Deuterium isotope effect on ^{1}H and ^{13}C chemical shifts of intramolecularly hydrogen bonded perylenequinones

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The primary and secondary deuterium isotope effects on proton and carbon chemical shifts were measured for a number of natural pigments and their derivatives, which contain the perylenequinone system and present a phenol–quinone tautomerism, cercosporin 1, isocercosporin 2, phleichrome 3, isophleichrome 4, cladochrome E 5, elsinochromes 6–8, hypocrellin 9, noranhydrocercosporin 10 and noranhydrophleichrome 11. Deuterium isotope effects on protons were also measured for 1,4-dihydroxyanthraquinone 12, methyl 4-(1,4-dihydroxy-9,10-dioxo-9,10-dihydroanthracen-2-yl)butanoate 13, *N*-acetyldaunomycin 15, daunomycinone 16, naphthazarin 17 and a number of intramolecularly hydrogen-bonded enols from β-diketones, β-ketoesters and *o*-hydroxyacyl aromatic compounds 19–24. The primary isotope effects $\Delta\delta$ (^{1}H,^{2}H) on OH proton shift and ^{1}H chemical shifts of OH groups are correlated and can be used to estimate the strength of the hydrogen bonds in solution. The secondary isotope effects on proton and carbon nuclei are transmitted along the whole extended conjugated perylenequinone system. Long-range effects over eleven bonds and over seven bonds were observed in compounds 1–11 and in 12–18.

The perturbation of the equilibrium due to the presence of deuterium was considered and calculations were performed in order to evaluate the amount of this contribution to the isotope effect. The variation with temperature of primary and secondary effects, from 25 °C down to −70 °C, was studied for compounds 1, 3, 6, 12 and 17, and for acetylacetone 22 and benzoylacetone 23. Parameters from a number of X-ray analyses, for example the O · · · O distances (as suggested by Gilli *et al.*, *J. Am. Chem. Soc.*, 1991, 113, 4917), gave evidence of a substantial parallelism between the liquid and the solid phase. The strength of the hydrogen bond in perylenequinones depends on the planarity of the two naphthalene units rather than on the distortion of the polycyclic ring.

Introduction

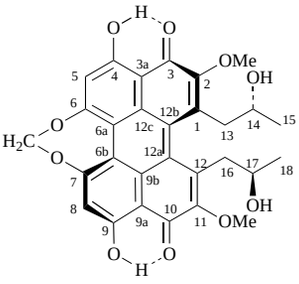
Recently, deuterium isotope effects on nuclear shielding have received renewed attention.[1–7] The interest covers both the theoretical aspects[5] and applications to organic chemistry.[6] A great number of applications treat this phenomenon mainly as a substituent effect.[3] On the other hand, the theory has the intriguing task of reconciling the vibrational origin with the transmission of this effect through a large molecule and of finding agreement with the experimental data. Long-range isotope effects on carbon atoms over five and six bonds were found in hydroxyquinones[8] and hydroxyacyl aromatics;[1] in phenalenone derivatives[7] this effect is transmitted over seven bonds and in conjugated polyenic systems over eleven bonds.[6] The presence of intramolecular hydrogen bonds is known[1] to enhance this phenomenon and correlations were found between isotope effects and hydrogen-bond potential, as well as between primary[9] and secondary[1b] deuterium effects and chemical shifts of the hydrogen-bonded OH protons. There has been increasing interest in the last few years in the study of tautomeric processes which involve hydrogen bonding. In spite of the fact that hydrogen bonds are responsible for the structures and properties of important biological systems (and many publications have appeared on the subject), the real nature of the hydrogen bond and of the factors determining its strength is almost unknown and is still a matter of study and discussion.[3,4,10,11]

We have investigated[12,13] a number of perylenequinones of natural origin and their derivatives **1**–**11**, which display intramolecular hydrogen bonds and phenol–quionone tautomerism, either in solution or in the solid phase. 4,9-Dihydroxyperylene-3,10-dione (tautomer **A**) and 3,10-dihydroxyperylene-4,9-dione (tautomer **B**) (see Scheme 1) have been recognized as present in solution in fast equilibrium with respect to the NMR time-scale. The populations of each tautomer have been obtained from the coupling constants between the proton of the hydrogen-bonded OH groups and the adjacent carbon atoms.[13]

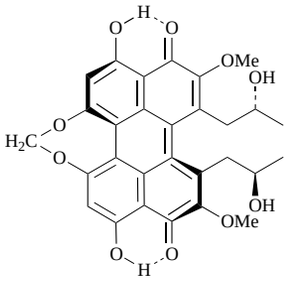
We report here on primary and secondary deuterium isotope effects in these compounds (**1**–**11**), and on their correlation with the strength of hydrogen bonding, with the distances between the oxygen atoms and with the tautomeric process. Isotope effects on protons were also measured for 1,4-dihydroxyanthraquinone **12**, methyl 4-(1,4-dihydroxy-9,10-dioxo-9,10-dihydroanthracen-2-yl)butanoate **13**, *N*-acetyldaunomycin **15**, daunomycinone **16** and other model compounds **17**–**24**, in order to check their dependence on the molecular structure.

Experimental

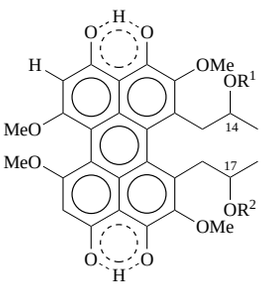
The compounds used for NMR measurements were spectroscopically and analytically pure, as reported in ref. 10. Hypocrellin **9** was a generous gift of Professor E. Breitmaier, daunomycin **14** and derivatives **15** and **16** were a gift of Farmitalia. Compounds **12**, **17** and **19**–**24** were commercial samples. ^{1}H, ^{13}C and ^{2}H NMR spectra were measured in $CDCl_3$ and in [$^{2}H_6$]acetone with a Bruker AMX-600 spectrometer, with a digital resolution of 0.0003 ppm for ^{1}H and 0.002 ppm for ^{13}C and ^{2}H. The concentration was 0.8–1.3 × 10^{-1} mol dm^{-3} for all



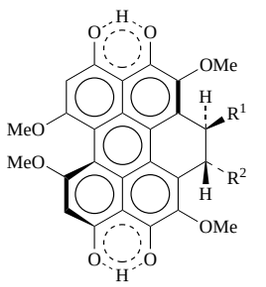
1 (Helix *M*)



