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VITAMINS AND ANTIVITAMINS K: TAUTOMERISM OF DICOUMAROL

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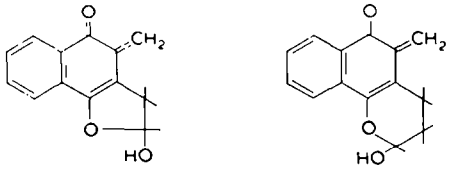
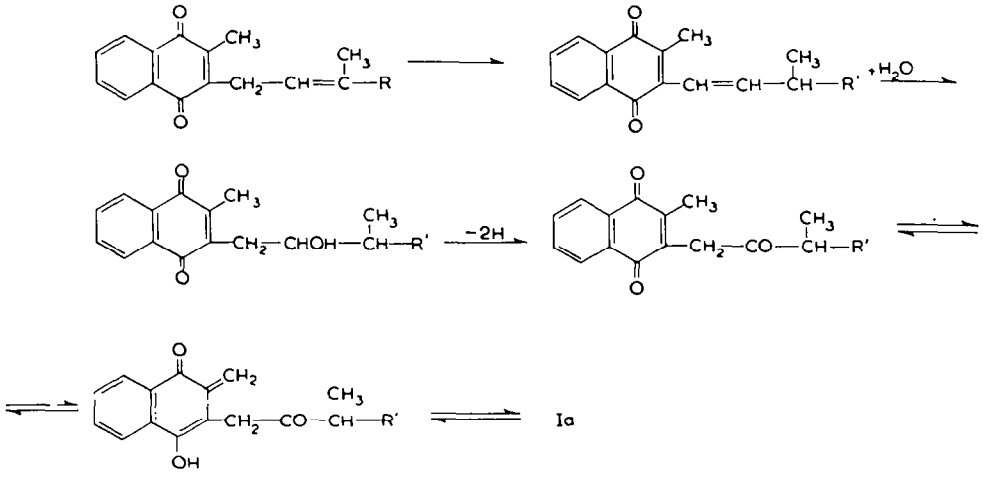
Abstract—Two isomeric dimethyl ethers of dicoumarol and derivatives substituted in the methylene group [formulae (XX) and (XXI)] were obtained. This result proves the tautomerism of dicoumarol and suggests for it the coumarin-chromone structure (XXIIa).

ACCORDING to our hypothesis, the structure necessary for vitamin-K activity is expressed by formulae (Ia) or (Ib), in which the *ortho*-quinoid group $O{=}\overset{|}{C}{-}\overset{|}{C}{=}CH_2$ acts as an active centre, while the semiketal group combines the active molecule with the protein part of the enzyme.[1]



Ia Ib

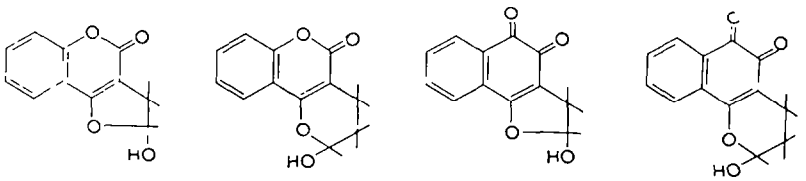
In biological conditions, the molecules represented by formula (Ia) might be formed as a result of oxidation of the phytyl or difarnesyl chain of vitamins K according to Scheme 1:



Scheme 1

For the antivitamins K we suggest the structures (IIa), (IIb), (IIIa), (IIIb), similar in shape to (Ia) or (Ib), but devoid of the active centre.[1]

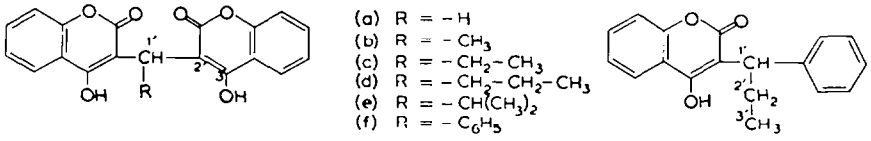
[1] I. Chmielewska, *Przem. Chem.* **29**, 740 (1950); C. Bełzecki, B. Jurecka and I. Chmielewska, *Acta Physiol. Pol.* **1**, suppl., 126 (1950).



IIa IIb IIIa IIIb

The structure (IIIa), (IIIb) can be formed from the 3-substituted derivatives of 2-hydroxy-1:4-naphthoquinone. The haemorrhagic activity of several compounds of this group is known.[2,3]

The structures (IIa), (IIb) are derived from the 3-substituted-4-hydroxycoumarins. All synthetic mono-compounds belonging to this group, known before 1950, and endowed with anticoagulant activity, answered the postulates of our hypothesis, concerned the antivitamin-K activity. However, the most active compound of the group, viz., dicoumarol (IVa), as well as 3-(1'-phenyl-*n*-propyl)-4-hydroxycoumarin (V), synthesised in 1953,[4] do not fit our hypothesis. The symmetrical molecule of dicoumarol (IVa) cannot change into (IIb), and the compound (V), lacking the ketone group in position 2', cannot form the structure (IIa).

