

4-Hydroxycoumarin/2-Hydroxychromone Tautomerism:
Infrared Spectra of 2-¹³C and 3-D Labeled
4-Hydroxycoumarin and Its Anion

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Bands with primarily ν (C=O) and ν (C=C) character in the spectra of 4-hydroxycoumarin and its anion were identified by isotopic substitution with either ¹³C or deuterium. Two bands of each type were found for spectra of 4-hydroxycoumarin in solution in chloroform, dioxane, or dimethylsulfoxide, with ν (C=O) at 1704-1733 cm⁻¹ and ~1567 cm⁻¹. Two bands, at 1618 and 1559 cm⁻¹, are associated with ν (C=C) in the spectrum of crystalline 4-hydroxycoumarin monohydrate, but only a single ν (C=O) band at ~1655 cm⁻¹ was observed. Anhydrous 4-hydroxycoumarin has ν (C=O) bands at ~1700 cm⁻¹ and a shoulder at ~1670 cm⁻¹. The strong band at 1660 cm⁻¹ in the spectrum of 4-hydroxycoumarin anion in dimethylsulfoxide solution is due to a delocalized ν (O=C=O) vibration, whereas the band at 1555 cm⁻¹ has partial ν (C=C) character and involves C(3) but not C(2), supporting a fully delocalized charge structure for the anion. No evidence for the existence of the 2-hydroxychromone tautomer was found, except in the case of anhydrous 4-hydroxycoumarin in the solid state.

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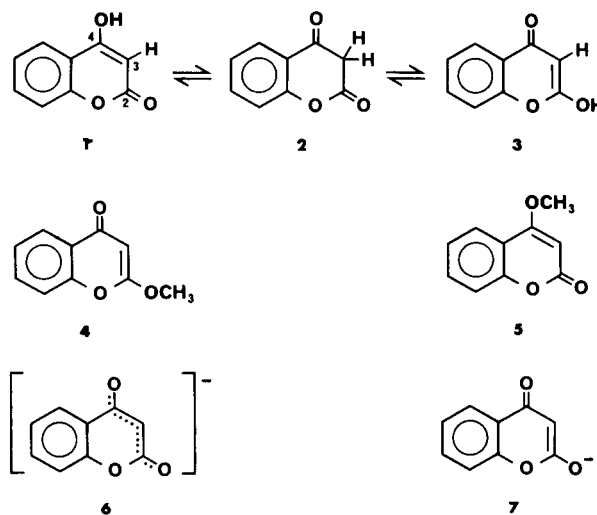
4-Hydroxycoumarin comprises the structural nucleus of many natural products, drugs and pesticides (1). Even during the initial chemical characterization of 4-hydroxycoumarin (**1**) by Anschutz (2), it was recognized to be the enol tautomer of 2,4-chromandione (**2**) (Scheme 1). Arndt and co-workers (3) later reported that 4-hydroxycoumarin (**1**) could also react as the 2-hydroxychromone tautomer (**3**) with diazomethane to yield small amounts of 2-methoxychromone (**4**) as well as larger amounts of 4-methoxycoumarin (**5**). Similarly, treatment of the silver salt of **1** with iodomethane (4) yields mainly **5** with small amounts of **4**. Alkylation of **1** with reagents capable of forming stable carbonium ion intermediates, however, yields C-alkylated products at C(3) as well as O-alkylated products at O(4) (5,6). The vinylic hydrogen at C(3) of **1** is readily exchanged for deuterium (7), an indication that the chromandione tautomer (**2**) can exist at least transiently in solution, although this tautomer is undetectable using proton magnetic resonance techniques (8).

