NMR Study of Tautomerism in Natural Perylenequinones

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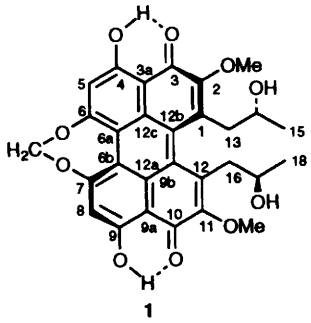
The keto–enol tautomerism of the dihydroxyperylenequinone system of a number of natural compounds, cercosporin **1**, isocercosporin **2**, phleichrome **3**, isophleichrome **4**, elsinochromes **5–9**, cladochrome C **10**, and hypocrellin **11** was studied by ^1H, ^2H and ^{13}C NMR spectroscopy. 4,9-Dihydroxyperylene-3,10-dione and 3,10-dihydroxyperylene-4,9-dione tautomers were recognized as present in fast equilibrium in $CDCl_3$ and [$^2H_6$]acetone solutions. The populations of each tautomer were obtained from the coupling between the proton of the hydrogen-bonded OH groups and the adjacent carbon atoms, *i.e.* J(C3, OH) and J(C4, OH). The most important factors governing the tautomeric equilibrium in these helix-shaped compounds appeared to be the substituent effects, the strength of the intramolecular phenol–quinone hydrogen bond, the distortion from the planarity of the perylene-quinone system, solvation and aggregation effects. The strength of the hydrogen bonds, which is related to the distortion from planarity, was evaluated from ^1H chemical shifts and primary deuterium isotope effects. Proton shifts of OH groups and isotope effects are linearly correlated. The influence of solvents and concentration on the tautomeric equilibrium was studied in the case of **4**, for which a model of vertical stacking-type dimerisation is proposed.

Perylenequinones form a relatively small, but increasing group of biologically active pigments obtainable from natural sources.[1] Besides the group of aphins, which are responsible for the pigmentation of some species of aphids, a series of 4,9-dihydroxyperylene-3,10-quinones have been isolated from fungi. Similar compounds have been recently found also in marine organisms.[2]

Interest for these compounds stems not only from their peculiar structure features, but also from their photodynamic activity. One of them, cercosporin **1**, is an efficient singlet oxygen producer,[3] this property probably being related with its function as a toxin of many phytopathogenic strains of *Cercospora*. Other compounds, belonging to the cladochrome group,[4–6] have been shown recently[6,7] to be potent inhibitors of protein kinase C, thus hinting at possible antitumour[8] and antiviral activity,[9] this latter exhibited by similar, more extended, quinonoid systems.[10]

The 4,9-dihydroxyperylene-3,10-quinone structure gives rise to different tautomeric forms. This paper reports a NMR study of tautomerism in some of the natural compounds and their derivatives, namely cercosporin **1**,[11] isocercosporin **2**,[11] phleichrome **3**,[12] isophleichrome **4**,[13] elsinochrome A **5**,[14] elsinochromes B_1 **6** and B_2 **7**,[15] elsinochromes C_1 **8** and C_2 **9**,[15] cladochrome C **10**[5] and hypocrellin **11**.[16]

All these compounds are characterized by a 4,9-dihydroxyperylene-3,10-quinone nucleus substituted in positions 2,11 by two methoxy groups, in positions 1,12 by a 6- or 7-membered ring or by two 2-hydroxy- or 2-acyloxy-propyl side chains, and in positions 6,7 by a methylenedioxy or by two methoxy groups. These substituents induce enough steric strain to force the polycyclic ring to assume a helical shape. This helicity generates axial chirality, which, when associated to the chirality of the asymmetric carbon atoms of the chains, gives rise to diastereoisomerism.[1] Thus, cercosporin can be interconverted thermally with isocercosporin, which has opposite helicity and a different conformation of the side chains. The same happens for phleichrome and isophleichrome, whereas this conversion does not seem to be possible for the elsinochromes and hypocrellin, although they are also helical molecules.



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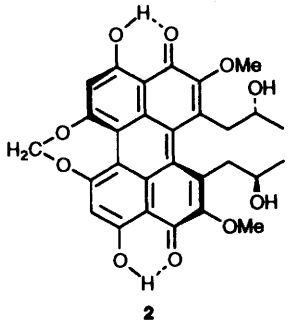

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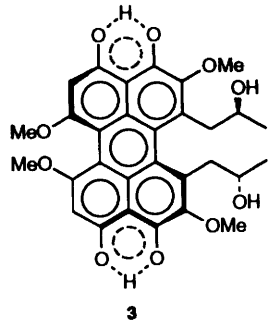

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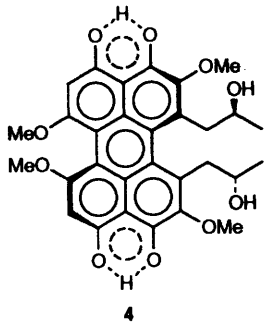

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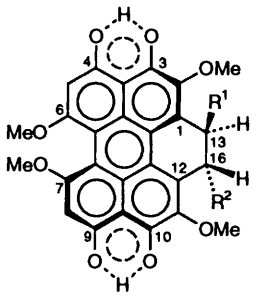
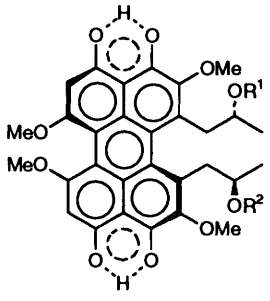
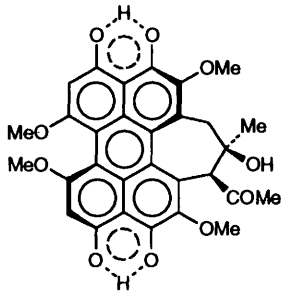
The phenol–quinone tautomerism of the derivatives of 4,9-dihydroxyperylene-3,10-quinone here studied is represented by an equilibrium between the four tautomers **A**, **B**, **C** and **D** (Scheme 1). Depending on the substitution on the ring, some of them may be identical: in particular, compounds **1–5** and **8**, **9** possess a C_2 axis of symmetry lying in the main plane of the