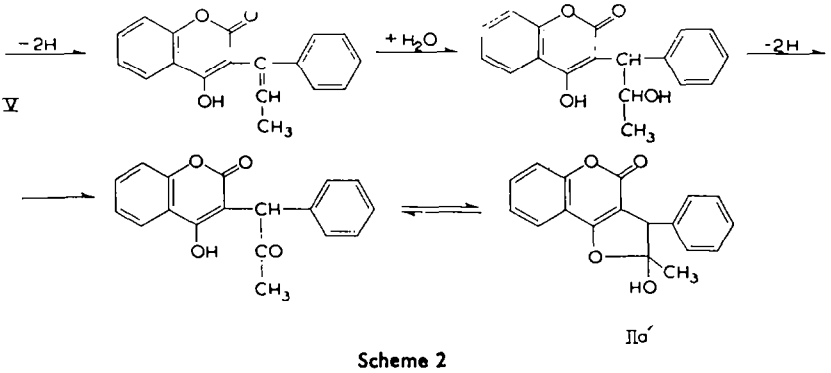


(a) R = -H
(b) R = $-CH_3$
(c) R = $-CH_2-CH_3$
(d) R = $-CH_2-CH_2-CH_3$
(e) R = $-CH(CH_3)_2$
(f) R = $-C_6H_5$

IV V

Therefore our investigations on the anticoagulant activity of derivatives of 4-hydroxycoumarin were intended to disentangle the inconsistencies referred to above. Consequently, we tried (*i*) to investigate whether compound (V) may in the living organism undergo changes resulting in structure (IIa) and (*ii*) to find the tautomeric form of dicoumarol capable of assuming structure (IIb).

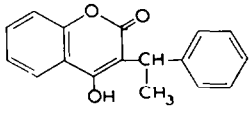
Compound (V) appeared likely to be transformed to the type (IIa) on biological oxidation according to Scheme 2:



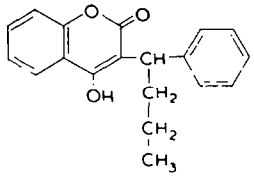
Scheme 2

[2] C. C. Smith, R. Fradkin and M. Lackey, *Proc. Soc. Exp. Biol., N.Y.* **61**, 398 (1946); C. C. Smith, *Proc. Soc. Exp., Biol. N.Y.* **64**, 45 (1947).
[3] I. Chmielewska and B. Jurecka, *Przem. Chem.* **29**, 5 (1950); I. Chmielewska, J. Hennig and M. Miodkowska-Iwaszkiewicz, *Przem. Chem.* **30**, 111 (1951); I. Chmielewska, H. Kowarzyk, B. Jurecka and A. Pachecka, *Prace Wroclawsklego Towarzystwa Naukowego S. B.* No. 40 (1951).
[4] R. Jürgens, *Schweiz. Med. Wschr.* **83**, 471 (1953); F. Koller and H. Jacob, *Schweiz. Med. Wschr.* **83**, 476 (1953).

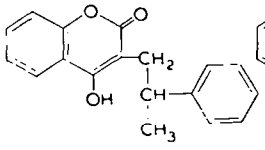
The possibility of biological oxidation in the side-chain of 3-substituted-4-hydroxycoumarins has not yet been proved. Difficulties arising in obtaining direct evidence of such a process suggested the indirect way: examination of the anticoagulant activity of the following 3-substituted-4-hydroxycoumarins (VI), (VII), (VIII) and (IX).[5]



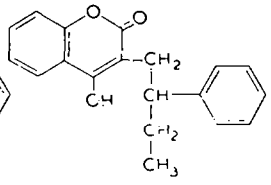
VI



VII



VIII



IX

Of the four compounds investigated only (VII) [the homologue of (V)] has the methylene group in the 2′-position and should undergo transformations according to Scheme 2. Therefore we expected its pronounced anticoagulant activity, comparable with that of dicoumarol. The remaining compounds—(VI) with a chain of two carbon atoms, (VIII) and (IX) [isomers of (V) and (VII)] with the 2′-position blocked by a phenyl residue—should not have any appreciable anticoagulant activity.

Prothrombin time in rabbits after administration of the compounds proved the activity of (VIII) and (IX) to be nil, that of (VI) negligible and that of (VII) superior to the activity of dicoumarol.[6]

The results, indicating that the 2′-methylene group is necessary for the activity of compound (V) and its analogues, seemed to confirm our assumption concerning its biological oxidation.