Tautomerism between 4-hydroxycoumarin (**1**) and 2-hydroxychromone (**3**) has been studied by chemical methods (3,4,9-12), ultraviolet spectroscopy (11,13-18), and infrared spectroscopy (11,12,15,17-23). Infrared spectral assignments of structures **1** and **3** by comparison to **4** and **5** has been the most widely accepted technique for structural elucidation of 4-hydroxycoumarin derivatives. Recent x-ray diffraction studies of a number of these derivatives (24-37) have confirmed that every compound studied to date crystallizes as the coumarin tautomer, including 4-hydroxycoumarin (**1**) monohydrate (25). X-ray studies of **1**·H₂O reveal that it crystallizes with four-molecules of 4-hydroxycoumarin and four water molecules

in an orthorhombic unit cell. Each water molecule is hydrogen-bonded to the carbonyl oxygen of two adjacent 4-hydroxycoumarin molecules with inter-oxygen distances of 2.59 Å and 2.73 Å. The oxygen of each water molecule is in turn hydrogen-bonded to the enolic hydroxyl group of a third 4-hydroxycoumarin molecule with an interoxygen distance of 2.80 Å (25).

Despite the consistent finding of a 4-hydroxycoumarin structure by x-ray crystallographic methods, certain 4-hydroxycoumarin derivatives have been claimed to crystallize as 2-hydroxychromones based on infrared spectral evidence (38,39) and many have been claimed to exist at least to some extent as the 2-hydroxychromone tautomer in solution (3,11-13,15,17,19). Reliable techniques to

Scheme 1



distinguish the various tautomeric forms of 4-hydroxycoumarin derivatives would be valuable in clarifying the behavior of these compounds.

4-Hydroxycoumarin (**1**) was prepared with the C(2) carbon of the coumarin ring replaced by ^{13}C using a specific synthetic method (40); in addition, the 3,4-dideutero analog (7) was prepared. Treatment with base yielded the $2\text{-}^{13}\text{C}$ or 3-*d* labeled anion (6). Comparison of the spectra of the unlabeled and ^{13}C -labeled compounds permitted identification of the vibrations involving C(2) (41-43). Deuteration of 4-hydroxycoumarin (**1**) also permitted identification of vibrations involving C(3), C(4), and O(4).

Results and Discussion.

Crystalline 4-Hydroxycoumarin Monohydrate ($\text{1}\cdot\text{H}_2\text{O}$) and Anhydrous 4-Hydroxycoumarin (**1**).

The infrared spectrum of anhydrous 4-hydroxycoumarin (**1**) in a potassium bromide disc matched published spectra (19,20,44,45), whereas the spectrum of the monohydrate resembled a spectrum reported for 4-hydroxycoumarin in a paraffin oil mull (46). The infrared spectrum of **1** has been extensively discussed by Farmer (20), who compared partially and fully deuterated preparations in making band assignments. Unfortunately, Farmer compared anhydrous 4-hydroxycoumarin (**1**) with what appears to be a mixture of 4-hydroxycoumarin hydrate ($\text{1}\cdot\text{H}_2\text{O}$) and 4-hydroxycoumarin-4-*d*-deuterate ($\text{1-4-}d\text{-D}_2\text{O}$) and with 4-hydroxycoumarin-4-*d*-deuterated ($\text{1-4-}d\text{-D}_2\text{O}$) in reasonably pure form. This is evident from the appearance of bands at $\sim 2220\text{ cm}^{-1}$ (ν O-D) and at $\sim 1075\text{ cm}^{-1}$ (δ O-D) in the reported spectra (20), which most likely arise from water of crystallization. As a consequence of comparing the anhydrous to hydrated material, some of Farmer's assignments appear in error.

