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STUDIES ON A CHIRALITY OF ORELLANINE. SPECTRAL NONEQUIVALENCE OF ATROPISOMERS OF TETRA-*O*-METHYLORELLANINE AND RELATED COMPOUNDS INDUCED BY CHIRAL SOLVATING AGENTS

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Dedicated to Professor A. I. Meyers on the occasion of his 70th birthday.

Abstract – The precise carbon-hydrogen correlations of orellanine tetramethyl ether were established by NMR techniques and the methylated alkaloid, as well as some of its structural analogues, was subjected to ^{1}H NMR experiments with the use of (*R*)-(+)-*t*-butylphenylphosphinothioic acid, BINOL or TADDOL as the chiral solvating agents. It was found that tetramethylorellanine, as well as 3,3'-dimethoxy-2,2'-bipyridine-*N*,*N'*-dioxide, and some other model compounds gave, in such conditions, proton spectra being composed of separated patterns, each of which corresponds to the individual enantiomer.

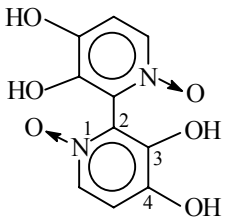
Orellanine, 3,3',4,4'-tetrahydroxy-2,2'-bipyridine-1,1'-dioxide (**1**), a toxic metabolite isolated, first, from the mushroom *Cortinarius orellanus*, and, later, also from other species of that genus, was intensively studied, especially during the last three decades. As a result, the isolation,[1-3] structure elucidation,[3] synthesis,[4-8] mechanism of thermal, photochemical, and possibly physiological deoxidation[9,10] and toxic mechanism proposals[11-13] were described, and the chemical and physiological problems of orellanine were the subjects of several reviewing articles.[11,14-17]

Surprisingly, orellanine, isolated from the natural source, was never reported to be optically active, though the molecular structure suggests a possible chirality, due to the expectable existence of atropisomerism. It was shown that, similar to biphenyl derivatives, also 2,2'-bipyridine-1,1'-dioxide and 2,2'-biquinoline-1,1'-dioxide substituted in 3,3'-positions with carboxylic or methyl groups,[18] can exist as

stable enantiomers, but no evidence was reported of stable atropisomers of this system bearing like orellanine hydroxyl groups or other substituents attached by oxygen in 3,3'-positions .

The X-Ray estimations showed that, in a crystalline state, orellanine molecule is composed of two pyridine rings, arranged to each other, in the perpendicular planes and such a conformation is additionally stiffened by a water molecule attached to both *N*-oxide oxygens.[19] In the case of a salt, orellanine trifluoroacetate, the proton of trifluoroacetic acid is shared by the two *N*-oxide functions, which additionally binds the two rings remaining thus in planes making an angle of 80º.[20] It also follows from these results that orellanine or its protonated form exist in the crystal structure as a mixture of conformational enantiomers in both cases, each of which is additionally stabilized by the intermolecular hydrogen bonds with a contribution of the water or trifluoroacetic acid molecule, respectively.

Yet the existence of orellanine in stable enantiomeric forms in solution was never demonstrated. Most likely this is mainly the result of the very poor solubility of the alkaloid, which is practically insoluble in organic solvents and water, except for DMSO, TFA, pyridine, aqueous alkalies and acids, and sparingly MeOH. The poor solubility of orellanine limited not only the number of available measurement techniques, but also the number of different solvents, required in the isolation and purification procedures, to MeOH in reflux and acidic or basic aqueous solutions, the use of which can cause a change in the molecular electron distribution, which, in turn, together with heating, might be responsible for a conformational change leading to a racemization during the isolation. Therefore, the hitherto laboratory observations regarding the racemic form of orellanine might not reflect the real steric structure of the biosynthesis product.



