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Deuterium Isotope Effects on ^{13}C Chemical Shifts of *o*-Hydroxyacyl Aromatics. Intramolecular Hydrogen Bonding

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The interesting deuterium isotope effects of gossypols have been reinvestigated and the very large two-bond isotope effect, $^{2}\Delta$C-6(OD), is ascribed to electric field effects. Common to the investigated compounds is the presence of intramolecular hydrogen bonds. A feature strongly related to the strength of the intramolecular hydrogen bond is intermolecular OH exchange. Electron-attracting substituents at the 3- and 5-positions of 2-hydroxyacyl aromatics increase the acidity of the 2-OH proton and therefore the intermolecular exchange, but not the hydrogen bond strength, whereas alkyl groups *ortho* to the intramolecularly hydrogen bonded OH prevent the OH group from swinging out and therefore prevent intermolecular exchange. Conformational equilibria were studied in 2-hydroxy-3-nitro-6-methoxyacetophenone. Surprisingly, the form with the weaker intramolecular hydrogen bond to the nitro group is dominant at ambient temperature, whereas it is the opposite at 160 K. For 2-hydroxy-5-methyl-3-nitroacetophenone a similar pattern is seen, but with much less of the form having hydrogen bonding to the nitro group at ambient temperature. 2-Acetyl-1, 8-dihydroxy-3,6-dimethylnaphthalene is involved in tautomerism of the enolic β-diketone type and large deuterium isotope effects on the ^{13}C and O*H* chemical shifts are observed. © **1997 by John Wiley & Sons, Ltd.**

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INTRODUCTION

Deuteriation of the OH groups of gossypols and related compounds led to interesting signs of long-range isotope effects on ^{13}C chemical shifts and to large two-bond isotope effects,[1] and therefore generated interest in the study of deuterium isotope effects on ^{13}C chemical shifts.[2–6] In the meantime these isotope effects of *o*-hydroxyacyl aromatics have been thoroughly investigated.[7,8] The effects of substituents have been mapped and the transmission mechanisms looked into.[7] Long-range effects are seen in conjugated systems.[4,9] Strain effects as found in, e.g., 1-acetyl-2-hydroxynaphthalene (2-hydroxyacenaphthone), have been discussed, but the corresponding aldehydes did not show such effects.[10] The very large effects found for the gossypols[1] are therefore to a large extent still unexplained.

Tautomerism of β-diketones and similar systems using deuterium isotope effects on ^{13}C chemical shifts have also been treated extensively[11–14] and the relationship between $^{2}\Delta$C(OD) and $^{4}\Delta$C(OD)[14] or $^{2}\Delta$C(ND) and δNH[15] has been presented as a way of identifying tautomeric systems.

Isotopic perturbation of equilibrium showed interesting effects in symmetrical 2,6-dihydroxyacyl aromatics,[16,17] but also in the asymmetric compounds.[5,16] For the 2,6-dihydroxy compounds one OH group is momentarily non-hydrogen bonded and thus exposed to the solvent and prone to exchange.[17]

For weaker intramolecular hydrogen bonds, the influence of solvent becomes important. Some solvents such as DMSO are termed hydrogen bond breaking, really meaning that a hydrogen bond is formed to the solvent and the hydrogen bond is changed from an intra- to an intermolecular hydrogen bond.

One remarkable feature of some of the intramolecularly hydrogen bonded systems is the broadness of the XH resonance despite the fact that the δXH indicates a strong or even a very strong hydrogen bond. This feature is seen in 5-nitrosalicylaldehydes,[7] 3- and 5-acetyl-6-methyl-2*H*-pyran-2,4(3*H*)-diones[18] and the enol form of 2-acylindane-1,3-dione.[19,20] This can be ascribed to increased acidity of the OH proton. The finding that for 5-substituted *o*-hydroxyacyl compounds $^{2}\Delta$C(OD) did not change appreciably, but δOH did, raises the important question of whether the hydrogen bond increases in strength with higher acidity or not. Furthermore, for the 2-acylindane-1,3-diones the broadness was diminished by a bulky acyl group at the C-2 position.[19] For aromatic systems the influence of bulky substituents can be investigated by substitution at the carbon *ortho* to the hydroxy group.

Deuterium isotope effects on ^{13}C chemical shifts represent a useful tool for characterizing the structure and dynamics of *o*-hydroxyacyl aromatics and alkenes covering a broad range of compounds, e.g. *o*-

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Table 1. ^{13}C chemical shifts (ppm) obtained in $CDCl_3$ using TMS as internal reference

