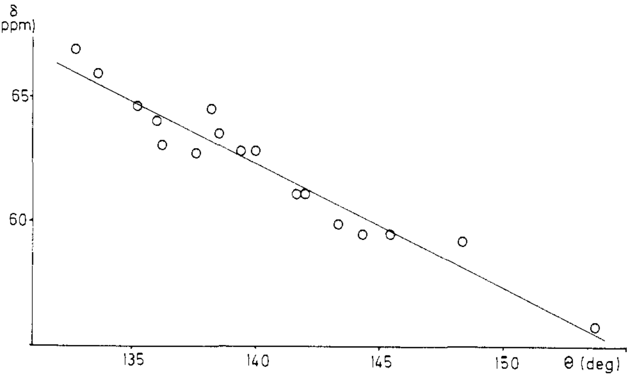


Figure 5. The dependence of ^{27}Al chemical shifts on the mean [Al-O-Si] bond angles in framework aluminosilicates.

A very similar relationship holds between the ^{27}Al chemical shifts (see Table III) and the mean bond angles $\theta' = \langle \text{Al-O-Si} \rangle$ as well (see Figure 5). The values of mean ⟨Al-O-Si⟩ bond angles presented in Table III are based on the latest crystallographic studies available to us. For natrolite and scolecite they were derived from data, presented in ref 41 and 53. A linear regression, based on 17 data points, yields

$$\delta_{CS}(\text{Al}) = -0.50\theta' + 132 \text{ (ppm)} \qquad (9)$$

with $r = 0.95$. A tentative assignment of ^{27}Al resonances in scolecite and thomsonite, based on the mean bond angles, fits this correlation.

A very similar correlation with a −0.61 ppm/deg increment between the ^{27}Al chemical shifts and the mean bond angles has been found in $AlPO_4$ polymorphs.[20] The actual ^{27}Al chemical shifts, refined through MAS NMR curve fitting and stationary sample measurements at a low field, were used.

It appears to be well established now that accurate isotropic, field-independent solid-state ^{27}Al chemical shifts can be determined with modern instrumentation and the new techniques even in powder samples and should be used in all MAS NMR experiments. There is much analogy with the ^{29}Si chemical shifts which display a closely similar dependence upon changes in both the first and second coordination sphere and in correlations with the mean bond angles.

Acknowledgment. We are grateful to Dr. G. Engelhardt (Central Institute of Physical Chemistry, Berlin) for graciously providing us with many of the aluminosilicate samples used.

Registry No. Al, 7429-90-5.

---

(44) Schlenker, J. L.; Pluth, J. J.; Smith, J. V. *Mat. Res. Bull.* **1979**, *14*, 849.

(45) Gramlich, V.; Meier, V. M. *Z. Kristallogr.* **1971**, *133*, 134.

(46) Ferraris, G.; Jones, D. W. Yerkess, J. *Z. Kristallogr.* **1972**, *135*, 240.

(47) Smith, J. V.; Knowles, C. R.; Rinaldi, F. *Acta Crystallogr.* **1964**, *17*, 374.

(48) Fischer, K. *N. Jahrb. Mineral. Monatsh.* **1966**, 1.

(49) Smith, J. V. "Feldspar Minerals"; Springer-Verlag: Berlin, 1974; Vol. 1.

(50) Hahn, T.; Buerger, H. J. *Z. Kristallogr.* **1955**, *106*, 308.

(51) Olson, D. H. *J. Phys. Chem.* **1970**, *74*, 2758.

(52) Mortier, W. J.; Bosmans, H. J. *J. Phys. Chem.* **1971**, *75*, 3327.

(53) Pechar, F. *Acta Crystallogr.* **1981**, *B37*, 1909.

(54) Hassan, I.; Grundy, H. D. *Acta Crystallogr.* **1984**, *B40*, 6.

(55) Wadsworth, M.; France, P. *J. Magn. Reson.* **1983**, *51*, 424.

