2H), 7.42 (2H), 4.95 (1H), 2.4 - 3.0 (7H, complex multiplet), 1.7 - 1.9 (1H, complex multiplet), - 0.10 (1H). I.r. spectrum as Nujol mull, 1672, 1640, 1625, 1609, 1583, 1569. I.r. spectrum in chloroform, 1711, 1646, 1623, 1572. U.v. spectrum, λmax; 308 (16,800), 284 (16,400), 273 (16,500); sh; 323, 320. In alkali, λmax; 303; sh; 293.

4-hydroxycarbostyril addition product. 3-o-hydroxybenzyl-2, 4-diketochroman (0.266 g., 0.001 mole) was boiled under reflux in ethanol (18 ml.) with 4-hydroxycarbostyril (0.161 g., 0.001 mole) for 24 hours. On cooling and filtering, an amorphous solid was obtained which was recrystallised from benzyl alcohol as colourless microcrystalline material, m.p. 295 - 297° $C_{25}H_{15}O_5N$ requires C: 73.34; H: 3.69; N. 3.42. Found, C: 73.05; H: 3.85; N: 3.12. N.m.r. spectrum in trifluoro-acetic acid, 4.73 (1H), 2.1 - 3.6 (12H, complex multiplet). I.r. spectrum as Nujol mull, 1689, 1634, 1646, 1611, 1590, 1577, 1560.

U.v. spectrum, λmax; 318, 292, 267; sh; 335, 300, 258. In alkali, λmax; 303; sh; 335, 318, 255.

3-(2-hydroxy, 3-methoxybenzylidene)-2,4-diketochroman. 4-hydroxycoumarin (1.62 g., 0.01 mole) and o-vanillin (1.42 g., 0.01 mole) were boiled under reflux in ethanol (20 ml.) for 10 minutes. A solid separated on cooling, which was filtered off and recrystallised from an ethanol-chloroform mixture as feathery yellow needles, m.p. 200 - 201°. Yield, 30% theoretical. $C_{17}O_{12}H_5$ requires C: 68.91; H: 4.08. Found, C: 69.03; H: 4.19. The compound was insoluble in chloroform and decomposed by trifluoro-acetic acid. I.r. spectrum as Nujol mull, 1720, 1631, 1614, 1597, 1579. U.v. spectrum, λmax; 308, 288; sh; 265, 243. In alkali, λmax; 308; sh; 291.

Acetyl derivative. The above compound (0.592 g., 0.0002 mole) was dissovled in 40% acetic anhydride-pyridine solution (10 ml.) and allowed to stand for 3 minutes. The solution was poured into water, and the precipitate collected by filtration, dried, and twice recrystallised from ethanol as pale yellow needles, m.p. 151-152°, after treatment with animal charcoal. Yield, 55% theoretical. $C_{19}H_{14}O_6$ requires C: 67.45; H: 4.16. Found, C: 66.97, H: 4.08. N.m.r. spectrum, 8.00 (3H), 6.08 (3H), 2.2 - 3.0 (7H, complex multiplet). 1.96 (1H). I.r. spectrum as Nujol mull, 1758, 1742, 1672, 1613, 1580. U.v. spectrum, λmax; 310 (20,200), 252 (15,100).

Dimedone addition product. The above compound (0.296 g., 0.001 mole) was boiled under reflux with dimedone (0.140 g., 0.001 mole) in

ethanol (10 ml.) for 5 hours. On cooling, a colourless precipitate formed, which was filtered off and recrystallised from ethanol as colourless needles, m.p. 247 (d). Yield 59% theoretical. $C_{25}H_{22}O_6$ requires C: 71.76; H: 5.30. Found, C: 71.16; H: 5.09. N.m.r. spectrum, 8.97 (3H), 8.88 (3H), 7.65 (2H), 7.34 (2H), 6.11 (3H), 4.95 (1H), 2.2 - 3.5 (6H, complex multiplet), 1.7 - 1.9 (1H, complex multiplet), - 1.0 (1H). I.r. spectrum in chloroform, 1704, 1608, 1575. I.r. spectrum as Nujol mull, 1672, 1640, 1621, 1610, 1584. U.v. spectrum, λmax; 312 (18,400), 274 (17,500); sh; 328, 300, 283. In alkali, λmax; 311; sh; 280.