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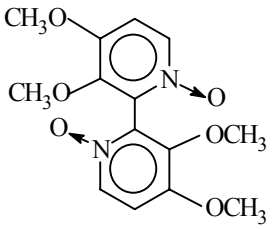

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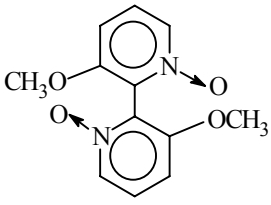

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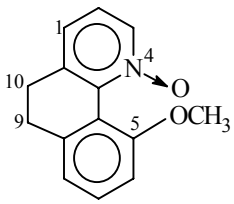

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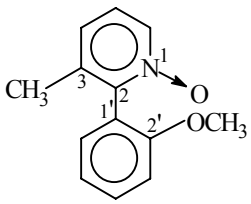

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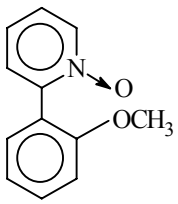
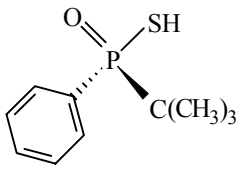


6 (ref. 9)

In the attempts aimed to check up on the existence in solution of stable atropisomers of orellanine (**1**) and compounds of a related structure (**2-6**), we applied NMR techniques with the use of chiral solvating

agents (CSAs), from which (*R*)-(+)-*t*-butylphenylphosphinothioic acid (**7**), the compound widely applied in stereochemistry by Mikołajczyk's group,[23] was found to be the especially effective one for our purposes, though an assymmetric molecular recognition in solution by NMR spectrometry was also achieved when either 2,2'-dihydroxy-1,1'-binaphthyl (BINOL) (**8**) or 2,2-dimethyl-4,5-bis(diphenylhydroxymethyl)-1,3-dioxolane (TADDOL) (**9**) was used.[24]



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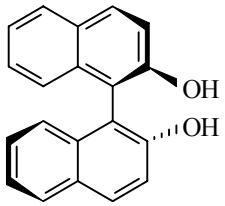

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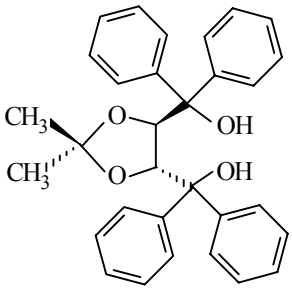

9 (ref. 24)

Table 1. The carbon-proton correlation of the selected bipyridine derivatives (**1**, **2** and **3**) (in DMSO-d_6).

Position of carbon	**1**		**2**		**3**	
	δ_C (δ_H) [ppm]	HMBC correlation $^nJ_{C-H}$ (n >1)	δ_C (δ_H) [ppm]	HMBC correlation $^nJ_{C-H}$ (n >1)	δ_C (δ_H) [ppm]	HMBC correlation $^nJ_{C-H}$ (n >1)
2,2'	130.14	$\Rightarrow H_{6,6'}(^3J)$	135.64	$\Rightarrow H_{6,6'}(^3J)$	131.30	$\Rightarrow H_{4,4'}(^3J)$, $H_{5,5'}(^4J)$, $H_{6,6'}(^3J)$
3,3'	150.01	$\Rightarrow H_{5,5'}(^3J)$	145.22	$\Rightarrow H_{5,5'}(^3J)$, $OC\underline{H}_3(^3J)$	157.09	$\Rightarrow H_{4,4'}(^2J)$, $H_{5,5'}(^3J)$, $H_{6,6'}(^4J)$, $OC\underline{H}_3(^3J)$
4,4'	155.01	$\Rightarrow H_{6,6'}(^3J)$	150.05	$\Rightarrow H_{6,6'}(^3J)$, $OC\underline{H}_3(^3J)$	126.42 (7.19)	$\Rightarrow H_{6,6'}(^3J)$, $OC\underline{H}_3(^4J)$
5,5'	110.08 (7.15)	$\Rightarrow H_{6,6'}(^2J)$	110.28 (7.27)	$\Rightarrow H_{6,6'}(^2J)$	108.86 (7.46)	$\Rightarrow H_{6,6'}(^2J)$
6,6'	131.88 (8.26)	$\Rightarrow H_{5,5'}(^2J)$	134.95 (8.17)	–	132.57 (7.99)	$\Rightarrow H_{4,4'}(^3J)$, $H_{5,5'}(^2J)$
3,3'-O$\underline{C}H_3$	–	–	60.68 (3.71)	–	56.75 (4.02)	–
4,4'-O$\underline{C}H_3$	–	–	56.40 (3.94)	–	–	–

It appeared, however, that the method cannot be applied to orellanine itself, because of the alkaloid insolubility in $CHCl_3$, CH_2Cl_2 and C_6H_6, while using DMSO or pyridine or a protic solvent, like CD_3OD

added to $CDCl_3$, caused a deactivation of each CSA towards the alkaloid. Therefore, we decided to carry out the experiments on a tetra-*O*-methylorellanine (**2**) showing a better solubility in organic solvents. The methylation should not cause a significant energetic change of the molecule determining its conformation, because, on the one hand, replacing a hydrogen of OH by a little more bulky methyl group will indeed

Table 2. The long range carbon–proton correlation of some 2-phenylpyridine derivatives in $CDCl_3$.