Intramolecular Hydrogen Bonding as Reflected in the Deuterium Isotope Effects on Carbon-13 Chemical Shifts. Correlation with Hydrogen Bond Energies

Jacques Reuben

Contribution No. 1863 from Hercules Incorporated, Research Center, Wilmington, Delaware 19894. *Received September 16, 1985*

Abstract: The carbon-13 resonances of atoms bearing phenolic or enolic hydroxyl groups, that are engaged in intramolecular hydrogen bonds, experience large ($^2\Delta$ may exceed 1 ppm) upfield deuterium isotope effects. The magnitude of the two-bond isotope effect, $^2\Delta$, correlates with the hydrogen bond energy as obtained from the hydroxyl proton chemical shift. In the conjugated systems investigated in this work, the isotope effects extend over several (up to six) chemical bonds. The signs and magnitudes of the long-range isotope effects are related to molecular structure.

Hydrogen bonding is an important phenomenon ubiquitous in cellular matter.[1] Intramolecular hydrogen bonding plays a key role in maintaining the functional structure of biological macromolecules. Recently a possible relation between intramolecular hydrogen bonds and activity of anthracycline antitumor agents has been indicated.[2] Manifestations of hydrogen bonding can be observed by almost all of the available spectroscopic techniques.[3] The isotopic multiplets in carbon-13 NMR spectra of partially deuterated molecules comprise a new spectroscopic approach to the elucidation of molecular structure, dynamics, and equilibria.[4] In a continuation of the efforts[5,6] directed toward unraveling the information content of these multiplets, a series of substances with hydroxyl groups engaged in intramolecular hydrogen bonds were investigated. Reported here are the results of this investigation.

Carbon atoms in the vicinity of partially deuterated hydroxyl groups exhibit multiplet structure in the proton-decoupled car-

---

(1) Watson, J. D. "Molecular Biology of the Gene"; W. A. Benjamin, Inc.: New York, 1965; Chapter 4.

(2) Ashnagar, A.; Bruce, J. M.; Dutton, P. L.; Prince, R. C. *Biochim. Biophys. Acta* **1984**, *801*, 351-359.

(3) Vinogradov, S. N.; Linnell, R. H. "Hydrogen Bonding"; Van Nostrand-Reinhold Co.: New York, 1971.

(4) For a review see: Hansen, P. E. *Annu. Rep. NMR Spectrosc.* **1983**, *15*, 105-234.

(5) Reuben, J. *J. Am. Chem. Soc.* **1983**, *105*, 3711-3713; **1984**, *106*, 2461-2462; **1985**, *107*, 1433-1435.

(6) Reuben, J. *J. Am. Chem. Soc.* **1984**, *106*, 6180-6186; **1985**, *107*, 1747-1755; **1985**, *107*, 1756-1759.

0002-7863/86/1508-1735$01.50/0

bon-13 NMR spectrum.[4-9] These multiplets result from small upfield deuterium isotope effects on the carbon-13 chemical shifts and are observable under conditions of slow (relative to the magnitude of the isotope effect) chemical exchange between the protio and deuterio forms. For alcohols and carbohydrates the isotope effect (across two bonds) from a directly bonded hydroxyl, $^2\Delta$, is in the range 0.09–0.12 ppm. If the hydroxyl is on a vicinal carbon the effect is smaller: $^3\Delta \leq 0.07$ ppm. Somewhat larger values have been observed in simple phenols.[8] Isotope effects from groups beyond the immediate hydroxylic environment, i.e., $^n\Delta$ with $n > 2$, have been observed in a number of carbohydrates and β-diols, for which intramolecular hydrogen bonding is possible.[6,7] These long-range effects are thought to be transmitted through hydrogen bonds of given and fixed spatial orientation[7] or to be due to isotopic perturbations of chemical equilibria involving hydrogen bonds.[6] In systems with intramolecular hydrogen bonds involving phenolic groups or the enol forms of β-diketones, $^2\Delta$ can be very large: a recent survey of the literature lists values of up to 1.5 ppm.[4] However, most of the literature results were obtained by comparing the chemical shifts of the protio and deuterio forms in separate samples (and separate experiments) rather than directly from the multiplets in the spectra of partially deuterated materials. Unfortunately, the values thus obtained may contain contributions from other phenomena, e.g., concentration-dependent intermolecular interactions, thereby hampering quantitative interpretations. Indeed, Hansen in this comprehensive review[4] could conclude only that large values of $^2\Delta$ are good indicators of hydrogen bonding. In the present work all of the data were obtained directly from the isotopic multiplets under comparable experimental conditions. A meaningful correlation with the hydrogen bond energy (as measured by the hydroxyl proton chemical shift[10]) was obtained. In addition to the effects transmitted across two and three bonds, long-range isotope effects extending over up to six covalent bonds from the hydrogen atoms were also discerned.