3-2'-hydroxy-1'-naphthylidene-2,4-diketochroman

1-formyl-2-hydroxynaphthalene (3.44 g., 0.02 mole) and 4-hydroxycoumarin (3.24 g., 0.02 mole) were dissolved in ethanol (50 ml.). The solution was boiled under reflux for 1 minute, after which time the product began to separate. On cooling and filtration, the yellow feathery crystals of the diketochroman were obtained, m.p. 245-247°. Yield, 75% theoretical. $C_{20}H_{12}O_4$ requires C: 75.94; H: 3.82. Found, C: 76.01; H: 4.00. I.r. spectrum as Nujol mull, 1702, 1624, 1597, 1567. U.v. spectrum, λ max; 357, 319, 257; sh; 328, 280, 264, 251. In alkali, λmax; 292, sh; 350, 309, 263, 257, 250.

Acetyl derivative. The above compound (0.632 g., 0.002 mole) was suspended in pyridine (5 ml.) and acetyl chloride (1ml.) added. After two hours the reaction mixture was poured into water, crystallisation of the acetyl compound occurring at once.

The product was recrystallised from cyclohexanone as yellow needles, m.p. 225-229°. Yield, 60% theoretical. $C_{22}H_{14}O_5$ requires C: 73.74; H: 3.94. Found, C: 73.57; H: 4.14. N.m.r. spectrum, 7.97 (3H), 1.5 - 2.7 (10H, complex multiplet), 0.86 (1H). I.r. spectrum as Nujol mull, 1760, 1722, 1663, 1604, 1561. U.v. spectrum, λmax; 380 (12,100), 260 (15,900); sh; 333.

Dimedone derivative. This diketochroman was found not to react with dimedone to an appreciable extent under the same conditions as those in which other diketochromans reacted.

3-(o-hydroxybenzylidene)-4-keto-6-methyl-3,4-dihydro-α-pyrone. 4-hydroxy-6-methyl-α-pyrone (0.126 g., 0.001 mole) was boiled under reflux in ethanol (10 ml.) with salicylaldehyde (0.112 g., 0.001 mole) for 10 minutes. On standing, the product separated as orange-yellow prisms, m.p. 149-150°. Yield, 74% theoretical. $C_{13}H_{10}O_4$ requires C: 67.82; H: 4.38. Found, C: 67.43; H: 4.46. N.m.r. spectrum, 7.76 (3H), 3.05 (1H, 5-proton), 2.2 - 2.9 (4H, complex multiplet), 1.43 (1H, benzylidene proton) - 5.69 (1H). I.r. spectrum in chloroform, 1728, 1608, 1590. I.r. spectrum as Nujol mull, 1727, 1603, 1581. U.v. spectrum λmax; 353 (13,900), 301 (10,000); sh; 397, 335, 246. In alkali, λmax; 361, 293; sh; 325.

Acetyl derivative. This compound did not give a stable acetyl derivative when treated with a) boiling acetic anhydride, b) acetyl chloride in pyridine, c) acetic anhydride in pyridine. In all the reactions tried the compound remained unreactive,

though with acetyl chloride and pyridine an intractable tar was obtained on standing for a long period at room temperature.