To clarify the ambiguities in the reported assignments and to gain additional information for confirming other band assignments, we prepared a more highly deuterated analog. Facile exchange of hydrogen for deuterium at both C(3) and O(4) is accomplished by heating 4-hydroxycoumarin (**1**) in acetone or dioxane solutions containing deuterium oxide or by recrystallization from boiling deuterium oxide. 4-Hydroxycoumarin-3,4-*d*₂ monodeuterate ($\text{1-3,4-}d_2\text{-D}_2\text{O}$) crystallizes from the cooled reaction mixture. Anhydrous material may be obtained by crystallization from water-free dioxane. Both deuterated materials had the same melting points as the unlabeled analogs. Both the monodeuterate and anhydrous product readily exchange deuterium for protium upon exposure to atmospheric water or hydroxylic solvents; spectra of solutions could only be obtained using solvents previously treated with small amounts of deuterium oxide. Solutions were prepared in this was using deuterated solvents and analysed for protium exchange by proton magnetic

resonance spectroscopy. No exchange was observed. Additionally, the infrared spectra in the region $3200\text{-}3700\text{ cm}^{-1}$ showed no evidence for significant contamination with protium oxide.

4-Hydroxycoumarin monohydrate ($\text{1}\cdot\text{H}_2\text{O}$) has several absorption bands which can be identified with the water of crystallization (Table 1). A band at $\sim 3370\text{ cm}^{-1}$ shifts upon deuteration to $\sim 2540\text{ cm}^{-1}$, a deuterium/protium isotope effect of 75.3%. This band must therefore be the ν (O-H) band of the water of crystallization as it is absent in the anhydrous material. The isotope effect is close to that predicted by theory; substitution of deuterium for protium should decrease the vibrational frequency to 72.8% of that observed in the protium analog (47), if only oxygen in the water of crystallization participates as the other atom in

the stretching vibration. A band at 1342 cm^{-1} in the spectrum of the monohydrate shifts to 1078 cm^{-1} upon deuteration, 80.3% of the initial value. This band was not observable in the spectrum of the anhydrous material so this may represent a δ (OH) band for the water of crystallization. Another band at 1406 cm^{-1} in the spectrum of the deuterated monohydrate has no counterpart in the spectrum of the protium analog, but also possibly represents a δ (OH) band for the water of crystallization; the corresponding protium band must be in the $1600\text{-}1650\text{ cm}^{-1}$ region but is obscured by other strong bands. Two δ (OH) bands are expected for the monohydrate since two different hydrogen bond lengths are present in the crystal lattice. Finally, the anhydrous material has a band at 3080 cm^{-1} which shifts to $\sim 2240\text{ cm}^{-1}$ in the deuterated analog, 72.7% of the initial value, corresponding to ν (O-H) for the enolic hydroxyl group. Since a weak band at $\sim 3100\text{ cm}^{-1}$ remained in the spectrum of the anhydrous deuterated analog, some exchange of protium for deuterium must have occurred during sample preparation.

Isotopic replacement of C(2) with ^{13}C should measurably shift several absorption bands. An isolated ν (C=O) band should shift $36\text{-}38\text{ cm}^{-1}$ to a lower frequency (47); smaller shifts are indicative of more complex multiatom stretching or bending vibrations. Deuteration can produce a similar shift in ν (C=C) band for the C(3) = C(4) bond. Again, smaller shifts indicate more complex vibrations. Isolated ν (C=O) bands may show a deuterium isotope effect of $10\text{-}20\text{ cm}^{-1}$ due to hydrogen bonding, but isolated ν (C=C) bands due to C(3) and C(4) should be unaffected by replacement of C(2) with ^{13}C .

The solution spectra (Table 2) of 4-hydroxycoumarin (**1**) all have five bands in the $1550\text{-}1750\text{ cm}^{-1}$ region, but the crystalline materials, both anhydrous and the monohydrate, have only four bands in this region. The possibility that a fifth weak band in this region may be obscured should not be discounted, as some of the bands for the crystalline material are asymmetric and broad.

