Conformation and tautomerism of hypocrellins. Revised structure of shiraiachrome A

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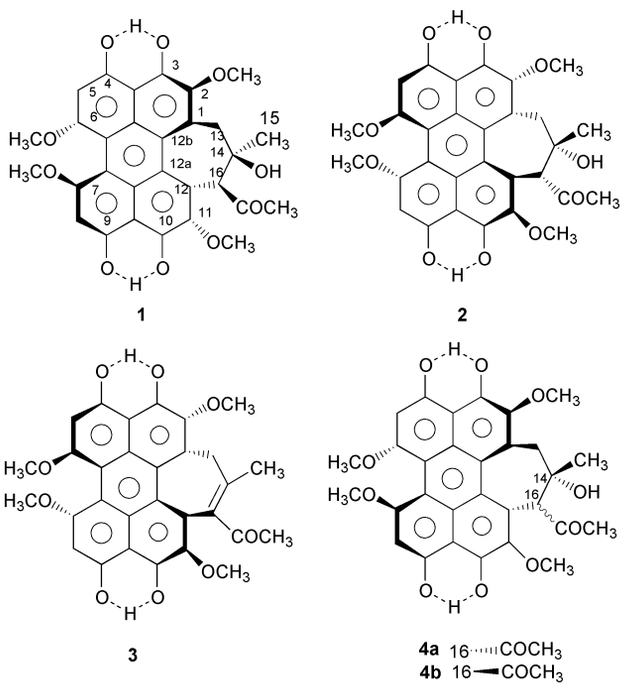
Hypocrellins, natural perylenequinones from the fungi *Hypocrella bambusae* and *Shiraia bambusicola*, are being intensively studied for their photodynamic activity. As a recent paper reported the existence of a slow tautomeric equilibrium for hypocrellin A, the behaviour in solution of hypocrellin and hypocrellin A was reinvestigated by NMR. 1D and 2D ROESY spectra at different temperatures and in different solvents have been measured and quantitative NOE experiments have been performed to obtain the cross-relaxation rates and then the interproton distances, to be used for MM and MD calculations. This allowed us to confirm that tautomerism in these compounds is a fast process on the NMR time scale, and to establish the relative population of the two principal tautomers and the preferred conformation of hypocrellin, hypocrellin A and of their atropisomers. The atropisomeric interconversion process is fast enough to be studied by NMR; the rate constants, obtained by ROESY-exchange experiments, gave the activation parameters. The helix inversion also induces the inversion of the seven-membered ring, which adopts a twist–boat conformation in both atropisomers. The values of the dihedral angle C(1)–C(12b)–C(12a)–C(12), which shows the distortion of the perylenequinone system, have been obtained from energy minimisation of the structures derived from NOE data. They are in the range 25–29° for all stereoisomers. The structure of shiraiachrome A, another member of the series, has been revised to *M*(*R*),14*S*,16*S*. The conformation and the tautomeric equilibrium of this compound have been similarly determined.

Introduction

Perylenequinones are a group of natural substances mostly of fungal origin, which exhibit intriguing stereochemical features and interesting biological activity, especially due to their photodynamic activity.[1] Among them, the hypocrellins, compounds extracted from the Chinese fungi *Hypocrella bambusae* and *Shiraia bambusicola*, have been intensively investigated for their light-induced antitumor and antiviral activity.[2] In particular, the energetic and dynamic properties of their electronic excited states have been the subject of numerous studies.[3]

The first compound to be isolated and characterized was hypocrellin **1** from *H. bambusae*.[4] Then Wu *et al.*[5] and Kishi *et al.*[6] isolated compounds **2** and **3** from *S. bambusicola*, and a compound (called shiraiachrome A[5] or hypocrellin B[6]) to which the structure **4a** was attributed. †

Unfortunately, there has been much confusion in the naming of the same compounds extracted from different sources. Although Thomson has attempted in the last edition of his book on natural quinones[7] to review the literature and to systematise the nomenclature, his suggestions have not been followed by others, in particular by the several authors who have studied the biological and photoelectronic properties of hypocrellins. The two most studied compounds are **2**, the enantiomer of **1**, which is called hypocrellin A, and the product of its dehydration **3**, which is called hypocrellin B, notwithstanding that other authors gave this name to another compound. Due to the large number of studies that use the names of hypocrellin A and B for **2** and **3** respectively, we would suggest keeping these names for these compounds, as we shall do throughout the paper, using the name shiraiachrome A for compound **4** according to Thomson and to some of the authors who isolated it, and abandoning the name hypocrellin C or shiraiachrome C for **3**.



As it appears from the formulae, hypocrellins share with many other natural perylenequinones an axial chirality due to the steric hindrance of the methoxy groups in the "left" moiety and of the seven-membered ring (or side chains in the other compounds) in the "right" moiety, which leads to atropisomerism. The existence of stereogenic centers in the

† For the sake of comparison, the numbering of hypocrellins is that adopted by Petrich *et al.*,[3a] which anyway is the same "biosynthetic" numbering used for all other natural perylenequinones.

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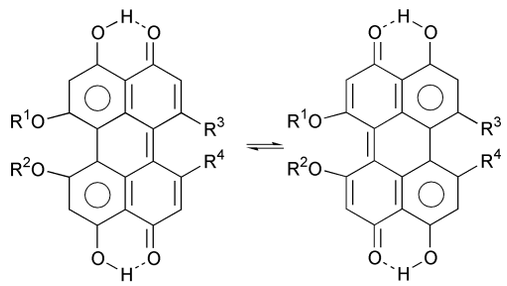
seven-membered ring, combined with the axial chirality, generates diastereoisomerism. In fact, other perylenequinones, such as cercosporin or phleichrome, undergo a thermal interconversion of the atropo/diastereoisomers at elevated temperatures, due to the high barrier to interconversion.[1,8–10] Furthermore, the 3,10-dihydroxy-4,9-perylenequinone system can exist in two main tautomeric forms, which we have called tautomer I and tautomer II, in fast equilibrium. A detailed study of the tautomerism of cercosporin and other natural perylenequinones, including hypocrellin **1** has been reported by us.[9,10]



I II

The helix interconversion was never observed for hypocrellins until recently, when a paper by Petrich and co-workers[3a] reported a study of the equilibrium between atropisomers and also tautomers of hypocrellin A **2**. As a part of our ongoing studies on stereochemistry and tautomerism of natural perylenequinones, we report here the results of our investigation on hypocrellins that correct some of the results of Petrich[3a] and require a revision of the structure of shiraiachrome A to **4b**.