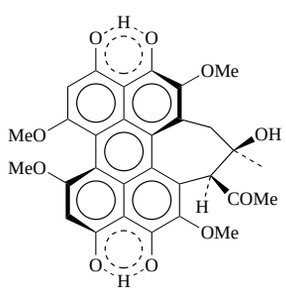
2 (Helix *P*)



3 $R^1 = R^2 = H$, (Helix *P*, 14*S*, 17*S*)
4 $R^1 = R^2 = H$, (Helix *M*, 14*S*, 17*S*)
5 $R^1 = COO(4\text{-}OHPh)$, $R^2 = COPh$ (Helix *P*, 14*R*, 17*R*)



6 $R^1 = COMe$, $R^2 = COMe$
7 $R^1 = R^2 = CHOHCH_3$, (14*S*, 17*S*)
8 $R^1 = R^2 = CHOHCH_3$, (14*R*, 17*R*)



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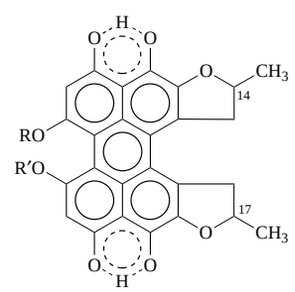
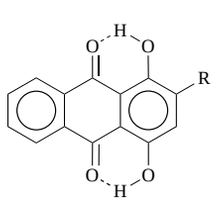
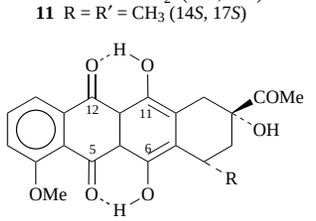


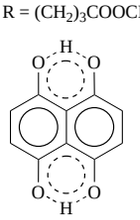
10 R = R′ = -CH_2- (14*R*, 17*R*)
11 R = R′ = CH_3 (14*S*, 17*S*)



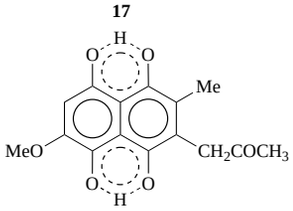
12 R = H
13 R = $(CH_2)_3COOCH_3$



14 R = Me, HO, NH_3^+ Cl^-
15 R = Me, HO, NHCOMe
16 R = OH



17



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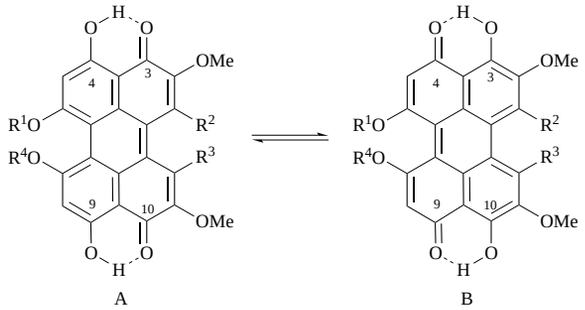


A B

Scheme 1 Tautomeric forms of perylenequinones **1**–**11**. Compounds **26** and **27** are the corresponding hexamethyl ethers.

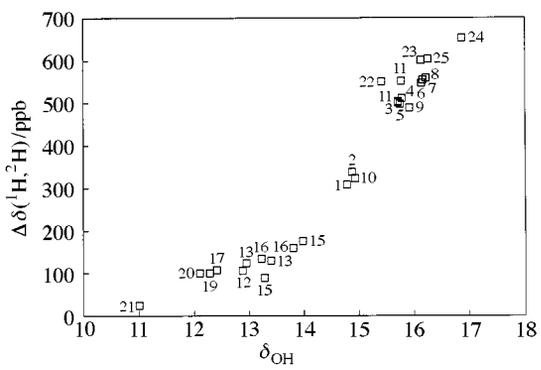


Fig. 1 Plot of the primary isotope effect on the chemical shift of phenolic OH protons $\Delta\delta(^1H,^2H)$ *vs.* ^1H chemical shift of OH groups δ_{OH} for perylenequinones **1**–**11**, anthraquinones **12**–**16**, naphthoquinones **17** and **18**, *o*-hydroxyacetophenone **19**, ethyl acetoacetate **20**, salicylaldehyde **21**, acetylacetone **22**, benzoylacetone **23**, dibenzoylmethane **24** and 9-hydroxyphenalenone **25**. The data for **19** and **25** are from ref. 9(*a*) (solv. CH_2Cl_2) and ref. 7 (solv. acetone) respectively.

experiments except for compounds **5**, **13** (0.3×10^{-1}), **15** and **16** (2×10^{-2} mol dm^{-3}). The temperature was 25 °C, except for compounds **1**, **3**, **6**, **11**, **12**, **17**, **22** and **23**, which were also measured at lower temperatures. ^1H chemical shifts are given as δ values from internal $SiMe_4$ and are accurate to within ±0.005 ppm. The C–H coupling constants were measured from undecoupled spectra and are given as absolute values with an estimated accuracy of ±0.2 Hz. The primary deuterium isotope effect $\Delta\delta(^1H,^2H)$ was measured on the phenolic groups, by using the chemical shift difference between the ^1H signal and ^2H signal in a solution containing hydrogen and deuterium compounds. $CDCl_3$ and $CHCl_3$ signals were used as internal standards, according to ref. 9(*b*). Estimated accuracy ±10 ppb. The deuterium isotope effects on ^{13}C chemical shifts were obtained for most compounds by two measurements from solutions containing different amounts of deuterated species, and are estimated to be accurate within ±5 ppb. The solutions were prepared by adding *ca.* 0.03 cm^3 of a 2:1 or 1:1 H_2O–D_2O mixture to 0.4 cm^3 of a 10^{-1} mol dm^{-3} solution of the compound in $CDCl_3$. The solutions for measurement of coupling constants were prepared by using a 1:4 H_2O–D_2O mixture. The assignment of the signals of monodeuterated (D_1), bideuterated (D_2) and non-deuterated (D_0) species were usually performed by following the change of the relative intensities of the signals. The secondary deuterium effects on ^1H chemical shifts were measured from the same solutions used for ^{13}C, except for a few titrations which were performed by adding increasing amounts of D_2O (up to 60 mm^3) to a solution of the quinone, and then by adding increasing amounts of H_2O to the solution containing the deuterated species. The estimated accuracy of the secondary effects on ^1H is ±1 ppb.