The monohydrate ($\mathbf{1}\cdot\text{H}_2\text{O}$) has three bands in the 1550-1750 cm^{-1} region which are more strongly affected by isotopic replacement of C(2) with ^{13}C than by deuteration at C(3) and O(4). The highest frequency band, at $\sim 1655 \text{ cm}^{-1}$, shifts by over 40 cm^{-1} , and so must have substantial $\nu(\text{C}=\text{O})$ character. Farmer (20) assigned this band to $\nu(\text{C}=\text{O})$ as well. The band at 1618 cm^{-1} is shifted either to 1587 or 1577 cm^{-1} after ^{13}C substitution; the band at 1600 cm^{-1} is shifted to whichever of the 1587 and 1577 cm^{-1} bands that remains. Since the 1600 cm^{-1} band is unaffected by deuteration, this band must represent a coupled pyrone ring vibration not involving O(2), but involving C(2), O(1) and possibly the benzene ring. The 1618 cm^{-1} band has substantial $\nu(\text{C}=\text{O})$ character. The remaining band at 1559 cm^{-1} is more strongly affected by deuteration than by ^{13}C substitution; it must have substantial $\nu(\text{C}=\text{C})$ character but is perhaps better described as a pyrone ring stretching vibration, as assigned by Farmer (20).

These assignments require knowledge that 4-hydroxycoumarin hydrate ($\mathbf{1}\cdot\text{H}_2\text{O}$) crystallizes as the coumarin tautomer and that the crystal structure is not affected by deuteration. The first has been shown by x-ray diffraction (25), whereas the second can be inferred from the identical melting point behavior of the labeled and unlabeled analogs, separately and mixed with each other.

Similar assignments cannot be made as readily for anhydrous 4-hydroxycoumarin ($\mathbf{1}$), as no independent evidence that it does not crystallize as 2-hydroxychromone ($\mathbf{3}$)

is available. Furthermore, the deuterated analog could not be obtained free of protium contamination and is most likely a mixture of mono- and dideuterated products ($\mathbf{1}\text{-}3\text{-}d$ and $\mathbf{1}\text{-}3,4\text{-}d_2$) and indicated by the presence of both $\nu(\text{O-H})$ and $\nu(\text{O-D})$ bands. Finally, three of the four bands in the 1550-1750 cm^{-1} region are broad and asymmetric; although a distinct shoulder was repeatedly observed for the highest frequency band, the other broad bands might also conceal weaker additional bands. With these caveats, the lowest frequency band at $\sim 1555 \text{ cm}^{-1}$ no longer seems to be associated with C(2) and C(3), as it remains unaffected by either deuteration at C(3) or substitution with ^{13}C at C(2). The band at 1608 cm^{-1} , which is affected by ^{13}C substitution but not by deuteration, seems to be the same C(2), O(1) ring vibration that occurs at 1600 cm^{-1} for the monohydrate. The band at $\sim 1700 \text{ cm}^{-1}$ and its shoulder at $\sim 1670 \text{ cm}^{-1}$ both primarily involve a double bond stretching vibration involving C(2). That these are $\nu(\text{C}=\text{O})$ of the 4-hydroxycoumarin form and not $\nu(\text{C}=\text{C})$ of the 2-hydroxychromone form is suggested by the almost negligible effect of deuteration on the high frequency band. However, the appearance of a band at 1623 cm^{-1} in the spectrum of the deuterated analog leads to two possible assignments. The anhydrous material may have different hydrogen bonding schemes in the crystal lattice so that some molecules have loosely bound carbonyl groups with $\nu(\text{C}=\text{O})$ at $\sim 1700 \text{ cm}^{-1}$, and others have strongly bound carbonyl groups, possibly interacting with two neighboring molecules, with $\nu(\text{C}=\text{O})$ at $\sim 1670 \text{ cm}^{-1}$. The se-

Table 1
Effect of Isotopic Substitution and Hydration on the Infrared Spectrum of Crystalline 4-Hydroxycoumarin ($\mathbf{1}$) (a)

