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THE STRUCTURE OF DICOUMAROL AND RELATED COMPOUNDS

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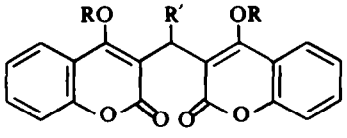
Abstract—From a study of nuclear magnetic resonance and infrared spectra, intramolecularly hydrogen-bonded structures are proposed for dicoumarols (**9**), 3-(α-acetonylbenzyl)-4-hydroxycoumarin (Warfarin) (**13**) and dimethones (**8**). A possible relationship between such hydrogen-bonded structures and the biological activities of (**9**) and (**13**) is suggested.

DICOUMAROL,3,3′-methylenebis-(4-hydroxycoumarin), (**1**, R = R′ = H) has been identified[1] as the haemorrhagic agent in the spoiled sweet clover disease of cattle and together with related compounds, e.g. pelantan (**1**, R = H, R′ = $COOC_2H_5$) and 3-(α-acetonylbenzyl)-4-hydroxycoumarin (Warfarin) (**2**) it has found considerable



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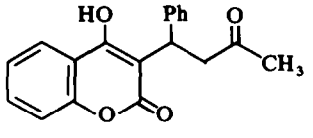

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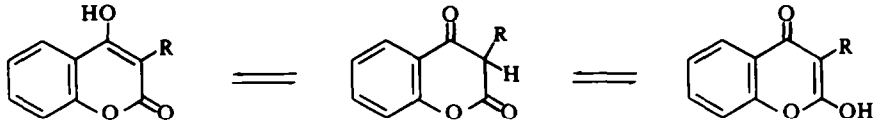
use as a clinical anticoagulant.[2,3] Dicoumarol is also an effective uncoupler of mitochondrial oxidative phosphorylation,[4] and a relationship has been indicated[5,6] between its anticoagulant properties and its role as an uncoupler of oxidative phosphorylation. Since the tautomeric structures of these compounds may have a considerable bearing on their biological activities, their structures have been re-investigated in the present work.

Both **1** and **2** are cyclic β-ketoesters; the parent compound 4-hydroxycoumarin (**3**, R = H) can be represented as one of three tautomeric structures viz. **3**, **4** or **5**.



3 **4** **5**

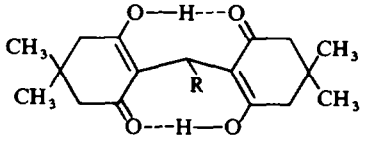
This tautomerism has been discussed by Arndt *et al.*[7] who concluded from methylation studies that 4-hydroxycoumarin was preponderantly in the coumarin form **3**, both in the solid state and in solution in polar solvents. Support for this conclusion has been provided by an investigation[8] of the IR spectra of 4-hydroxycoumarins.

Cyclic β-diketones, e.g. dimedone (**6**) also exist wholly as the enolic tautomer (**7**) in the solid state[9–11] and in aqueous solution.[12] In an NMR study[13] of the keto-enol

6 **7**

tautomerism of cyclohexane-1,3-diones equilibrium constants have been derived from the variation with concentration in the chemical shift of the signal due to the proton of the enolic hydroxyl group. Although the formation of intramolecular hydrogen-bonds is precluded owing to steric considerations, **7** can readily form intermolecularly hydrogen-bonded dimers in solution.

2,2′-Alkylidenebis-(5,5-dimethylcyclohexane-1,3-diones) (dimethones) (**8**), the condensation products of **7** and aldehydes, are analogues of **1**. The IR spectra of **8**[11,14,15] indicate that they contain intramolecular hydrogen-bonds between the carbonyl and



