4-Hydroxycoumarin/2-Hydroxychromone Tautomerism: Infrared Spectra of 3-Substituted-2-^{13}C-4-hydroxycoumarins

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By isotopic replacement of the carbonyl carbon with ^{13}C, the C=O stretching frequency was identified as the highest frequency strongly absorbing band in the 1550-1750 cm^{-1} region of the infrared spectra of several 3-substituted 4-hydroxycoumarins and 3-substituted 4-alkoxycoumarins. The compounds selected for study were either known to crystallize as the coumarin tautomeric form by x-ray diffraction studies or were congeners of such compounds. The carbonyl band varied from 1664 cm^{-1} in inter- or intramolecularly hydrogen bonded derivatives to 1718 cm^{-1}.

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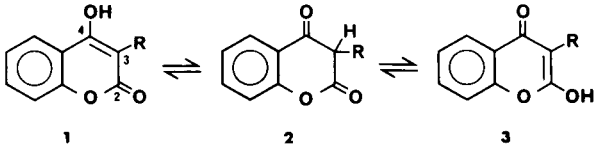
Numerous 3-substituted 4-hydroxycoumarin derivatives have found clinical use as anticoagulant drugs for the treatment of thromboembolism (1-3). They are also extensively employed in agriculture as rodenticides (4). All coumarin anticoagulants exert their therapeutic and toxic effects by interfering with the vitamin K dependent synthesis of clotting factors required for the coagulation of blood (5). Coumarin anticoagulants apparently interfere with the regeneration of vitamin K from vitamin K epoxide, formed during the carboxylation of glutamic acid residues on clotting factor precursor proteins (6).

A detailed molecular understanding of the mode of action of coumarin anticoagulants is hampered by the failure of many investigators to consider the various tautomeric forms in which these drugs may exist. 3-Substituted 4-hydroxycoumarins (**1**) may exist in solution as such, or as 3-substituted 2,4-chromandiones (**2**) or 3-substituted 2-hydroxychromones (**3**) (Scheme 1).

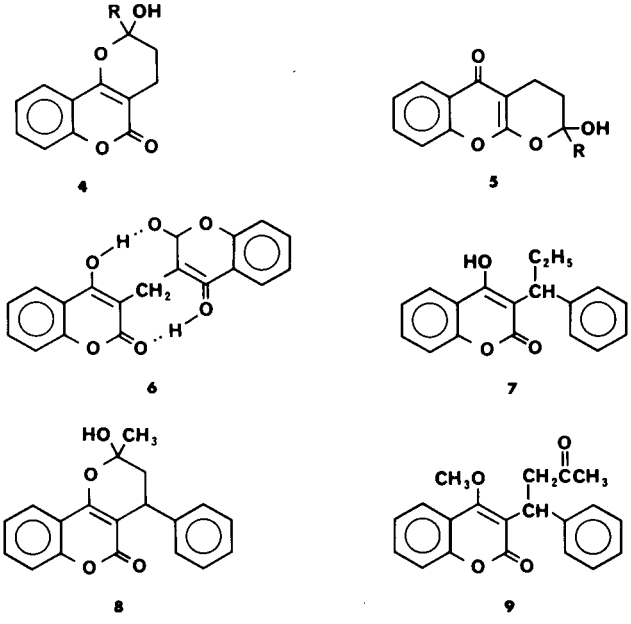
Scheme 1



1 **2** **3**

In addition, 3-γ-keto-4-hydroxycoumarins can display ring-chain tautomerism, with the formation of coumarin (**4**) and chromone (**5**) cyclic hemiketals. Numerous chemical and physical methods have been used to determine the dominant tautomeric forms of these compounds, including studies of chemical reactivity, and ultraviolet, infrared, and nuclear magnetic resonance spectroscopy (7-10). These methods, except for infrared spectral studies of isotopically labeled 4-hydroxycoumarin (7), have based structural assignments on comparisons to structurally rigid model compounds. Recently, however, the structures of many 3-substituted 4-hydroxycoumarins have been determined directly in the crystalline state by x-ray diffraction (8,11-20). All of the compounds studied crystallize as either 4-hydroxycoumarins (8,11-16) or as coumarin cyclic ketals or hemiketals (8,17-20).