5 R^1 = COMe, R^2 = COMe
6 R^1 = $C^{14}O^{15}Me$, R^2 = $C^{17}HOH^{18}Me$ 17*S*
7 R^1 = COMe, R^2 = CHOHMe 17*R*
8 R^1 = $C^{14}HOH^{15}Me$, R^2 = $C^{17}HOH^{18}Me$ 14*S*, 17*S*
9 R^1 = CHOHMe, R^2 = CHOHMe 14*R*, 17*R*



10 R^1 = COPh, R^2 = COPh-4-OH



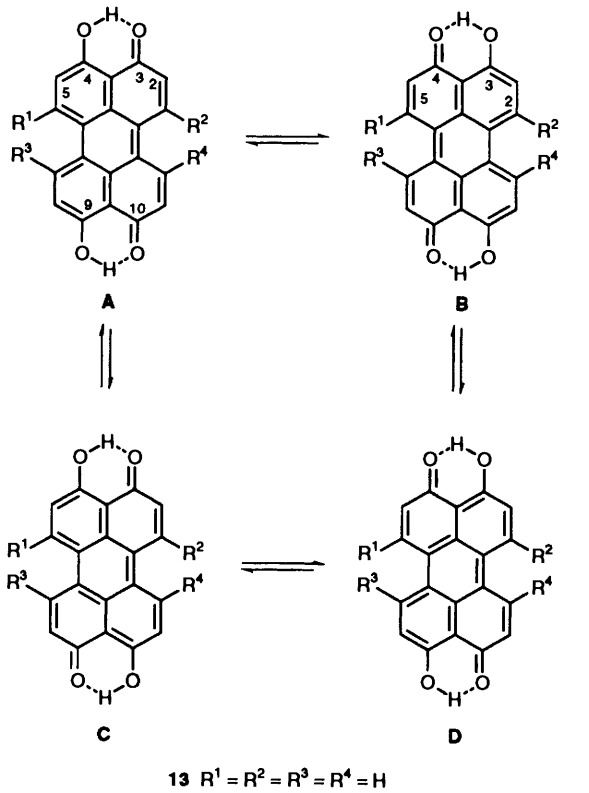
11

molecule, which makes the two tautomers **C** and **D** identical.*

The simplest model for these compounds is naphthazarin **12**, which has been shown to exist, both in solution[17] and in the solid phase,[18] as a fast equilibrium mixture where the two tautomers **I** and **II** predominate. They are the most stable ones, the two 1,5-quinonoid forms (**III**) possessing an *ab initio* calculated[17b] additional energy of 104.75 kJ mol^{-1} (Scheme 2).

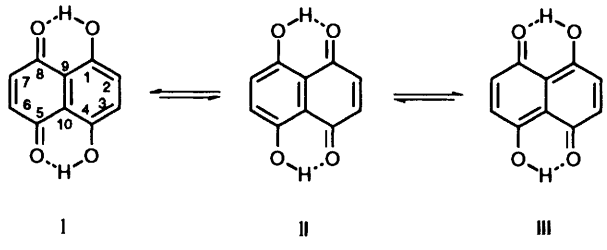
The parent compound 4,9-dihydroxyperylene-3,10-quinone **13**[19] had not been previously studied in this respect, since it is practically insoluble in any solvent, thus precluding both UV and NMR measurements. The ^{13}C NMR spectrum in the solid phase showed six signals: one resonance at δ 180 was assigned to the four oxygen-bound carbon atoms, one resonance at δ 110 to C-3a and C-9a, two resonances at δ 135–140 were assigned to the other six quaternary carbons, specifically two sets of four equivalent and two equivalent nuclei respectively. Finally a strong signal between δ 120 and 125 is due to the eight hydrogen-bound carbons. This spectrum indicates that perylenequinone **13** exists as a fast equilibrium mixture of tautomers, similarly to naphthazarin. Semiempirical AM1[20] calculations indicate a higher stability of the 4,9-dihydroxyperylene-3,10-dione tautomer (heat of formation = −240.1 kJ mol^{-1}) with respect to the 3,9-dihydroxyperylene-4,10-dione (−227.5 kJ mol^{-1}). The same calculations performed on naphthazarin give a difference of *ca.* 33.5 kJ mol^{-1} in favour of the tautomers **I** and **II** with respect to the 1,5-quinonoid one (in qualitative agreement with the *ab initio* calculations[17b].)

For cercosporin **1**,[11c,d] elsinochrome A **5**[21,22] and hypocrellin **11**[16,23] X-ray data are available and show that in the solid state cercosporin is in the tautomeric form **A**, a form which prevails also for elsinochrome A, whereas hypocrellin has structure **B**.



13 R^1 = R^2 = R^3 = R^4 = H

Scheme 1 Tautomeric forms of 4,9-dihydroxyperylene-3,10-quinones



Scheme 2 Tautomeric forms of naphthazarin **12**

^1H and ^{13}C NMR chemical shifts should provide information about the tautomerism in solution, as shielding effects are different for nuclei in an aromatic ring or in a quinonoid one. In fact the chemical shift of 5-H (Table 1) varies for the compounds studied from δ 7.03 for cercosporin **1** to δ 5.28 for isophleichrome **4** in $CDCl_3$. A similar trend, in the opposite sense, is shown by the ^{13}C chemical shifts (Table 2). The values, reported in Tables 1 and 2, might suggest that cercosporin exists predominantly in the **A** form and isophleichrome in the **B** form. All the other compounds, showing intermediate values, should exist as a fast equilibrium of tautomers. The 5-H shift for the two permethyl ethers of phleichrome **14** and **15**,[13] derived from tautomer **A** (δ 6.74) and tautomer **B** (δ 6.03), respectively, should have values at the two extremes of the range. This is not the case, *cf. e.g.* the 5-H shift for **4** and **15**. Actually the chemical shift values might be misleading, as they are affected by magnetic anisotropy effects, that are especially important for protons in such extended aromatic systems. On the other hand, the ^{13}C chemical shift of C-3 and C-4 for the permethyl ethers **14** and **15** is sensitive also to the substituent effects of the methyl group.