Dimedone derivative. The above compound (0.115 g., 0.0005 mole) was dissolved in hot ethanol (5 ml.) and boiled under reflux with dimedone (0.140 g., 0.001 mole) for 6 hours. On cooling and addition of water to the solution, a colourless material separated. This material was filtered off, and recrystallised from aqueous ethanol as colourless prisms, m.p. 241-243°. Yield, 51% theoretical. $C_{21}H_{20}O_5$ requires C: 71.58; H: 5.72. Found, C: 72.20; H: 5.97. N.m.r. spectrum, 8.94 (3H), 8.87 (3H), 7.94 (3H, 6-methyl group of α-pyrone residue), 7.74 (2H), 7.67 (2H), 5.06 (1H), 4.14 (1H, 5-proton of α-pyrone residue), 2.96 (4H, tight multiplet), - 0.42 (1H)

4-hydroxy-6-methyl-α-pyrone addition product.

4-hydroxy-6-methyl-α-pyrone (1.26 g., 0.01 mole) and salicylaldehyde (1.12 g., 0.01 mole) were boiled under reflux in ethanol (10 ml.) for two hours. The resulting precipitate was filtered off and recrystallised from benzyl alcohol as a colourless, microcrystalline solid, m.p. 267 (d). Yield, 80% theoretical, $C_{19}H_{14}O_6$ requires C: 67.45; H: 4.17. Found, C: 67.00; H: 4.28. N.m.r. spectrum in trifluoro-acetic acid, 7.72 (3H), 7.67 (3H), 4.49 (1H), 3.66 (1H), 3.61 (1H), 2.2 - 3.1 (4H, complex multiplet). On formation of this compound a molecule of water is lost between the hydroxyl group of the salicylaldehyde residue and the hydroxyl group of one of the α-pyrone residues. (The same reaction occurs on formation of salicylaldehyde dicoumarol.)

For this reason the n.m.r. spectrum shows the 5-proton and 5' -proton, the 6-methyl group and the 6' -methyl group, are in different chemical environments. I.r. spectrum as Nujol mull, 1670, 1615, 1572. U.v. spectrum, λmax; 299, 260; sh; 282, 274. In alkali, λmax; 380, 265; sh; 317, 305.

3-(2'-hydroxy-3'-methoxybenzylidene)-4-keto-6-methyl-3,4-dihydro-α-pyrone. 4-hydroxy-6-methyl-α-pyrone (0.252 g., 0.002 mole) and o-vanillin, (0.284 g., 0.002 mole) were dissolved in 50% aqueous ethanol (10 ml.) and allowed to stand for 24 hours. The product, which had separated, was filtered under suction and recrystallised from an ethanol-chloroform mixture as yellow needles, m.p. 170-171°. Yield, 71% theoretical. $C_{14}H_{12}O_5$ requires C: 64.61; H: 4.65. Found, C: 64.46; H: 4.68. N.m.r. spectrum, 7.76 (3H), 6.05 (3H), 2.99 (1H, 5-proton of α-pyrone ring), 2.80 (3H, tight multiplet), 1.42 (1H, benzylidene proton), - 5.73 (1H). I.r. spectrum in chloroform, 1728, 1600 (broad band). U.v. spectrum, λmax; 345 (9,800), 254 (5,100); sh; 397. In alkali, λmax; 359, 296; sh; 254.

Dimedone addition product. The above compound (0.260 g., 0.001 mole) was boiled under reflux with dimedone (0.140 g., 0.001 mole) in ethanol (10 ml.) for 24 hours. The resulting precipitate was filtered off and recrystallised from an ethanol-chloroform mixture, as colourless needles, m.p. 239-241°. N.m.r. spectrum, 8.99 (3H), 8.92 (3H), 7.94 (3H), 7.70 (2H), 7.41 (2H), 6.14 (3H, protons of methoxy group) 5.09 (1H), 4.13 (1H), 2.9 - 3.2 (3H, complex multiplet), - 0.41 (1H). U.v. spectrum, λmax;

301 (14,600), 280 (10,900). In alkali, λmax; 302, 282.

3-(2,3-dihydroxybenzylidene)-2,4-diketo-3,4-dihydro-α-pyrone.