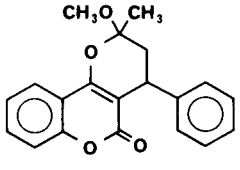
Of the methods which have been used for studying 4-hydroxycoumarin/2-hydroxychromone tautomerism, only infrared spectroscopy has the capibility to distinguish tautomeric substances both in solution and in the crystalline state. To test the strengths and weaknesses of this method, we studied the infrared spectra of selected 4-hydroxycoumarin derivatives known to crystallize as the 4-hydroxycoumarin tautomer as determined by x-ray diffraction studies. These included dicumarol (3,3'-methylenebis [4-hydroxycoumarin], (**6**) (12), phenprocoumon (**7**) (13,16) and warfarin (**8**) (18). Warfarin 4-methyl ether (**9**), which structure has been unequivocably established by x-ray diffraction studies (8), was included as a model 4-alkoxy derivative.



4 **5** **6** **7** **8** **9**

Each 4-hydroxy or 4-alkoxycoumarin derivative was prepared with the C(2) carbon of the coumarin ring replaced by ^{13}C using a specific synthetic method (31). Comparison of the spectra of the unlabeled and ^{13}C-labeled compounds permitted identification of the vibrations involving C(2) (22-24). Since all of the compounds crystallize as the 4-hydroxycoumarin tautomer, isotopic substitution at C(2) permitted identification of ν (C=O) in spectra of the crystalline samples. Vibrations involving C(2) could also be identified in the spectra of samples dissolved in the three solvents studied: chloroform, a hydrogen bond donating solvent, dimethylsulfoxide, a powerful hydrogen bond accepting solvent, and dioxane, a weaker hydrogen bond accepting solvent which disrupts intermolecular hydrogen bonds (25) but does not disrupt the intramolecular hydrogen bonds in dicumarol (10).

Two additional compounds, the major diastereomer of cyclocoumarol (**10**), and warfarin ethylenedithioketal (**11**), were also studied. The latter compound (**11**) has been claimed to crystallize as a chromone (26).



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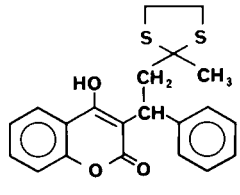

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Results and Discussion.

Crystalline Dicumarol (**6**) and Phenprocoumon (**7**).

The infrared spectra of both of these crystalline 3-substituted 4-hydroxycoumarins (12,13) have a strong band at 1664 cm^{-1} which disappears upon replacement of C(2) with ^{13}C. The band apparently shifts and is superimposed on an existing band at 1631 or 1621 cm^{-1} (Table 1). The band at 1664 cm^{-1} therefore had substantial ν (C=O) character. Bands at 1605 and 1572 cm^{-1} for dicumarol (**6**) and bands at 1611 and 1569 cm^{-1} in the spectrum of phenprocoumon (**7**) are shifted by ~ 10 and ~ 5 cm^{-1}, respectively, upon replacement of C(2) by ^{13}C. These bands represent involvement of C(2) with pyrone or benzene ring stretching vibrations. Bands at 1399 cm^{-1} for phenprocoumon (**7**) and 1112 cm^{-1} for dicumarol (**6**) are significantly shifted after isotopic substitution and presumably are ν (C-C) or ν (C-O) bands of the pyrone ring. A weak band at ~ 755 cm^{-1} in both spectra is also shifted; presumably this is a pyrone ring bending vibration.

Table 1

Effect of Isotopic Substitution on the Infrared Spectra of Crystalline Dicumarol (**6**) and Phenprocoumon (**7**) (a)

Absorption Band, cm^{-1} (Potassium bromide pellet) (b)			
6	**6**-2,2'-^{13}C	(±)-**7**	(±)-**7**-2-^{13}C
1664 (S) (c)		1664 (S)	
1631 (S)	1631 (S)	1621 (S)	1621 (VS)
1604 (S)	1595 (VS)	1611 (S)	1600 (VS)
1572 (S)	1567 (VS)	1569 (M)	1563 (M)
		1399 (M)	1391 (M)
1112 (S)	1098 (S)		
746 (M)	731 (M)	744 (W)	733 (W)

(a) Only those bands which are affected by isotopic substitution are listed. (b) ± 3 cm^{-1}. (c) VS = very strong, S = strong, M = medium, W = weak.

