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CARBON-13 NUCLEAR MAGNETIC RESONANCE STUDIES OF COUMARIN AND RELATED COMPOUNDS†‡

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Abstract—Fourier-transform ^{13}C NMR spectra of nine coumarinoid compounds of medicinal interest are reported. All of the carbon resonances are assigned with the aid of various spectral techniques and stable isotopic labeling. The substituent effects on the chemical shifts in several systems are also discussed.

With the advent of Fourier-transform methods and computer technology, natural-abundance ^{13}C NMR (FT-^{13}C NMR) has become a sensitive and powerful tool in the structural elucidation of natural products and studies of chemical conformation. The availability of ^{13}C NMR data on coumarinoids in the literature is still scarce, despite the abundance of this moiety in plant natural products and its important use in pharmaceuticals. Among the latter, the 4-hydroxycoumarin moiety is present in many of the leading anticoagulants used clinically today. Part of the scarcity of data can be attributed to the general difficulties in shielding assignments especially for a new class of compounds. Assignments of shieldings§ on the basis of chemical intuition alone are often equivocal, although confidence can be gained if one compares the data with analogs or similar systems. Unequivocal carbon shielding assignments can, however, be obtained by suitable instrumental techniques and with the aid of specific isotopic labelling. In this paper, carbon assignments on a number of coumarinoids are reported using FT NMR spectroscopy, off-resonance and gated decoupling, along with specific deuterium and ^{13}C labelling.

Spectral assignments. The shielding assignments of all carbons for coumarinoid compounds **1–9** are shown on Tables 1 and 2. The carbon shieldings of coumarin, **1**, have been previously assigned.[1,2] The shieldings of 4-hydroxycoumarin, **2**, were assigned as follows. The positions of C-9, C-8 and C-10 were first tentatively located with reference to anisole[3] and to *o*-cresol.[4] The general regions for absorption by C-2, C-3 and C-4 could be deduced from the shifts of methyl vinyl ether,[5] $\Delta^{2,3}$-cyclohexenone,[6] saturated ϵ-lactone[7] and coumarin. The differentiation of quaternary carbons (C-2, C-4, C-9 and C-10) from the tertiary carbons (C-3 and C-8) was accomplished by the off-resonance decoupled techniques. The off-resonance decoupled ^{13}C spectrum of **2** (Fig. 1) indicates that those signals tentatively assigned by C-2, C-4, C-9 and C-10 remain as singlets, although C-9 and C-10 display a small coupling attributable to the vicinal protons. This small coupling serves to differentiate the signals from C-8. Furthermore, C-9 and C-10 could be easily differentiated by the downfield shift of C-9 arising from the attached oxygen.

The assignments of C-5, C-6 and C-7 could not be clearly made by direct comparison with anisole and *o*-cresol alone. However, the 4-OH group is expected to make a larger change in the shift of C-5 relative to the same carbon in coumarin than of C-6 or C-7. On this basis, the signal at 123.01 ppm rather than 132.34 ppm was assigned as arising from C-5. Confirmation of this assignment was obtained from the spectrum of the partially deuterated 6,7-dideuterio-4-hydroxycoumarin, **3** (Fig. 2). Here decreases were observed in the intensities of the signals of C-6 and C-7 because of deuterium-^{13}C couplings and the nuclear Overhauser effect. The signals for C-6 and C-7 were differentiated on the basis of the additive effect of methyl substitution in *o*-cresol. The shielding value at C-4 (equivalent to C-6 in **2**) of anisole is only slightly downfield of the same carbon in *o*-cresol, whereas C-5 (equivalent to C-7 in **2**) is 2.4 ppm upfield of the corresponding carbon in *o*-cresol. On this basis, the signals for C-6 and C-7 are those at 124.2 ppm and at 131.7 ppm, respectively. The assignment of C-3 was confirmed by D–H exchange, because the C-3H and C-4H protons can be readily exchanged. The diminished intensity of the signal at 90.98 ppm confirmed the assignment of C-3 (Fig. 3). The remaining assignments were C-2 and C-4. A gated-decoupled spectrum (Fig. 4) of these carbons exhibits a broad multiplet for the downfield signal and sharp doublet for the upfield signal. A multiplet

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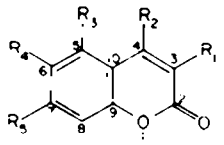
§The term "shieldings" used in this context is from Stothers, Ref. 11.