The structure of 3:3′-methylenebis-(4-hydroxycoumarin) (IVa) was accepted for dicoumarol,[7,8] but the tautomeric keto–enol and 2:4′-dienol structures were not impossible on theoretical grounds. However, experiments designed to prove the tautomeric forms have been unsuccessful for a long time.

The first indication of such a possibility was the discovery by Arndt *et al.* in 1951 of the coumarin–chromone tautomerism of 4-hydroxycoumarin by obtaining two isomeric methyl ethers: 4-methoxycoumarin (X) and 2-methoxychromone (XI).[9]

[5] I. Chmielewska, J. Cieślak and K. Szpalerska, *Roczn. Chem.* **30**, 813 (1956).

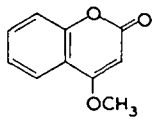
[6] Z. Latallo, J. Panasewicz, J. Cieślak and I. Chmielewska, *Bull. Acad. Pol. Sci. Cl II* **5**, 5 (1957).

[7] R. Anschütz and H. Fresenius, *Ber. Dtsch. Chem. Ges.* **36**, 465 (1903); *Liebigs Ann.* **367**, 212 (1903); *Ibid.* **379**, 336 (1916).

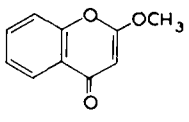
[8] R. S. Owerman, M. A. Stahmann, C. F. Huebner, W. R. Sullivan, L. Spiro, D. C. Doherty M. Ikawa, L. Graf, S. Roseman and K. P. Link, *J. Biol. Chem.* **153**, 5 (1944).

[9] F. Arndt, L. Loewe, R. Ün and E. Ayça, *Chem. Ber.* **84**, 319 (1951).

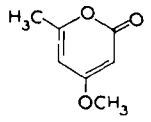
In 1952 we isolated two isomeric methyl ethers, 4-methoxy-6-methyl-2-pyrone (XII) and 2-methoxy-6-methyl-4-pyrone (XIII), and thus proved the 2:4-pyrone tautomerism of 6-methyl-2H-pyran-2:4-(3H)-dione.[10]



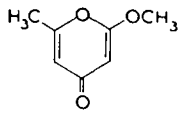
X



XI



XII



XIII

We found also a convenient method for quantitative separation of the isomeric ethers (XII) and (XIII); it consisted in adding hydrogen chloride to a cold solution of the isomers in anhydrous ether till the insoluble hydrochloride of (XIII) settled out. (XII) remained in solution and was separated after evaporation of the solvent. (XIII) was obtained from its hydrochloride by treatment with 1 equivalent of diethylamine in ether. The ratio of isomeric ethers (XII) and (XIII) was the same as that of the isometric ethers (X) and (XI) i.e., 3:1.[11]

Isomeric methyl ethers of 3:3′-*methylene bis*-(2H-*pyran*-2:4(3H)-*dione*)

The next step was the methylation of 3:3′-methylenebis-(2H-pyran-2:4-(3*H*)-dione) (XIV) with diazomethane.[12,13] This compound was used as a model in investing the possible tautomerism due to the differences in position of enolisation. The product of methylation was shown to be an equimolecular mixture of two compounds *A* and *B*. Compound *A*, m.p. 199–201°, forming no hydrochloride, was a dimethyl ether. Its properties agreed with those of (XII) and consequently structure (XVI) of 2:2′-pyrone was assigned to it. Compound *B*, m.p. 152–153°, isolated as a hydrochloride, was a monomethyl ether. Its properties corresponded to those of (XI) and (XIII), indicating the methoxy group in position 2. Since *B* could not be methylated by diazomethane, it appears reasonable to assume for it the structure (XVII) without the free enol group (Scheme 3).

Another monomethyl ether, *C*, m.p. 147–149°, isomeric with *B*, was obtained on methylation of the mono-sodium salt of (XIV) with diazomethane in methanol–ether solution. The ether *C* does not form a salt with hydrogen chloride. Further methylation furnished the dimethyl ether *A*, indicating the existence in monomethyl ether *C* of the methoxy group in the 4-position and of the free enol group (XV).

The results of methylation described above, as well as the properties of the monoethers *B* and *C*, prove the tautomerism of (XIV) and suggest that in the solid state and in ether solution the tautomeric form of a dipole structure (XVIII) predominates.