Crystalline (−)-(*S*)-Warfarin (**8**) and (±)-Warfarin 4-Methyl Ether (**9**).

Table 2

Infrared Spectra and Effect of Isotopic Substitution at C-2 of Crystalline (−)-(*S*)-Warfarin (**8**) and (±)-Warfarin 4-Methyl Ether (**9**)

Absorption Band, cm^{-1} (Potassium bromide pellet) (a)			
8	**8**-2-^{13}C	**9**	**9**-2-^{13}C
3425 (S) (b)	3425 (S)		
3106 (W)	3106 (W)	3106 (W)	3106 (W)
3067 (W)	3067 (W)	3030 (W)	3030 (W)
3030 (W)	3030 (W)	2959 (W)	2959 (W)
2959 (W)	2959 (W)	2865 (W)	2865 (W)
1686 (S)	1661 (S)	1718 (VS, br)	1718 (VS, br)
			1664 (S)
1621 (S)	1621 (S)	1616 (S)	1616 (S)
1570 (M)	1570 (M)	1575 (M)	1567 (M)
1495 (M)	1495 (M)	1497 (M)	1497 (M)
1475 (M)	1475 (M)	1460 (M)	1460 (M)
		1420 (M)	1420 (M)
1389 (S)	1389 (S)	1372 (W)	1372 (W)
		1355 (S)	1355 (S)
		1344 (S)	1344 (S)
1330 (W)	1330 (W)	1330 (W)	1330 (W)
1277 (M)	1277 (M)	1285 (W)	1285 (W)
1252 (M)	1245 (M)	1266 (W)	1266 (W)
1221 (W)	1221 (W)	1230 (W)	1230 (W)
1198 (M)	1198 (M)	1189 (W)	1189 (W)
1176 (M)	1176 (M)	1174 (M)	1174 (M)
	1157 (W)	1139 (W)	1139 (W)
1106 (M)	1106 (M)	1100 (S)	1088 (W)
1075 (S)	1075 (S)	1076 (M)	1076 (M)
1032 (W)	1032 (W)	1055 (M)	1055 (M)
1010 (M)	1010 (M)	1033 (W)	1033 (W)
995 (W)	995 (W)	1008 (S)	1008 (S)
950 (W)	950 (W)		
918 (W)	918 (W)	961 (S)	961 (S)
901 (M)	901 (M)	922 (W)	922 (W)
876 (M)	876 (M)	907 (W)	907 (W)
811 (W)	811 (W)	864 (W)	864 (W)
782 (W)	782 (W)	834 (W)	834 (W)
		784 (W)	784 (W)
765 (S)	755 (S)	767 (M)	767 (M)
755 (S)	741 (W)	760 (S)	760 (S)
697 (S)	697 (S)	750 (S)	737 (S)
		704 (S)	704 (S)

(a) ±10 cm^{-1}, $\nu > 2000$ cm^{-1}; ±3 cm^{-1} $\nu < 2000$ cm^{-1}. (b) VS = very strong, S = strong, M = medium, W = weak, br = broad.

Both of these compounds contain alkyl substituents at O(4) in the crystalline state (8,18). The infrared spectral bands of both compounds are listed in Table 2.

Warfarin (**8**) has a band at 1686 cm^{-1} which is shifted 25 cm^{-1} by replacement of C(2) with ^{13}C; this must be the ν (C=O) band, possibly coupled to other ring stretching vibrations. Warfarin 4-methyl ether (**9**) has a broad strong band at 1718 cm^{-1} which is reduced in intensity and sharpened upon replacement of C(2) by ^{13}C; additionally, a new band, also strong and sharp, appears at 1664 cm^{-1}. The methyl ether has two carbonyl groups: C(2)=O(2) in the coumarin ring and a second group in the side chain. The ν (C=O) for both groups must be at 1718 cm^{-1} in the unlabeled compound, while ν (C=O) for the coumarin ring is shifted by labeling to 1664 cm^{-1}.

Warfarin 4-methyl ether (**9**) has bands at 1420, 1355, 1344 and 767 cm^{-1} without counterparts for warfarin (**8**). These must be ν (C-O) and δ (C-H) vibrations from the added methyl group. Warfarin has a band at 995 cm^{-1} not in the spectrum of the methyl ether which may be associated with the hemiketal ring.