More information about the tautomerism appeared from the

* Due to this symmetry, only half of the signals belonging to H or C atoms appear in the NMR spectra of these compounds. Therefore, in the following discussion, mention of a particular H or C atom of the 'upper' part will include the corresponding atom of the 'lower' part.

Table 1 ^{1}H Chemical shift values (δ) for compounds **1**–**11**[a]

	1		**2**		**3**		**4**		**5**		**6**	**7**		**8**	**9**	**10**		**11**	
	b	*c*	*b*	*c*	*b*	*c*	*b*, *d*	*c*	*b*	*c*	*b*	*b*	*c*	*b*	*c*	*b*	*c*	*b*	*c*
4-OH	14.82	14.90	14.90	14.98	15.78	15.91	15.72	15.95	16.14	16.34	16.07	16.13	16.40	16.12	16.22	15.79	16.04	15.90	16.04
5-H	7.03	7.05	6.67	7.02	6.58	6.76	5.28	6.73	6.61	6.80	6.57	6.57	6.74	6.56	6.62	6.29	6.52	6.50	6.61
13-Ha	3.58	3.65	3.49	3.51	3.60	3.65	3.64	3.49	5.20	5.27	5.14	4.82	5.05	4.16	3.88	3.65	3.75	3.50	3.41
13-Hb	2.88	2.93	2.86	2.97	2.95	2.91	3.09	2.95			4.21[f]	4.25[f]	4.32[f]			3.10[h]	3.18[h]	2.63	2.61
14-H	3.38	3.42	3.69	3.61	3.42	3.44	3.81	3.64			3.64[g]	3.84[g]	4.02[g]	3.68	3.76	4.71[h]	4.80[h]	3.47[f]	3.84[f]
2-OMe	4.18	4.16	4.20	4.19	4.20	4.19	4.33	4.17	4.36	4.31	4.25	4.26	4.20	4.21	4.25	4.29	4.29	4.10	4.12
6-OMe	5.75[e]	5.93[e]	5.62[e]	5.90[e]	4.06	4.15	3.26	4.18	4.06	4.21	4.04	4.04	4.16	4.04	4.08	3.69[h]	3.93[h]	4.05	4.12
15-H_3	0.60	0.47	0.96	0.99	0.53	0.31	1.13	0.98	2.04	2.12	2.09	2.10	2.09	1.11	1.19	(1.29)	(1.32)	(1.70)	(1.66)
18-H_3	0.60	0.47	0.96	0.99	0.53	0.31	1.13	0.98	2.04	2.12	1.14	1.14	0.96	1.11	1.19	(1.19)	(1.20)	(1.89)	(1.90)

[a] Measured at 25 °C from internal Me_4Si. Accuracy within ±0.01 ppm. Concentration *ca.* 10^{-1} mol dm^{-3}, except for **3** in $CDCl_3$ and **10** (*ca.* 10^{-2} mol dm^{-3}). Only values for one half of the molecule are given, also for the asymmetric compounds **6**, **7**, **10** and **11**. For these latter, the signals are coincident or separated by 0.01–0.04 ppm, unless specified; the values given are the upfield ones. Values in parentheses may be interchanged. [b] $CDCl_3$. [c] [$^2H_6$]Acetone. [d] Concentration 2.8 × 10^{-1} mol dm^{-3} (saturated solution); the shifts change considerably on dilution (see text). [e] Value for 6-OCH_2O. [f] Values for 16-H. [g] Value for 17-H. [h] The values for the other half of the molecule are δ 3.17 and 3.30 (16-H), δ 5.04 and 5.12 (17-H), δ 3.86 and 4.07 (6-OMe) in $CDCl_3$ and in acetone respectively.

Table 3 Selected C, H coupling constant values (Hz) for compounds **1**–**11**[a]

	1		**2**		**3**		**4**		**5**		**6**	**7**		**8**	**9**	**10**	**11**
J(C,H)	*b*	*c*	*b*	*c*	*b*	*c*	*b*	*c*	*b*	*c*	*b*	*b*	*c*	*b*	*b*	*b*	*b*
C2, H13a	3.4[d]	*e*	3.0[d,f]	*e*	*e*	*e*	3.0[d,f]	*e*	*e*	*e*	3.6	3.6	*e*	3.5[f,i]	3.6[f,i]	*e*	*e*
C2, H13b	5.9[d]	*e*	6.0[d,f]	*e*	*e*	*e*	6.0[d,f]	*e*								*e*	*e*
C2, OMe	3.4	*e*	3.0[f]	*e*	*e*	*e*	3.0[f]	*e*	3.3	*e*	3.6	3.6	*e*	3.5[f]	3.6[f]	*e*	*e*
C2, OH	⩽0.5	*e*	⩽0.3	*e*	*e*	*e*	3.5[f]	*e*	3.3	*e*	3.0[f]	3.0[f]	*e*	3.0[f]	*e*	*e*	*e*
C3, OH	⩽0.3[g]	⩽0.3	⩽0.3	⩽0.3	2.0	1.9	4.0[h]	2.0	3.0[g]	2.2	2.7	2.5	2.4	2.4	2.8	2.6	2.7
C3a, OH	5.5	5.8	5.8	5.5	5.7	5.7	5.3	6.2	6.0	5.5	6.0	6.0	6.0	5.9	6.0	5.9	6.0
C3a, H5	5.2	5.0	5.2	5.7	4.2	4.6	4.2	4.2	4.4	4.0	4.3	4.3	4.5	4.4	4.4	4.5	4.4
C4, H5	3.3	3.3	3.4	3.3	3.2	3.5	3.2	3.2	3.4	3.6	3.4	3.4	3.5	3.4	3.3[f]	3.4[f]	3.4
C4, OH	5.2	5.2	5.2	5.3	3.8	4.0	1.6[h]	4.2	3.0	3.9	3.2	3.2	3.5	3.0	3.3[f]	3.4[f]	3.1
C5, OH	6.9	6.7	6.7	6.5	4.5	5.1	2.0	5.2	3.8	4.5	3.7	3.6	4.3	3.6	3.7	4.0	3.7