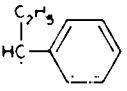
1: Coumarin, R_1 = R_2 = R_3 = R_4 = R_5 = H

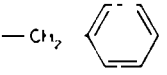
2: 4-Hydroxycoumarin, R_1 = R_3 = R_4 = R_5 = H, R_2 = OH

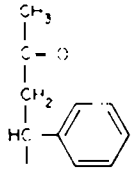
3: 6,7-Dideuterio-4-hydroxycoumarin, R_1 = R_3 = H, R_2 = OH, R_4 = R_5 = D

4: **4-Methoxycoumarin**, R_1 = R_3 = R_4 = R_5 = H, R_2 = OCH_3

5: **3-Bromo-4-hydroxycoumarin**, R_1 = Br, R_2 = OH, R_3 = R_4 = R_5 = H

6: **Phenprocoumon**, R_1 =  , R_2 = OH, R_3 = R_4 = R_5 = H

7: 3-Benzyl-4,5-dihydroxycoumarin, R_1 =  , R_2 = R_3 = OH, R_4 = R_5 = H

8: **4-Warfarin methyl ether**, R_1 =  , R_2 = $-OCH_3$, R_3 = R_4 = R_5 = H

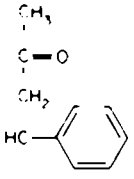
9: Sodium warfarin, R_1 =  , R_2 = O Na^+, R_3 = R_4 = R_5 = H

Table 1. Carbon shieldings of coumarin and related compounds

Carbon*	Coumarin 1	4-Hydroxy-coumarin 2	4-Methoxy-coumarin 4	3-Bromo-4-hydroxycoumarin 5
C-4	143.93	165.45	165.50	162.18
C-2	159.61	161.75	161.32	158.40
C-9	153.33	153.38	152.47	151.60
C-7	131.69	132.34	132.41	132.65
C-6	124.23	123.62	123.86	124.26
C-5	128.22	123.01	122.51	123.37
C-8	116.04	116.14	116.18	116.33
C-10	118.59	115.73	114.99	115.82
C-3	116.04	90.98	90.06	89.13
C-11	–	—	—	—
C-12	—	–	–	—
C-13	—	–	–	—
C-14	—	—	—	—
C-1′	–	—	—	—
C-2′	—	–	—	—
C-3′	—	–	–	–
C-4′	—	—		–
O-ME	—	—	56.88	—

*Chemical shifts are in ppm from internal TMS except sodium warfarin whose chemical shifts were measured with external 1,4-dioxane and converted to the TMS scale using the factor 67.4 ppm. Compounds **1**, **2**, **4** were recorded by a Varian XL-100, compounds **3**, **5**, **6**, **7**, **9** by a Brukarian, and compound **5** by a Varian CFT-20 spectrometer.

is expected for C-4 because of coupling with several protons while a sharp doublet is expected for C-2 from coupling with the C-3 proton. Therefore, the upfield signal was assigned to be C-4 and the downfield one to be C-2. Recently, identical carbon shieldings for **2**[11] and **4**[11] have been published elsewhere employing alternate methods for assigning these spectra.

The carbon shieldings for **2** are substantially different from **1** itself. The 4-OH causes C-4 to shift downfield by 21.5 ppm but C-3 upfield by 25.16 ppm. Similar effects arising from electronegative groups are known for alkenes.[8] The C-5 resonance of **1** is shifted upfield more than 5 ppm in **2** whereas C-9 is essentially unchanged. This result suggests a γ effect between the 4-OH group and the proton at C-5.[12] The cause of the γ effect in this system may be attributable to the steric interaction of

Table 2. Carbon shieldings of coumarin and related compounds

Carbon†	Phenprocoumon 6	3-Benzyl-4,5-dihydroxycoumarin 7	Warfarin 4-methyl ether 8	Sodium warfarin 9
C-4	160.69	162.63	164.21	175.74
C-2	161.55	162.45	162.30	167.92
C-9	152.21	153.11	153.01	153.49
C-7	131.68	132.05	131.39	131.62
C-6	123.67	110.21	123.53	123.94
C-5	123.44	154.57	126.69	126.60
C-8	116.25	107.86	116.55	116.65
C-10	116.11	103.80	116.76	122.33
C-3	107.88	102.41	119.76	103.4
C-11	41.74	28.69	36.38	36.1
C-12	23.59	-	45.71	46.6
C-13	12.68	—	207.06	216.46
C-14	- -	—	30.13	30.3
C-1'	143.65	140.17	142.21	145.0
C-2'	127.87	128.13	127.93	128.04
C-3'	128.87	128.28	128.44	128.9
C-4'	125.78	125.78	123.93	125.02
O-ME	—	-	61.89	

†Chemical shifts are in ppm from internal TMS except sodium warfarin whose chemical shifts were measured with external 1,4-dioxane and converted to the TMS scale using the factor 67.4 ppm. Compounds **1**, **2**, **4** were recorded by a Varian XL-100, compounds **3**, **5**, **6**, **7**, **9** by a Brukarian and compound **8** by a Varian CFT-20 spectrometer.