4-hydroxy-6-methyl-α-pyrone (0.252 g., 0.002 mole) was dissolved in ethanol (10 ml.) and 2, 3-dihydroxybenzaldehyde (9.276 g., 0.002 mole) added. The product crystallised from the reaction mixture after 2 days as yellow needles, m.p. 229°(d). Yield, 51% theoretical. $C_{13}H_{10}O_5$ requires C: 63.41; H: 4.09. Found, C: 63.15; H: 3.96. U.v. spectrum, λmax; 351 (24,000), 261 (8,500); sh; 396. In alkali, λmax; 357, 326, 287; sh; 238.

Acetyl derivative The above compound (0.147 g., 0.0006 mole) was boiled under reflux in acetic anhydride (3 ml.) for 30 minutes. On cooling and decomposition of excess acetic anhydride with water, the product separated. It was collected by filtration and recrystallised from an ethanol-chloroform mixture as pale yellow needles, m.p. 172-174°. Yield, 84% theoretical $C_{15}H_{12}O_6$ requires C: 62.50; H: 4.20. Found, C: 62.45; H: 4.16. N.m.r. spectrum, 7.75 (3H), 7.59 (3H), 3.04 (1H), 2.4 - 2.8 (3H, complex multiplet), 1.40 (1H), 5.78 (1H). I.r. spectrum in chloroform, 1776, 1746, 1617, 1591. U.v. spectrum, λmax; 352 (19,500), 270 (4,500); sh; 395, 360, 342, 305, 257. In alkali, λmax; 357, 325, 282; sh; 240.

Oxidation of 3-o-hydroxybenzylidene-2,4-diketochroman.

This compound (0.266 g., 0.001 mole) was boiled under reflux in chloroform (10 ml.) with activated manganese dioxide (0.087 g.)

for 3 hours. Filtration of the reaction mixture through Kieselguhr, and evaporation of the chloroform yielded starting material, m.p. 173-174°. Yield, 95% theoretical

Reduction of 3-o-hydroxybenzylidene-2,4-diketochroman.

This compound (0.512 g., 0.002 mole) was suspended with 10% palladium on charcoal (0.001 g.) in ethanol (20 ml.). The mixture was shaken under an atmosphere of hydrogen for 24 hours, by which time hydrogen uptake had ceased at 1 mole of hydrogen per mole of starting material, and the yellow colour of the starting material had disappeared. The reaction mixture was filtered through Kieselguhr, and the alcohol evaporated to yield a colourless product. The product was recrystallised from aqueous ethanol as colourless prisms, m.p. 229-231. Yield, 68% theoretical. $C_{16}H_{12}O_4$ requires C: 71.63; H: 4.51. Found, C: 71.28; H: 4.50. I.r. spectrum as Nujol mull, 1660, 1620, 1604, 1573. U.v. spectrum, λmax; 323 (8,700), 309 (13,000), 284 (12,900), 273 (12,700); sh; 318, 297. In alkali, λmax; 313; sh; 292, 281, 242.

Diacetyl derivative. The above product (0.100 g.) was boiled under reflux for 1 hour in acetic anhydride (2 ml.) and the reaction mixture poured into water. The product, which separated, was recrystallised from aqueous ethanol as colourless needles, m.p. 151-153°. $C_{20}H_{16}O_6$ requires C: 68.18; H: 4.58. Found, C: 67.77; H: 4.58. N.m.r. spectrum, 7.65 (3H), 7.64 (3H), 6.11 (2H), 2.3 - 2.9 (4H, complex multiplet). I.r. spectrum as Nujol mull, 1777, 1750, 1714, 1634, 1611, 1577.

U.v. spectrum, λmax; 313, (11,200), 275 (18,200); sh; 283.

Reduction of 3-o-hydroxybenzylidene-2,4-diketo-3,4-dihydro-α-pyrone.