[a] Estimated accuracy ±0.1 Hz, unless specified. Concentration as in Table 1. Only values for one half of the molecule are given also for the asymmetric compounds **6**, **7**, **10**, and **11**, because they are within the estimated accuracy. [b] $CDCl_3$. [c] [$^2H_6$]Acetone. [d] The values may be interchanged. [e] Not measured. [f] Accuracy ±0.2 Hz. [g] For this compound a coupling constant of 1.0 Hz was detected between C-3 and 5-H, which are related by a W geometry; a similar coupling is presumably present also in the other compounds. [h] These values change on dilution, *i.e.* *J*(C3, OH) = 3.4 Hz (1.2 × 10^{-1} mol dm^{-3}) and 2.7 Hz (1.5 × 10^{-2} mol dm^{-3}); *J*(C4, OH) = 2.2 Hz (1.2 × 10^{-1} mol dm^{-3}) and 3.2 Hz (1.5 × 10^{-2} mol dm^{-3}). [i] Estimated value from the 2nd order spectrum.

Table 2 ^{13}C chemical shift values (δ) for compounds **1**–**4**, **10** and **11**[a]

	1[b]	2[b]	3[b]	4[b]	4[c]	10[b]	11[b]
C-1	135.35	136.93	135.34	137.91	139.08	133.59	133.20
C-2	152.86	152.11	151.82	150.53	152.45	153.38	150.69
C-3	181.82	181.57	173.49	167.70	176.14	172.29	171.03
C-3a	108.28	108.33	105.95	106.40	106.34	106.36	106.72
C-4	167.49	167.46	177.69	182.41	176.98	178.13	179.79
C-5	109.30	108.93	101.51	101.07	101.23	101.16	102.02
C-6	163.41	163.34	166.85	167.14	167.70	166.61	167.46
C-6a	112.93	113.02	117.09	119.02	116.56	116.73	117.70
C-12b	130.65	131.66	127.11	127.26	130.54	127.22	127.70
C-12c	127.96	127.51	126.18	124.77	127.20	125.38	125.00
C-13	42.21	42.54	42.21	43.40	43.93	38.75	41.87[f]
C-14	68.09	69.00	68.40	68.76	69.02	*e*	78.74
C-15	23.31	23.77	23.07	24.62	24.47	20.65	26.96[g]
2-OMe	61.20	60.95	61.44	61.34	61.13	61.20	62.05
6-OMe	92.65[d]	92.59[d]	56.45	55.94	57.25	55.99	56.45

[a] See footnote *a* to Table 1. In the case of the asymmetric compounds **10** and **11** the signals of the other half of the molecule are within 1 ppm lowfield, unless specified. The chemical shift values for elsinochromes **5**–**9** are reported in ref. 15. [b] $CDCl_3$. [c] [$^2H_6$]Acetone; the values in this solvent are reported only for **4**, which shows the largest variations. [d] Value for 6-OCH_2O. [e] δ 72.33 or 76.25, C-14 was not assigned *vs.* C-17. [f] C-16 is at δ61.68. [g] C-18 is at δ 30.09.

coupling constants between the proton of the hydrogen-bonded OH groups and the adjacent carbon atoms (Table 3). For naphthazarin we measured a coupling constant across two bonds J(C1, OH) of 3.2 Hz and two couplings across three-bonds, *i.e.* J(C2, OH) and J(C9, OH) of 3.8 and 5.0 Hz respectively (see Scheme 2). These values are averages of those corresponding to each tautomer, as the tautomeric process is fast with respect to the NMR time scale.

In the perylenequinone system the process is also fast, but there is less symmetry in the derivatives than in the parent compound. Thus the carbon atoms C-3 and C-4, as well as C-2 and C-5, are not equivalent and the coupling constants involving these nuclei and the OH protons are significant for each tautomer. In particular J(C3, OH) must be zero in tautomer **A**, and the same holds for J(C4, OH) in tautomer **B**, since the interaction of a carbon atom with the *cis* OH proton *via* a four-bond pathway is null.[24] The same occurs for the couplings across three bonds J(C2, OH) and J(C5, OH), which must be zero in tautomer **A** and **B** respectively, whereas J(C3a, OH) is never null (see Scheme 1).

Cercosporin **1** and isocercosporin **2** show negligible coupling for both C-2 and C-3 atoms (the signals are very sharp, with line-width of 1.5 Hz), whereas C-4 and C-5 display significant interactions, which amount to 5.2–6.9 and 6.5–6.9 Hz respectively. This indicates that cercosporin and its isomer **2** exist almost exclusively as tautomer **A** both in chloroform and acetone solution. The zero coupling observed for C-2 and C-3 excludes a significant presence not only of tautomer **B**, but of **C** and **D** as well.

Phleichromes **3**, **4**, elsinochromes **5**–**9**, cladochrome C **10** and hypocrellin **11** show a different pattern: all four carbon atoms C-2 to C-5 are coupled with the hydroxy proton. In a few cases, *i.e.* for **3**, **4** in acetone and **11** in $CDCl_3$, the C-2 resonance was not analysed; actually the multiplicity of this signal is complex, as C-2 is coupled to OH, 13-H and to the methoxy protons. However the data reported in Table 3 clearly show that for **3**–**11** both tautomers **A** and **B** are present at the equilibrium. Tautomers **C** and **D** cannot be excluded on this basis, but the calculated higher energy of these forms in the parent compound and in some derivatives let us suppose that their contribution is negligible.