Compound	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8	Others	Temperature (K)
3	117.6	162.5	118.4	148.0	120.2	130.6	203.9	26.5	21.9 (CH_3)	300
4	111.1	161.8	110.9	152.3	101.7	130.8	201.3	25.7	37.9/14.8 ($HNCH_2CH_3$), 14.3/20.9/24.6 ($CH_2CH_2CH_3$)	300
5	118.8	160.1	138.1	131.4	140.1	124.4	205.2	27.1	35.2/34.3 (C), 31.4/29.4 [$(CH_3)_3C$]	300
6	112.1	~167.5	101.4	159.6	130.9	130.4	202.5	26.2	56.9 (OCH_3)	300
7	116.9	156.9	129.3	131.1	103.3	164.8	202.6	33.2	57.2 (OCH_3)	300
7	115.3	157.6	130.2	131.8	103.1	165.0	203.7	33.7	57.2 (OCH_3)	250
7	n.o.[a]	br.[b]	130.2	132.2	103.0	165.2	204.2	33.9	57.3 (OCH_3)	230
7A	110.5	161.3	128.8	134.9	101.9	166.8	207.2	—	—	160
7B	119.2	152.9	~134.9	130.7	104.8	163.1	201.9	—	—	160
8	122.3	154.0	137.0	128.2	131.6	137.0	202.8	28.1	20.2 (CH_3)	300
10	113.1	152.5	151.4	151.4	107.3	126.3	200.5	—	138.3 C-1′, 128.9 C-2′, 128.2 C-3′, 131.4 C-4′	300
11	112.6	165.8	103.3	164.5	107.8	135.5	198.6	–	129.5 C-1′, 131.5 C-2′, 115.2 C-3′, 160.9 C-4′	300
12	118.1	161.7	120.5	145.6	124.1	132.8	200.2	—	20.8 (CH_3), 137.5 C-1′, 129.0 C-2′, 128.5 C-3′, 132.1 C-4′	300
13	169.0	113.2[c]	133.4	117.4	121.4	138.5	112.7	158.2	143.9 C-4a, 111.1[c] C-8a, 204.8 C=O, 32.1 ($COCH_3$), 22.0[c] (CH_3), 25.3[c] (CH_3)	300
14	—	159.5	111.2	153.1	131.3	115.2	166.7	109.5	112.0 C-4a, 155.3 C-8a, 204.5 C=O, 34.0 ($COCH_3$), 19.2 (CH_3)	300
15	110.2	160.3	118.4	136.8[c]	107.1	159.0	205.8	33.9[c]	33.5[c]/136.4[c]/116.4 ($CH_2CH{=}CH_2$)	300
16	110.0	160.8	121.8	136.6	106.3	157.6	205.9	33.5	31.2/22.7/13.9 ($CH_2CH_2CH_3$)	300
17	122.8	161.6	122.8	137.8	129.4	137.8	192.1	—	19.9 (CH_3)	300
18	111.2	165.7	116.0	150.5	n.o.[a]	130.4	202.4	25.9	42.2/16.3 ($HNCH_2CH_3$)	300
19	155.2	133.7	125.1	120.2	137.6	120.0	—	—	—	300
20	148.7	136.0	131.7	118.5	131.7	136.0	—	—	—	180
21	144.5	182.5	125.5	147.7	129.5	130.8	129.4	122.8	128.3 C-4a, 130.4 C-8a	300

[a] n.o. means not observed.
[b] br. means broad.
[c] May be interchanged.

hydroxyacetophenones, *o*-hydroxybenzaldehydes and *o*-hydroxybenzoic esters,[5,7,10,17] 2-acylindane-1,3-diones,[19,20] linderones and lucidones,[6] enaminones[15,20,21] and enamino esters.[15,20]

The present paper demonstrates how the above-described phenomena, steric strain, isotopic perturbation of equilibria, tautomerism and ordinary hydrogen bonded systems may be distinguished by means of deuterium isotope effects on ^{13}C chemical shifts.

ASSIGNMENTS

The ^{13}C chemical shifts are given in Table 1. The assignment of the ^{13}C spectra of **1** is that in Ref. 1 and the assignment of gossypolone (**2**) is similar to those of the hemigossypolones in Ref. 1. The assignments of **3–12**, **14–16** and **17–20** are based on substituent effects. In the case of **14**, the starting parameters were those of 7-hydroxycoumarin.[22] The spectra of **7** at ambient temperature are clearly the result of an equilibrium. The low-temperature spectra of **7** showed two sets of resonances, an intense set, **7A** (see Results) and a very weak one, the **B** form. The OH resonances were assigned in the same way. The assignment of **13** is based on HETCOR spectra.[23] The OH resonances were assigned so that 1-OH is at highest frequency. The C-3 resonance of **17** can be assigned unambiguously as it shows two equally large isotope effects due to symmetry. The assignment of **21** was made in analogy with that of 1-phenylazo-2-naphthol.[24]

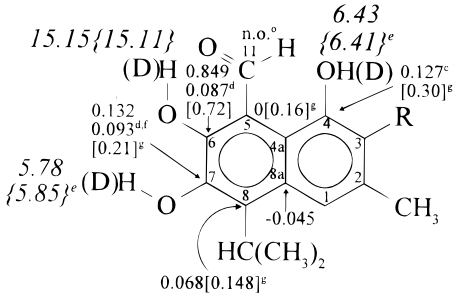
The determinations of the signs of isotope effects were performed by varying the degree of deuteriation. For those compounds with more than one exchangeable hydrogen the assignment of isotope effects due to specific deuteria could be made for **4** and **18**. In the former case the ND group did not give rise to an isotope effect and for **18** the degree of incorporation was distinctly different from that of the OH group. For **1**, **2**, **10**, **11** and **13**, comparisons were made with well established substitutent effects.