This reduction was carried out in the same manner as that of the 3-o-hydroxybenzylidene-2,4-diketochroman above. 1 mole of hydrogen per mole of compound was taken up after 24 hours. The product was washed with chloroform and recrystallised from aqueous ethanol as colourless plates, m.p. 173-177°. $C_{13}H_{12}O_4$ requires C: 67.23; H: 5.21. Found, C: 67.53; H: 5.44.

I.r. spectrum as Nujol mull, 1665, 1633, 1609, 1564.

U.v. spectrum, λmax; 291 (7,500); sh; 281, 273. In alkali, λmax; 283; sh; 292, 275.

Diacetyl derivative. Prepared in an analogous manner to the diacetyl derivative above, it was obtained from aqueous ethanol as colourless needles, m.p. 80-81°. Yield, 47% theoretical. $C_{17}H_{16}O_6$ requires C: 64.55; H: 5.10. Found, C: 64.11; H: 5.21. N.m.r. spectrum, 7.90 (3H), 7.78 (3H), 7.70 (3H), 6.32 (2H), 4.05 (1H), 2.8 - 2.9 (4H, complex multiplet).

I.r. spectrum in chloroform, 1765, 1716, 1654, 1592.

U.v. spectrum, λmax; 297 (7,700).

Oxidation of Dicoumarol.

Dicoumarol was found to be inert to the following oxidising agents when suspended in contact with their aqueous solutions at room temperature. a) Ferric chloride, b) thallic oxide, c) alkaline potassium ferricyanide.

Dicoumarol (0.336 g., 0.001 mole) was suspended in ceric ammonium nitrate solution (total quantity of ceric ammonium nitrate

equivalent to dicoumarol as 2 electron oxidising agent and the mixture stirred for 24 hours. On filtration, dicoumarol (0.168 g., 0.0005 mole) was obtained. The solution was extracted with ether, and on evaporation of the ether an oil was given which had a smell of salicyl derivatives, but was not a single compound.

Reaction of acetone and 4-hydroxycoumarin.

4-hydroxycoumarin and acetone were heated together in a sealed tube at 140° for 24 hours. The only compounds that could be identified from the resulting solution were unreacted 4-hydroxycoumarin, and the addition products of 4-hydroxycoumarin and mesityl oxide.

Reaction of benzaldehyde dicoumarol and N-bromosuccinimide.

No reaction occurred when benzaldehyde dicoumarol was boiled under reflux in carbon tetrachloride with an equivalent of N-bromosuccinimide.

Reaction of ethyl magnesium bromide and 3-acetyl-4-hydroxycoumarin.

On hydrolysis of the products of the reaction of 2 equivalents ethyl magnesium bromide and 3-acetyl-4-hydroxycoumarin in ether, only starting material was obtained.

Measurement of Pka Values.

The following tables of data were prepared from spectra obtained on a Carey u.v. recording spectrophotometer. Buffers were 0.1 molar, and the temperature at which the investigation was carried out was 21°. The method of calculation was that described by Albert and Serjeant.[101] Owing to the extreme insolubility of the dicoumarols

in acidic solutions, pKa_1 values were obtained using a 0-0.1 O.D. slide wire.

Solubility of acetaldehyde dicoumarol.

Acetaldehyde dicoumarol was added to a series of buffers, and the solid allowed to equilibrate with its solution in a constant temperature bath at 21°. The buffers were 0.01 molar, made up in 0.1 molar sodium chloride solution. The solubility was estimated spectrophotometrically, using the wavelength 289 mu, an isosbestic point for the dissociation of the molecule into its mono-anion. Using the method of calculation of Albert and Serjeant[101] an approximate pKa value of 2.6 was obtained. The solubility of the neutral molecule was found to be 6×10^{-6} molar under these conditions.

Dicoumarol itself was found to be too insoluble for an accurate determination of its pKa_1. The value is probably between 3.7 and 4.1 pKa units.

pKa values of mono-basic 4-hydroxycoumarin derivatives.