Experimental

The materials that we have investigated are a sample of hypocrellin **1** kindly provided years ago by Professor Breitmaier, and a 2 mg sample of hypocrellin A **2**, purchased from Molecular Probes Inc., which is also the source of the material examined by Petrich *et al.*[3a]

HPLC analysis was performed using a Hewlett Packard HPLC equipped with Rheodyne injector (0.02 cm^3 loop), diode array and an RP-18 reverse phase column with isocratic conditions; solvent MeOH–H_2O (85 : 15); flow rate 1 cm^3 min^{-1}; temperature 24 °C; detector set at $\lambda = 450$, 550 and 580 nm.

^1H NMR spectra were measured in $CDCl_3$ and in [$^2H_6$]-acetone with a Bruker AMX-600 spectrometer. The concentrations for **1** were *ca.* 1.8×10^{-3} mol dm^{-3}; for **2** *ca.* 3×10^{-3} mol dm^{-3}. Chemical shifts are given in δ values from internal Me_4Si and are accurate within ±0.01 ppm. The coupling constants values are in Hz, with an estimated accuracy of ±0.1 Hz. 2D NOESY and ROESY spectra were acquired in the phase sensitive TPPI mode, with 2K × 256–512 complex FIDs, spectral width of 12100 Hz, recycling delay 2 s, scans 48, at temperatures −15 °C, −5 °C, +5 °C, +15 °C and +25 °C, with mixing times from 20 ms to 300 ms. A different set of ROESY experiments was performed in order to obtain high resolution spectra of the downfield hydroxy protons (16.0–16.6 ppm), with spectral width of 1500 Hz and offset at 15.5 ppm, at temperatures of +5 °C and −15 °C. All spectra were transformed and weighted with a 90° shifted sine-bell squared function to 2K × 512 real data points The rate constants (k) of the conversion of **1** into **6** were calculated from the ROESY-exchange experiments by using eqns. (1) and (2):

$$I_{AA} = \tfrac{1}{2}[1 + \exp(-2kt_{mix})]\exp(-t_{mix}/T_1^{A})M_{AO} \quad (1)$$

$$I_{AB} = \tfrac{1}{2}[1 - \exp(-2kt_{mix})]\exp(-t_{mix}/T_1^{B})M_{BO} \quad (2)$$

where I_{AA} and I_{BB} are the intensities of diagonal and cross-peaks respectively, t_{mix} is the mixing time and M_{AO}, M_{BO} are the equilibrium magnetisations. T_1^{A} and T_1^{B} are the longitudinal relaxation times of protons at sites A and B respectively.

The inter-proton distances were obtained by use of the Felix software included in the Insight II & Discover programs (v. 2.3.5 Biosym Technologies, San Diego, USA). Molecular models were built using a Silicon Graphics 4D35GT workstation running the Insight II & Discover software. Molecular mechanics (MM) and molecular dynamics (MD) were carried out using CVFF as forcefield. The starting geometry of hypocrellins was generated using standard bond lengths and angles and the dihedral angle C(1)–C(12b)–C(12a)–C(12), named "twist angle", with a value of 30°. The models were then energy minimised without and with NOE constraints, using steepest and conjugated gradients until a maximum energy derivative of 4.18×10^{-3} kJ mol^{-1} $Å^{-1}$ was reached. The twist angle decreases to values of 25–27°, which are very similar to the dihedral angle between the two averaged planes of the naphthalene moieties obtained[9] from X-ray data[11] of hypocrellin, *i.e.* 24.9°. The subsequent molecular dynamics calculation, performed for 5 ps at 1000 K temperature and sampling the trajectory every 1 ps, followed by minimisation with and without NOE constraints, led to similar results, with twist angles ranging from 26° to 29°.

Results and discussion

The NMR spectra of the sample of hypocrellin A (Fig. 1 and 2a) showed the presence of four compounds, whereas the HPLC analysis showed only three components. The chemical shift values, reported in Table 1, indicate that all the four compounds have a hypocrellin type structure. The most abundant one, **2** (60%), is hypocrellin A and the least abundant, **3** (8%), was easily recognised as the known[5,6] anhydro derivative or hypocrellin B. Of the other two compounds, one (**5**) is present at 18% and is in dynamic exchange with hypocrellin A. On the contrary the fourth compound, **4** (14%), is not in dynamic exchange with either **2** or **5**. The compound **5** was thus identified as the atropisomer of hypocrellin A (**2**).

Hypocrellin A (**2**) is the enantiomer of the original hypocrellin (**1**), isolated from *Hypocrella bambusae*,[4] which exhibits axial chirality *M*(*R*) and configuration 14*R*,16*S*. The structure of hypocrellin **1** follows from X-ray crystallographic analysis,[4,11] whereas the absolute configuration of hypocrellin A was deduced on the basis of CD spectra.[5,6] The spectrum of hypocrellin **1** is reported in Fig. 2b. The sample is very pure; the absence of compounds **3** and **4** can be noted. A direct comparison of hypocrellin **1** and hypocrellin A **2** was deemed useful by Thomson.[7] We thus performed NMR experiments on both compounds **1** and **2** and the NMR data are identical. Since they show opposite CD spectra,[5] we can thus confirm for hypocrellin A (**2**) the configuration 14*S*,16*R* and the axial chirality *P*(*S*). The atropisomer **6** of hypocrellin **1** is consequently the enantiomer of **5**, as can be seen from the identical NMR spectra of **5** and **6**. Also **1** and **6** are in dynamic exchange.