1	Absorption Band, cm^{-1} (Potassium Bromide pellet) (b)				
	1- ^{13}C	1-3,4- d_2	1- H_2O	1-2- ^{13}C - H_2O	1-3,4- d_2 - D_2O
3080 (M, br) (S)	3100 (M, br)	~ 3100 (W, br)	3370 (S) (c)	3380	
		~ 2240 (W, br)			2540 (M)
2930 (M, br)	2920 (M, br)	2920 (M, br)	2900 (M, br)	2900 (M, br)	2120 (W, br)
2770 (M, br)	2770 (W, br)	2730 (W, br)	2730 (M, br)	2730 (M, br)	2020 (W, br)
2560 (M, br)	2560 (W, br)	2570 (W, br)	2570 (M, br)	2570 (M, br)	1930 (W, br)
1700 (S, br)	1658 (M, sh)	1690 (S, br)	1655 (S, br)	1610 (S)	1640 (S, br)
~ 1670 (W, sh)	1629 (S)	1623 (M)	1618 (S)	1587 (s)	1607 (S)
1608 (S)	1595 (S)	1606 (S)	1600 (S)	1577 (S)	1597 (S)
~ 1555 (M, br)	~ 1555 (M, br)	~ 1555 (M, br)	1559 (S)	1548 (S)	1540 (S)
1504 (M)	1502 (M)	1501 (M)	1515 (M)	1515 (W)	1492 (M)
1458 (W)	1458 (W)	1445 (W)	1468 (W)		1443 (W)
					1406 (S)
			1342 (S)	1342 (S)	1078 (M)
1305 (M, br)	1316 (M)	1310 (M, br)	1326 (S)	1325 (S)	1328 (M)
1278 (M)	1270 (M)	1275 (M)	1297 (S)	? (W)	1293 (M)
1240 (M)	1233 (M)	1239 (M)	1277 (S)	1276 (S)	1269 (W)
1192 (M)	1185 (M)	1200 (M)	1203 (M)	1190 (M)	1205 (M)
871 (W)	875 (W)	911 (M)	878 (M)	873 (M)	910 (W)
813 (M)					
720 (W)	700 (W)		738 (W)	716 (W)	672 (W)

(a) Only those bands affected by isotopic substitution or hydration are listed. (b) $\pm 10 \text{ cm}^{-1}$, $\nu > 2000 \text{ cm}^{-1}$; $\pm 3 \text{ cm}^{-1}$, $\nu < 2000 \text{ cm}^{-1}$. (c) S = strong, M = medium, W = weak, br = broad, sh = shoulder.

cond possibility is that some of the molecules exist in the crystal lattice as 4-hydroxycoumarin (**1**) with ν (C=O) at ~ 1700 cm^{-1} , and other molecules exist as 2-hydroxychromone (**3**) with ν (C=O) at ~ 1670 cm^{-1} .

Clarification of the ambiguities in assigning the bands for anhydrous 4-hydroxycoumarin could be resolved either by crystallographic structure determination or by infrared spectral studies of analogs labeled with ^{13}C at C(3) or C(4).

Some additional bands, presumable ν (C-C), ν (C-O) and δ (C-H) bands, are shifted slightly by isotopic substitution. The largest shift was observed for a very weak band at 720-740 cm^{-1} , presumably a pyrone ring bonding vibration.

4-Hydroxycoumarin (**1**) in Solution.

The infrared spectrum of **1** has previously been reported in dioxane solution (17,20,21). Perel'son and co-workers have demonstrated that the integrated intensity of the band at ~ 1732 cm^{-1} in the dioxane solution spectra of **1** and **5** were identical. They concluded that no 2-hydroxychromone (**3**) was present in solution.

To examine further the effect of solvent properties on the infrared spectrum of 4-hydroxycoumarin (**1**), spectra in the 1550-1750 cm^{-1} region were compared in chloroform, dioxane, and dimethylsulfoxide (Table 2). Chloroform and dimethylsulfoxide were chosen because of their utility for nuclear magnetic resonance studies (22,34), in addition to their opposing effects on hydrogen bonding.