Monomethyl ether of dicoumarol

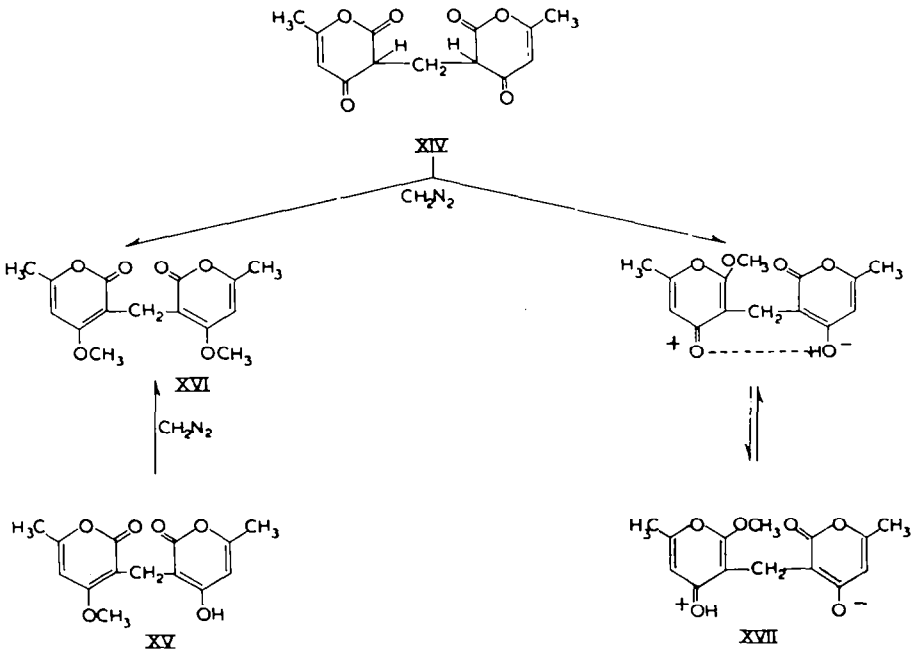
The existence of two isomeric monomethyl ethers of compound (XIV) suggested the possibility of analogous monomethyl ethers for dicoumarol. One of them,

[10] I. Chmielewska and J. Cieślak, *Przem. Chem.* **31**, 196 (1952); I. Chmielewska, J. Cieślak and T. Kraczkiewicz *Roczn. Chem.* **30**, 1009 (1956).

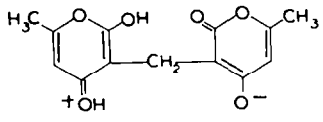
[11] J. Cieślak, *Roczn. Chem.* **26**, 483 (1952).

[12] I. Chmielewska and J. Cieślak, *Bull. Acad. Pol. Sci Cl III* **2**, 149 (1954); *Roczn. Chem.* **30**, 825 (1956).

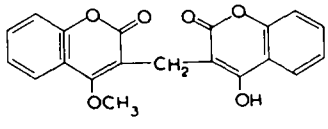
[13] J. Cieślak, Thesis, Warsaw University (1955).



Scheme 3



(XIX), m.p. 172–174°, was obtained by Link *et al.* in the reaction of epoxydicoumarol with sodium methoxide.[14] Since the methylation of dicoumarol with diazomethane furnished only a dimethyl ether, m.p. 156–158°, obtained for the first time by Link *et al.*[15] we attempted to prepare monoethers, (*a*) by demethylation of Link's dimethyl ether in methanolic hydrogen chloride or sodium hydroxide solutions; (*b*) methylation of dicoumarol with dimethyl sulphate; and (*c*) methylation of the monosodium salt of dicoumarol with diazomethane. In all cases the products obtained proved to be identical with Link's monomethyl ether. Its properties confirmed the formula (XIX), proposed by Link.



Isomeric dimethyl ethers of dicoumarol

The structure (XXa), accepted for Link's dimethyl ether of dicoumarol, agrees perfectly with many properties of this compound, except for partial demethylation in methanolic hydrogen chloride solution with formation of a monoether (XIX).

[14] C. F. Huebner, W. R. Sullivan, M. A. Stahmann and K. P. Link, *J. Amer. Chem. Soc.* **65**, 2292 (1943).
[15] H. A. Campbell, W. Roberts, W. Smith and K. P. Link, *J. Biol. Chem.* **138**, 21 (1941).