Helix inversion process

The inversion process for these compounds is slow enough to be studied by NOESY-exchange NMR. The interconversion rate constants (k) of **1** into the atropisomer **6** were directly determined by quantification of the signal intensities of diagonal and cross-peaks of the ROESY spectra, performed at different mixing times and temperatures. The k values are derived by fitting the experimental data into the appropriate equations (see Experimental). The rate constant values and the resulting activation parameters are reported in Table 2. These data are in line with those reported by Petrich *et al.*[3a] for two species only (named A and B in their paper) and obtained by line-shape analysis. The barrier of the helix inversion process is lower with respect to other perylenequinones such as cercosporin and

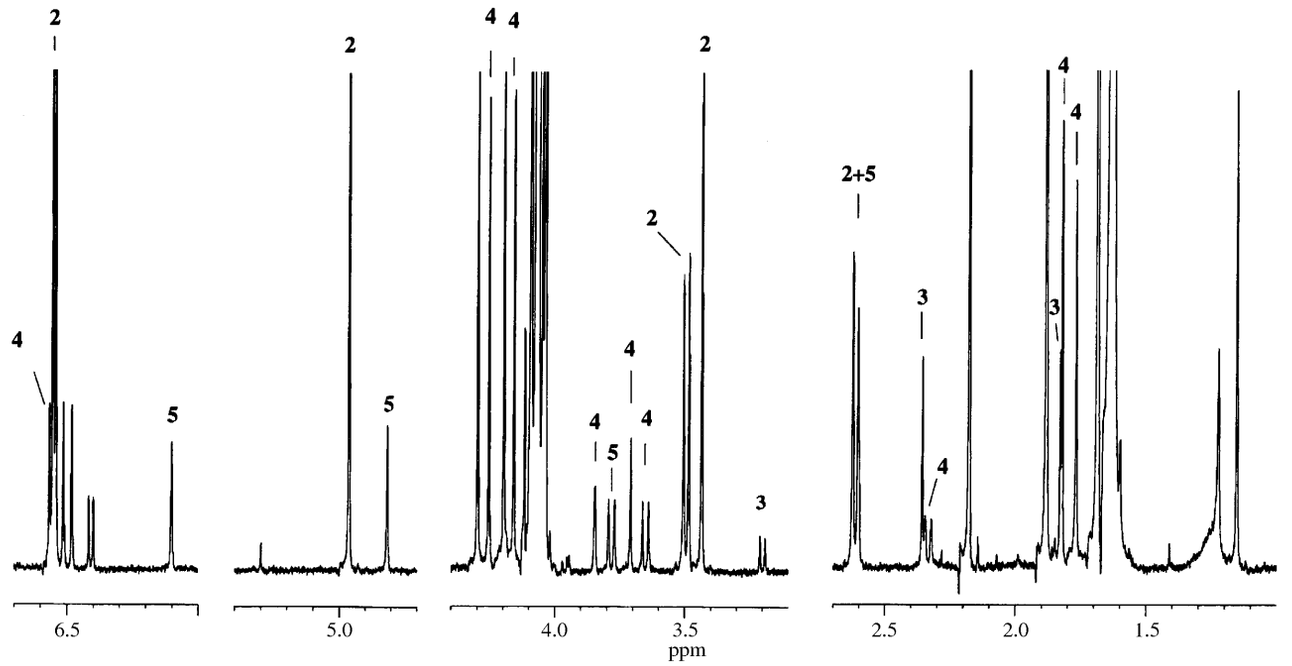


Fig. 1 ^{1}H NMR spectrum in $CDCl_3$ at 0 °C of the mixture of **2**, **3**, **4** and **5** (sample from Molecular Probes). Only selected signals are marked.