Table 2

Effect of Isotopic Substitution on the Infrared Spectra in the Region 1550-1750 cm^{-1} of 4-Hydroxycoumarin in Chloroform, Dioxane and Dimethylsulfoxide

Solvent	Absorption Band, cm^{-1} (a)		
	1	1-2- ^{13}C	1-3,4- d_2
Chloroform	1704 (M)	1664 (M)	1701 (S) (c)
	1681 (M)	1653 (M)	1680 (W)
	1631 (S)	1634 (S)	1628 (S)
	1608 (M)	1608 (M)	1603 (S)
	1567 (M)	1565 (M)	1561 (W)
Dioxane	1733 (S)	1689 (M)	1719 (S)
	1706 (W)	1675 (W)	1698 (M)
	1634 (S)	1634 (S)	1612 (M)
	1608 (W)	1608 (W)	1603 (W)
	1567 (W)	1567 (W)	1551 (W)
Dimethylsulfoxide	1727 (S)	1684 (W)	1720 (M)
	1708 (S)	1672 (S)	1699 (S)
	1628 (S)	1631 (S)	1617 (S)
	1611 (M)	1613 (M)	1610 (S)
	1567 (M)	1567 (M)	1557 (M)

(a) ± 3 cm^{-1} . (b) S = strong, M = medium, W = weak. (c) Spectra of deuterated derivative were recorded in solvents containing 0.5-1% deuterium oxide.

Five bands were observed in the solution spectra in this region, as compared to only three or four bands for the crystalline products. The two highest frequency bands are

shifted 30-40 cm^{-1} by replacement of C(2) by ^{13}C whereas deuteration at C(3) and O(4) more strongly affects the third and fifth bands at ~ 1630 and ~ 1570 cm^{-1} in dioxane and dimethylsulfoxide. In chloroform, the effect of deuteration is less pronounced, but the ~ 1570 cm^{-1} band is still shifted more than the remaining bands. These data are consistent with assignment of the two highest frequency bands to ν (C=O) of a coumarin carbonyl group and the third and fifth bands to pyrone ring stretching vibrations with considerable ν (C=C) character from the C(3)=C(4) bond. A 2-hydroxychromone structure (**3**) would require that deuteration and ^{13}C replacement both affect the same bands most strongly.

The origin of the split ν (C=O) bands have been reported for other unsaturated lactones (48-50), and have been attributed to anharmonic Fermi resonance with the α -vinyl δ (C-H). Deuteration usually abolishes the splitting. Such an effect was not observed in this case, so the origin of the splitting remains unclear. Possibly symmetric and assymetric coupling of ν (C=O) with pyrone ring stretching vibrations may occur.

4-Hydroxycoumarin Anion (**6**).

The infrared spectrum of 4-hydroxycoumarin anion has previously been investigated by Perel'son and Sheinker (17). Three bands were observed in the region 1550-1750 cm^{-1} at ~ 1658 , ~ 1623 and ~ 1557 cm^{-1} in the solid state. These bands shifted to ~ 1630 , ~ 1610 and ~ 1531 cm^{-1} after dissolution in deuterium oxide. Under these conditions, however, 4-hydroxycoumarin anion rapidly undergoes deuteration at C(3) (7). Nevertheless, these authors claimed that the band at ~ 1557 cm^{-1} was due to ν (C-O $^-$) whereas the bands at ~ 1623 and ~ 1658 cm^{-1} were due to ν (C=O) and ring vibrations. Later the same authors (18) postulated that 4-hydroxycoumarin anion (**6**) was better described by the charge-localized structure corresponding to 2-hydroxychromone $^-$ anion (7), based on Hückel LCAO calculations.

Isotopic replacement of C(2) with ^{13}C caused the band observed at 1660 cm^{-1} in dimethylsulfoxide solution (Table 3) to shift to ~ 1635 cm^{-1} . This shift is somewhat smaller (~ 25 cm^{-1}) than the expected isotope effect of 36-38 cm^{-1} for an isolated ν (C=O) (4). The remaining bands in this region were unaffected.