An estimate of the ratio of tautomers **A** and **B** requires discussion of the values of the coupling constants. Many compounds with intramolecular hydrogen-bonded phenolic hydroxy groups show coupling constants between OH protons and aromatic carbons in the following ranges:[25] ^2J = 4.5–5.0; $^3J_{cis}$ = 4.5–5.7; $^3J_{trans}$ = 6.5–7.3 Hz. The values of ^3J(C3a, OH) = 5–6 Hz (Table 2) are comparable with the literature data for $^3J_{cis}$. In the case of ^2J and $^3J_{trans}$ we must consider for our compounds the sum of two coupling constants, involving the carbon atoms of the tautomers present in solution, *i.e.*: ^2J(C3, OH) + ^2J(C4, OH) = 5.0–6.2; ^3J(C2, OH) + ^3J(C5, OH) = 5.5–7.4 Hz. These values are also in line with the data of the literature.

In order to examine the values of the single coupling constants, it was necessary to evaluate the effect of the 2-OMe group on J(C3, OH) and J(C2, OH). Therefore we measured the ^{13}C NMR spectrum of 3-methoxysalicylaldehyde. The value of 4.7 Hz obtained for ^2J(C2, OH) and for ^3J(C1, OH) is very similar to the corresponding ones in salicylaldehyde, 4.77 Hz and 4.60 Hz respectively.[25b] This proves that the substituent effect of the 2-OMe group on ^2J(C3,OH) and ^3J(C3a, OH) of the perylenequinones can be neglected. As the C-3 signal of 3-methoxysalicylaldehyde could not be analysed, due to couplings with the methyl and ring protons (the same holds for other possible models), we have at present no direct experimental data to quantify the effect on ^3J(C2, OH), although it can be expected to decrease the coupling by *ca.* 1 Hz. Therefore the relative populations of the tautomers **A** and **B** reported in Table 4 were calculated by using only the two-bond coupling.

It appears from Table 4 that the predominant tautomeric form(s) are different for each compound. Cercosporin **1** and isocercosporin **2** exist only as tautomer **A**, although a small amount (less than 10%) of tautomer **B** cannot be excluded. Tautomer **A** is still the most abundant (65–70%) in phleichrome **3** and cladochrome C **10**. The same holds for isophleichrome **4** in acetone; in $CDCl_3$ the tautomeric equilibrium is strongly dependent on the concentration (see later). Elsinochromes **5**–**9** and hypocrellin **11** appear as *ca.* 50% mixtures of **A** and **B** in chloroform solution. For elsinochromes **5** and **7** in acetone there is a slight increase of tautomer **A** (*ca.* 60%).

An interpretation of these results is not easy, as it implies the analysis of all the factors which may affect the tautomeric equilibrium in these rather complex molecules. These factors include the electronic effects of the substituents, the strength of the intramolecular phenol–quinone hydrogen bonds, the distortion from the planarity of the perylenequinone system, solvation and aggregation effects. In all compounds the substituents on the ring differ only in being open chains or rings, so that their electronic effects can be estimated to be very similar.

Table 4 Relative population (%) of tautomers **A** and **B** for compounds **1–11**[a]

Solvent	Tautomer	1	2	3		4		5	6	7	8	9	10	11
$CDCl_3$	**A**	95	95	66	54[b]	39[c]	29[d]	50	54	56	56	54	57	53
	B	5	5	34	46	61	71	50	46	44	44	46	43	47
$[^2H_6]$Acetone	**A**	95	95	68		68		64	—	59				
	B	5	5	32		32		36	—	41				

[a] Obtained from J(C3, OH) and J(C4, OH). Estimated error within ±6%. [b,c,d] Value for different concentrations, *i.e.* 1.5×10^{-2} mol dm^{-3} (*b*), 1.2×10^{-1} mol dm^{-3} (*c*) and 2.8×10^{-1} mol dm^{-3} (saturated solution) (*d*).

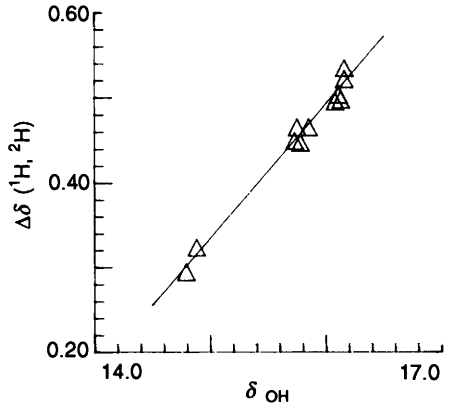


Fig. 1 Plot of the primary deuterium isotope effect on the chemical shift of phenolic groups $\Delta\delta(^1H,^2H)$ *vs.* ^1H chemical shift of OH protons for **1–11**; correlation coefficient $r = 0.992$

Table 5 Some distances (Å) and angles (°) obtained from X-ray data[a] for compounds **1**, **11** and **5**

	d_1[b]	d_2[b]	d_3[c]	φ[d]
1	0.086	0.109	0.251	18.8
11	0.061	0.054	0.315	24.9
5	0.046	0.043	0.181	14.7

[a] Refs. 11(d), 21, 23. [b] d_1 and d_2 are the average distances from the naphthalene least square planes of the ten carbon atoms of the 'upper' (C-1 → C-12b) and 'lower' (C-6b → C-9b) naphthalene moieties respectively. [c] d_3 is the average distance of the 20 perylenequinone carbon atoms from the perylenequinone least-square plane. [d] φ is the dihedral angle between the two averaged planes of the naphthalene moieties.