Experimental Section

The materials investigated in this work were obtained from commercial sources,[11] except for the diethyl ester of 1,4-dihydroxy-2,5-dicarboxy-1,4-cyclohexadiene (**2**), which was a gift from Dr. W. J. Freeman. Carbon-13 NMR spectra were recorded at ambient temperature (24 ± 1 °C) with a Nicolet 360WB spectrometer operating at 90.56 MHz in the pulsed Fourier transform mode. Low-power broad-band proton decoupling was achieved with the MLEV-64 sequence.[12] When necessary, spectral resolution was enhanced with apodization routines supplied by the instrument manufacturer. The solvent for salicylaldehyde (**1**) was $CDCl_3$. A 1:1 $CDCl_3/Me_2SO\text{-}d_6$ mixture was used for compound **2**. All other compounds were dissolved in $Me_2SO\text{-}d_6$. The concentration was ca. 0.2 M. Partial deuteration was achieved by the addition of a calculated amount of D_2O followed by treatment with $CaSO_4$ or molecular sieves and filtering.

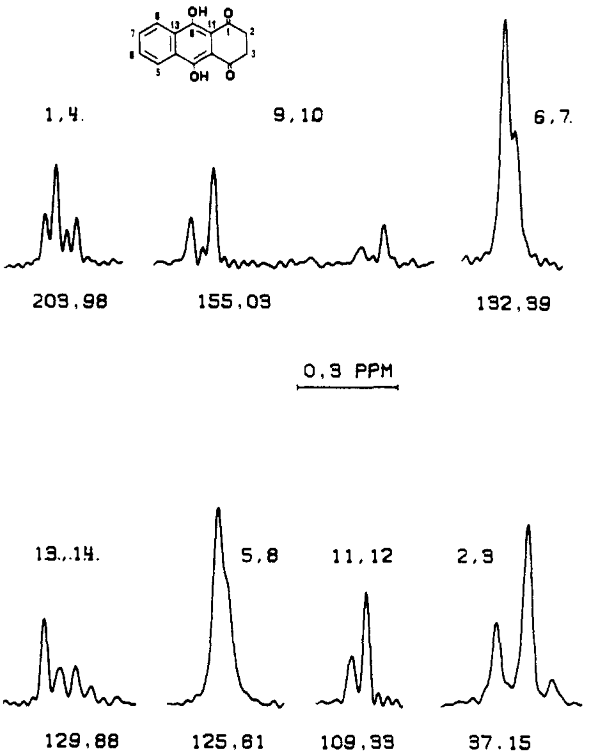


Figure 1. Isotopic multiplets in the carbon-13 NMR spectrum of leucoquinizarin (**4**) with partially deuterated hydroxyls (H/D > 1). Four isotopic isomers are possible: O-9-H, O-10-H (**4a**), O-9-D, O-10-H (**4b**), O-9-H, O-10-D (**4c**), and O-9-D, O-10-D (**4d**). As a result the resonances of carbon atoms affected by isotope substitution at both hydroxyls appear as quartets (C-1,4, C-9,10, and C-13,14), while those affected by substitution at only one hydroxyl appear as doublets (C-2,3, C-5,8, C-6,7, and C-11,12). The chemical shifts (in ppm) of the protio form (**4a**) are given under the spectral bands.

The spectral assignments for salicylaldehyde (**1**),[13] leucoquinizarin (**4**),[14] chrysin (**5**),[15] 1,4-dihydroxyanthraquinone (quinizarin, **6**),[16] 1,8-dihydroxy-1,10-anthraquinone (**7**),[16] tetracycline hydrochloride (**8**),[17] and chlortetracycline hydrochloride (**9**)[17] were taken from the literature. The chemical shifts (in ppm with the central component of $Me_2SO\text{-}d_6$ at 41.1 ppm) for the diethyl ester of 1,4-dihydroxy-2,5-dicarboxy-1,4-cyclohexadiene (**2**) are the following: 172.26 (CO), 169.35 (C-1,4), 94.44 (C-2,5), 62.14 (CH_2O), 29.66 (C-3,6), 15.43 (CH_3). Those for 2-methyl-5-hydroxy-1,4-naphthoquinone (plumbagin, **3**) are the following: 191.33 (C-4), 185.03 (C-1), 161.78 (C-5), 150.57 (C-2), 137.10 (C-7), 135.91 (C-3), 133.05 (C-9), 124.41 (C-6), 119.47 (C-8), 115.84 (C-10), 16.28 (CH_3).