Results and discussion

Primary deuterium isotope effect, $\Delta\delta(^1H,^2H)$

The values of the primary isotope effect on the chemical shift of phenolic OH groups for perylenequinones **1**–**11** are reported in Table 1, together with those for anthraquinones **12**–**16** and naphthoquinones **17**–**18**. The values for other intramolecularly hydrogen-bonded compounds **19**–**25** are given in Fig. 1. A few

Table 1 Primary and secondary deuterium isotope effects (ppb) and chemical shifts (ppm) of hydrogen bonded OH proton for perylenequinones **1**–**11**, anthraquinones **12**–**16** and naphthoquinones **17** and **18**[a]

Compounds	**1**	**2**	**3**	**4**[b]	**5**	**6**	**7**	**8**	**9**	**10**[c]	**11**[d]	**12**	**13**	**15**	**16**	**17**	**18**
$\Delta\delta(^1H,^2H)$[e]	307	337	503	510	497	536	553	558	487	322	500 550	106	123 130	88 176	134 160	117	[h]
$\Delta\delta H(O^2H)$[f]	24	26	64	63	63	55	56	57	45	35	63	2[i]	3	6	6	8	9[j]
δ_{OH}[g]	14.79	14.88	15.72	15.78	15.78[k]	16.14	16.16	16.22	15.92[k]	14.94	15.74	12.88	12.95 13.39	13.28 13.98	13.22 13.81	12.41	13.30 12.87

[a] Measured at 25 °C in $CDCl_3$ unless specified. In the case of asymmetric compounds **5**, **9**, **13**, **15**, **16**, **18** two distinct values were obtained for each parameter, but only one is reported when their difference is within the accuracy. [b] Conc. 1×10^{-2} M. [c] Two atropoisomers in fast equilibrium. [d] Measured at −5 °C where the two atropoisomers are well separated; the accuracy for the primary effects is ±30 ppb, the secondary effects are equal, the OH proton shifts are 15.71 and 15.77. [e] Primary effect, accuracy ±10 ppb. [f] Accuracy ±1 ppb. [g] Accuracy ±0.005 ppm. [h] Not measured. [i] The signal is broad. [j] Measured in dioxane. [k] Average of two distinct values: 15.81, 15.75 for **5** and 15.94, 15.91 for **9**.

data were obtained from the literature,[7,9] but in many cases spectra were measured *de novo*, in order to compare values under the same experimental conditions. These isotope effects were found always to have a positive sign, which is an indication[9] of a double minimum in the hydrogen bond potential. We plotted in Fig. 1 the values of $\Delta\delta(^1H,^2H)$ *vs.* the proton shift of OH groups for all compounds **1**–**25**, in order to check whether the correlation previously found by Forsén *et al.*[9] still holds, when data from structurally different keto–enol compounds are included.

The perylenequinone system in the studied molecules is not planar, but assumes a helical shape, owing to the steric strain of the substituents on the polycyclic ring. The helicity generates axial chirality, which, when associated with the chirality of the asymmetric carbon atoms of the chains, gives rise to diastereoisomerism (atropoisomerism). Thus cercosporin **1** can be interconverted thermally with isocercosporin **2**, which has opposite helicity and a different conformation of the side chains. The same happens for phleichrome **3** and isophleichrome **4**, whereas this conversion does not seem to be possible for the elsinochromes **6**–**8** and hypocrellin **9**, although they are also helical molecules.[12]

The results shown in Fig. 1 appear interesting for two reasons: (*i*) the perylenequinones actually fill up an empty area between the very strong hydrogen bonds of β-diketones and bonds of medium strength such as those of hydroxyanthraquinones, β-ketoesters and *o*-hydroxyacyl aromatics, thus completing such a relationship; (*ii*) the correlation between $\Delta\delta(^1H,^2H)$ and δ_{OH} still holds, the new data giving additional experimental evidence. An inspection of Fig. 1 shows that the lower chemical shift values of cercosporin **1** and isocercosporin **2** with respect to the other perylenequinones is paralleled by smaller isotope effects and must be related to a weaker hydrogen bond in **1** and **2**. This was explained[13] as a consequence of the significant distortion of the perylenequinone ring.

Cercosporin **1** in the solid phase[12b,14] is a true helical compound, whereas elsinochrome A **6**[15] and hypocrellin **9**[16] assume an X shape, made of the two planes of the naphthalene moieties. The geometry nearer to planarity of the two naphthalene moieties in **6** and **9** should favour the formation of stronger hydrogen bonds. This conclusion is supported by the values of the distance $d(O\cdots O)$, which is slightly shorter for hypocrellin and elsinochrome (2.49 Å) than for cercosporin (2.53 Å).† Gilli and co-workers[11b] proposed the use of the interatomic distance between the two oxygen atoms, $d(O\cdots O)$, as an indicator of the hydrogen bond strength, thus allowing their classification according to the distance $d(O\cdots O)$, as very strong (<2.50 Å), strong (2.50–2.65 Å), medium (2.65–2.80 Å) and weak (>2.80 Å). The attempt to extend to our compounds the linear correlation between δ_{OH} and $d(O\cdots O)$, found[11b] for 1,3-diarylpropane-1,3-dione enols was satisfactory for perylenequinones, but not as good for daunomycin **14** (Fig. 2). It must be said that the accuracy of the earlier X-ray analysis[17] of daunomycin is not comparable with the other ones.

The examined hydroxyanthraquinones **12**, **13**, **15** and **16** show a primary isotope effect between 0.100 and 0.200 ppm, in line with the shift of the OH protons (13–14 ppm), as appears from Fig. 1. It is interesting to note that in the case of

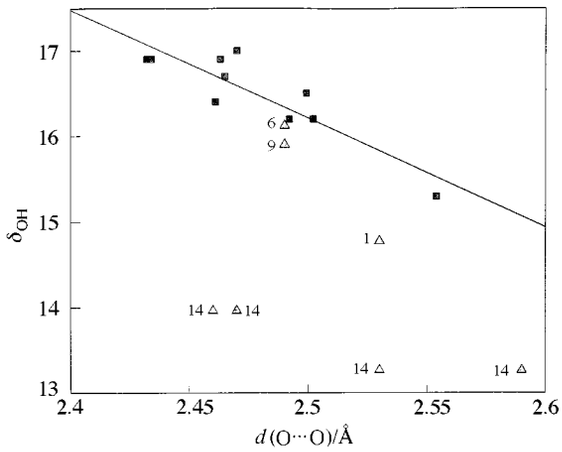
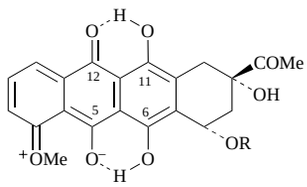