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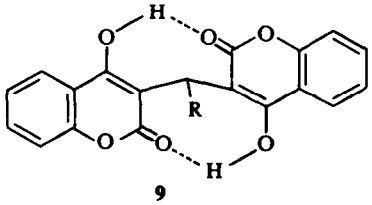
the enolic hydroxyl groups of the adjacent rings; an early NMR investigation,[16] however, failed to show the hydrogen-bonded protons. We have found that the NMR spectrum of a solution of (**8**, R = H) in deuterochloroform shows four singlets at 8·92 ($C_{(5)}CH_3$: 12H), 7·74 ($C_{(4)}$, $C_{(6)}CH_2$: 8H), 6·92 (bridge CH_2: 2H) and −1·31 τ (2H). The last singlet is independent of concentration and can be removed on the addition of a trace of deuterium oxide. We assign this signal to the two strongly deshielded protons of the enolic hydroxyl group which are intramolecularly hydrogen-bonded to carbonyl groups and we consider that this NMR evidence confirms the structural assignment for **8** mentioned above.[11,14,15] The protons of the ring methylene groups give rise to a single resonance, probably due to a rapid tautomeric exchange which averages their chemical shifts.

The NMR spectra of dicoumarols in solution in deuterochloroform also show signals due to two strongly deshielded protons which are also independent of concentration and which can also be removed by the addition of a trace of deuterium oxide to the deuterochloroform.

This indicates the presence of intramolecularly hydrogen-bonded protons and suggest the structure **9** for dicoumarols.



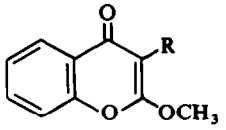
9

For the parent compound (**9**, R = H) the two hydrogen-bonded protons give rise to a single resonance signal at $-1{\cdot}31\ \tau$; however, when the bridge methylene group bears either an alkyl or an aryl group each proton gives rise to a separate singlet.

Examination of molecular models shows that in this case the alkyl or aryl substituent lies close to one of the hydroxyl protons accounting for the difference in their magnetic moments. This behaviour is also apparent for (**8**, R = CH_3 or C_6H_5) when both enolic protons can be distinguished in the NMR spectrum.

For all dicoumarols there is a complex multiplet in the NMR spectrum between 1·5 and 2·0 τ due to two protons as well as a multiplet due to six protons in the region 2·2 to 3·0 τ. The low field multiplet is presumably due to the deshielded protons on $C_{(5)}$ and $C'_{(5)}$[17] and is evidence for the partial C=O character of the $C_{(4)}$—oxygen bond caused by the formation of a hydrogen bond between the hydroxyl group and the carbonyl group in the adjacent ring. When such hydrogen-bonding does not occur, e.g. in (**3**, R = H, CH_3 or C_6H_5), the resonance of the $C_{(5)}$ proton is apparently at a higher field, being lost in the aromatic proton region around 3 τ.

In the IR spectrum of a solution (**9**, R = H) in chloroform or dioxan the carbonyl stretching frequency occurs at 1660 cm^{-1}. The position of this band does not alter when the infrared spectrum is taken in the solid state (Nujol mull or potassium chloride disc), while the carbonyl stretching frequencies of (**3**, R = H or CH_3) vary from 1700 cm^{-1} (Nujol mull) to 1730 cm^{-1} (dioxan solution), as would be expected if intermolecular hydrogen bonding occurred. Moreover, the carbonyl stretching frequencies of 3,3′-methylenebis-(4-methoxycoumarin) (**1** R = CH_3, R′ = H), 4-methoxycoumarin and 4-methoxy-3-methylcoumarin occur at 1725, 1710 and 1710 cm^{-1} (chloroform solution). On the other hand 2-methoxychromone (**10**, R = H) and 2-methoxy-3-methylchromone (**10**, R = CH_3) both have carbonyl stretching frequencies at ca. 1630 cm^{-1} (chloroform solution). As this value is 30 cm^{-1} *lower* than that for **9**, we believe that a dichromone structure (**11**) for discoumarol, as proposed by



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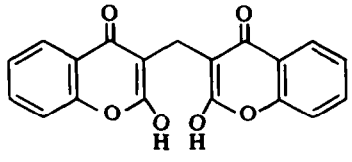