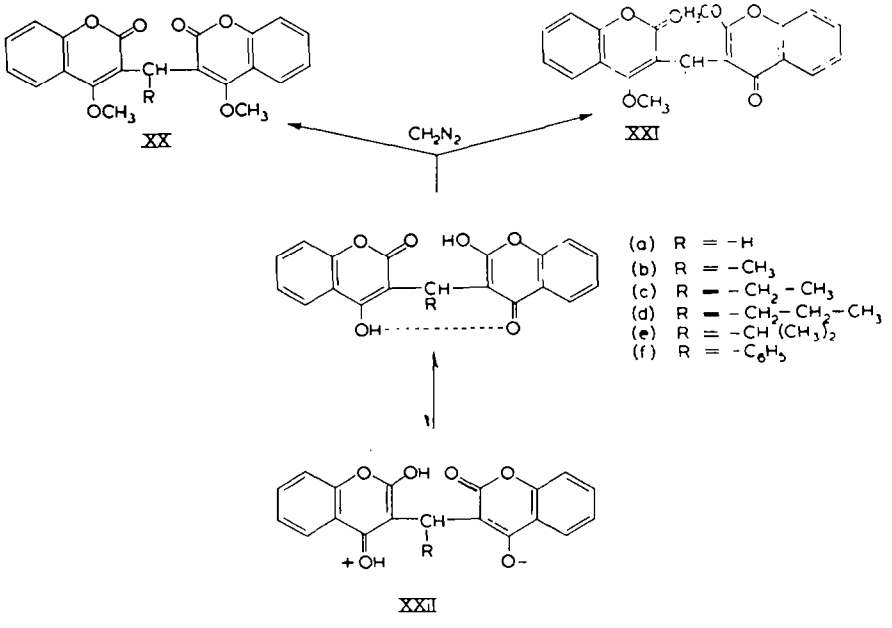
This behaviour, characteristic for 2-methyl ethers (XI), (XIII) and (XVII), was unexpected considering that Link's dimethyl ether appeared to be pure (XXa). The attempt to separate this substance into two fractions by the action of hydrogen chloride was unsuccessful. However, separation was effected through the use of perchloric acid, and the "dimethyl ether of dicoumarol" was shown to be a mixture of two isomers: D, m.p. 154–156°, which did not form a salt with perchloric acid, and E, m.p. 159–161°, perchlorate, m.p. 141–142°.

The ratio of the isomeric dimethyl ethers D and E in the methylation product of dicoumarol monomethyl ether was 5:1[16] and that in the methylation product of dicoumarol 1:1.

The properties of the dimethyl ether D agreed with the structure of 3:3'-methylene-bis-(4-methoxycoumarin) (XXa); those of E indicated the presence of the 2-methoxy-chromone ring in the molecule. It is important to note that E results on methylation of dicoumarol as well as of dicoumarol monomethyl ether (XIX). We concluded that E is 3'-(4'-methoxycoumarin)-methylene-3-(2-methoxychromone) (XXIa).

(XXIa), unlike the isomer (XXa), is not stable in methanolic hydrogen chloride solution and demethylates in the 2-position to yield the monomethyl ether (XIX).

Formation of two isomeric dimethyl ethers (XXa) and (XXIa) proves the tautomerism of dicoumarol. The 1:1 ratio of the ethers obtained by direct methylation of this compound suggests that in the solid state (the methylation with diazomethane takes place in ether suspension) the coumarin–chromone form (XXIIa) predominates.



It is interesting to note the close analogy in the structures of two "bis" compounds investigated, namely (XIV) and dicoumarol. The differences observed in the formation of its methyl ethers seems to be explained as follows. From (XIV), after introduction of the methyl group in the 2-position, the stable onium salt of the monomethyl ether

[16] J. Chmielewska and J. Cieślak, *Roczn. Chem.* **31**, 1079 (1957).

(XVII) is formed which is resistant to further methylation. Since in dicoumarol the pyrone ring is condensed with the benzene ring, the ability to form an 'onium salt after introduction of the methyl group in the 2-position is reduced. Therefore the second methyl group can be introduced to form (XXIa).

Isomeric dimethyl ethers of dicoumarol derivatives

The existence of coumarin–chromone tautomerism was then proved for several derivatives of dicoumarol substituted in the methylene bridge, namely (IVb), (IVc), (IVd), (IVe) and (IVf). The products were methylated with diazomethane and the isomeric dimethyl ethers were separated by the perchlorate method. In such a way two groups of isomeric ethers (XX) and (XXI) were obtained. Their behaviour was similar to that of the isomeric dimethyl ethers of dicoumarol.

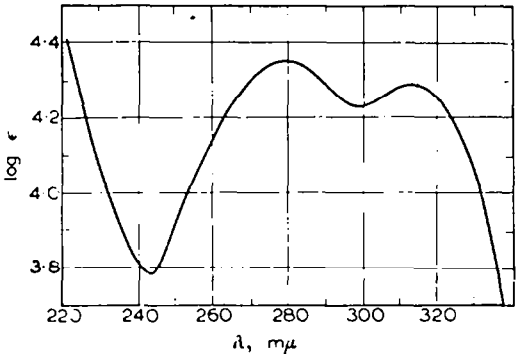


FIG. 1. Ultra-violet spectrum of compounds (XX).