Deuteration at C(3) caused shifts in both the ~ 1660 and 1555 cm^{-1} bands by 10-13 cm^{-1} to lower frequencies, but also shifted the band at 1615 cm^{-1} to a lesser extent. All three of these bands must therefore involve C(3). The small isotope effects are indicative of involvement of C(3) in multiatom vibrational modes. Additionally, the fact that the band at ~ 1660 cm^{-1} is affected both by deuteration at C(3) and substitution of ^{13}C at C(2) indicates that both C(2) and C(3) are involved in this vibration.

The effects of isotopic substitution on the spectrum of

Table 3

Effect of Isotopic Substitution on the Infrared Spectrum in the Region 1500-1750 cm^{-1} of 4-Hydroxycoumarin Anion in Dimethylsulfoxide

6	Absorption Band, cm^{-1} (a)	
	6- ^{13}C	6-3-d
1660 (S) (b)	1635 (M, sh)	1650 (S) (c)
1615 (VS)	1615 (VS)	1610 (VS)
1555 (S)	1555 (S)	1542 (S)

(a) $\pm 3 \text{ cm}^{-1}$. (b) VS = very strong, S = strong, M = medium, sh = shoulder. (c) Spectrum recorded in dimethylsulfoxide containing 0.5-1% deuterium oxide.

the anion (6) in dimethylsulfoxide are comparable to the effects observed for unionized 4-hydroxycoumarin. The band at $\sim 1660 \text{ cm}^{-1}$ is therefore most likely due to a stretching vibration involving C(2), C(3), and O(2), while the band at $\sim 1555 \text{ cm}^{-1}$ most likely represents a stretching vibration involving C(3), C(4) and possibly O(4). The involvement of O(2) and O(4) in these vibrations is supported by the data in deuterium oxide (17). In this solvent, hydrogen bonding from the solvent should cause an additional shift of 10-20 cm^{-1} in the bands at ~ 1660 and $\sim 1555 \text{ cm}^{-1}$ and indeed shifts of this size were observed.

The band at 1615 cm^{-1} is only slightly affected by deuteration and remains unchanged after replacement of C(2) by ^{13}C , so it is most likely a ring vibration.

Conclusions.

The use of both ^{13}C and deuterium labeling has permitted a re-examination of the questionable assignments of certain absorption bands observed in the infrared spectrum of 4-hydroxycoumarin (1). Because 4-hydroxycoumarin monohydrate ($1 \cdot \text{H}_2\text{O}$) is known to have a 4-hydroxycoumarin structure and not a 2-hydroxychromone structure in the crystalline state, assignments made with the aid of ^{13}C and deuterium labeling for this material could be made readily. The information gained could then be used to assign corresponding vibrational bands in the spectrum of anhydrous 4-hydroxycoumarin (1).

Although dual isotopic labeling was sufficient to demonstrate that 4-hydroxycoumarin exists as such, and not as the 2-hydroxychromone tautomer (3) in solution in chloroform, dioxane, and dimethylsulfoxide, it could not be used to unambiguously assign the structure of anhydrous 4-hydroxycoumarin (1) in the solid state because of the unknown effects of intermolecular hydrogen bonding. Dual isotopic labeling with ^{13}C in different positions would resolve this issue.

The claim of Vanhaelen and Vanhaelen-Fastre (45) to have isolated "2-hydroxychromone" and to have studied its properties in solution could not be substantiated by us, although it is possible that some of the molecules in anhydrous 4-hydroxycoumarin in the solid state may exist as this tautomer.

The solvent and isotopic effects on the spectrum of 4-hydroxycoumarin anion (6) are consistent with a structure in which the negative charge is delocalized. The solvent effects upon going from the hydrogen-bond acceptor dimethylsulfoxide to the hydrogen-bond donor deuterium oxide strongly suggest substantial involvement of O(2) with the C(2) - C(3) vibration and of O(4) with the C(3) - C(4) vibration but conflict with the 2-hydroxychromone anion structure (7) deduced from theoretical calculations. We are currently investigating the nuclear magnetic resonance spectral properties of 4-hydroxycoumarin anion to clarify this structural assignment.