An important factor which could be related to the tautomeric equilibrium is the strength of the intramolecular hydrogen bonds. Information on the strength of a hydrogen bond can be obtained from the ^1H chemical shift values for the phenolic OH groups and from the deuterium or tritium isotope effects on chemical shifts.[26] In particular in the case of tautomeric systems deuterium isotope effects on ^{13}C nuclear shielding have been studied.[26b]

We performed deuteration experiments at the hydroxy groups, and observed extensive effects on the hydrogen and carbon atoms of the whole perylenequinone system. Either the phenolic or carbonyl carbon atoms (C-3, 4) can display strong two-bond effects ($^2\Delta$) as a consequence of the tautomeric equilibrium, which 'averages' the single- and double-bond character of the carbon–oxygen bonds of these atoms. In addition these carbons show significant long-range effects over nine bonds, due to deuteration of the hydroxy at C-9, 10. For compounds **1** and **2**, which exist predominantly as tautomer **A**, the double splitting observed for both C-3 and C-4 corresponds to $^2\Delta$ and $^9\Delta$ effects at C-4, and to $^4\Delta$ and $^9\Delta$ effects at C-3. The $^2\Delta$ shift is strong and positive, *i.e.* +0.680 ppm for **1** and +0.731 ppm for **2**; the $^4\Delta$ shift is negative, *i.e.* −0.139 ppm for **1** and −0.189 for **2**. The $^9\Delta$ shifts are +0.070 and +0.090 ppm at C-4, −0.037 and −0.048 ppm at C-3 for **1** and **2** respectively.*

When the population of tautomer **B** becomes relevant, the splittings observed at these nuclei result from the combination of two effects, *i.e.* $^2\Delta + {}^4\Delta$ and $^9\Delta + {}^9\Delta$ respectively. Therefore the comparison of the values in the perylenequinone series is difficult, and the usefulness of the $^2\Delta$ effect in the evaluation of the hydrogen bond strength in this case is poor.

On the contrary the primary deuterium isotope effect $\Delta\delta(^1H,^2H)$ measured on the phenolic groups does not suffer from such long-range effects. A correlation between the primary isotope effect and the value of the proton chemical shift of the hydrogen-bonded nucleus has been observed and discussed.[26a,b] We measured such a primary deuterium isotope shift in all compounds **1–11**. The positive sign of $\Delta\delta(^1H,^2H)$ observed is an indication of a double-minimum in the hydrogen bond potential.[26a] The correlation of such effects with the proton shifts of the hydrogen-bonded phenolic groups appears very interesting (Fig. 1).

Cercosporin **1** and isocercosporin **2** show upper field values in the proton chemical shift (δ 14.82 and 14.90) with respect to the other perylenequinones (δ 15.7–16.2), indicating weaker hydrogen bonds. This is paralleled by smaller isotope effects (0.3 *vs.* 0.4–0.5 ppm) and might be related to differences in molecular shape. The weaker hydrogen bond in **1** and **2** could be a consequence of the significant distortion of the perylenequinone ring. This distortion is indicated by the value of the average distance from the least square plane of the perimetral 20 atoms (d_3 in Table 5), obtained from the solid phase structure. The deviation is larger for hypocrellin and cercosporin than for elsinochrome A. However, this parameter is perhaps not the best descriptor of the ring distortion in this case, because the three molecules have quite different shapes. Cercosporin **1** is almost a true helical compound, whereas **5** and **11** assume an **X** shape, made of the two planes of the naphthalene moieties; the angle φ between the planes is shown in Table 5. Therefore a better parameter could be found in the average distance from the least-square planes of the ten C atoms of each naphthalene moiety in the ring (d_1 and d_2 in Table 5). The geometry nearer to planarity of the two naphthalene moieties in **5** and **11** should favour the formation of strong hydrogen bonds. Indeed, among the three compounds examined, **5** exhibits the strongest hydrogen bond in solution, and values of d_1 and d_2 in the solid phase correlate with the strength of the hydrogen bond. An inspection of Fig. 1 shows that the elsinochromes **6–9** are similar to **5** and display the strongest isotope effects, whereas phleichromes **3**, **4** and cladochrome C **10** (which is actually a diester of *ent*-**4**) show intermediate values. The correlation of primary isotope effects

* The sign of the four-bond effect ($^4\Delta$) is opposite to that found for the same fragment in hydroxyanthraquinones.[26c] A detailed discussion of these isotope effect experiments on hydroxyanthraquinones and hydroxyperylenequinones will be reported elsewhere.

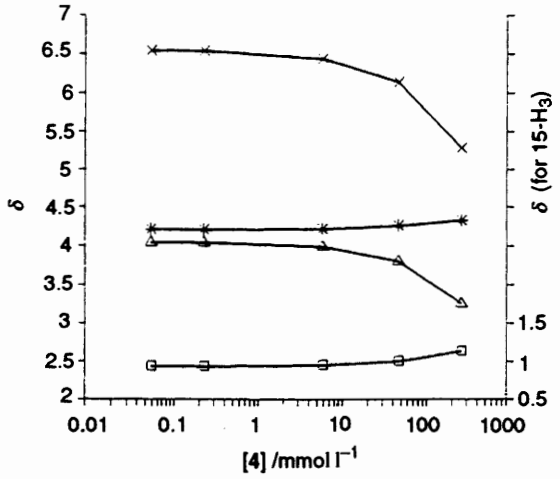


Fig. 2 ^{1}H chemical shift variation for isophleichrome **4** as a function of concentration in $CDCl_3$

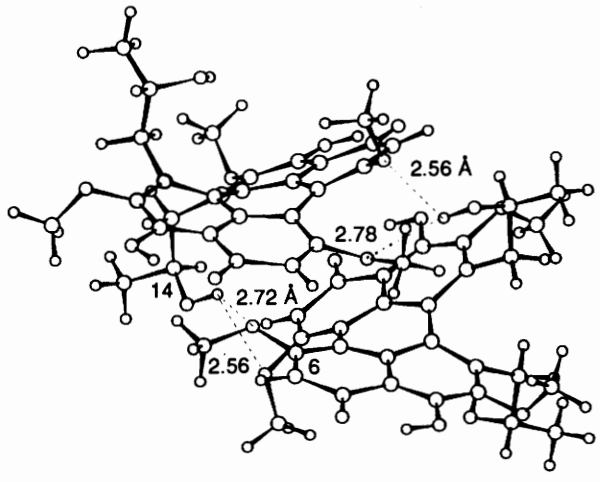


Fig. 3 Model of a dimer of **4**. The indicated interatomic distances are those between the alcoholic OH protons at C-14 and the oxygen atoms of the methoxy groups at C-6 and C-7.

and proton shifts for compounds **1**–**11** is linear, with a correlation coefficient r of 0.992.