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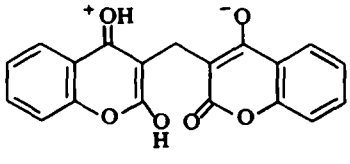
Knobloch *et al.*[18] is unlikely as any inter- or intramolecular hydrogen-bonding in **11** would cause the carbonyl band to appear at even lower frequencies. We also



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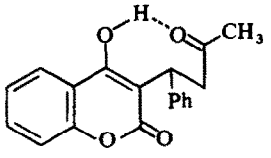
believe that the spectroscopic evidence is inconsistent with a mixed coumarin-chromone structure (**12**) which has been put forward for dicoumarol by Chmielewska[19]

on the basis of methylation studies. A preliminary X-ray crystallographic investigation[20] of 3,3'-methylenebis-(6-bromo-4-hydroxycoumarin) indicates that there is a two-fold axis of symmetry in this molecule, consistent with an intramolecularly hydrogen-bonded structure similar to **9**.

The IR spectra of 3-(α-acetonylbenzyl)-4-hydroxycoumarin (**13**) and 3-(α,α-dimethyl-β-acetylethyl-4-hydroxycoumarin[21] (**14**) show carbonyl stretching frequencies in the region 1670–1680 cm^{-1}. These are due to the carbonyl groups in the side chains which are hydrogen-bonded to the 4-hydroxy groups of the coumarin moieties. Although **13** is only poorly soluble in non-polar solvents, its NMR spectrum in deuterochloroform was recorded with the aid of a computer of average transients when a signal due to a strongly deshielded proton at 0·5 τ could be observed.



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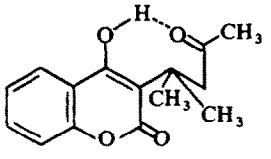

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Furthermore, the proton on $C_{(5)}$ of the coumarin moiety now appears as a multiplet at 1·9 τ; and by analogy with the spectroscopic avidence for **9**, an intramolecularly hydrogen-bonded structure is likely for **13**.

In biological systems, the formation of these intramolecular hydrogen-bonds may hold both **9** and **13** in a suitable configuration for binding to an enzyme and hence may be an important factor in the biological activities of these compounds. It is of interest the (−)-*S*-3-(α-acetonylbenzyl)-4-hydroxycoumarin (**13**)[22] and (−)-*S*-3-(α-ethylbenzyl)-4-hydroxycoumarin (Marcumar)[23] are more active as anticoagulants than their enantiomers.[23, 24] Examination of molecular models of the two enantiomers of **13** shows that while both can form the intramolecular hydrogen-bond, there is less steric interaction between the phenyl ring and the carbonyl group of the coumarin in the *S*- than the *R*-isomer. It is possible that Marcumar is oxidized *in vivo* to a derivative containing a carbonyl group at $C_{(3)}$ of the side chain, this derivative could then assume a suitable configuration for biological activity by virtue of formation of a hydrogen-bonded structure similar to **13**. Moreover, we have found[25] that compounds which contain intramolecular hydrogen-bonds, e.g. **9** and **13** are both uncouplers and inhibitors of mitochondrial oxidative phosphorylation, while compounds which can only form intermolecular hydrogen-bonds, e.g. **3** are only uncouplers of oxidative phosphorylation. Here again, the formation of hydrogen-bonds may be an important factor in assisting the molecule to attain the correct configuration for biological activity.

EXPERIMENTAL

Proton NMR spectra were taken in deuterochloroform solution at 60 Mc/s on a Perkin–Elmer R10 spectrometer, and the chemical shifts were recorded against tetramethylsilane as internal standard. The accumulated NMR spectrum was taken with the aid of a Perkin–Elmer—Northern NS 544 digital memory oscilloscope.

Starting materials

Dicoumarol (**9**, R = H), pelantan (ethyl glyoxylate dicoumarol) (**9**, R = $COOC_2H_5$) dimedone (**7**) and 4-hydroxycoumarin (**3**, R = H) were commercially available and were used without further purification. (3-α-acetonylbenzyl)-4-hydroxycoumarin (**13**) (m.p. 161°) was prepared by the acidification of the commercially available sodium salt.