RESULTS

As indicated above, the deuterium isotope effects on chemical shifts and OH chemical shifts are the central theme of this paper (Scheme 1). The isotope effects are defined as $^{n}\Delta C(D) = \delta C(H) - \delta C(D)$, n being the number of intervening bonds between the observed nuclei and the isotope in use. The deuteriation is done at the OH positions leading to OD groups. A more specific notation in this case is $^{n}\Delta C(OD)$. The isotope effects were measured in one tube experiment with both isotopomers present and the experiments were repeated if necessary with different H : D ratios.

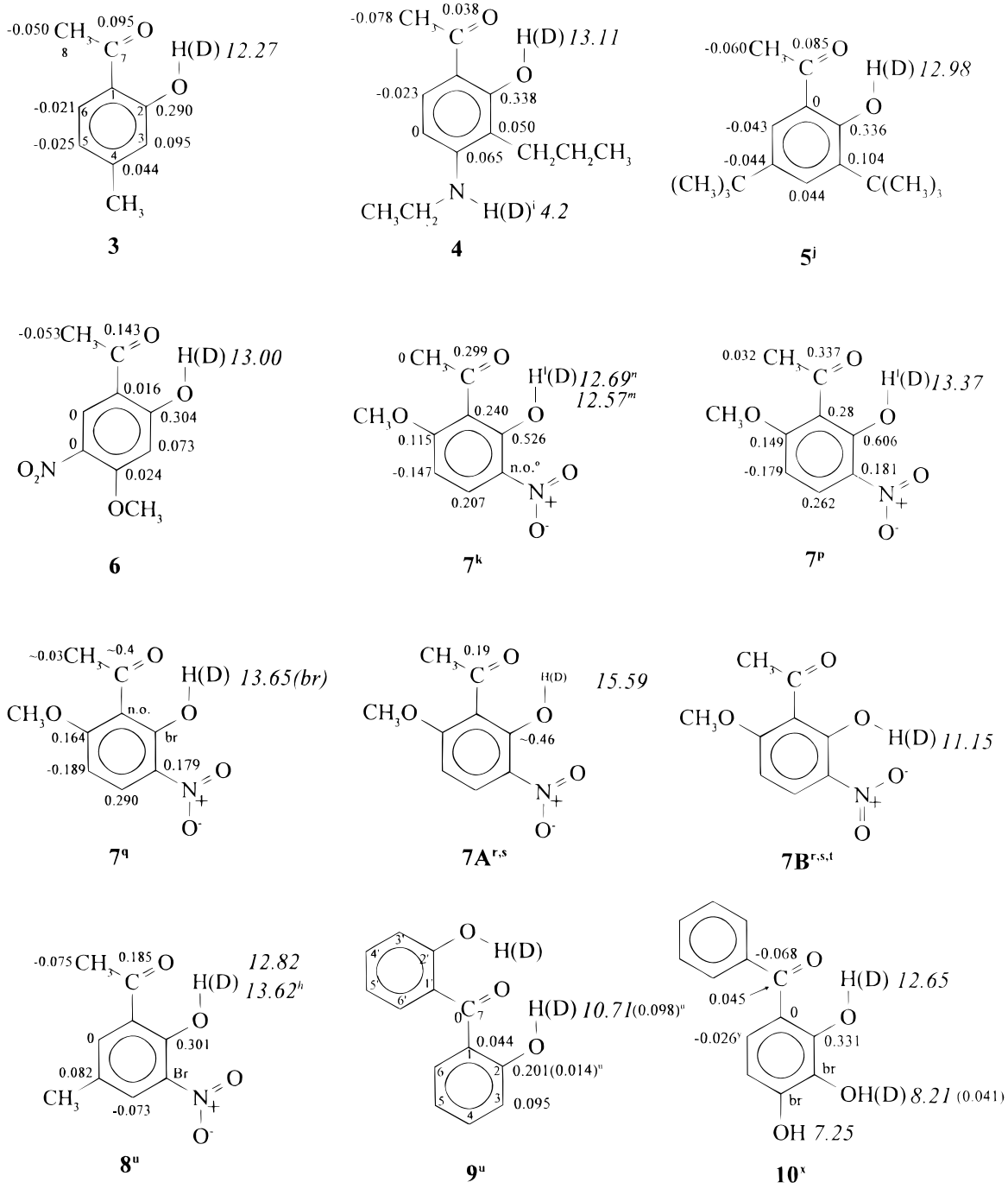
The isotope effects of **1** were reinvestigated. Compound **1** is not very soluble in either $CDCl_3$ or CD_2Cl_2, so the data for **1** were obtained in acetone-d_6 at low temperature in a mixture of the isotopomers. This is in contrast to those in Ref. 1, which were recorded at ambient temperature. At ambient temperature fast intermolecular exchanges occur and one obtains the sum of isotope effects, not the individual contributions from each deuterium. Except for C-7 the isotope effects differ considerably (Scheme 1). The very large $^{2}\Delta C$-6(OD) isotope effect (0.85 ppm) is remarkable as the corresponding isotope effect of 2-hydroxy-1-napthaldehyde is 0.41 ppm.[7] A number of long-range effects due to 6-OD are also seen. The two other OD groups give rise to isotope effects over two and three bonds (Scheme 1). Gossypolone (**2**) was too insoluble at low temperature to be able to measure deuterium isotope effects on ^{13}C chemical shifts.