Proton chemical shifts of the hydroxyl groups in the same solutions were measured at 90 MHz with a Varian EM-390 spectrometer. The characteristic low-field position of these resonances facilitates their assignment. Asleson's assignments were used for the tetracyclines.[18]

Results and Discussion

Spectral Features. For phenolic systems both upfield (positive) and downfield (negative) isotope shifts have been reported.[4,8] The direction of the isotope shift can be readily determined from the relative intensities of the multiplet components provided the isotope ratio differs from unity. In this work this ratio was adjusted to be H/D > 1, giving more intense protio components. An example is presented in Figure 1, where the isotopic multiplets in the carbon-13 NMR spectrum of leucoquinizarin (**4**) are shown. It is seen that all of the carbon resonances exhibit multiplet structure. The very large $^2\Delta$ for the hydroxylated carbons 9 and 10 is rather conspicuous. This resonance is further split by a long-range isotope effect extending over five bonds. Judging from the relative intensities, this effect is of a negative sign. Thus, the assignments of the four components in this band are (from low to high field, see Figure 1) the following: C-9 of **4c** and C-10 of **4b**, C-9 and C-10 of **4a**, C-9 and C-10 of **4d**, C-9 of **4b** and C-10 of **4c**. The resonance of carbons 1 and 4 also exhibits two isotope effects of opposite signs, whereas the two isotope effects for carbons 13 and 14 are of the same sign. Note the different intensity patterns of these quartets. These patterns are rather characteristic and can

---

(7) Christofides, J. C.; Davies, D. B. *J. Am. Chem. Soc.* **1983**, *105*, 5099–5105. Christofides, J. C.; Davies, D. B. *J. Chem. Soc., Chem. Commun.* **1983**, 324–326. Christofides, J. C.; Davies, D. B. *J. Chem. Soc., Perkin Trans. 2* **1984**, 481–488.

(8) Newmark, R. A.; Hill, J. R. *Org. Magn. Reson.* **1980**, *13*, 40–44.

(9) Gagnaire, D.; Vincendon, M. *J. Chem. Soc., Chem. Commun.* **1977**, 509–510.

(10) Schaefer, T. *J. Phys. Chem.* **1975**, *79*, 1888–1890.

(11) Leucoquinizarin (**4**) is sold by Aldrich under the name 1,4,9,10-tetrahydroxyanthracene.

(12) Levitt, M. H.; Freeman, R.; Frenkiel, T. *J. Magn. Reson.* **1982**, *47*, 328–330.

(13) Chang, C. *J. Org. Chem.* **1976**, *41*, 1881–1883.

(14) Kikuchi, M.; Yamagishi, T.; Hida, M. *Dyes Pigm.* **1981**, *2*, 143–151.

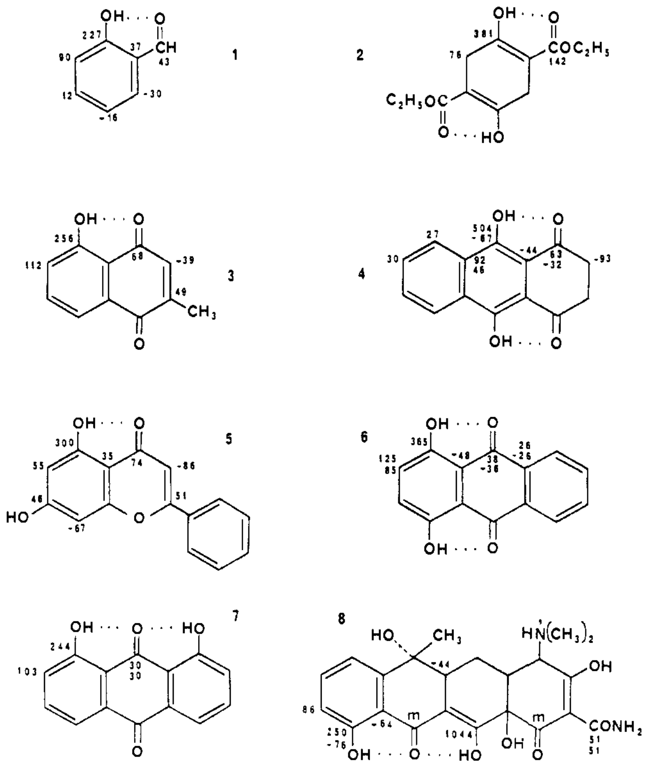
(15) Markham, K. R.; Chari, V. M.; Mabry, T. J. In "The Flavonoids: Advances in Research"; Harborne, J. B., Mabry, T. J., Eds.; Chapman and Hall Ltd.: London, 1982; Chapter 2.