‡In $CDCl_3$.

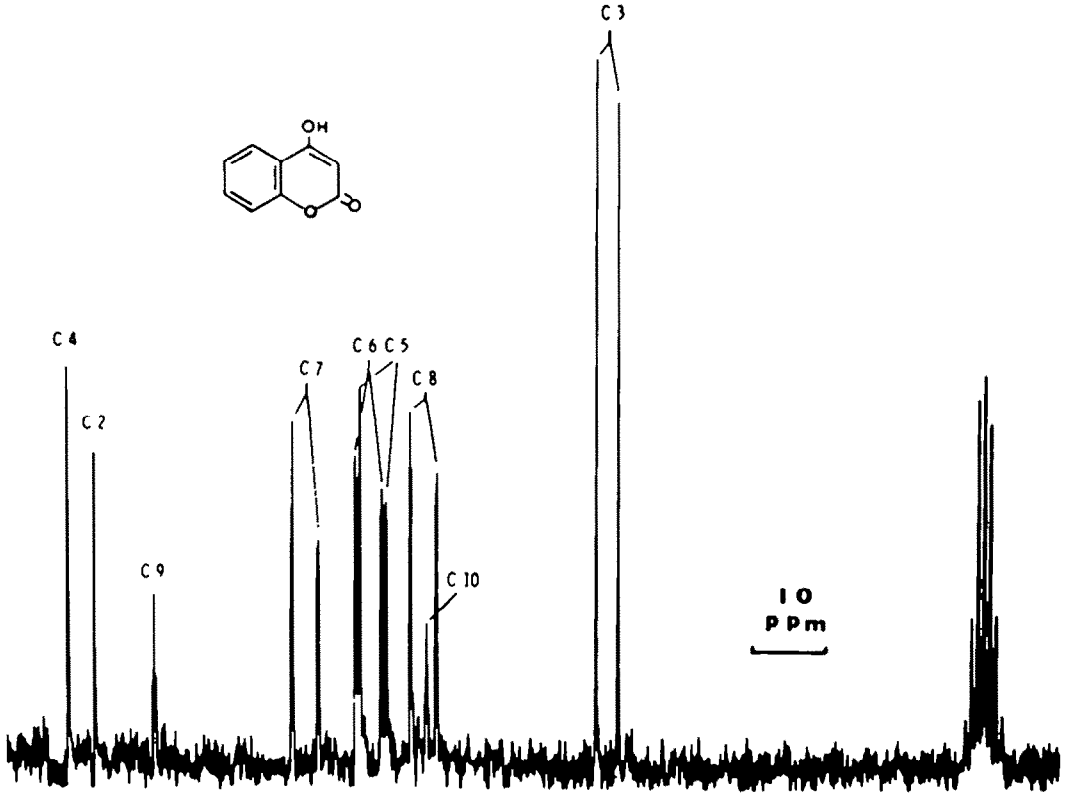


Fig. 1. Off-resonance FT ^{13}C NMR spectrum of **2** in DMSO-d_6. The septet on the far right was derived from the solvent. Concentration 0.7 M.

†An alternative explanation of the upfield effect is that it may be due to the hyperconjugative effect of the free electron pair of oxygen transmitted through the π bond system similar to the γ-anti effect suggested by Eliel *et al.* (E. L. Eliel *et al.*, *J. Am. Chem. Soc.* **97**, 322 (1975)). The authors wish to thank one of the referees for pointing this out

these two groups similar to the 1-alkyl substituted naphthalene systems.[†][13,14]

The carbon shielding of 4-methoxycoumarin, **4**, closely resemble those of **2** as expected, and the assignments are straightforward.

The carbon assignments of 3-bromo-4-hydroxy-

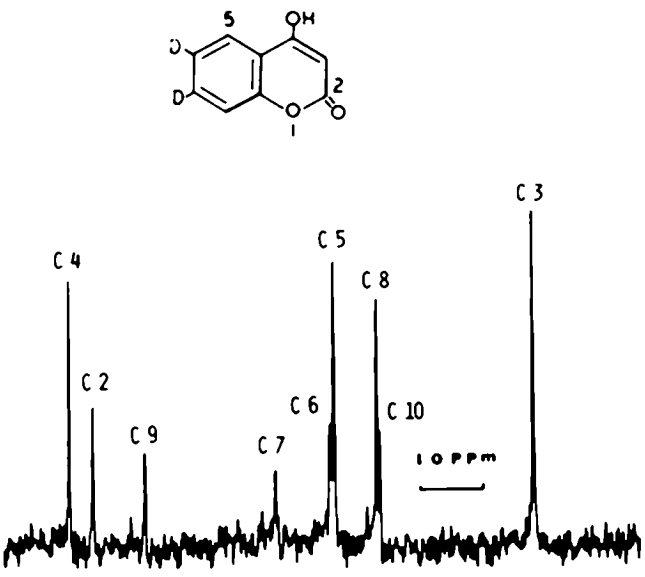


Fig. 2. FT ^{13}C NMR spectrum of 27% 3* in 2 dissolved in DMSO-d_6. The septet on the far right was derived from the solvent. Concentration 0.3 M.