Fig. 2 Plot of ^1H chemical shift of the hydrogen-bonded OH groups *vs.* the interatomic distance $d(O\cdots O)$ for (■) cercosporin **1**,[12a,14] elsinochrome A **6**,[15] hypocrellin **9**,[16] daunomycin **14**[17] and (△) a series of 1,3-diarylpropane-1,3-dione enols from ref. 11(*b*)

anthraquinones **13**, **15** and **16**, the presence of two hydrogen-bonded phenol hydroxy groups of different strength on the same molecule is precisely indicated by both the chemical shift and the isotope effect. For daunomycin **14**, the $O^5\cdots H{-}O^6$ hydrogen bond is stronger than $O^{11}\cdots HO^{12}$, in agreement with a shorter distance found between O^5 and O^6 from X-ray studies.[17a,b] $d(O^5\cdots O^6) = 2.46$ and 2.47 Å, $d(O^{11}\cdots O^{12}) = 2.59$ and 2.53 Å, respectively. The electron donating methoxy group at C-4 induces a partial negative charge on the carbonyl at C-5, but not on the carbonyl at C-6, giving rise to the resonance structure shown. This is expected to enhance the strength of hydrogen bond $O^5\cdots H{-}O^6$ on the basis of the resonance-assisted hydrogen bonding model.[11c] An additional shortening of this bond comes from the steric effect[11c] of the methoxy group at the *peri* position. The steric factor on the other hand must be the only one responsible for the stronger hydrogen bond $O^9\cdots H{-}O^1$, with respect to $O^{10}\cdots H{-}O^4$, found in **13**, while in the case of **16** the same reasons given for daunomycins **14** and **15** apply. These results show a substantial parallelism between the solid and the liquid phases: that is the relative strength of the hydrogen bonds appears similar in the two phases.



Resonance structure of anthraquinones **14**–**16**

One last comment must be given on Fig. 1 for noranhydrocercosporin **10** and noranhydrophleichrome **11**, two derivatives of the natural substances **1** and **3**, respectively. The perylenequinone system in compound **10** was deemed[12b] to be almost planar on the basis of CD spectra. The relief of steric hindrance due to the folding of the side chains to form the dihydrofuran rings lowers the inversion barrier. The variable temperature NMR spectra gave evidence of the helical structure for both derivatives, showing in addition that the barrier is lower with respect to the parent compounds **1** and **3**. At room temperature **10** is a mixture of two atropoisomers in fast equilibrium, whereas **11** gave broad signals. Separate and sharp resonances were obtained at −80 °C for **10** and −5 °C for **11**.[18]

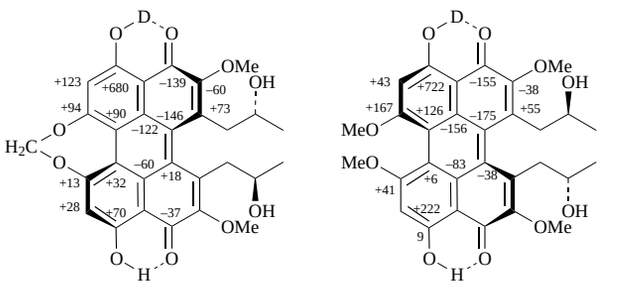
Both the primary isotope effect and the shift of the phenolic OH protons are comparable to the values of the parent compounds **1** and **3** respectively (Table 1), although the values in the case of **11** were measured at different temperatures. These

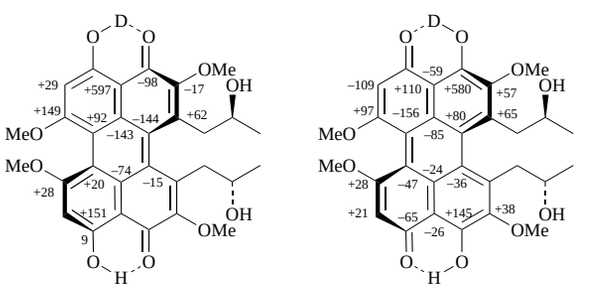
† The accuracy of these parameters is of the same order of that required by Gilli and co-workers [Ref. 11(*a*)]. The X-ray analysis of elsinochrome A **6** (crystallographic *R* factor 0.046, average standard deviation 0.005), showed that the symmetry around the C_2 axis is lost: while deviations from such symmetry are not significant in the central region of the molecule, they become more important at the periphery and $d(O\cdots O)$ values in the two halves are not equal (2.47 and 2.52 Å). The averaged value is the same found in hypocrellin **9** (2.49 Å, $R = 0.057$, stand. dev. 0.014).[16] The X-ray results on cercosporin **1** gave $d(O\cdots O) = 2.51$ Å ($R = 0.043$)[14] for both units, while for a non-symmetric derivative of **1** (14-*o*-acetate, 17-*o*-benzoate) it was found to be 2.50 and 2.53 Å ($R = 0.044$, stand. dev. 0.005).[12b]

results lead to the conclusion that the strength of the hydrogen bond does not depend on the distortion of the bonds connecting the two naphthalene units. X-Ray analyses of **10** and **11** are not available at present and the same holds for **3**; however the results in the solid phase for **1**, **6** and **9** showed different structures of the naphthalene units, leading to the hypothesis[13] that the strength of the hydrogen bond is dependant on the planarity of these moieties rather than on the distortion of the polycyclic ring. This is now confirmed by the results obtained with derivatives **10** and **11**.

Secondary deuterium isotope effect on carbon and proton shifts

The isotope effect on carbon atoms is spread over the whole perylenequinone system (Table 2 and Scheme 2). Either the





Diluted (1.5 x 10^{-2} M) solution	Concentrated (2.8 x 10^{-1} M) solution
Tautomer A prevailing	Tautomer B prevailing

Scheme 2 Secondary deuterium isotope effect on ^{13}C chemical shift of some perylenequinones, in $CDCl_3$ solution

C-3 or the C-4 carbon atoms can experience strong two-bond effects ($^2\Delta$), as a consequence of the tautomeric equilibrium (see Scheme 1). The effects on C-3 and C-4 are both positive for elsinochrome A **6** and hypocrellin **9**, whereas in the case of **1**, **2**, **3** and **10** the values are positive for C-4 and negative for C-3.