Warfarin (**8**) has bands at 1252, 1176, 765 and 741 cm^{-1} which are affected by isotopic replacement of C(2). Warfarin 4-methyl ether (**9**) has bands at 1100 and 750 cm^{-1} which are similarly shifted by isotopic replacement of C(2). These bands must be pyrone ring stretching and bending vibrations.

The 32 cm^{-1} difference between the ν (C=O) of (−)-(*S*)-warfarin (**8**), which is intermolecularly hydrogen bonded in the crystalline state (18), and (±)-warfarin 4-methyl ether (**9**), which is not (8), indicates the magnitude of the shift induced in the carbonyl stretching vibration by hydrogen bonding. The effect in the spectrum of (±)-phenprocoumon (**7**) is even greater, as might be expected since the hydrogen bond in this case involves the strongly acidic enol hydroxyl group (13). In fact, the effect for phenprocoumon is as large as the shift seen for dicumarol (**6**), which is intramolecularly hydrogen bonded in the crystal (12).

Crystalline (*R,R/S,S*)-Cyclocoumarol (**10**) and (±)-Warfarin Ethylenedithioketal (**11**).

The major isomer of cyclocoumarol (**10**) is diastereomeric to the minor (*R,S/S,R*) isomer, which crystallizes as a coumarin ketal (8). The infrared spectrum (Table 3) of crystalline **10** has elements of similarity to both warfarin (**8**) and its 4-methyl ether (**9**). Bands at 1712, 1631, 1082 and 764 cm^{-1} are shifted by isotopic replacement of C(2) with ^{13}C. Similarly, the infrared spectrum of (±)-warfarin ethylenedithioketal (**11**) reveals only bands at 1669, 1616, 1094 and 756 cm^{-1} that are sensitive to isotopic substitution at C(2) (Table 3). The difference between the highest frequency bands at 1712 and 1669 cm^{-1} are similar to the differences between the known coumarins, warfarin

Table 3

Infrared Spectra and Effect of Isotopic Substitution at C-2 of Crystalline (*R,R/S,S*)-Cyclocoumarol (**10**) and (±)-Warfarin Ethylenedithioketal (**11**)

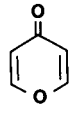
Absorption Band, cm^{-1} (Potassium bromide pellet) (a)			
10	**10**-2-^{13}C	**11**	**11**-2-^{13}C
3030 (W, br) (b)	3030 (W, br)	3280 (M)	3280 (M)
2960 (W, br)	2960 (W, br)	3010 (W, br)	3010 (W, br)
2850 (W, br)	2850 (W, br)	2960 (W, br)	2960 (W, br)
1712 (S)	1681 (S)	1669 (S)	1629 (S)
1631 (S)	1626 (S)	1616 (S)	1608 (S)
1575 (M)	1575 (M)	1567 (M)	1567 (M)
1493 (M)	1493 (M)	1497 (M)	1497 (M)
1456 (M)	1456 (M)	1456 (M)	1456 (M)
1383 (S)	1383 (S)	1389 (M)	1389 (M)
1330 (W)	1330 (W)	1333 (W)	1333 (W)
1316 (W)	1316 (W)		
1285 (W)	1285 (W)	1280 (W)	1280 (W)
1269 (W)	1269 (W)	1248 (W)	1248 (W)
1238 (M)	1238 (M)		
1218 (M)	1218 (M)	1220 (M, br)	1220 (M, br)
1190 (M)	1190 (M)		
1183 (M)	1183 (M)	1171 (M)	1171 (M)
1172 (M)	1172 (M)	1152 (W)	1152 (W)
1147 (M)	1147 (M)	1111 (M)	1111 (M)
1101 (M)	1101 (M)	1094 (W)	1071 (W)
1082 (S)		1057 (W)	1057 (W)
1071 (S)	1071 (M, br)	1035 (W)	1035 (W)
1034 (S)	1034 (S)	1001 (W)	1001 (W)
1005 (W)	1005 (W)	947 (M)	946 (W)
979 (M)	979 (M)		
919 (M)	914 (M, br)	916 (W)	916 (W)
912 (M)		892 (W)	892 (W)
898 (M)	898 (M)	879 (W)	879 (W)
838 (M)	838 (M)	806 (W)	806 (W)
808 (S)	808 (S)	756 (S, br)	756 (S, br)
782 (M)	782 (M)		734 (S)
764 (S)	757 (S)	699 (S)	699 (S)
753 (S)	753 (S)		
699 (S)	699 (S)		

(a) ±10 cm^{-1}, $\nu > 2000$ cm^{-1}; ±3 cm^{-1} $\nu < 2000$ cm^{-1}. (b) S = strong, M = medium, W = weak, br = broad.