*The carbon shieldings of 3 could not be accurately distinquished from 2 with the experimental condition employed since only 27% 3 in 2 was used for the spectral measurement.

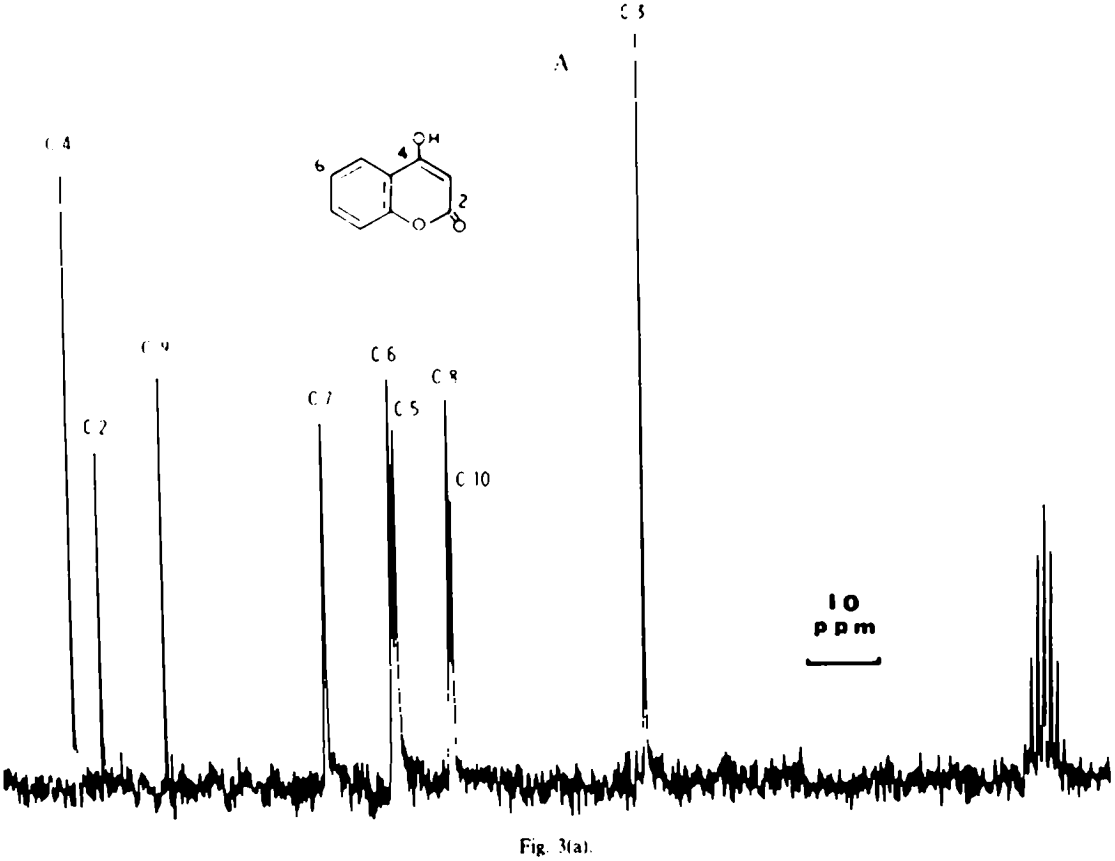


Fig. 3(a).

coumarin, 5, were also deduced by a direct comparison of its spectrum with that of 2. Substitution of bromine on C-3 produces perturbations on the shielding of the carbon being substituted and of the neighboring C atoms. Both C-2 and C-4 exhibit upfield shifts in excess of 3 ppm while C-3 is shifted about 1 ppm in the same direction. The influence on the rest of the ring system is small except on C-9 which shows an upfield shift of 1.78 ppm. These