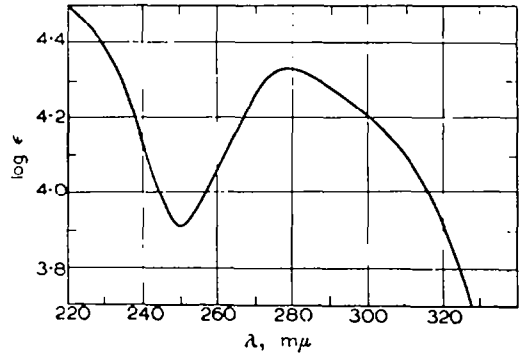


FIG. 2. Ultra-violet spectrum of compounds (XXI).

Ultra-violet spectra

All compounds within a given group have identical ultra-violet spectra, which differ from those in the other group (Figs. 1 and 2). The dimethyl ethers (XXI) show merely one maximum at 275 mμ (log ε 4·32) and the dimethyl ethers (XX) show two maxima, at 284 mμ (log ε 4·33) and at 321 mμ (log ε 4·28).

Infra-red spectra

In order to compare infra-red absorption of dimethyl ethers (XX) and (XXI),

we measured spectra of both 4-methoxycoumarin (X) and 2-methoxychromone (XI). The results are given in Table 1.

TABLE 1. INFRA-RED SPECTRA IN CARBON TETRACHLORIDE

4-Methoxycoumarin (μ)	5·78; 6·13; 6·40; 6·70; 6·85; 7·20; 7·55; 7·85; 8·10; 8·48; 8·75; 9·00; 9·70; 10·15; 10·75; 11·60;
2-Methoxychromone (μ)	6·05; 6·15; 6·38; 6·80; 6·90; 7·20; 7·65; 8·05; 8·48; 8·85; 9·45; 9·78; 10·45; 11·68

TABLE 2. INFRA-RED SPECTRA IN CARBON TETRACHLORIDE OF COMPOUNDS OF GENERAL FORMULAE (XX) AND (XXI)

(XXa) (μ)	(XXb) (μ)	(XXc) (μ)	(XXf) (μ)	(XXIa) (μ)	(XXIb) (μ)	(XXIc) (μ)	(XXIf) (μ)
3·38	3·40	3·40	3·40	3·40	3·40	3·40	3·40
5·80	5·80	5·80	5·82	5·80	5·85	5·83	5·82
6·13	6·18	6·18	6·18	6·15	6·18	6·18	6·18
6·35	6·38	6·40	6·40	6·36	6·40	6·40	6·43
6·70	6·70	6·70	6·72				
6·85	6·88	6·88	6·88	6·85	6·85	6·85	6·85
				7·20	7·25	7·30	7·28
7·40	7·43	7·43	7·42	7·40	7·40	7·45	7·42
7·55	7·55	7·85	7·80	7·55	7·70	7·70	7·67
7·83	7·65	8·18	8·18	7·70	8·03	7·90	8·20
8·18	7·82	8·38	8·38	8·20	8·18	8·20	8·78
8·75	8·18	8·68	8·75	8·53	8·60	8·75	9·15
9·10	8·35	8·93	9·05	8·75	9·15	9·15	9·63
9·45	8·65	9·13	9·55	8·90	9·65	9·60	10·43
10·35	9·25	9·60	10·45	9·10	10·20	10·20	
11·05	9·43	10·33		9·55	10·55	10·60	
	9·70	10·45		10·25			
	9·98	10·60		10·35			
	10·43			10·48			

In agreement with the structures assumed, 4-methoxycoumarin shows carbonyl frequency of a lactone group at 5·78 μ, and 2-methoxychromone, that of an unsaturated ketone at 6·05 μ. The same frequencies were shown, respectively, in (XII) and (XIII), as well as in the isomeric methyl ethers of other 6-substituted-2H-pyran-2: 4(3H)-diones.[12,17]

Dimethyl ethers (XX) show carbonyl frequency at 5·85 μ in agreement with the spectrum of 4-methoxycoumarin. We expected the dimethyl ether (XXI) to show carbonyl frequencies of coumarin at 5·8 μ and of chromone at 6·0 μ; unexpectedly the band at 6·0 μ did not appear in the spectra of (XXI). No explanation of this behaviour can as yet be offered.

[17] I. Chmielewska, J. Cieślak, K. Gorczyńska, B. Kontnik and K. Pistkowska, *Tetrahedron*, **4**, 36 (1958).