Compound **3** showed that methyl substitution at position 4 had a very moderate effect at the isotope effects compared with 2-hydroxyacetophenone.[7] The isotope effects of **4** were changed as expected for an electron-donating substitutent at position 4 and are



1[b]

Scheme 1. Deuterium isotope effects on ^{1}H and ^{13}C chemical shifts and OH chemical shifts (in italics) for **1**, **3–14** and **17–21**.[a] [a] Hydrogen bonds not shown to avoid overcrowding. [b] Temperature 220 K. Values are due to deuteriation at 6-OH if nothing else is given. [c] Due to deuteriation at 4-OH. [d] Due to deuteriation at 7-OH. [e] Temperature 200 K. [f] Assignment tentative. [g] Values in square brackets from Ref. 1. [h] Temperature 180 K. [i] No deuterium isotope effects on chemical shifts could be observed due to deuteriation at this NH group. [j] Temperature 220 K. [k] Temperature 300 K. [l] For hydrogen bonding of OH group, see text. [m] Solvent $CDCl_3$. [n] Solvent CD_2Cl_2. [o] n. o. means not observed. [p] Temperature 250 K. [q] Temperature 220 K. [r] Temperature 160 K. [s] Not all isotope effects could be observed owing to the poor signal-to-noise ratio caused by the low solubility of the compound at 160 K. [t] Isotope effects could not be measured owing to effects of footnote s and very low occurrence of this isomer. [u] Temperature 260 K. [v] Numbers in parentheses are isotope effects due to deuteriation at 2-OH. [x] Trace of DMSO-d_6 added to improve the solubility. [y] Assignment tentative. [z] Isotope effect due to deuteriation at 2-OH. [aa] Only the very large isotope effects are observed, probably because of OH exchange. [bb] Compound showed tautomerism. See text. Isotope effects are due to deuteriation at 1-OH, if nothing else is given. [cc] Isotope effect due to deuteriation at 8-OH. [dd] Values in parentheses are deuterium isotope effects on OH chemical shifts caused by deuteriation at the other OH group. [ee] Isotope effects could not be measured in CD_2Cl_2 owing to insolubility at low temperature. [ff] Temperature 310 K.