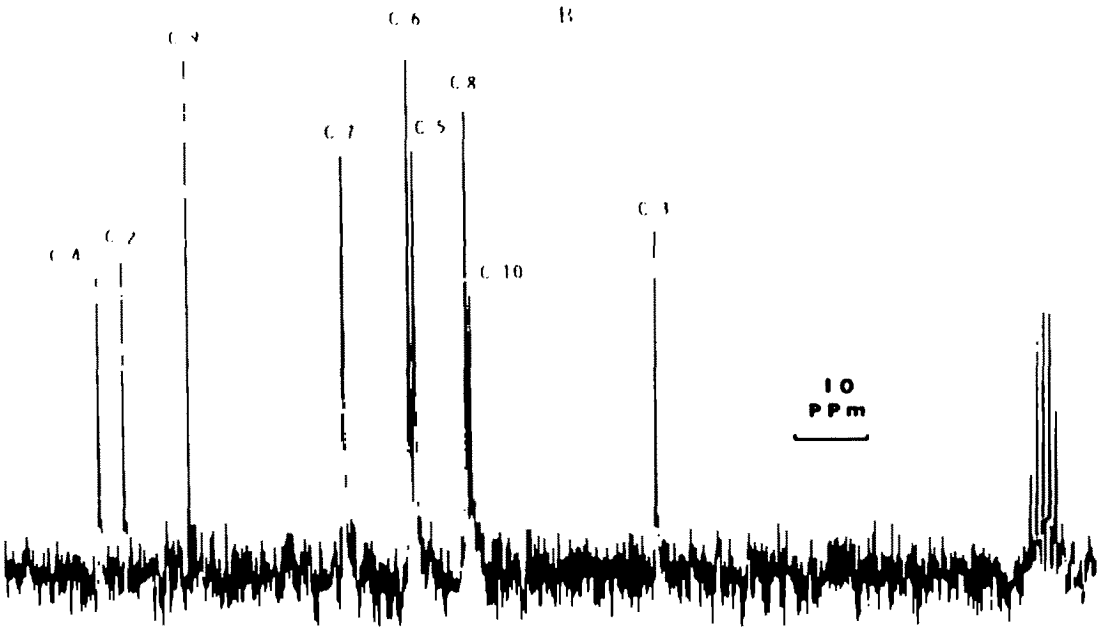


Fig. 3(b).

Fig. 3. (a) FT ^{13}C NMR spectrum of **2** in DMSO-d_6. (b) FT ^{13}C NMR spectrum of **2** in DMSO-d_6 after equilibration with 0.1 ml of D_2O for 2 hr.

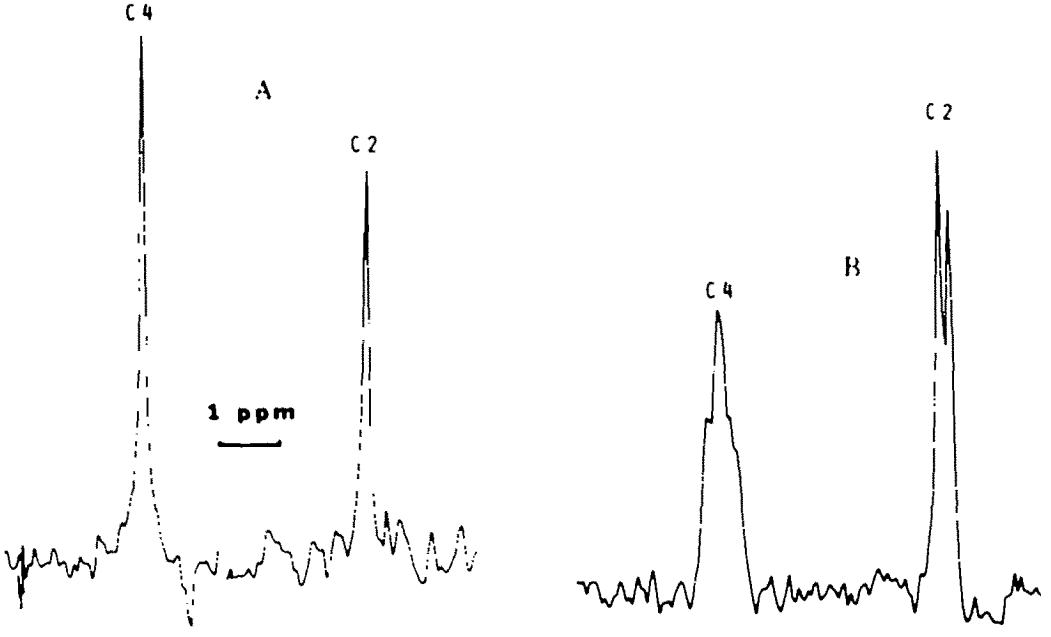


Fig. 4. (a) Gated decoupled FT ^{13}C NMR spectrum of **2** in DMSO-d_6. Pulse delay, 4.2 secs. Only C-2 and C-4 expanded scale are shown. (b) Expanded FT ^{13}C NMR spectrum of **2** in DMSO-d_6. Pulse delay 0.4 secs. Only C-2 and C-4 are shown.