4-methyl ether and (±)-phenprocoumon, for which the shift can be explained as the result of strong intermolecular hydrogen bonding.

Identification of 4-hydroxycoumarin and 2-hydroxychromone structures by means of examination of the effects of isotopic replacement of C(2) by ^{13}C would be straightforward if the ν (C=O) and ν (C=C) bands were distinct and if it were known that ν (C=O) always was to be found at a higher frequency than ν (C=C). These assumptions, though frequently made by investigators in this field, may not be warranted. Simple γ-pyrones (**12**) exhibit three strong bands (27) between 1560 and 1680 cm^{-1}. However, complexation with Lewis acids (28) causes a greater shift in the lower frequency band for 2,6-dimethyl-4-pyrone (**12**, $R_3 = R_5 = H$, $R_2 = R_6 = CH_3$) than the band near 1670 cm^{-1}. This has been interpreted as indication that ν (C=C) is the higher frequency band. On the

other hand, the structurally related 5-hydroxy-2-methylchromones (**13**), which are strongly intramolecularly hydrogen bonded, have been shown (29) to have two bands at ~ 1665 and ~ 1630 cm^{-1} which have a high degree of ν (C=O) character on the basis of isotope substitution experiments with deuterium and ^{18}O. Thus the relative positions of the ν (C=O) and ν (C=C) bonds in 2-hydroxychromones (**3**) are uncertain.



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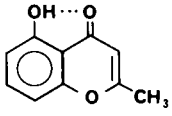

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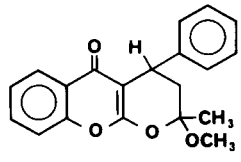

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(*R,R/S,S*)-Cyclocoumarol might be postulated to have the chromone ketal structure (**14**) instead of the coumarin ketal structure (**10**). If this were true, both bending and stretching vibrations in the pyrone ring and the ketal would be expected to be affected by isotopic replacement of C(2) by ^{13}C. Since only two bands can be identified in the 600-1550 cm^{-1} region which are so affected, structure **14** is unlikely, and structure **10** is preferred for (*R,R/S,S*)-cyclocoumarol. Support for this assignment comes from ^{13}C magnetic resonance studies (8). The chemical shifts for C(2) and C(4) are nearly identical with reported chemical shifts for crystallographically proven 4-alkoxycoumarins and totally dissimilar from those observed for 2-methoxychromone (30).

The infrared spectrum of the dithioketal (**11**) is very similar to the known 4-hydroxycoumarins. If it can be assumed that ν (C=O) will be found at a higher frequency than ν (C=C) or that more complex ring stretching vibrations are required for 2-hydroxychromones, then the dithioketal must be a 4-hydroxycoumarin derivative. Proof of this assignment requires assignment of the bands in legitimate 2-hydroxychromones or synthesis of **11** with a ^{13}C label at C(3) or C(4).

3-Substituted-4-hydroxycoumarin Derivatives in Solution.

All of the 3-substituted derivatives have only one band in the 1550-1750 cm^{-1} region which is significantly shifted by replacement of C(2) with ^{13}C; in each case a shift to lower frequency of 30-40 cm^{-1} is observed independent of solvent. The position of this band is highly solvent dependent, but it is always the highest frequency strong band in this region (Table 4). This band displays the same solvent dependent shift for all compounds studied except

Table 4

Infrared Spectra in the Region 1550-1750 cm^{-1} of Selected 3- Substituted 4-Hydroxycoumarin Derivatives in Chloroform, Dioxane and Dimethylsulfoxide.