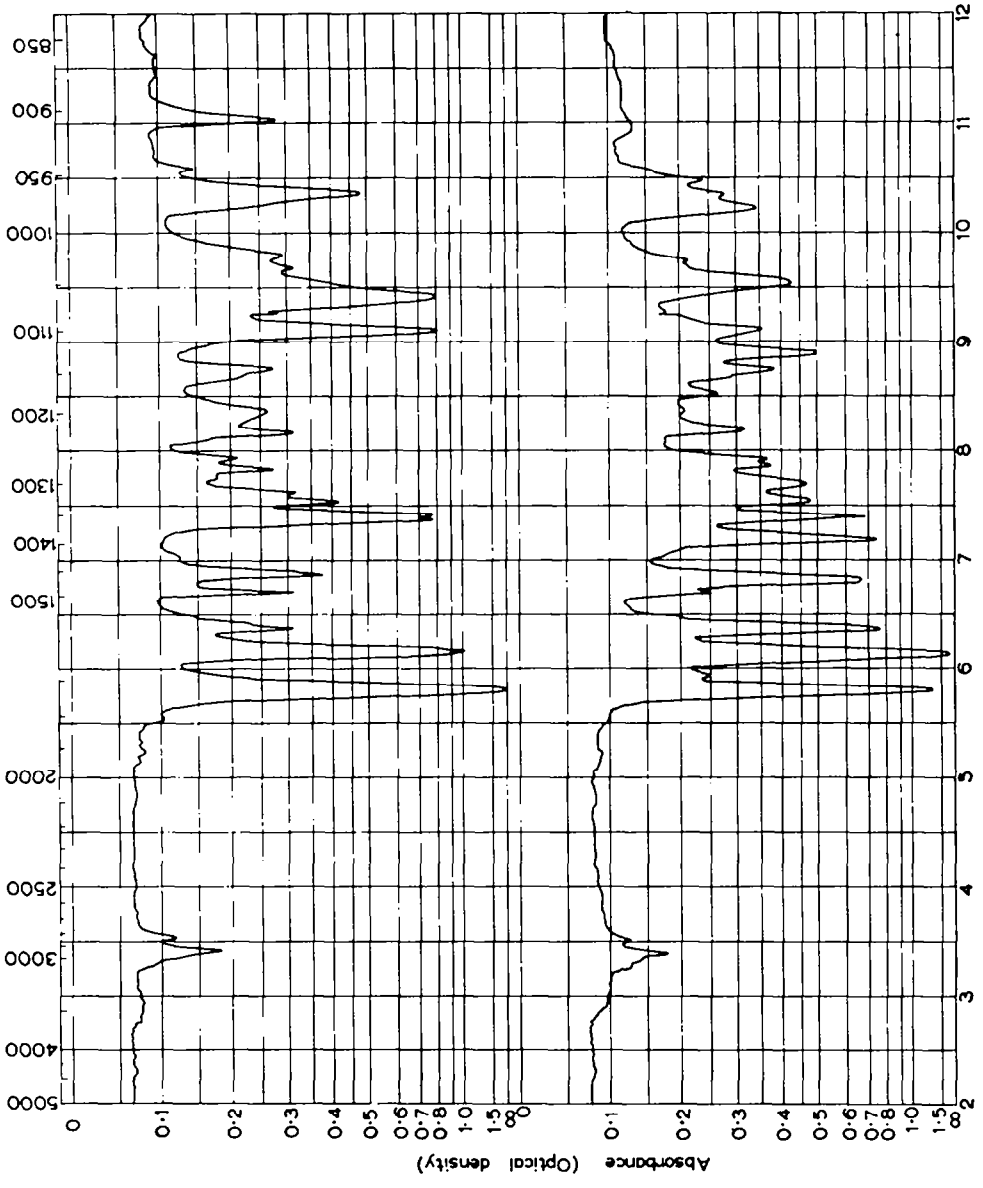


FIG. 3. Infra-red spectra in carbon tetrachloride: (1) 4:4'-dimethyl ether of dicoumarol (XXa); (2) 2,4'-dimethyl ether of dicoumarol (XXIa).

All dimethyl ethers (XX) have bands in the same position and of identical relative intensity in the region between 5·5 and 8·0 μ. Similar relationships were noted within the group of dimethyl ethers (XXI). Nevertheless, the two groups differ in absorption spectra. The relative intensity of bands in the region of 6 μ of 4:4′-diethers [5·85 μ (strong), 6·18 μ (medium)] is reversed in 2:4′-diethers [5·85 μ (medium), 6·18 μ (strong)], the band at 6·70 μ is present only in spectra of 4:4′-diethers, and the band at 7·25 μ only in those of 2:4′-diethers. Results are shown in Fig. 3 and Table 2.

Conclusion

The establishment of the tautomerism of dicoumarol appears to afford some evidence for the possibility of its transformation into the structure (Ib), necessary for the antivitamin-K activity, according to our hypothesis.

EXPERIMENTAL

Ultra-violet absorption spectra were determined in 96% ethanol with a Beckmann DU spectrophotometer. Infra-red absorption spectra were measured with a Perkin–Elmer model 21 spectrometer with a prism of sodium chloride. The compounds were examined in carbon tetrachloride solution.