results are not consistent with the shielding parameters observed for simple bromine-substituted alkenic or aromatic systems.[15]

The carbon shieldings of the 4-hydroxycoumarin ring in phenprocoumon, **6**, were assigned by a direct comparison of its spectrum with that of **2**. The shielding assignments are straightforward (Table 1) except for C-2 and C-4 which are too close to be readily differentiated. The spectrum of **6** enriched with ^{13}C† at C-2 (Fig. 5) indicated that the resonance for this carbon, unlike the corresponding signal in **2**, occurs downfield of C-4 by 0.86 ppm.[16] The assignments for C-11, C-12, C-13 were also reported previously,[16] and were substantiated by an off-resonance decoupled spectrum (Fig. 6). The resonances for C-1', C-4' were assigned by analogy to those of monoalkyl-substituted benzenes.[17]

† ^{13}C phenprocoumon labelled at C-2 was synthesized by ring opening and decarboxylation of phenprocoumon followed by recarboxylation and ring closure with barium ^{13}C-carbonate. The synthetic procedure will be published elsewhere (W. R. Porter and W. F. Trager).

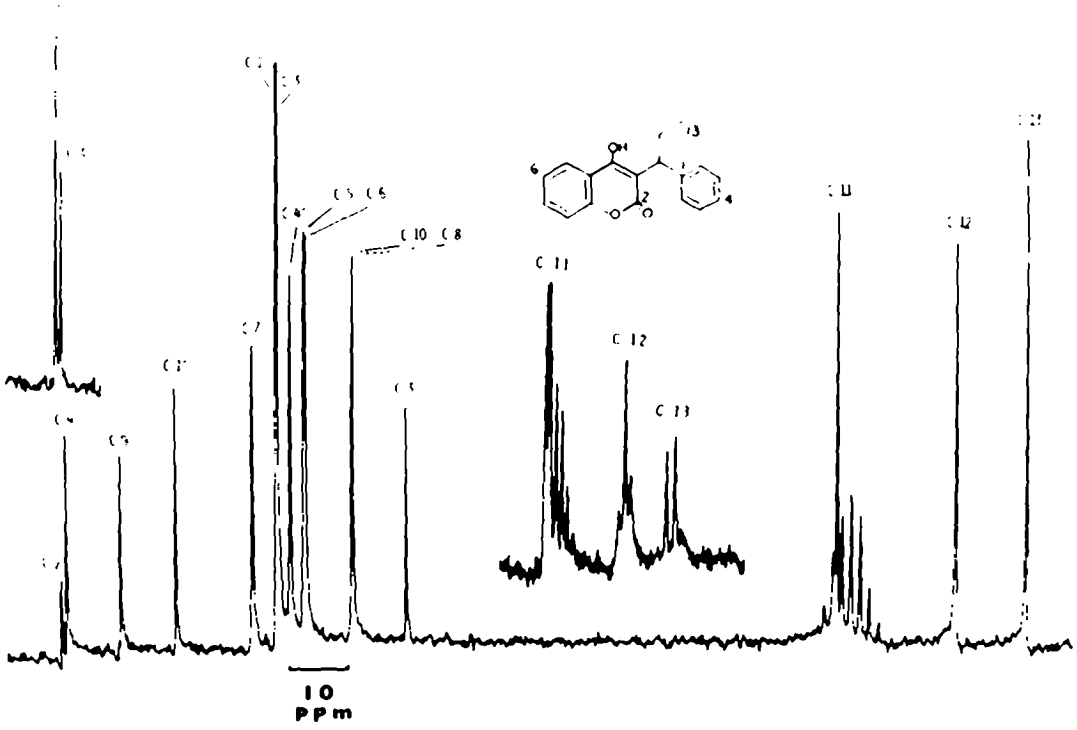


Fig. 5. FT ^{13}C NMR spectrum of **6** in DMSO-d_6. Left top shows enhancement of C-2 signal by ^{13}C enrichment. Middle shows portion of its off-resonance spectrum. Concentration 0.75 M.