4			**5**		
Position of carbon	δ_C (δ_H) [ppm]	HMBC Correlation $^nJ_{C-H}$ (n >1)	Position of carbon	δ_C (δ_H) [ppm]	HMBC Correlation $^nJ_{C-H}$ (n >1)
1	122.49 (7.08)	⇒ $H_3(^3J)$, $H_{10}(^3J)$	2	147.45	⇒ $H_{3'}(^4J)$, $H_5(^4J)$, $H_{6'}(^3J)$, $H_6(^3J)$, $CH_3(^3J)$
2	122.45 (7.05)	–	3	136.58	⇒ $H_4(^2J)$,$H_5(^3J)$, $H_6(^4J)$,$CH_3(^2J)$
3	138.75 (8.22)	–	4	126.55 (7.143)	⇒ $H_6(^3J)$, $CH_3(^3J)$
4a	144.29	⇒ $H_1(^3J)$, $H_2(^4J)$, H_3 (^3J), $H_6(^4J)$, $H_8(^4J)$, H_9 (^4J), $H_{10}(^3J)$	5	123.60 (7.136)	⇒ $H_6(^2J)$
4b	117.13	⇒ $H_6(^3J)$, $H_8(^3J)$, $H_7(^4J)$, $H_{10}(^4J)$	6	137.34 (8.22)	⇒ $H_4(^3J)$, $H_5(^2J)$
5	158.29	⇒ $H_6(^2J)$, $H_7(^3J)$, $H_8(^4J)$,$OC\underline{H}_3(^3J)$	1'	121.27	⇒ $H_{3'}$ (^3J), $H_{5'}$ (^3J)
6	111.16 (6.98)	⇒ $H_7(^2J)$, $H_8(^3J)$	2'	156.60	⇒ $H_{3'}$ (^2J), $H_{6'}$ (^3J), $H_{4'}$ (^3J), $H_{5'}$ (^4J), $OCH_3(^3J)$
7	130.46 (7.38)	⇒ $H_8(^2J)$	3'	111.30 (7.03)	⇒ $H_{4'}$ (^2J), $H_{5'}$ (^3J)
8	119.12 (6.89)	⇒ $H_6(^3J)$, H_{10} (^4J)	4'	130.49 (7.44)	⇒ $H_{6'}$ (^3J), $H_{3'}$ (^2J)
8a	142.23	⇒ $H_6(^4J)$, $H_7(^3J)$, $H_8(^2J)$, $H_9(^2J)$, $H_{10}(^3J)$	5'	120.91 (7.08)	⇒ $H_{3'}$ (^3J)
9	29.52 (2.69)	⇒ $H_8(^3J)$	6'	130.05 (7.20)	⇒ $H_{4'}$ (^3J)
10	29.85 (2.79)	⇒ $H_1(^3J)$	2'-$O\underline{C}H_3$	55.70 (3.78)	–
10a	139.19	⇒ $H_1(^2J)$, $H_2(^3J)$, $H_{10}(^2J)$	3-$\underline{C}H_3$	19.36 (2.06)	–
5-$O\underline{C}H_3$	55.96 (3.86)	–			

cause an increase of steric hindrance, but, on the other hand, the methylation simultaneously removes the hydrogen bonds stabilizing the molecular conformation.