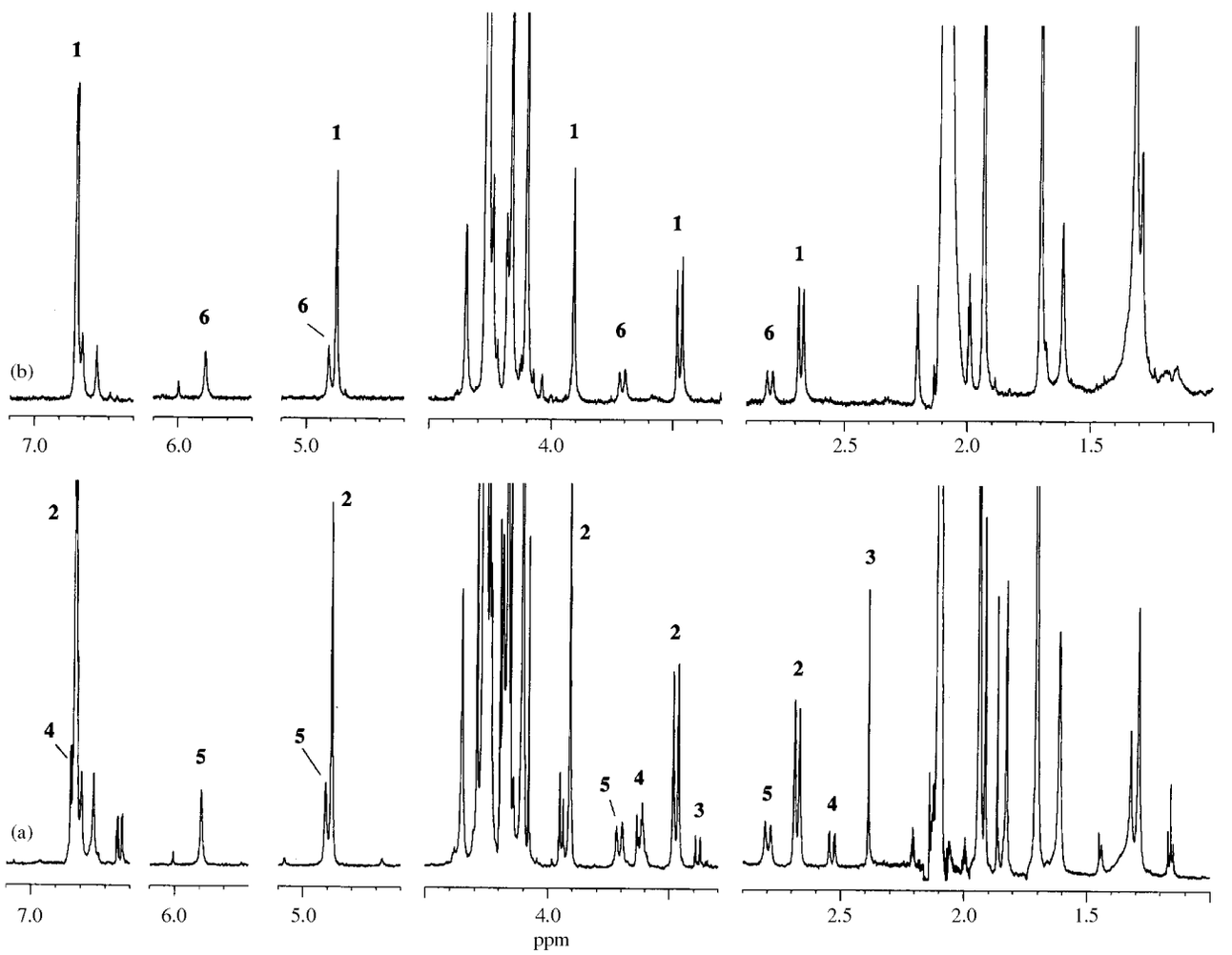


Fig. 2 ^{1}H NMR spectra in $[^2H]_6$acetone at +5 °C of (a) the Molecular Probes sample and (b) hypocrellin **1** and the atropisomer **6**.

phleichrome,[8a,b] because the presence of the seven-membered ring instead of the two chains reduces the steric hindrance and the conformational mobility. Although $\Delta G^{\ddagger}$ and $\Delta H^{\ddagger}$ are high enough, the entropy change, $\Delta S^{\ddagger}$, is positive, thus favouring the inversion process.

Conformation of hypocrellins (1, 2) and of their atropisomers 5 and 6

The atropisomers of **2** and **1**, *i.e.* **5** (*M*,14*S*,16*R*) and **6** (*P*,14*R*,16*S*) are less stable than **2** and **1**, as follows from their relative populations at the equilibrium. The conformational

Table 1 Chemical shift values (δ) for compounds **1**–**6**[a]

	1, **2**			**3**			**4**			**5**, **6**		
	$CDCl_3$, 25 °C	[$^2H_6$]Acetone, 25 °C	[$^2H_6$]Acetone, 5 °C	$CDCl_3$, 25 °C	[$^2H_6$]Acetone, 25 °C	[$^2H_6$]Acetone, 5 °C	$CDCl_3$, 25 °C	[$^2H_6$]Acetone, 25 °C	[$^2H_6$]Acetone, 5 °C	$CDCl_3$, 25 °C	[$^2H_6$]Acetone, 25 °C	[$^2H_6$]Acetone, 5 °C
5-H	(6.55)	6.81	6.82	(6.42)	(6.64)	(6.64)	(6.55)	6.7[b]		(6.48)	(6.73)	(6.74)
8-H	(6.56)	6.81	6.82	(6.40)	(6.62)	(6.62)	(6.56)	6.7[b]		(6.52)	(6.81)	(6.79)
13-Ha	3.50	3.49	3.46	4.02	4.03	4.09	3.66	3.60	3.61	3.78	3.64	3.70
13-Hb	2.61	2.68	2.67	3.21	3.38	3.38	2.33	2.52	2.53	2.61	2.81	2.79
14-OH	4.88	4.85	4.88	—	—	—	3.78	4.20	4.24	6.00	5.83	5.89
15-H_3	1.69	1.71	1.70	1.82	1.86	1.86	1.77	1.83	1.82	1.62	1.62	1.60
16-H	3.43	3.88	3.91	—	—	—	3.71	[c]	3.94	4.81	4.91	4.90
16-COMe	1.87	1.94	1.93	2.36	2.40	2.38	1.82	1.86	1.91	1.16	1.29	1.28
2-OMe	4.08[d]	4.18	4.16	(4.12)	[c]	[c]	4.17	(4.09)	[c]	4.21	4.36	4.35
11-OMe	4.10[d]	4.11	4.10	(4.04)	[c]	[c]	4.26	(4.20)	[c]	4.30	4.19	4.18
6-OMe	4.06	4.26	4.27[e]	(4.05)	[c]	[c]	(4.03)	[c]	[c]	[c]	4.26	4.26
7-OMe	4.06	4.26	4.26[e]	(4.05)	[c]	[c]	(4.04)	[c]	[c]	[c]	4.26	4.26
3,4-OH	15.94[f]	16.19	16.22	(16.01)	(16.38)	(16.41)	(15.96)	(16.19)	(16.21)	16.02	16.36	16.41
9,10-OH	15.89[f]	16.11	16.14	(15.99)	(16.41)	(16.41)	(16.06)	(16.21)	(16.25)	16.22	16.46	16.50

[a] Measured from internal Me_4Si. Accuracy within ±0.01 ppm. Concentration *ca.* 3×10^{-3} mol dm^{-3}. Similar values in parentheses may be interchanged; a and b stand for lowfield and upfield proton, respectively. [b] 6.7–6.8 ppm. [c] Not detected. [d] Values obtained at −5 °C, because the signals are overlapped at 25 °C. [e] Values obtained at −15 °C. [f] Assigned by the NOE interactions with 2-OMe and 11-OMe. respectively.