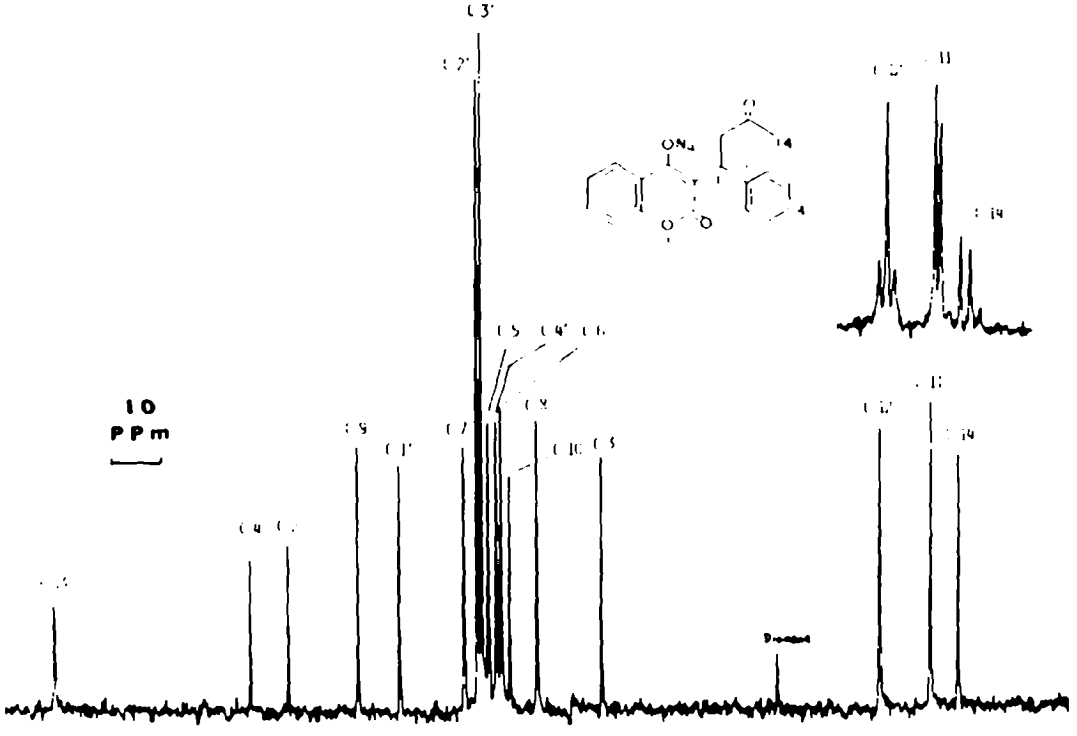


Fig. 6. FT ^{13}C NMR spectrum of **9** in D_2O. Right top shows portion of its off-resonance decoupled spectrum. Concentration 0.7 M.

The assignments of the carbon shieldings of 3-benzyl-4,5-dihydroxycoumarin, **7**, were made with reference to those of **6** and **2**. The closeness of the C-2 and C-4 resonances lead to an arbitrary assignment.

The resonances of the ether formed by treatment of sodium warfarin, the commonly used oral anticoagulant, with methyl iodide (4-warfarin-methyl ether, **8**) were assigned by comparison with several model compounds.

The resonance 207.06 ppm is near the expected value for a methyl ketone (206.25 ppm in 2-butanone[18]) and is therefore assigned by C-13. The resonances for C-11, C-12, C-14 and the OMe carbon were assigned on the basis of off-resonance decoupling. The resonances for C-1′–C-4′ on the phenyl ring were assigned as before. The remainder of the spectrum was assigned by analogy to **2**. The large downfield shift of C-3 to 110.76 ppm was strikingly different from the resonance of C-3 in **4** at 90.06 ppm and in **6** at 107.88 ppm. Indeed, the position of the C-3 resonance of **8** is even downfield of the corresponding signal in coumarin itself (Table 1). It seems likely that in **2** or **4**, C-3 experiences a large upfield shift due to conjugation. Whereas in **8**, and to a lesser extent in **6**, this same effect is not seen because the coplanarity of the methoxyl substituent with the α,β-unsaturated lactone system necessary for a strong resonance interaction is inhibited through steric hindrance with the substituent at C-3. The downfield position of C-3 relative to **1** is presumably due to the electronegativity of the OMe group.

Sodium warfarin, **9**, is poorly soluble in DMSO. Consequently, its ^{13}C NMR spectrum was recorded in water with 1,4-dioxane as the reference. The observed resonances were converted to the TMS scale by adding a factor of 67.4 ppm.* The carbon shielding assignments were derived from comparisons of the spectrum with those of **6** and **8**. The shielding for C-6 through C-9 of **9** are essentially identical to those of the model compounds while C-3 is shifted upfield with respect to **6** and downfield with respect to **2**, an effect consistent with the previous discussion. Both C-5 and C-10 are shifted downfield with respect to both model compounds. These shifts are presumably due to a combination of solvent effects and the influence of the anionic nature of warfarin. Major changes in the shieldings are seen for C-2 and C-4. The signals of these two carbons were differentiated by the enhancement of the C-2 signal intensity via addition of **9** labelled with ^{13}C at C-2.† The reason for the large downfield shift of C-4 relative to the position of the analogous carbon in **8** is not readily apparent. However, recent work in our laboratories suggests that the effect may be due to a substantial contribution of the chromone resonance structure to the anion.