Preparation of dimethones (**8**) *and dicoumarols* (**9**)

Dimedone was heated under reflux in 50% aqueous ethanol with the aldehyde (1·5-fold excess), the dimethones were precipitated and were recrystallized from aqueous ethanol. Dicoumarols (**9**) were prepared by the method of Link *et al.*,[26] their physical properties are shown in Table 1.

TABLE 1. PHYSICAL PROPERTIES OF DICOUMAROLS (**9**)

9, R =	Yield (%)	m.p. (°C)	Molecular formula	Analysis Calc. (%)		Analysis (Found) (%)	
				C	H	C	H
CH_3	70	172–174	$C_{20}H_{14}O_6$	68·6	4·03	68·7	4·15
C_2H_5	60	142–144	$C_{21}H_{16}O_6$	69·2	4·40	69·6	4·68
C_6H_5	70	228–230	$C_{25}H_{16}O_6$	72·8	3·91	72·8	3·87
4-$NO_2C_6H_4$	50	248–250	$C_{25}H_{15}NO_8$	65·6	3·30	65·5	3·45
4-$CH_3C_6H_4$	80	269–271	$C_{26}H_{18}O_6$	73·2	4·26	72·9	4·30
4-$CH_3OC_6H_4$	90	248–252	$C_{26}H_{18}O_7$	70·6	4·10	70·0	4·40
4-ClC_6H_4	75	239–240	$C_{25}H_{15}ClO_6$	67·2	3·36	67·0	3·52

4-Hydroxy-3-methylcoumarin (**3**, R = CH_3) was prepared from methyl salicylate,[27] m.p. 229–230 (from ethanol). (Found: C, 68·4; H, 4·68; Calc. for $C_{10}H_8O_3$: C, 68·2, H, 4·58%). IR ν_{max} 1670 cm^{-1} (Nujol mull).

4-Hydroxy-3-phenylcoumarin (**3**, R = C_6H_5) was prepared as above m.p. 264–266° (from ethanol). (Found: C, 75·3; H, 4·49; Calc. for $C_{15}H_{10}O_3$: C, 75·6; H, 4·23%). IR ν_{max} 1670 cm^{-1} (Nujol mull).

Methylation of 4-hydroxycoumarin

An excess of diazomethane was added to a suspension of 4-hydroxycoumarin (3·0 g) in ether (200 ml). After 24 hr, the precipitated 4-methoxycoumarin (1·5 g, m.p. 125°, Lit.[7] m.p. 125°, IR ν_{max} 1710 cm^{-1} ($CHCl_3$)) was collected, the filtrate evaporated to dryness and the residue extracted with ice-cold 20% hydrochloric acid (3 × 10 ml). The combined acid extracts were neutralised with sodium carbonate and extracted with ether (3 × 50 ml). The ethereal extracts were washed with 2N sodium hydroxide solution (100 ml) then water and finally dried. After removal of the ether the remaining solid (250 mg) was purified by TLC on silica when development with ether gave 2-methoxychromone [200 mg, m.p. 103–104°, Lit.[7] m.p. 108°; IR ν_{max} 1635 cm^{-1} ($CHCl_3$)].

Methylation of 4-hydroxy-3-methylcoumarin

This was carried out as described above for 4-hydroxycoumarin to give 4-methoxy-3-methylcoumarin [m.p. 43–44°, Lit.[28] m.p. 43°; IR ν_{max} 1710 cm^{-1} ($CHCl_3$)].

3,3'-Methylenebis-4-methoxycoumarin (**1**, R = CH_3, R' = H). A suspension of dicoumarol (1·2 g) in ether was stirred with an excess of diazomethane in ether for 2 hr. Acetic acid was added to destroy the excess diazomethane and the ether was evaporated. The residue (1·27 g) was purified by chromatography on a silica column when 3,3'-methylenebis-(4-methoxycoumarin) (0·61 g, m.p. 155°, Lit.[19] m.p. 154–156°) was obtained following elution with ether. The NMR spectrum showed signals at 5·95 (2Hs) 5·87 (6Hs) and 2·2–2·9 τ (8Hm), the IR spectrum showed a band at 1725 cm^{-1} ($CHCl_3$).

