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Carbon-13 Nuclear Magnetic Resonance Spectroscopy. Structure of the Anticoagulant Warfarin and Related Compounds in Solution

(coumarin-related compounds/phenprocoumon)

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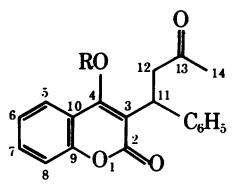
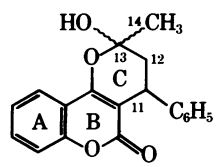
ABSTRACT **Carbon-13 nuclear magnetic resonance spectra have been obtained for the coumarin-related compounds warfarin and phenprocoumon. The spectral assignments indicate that warfarin exists as a mixture of cyclic hemiketal diastereomers in dimethyl sulfoxide solution. The sodium salt of warfarin exists as the ring-open form in water solution.**

Warfarin has been used as a rodenticide and anticoagulant since the late 1940's, while the sodium salt of warfarin (Coumadin®) has become the most widely used anticoagulant in many hematological diseases and surgical procedures. The rationale for structures 1 and 2 assigned to warfarin and its sodium salt, respectively, has come from chemical synthesis (1) and spectroscopic methods (2). These structures have been assumed to be correct for many years, although no rigorous structural proof has as yet been presented in the literature. Recently, it has been suggested that crystalline warfarin exists as the hemiketal, 3. (Diffraction data indicates a cyclic hemiketal structure in the solid state; E. Valente, W. F. Trager, and L. H. Jenson, personal communication and paper submitted for publication.)



1, R = H
2, R = Na



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Clearly, structural information for the dissolved substance is of great interest and importance to those concerned with the mode of action of warfarin.

Solution studies of warfarin utilizing infrared and proton nuclear magnetic resonance (NMR) spectra, are difficult to interpret and are ambiguous due to the complexity of the spectra, although the proton NMR spectrum of warfarin is similar to that of the cyclocoumarol, 4 (K. K. Chan, unpublished data). This fact suggests that the keto structure 1 for warfarin in solution may also be incorrect. Much more definitive information seemed possible through use of carbon-13 NMR to differentiate between structures 1 and 3 because the hemiketal carbon (3, position 13) would have a grossly different chemical shift than a keto carbon (1, position 13; 2, position 13).

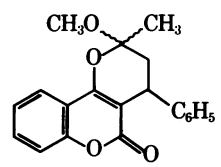
EXPERIMENTAL

The samples used in this work were synthesized or were gifts of Dr. W. F. Trager.‡ Most solutions were 0.75 M in dimethyl sulfoxide-d_6. Tetramethylsilane (TMS) was used as the internal standard. Sodium warfarin was, however, investigated in water because of its low solubility in dimethyl sulfoxide. For it, the field-frequency stability was achieved by locking onto an external deuterium oxide sample which contained dioxane (13% v/v) to act as the external standard.

Chemical-shift measurements were made using a Brukarian, pulsed FT spectrometer (3) operating at 15.09 MHz for carbon-13. The temperature was maintained at 37.0 ± 1° with a Bruker B-ST temperature control unit. Off-resonance and a gated-decoupling (4) technique which turns off the proton noise decoupler during acquisition of data were used in facilitating spectral assignments. A typical spectrum was run with an acquisition time of 0.8 sec, pulse delay of 1.0 sec, sweep width of 5000 Hz and 8K data points.

RESULTS AND DISCUSSION

The assignment of spectral peaks is based on several well-established methods which are now common in ^{13}C spectroscopy (for discussion see refs. 5 and 6). For example, the alkyl carbons, C11, C12, and C14, are known to have characteristic chemical shifts (4) based upon model studies with hydrocarbons. The peaks can be further differentiated by the multiplicity patterns obtained from off-resonance experiments. However, ambiguities were encountered for warfarin and, for example, the C2 and C4 peaks were not assignable by analogy with the resonances of coumarin or 4-hydroxycoumarin. Also, off-resonance did not provide sufficient information to differentiate between the peaks of C3 and C13. It is known that proton-carbon spin-spin splittings for a given carbon-13 nucleus can be retained while maintaining high nuclear Overhauser enhancement by using gated decoupling (4). And,



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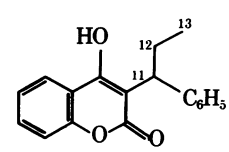

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Abbreviations: NMR, nuclear magnetic resonance; TMS, tetramethylsilane; Me_2SO, dimethyl sulfoxide.