Let us examine first cercosporin **1** and its derivatives **2**[13] and **10**,[18] as they exist in solution as a single tautomeric form (**A**, shown in Scheme 1, 95–100%). The strong positive $^2\Delta$ effect for C-4 is in agreement with the structure of tautomer **A** and with a relatively strong intramolecular hydrogen bond. The negative $^4\Delta$ effect for C-3 could be explained with the distortion of the naphthalene units (as discussed in the previous paragraph), that leads to a small twisting of the carbonyl groups out of the naphthalene planes. Similar unusual negative $^4\Delta$ effects were found in a number of sterically hindered intramolecularly hydrogen-bonded aromatic ketones and interpreted in terms of a twist of the carbonyl group.[1a] The finding that the isotope effect has a positive sign in elsinochrome A **6** and hypocrellin **9**, where the two naphthalene units are more planar, should confirm these results, but unfortunately both **6** and **9** exist in solution as a *ca.* 50% mixture of two tautomers[13] and the measured values are actually the sum of $^4\Delta$ and $^2\Delta$ isotope effects. The positive value found for C-4 cannot be attributed only to a possible smaller steric strain,[1b] but also to the averaging between positive $^2\Delta$ and negative $^4\Delta$ effects, due to the tautomeric equilibrium.

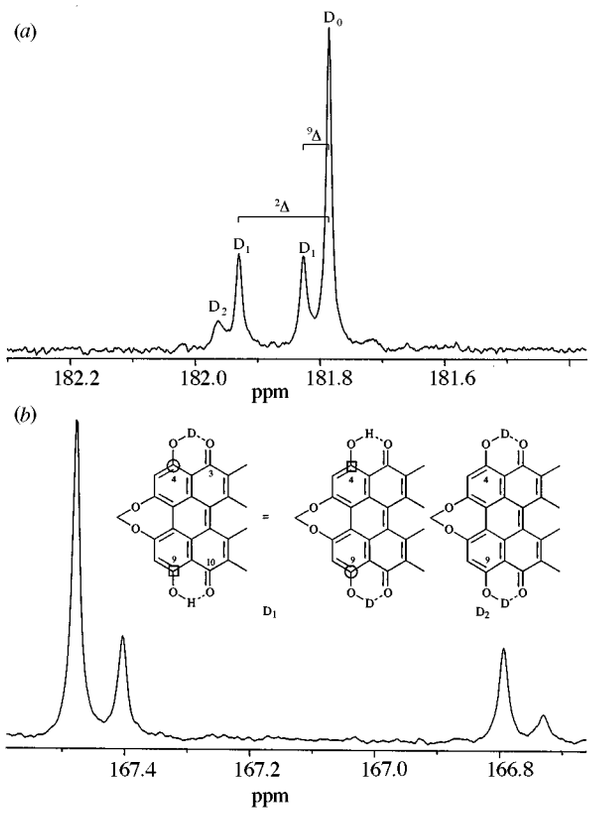


Fig. 3 ^{13}C NMR spectrum of a 10^{-1} mol dm^{-3} $CDCl_3$ solution of cercosporin **1** added with a 1:1 D_2O–H_2O mixture. (*a*) C-3, C-10 (*b*) C-4, C-9 signals. D_0, D_1 and D_2 are the resonances of non-deuterated, monodeuterated and bideuterated species respectively. The D_1 species has lost the C_2 symmetry axis.

In such equilibrating systems it is difficult to ascertain whether isotopic effects on chemical shifts are caused by intrinsic (involving a single species) or equilibrium effects.[19] As the chemical shift difference between the two tautomeric species is large for the oxygen-bound carbon atoms (*ca.* 17 ppm), the long range effects on C-3,10 and C-4,9 might be due to a variation of the tautomeric equilibrium, caused by the presence of deuterium. In order to check whether the equilibrium perturbation is responsible for the experimental long-range isotope effects, we calculated the equilibrium constant modified for the isotopic substitution at one of the hydroxy groups, by using eqn. (1)[20] where $\Delta\delta$ is the observed isotope effect, k is the factor

$$\Delta\delta = \frac{K(k-1)(\delta_B - \delta_A)}{(1+K)(1+kK)} \qquad (1)$$

modifying the equilibrium constant $K = [A]/[B]$ and δ_B, δ_A are the chemical shifts for the pure tautomers **A** and **B**. These were estimated from values for the hexamethyl ethers **26** and **27** of tautomers A and B respectively. In the case of cercosporin **1**, with $\delta_B - \delta_A = 17$ ppm and isotope effects of $+0.075$ and -0.037 ppm, we obtained for C-4,9 and C-3,10 respectively $k = 1.1095$ and 1.048, corresponding to an increment of tautomer **A** of the order of 0.4–0.2%. This might be reasonable, as we found[13] that the population of tautomer **A** in the non-deuterated equilibrium mixture is 95–100%. However, the same calculation for C-9b,12c performed with the k values thus obtained gave $\delta_B - \delta_A = -14.73$ and -27.97 ppm, which are not compatible with the chemical shift difference measured for the same carbon atoms of **26** and **27**, *i.e.* -4.43 ppm. A similar procedure performed for C-12b,12a gave $\delta_B - \delta_A = +4.30$ ppm, also not compatible with data from the models, *i.e.* -5.74 ppm. Consequently we must conclude that the contribution of the equilibrium shift to the isotope effects for cercosporin **1**, if any, is very low, and therefore the long-range effects on ^{13}C must be

Table 2 Secondary deuterium isotope effects (ppb) on ^{13}C chemical shifts for perylenequinones **2**, **3**, **4**, **6**, **9** and **10**[a]