Spectroscopic properties of dimethones (**8**)

The proton NMR spectrum of (**8**, R = H) showed singlets at 8·92 (12H), 7·74 (8H), 6·92 (2H) and −1·31 τ

(2H). The position of the signal at $-1{\cdot}3$ τ did not vary over a wide range of concentrations. The proton NMR spectra of (8, R = CH_3) and (8, R = C_6H_5) was similar to that described above apart from changes due to the introduction of substituent on the bridge methylene group. For (8, R = CH_3) the enolic protons appeared at $-0{\cdot}4$ and $-2{\cdot}74$ τ and for (8, R = C_6H_5) the enolic protons were at $-0{\cdot}6$ and $-1{\cdot}76$ τ. The IR spectra of (8, R = H, CH_3 and C_6H_5). All showed carbonyl stretching frequency bands in the region 1605–1595 cm^{-1} (Nujol mull) and none at higher frequencies.

Spectroscopic properties of dicoumarols (9)

(a) *Proton NMR spectra.* The NMR spectrum of (9, R = H) in deuterochloroform solution showed the following resonance signals 6·46 (2Hs) 2·2–3·0 (6Hm), 1·8–2·0 (2Hm) and $-1{\cdot}7$ τ (2Hs). The spectra of other 9 were similar except for variations caused by changes in R and for changes in the chemical shifts of the hydroxyl protons which are shown in Table 2.

TABLE 2. CHEMICAL SHIFTS OF HYDROXYL PROTONS IN (9)

R	τ	
H	$-1{\cdot}7$	(2H)
CH_3	$-1{\cdot}44$	$-2{\cdot}14$
C_2H_5	$-1{\cdot}84$	$-2{\cdot}66$
C_6H_5	$-1{\cdot}66$	$-1{\cdot}52$
$4NO_2$–C_6H_4	$-1{\cdot}35$	$-1{\cdot}53$
$4CH_3$–C_6H_4	$-1{\cdot}3$*	
$4CH_3O$–C_6H_4	$-1{\cdot}25$	$-1{\cdot}35$
4Cl–C_6H_4	0·2	$-1{\cdot}58$

* Broad asymmetric peak due to two protons.

The positions of the hydroxyl protons of (9 R = C_2H_5) did not vary over a wide range of concentrations and the signals disappeared on the addition of a trace of deuterium oxide to the NMR tube.

(b) *Infrared spectra.* The IR spectra of (9) in solution in chloroform are shown in Table 3.

TABLE 3. MAIN BANDS IN IR SPECTRA OF (9)

9, R =	cm^{-1}
H	1659, 1630, 1603, 1573
H	1655, 1630, 1602, 1569 (a)
H	1655, 1630, 1600, 1570 (b)
H	1659, 1627, 1599, 1569 (c)
CH_3	1659, 1622, 1607, 1569
C_2H_5	1660, 1622, 1609, 1571
C_6H_5	1660, 1620, 1604, 1579
$4NO_2$–C_6H_4	1660, 1620, 1603, 1578
$4CH_3$–C_6H_4	1660, 1620, 1604, 1579
$4CH_3O$–C_6H_4	1660, 1620, 1604, 1580
4Cl–C_6H_4	1660, 1620, 1603, 1589
$COOC_2H_5$	1735, 1658, 1618, 1600, 1568

(a) Nujol mull (b) KCl disc (c) Dioxan

NMR spectrum of 3-(α-*acetonylbenzyl*)-4-*hydroxycoumarin* (13)

The NMR spectrum of (13) in solution of deuterochloroform was taken with the aid of a computer of average transients, after 64 scans the following peaks were discernible 8·3 (3Hs) 7·4 (2Hd) 5·6 (1Ht), 2·5 (8Hm), 1·9 (1Hm) and 0·5 τ (1Hs).

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