Table 2 Rate constants and activation parameters for the helix inversion process of **1** into **6**

T/°C	k/s^{-1}	$\Delta G^{\ddagger}$/ kJ mol^{-1}	$\Delta H^{\ddagger}$/ kJ mol^{-1}	$\Delta S^{\ddagger}$/ J mol^{-1} K^{-1}
22	15.3	65.5		
15	6.1	66.1	76	37
5	2.1	66.2		
−5	0.6	66.6		

Table 3 NOE interactions for hypocrellins[a]

1, **2**	**4**	**5**, **6**
13-Ha · · · 13-Hb	13-Ha · · · 13-Hb	13-Ha · · · 13-Hb
13-Ha · · · 2-OMe	13-Ha · · · 2-OMe	13-Ha · · · 15-H_3
13-Hb · · · 14-OH	COMe · · · 11-OMe	13-Hb · · · 15-H_3
16-H · · · 14-OH	COMe · · · 14-OH	13-Ha · · · 14-OH
16-H · · · COMe	16-H · · · 14-OH	14-OH · · · 2-OMe
COMe · · · 11-OMe	16-H · · · COMe	14-OH · · · 16-H
15-H_3 · · · 13-Ha	15-H_3 · · · 11-OMe	16-H · · · 15-H_3
15-H_3 · · · 16-H	15-H_3 · · · 13-Ha	16-H · · · COMe
15-H_3 · · · 14-OH	15-H_3 · · · 13-Hb	16-H · · · 11-OMe
5,8-H · · · 6,7-OMe	15-H_3 · · · 14-OH	15-H_3 · · · 11-OMe
5,8-H · · · 3,9-OH	5,8-H · · · 6,7-OMe	5,8-H · · · 6,7-OMe
3,4-OH · · · 2-OMe		
9,10-OH · · · 11-OMe		

[a] Obtained in $CDCl_3$ and in [$^2H_6$]acetone at +5 °C and 25 °C; a and b stand for lowfield and upfield proton respectively.

change of the seven-membered ring is tightly connected with the helix inversion process, as observed by Petrich *et al.*[3a] A *gauche* relationship of the methyl and acetyl groups was also suggested as the most probable for **2**, and an *anti* relationship was likewise suggested for **5**.

We performed the conformational analysis of **1** and **2** by using quantitative NOE experiments and MM/MD calculations. Firstly, we started with the stereospecific assignment of 13-Ha and 13-Hb on the basis of the NOE interactions with a group directly linked to the aromatic ring, *i.e.* the methoxy at C-2. The OMe signal at 4.18 ppm showed an NOE interaction with the lowfield H-13 proton, labelled 13-Ha, while the OMe signal at 4.11 ppm showed an interaction with the acetyl group (see Table 3). This allows the stereospecific assignment of the methylene protons at C-13 and at the same time the attribution of 2-OMe at 4.18 ppm and 11-OMe at 4.11 ppm. Therefore the

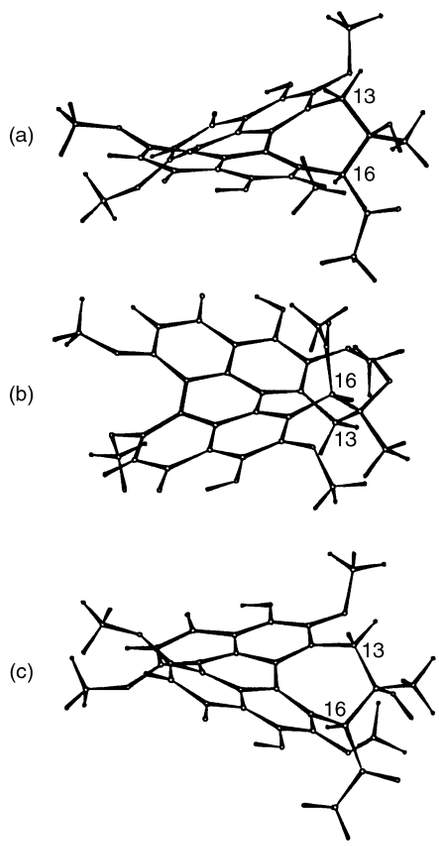
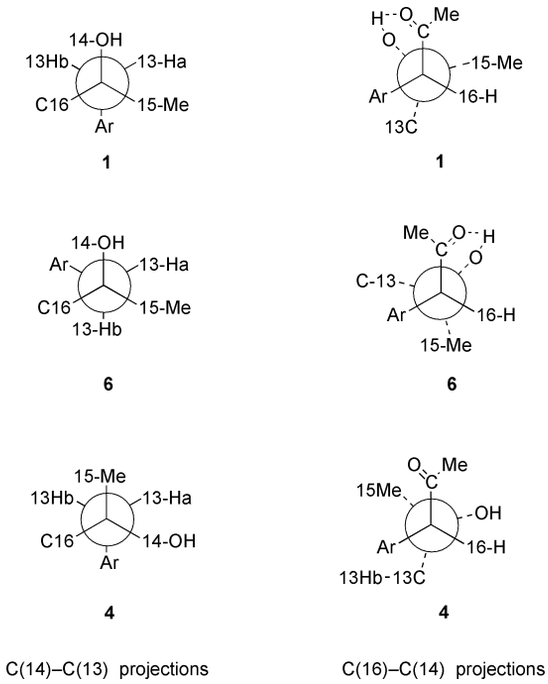


Fig. 3 Preferred conformation of (a) hypocrellin **1**, (b) the atropisomer **6** and (c) shiraiachrome A (**4**).



lowfield 13-Ha is oriented toward the methoxy group at C-2 and consequently is *pro-S* in **1**, whereas it is *pro-R* in the enantiomer **2**.

The methyl group 15-H_3 showed strong NOE interactions with 16-H and 13-Ha, but not with 13-Hb. This indicates that the methyl is antiperiplanar to 13-Hb and is close to 16-H. However a quantitative measurement of the NOE cross-peaks is required in order to obtain inter-proton distances for defining the conformation of the seven-membered ring. From the cross-relaxation rates, measured by experiments with different mixing times and referred to the distance between 13-Ha and 13-Hb, we obtained the distance values to be used for the calculations.

The most stable conformation for the enantiomeric hypocrellins **1**, **2** obtained from energy minimisation has a twist angle of 27°, which is in line with the X-ray results.[4,11] From MD calculations we obtained the same twist angle. The seven-membered ring is in a twist–boat [12] conformation (Fig. 3a), which is not so flexible because of the lock to the aromatic ring. The methyl and acetyl groups are *trans* and pseudo equatorial and are both projected outward of the aromatic system. The conformation around C(16)–C(14) bond is not a regular staggered form, 16-H being very close to the methyl 15-H_3, as shown in the Newman projection. This might also be induced by the possible formation of an intramolecular hydrogen bond between the hydroxy proton at C-14 and the carbonyl of the acetyl group. The shift of the alcoholic proton in $CDCl_3$ supports this hypothesis. A distance of 1.9 Å between 14-OH proton and the carbonyl was obtained from the calculations.