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‡ Samples of phenprocoumon and 30% carbon-13 enriched C2-phenprocoumon were generously provided by Prof. W. F. Trager and Mr. William Porter, University of Washington, Seattle.

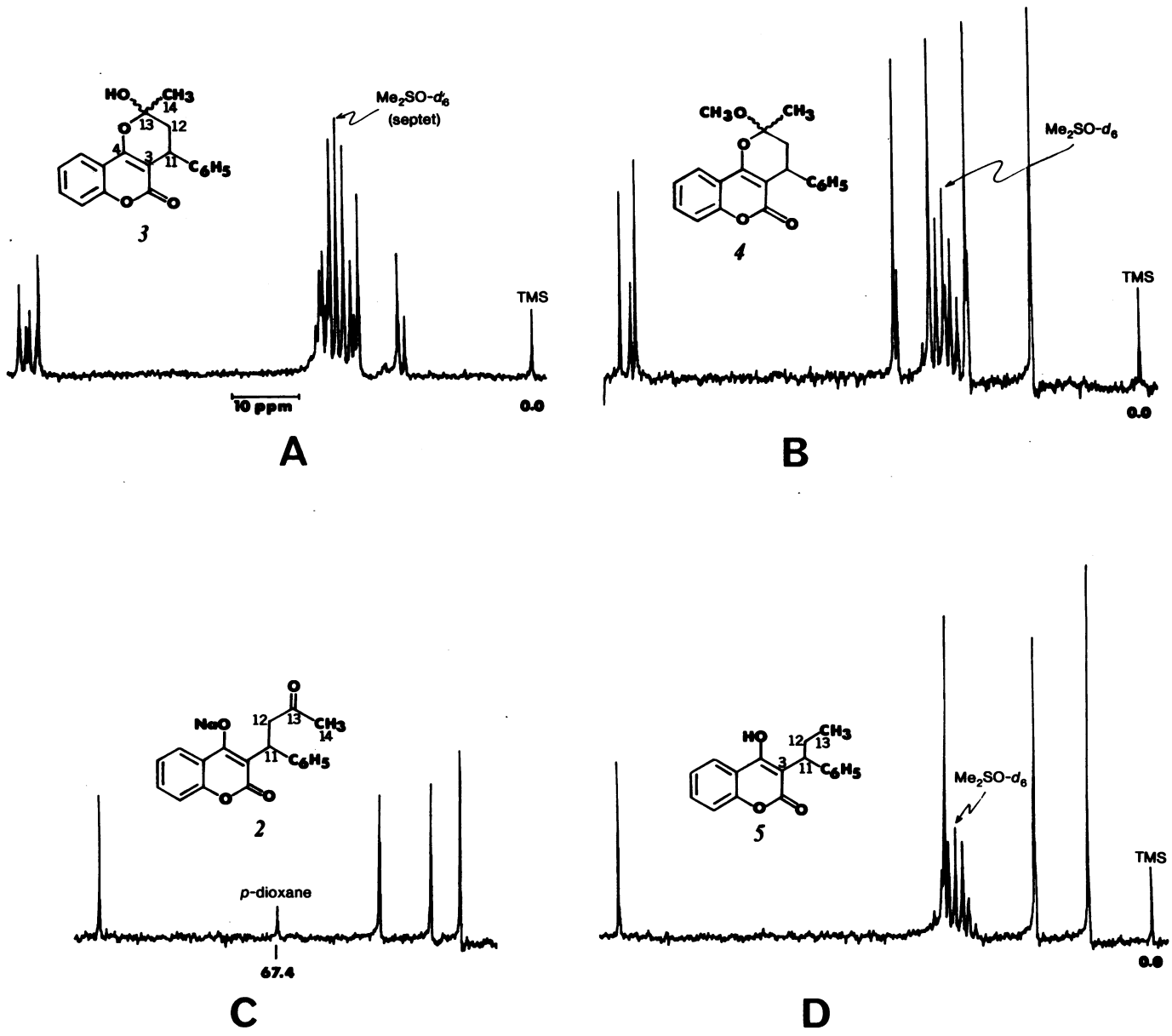

FIG. 1. Upfield part of the carbon-13 spectra of warfarin (A), cyclocoumarol (B), sodium warfarin (C), and phenprocoumon (D). The septet centered at 39.5 ppm in spectra A, B, and D arises from the dimethyl sulfoxide-d_6 (Me_2SO-d_6) used as solvent.

with this technique, C2 and C4 could be differentiated, because C4 is coupled to more protons than C2 and thus gives a broad singlet. The resonances of C3 and C13 were similarly assigned by correlating the multiplicities of their resonances with the number of neighboring protons for structure **3**. The assignment of C2 and C4 in phenprocoumon, **5**, was made by comparing natural-abundance **5** with the material labeled with ^{13}C at C2.