The long-range deuterium effects on ^{13}C shift, for instance $^9\Delta$, should give information on the planarity of the perylenequinone system. Actually the $^9\Delta$ effect is smaller for **1** and **2**, with respect to the other compounds, but the interpretation of the results is difficult because it is complicated by the tautomeric process, which implies changes in the distribution of the π bonds. As the long-range deuterium effects in aromatic systems are interpreted[26d] on the basis of direct and extended π-polarisation, the presence of two tautomers in fast exchange makes even more difficult the identification of the pathway and therefore the interpretation of the experimental long-range effects.

However, from the above results, the planarity of the two naphthalene moieties, and consequently also the strength of the hydrogen bonds, can be considered an important factor governing the tautomeric equilibrium in our compounds. Cercosporin **1** and isocercosporin **2** show the weakest hydrogen bonds and exist almost exclusively as a single tautomer (**A**); the population of the second tautomer (**B**) increases with the strength of the hydrogen bonds from phleichromes **3**, **4** and cladochrome C **10** to hypocrellin **11** and elsinochromes **5**–**9**.

The influence of solvents and of concentration on tautomeric equilibria is well-known. In particular, electron-donating solvents such as acetone are expected to interfere with the intramolecular hydrogen bonding. Actually the ^{1}H shift of the hydrogen bonded OH groups of all compounds changes slightly from $CDCl_3$ to acetone (see Table 1), the largest lowfield shift (δ 0.2–0.3) being observed for phleichromes and elsinochromes. However a remarkable change was found in the case of isophleichrome **4** for 5-H and 6-OMe, which are strongly deshielded in acetone (δ 1.45 and 0.92), whereas all the other protons are shielded by *ca.* 0.15. Very similar variations were observed, again only for **4**, on dilution in $CDCl_3$ solution: 5-H and 6-OMe resonances shift progressively to low field, up to reach δ values of 1.26 and 0.78 for the most dilute solution, *i.e.* 5.8×10^{-5} mol dm^{-3} (Fig. 2). These results indicate that isophleichrome **4** is involved in an association process in $CDCl_3$ solution. The association constant, calculated for a dimerisation process, is low (180 dm^3 mol^{-1}). It is well known also that non-ionic solvents, such as chloroform or CCl_4, may allow the formation of aggregates, whereas acetone and dimethylsulfoxide are disaggregating agents.

We have envisaged a model of vertical stacking-type aggregation of **4** (Fig. 3). The energy of interaction between two molecules of **4**, oriented as in Fig. 3, is attractive. The van der Waals and the electrostatic components, calculated by molecular mechanics, are −64.9 kJ mol^{-1} and −10.1 kJ mol^{-1}, respectively. In this model the aromatic rings of the two molecules are partially superimposed, so that 5-H and 6-OMe experience a strong shielding by the ring-current effect, thus explaining the lowfield shift upon dilution. Conversely, the protons of 2-OMe and of the side-chains on the opposite part of the molecule are outside the shielding area of the aromatic system. Therefore they are expected to be unaffected or slightly deshielded by the magnetic anisotropic effect. For these protons we found a shielding of *ca.* 0.15–0.20 ppm on dilution in $CDCl_3$, or with the change from this solvent to acetone, in agreement with the predictions based on the model. Similar results were obtained on heating the $CDCl_3$ solution from 30 to 70 °C. In the model of Fig. 3 we have used the side-chains' conformation of **4** previously suggested,[1] which allows an interaction of the alcoholic OH protons at C-14, 17 with the 6,7-OMe groups of the adjacent unit. However, the chemical shift value of the alcoholic OH proton is not significant in order to recognize a possible hydrogen bond, as it exchanges with water, which is always present in traces in $CDCl_3$. For the same reason, the NOE interactions found in the ROESY experiment between the phenolic OH protons and the hydrogen nuclei of the side chains (13-H and 14-H) are explained by transferred NOE to the phenolic OH through the alcoholic OH and water, by chemical exchange. This also explains the small interaction observed between 5-H and 14-H, as 5-H shows a strong NOE with the phenolic OH proton. Intermolecular NOE interactions, for instance those involving 6-OMe protons, were not detected, but the association constant is too low to allow the detection of intermolecular NOE.

The different conformation of the side chains in the stereoisomer **3** with respect to **4**, which appears to be related[1] to the opposite chirality of the ring helix, might explain why the association does not occur in phleichrome **3**. Dilution experiments performed on the other compounds showed a deshielding of 5-H (δ 0.39) only for isocercosporin **2**. The same proton showed the same deshielding (δ 0.35) in acetone *vs.* $CDCl_3$, but such effects are too low to be a proof of a significant association, even if the conformation of the side chains[1] is the same for **2** and **4**. The presence of the methylenedioxy group instead of the two methoxys at C-6, 7 is probably determinant for the lack of aggregates.

The effect of association on the tautomeric equilibrium appears in the case of isophleichrome **4**: the population of tautomer **B** increases with aggregation. As it is shown in Table 4, with the increase of concentration in $CDCl_3$, the population of