	C-1 $^{6}\Delta$	C-2 $^{5}\Delta$	C-3 $^{4}\Delta/^{2}\Delta$	C-3a $^{3}\Delta$	C-4 $^{2}\Delta/^{4}\Delta$	C-5 $^{3}\Delta$	C-6 $^{4}\Delta$	C-6a $^{5}\Delta$	C-6b $^{6}\Delta$	C-7 $^{7}\Delta$	C-8 $^{8}\Delta$	C-9 $^{9}\Delta$	C-9b $^{7}\Delta$	C-10 $^{9}\Delta$	C-12 $^{7}\Delta$	C-12a $^{6}\Delta$	C-12b $^{5}\Delta$	C-12c $^{4}\Delta$
2[b]	87	−67	−177	[d]	718	110	101	102	34	17	32	85	−67	−46	12	31	−165	−137
6[b]	47	−22	219	−25	554	23	152	72	−28	32	−8	122	−50	−51	[d]	[d]	−110	−119
9[b,e]	51	36	191	−27	489	34	144	78	−43	[d]	[d]	71	−54	[d]	[d]	[d]	−116	−121
			258		521							143						
10[b,f]	111	−62	−217	22	723	128	96	91	41	27	+32	111	−79	−80	+22	17	−116	−144
3[c]	76	−62	−229	[d]	890	58	193	172	107	40	[d]	294	−107	−160	[d]	−52	−213	−179
4[c]	83	−68	184	18	884	41	192	152	101	50	[d]	299	−104	−45	[d]	−36	−208	−182

[a] Data for **1**, **3** and **4** in $CDCl_3$ are reported in Scheme 2. All compounds, except for **9** have a C_2 symmetry axis. For missing atoms the isotope effect was not detected. [b] In $CDCl_3$. [c] In acetone. [d] Not detected or not resolved. [e] The data reported are the average of the values for the corresponding nuclei in the two halves, when the single resonances are detected and their difference is within the error margins. Otherwise the two values are reported. [f] Averaged values of the two atropoisomers in fast equilibrium.

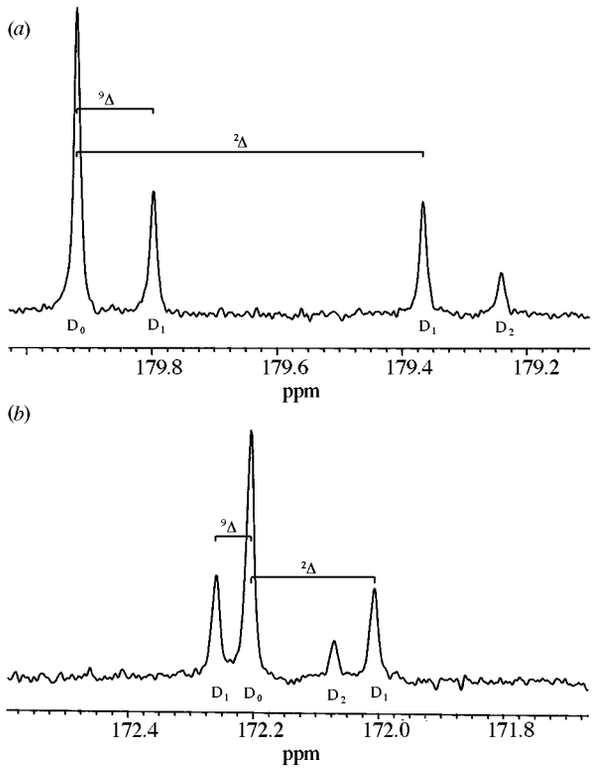


Fig. 4 ^{13}C NMR spectrum of a $CDCl_3$–D_2O solution of elsinochrome A **6** prepared as described in Fig. 3. (*a*) C-3, C-10 and (*b*) C-4, C-9 signals.

largely intrinsic. This conclusion is in agreement with the results obtained for 9-hydroxyphenalen-1-one,[7] which has a symmetric energy profile. It is further strengthened by the fact that both ^{13}C and ^{1}H chemical shifts and the primary and secondary isotope effects for cercosporin **1** do not change with solvent and temperature.

In contrast, for phleichrome **3** and the stereoisomer **4** the tautomeric equilibrium depends on these factors.[13] In the case of isophleichrome **4**, this dependence is relevant as shown by $J_{C,OH}$ values[13] and is also reflected in the isotope effects reported in Scheme 2. In 10^{-2} mol dm^{-3} $CDCl_3$ solution, tautomer **A** is predominant, as well as in acetone (55–70%),[13] and the isotope effects are strong and positive for C-4 and negative for C-3 respectively. In contrast, in more concentrated $CDCl_3$ solution (10^{-1} mol dm^{-3}), tautomer **B** is predominant (*ca.* 70%),[13] and a strong positive value, mainly due to the $^2\Delta$ effect, was observed for C-3, in agreement with the structure of tautomer **B**. The value for C-4 is also positive, but smaller. In the case of phleichrome **3**, the calculation performed through eqn. (1) gave a 1.9% increment of tautomer **A**. With the modified equilibrium constant, the calculated chemical shift difference $\delta_B - \delta_A$ for C-9b,12c in $CDCl_3$ is −6.21 ppm, which could be considered compatible with the value of −4.43 ppm, measured for **26** and **27**. But in acetone the discrepancy becomes larger (−8.26 ppm). However, a contribution of the equilibrium shift to the isotope effect cannot be excluded in this case. For phleichrome **3**, we measured the coupling constants between the OH proton and the oxygen-bound carbon atoms in the monodeuterated species. The results were substantially the same, within experimental error, as those obtained both from the signal of the non-deuterated species present in the same solution, and the spectrum of **3** without D_2O. As the error in this measurement (±0.2 Hz) is reflected in the population of tautomers by an amount of *ca.* 8% a small increment of tautomer **A** by deuteration is thus possible. For elsinochrome A **6** similar results were obtained.

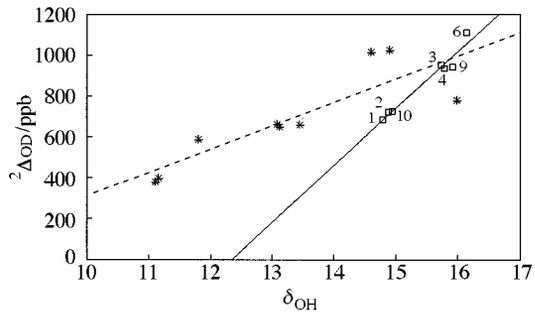


Fig. 5 Plot of the secondary deuterium isotope effect on the chemical shift of C-4, $^2\Delta$(OD), *vs.* OH proton shift, δ_{OH}, for (□) perylenequinones **1**–**4**, **6**, **9**, **10** (present work) and (*) some *o*-hydroxyacylaromatics [data taken from ref. 1(*a*), Fig. 2 and from ref. 7]. The □ values were obtained from the data in Table 1 corrected for 100% of tautomer **A**.