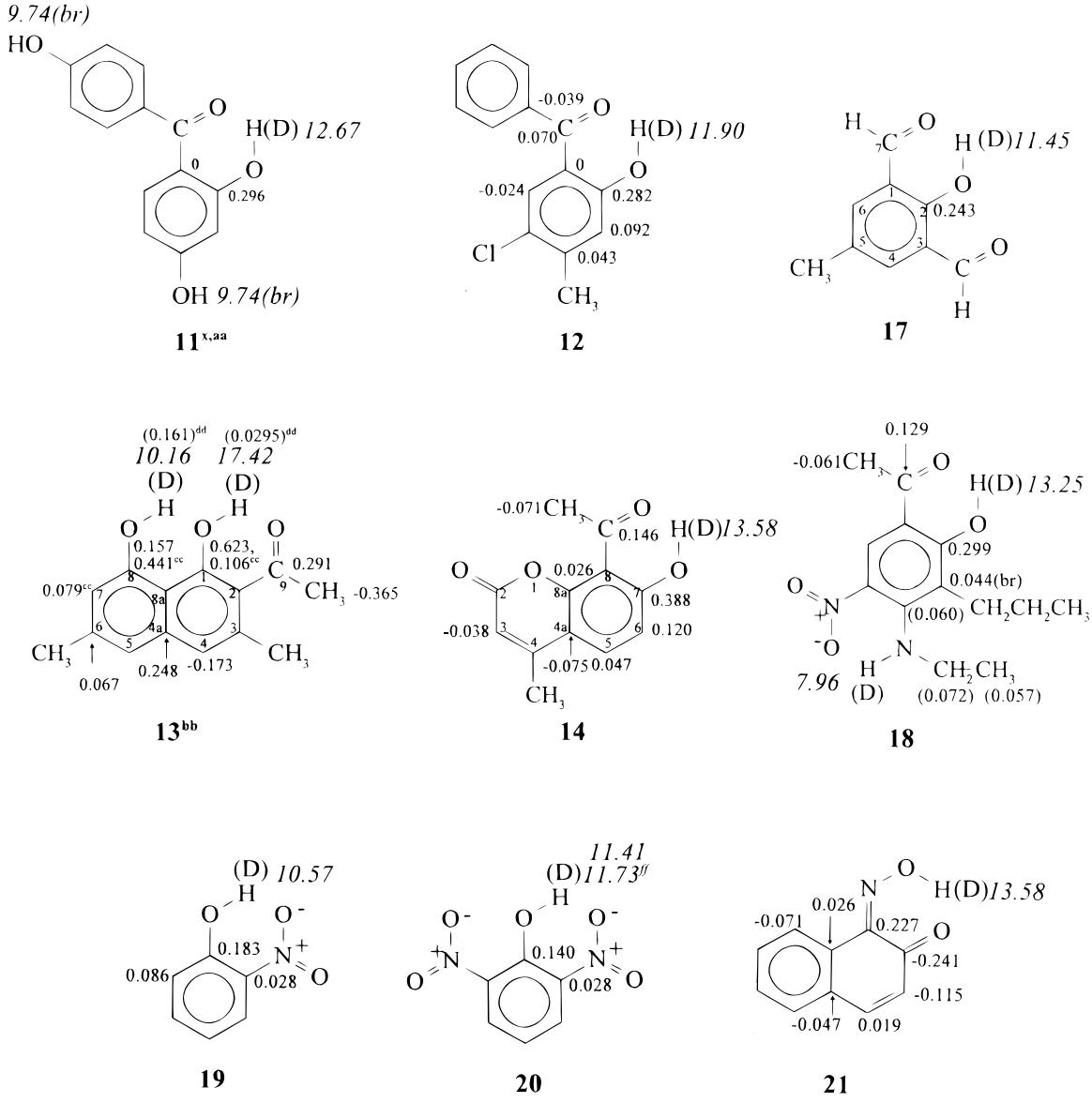


Scheme 1—(*Continued*)

similar to those of 4-methoxy-2-hydroxyacetophenone.[7] Compound **6** had to be cooled to show isotope effects on ^{13}C chemical shifts.

The OH chemical shift of **5** was to higher frequency compared with 2-hydroxyacetophenone. $^{2}\Delta$C-2(OD) was likewise increased. This can be ascribed to the steric compression effect of the *tert*-butyl group at C-3. The effect of the *tert*-butyl group at C-5 is likely to be unimportant.

The ambient temperature spectra of **7** gave sharp resonances, but unusual deuterium isotope effects on ^{13}C chemical shifts. Both the OH and the ^{13}C chemical shifts varied considerably with temperature up to 170 K, at which temperature two sets of resonances appeared. The low-frequency OH resonance at 11.15 ppm was broad and not very strong. The intensity ratio was 10:1 at 170 K. At 160 K, the low-frequency OH resonance sharpened. The ^{13}C spectrum at 160 K also showed two sets of resonances (Table 1). The resonances of the minor form are broad and not very intense and no isotope effects could be determined for this form. The ^{13}C chemical shifts of the two forms are rather different, and with those of the minor form generally to lower frequency (Table 1). The two sets of resonances can be ascribed to the two isomers **7A** and **7B** (Fig. 1). The C=O resonance of the major form showed a relatively large isotope effect of 0.19 ppm at 160 K and this effect demonstrates clearly that this has to be the isomer with an intramolecular hydrogen bond to the carbonyl group (**7A**). Analysis of the OH and ^{13}C chemical shifts



Scheme 1—(*Continued*)

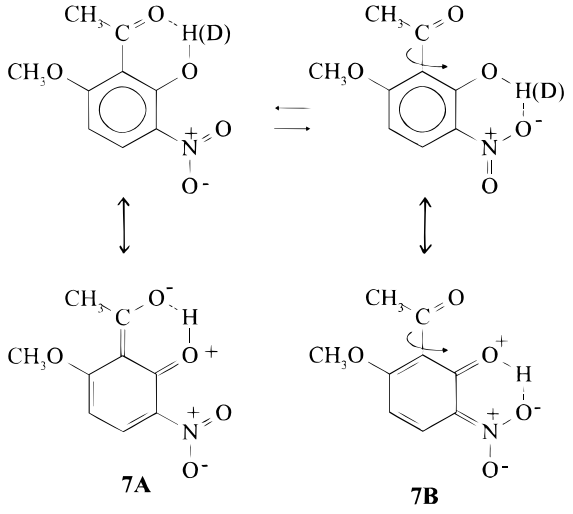


Figure 1. Rotamers and resonance forms of **7**.

at 300 K revealed that the form **7B** with a hydrogen bond to the nitro group was dominant (64%). At 250 K the two forms were present in almost equal amounts. Further cooling broadened the OH resonance and moved it further to higher frequency. At 220 K the **7A** form dominated (56%).

Knowing the chemical shift differences between carbon resonances of forms **A** and **B** and the isotope effects of form **A**, and being able to estimate those of form **B** based on the data for **19** and **20**, the shifts in the equilibrium upon deuteriation can be estimated to be *ca.* 4% at 300 K and *ca.* 4.6% at 250 K.

The ^{1}H spectrum of **8** showed an increase in the chemical shift of the OH resonance from 12.82 ppm at 300 K to 13.62 ppm at 180 K, but also line broadening in the temperature range 220–200 K. In the temperature range 300–230 K, a linear dependence with temperature was found with a coefficient of 0.008 ppm K^{-1}. The ^{13}C

spectrum likewise showed a modest temperature dependence. The C=O, C-1 and C-3 resonances broadened around 220–200 K like the OH resonance to become sharp at 180 K.