Compound	Spectrum of molecule	Isosbestic points	Spectrum of anion	A.W.L.	pKa
4-hydroxycoumarin	λmax; 303 (6,800) 281 (9,900), 270 (9,200); sh; 315	282 (9,600) 246 (1,900)	λmax; 286 (14,500); sh; 296, 242	290	4.20 ±0.02
3-methyl-4-hydroxycoumarin	λmax; 305 (10,800), 282 (12,000), 273 (10,700)	286 (10,100) 253 (2,600)	λmax; 307 (14,600)	270	4.17 ±0.04
3-phenyl-4-hydroxycoumarin	λmax; 307 (12,300), 284 (11,900); sh; 320, 270	296 (11,000) 257 (5,600)	λmax; 308 (13,300); sh; 288, 257, 243	280	3.76 ±0.02
Warfarin	λmax; 304 (10,700 283 (12,600), 276 (11,300); sh; 318	288 (10,100) 249 (3,500)	λmax; 308 (13,300); sh; 292	272	5.03 ±0.04
3-formyl-4-hydroxycoumarin dimethone	λmax; 259 (16,800); sh; 310, 289	289 (9,200) 256 (14,500)	λmax; 310 (11,500), 238 (16,700); sh; 289	265	4.98 ±0.03
Salicylaldehyde semidimethone-dicoumarol	λmax; 312 (13,000), 278 (13,800); sh; 324, 288	281 (12,400) 252 (5,400)	λmax; 305 (17,300); sh; 290, 282	305	4.36 ±0.08

pKa values of Dicoumarols

	undissoc. species	Isosbestic points	dissoc. species	A.W.L.	pKa
Dicoumarol pKa 2	λmax, 302 (19,100) 276 (20,600); sh; 320	287 (17,800) 253 (7,300) 232 (23,200)	λmax; 314 (30,700)	313	8.01 ±0.05
Acetaldehyde dicoumarol pKa 1	λmax, 313 (19,800); sh; 328, 290	290 (16,100) 263 (13,400) +	λmax; 303 (17,700), 278 (18,100)	315 277	2.40 ±0.10
Acetaldehyde dicoumarol pKa 2	λmax, 303 (17,700) 278 (18,100)	289 (16,500) 256 (6,600)	λmax; 313 (24,800), sh; 295	315	9.16 ±0.04
Benzaldehyde dicoumarol pKa 1	λmax, 315 (24,700) sh; 328, 292	290 (18,000) 260 (12,600)	λmax; 303 (18,700), 278 (19,600)	315 278	2.40 ±0.10
Benzaldehyde dicoumarol pKa 2	λmax, 303 (18,700) 278 (19,600)	287 (17,600) 256 (7,400) 235 (24,200)	λmax, 314 (29,800); sh; 292, 243	315	9.11 ±0.05
Pelantan pKa 1	λmax, 319 (22,300); sh; 323, 275	325 (11,300) 280 (17,500)	λmax, 303 (16,900), 276 (19,000) sh; 310, 286	319	<1.5
Pelantan pKa 2	λmax, 303 (16,900) 276 (19,000); sh; 310, 286	282 (16,600) 252 (6,500)	λmax, 308 (25,700), sh; 291, 240	305	7.58 ±0.03

+ Possibly 2 more isosbestic points lying ± 1 mμ either side of 301 (17,600)

pKa values of monobasic dimedone derivatives

Compound	Spectrum of molecule	Isosbestic points	Spectrum of anion	A.W.L.	pKa
Dimedone	λmax, 259 (16,700)	267 (13,000)	λmax, 282 (27,500)	280	5.05 ±0.03
Salicylaldehyde	λmax, 271 (16,400)	274 (15,900) 241 (7,800)	λmax, 288 (23,200)	290	6.10 ±0.03
2-formyldimedone dimethone	λmax, 271 (8,500), 240 (21,800)	253 (15,000) 222 (8,100)	λmax, 290 (15,400), 241 (15,500)	290	6.95 ±0.04