Hansen[1a,b] found a linear correlation between $^2\Delta$ isotope effects and the shift of OH protons in intramolecularly hydrogen-bonded acyl aromatics, but the slope varies for different classes of compounds. As our perylenequinones show different amounts of tautomers **A** and **B**, such a correlation does not hold, because the $^2\Delta$ effect is strongly dependent on the composition at equilibrium. However, with the $^2\Delta$ values corrected to 100% of tautomer **A**, and taking into account a possible error, estimated within 10% of the population of tautomer **A**, we obtained the plot shown in Fig. 5. The perylenequinones **1**–**4**, **6**, **9** and **10** lie on a straight line, which on the other hand is different, as expected, from that for another group of compounds taken from the literature.[1a] The variation of $^2\Delta$ isotope effects in our perylenequinones follows qualitatively the predicted trend, which indicates that elsinochrome A **6** displays the strongest hydrogen bond.

The secondary effects of deuteration are transmitted along the whole extended conjugated system. An inspection of Scheme 2 and Table 2 shows that the effects on ^{13}C shifts are positive and negative, but not distributed in an alternating way. A recent theoretical study[5] of the isotope effect shows that two factors are mainly responsible for the change in chemical shifts, which can be described by a vibrational term and an electronic term. This latter, governing the transmission along the electronic pathway, *i.e.* through the bonds, is the most important in the case of long-range effects. This is the reason why in extended π-conjugated systems, like perylenequinones, such effects are relevant. Although it is difficult to correlate their values with the electronic charge distribution, one observation is relevant: the positive values appear more concentrated on carbon atoms of benzenoid rings, whereas the negative ones are more frequent for atoms with a quinone character. This is clearly shown by cercosporin **1** and its derivatives **2** and **10**, because these molecules exist as single tautomers. However, in the case of compounds **3** and **4** also the values of the long-range effects follow the same trend, which consequently indicates that tautomer **A** is predominant. It is significant that the variation of these isotope effects observed in isophleichrome **4** with solvent and concentration is in line with the change of the tautomeric equilibrium. These long-range effects on ^{13}C shifts can be used as additional evidence that tautomers of 'cross-quinone' structure like 3,9-dihydroxyperylene-4,10-dione[13] are not significantly present. On the other hand, these tautomers, less stable than tautomers **A** and **B**, have been excluded[13] for compounds **1** and **2**, but there was no experimental evidence to exclude them for the other perylenequinones. These results now support the conclusion that tautomers **A** and **B** are actually the only significant components of the tautomeric equilibrium.

The secondary long-range isotope effects on protons are small (4–8 ppb at 5-H and 2-OMe), except for one, surprisingly large, over 11 bonds, found at the phenolic OH protons. Similar

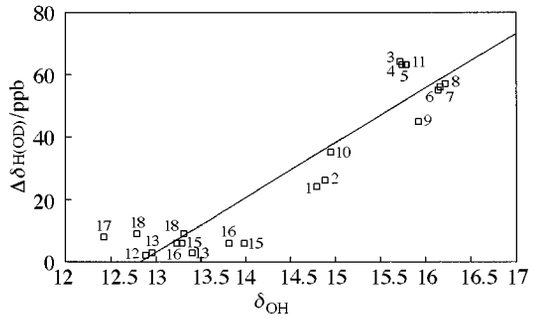


Fig. 6 Plot of the long range deuterium isotope effect on the chemical shift of phenolic OH protons $\Delta\delta$H (OD) *vs.* OH proton shift, δ_{OH}, for perylenequinones **1**–**11** ($^{11}\Delta$) anthraquinones **12**–**16** and naphthoquinones **17**, **18** ($^{7}\Delta$)

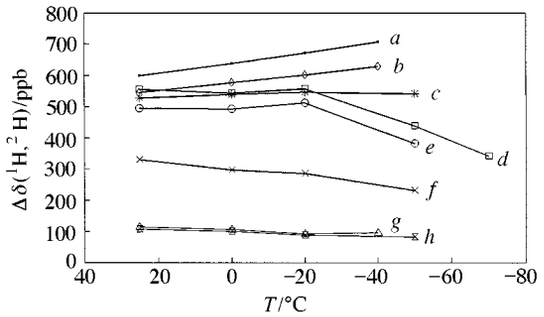


Fig. 7 Plot of the primary isotope effect on the chemical shift of OH proton $\Delta\delta$ (^{1}H,^{2}H) *vs.* temperature for (*a*) benzoylacetone **23**, (*b*) acetylacetone **22**, (*c*) elsinochrome A **6** in $CDCl_3$, (*d*) and (*e*) phleichrome **3** in acetone and $CDCl_3$ respectively, (*f*) cercosporin **1** in acetone (*g*) anthraquinone **12** and (*h*) naphthazarin **17** in $CDCl_3$

positive effects, but smaller in magnitude were measured in naphthazarin and in other naphthoquinones and anthraquinones (Table 1). All compounds show two phenolic OH signals, which are well separated and do not reveal significant chemical exchange at room temperature. The observation of a negative long-range effect, $^{8}\Delta$, on the OH shift in 1,8-dihydroxyanthraquinones was explained by Hansen [1a] with a steric strain associated with a lengthening of the intramolecular hydrogen bonding. The steric strain cannot be invoked to explain the positive $^{7}\Delta$ and $^{11}\Delta$ effects found in the present study. On the other hand positive or negative long range effects (over up to nine bonds) were found for all the carbon atoms of the perylenequinone system. The effect can thus be transmitted through the π-conjugated system as far as to the opposite side of the polycyclic ring, at the OH proton. Alternatively, this effect might also be explained with a variation of the tautomeric equilibrium, due to the presence of deuterium. However calculation through eqn. (1) by using the modified equilibrium constant values obtained for **1** and **3** from ^{13}C isotope effects, gave a chemical shift difference of 5–6 ppm between tautomers **A** and **B**. These values are not reliable, if we consider that δ_{OH} for **1** (tautomer **A**) is 14.79 and for **4** (70% tautomer **B** in concentrated $CDCl_3$ solution) is 15.73 ppm. An inspection of Table 1 shows that this $^{11}\Delta$ effect is larger for compounds **3**–**9** (55–77 ppb) than for cercosporin **1** and derivatives **2** and **10** (25–35 ppb). The values decrease to 7–9 ppb in anthraquinones and naphthoquinones **12**–**17**. We have plotted in Fig. 6 these long range values *vs.* δ_{OH} and a correlation with the OH proton shift seems to occur. Such effects, as well as those discussed above, appear to depend on the strength of the hydrogen bond and they are larger in those compounds which display a significant amount of both tautomers. It might be suggested that a high π-electron mobility favours the transmission through the conjugated system.