The isotope effects of **9** have been published previously.[5,10] In this study, the compound was cooled to slow OH exchange and give precise isotope effects. For **10** and **12**, substitution at positions 3–5 were further investigated. The data for **10** showed that an extra OH group at position 3 had only a small effect on $^2\Delta$C(OD) (see discussion of gossypols).

The very high frequency OH shift of **13** (17.42 ppm) is remarkable (Scheme 1) as the corresponding 1-hydroxy-2-acenaphthone has a δOH of 13.99 ppm.[7] Also, the second OH was sharp (10.16 ppm). Likewise, both OH resonances of **13** showed very large isotope effects when the other OH group was deuteriated (Scheme 1). The situation is somewhat akin to that seen for 2-hydroxydibenzoylmethane.[8]

The $^2\Delta$C(OD) isotope effect for **14** is interesting as **14** is structurally similar to 2-hydroxy-1-acenaphthone[10] with the only difference that no steric interaction exists between the CH_3C=O group and 0-1 in **14**. The long-range effects of **14** are to a certain extent similar to those observed for khellinone,[7] showing that the transmission of the isotope effect into the double bond is a common feature.

The C-1, C-2, C-3 and C-5 resonances of the ^{13}C spectrum of **17** are sharp at ambient temperature, whereas those of the C=O and the C-4 and C-6 carbon resonances are broad. The $^2\Delta$C(OD) isotope effect is only marginally larger than that of salicylaldehyde.[1,4,5,7,10]

As reference parameters for **7**, the *o*-nitrophenols **19** and **20** were recorded. Both showed small two-bond isotope effects of the order of 0.183 ppm (**19**) and 0.140 ppm (**20**) and the latter showed broad resonances at 180 K. The latter value is in line with the finding for *o*-hydroxyacyl aromatics that an extra nitro group leads to a decrease in $^2\Delta$C(OD) (see later).

Finally, 1-nitroso-2-hydroxynaphthalene (**21**) was investigated. The results showed that this compound existed as the keto-imine–hydroxy-nitroso tautomeric mixture. The isotope effects are almost constant with temperature.

^{1}H OH chemical shifts

Most OH chemical shifts are given in Scheme 1. For those compounds not mentioned in Scheme 1, the OH chemical shifts are as follows: gossypolone (**2**), δ6-OH = 12.65 ppm and δOH = 10.18 ppm at 193 K in acetone-d_6 ; 3-allyl-2,6-dihydroxyacetophenone (**15**), δ2-OH = 10.84 ppm and δ6-OH = 8.62 ppm at 220 K in CD_2Cl_2 ; and 3-propyl-2,6-dihydroxyacetophenone (**16**), δ2-OH = 11.92 ppm and δ6-OH = 7.85 ppm at 300 K in $CDCl_3$.

DISCUSSION

Gossypols

Gossypol has been investigated in great detail because of its interesting biological properties.[1,26,27] It has been established that gossypol can exist as a lactol in DMSO, acetonitrile, ethanol and methanol,[26–28] but also in a tautomeric equilibrium with the aldehyde (Fig. 2).[26,28] In chloroform a tautomeric equilibrium between the aldehyde, the ketol and aldol forms in Fig. 2 has been suggested.[28,30] In basic solution the ketol form has been established.[26,31] The δ6-OH of gossypol and hemigossypol[1] (*ca.* 15.3 ppm in acetone-d_6) and a large $^2\Delta$C-6(OD) of 0.8 ppm could suggest a tautomeric equilibrium,[11–14] but the lack of an isotope effect at C-11 is against a tautomeric equilibrium. *o*-Hydroxy aromatic aldehydes generally show very small isotope effects at the aldehyde carbon.[3,9] For tautomeric systems, as found in Ref. 2, a large effect is clearly seen both at the C—OH and the aldehyde carbon. Both findings suggest that **1** is not part of a tautomeric equilibrium in chloroform. AM1 and PM3 calculations of the monomer of gossypol confirm that *in vacuo* the aldehyde form is dominant ($\Delta\Delta H$ = 4.4 and 6.5 kcal, respectively; 1 kcal = 4.184 kJ). $\Delta\Delta S$ was not calculated, but has been found to be insignificant in similar systems.[32] Another explanation for the large two-bond isotope effects emerges from a comparison of the ^{13}C chemical shifts of hemideoxygossypol[33] and hemigossypol.[1] No major differences in chemical behaviour are found between gossypol and hemigossypol.[1] The chemical shifts of C-5, C-6 and C-7 are very similar, whereas that of C-11 in hemigossypol is 5.1 ppm to higher frequency. This suggests a local interaction involving the aldehyde group. The OH group at C-4 may lead to a mesomeric strengthening of the acidity of 6-OH. This is also supported by the tendency for intermolecular exchange of this proton. However, the very large isotope effect cannot be explained by this effect,[10] as this is also found in the 7-methoxy derivative. However, the OH position at high frequency and the large $^2\Delta$C-6(OD) of hemigossypol and gossypol can be understood as the OH group at position 4 leads to a more acidic 6-OH group, but more importantly to polarization of the C=O aldehyde group due to an electric field and maybe steric effects, thus effectively leading to more of the resonance form **C**, shown if Fig. 2.