Compound	Absorption Band, cm^{-1} (a)					
	Chloroform		Dioxane		Dimethylsulfoxide	
	Unlabeled	2-^{13}C-Labeled	Unlabeled	2-^{13}C-Labeled	Unlabeled	2-^{13}C Labeled
Dicumarol (**6**)	1664 (S) (b)		1669 (S)		1701 (S)	1669 (S)
	1637 (M)	1637 (VS)	1637 (W)	1637 (W)		
	1608 (W)	1592 (M)	1608 (W)	1600 (W)	1621 (S)	1621 (S)
	1577 (W)	1572 (W)	1575 (W)	1575 (W)	1572 (W)	1572 (W)
(±)-Phenprocoumon (**7**)	1698 (S)	1664 (S)	1718 (S)	1678 (S)	1705 (S)	1669 (S)
	1634 (S)	1637 (S)	1626 (M)	1623 (W)	1616 (S)	1616 (S)
	1577 (W)	1577 (W)	1575 (W)	1572 (W)	1569 (W)	1569 (W)
(−)-(*S*)-Warfarin (**8**)	1709 (S)	1669 (S)	1724 (S)	1684 (S)	1717 (S)	1669 (S)
	1629 (S)	1629 (S)	1629 (S)	1629 (S)	1627 (S)	1621 (S)
				1615 (W)		
	1572 (W)	1575 (W)	1572 (W)	1572 (W)	1576 (W)	1572 (W)
(±)-Warfarin 4-methyl ether (**9**)	1718 (S)	1672 (S)	1724 (S)	1680 (S)	1718 (S)	1678 (S)
	1616 (S)	1618 (S)	1618 (S)	1618 (S)	1618 (S)	1618 (S)
	1570 (W)	1570 (W)	1572 (W)	1572 (W)	1570 (W)	1570 (W)
(*R,R/S,S*)-Cyclocoumarol (**10**)	1721 (S)	1678 (S)	1727 (S)	1692 (S)	1721 (S)	1678 (S)
	1631 (S)	1631 (S)	1634 (S)	1637 (S)	1629 (S)	1629 (S)
	1577 (W)	1577 (W)	1580 (W)	1580 (W)	1575 (W)	1575 (W)
(±)-Warfarin ethylenedithioketal (**11**)	1709 (S)	1667 (S)	1712 (S)	1672 (S)	1701 (S)	1664 (S)
	1626 (S)	1626 (S)	1637 (S)	1637 (S)	1618 (S)	1618 (S)
	1575 (W)	1575 (W)	1575 (W)	1575 (W)	1569 (W)	1569 (W)

(a) ±3 cm^{-1}. (b) VS = very strong, S = strong, M = medium, W = weak.

dicumarol (**6**) as the ν (C=O) bands for 4-hydroxycoumarin (**7**). As discussed above, single isotopic substitution is insufficient to prove that this band represents primarily the ν (C=O) of a 4-hydroxycoumarin and not the ν (C=C) of a 2-hydroxychromone, but the similarity in behavior to 4-hydroxycoumarin in the different solvents and the high frequency - greater than 1700 cm^{-1} in dimethyl sulfoxide and greater than 1710 cm^{-1} in dioxane except for dicumarol (**6**) - seem inconsistent with a 2-hydroxychromone structure.

Even dicumarol (**6**), which has a lower frequency for this band in chloroform and dioxane, cannot be claimed to have a 2-hydroxychromone structure, since it remains intramolecularly hydrogen bonded both in chloroform and dioxane solution (10). Its spectra in these solvents does not differ appreciably from that observed for crystalline **6**, which has a 4-hydroxycoumarin structure (12).

Definitive proof of the absence of a 2-hydroxychromone structure requires additional isotopic labeling studies with replacement of C(3) or C(4) or evidence that ν (C=O) for 2-hydroxychromone (**3**) is the highest frequency band in the 1550-1750 cm^{-1} region.

Conclusions.

Isotopic substitution at C(2) with ^{13}C permits identification of a band with primarily ν (C=O) character in the infrared spectra of several 3-substituted-4-hydroxycoumarins. This band ranges from 1664-1718 cm^{-1}, depending on the extent of hydrogen bonding in the crystal. The highest frequency band in the 1550-1750 cm^{-1} region in the solution spectra of these compounds also arises from a double bond involving C(2). The position of this band is consistent within the group of 4-hydroxy and 4-alkoxy derivatives studied. It is also consistent with the reported frequency for the model compound, 4-methoxycoumarin, but not with that for 2-methoxychromone (31).