(16) Berger, Y.; Castonguay, A. *Org. Magn. Reson.* **1978**, *11*, 575–577.

(17) Asleson, G. L.; Frank, C. W. *J. Am. Chem. Soc.* **1975**, *97*, 6246–6248. See also: Casy, A. F.; Yasin, A. *J. Pharm. Biomed. Anal.* **1984**, *2*, 19–36.

(18) Asleson, G. L. Ph.D. Thesis, The University of Iowa, 1975.

Scheme I. Deuterium Isotope Effects (in ppb) on Carbon-13 Chemical Shifts

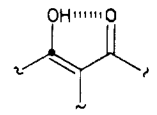


be readily recognized in the carbon-13 spectra of partially deuterated substances.

The isotope effects determined from the multiplet separations are listed (in units of ppb) on the structural formulas in Scheme I. For symmetrical molecules (**2**, **4**, **6**, and **7**) one set of values is given at one of the pair of equivalent carbon atoms. The multiplet patterns for the two ring carbonyls of tetracycline hydrochloride (**8**) could not be analyzed due to substantial overlap and line broadening. Also, no isotopic multiplets were observed arising from the other hydroxyls and the tertiary ammonium group in **8**, probably due to rapid chemical exchange. Rapid exchange prevails also for the second hydroxyl of chrysin (**5**), which is not engaged in an intramolecular hydrogen bond. Chlortetracycline hydrochloride (**9**) exhibited broad lines, probably due to the presence of substantial amounts (ca. 10%) of impurities. However, in the spectrum of the partially deuterated material the phenolic and enolic carbons were readily identified as doublets with separations of 280 and 1000 ppb, respectively.

In the following paragraphs an attempt is made to identify the positional origin of the isotope effects in order to relate them to specific structural fragments. A more detailed discussion is given for the two-bond isotope effect.

$^2\Delta$. The magnitude of the two-bond isotope effects spans the range 227–1044 ppb. This range exceeds the magnitude of *one-bond* isotope effects[4] and is characteristic of the structural fragment A. O'Brien and Stipanovic, in their work on cotton terpenoids, were the first to recognize that large isotope shifts in phenolic systems are associated with intramolecular hydrogen bonds.[19] It is of interest to find whether these effects are related in a more quantitative manner to such features as the energy of the hydrogen bond. Schaefer has shown that the chemical shift of the hydroxyl *proton* of ortho-substituted phenol derivatives is related to the hydrogen bond energy, viz.:



A

$^2\Delta$: 227-1044 ppb

$$\Delta\delta_{OH} = -0.4 + E \tag{1}$$

where $\Delta\delta_{OH}$ is the chemical shift in ppm relative to phenol and E is the hydrogen bond energy in kcal/mol.[10] Hydrogen bond energies obtained from this relationship correlate well with infrared data and theoretical calculations.[20] A plot of $^2\Delta$ (on a logarithmic scale) against E (as obtained from eq 1)[21] is shown in Figure 2. A linear regression analysis yields the following empirical relation:

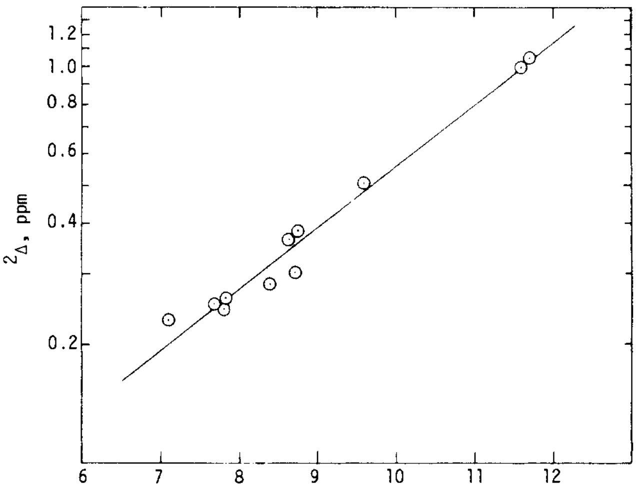


Figure 2. A plot of the two-bond deuterium isotope effect (on a logarithmic scale) against the hydrogen bond energy as obtained from the hydroxyl proton shifts (eq 1). The line is described by eq 2.

$$\ln(^2\Delta) = 2.783 + 0.354E \tag{2}$$

where $^2\Delta$ is in ppb and E in kcal/mol. The correlation coefficient is 0.988. From eq 2 one obtains $^2\Delta$ = 16 ppb at E = 0. This suggests that the two-bond isotope effects from hydroxyl groups are largely due to hydrogen-bonding interactions. In the absence of suitable ortho substituents these would be *inter*molecular interactions of the solute–solvent and solute–solute types. With the average value of $^2\Delta$ for simple phenols (140 ppb)[8] and eq 2 one obtains E = 6.1 kcal/mol, an energy typical for such interactions.[3]

Current theories of isotope shifts consider two main mass dependent contributions to nuclear shielding: bond-length displacement and bond-angle distortion.[22] Although only molecules containing a few atoms have been treated so far, the importance of the latter contribution has been recognized as leading to larger isotope shifts.[23] It seems that the mechanism for isotope shifts in the hydrogen-bonded fragment A must involve the in-plane C–O(H) bending vibration and the associated C–C–O(H) bond-angle distortion. Such a distortion may be regarded as a perturbation on the hybridization, which in aromatic and conjugated systems is likely to spread over the whole molecule. In this way the long-range isotope effects observed in this work can also be rationalized.

$^3\Delta$. Three-bond isotope effects at carbon atoms anti to the hydrogen bond (fragment B) are observed in all cases except at the hydroxylated carbon next to the enol of tetracycline (**8**). Such quaternary carbons are known to exhibit very small isotope effects.[6]

---

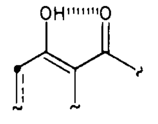
(19) O'Brien, D. H.; Stipanovic, R. D. *J. Org. Chem.* **1978**, *43*, 1105–1111.

(20) Takasuka, M.; Matsui, Y. *J. Chem. Soc., Perkin Trans. 2* **1979**, 1743–1750.

(21) In calculating $\Delta\delta_{OH}$ the chemical shift of phenol was taken as 4.4 ppm. A correction of 0.72 ppm was applied to the shift of the enolic hydroxyls of compounds **2**, **8**, and **9** in order to make them comparable to the shifts of phenolic hydroxyls. This correction was estimated from the difference between the methyl proton shifts of 1-methylcyclohexene and toluene.

(22) Jameson, C. J. *Bull. Magn. Reson.* **1981**, *3*, 3–28 and references therein.

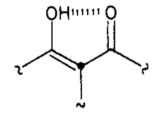
(23) Osten, H. J.; Jameson, C. J. *J. Chem. Phys.* **1984**, *81*, 4288–4292; **1985**, *82*, 4595–4606.



B

$^3\Delta$: 55-125 ppb

In **4** and **6**, with two hydroxyls para to each other, two isotope effects are observed for the same carbon atom. The larger among them is assigned to fragment B and the smaller to $^4\Delta$ in an anti-meta structure (fragment D). The range of $^3\Delta$ for fragment B observed in this work (55–125 ppb) is comparable to that in substituted phenols (50–110 ppb).[8] The situation is more complex for the carbon atom syn to the hydrogen bond (fragment C). In cases with only one source for an isotope effect at this position,

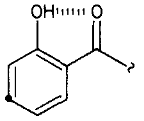


C

$^3\Delta$: 0-37 ppb

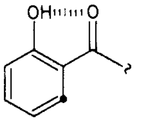
the effect is either small and positive (**1** and **5**) or nil (**3**). Therefore the negative values observed in **4**, **6**, and **8** are assigned to $^4\Delta$ in structure E (**4** and **6**) or $^5\Delta$ in structure H (vide infra).