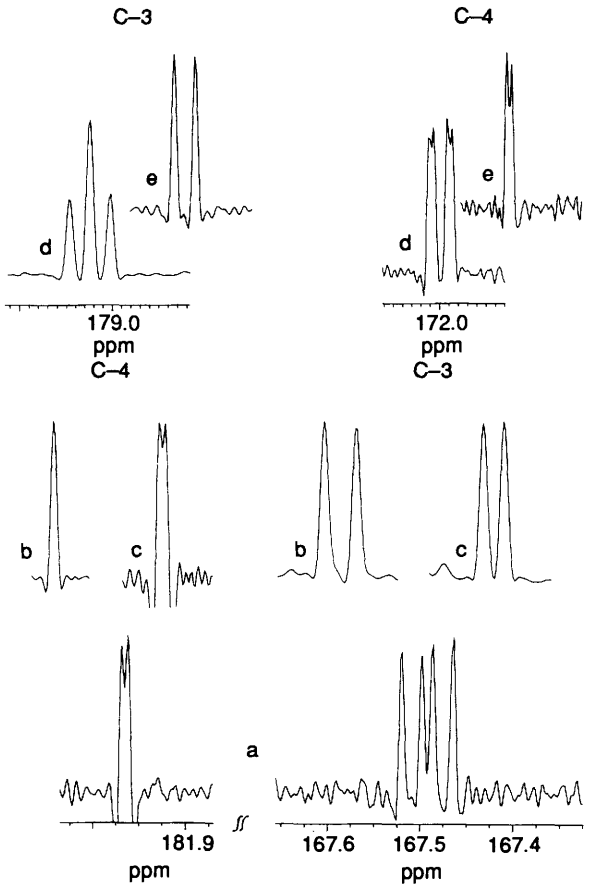


Fig. 4 Signals of C-3 and C-4 in the ^{13}C NMR spectra in $CDCl_3$ solution of (*a*, *b*, *c*) cercosporin **1** and (*d*, *e*) elsinochrome A **5**. (*a*, *d*) without ^1H decoupling; (*b*) with decoupling of 5-H; (*c*, *e*) by adding D_2O. C-3 and C-4 signals show the interactions with the phenolic OH proton and with 5-H.

tautomer **B** increases progressively from 46 to 71%. In acetone, where aggregates cannot be formed, the population of tautomer **B** is similar (35%) to that of phleichrome **3**; in fact the monomeric species appears at 10^{-4} mol dm^{-3} concentration, as it is shown by the invariance of the proton chemical shift values. Consequently some association must still occur in 10^{-2} mol dm^{-3} $CDCl_3$ solution, where tautomer **B** amounts to 46%.

We do not have at present an explanation either for this influence of the aggregation on the tautomeric equilibrium, or for the prevalence of tautomer **B** in hypocrellin in the solid state,[16] whereas in solution both tautomers are equally populated.

Therefore we have to conclude that the important factors governing the tautomeric equilibrium in these perylenequinones are not only the planarity of the naphthalene moieties and consequently the strength of the hydrogen bonds, but also solvent and concentration effects, which are connected with the association phenomena, or the packing energy in the solid state, able to overcome the small energy difference between the extreme forms.

Experimental

The compounds used for measurements of the NMR spectra were spectroscopically and analytically pure, as reported in ref. 11 for **1** and **2**, ref. 13 for **3** and **4**, ref. 15 for **5**–**9**, and ref. 5 for **10**. Hypocrellin **11** [16] was a generous gift of Professor E. Breitmaier. Naphthazarin **12** was a commercial sample (Aldrich), whereas **13** was prepared according to ref. 19.

NMR spectra were measured in $CDCl_3$ and in [$^2H_6$]acetone with Bruker AMX-600 and CPX-300 spectrometers. The concentrations for ^1H, ^{13}C and ^2H spectra were *ca.* 10^{-1} mol dm^{-3}, unless specified otherwise (see Tables 1 and 3). For dilution experiments, the concentration was progressively decreased down to 3.0×10^{-5} mol dm^{-3}. Chemical shifts are in δ values from internal Me_4Si and are accurate within ± 0.01 ppm. The assignments of carbon signals were performed by heteronuclear selective decoupling. Examples are given in Fig. 4. The methoxy groups were assigned from the NOE interaction between 2-OMe and 13-H. The C, H coupling constants were measured from undecoupled one-dimensional spectra, except for those involving C-2, and are given as absolute values.

2D-ROESY spectra were measured at 600.13 MHz with a continuous spin-lock (duration 0.4 s, 16 dB attenuation, corresponding to a field strength of 6500 Hz, pulse program ROESYTP) and the carrier set at 6.6 ppm. 512 × 1024 FIDS were collected (9100 Hz spectral width, recycling time 1.5 s, 64 scans) in TPPI (time proportional phase increments) mode and transformed phase-sensitive, with zero-filling in F1, after apodization with a 90° shifted sine-bell squared function. After the Fourier transform, the baseline of the 2D spectrum was corrected in both dimensions with a fifth degree polynomial.

Molecular modelling was performed on a Silicon Graphics 4D35-GT, equipped with 16 Mbyte memory, 1 Gbyte hard disk, running the package InsightII/Discover (BIOSYM Technologies, San Diego, California, version 2.1.0). The atomic potentials were taken from the software library, using the CVFF force field and the atomic charges were obtained from semiempirical calculations. Energy minimization was performed using a conjugate gradient algorithm, until the maximum energy derivative was less than 0.419 kJ $Å^{-2}$. Semiempirical calculations were performed with the AM1 method, using the AMPAC program (v. 5.0) included in the BIOSYM package. The minimized structure of **4** was obtained starting from an initial geometry with standard bond lengths and angles, with the side chains in the conformation previously suggested.[1,16] The dimer model of Fig. 3 resulted from energy minimization of the complex formed by two molecules of **4**.

The relative populations of tautomers **A** and **B** were calculated from the values of *J*(C-3, OH) and *J*(C-4, OH), using for each compound the sum of these two couplings as the value for a pure tautomer. If we consider the average over all compounds, we can estimate that the error is within ± 6%.

The primary deuterium isotope effect $\Delta\delta(^1H,^2H)$ was measured [26a] 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. 26(*a*). The deuterium isotope effect on ^{13}C chemical shifts was measured according to ref. 26(*c*). The solutions for the measurement of the isotope effects were prepared by adding 30 mm^3 of a 3:7 H_2O–D_2O mixture to 0.4 cm^3 of a 10^{-1} mol dm^{-3} solution in $CDCl_3$.

The association constant for **4** in $CDCl_3$ was calculated assuming a dimerization equilibrium and using the chemical shift values obtained from the dilution experiments reported in Fig. 2.

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