pKa values of Dibasic Dimethones

Compound	undissoc. species	Isosbestic points	dissoc. species	A.W.L.	pKa
formaldehyde dimethone pKa_1	λmax; 259 (25,200)	259 (25,200)	λmax; 267 (29,000); sh; 295	270	5.31 ±0.03
formaldehyde dimethone pKa_2	λmax; 267 (29,000); sh; 295	276 (21,900)	λmax; 292 (38,700)	290	10.01 ±0.05
acetaldehyde dimethone pKa_1	λmax; 262 (17,200)	261 (16,700)	λmax; 283 (41,100)	280	5.43 ±0.04
acetaldehyde dimethone pKa_2	λmax; 283 (41,100)	290 (35,000) 246 (3,400)	λmax; 288 (35,400)	-	> 13
benzaldehyde dimethone pKa_1	λmax; 263 (11,700)	none	λmax; 269 (18,400); sh; 295	275	4.76 ±0.04
benzaldehyde dimethone pKa_2	λmax; 269 (18,400) sh; 295	274 (17,600) 229 (5,000)	λmax; 287 (24,900)	290	12.2

Biochemistry Section.

Mitochondria were prepared from fresh beef heart by the Nagarse proteinase method.[102] Oxygen uptake was followed by means of a Clark Electrode coupled to a pen recorder. The medium used had the following composition. Tris base (0.1 molar buffered to pH 7.6 by addition of hydrochloric acid) sucrose (0.25 molar), inorganic orthophosphate (0.01 molar), magnesium chloride (0.006 molar). Mitochondria were added to the medium in the cell compartment (thermostated to 30°) with substrate (0.0027 molar glutamate and 0.0027 molar malate in the cell) and on allowing time for the system to settle (about 2 minutes) test substances were added. Changes in rate of oxygen uptake were determined from the pen recorder traces. The respiratory control of the mitochondria was found to be between 4 and 8. Protein was estimated by the Biuret method.[104] Graphs were plotted of the concentration of uncoupler against the rate of oxygen uptake, the concentration of oxygen per ml. of medium was taken to be 0.465 μ.g. atoms, as given by Haslam.[105] Some of the graphs obtained are given in the Discussion Section.

Alkali soluble test compounds were added in sodium hydroxide solution (minimum volume of 0.002 molar sodium hydroxide to give solubility, followed by dilution with water to stated volume.) 4-methoxycoumarin and 4-acetoxycoumarin were added in alcohol solution, a correction being made for the small uncoupling effect of the alcohol from "blank" runs.

The activities of the compounds are recorded in the tables overleaf, and the significance of the column headings are described in the Discussion Section, (See Diagram XXXVIII) Both 4-acetoxycoumarin and 4-methoxycoumarin were found to show a small amount of uncoupling activity at relatively large concentrations. 5×10^{-4} molar 4-acetoxycoumarin gave a rate of respiration 20% of the maximum rate induced by dicoumarol, and 10^{-3} molar 4-methoxycoumarin gave a rate of 30% on the same basis.

Activity of Dicoumarols.

Parent carbonyl compound	A	B	C	D	E	C.V.
formaldehyde	1.00	0.71	1.9	2.88	0.78	0
acetaldehyde	0.75	0.92	2.1	2.11	1.2	
propionaldehyde	0.76	1.62	2.5	1.21	2.2	
p-tolualdehyde	1.02	0.49	1.0	1.96	0.54	
p-methoxybenzaldehyde	0.97	0.68	1.3	1.49	0.69	5
p-chlorobenzaldehyde	0.87	0.86	1.4	1.00	1.1	10
benzaldehyde	0.65	1.6	2.8	0.99	2.6	20
p-nitrobenzaldehyde	0.57	1.5	2.3	0.78	2.9	20
p-dimethylaminobenzaldehyde	0.73	2.0	3.7	1.23	3.1	35
p-hydroxybenzaldehyde	Inactive at 10^{-3} molar					25
p-vanillin	0.46	3.7	5.4	0.54	8.7	
salicylaldehyde	0.71	2.2	4.3	1.00	3.6	
ethyl glyoxylate	0.79	14	21	0.84	20	
pyruvic acid	Inactive at 10^{-3} molar					

C.V. Coagulation valency, see page 70.