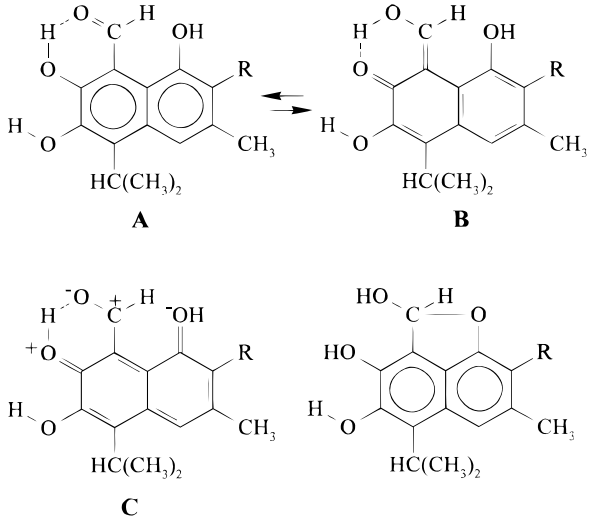


Figure 2. Tautomers and resonance forms of gossypol (**1**).

Equilibrium isotope effects

Compound **7** showed a sharp OH resonance and gave some unusual isotope effects that cannot be explained by straightforward effects of the substituent as discussed above. The very large effects at all carbons except the methyl group (relative to 2-hydroxyacetophenone) can be understood as an isotopic perturbation of the equilibrium as shown in Fig. 1. The deuterium isotope effects have to be analysed accordingly. Using the OH chemical shifts and the intensities at low temperature, the **B** form is dominant at ambient temperature, but the amount of this form is very small at 160 K. The spectra at 160 K gave a $^2\Delta$C(OD) of 0.46 ppm for the **7A** isomer. Most of the isotope effects at ambient temperature clearly have a positive equilibrium component. As the **A** form has chemical shifts to higher frequency than the **B** form (Table 1), the **B** form is increased upon deuteriation. This is even more so at 250 and 230 K although the amount of **B** isomer is decreasing. This can be explained by realizing that the mole ratio for **A** changes from 0.36 at 300 K to 0.56 at 220 K. This will increase the change in equilibrium upon deuteriation as seen by plotting these numbers on the graph in Fig. 7 in Ref. 14.

The finding that the amount of the dominant tautomer, **B**, at ambient temperature is not increased further at low temperature is unusual. This can be explained by assuming that ΔS for **7B** is positive and large enough to overcome the difference in ΔH due to a much weaker intramolecular hydrogen bond at ambient temperature. The more positive ΔS of **7B** can be understood as the nitro group of **7A** most likely is subject to hindered rotation due to conjugation (see later). The rotation of the acetyl group of **7B** therefore gives rise to a positive ΔS. For **8** the picture is less clear.

Intermolecular exchange

Line broadenings may occur in compounds such as those studied here because of either intermolecular exchange or averaging. An example of the latter is that of 2,4,6-trihydroxy-3,5-diacetylbenzene.[34]

From the study of 5-nitrosalicylaldehyde,[7] it became clear that this compound had to be cooled to show a sharp OH resonance and to give deuterium isotope effects on ^{13}C chemical shifts. This is most likely related to the higher acidity of the OH proton. Of the pair **19** and **20**, the former gave a sharp OH resonance at ambient temperature, whereas the latter had to be cooled. The pK_a values are 7.23 and 3.70, respectively.[35,36] It is interesting that the increase in δOH upon nitro group substitution is much smaller in **6** and **18** compared with the non-nitro-substituted compounds (4-methoxy-2-hydhydroxyacetophenone and **4**) than it is in 5-nitrosalicylaldehyde *vs.* salicylaldehyde.[7] For the first two a decrease in $^2\Delta$C(OD) with introduction of a nitro group is observed, whereas for 5-nitrosalicylaldehyde *vs.* salicylaldehyde $^2\Delta$C(OD) remains constant. For **7A** a large increase in δOH and a medium increase in $^2\Delta$C(OD) are observed resulting in a position below the correlation line in Fig. 3 (see later). Summarizing, a nitro group in the 3- or 5-position

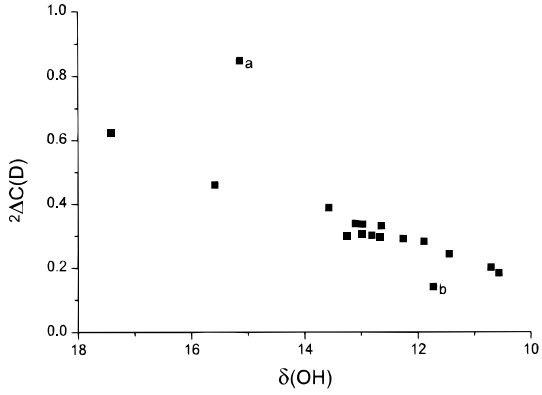


Figure 3. δOH *vs.* $^2\Delta$C(OD). a is **1** and b is **20**.