The conformation of the atropisomers **5** and **6** of **2** and **1** respectively was obtained as follows. The stereospecific assignment of the geminal protons at C-13 was made by use of the NOE-exchange cross-peaks connecting the two atropisomers, as no interaction with the 2-OMe protons was detected because of the low concentration of **5** and **6**. With the same procedure, the resonances of 2-OMe and 11-OMe were identified and assigned. The most significant NOE interactions, among those reported in Table 3, connect 15-H_3 with 13-Ha (strong) and 13-Hb (weak), then 16-H with 11-OMe and with 14-OH. The latter ones, not observed in the main atropisomer, indicate that 16-H is oriented toward the methoxy group at C-11 and is close to the 14-OH proton. The zero NOE observed between the methyl and the acetyl groups suggest that they are in a *trans* axial–axial relationship. The NOE data between 15-H_3 and the protons at C-13 support a "non classical" *gauche* orientation of the methyl with respect to both 13-Ha and 13-Hb, *i.e.* with dihedral angles different from 60°. A significant variation of the chemical shifts for **5** and **6**, with respect to **2** and **1**, was observed for the acetyl group, for 16-H and 14-OH protons, whereas the other resonances do not change. The 0.7 ppm upfield shift of COMe indicates that the methyl of the acetyl group is oriented toward the aromatic plane, and thus experiences the effect of the magnetic anisotropy of the electron system. This orientation might be forced by the hydrogen bond between the 14-OH and the carbonyl group, as suggested by the 1 ppm lowfield shift of the OH proton, with respect to **1** and **2**. The strong 1.0–1.4 ppm lowfield shift of 16-H is evidence of a significant ring current effect on 16-H, due to the parallel orientation of the C(16)–H bond with respect to the aromatic system, thus confirming the NOE results.

The distance values obtained from the cross-relaxation rates allowed us to build up a set of structures, which, after energy minimisation, led to a preferred twist–boat conformation for the seven-membered ring and a twist angle of 27°, as depicted in Fig. 3b. The distance between the 14-OH proton and the carbonyl oxygen (1.9 Å) is compatible with the formation of a hydrogen bond, which contributes to some stabilisation of the structure. This also explains why the 14-OH proton shows an NOE contact with 13-Ha, but not with 13-Hb. The hydroxy group and 13-Hb are *trans* in the twist–boat conformation, but are not coplanar, because the 14-OH proton is oriented toward the carbonyl group; this explains why we did not detect the four-bond coupling with 13-Hb, as found for **4**.

Structure and conformation of shiraiachrome A (4)

The stereoisomer **4** is not present in the sample of hypocrellin **1** that we have received from Professor Breitmaier. This is clear from both the NMR spectra and the HPLC analyses.

The structure of compound **4** was then determined by accurate NMR experiments performed on the sample of hypocrellin A, purchased from Molecular Probes Inc., which contains as an

impurity 14% of **4**. The most evident feature of the spectrum of **4** is the four-bond coupling of 2 Hz between 13-Hb and 14-OH, which proves the antiperiplanar orientation of 13-Hb and the OH proton. Significantly, the 0.8 ppm upfield shift of the OH resonance *vs.* **1** indicates a decrease of the hydrogen bond strength. The four-bond coupling is not present or is less than 0.5 Hz in the other compounds. These results suggest that the orientation of the hydroxy group at C-14 and consequently the configurational relationship of the two chiral centres C-14 and C-16 must be changed with respect to **1**. The strong NOE interactions found for 15-H_3 with both the protons at C-13 and the presence of an NOE contact of 15-H_3 with 11-OMe, but not with 2-OMe, confirm the above finding. The assignment of the two methoxy groups was made by the NOE interaction between 2-OMe and 13-Ha protons, as already described for **1** and **2**, which also led to the stereospecific assignment of the geminal protons at C-13. Furthermore the absence of NOE between the methyl 15-H_3 and 16-H indicates a *trans* axial–axial relationship between them; while the strong NOE connecting the acetyl group with 11-OMe confirms the orientation of the C-16 substituent. As this compound has exactly the same NMR spectrum as shiraiachrome A[5] and the sample of hypocrellin A derives from *Shiraia bambusicola*,[13] we can safely conclude that compound **4** is shiraiachrome A. Consequently the configuration of shiraiachrome A given by Wu[5] and quoted by Thomson[7] as *M*(*R*),14*S*,16*R* is not correct. On the other hand, Wu has measured the CD spectrum of shiraiachrome A in comparison with those of hypocrellins **1**, **2**, and found similar Cotton effects for shiraiachrome A and **1**, concluding that they must have the same axial chirality, *i.e.* *M*(*R*). Thus it follows that shiraiachrome A has the structure **4b**, *i.e.* *M*(*R*),14*S*,16*S*.

The distance values obtained by the NOE results led to a set of structures that after energy minimisation converge to the structure reported in Fig. 3c. The conformation of the seven-membered ring is a twist–boat and the twist angle is 27–28°, as for hypocrellins **1** and **2**. The conformation around the C(16)–C(14) bond is not a regular staggered form, as in **1**, 16-H being very close to 14-OH. From the distance value, there is no evidence of a hydrogen bond between 14-OH and the carbonyl group, in agreement with the chemical shift value of the OH proton. Finally, the atropisomer of **4b** is present in very low amounts and could be identified only in the lowfield region of the chelated OH protons (see later).