The structural assignment of neutral warfarin was especially facilitated by comparisons with the known cyclic compound, cyclocoumarol, **4**, and of the sodium salt, **2**, with phenprocoumon, **5**. The upfield region of the carbon-13 spectra (0–110 ppm) which is most informative for structure elucidation in these compounds is shown in Fig. 1 and the assignments for the various signals are presented in Table 1§.

§ A complete discussion of the assignments for the entire spectrum of each compound will be presented in a forthcoming paper.

The chemical shifts are also given for the ring-junction carbons, C3 and C4, which are far downfield and not shown in Fig. 1.

Cyclocoumarol **4** has two asymmetric centers (C11 and C13) and, therefore, two pairs of enantiomers are theoretically possible and two signals in the carbon-13 spectrum would be expected for each carbon. However, the bulky phenyl group at C11 should prefer an equatorial placement and thus, when the monomethoxy-ketal, **4**, is formed, one diastereomer is expected to be favored. It is indeed observed that there is a small second peak associated with each aliphatic carbon resonance in the carbon-13 spectrum. The methoxy "doublet" at 49.5 and 48.9 ppm indicates that the downfield peak of this pair is due to the major component. Axial methoxy carbons in cyclic systems have been observed to have upfield chemical shifts relative to equatorial methoxy carbons in carbon-13 spectroscopy (7). Because the larger peak (49.5 ppm) is down-

TABLE 1. *Carbon-13 chemical shifts of warfarin and related compounds*,†*

Carbon	2	3	4	5
C3	103.4	101.3 (102.0)	102.5 (102.7)	107.9
C4	175.7	158.8 (159.4)	157.8 (158.3)	160.7
C11	36.1	35.2 (36.1)	35.1 (34.8)	41.7
C12	46.6	42.9 (41.5)	42.5 (39.2)	23.6
C13	216.5	99.6 (103.4)	101.5 (104.7)	12.7
C14	30.3	27.3 (25.9)	21.7 (22.0)	—
$-OCH_3$			49.5 (48.9)	

* Chemical shifts are in ppm from internal TMS except for compound 2 whose chemical shifts were measured with external dioxane and converted to the TMS scale using the factor 67.4 ppm.

† Two chemical shifts are given for samples which contain a mixture of diastereomers. Those for the minor component are given in parentheses.

field for this pair it is concluded that the methoxy group on C13 is preferentially equatorial (3:1). The ratio of the peak intensities in the other doublets does not appear to be constant; this may in part be due to T_1 and nuclear Overhauser enhancement differences of the given carbons in each isomer.

The spectrum of warfarin (Fig. 1) is strikingly similar to the spectrum of cyclocoumarol, **4**, and this strongly supports structure **3** for warfarin in solution. Furthermore, the presence of two peaks for each carbon in ring C indicates that the substance is also a mixture of diastereomers. This would not be possible if warfarin were in the open-chain form **1**. The peaks assigned to C14 (27.3 and 25.9 ppm) have intensity ratios of about 2:1. The chemical shift of the isomeric methyl carbon (C14) is expected to be upfield when it occupies an axial instead of an equatorial position (8). That the major component (27.3 ppm) is the more downfield peak of this pair, indicates therefore that the methyl carbon is equatorial in the predominant isomer.

Further important evidence for the proposed structure **3** can be deduced from the spectrum of sodium warfarin at pH 9.2 which shows three alkyl peaks (Fig. 1). The doubling pattern which was apparent in compounds **3** and **4** is absent and this clearly demonstrates that the sodium salt is not a mixture of two diastereomers and must have the open-chain structure **2**. Further evidence of ring opening is provided by the peak at 216.5 ppm which is clearly a ketone carbon (see chapt. 5 of ref. 5).

Phenprocoumon, **5**, is closely related to the proposed structure for sodium warfarin and the substances do have similar carbon-13 spectra, provided account is taken of the absence of a carbonyl group at C13 in **5**. As expected, **5** shows no doubling of peaks as could be attributed to the presence of diastereomers.

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