The ethyl ether was dried over sodium. Some 0·05 N ethereal perchloric acid was prepared from 70% aqueous perchloric acid and dried over anhydrous magnesium sulphate. M.p. are not corrected. Compounds (IVb)–(IVf) were obtained by the method of Link *et al.*[18]

Perchlorate method. Powdered compound (IV) (0·01 mole) in ether suspension (1500 ml) was treated with diazomethane and left for 2–3 days in the cold, till a clear solution resulted. The excess of diazomethane was removed by distillation (a sample of the distillate did not react with perchloric acid) and adequate ether and 0·05 N ethereal perchloric acid (110 ml) was added in the cold. The perchlorate of (XXI) was filtered off and dried *in vacuo*.

The filtrate was washed with aqueous sodium hydrogen carbonate, then with water and dried over anhydrous magnesium sulphate. The solvent was removed and the residue (XX) was crystallised from aqueous acetone.

The perchlorate of (XXI) in methanol (50 ml) was treated with 1 equivalent of N methanolic potassium hydroxide, the potassium perchlorate was filtered off and the solvent was evaporated. Crude (XXI) was crystallised from light petroleum, boiling range 60–80°.

The yields and analytical data of compounds (XX) and (XXI) are given in Tables 3 and 4.

Demethylation of (XXIa). The dimethyl ether of dicoumarol (XXI a) (150 mg) was heated under reflux with 2% methanolic hydrogen chloride (5 ml) for 1 hr. The precipitate of epoxydicoumarol, m.p. 321–323° (30 mg), was filtered off and the filtrate was poured into water and extracted with ether. The ether extract was treated with 1% sodium hydroxide. Acidification gave dicoumarol monomethyl ether (XIX) (100 mg), m.p. 172–174°.

Dimethyl ethers (XXIb)–(XXIf) decomposed chiefly to epoxy compounds.

Dimethyl ethers (XXa)–(XXf) did not change under similar conditions.

[18] W. R. Sullivan, C. F. Huebner, H. A. Stahmann and K. P. Link, *J. Amer. Chem. Soc.* 65, 2288 (1943).

TABLE 3. YIELDS

Substrate		Perchlorate					2:4′-Diether		4:4′-Diether	
Compound taken	Amount (g)	yield (g)	m.p. (°C)	formula	OCH_3 required (%)	OCH_3 found (%)	yield of crude product (g)	m.p. of recrystallised product (°C)	yield of crude product (g)	m.p. of recrystallised product (°C)
(IVa)	3·36	2·30	141–142	$C_{21}H_{16}O_6.HClO_4$	13·35	13·10	1·80	159–161	1·80	154–156
(IVb)	3·50	2·36	144–145	$C_{22}H_{18}O_6.HClO_4$	12·96	12·75	1·86	133–135	1·90	152–153
(IVc)	3·64	2·24	150–151	$C_{23}H_{20}O_6.HClO_4$	12·59	12·38	1·78	96–98	2·11	128–129
(IVd)	3·78	2·26	163–164	$C_{24}H_{22}O_6.HClO_4$	12·24	11·97	1·80	48–51	2·24	117–119
(IVe)	3·78	1·67	160–161	$C_{24}H_{22}O_6.HClO_4$	12·24	12·02	1·67	146–148	2·70	203–204
(IVf)	4·12	1·85	161–163 (dec.)	$C_{27}H_{20}O_6.HClO_4$	11·47	11·19	1·49	200–201	2·90	182–183

TABLE 4. ANALYTICAL DATA

Compound	Formula	Required			Found		
		C (%)	H (%)	OCH_3 (%)	C (%)	H (%)	OCH_3 (%)
(XXa)	$C_{31}H_{16}O_6$	69·22	4·43	17·03	69·23	4·39	16·83
(XXIa)					69·35	4·51	17·21
(XXb)	$C_{33}H_{18}O_6$	69·83	4·79	16·40	70·00	4·59	16·25
(XXIb)					70·11	4·69	16·30
(XXc)	$C_{33}H_{20}O_6$	70·40	5·14	15·81	70·49	4·89	15·63
(XXIc)					70·50	5·04	15·59
(XXd)	$C_{34}H_{22}O_6$	70·92	5·43	15·27	71·13	5·29	15·11
(XXId)					70·70	5·59	15·08
(XXe)	$C_{34}H_{22}O_6$	70·92	5·43	15·27	71·05	5·22	15·27
(XXIe)					71·02	5·32	15·01
(XXf)	$C_{27}H_{20}O_6$	73·62	4·58	14·09	73·73	4·28	14·09
(XXIf)					73·65	4·45	13·81

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