Tautomerism in hypocrellins

It has been reported[3a] that three significantly populated species of hypocrellin A in the ground state are involved in an interconversion process. The three species have been identified[3a] as hypocrellin **2** in the tautomeric form II, the atropisomer **5** in the tautomeric form I and **2** in the tautomeric form I (called A, B and C respectively in Petrich's paper).[3a] This analysis implies that the tautomeric process is slow with respect to the NMR time scale. The three species are said to be in dynamic equilibrium, which is true only for hypocrellin **2** and its atropisomer **5**. The involvement of the third species ("C") in the equilibrium was based on the wrong assignment of the signals at 5.83 ppm and 4.85 ppm and on the finding of an NOE-exchange cross-peak between them. The two signals have been assigned[3a] to 16-H protons of species "C" and "B" respectively, whereas they belong to 14-OH of **5** (5.83 ppm) and to 14-OH of **2** overlapped with 16-H of **5** (4.85 ppm) (see Table 1 and Fig. 4). The overlapping of the latter signals was confirmed by changing temperature or solvent, both increasing the separation between the two resonances, as is shown by the spectrum in $CDCl_3$ (Fig. 1). Therefore the alleged[3a] exchange between 16-Hs of species "C" and "B" is in fact the exchange between 14-OHs of the two atropisomers **2** and **5**. This rules out the existence of the third species "C" and therefore of a slow exchange between tautomers.

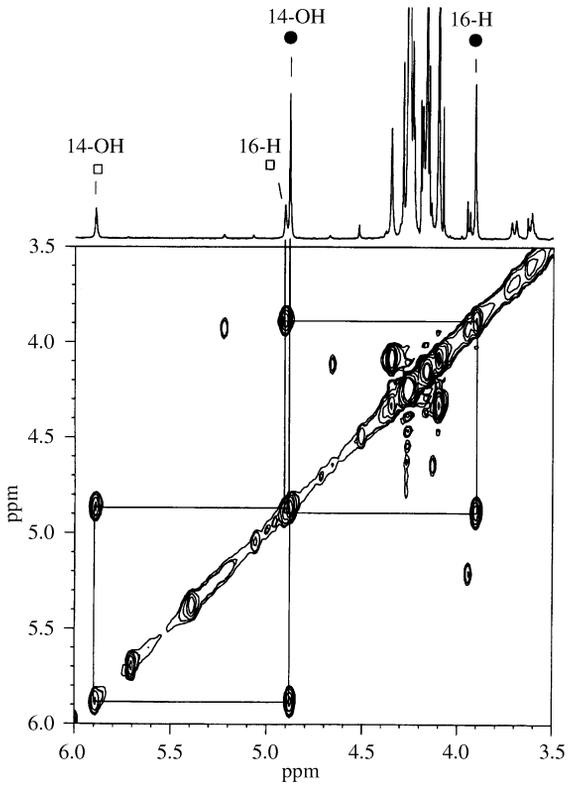


Fig. 4 1D and 2D ROESY spectra (4.0–6.0 ppm region) of (●) hypocrellin A (**2**) and (h) the atropisomer **5** in [$^2H_6$]acetone, +5 °C. The square traces connect the signals in dynamic exchange.

The lowfield portion of the NMR spectrum run in [$^2H_6$]-acetone on the Molecular Probes sample is shown in Fig. 5. The phenolic OH protons were assigned as follows: those of the atropisomers by the NOE-exchange cross-peaks; those of compounds **3** and **4b** by means of the signal intensities. The aromatic protons 5,8-H were assigned by the NOE contacts with 6,7-OMe, which in turn were recognised, with respect to 2,11-OMe, on the basis of the NOE interactions with the protons of the seven-membered ring. The assignment of 3,4-OH *vs.* 9,10-OH resonances in hypocrellin **1** was possible because they show NOE interactions with 2-OMe and 11-OMe respectively. This is additional evidence (see later) that the phenolic OH protons are rapidly exchanging between the two keto–enol oxygen atoms.

The OH resonances of **4b** do not exchange with either **2** or **5,** but with its atropisomer (OH signals thus identified at 16.56 ppm and 16.48 ppm) (Fig. 5a, b). In contrast, the signal at 16.06 ppm has been assigned by Petrich *et al.*[3a] to species C, in tautomeric exchange with species B, because it shows an NOE-exchange cross-peak with the signal at 16.42 ppm. Actually the signal at 16.06 ppm, together with that at 16.15 ppm (partially hidden) belong to the two monodeuterated species (d_1) of **2** at the phenolic OH protons. The deuteration process occurs slowly in [$^2H_6$]acetone solution, *via* the enol-tautomer of the solvent (Fig. 5a, b), but it can be improved by addition of D_2O[10] (Fig. 5c). The spectra in Fig. 5 clearly show how the signal intensities of the monodeuterated species increase with the concentration of D_2O. The monodeuterated species of **2** are in dynamic exchange with their atropisomers, as shown by the cross-peaks with the signals at 16.50 ppm and 16.42 ppm; the latter is partially overlapped by the resonances of **5** and **3**. This is confirmed by the spectrum at −15 °C (insert of Fig. 5c), where the signals around 16.4 ppm are well separated. The same experiments were performed on hypocrellin **1** (Fig. 5d, e).

These results confirm that there is no third species in dynamic equilibrium with **2** and **5**, and that the tautomeric process is too