EXPERIMENTAL

Infrared spectra were recorded on either a Beckman Model IR-20 or a Perkin-Elmer Model 599B 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 either 0.05 mm or 0.10 mm path length. A reference cell containing only pure solvent of matched pathlength 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. Polystyrene film was used as an external standard for calibration.

Melting points were determined on a Thomas-Hoover apparatus and were uncorrected. Proton magnetic resonance spectra were measured on a Bruker HX90E spectrometer. Isotopic composition was determined by electron impact mass spectrometry on a Finnigan Model 4000 mass spectrometer.

4-Hydroxycoumarin Monohydrate ($1 \cdot \text{H}_2\text{O}$).

4-Hydroxycoumarin (Aldrich) was recrystallized from aqueous dioxane (20), mp 209° ($-\text{H}_2\text{O}$), 213-214°.

4-Hydroxycoumarin-2- ^{13}C Monohydrate ($1 \cdot 2\text{-}^{13}\text{C}$).

o-Hydroxyacetophenone (Aldrich, 5 mmoles) was condensed with 90% enriched diethyl carbonate- ^{13}C (5.7 mmoles) as described for the synthesis of (-)-*S*-phenprocoumon (40). The reaction time required to complete the ring-closure reaction was 31 hours, yield, 1.5 mmoles (30%). The product crystallized from aqueous ethanol, mp 209° ($-\text{H}_2\text{O}$), 213-214°. The presence of 1 mole of water per mole of 4-hydroxycoumarin was confirmed by proton magnetic resonance spectroscopy in anhydrous dimethylsulfoxide.

4-Hydroxycoumarin-3,4- d_2 Monodeuterate ($1 \cdot 3,4\text{-d}_2 \cdot \text{D}_2\text{O}$).

4-Hydroxycoumarin (Aldrich), 1.0 g, was dissolved in 3.75 ml acetone mixed with 4.0 ml 98% enriched deuterium oxide. The mixture was boiled 1 hour with stirring. Stirring was continued and the mixture was cooled. Crystals of the monodeuterate separated and were collected by suction filtration, mp 209° ($-\text{D}_2\text{O}$), 213-214°, mixed mp with unlabeled 4-hydroxycoumarin hydrate 209° ($-\text{H}_2\text{O}$, $-\text{D}_2\text{O}$), 213-214°; nmr (DMSO- d_6): δ 5.95 (s, H, 0.036H), δ 7.3-7.9 (m, 4H), δ 10.34 (s, H, 0.048H). The mass spectrum showed evidence of considerable exchange with protium during sample preparation with ions at m/e 162, 163 and 164 prominent. Anhydrous 4-Hydroxycoumarin, Anhydrous 4-Hydroxycoumarin-2- ^{13}C , and Anhydrous 4-Hydroxycoumarin-3,4- d_2 .

The monohydrates were dissolved in anhydrous dioxane and evaporated under dry nitrogen. The process was repeated three times,

mp 213-214°. The absence of water of crystallization was confirmed by proton magnetic resonance spectroscopy in anhydrous dimethylsulfoxide.

4-Hydroxycoumarin Anion-2-¹³C (6-2-¹³C).

A solution of 4-hydroxycoumarin-2-¹³C in dimethylsulfoxide was treated with a stoichiometric amount of sodium methoxide.

4-Hydroxycoumarin Anion-3-d (6-3-d).

Anhydrous 4-hydroxycoumarin-3,4-d₂ (0.38 g, 2.34 mmoles) was dissolved in 70 ml anhydrous dioxane. Sodium deuterioxide in deuterium oxide (0.093 g, 2.34 mmoles) was added and the mixture was stirred 0.5 hours. The precipitated sodium salt was collected by filtration. Spectral analysis was performed immediately; nmr (DMSO-d₆): δ 7.1-7.8 (Ar-H, 4H). No vinyl H remained.

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