increases the acidity of the 2-OH proton and shifts this resonance to a higher frequency, but the hydrogen bond strength, as judged from $^2\Delta$C(OD), does not increase and at best it is unaltered. An acyl group is also expected to lead to similar effects although slightly smaller than those of a nitro group. This is seen for 2,4-dihydroxy-1,5-diacetylbenzene,[34] for which it was necessary to cool the sample, but not for the corresponding 3-ethyl derivative.[34] Likewise, for **18** isotope effects could be obtained at ambient temperature. A common feature for 2,4-dihydroxy-3-ethyl-1,5-diacetylbenzene[34] and **18** is the position of an alkyl group *ortho* to the OH group. According to the base-catalysed exchange of a hydrogen bonded OH group, the hydroxy group has to move away from the acceptor before exchange can take place.[37,38] In the present compounds the *o*-alkyl group makes this step much less likely and therefore slows intermolecular exchange. The effects of alkyl groups are also demonstrated in **5**, in which the hydrogen bond becomes stronger. The effects of alkyl groups are also seen in the nearly symmetrical compounds **15** and **16**. For **16** two broad OH resonances are observed at 11.92 and 7.75 ppm, indicating that the alkyl substituent in close to fixing the carbonyl in a hydrogen bond to 2-OH. Alkyl substitution at C-3 in the case of a *tert*-butyl group (**5**) was seen to increase both δOH and $^2\Delta$C-1(OD) and therefore also the hydrogen bond strength. A similar feature is found for **16** and to a lesser extent for **15**.

Tautomerism

The ^{13}C chemical shifts of **13** point towards a dominating carbonyl character of the C=O group. The deuterium isotope effects at the carbon chemical shifts are large for C-1, C=O CH_3C=O, C-8 and C-4a. The sum of the two- and four-bond isotope effects, $^2\Delta$C-1(OD) + $^4\Delta$C-9(OD), is 0.91 ppm, which points towards a tautomeric system.[14] Another feature of this compound is the steric interference between the CH_3C=O group and the methyl group at C-3. This could lead to strain effects.[10] However, a comparison of the effect for **13** with those for 2-hydroxy-1-acetophenone and similar compounds[10] reveals distinct differences. A comparison with *o*-hydroxydibenzoylmethane shows a resemblance.[8] In the latter case, the sum of $^2\Delta$C(OD) and $^4\Delta$C(OD) is also less than the 1.2 ppm which has been

suggested as a lower limit for tautomerism in six-membered rings.[14] δOH is much lower in the strained compounds and the isotope effect at the CH_3CO group is really much smaller in the strained compound. This, taken together with the remaining large and some negative isotope effects, points clearly towards a tautomeric system. This is also supported by plotting $^2\Delta$C(OD) *vs.* $^4\Delta$C(OD) in Fig. 7 in Ref. 20. This shows clearly that the point falls off the line. One favourable interaction is that between the OH group at C-8 and the enolic β-dicarbonyl system of C-1, C-2 and C-9.

The data for **21** can be compared with those of *o*-hydroxyazobenzenes and show clear tautomerism between keto-imine and hydroxy-nitroso forms.

CONCLUSION

The unusually large $^2\Delta$C-6(OD) of **1** has been explained as originating from an electric field polarization of the aldehyde C=O bond caused by the OH group at C-4.

Nitro or acetyl groups in *ortho* or *para* positions to the OH group clearly increase the acidity of the OH group and shift the OH resonance to higher frequency to an extent depending on the presence of other substituents. The $^2\Delta$C(OD) isotope effects are not increased and, therefore, the hydrogen bond is not strengthened.

An alkyl group *ortho* to the intramolecularly hydrogen bonded OH group diminishes intermolecular OH exchange and stabilizes the intramolecular hydrogen bond.

Tautomerism may be established using $^n\Delta$C(OD) isotope effects as found in **13**. Further, deuterium isotope effects are likewise found to reveal hydrogen bond dynamics not seen by ordinary NMR measurements as demonstrated for **7**. The rotamer equilibrium involving the OH group hydrogen bonding to both the acyl and the nitro group is immediately apparent.

EXPERIMENTAL

Compounds

Compounds **1** and **2** were purchased from Sigma (St Louis, MO, USA), **3–8**, **13–16** and **18** from Maybridge (Tintagel, UK), **8–12** and **19–21** from Aldrich (Weinheim, Germany) and **17** from Tokyo Kasei Kogyo (Chuo-ku, Japan). These products were used without further purification.

NMR

The ^{13}C NMR spectra of deuteriated species were recorded in $CDCl_3$ on a Bruker AC 250 NMR spectrometer at 62.896 MHz with a digital resolution of 0.55 Hz per point. Chemical shifts were measured relative to internal TMS. Spectra were recorded at 300 K in $CDCl_3$ unless stated otherwise. Spectra of both deuteriated and non-deuteriated species and of mixtures of the two species were recorded for all compounds. Most low-temperature spectra were recorded in CD_2Cl_2. The HETCOR spectra[23] were recorded as described previously.[17]

Deuteriation

Most of the compounds were deuteriated by dissolving the compounds in a mixture of CH_3OH and CH_3OD followed by evaporation of the solvent under reduced pressure. The degree of deuteriation could easily be varied this way.

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