The effect of temperature

Measurements were performed at low temperature down to −40 °C in $CDCl_3$ and to −70 °C in acetone, for perylenequinones **1**, **3**, **6**, for the anthraquinone **12**, naphthazarin **17**, acetylacetone **22** and benzoylacetone **23**. An inspection of Fig. 7 shows that the primary effect for **22** and **23** increases at low temperature, whereas all the other compounds display a different trend. Specifically in cercosporin **1** it slightly decreases, in **6**, **12** and **17** it appears almost constant, and in phleichrome **3** it is constant or slightly increasing up to −20 °C, then decreases significantly from −20 to −70 °C. A small change with temperature in the tautomeric equilibrium might be possible for those compounds which present an asymmetric double minimum potential curve, but it must be excluded for acetylacetone **22**. Intrinsic isotope shifts normally do not change with temperature; however in the case of a potential energy surface with shallow minima (which corresponds to a positive primary effect, as for **22**, **23** and also for perylenequinones) a temperature dependent intrinsic shift could be caused by changes in the vibrational energy levels.[19] The very similar trend of the curves for **22** and **23** suggests that the equilibrium in the latter does not change very much with temperature and thus the observed variation is mainly due to intrinsic effects.

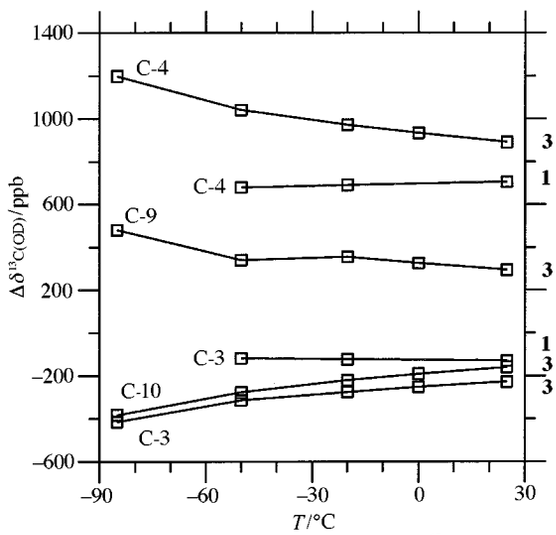


Fig. 8 Plot of the secondary isotope effect on ^{13}C chemical shift $\Delta\delta^{13}$C(OD) *vs.* temperature for cercosporin **1** in $CDCl_3$ and for phleichrome **3** in acetone

The proton chemical shift δ_{OH} does not show significant changes at low temperature. For all compounds the variation lies within 0.1–0.2 ppm. In contrast, measurements of the secondary isotope effect on ^{13}C shift were more interesting. Cercosporin **1** and elsinochrome A **6** are constant in both $CDCl_3$ and acetone, whereas phleichrome **3** shows significant changes at C-3,10 and C-4,9 sites (Fig. 8). The opposite trend found for C-4 and C-9 with respect to C-3 and C-10 clearly indicates an increase of the most stable tautomer **A** at low temperature. The results obtained with ^{13}C experiments might explain the behaviour of the primary effect with temperature. For perylenequinones the equilibrium contribution must be added to the intrinsic effect, and this induces the trend inversion at *ca.* −20 °C found for phleichrome **3**. The variation of the equilibrium with temperature for **1** and **6** is negligible, as well as for **12** and **17**. This is reasonable for cercosporin **1** and for 9,10-dihydroxyanthraquinone **12**, because a single tautomeric form is actually present. In naphthazarin **17** the two prevalent forms are equal, but for elsinochrome A **6** the absence of variation could be accidental.

Conclusions

The primary deuterium isotope effects on OH protons of hydroxyperylenequinones and hydroxyanthraquinones are large and positive and fit well with the correlation between such effects and the OH chemical shift found[9] for β-ketoesters and *o*-hydroxyacyl aromatics, thus giving additional evidence for the relationship. This effect can be successfully used to estimate the strength of the hydrogen bonds in solution, for all intramolecularly hydrogen bonded enol compounds, independently from the tautomeric process. In contrast, the secondary $^2\Delta$ effect on the carbonyl carbon shift depends on the tautomeric process and must be used with caution. The interaction distances d(O · · · O) obtained from X-ray analyses of **1**, **6** and **9** confirm the trend of the hydrogen bond strength in solution: **1**, **2**, **10** < **3**, **4**, **5**, **9** < **6**, **7**, **8**, showing a substantial parallelism between solid and liquid phase. These results lead to the conclusion that the strength of the hydrogen bond in perylenequinones depends on the planarity of the naphthalene units, rather than on the distortion of the polycyclic ring.

The secondary isotope effects on carbon nuclei in perylenequinones are transmitted along the whole extended π-conjugated system, with positive and negative, but not alternating, signs. The negative sign appears characteristic of the quinonoid and the positive one of the benzenoid ring; thus we could exclude a significant presence at equilibrium of cross-quinone tautomers, like 3,10-dihydroxyperylene-4,9-dione in phleichrome **3**, isophleichrome **4** as well as in **1**, **2** and **10**.

The long-range isotope effect over 11 bonds, found at the OH protons of perylenequinones, is analogous, but larger in magnitude, to the $^7\Delta$ effect detected in hydroxyanthraquinones and hydroxynaphthoquinones. Such effects, positive in sign, are correlated with the strength of the hydrogen bonds. From these results it appears that an extended conjugated system, with a high π-electron mobility favours the transmission of long-range effects on carbon as well as on proton chemical shifts.

In these systems it is difficult to know whether isotope effects are caused by intrinsic or equilibrium effects; however calculation of the equilibrium constant, following known procedures from the literature,[20] leads to results inconsistent with the experimental values. Thus we must conclude that the contribution of equilibrium effects is negligible or small.

The effect of temperature on the isotope effect is significant even for acetylacetone, where the symmetric structure excludes any contribution from equilibrium effects. This confirms the suggestion[19] that, in the case of compounds with strong primary positive effect, large temperature dependent intrinsic shifts could be expected.

The tautomeric equilibrium of cercosporin **1** and elsinochrome A **6** does not change with temperature, whereas in the case of phleichrome **3** an increase of the most stable tautomer **A** was found at low temperature. This is reflected in the variation of primary and secondary effects observed for **3**, but not for **1** and **6**.

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