Activity of other compounds tested.

Compound	A	B	C	D	E
coum-pyr	1.08	1.2	3.6	2.90	1.3
acetaldehyde bis-α-pyrone	Inactive at 10^{-3} molar				
warfarin	0.88	69	100	0.64	88
coum-sal.	0.55	10	18	1.23	21
4-monomethyl ether of dicoumarol	0.69	36	68	0.81	59
4-hydroxycoumarin	1.14				85
3-phenyl-4-hydroxycoumarin	1.08				56
3-methyl-4-hydroxycoumarin	1.17				115
4-hydroxycarbostyril	1.11				60
N-methyl-4-hydroxycarbostyril	Inactive at 5×10^{-3} molar				
4-hydroxy-6-methyl-α-pyrone	Inactive at 10^{-3} molar				

coum-pyr, is 3-methylene-(3',4'-hydroxycoumarinyl)-4-hydroxy-6-methyl-α-pyrone (diagram XXX).

acetaldehyde bis-α-pyrone, is 3,3'-ethylidene bis-4-hydroxy-6-methyl-α-pyrone.

coum-sal, is 3-o-hydroxybenzylidene-2,4-diketochroman.

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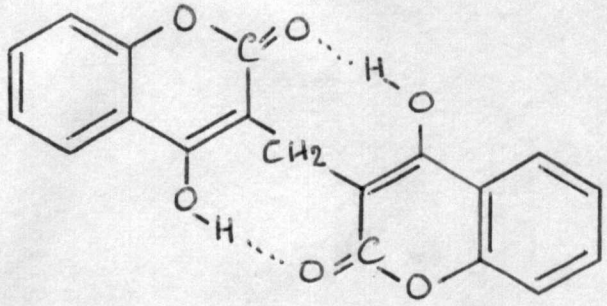
SUMMARY.

Previous
Analysis of the i.r. and u.v. spectra of dicoumarol (a powerful anticoagulant and uncoupler of oxidative phosphorylation) has failed to establish the detailed structure of the molecule.

In this thesis the i.r. and u.v. spectra of dicoumarol and its derivatives have been re-examined, and with the additional data available from n.m.r. spectroscopy the structure of the molecule has been found to be the hydrogen bonded structure below.



The pKa values of dicoumarols have been shown to be consistent with this formulation.

Other compounds (such as dimethones and 4-hydroxy-6-methyl-α-pyrones) have been shown to have a similar structure. The tautomerism of dimedone has been investigated by n.m.r. spectroscopy, and interpreted in terms of an equilibrium between keto, enol and enol dimers, the last mentioned involving hydrogen bonding between the hydroxyl protons and carbonyl groups of two enol tautomers.

A number of coumarin derivatives have been added to respiring beef heart mitochondria and variations in the rate of respiration of the mitochondria with changing concentration of coumarin noted. On the basis of the mode of interaction of the compound and the mitochondria, the compounds tested may be divided into three classes, a) those that uncouple and inhibit b) those that only uncouple, and c) those that are inactive. All compounds in the class a) are capable of intramolecular hydrogen bonding in which the hydrogen bonded hydroxyl proton and the carbonyl group to which it is bonded form part of an 8-membered ring. Those of the class b) are able to form only intermolecular hydrogen bonds. The mechanism of action of these compounds is consistent with their action at two different sites in the energy-linked oxidation reactions of respiring mitochondria. A comparison of the activity of the compounds as uncouplers and inhibitors of oxidative phosphorylation with their activity as anticoagulants suggests there may well be a connection between the two processes, though the present state of knowledge does not permit any common mechanism to be put forward.

COMPOUND INDEX

(Figures in parentheses refer to the Experimental Section).