$^4\Delta$. The four-bond isotope effect at a carbon atom meta to the hydroxyl and anti to the hydrogen bond (fragment D) appears to be rather small (12 ppb in **1**) or unresolved (**2**, **3**, **7**, **8**), unless there is another hydroxyl substitution on the ring (**4**, **5**, **6**). For the carbon atom syn to the hydrogen bond (fragment E) the



D

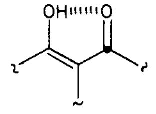
$^4\Delta$: 0-65 ppb



E

$^4\Delta$: -48-0 ppb

isotope effect is negative in **1** and **8** and also (vide supra) in **4** and **6**. However, the effect was not resolved in **2**, **3**, **5**, **7**, and the phenolic ring of **8**. Thus, the precise conditions for observing isotope effects in structure E remain obscure. The carbonyl carbon of the group involved in the hydrogen bond (fragment F) always exhibits a positive isotope effect. The range observed in this work

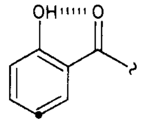


F

$^4\Delta$: 30-142 ppb

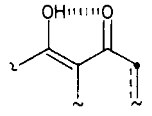
is 30–142 ppb, the largest value being for the ester carbonyl in **2**. In **4** and **6** the carbonyl carbon exhibits a second negative isotope effect, which is assigned to $^5\Delta$ arising from the second hydroxyl in the same molecule (structure I). Note also the four-bond isotope effect of 27 ppb for carbon 5(8) of leucoquinizarin (**4**).

$^5\Delta$. Negative isotope effects on the carbon atom para to the hydroxyl group (fragment G) have been observed in some substituted phenols.[8] In this work such effects were observed only in **1**, **4**, and **5**. With the exception of **7**, the carbon atom next to the carbonyl group (fragment H) exhibits a negative isotope



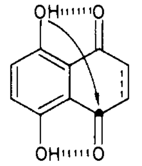
G

$^5\Delta$: -67-0 ppb



H

$^5\Delta$: -93-0 ppb

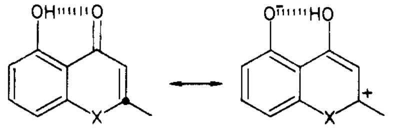


I

$^5\Delta$: -38, -32 ppb

effect. Also, the second carbonyl in the symmetrical structure I, present in **4** and **6**, shows a negative five-bond isotope effect. It is uncertain whether the isotope effect of 30 ppb on carbon 7(6) of leucoquinizarin (**4**) originates from the hydroxyl at position 9(10) or from the more distant hydroxyl at position 10(9). The fact that all other $^5\Delta$ values are negative would suggest the latter ($^6\Delta$) as the more likely alternative.

$^6\Delta$. A six-bond isotope effect was observed in four cases. The effect is positive for fragment J. It seems that the polar structure K is important for the transmittal of this long-range isotope effect.



J K

$^6\Delta$: 26-51 ppb

Indeed the effect is absent in **4**, which lacks the conjugated double bond, and in **7** and **8**, where the carbonyl is engaged in a second hydrogen bond. Note also the negative $^6\Delta$ from the enolic hydroxyl to the phenolic carbon in **8**.

Conclusions

Deuteration of hydroxyl groups involved in intramolecular hydrogen bonds leads to relatively large upfield isotope effects on the carbon-13 resonances of atoms bearing such groups. The magnitude of the isotope shift correlates with the hydrogen bond energy as obtained from the chemical shift of the hydroxyl proton. Thus, isotope shifts can be useful in estimating these energies and in assigning the proton resonances of hydroxyl groups. In the conjugated systems investigated in this work, the isotope effect spreads over up to six chemical bonds. Both positive (upfield) and negative (downfield) isotope effects are observed. The signs and magnitudes of the long-range isotope effects depend on the geometrical relationship between the affected carbon atom and the hydrogen bond. Thus, isotope effects should be useful in identifying structural fragments in such systems. The results presented here should stimulate further theoretical investigations of carbon-13 chemical shifts and isotope effects in systems with intramolecular hydrogen bonds.

Acknowledgment. The excellent technical assistance of D. S. Rice is greatly appreciated. I thank Dr. H. N. Cheng for helpful discussions.