The keto carbon on the side chain, C-13, is assigned to the most downfield signal by analogy to **8**. The shieldings for C-11, C-12 and C-14 were adduced by the off-resonance decoupled spectrum shown in Fig. 7, while the shieldings for the side chain aromatic ring were readily assigned by a comparison with **6** and with a monoalkyl-substituted benzene.[17]

EXPERIMENTAL

Shielding measurements were made using a Brukarian,‡ a Varian XL-100, or a Varian CFT-20 pulsed FT spectrometer operating at 15.09, at 25.2 and at 20 MHz, respectively for ^{13}C. For the Brukarian spectrometer, the temp. was maintained at $37.0 \pm 1.0°$ with a Bruker B-ST temp. control unit. Off-resonance and gated-decoupling[19] techniques were used in facilitating spectral acquisition. 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. The concentrations of most solns were 0.5–0.75 M (where indicated) DMSO-d_6 or in $CDCl_3$. TMS was used as the internal standard. Sodium warfarin, however, was investigated in water because of its low solubility in DMSO. For it, the field-frequency stability was achieved by locking onto an external deuterium oxide sample which contained 1,4 dioxane (13% v/v) to serve as the external standard and a field-frequency lock.

The samples used in this work were either gifts,‖ were synthesized, or were commercially available;§ all were used without further purification. The labeling of the hydroxy coumarins¶ was achieved by exchange with deuterium oxide over platinum oxide in a bomb, maintained at 130° for 2 hr. The positions of D substitution were verified by a detailed analysis of the ABCD-type proton spectra of 4-hydroxycoumarin at 60, 90, 100 and 220 MHz. The averages of the four chemical shifts in ppm relative to TMS and couplings in Hz obtained from the proton spectra at different frequencies were W1, 7.35 ± 0.01; W2, 7.33 ± 0.01; W3, 7.62 ± 0.01 and W4, 7.82 ± 0.01; and J12, 1.0 ± 0.4; J13, 8.3 ± 0.3; J14, 0.3 ± 0.2; J23, 7.5 ± 0.1; J24, 7.9 ± 0.1 and J34, 1.6 ± 0.1. The chemical shifts of the two protons with *one* large and *three* smaller couplings are clearly due to the protons at ring positions 5 and 8. These shifts correspond closely to the shifts of the residual protons (7.35 and 7.82 ppm) in the dideuterated 4-hydroxycoumarin. The need for a detailed analysis is evident from the fact that the two upfield protons, one of which must be either on the 5- or 8-ring carbon, have a very small chemical-shift difference (0.02 ppm).

4-Methoxycoumarin, **4**, was synthesized by methylation of 4-hydroxycoumarin with BF_3 in MeOH.[20] **5** was prepared by the lit. method[21] and **7** was synthesized by a condensation reaction using resorcinal and ethyl benzyl malonate.[22] **6**, was synthesized by a refined method of Schroeder *et al.*[23]

Compound **8** was prepared by iodomethane method outlined as follows. To a soln of 33 g (0.1 mole) of sodium warfarin, U.S.P., in 300 ml acetone was added 20 g (0.14 mole) iodomethane. The mixture was refluxed for 36 hr. Acetone was removed *in vacuo* and the residue was partitioned between 300 ml diethyl ether and 30 ml 1N NaOH. The ether soln was concentrated to give a white crystalline ppt, which was recovered by suction filtration to yield 15.5 g (48%), m.p. 124–126 (lit.[24] m.p. 127°) and a mixture of this and an authentic sample gave no m.p. depression. The IR spectrum showed a strong absorption at 1709 cm^{-1} and the PMR spectrum showed two sharp profiles at 2.14 ppm (side-chain Me) and at 4.00 ppm (MeO).

Note added in Proof: Recently, the assignment of the 5,8 protons have been further confirmed by the PMR spectrum of 6-deuterio-4-hydroxycoumarin synthesized by specific deuterium-halogen exchange.[25]

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*Sodium warfarin labelled at C-2 with ^{13}C was synthesized by desulfurization of 2-^{13}C-warfarin ethylene dithioketal. Details will be published elsewhere (W. R. Porter and W. F. Trager).

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§Compounds purchased commercially are coumarin and 4-hydroxycoumarin, Aldrich Chemical; sodium warfarin (Panwarfarin®), Abbott Laboratories.

¶W. F. Trager, unpublished data.

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