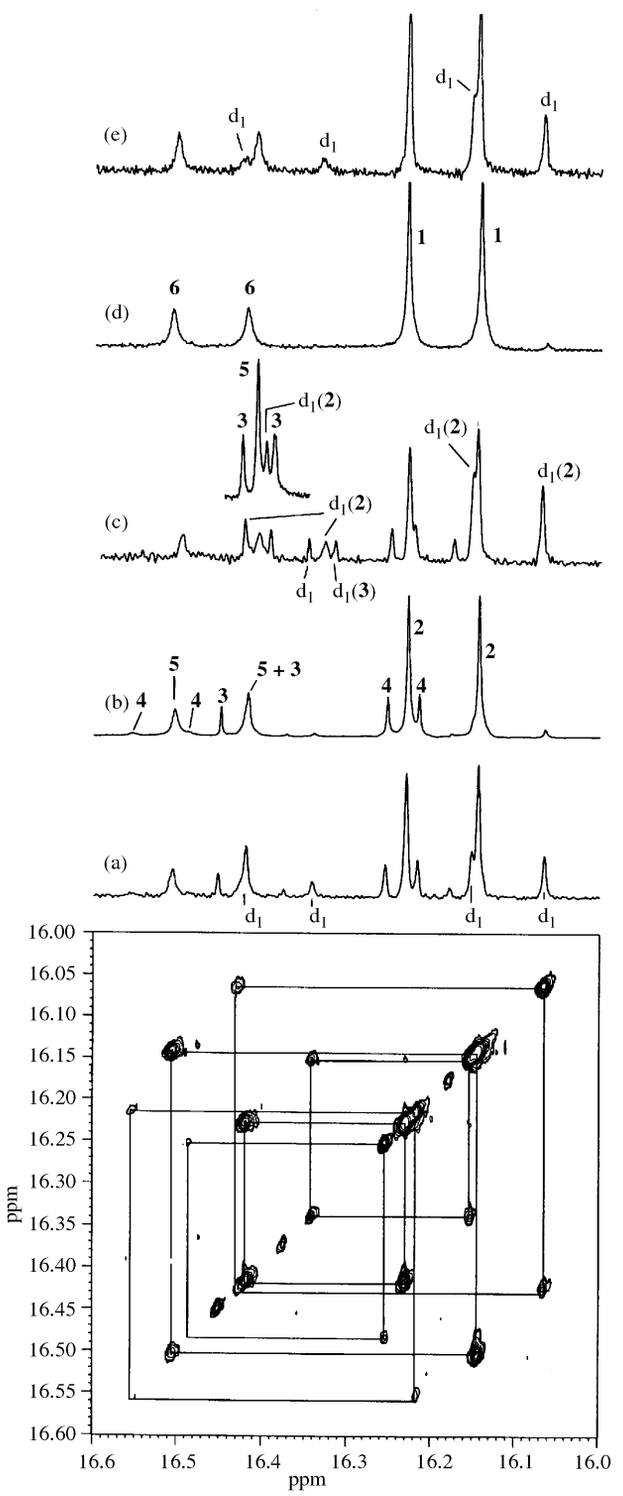


Fig. 5 NMR spectra (phenolic OH protons region) in [$^2H_6$]acetone at +5 °C of: (a, b, c) Molecular Probes sample; (a) 1D and ROESY spectra measured on a two-week old solution; (b) spectrum of a fresh solution; (c) the same after addition of D_2O; in the insert, the spectrum measured at −15 °C; (d, e) hypocrellin **1**; (d) fresh solution; (e) after addition of D_2O.

fast to give rise to different signals of the two tautomers in the NMR spectrum.

We have studied the tautomerism in perylenequinones,[9] and we found that (i) the process is fast even at low temperatures, and (ii) the tautomers I and II were recognised as present at *ca.* 50% in fast equilibrium, for hypocrellin **1**, as well as for elsinochromes. The populations of each tautomer were obtained from the coupling between the proton of the phenolic hydrogen-bonded OH groups and the adjacent carbon atoms, *i.e.* *J*(C3,OH) and *J*(C4,OH), which, in the specific case of **1** are 2.7 and 3.1 Hz respectively. For cercosporin, the first known perylenequinone, which exists in the tautomeric form I,[9] *J*(C4,OH) is 5.2 Hz and *J*(C3,OH) is zero. For isophleichrome in saturated $CDCl_3$ solution, the tautomer II prevails (70%) and the values of the coupling constants are reversed, *i.e.* 3.4 Hz and 2.2 Hz respectively. The secondary deuterium isotopic effect on carbon atoms, especially the strong $^2\Delta$ effect on C-3 or C-4, as well as the chemical shift of 5,8-H protons, also gives information on the tautomeric process.[10] The latter values, easier to obtain for compounds in low concentrations, are significant providing that important self-association processes are not occurring. The very similar chemical shift values of 5,8-H protons found for all compounds (Table 1) show that: (i) the populations of the two tautomers do not change as a consequence of the helix inversion process, (ii) the values found, 6.5–6.8 ppm, suggest a population of *ca.* 50% for each tautomer of **4**–**6** as well as for **1** and **2**.

Conclusions

The enantiomeric hypocrellins **1** and **2** adopt in solution a conformation with the seven-membered ring in the twist–boat form, with the two bulky groups at C-14 and C-16 in *trans* pseudo-equatorial orientation and both projected outward of the aromatic system. The dihedral angle C(1)–C(12b)–C(12a)–C(12), which shows the distortion of the perylenequinone system, is 27°, in agreement with the value found by X-ray analysis.

Hypocrellins **1** and **2** undergo the helix inversion process, which is fast enough to be observed by NMR, giving the atropisomers **6** and **5** respectively. The rate constants of the process, obtained by ROESY-exchange experiments, gave the activation parameters $\Delta G^{\ddagger}$, $\Delta H^{\ddagger}$, and $\Delta S^{\ddagger}$.

The helix inversion process also induces the inversion of the seven-membered ring, which adopts a twist–boat conformation with the two bulky groups in a pseudo axial–axial relationship. The methyl and the acetyl groups are projected toward the aromatic system, forced into this preferred orientation by the hydrogen-bond between the 14-OH and the carbonyl group. The twist angle shows that the distortion of the perylenequinone system is similar to that of **1** and **2**.

The atropisomers **5** and **6** are less stable than **1** and **2** in solution, as follows from their relative populations at equilibrium, obtained by the signal intensities (*ca.* 1 : 3) of the NMR spectrum.

The structure of shiraiachrome A (**4b**) was obtained by accurate NMR experiments performed on a Molecular Probes sample. They include the stereospecific assignment of the geminal protons at C-13 and NOE measurements connecting the protons of the seven-membered ring with each other and with the methoxy groups at C-2 and C-11. This allowed us to obtain the preferred conformation of **4b** and to conclude that the configurational relationship at the two chiral centres C-14 and C-16 must be changed, with respect to **1**. Thus the structure of shiraiachrome A given by Wu[5] as **4a**, *M*(*R*),14*S*,16*R*, must be revised to **4b**, *M*(*R*),14*S*,16*S*. The conformation of the seven-membered ring (twist–boat) and the distortion of the perylenequinone system (twist angle 27–28°) were obtained by NOE data and MM/MD calculations, as for the other stereoisomers.

The NMR study of hypocrellins **1** and **2** rules out the existence of a slow tautomeric equilibrium for these compounds, as reported by Petrich *et al.*[3a] on the basis of an incorrect assignment of some signals in the NMR spectrum of **2**.

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