Isotopic substitution at only one position cannot by itself prove that the hydroxy derivatives exist entirely or substantially as the coumarin tautomer. The reported high frequency ν (C=O) of 5-hydroxy-2-methylchromones casts doubt on the validity of assigning tautomeric forms to the hydroxy derivatives solely by comparison to model alkoxy derivatives. Additional evidence, for example the effect of isotopic substitution of ^{13}C at another position in the pyrone ring, is needed to permit unequivocable identification of ν (C=C) bands and assignment of 4-hydroxycoumarin or 2-hydroxychromone structures for the 3-substituted compounds.

Theoretical calculations (32-36) suggest that differences in both the extent of conjugation and in electron density at the pyrone ring carbon atoms should exist between 4-hydroxycoumarins and 2-hydroxychromones. We are currently investigating the ultraviolet and nuclear magnetic resonance spectral properties of selected 4-hydroxycoumarin derivatives and their coumarin and chromone alkyl ethers to test this hypothesis.

EXPERIMENTAL

Infrared spectra were recorded on a Beckman Model IR-20 grating spectrophotometer. Spectroscopic grade chloroform, dioxane, and dimethylsulfoxide were obtained from various commercial sources and purified before use by passage through a 10 cm column of activated alumina to remove traces of water and preservatives. Solutions containing 4% by weight, or saturated solutions of less soluble materials, were measured in cells with sodium chloride windows and 0.10 mm path length. A reference cell containing only pure solvent of matched path length was used to compensate for solvent absorbance. Solvent bands were observed as negative absorbances. Crystalline materials, 1% by weight, were mixed with purified anhydrous potassium bromide and compressed into discs approximately 1 mm thick. The reference beam was attenuated with an adjustable screen as required. Polystyrene film was used as an external standard for calibration.

Melting points were determined on a Thomas-Hoover apparatus and were uncorrected.

Dicumarol [2,2-methylenebis(4-hydroxycoumarin), **6**] was obtained from Nutritional Biochemicals and used without purification. The dicumarol gave only a single spot after chromatography on silica gel thin-layer plates developed in chloroform:acetic acid (9:1, v/v) and had mp 290-292°, in agreement with the reported value (37). All other materials were recrystallized to constant melting point prior to use. (±)-Phenprocoumon (**7**) was the gift of Dr. Lance R. Pohl.

Dicumarol-2,2'-^{13}C (**6**-2,2'-^{13}C).

This was prepared from 70 mg of (90% enriched) 4-hydroxycoumarin-2-^{13}C (7) and 2 ml 37% aqueous formaldehyde as described for the 2,2'-^{14}C analog (37). The product had mp 290-292°.

(±)-Phenprocoumon-2-^{13}C (**7**-2-^{13}C).

The racemate was prepared from equal amounts of the pure enantiomers (21) recrystallized from 70% aqueous ethanol (12 ml/g). The product had mp 178-180°; the ir spectrum was identical to that of the pure *S*-(−)-enantiomer.

(−)-Warfarin (**8**), (−)-Warfarin-2-^{13}C (**8**-2-^{13}C), (±)-Warfarin Ethylenedithioketal (**11**) and (±)-Warfarin Ethylenedithioketal-2-^{13}C (**11**-2-^{13}C).

The preparation of these compounds has been described previously (21).

(±)-Warfarin-4-methyl Ether (**9**) and (*R,R/S,S*)-cyclocoumarol (**10**).

The preparation of these compounds has been described previously (8).

(±)-Warfarin-4-methyl Ether-2-^{13}C (**9**-2-^{13}C) and (*R,R/S,S*)-Cyclocoumarol-2-^{13}C (**10**-2-^{13}C).

These compounds were prepared from (90% enriched) warfarin-2-^{13}C (21) as described for their unlabeled counterparts (8). The compounds had identical mp as their unlabeled counterparts (8): (±)-warfarin-4-methyl ether-2-^{13}C mp 126-127°; (*R,R/S,S*)-cyclocoumarol-2-^{13}C mp 167-168°.

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