The tetramethyl ether (**2**), and, for the sake of comparison, also orellanine (**1**) and its analogue 3,3'-dimethoxy-2,2'-bipyridine-*N,N'*-dioxide (**3**), all in DMSO-d_6, were first subjected to a precise NMR spectral analysis to determine unambiguously the hydrogen-carbon correlations and the chemical shift of each carbon of the molecule.[25] The COSY, HETCOR, HMQC and HMBC experiments allowed the assignment of the ring's proton-carbon correlations and methyl group-ring carbon to which the methoxy group is attached (Table 1). In the case of **2**, the evidence differentiating the 3-OCH_3 and 4-OCH_3 groups followed from a NOESY experiment. It was found by this method that the H-5,5' (7.27 ppm) signal is correlated with that at 3.94 ppm, which accordingly had to be assigned to the 4-OCH_3 group as being in close vicinity in space to that proton. The lack of NOE interaction of H-5 with the other methyl group absorbing at 3.71 ppm suggested their farther off location and, thus, the signal has to origin from 3-OCH_3. This is in accordance with the confirmed in the literature[22] rule indicating that the methoxy group, when displaced from the plane of the aromatic ring, reveals a higher value of the carbon chemical shift, and the 3-OCH_3 group, being in the crowdedness of the molecular bay-region, may be an object of distortion. Corresponding data for compounds (**4**, **5**) are collected in Table 2.[26]

The chloroform-d solution of the optically inactive tetramethyl ether (**2**) upon the addition of **7**, as well as of **8** or **9**, gave ^{1}H NMR patterns consisting of two nonequivalent , easily distinguishable spectra of the **2** enantiomers as the diastereomeric solvation complexes. Similar results were obtained with **3**, as well as with 5-methoxy-9,10-dihydro-4-azaphenanthrene-4-oxide (**4**) and 2-(2-methoxyphenyl)-3methylpyridine-1-oxide (**5**), while 2-(2-methoxyphenyl)pyridine oxide (**6**), the lower homologue of the last compound above, being not substituted at C-3, even at a low temperature getting down to –50°C, provided no resolution in such conditions. The results are summarized in Tables 3, 4 and 5.

Table 3. The effect of CSAs: **7**, **8** and **9** on the chemical shift of **3** in $CDCl_3$.

Proton position	Chemical shift of atropisomers A and B of **3** with **7** [ppm]		$\Delta\delta_{A-B}$ [ppm]	Chemical shift of atropisomers A and B of **3** with **8** [ppm]		$\Delta\delta_{A-B}$ [ppm]	Chemical shift of atropisomers A and B of **3** with **9** [ppm]		$\Delta\delta_{A-B}$ [ppm]
	δ A	δ B		δ A	δ B		δ A	δ B	
6,6'	8.36	8.27	**0.09**	7.99	7.93	0.06	7.90	7.81	0.09
5,5'	7.29	7.27	0.02	7.22	7.16	0.06	7.23	7.21	0.02
4,4'	6.99	7.04	-0.05	6.84	6.78	0.06	6.88	6.88	0.00
3,3'-OCH_3	3.71	3.84	**-0.13**	3.73	3.71	0.02	3.81	3.80	0.01

Table 4. ^{1}H NMR nonequivalences of diastereomeric solvation complexes of **2** with **7** in $CDCl_3$.

Proton Position	Chemical shift of **2** [ppm]	Chemical shift of atropisomers of **2**		$\Delta\delta_{A-B}$ [ppm]	$\Delta\delta_{2-A}$ [ppm]	$\Delta\delta_{2-B}$ [ppm]
		δA [ppm]	δB [ppm]			
6,6'	8.10	8.80	8.71	**0.09**	-0.70	-0.61
5,5'	7.00	6.82	6.94	-0.12	0.18	0.06
3,3'-OCH_3	3.90	3.74	3.87	**-0.13**	0.16	0.03
4,4'-OCH_3	4.00	3.94	3.96	-0.02	0.06	0.04

Table 5. ^{1}HNMR nonequivalences of diastereomeric solvation complexes of **4** and **5** with **7** in $CDCl_3$.

Proton position	Chemical shift [ppm] of atropisomers of **4**		$\Delta\delta_{A-B}$ [ppm]	Proton Position	Chemical shift [ppm] of atropisomers of **5**		$\Delta\delta_{A-B}$ [ppm]
	δA [ppm]	δB [ppm]			δA [ppm]	δB [ppm]	
1	7.30	7.29	0.01	4	7.37	7.35	0.02
2	7.08	7.13	-0.05	5	7.25	7.16	0.09
3	8.74	8.72	0.02	6	8.55	8.41	0.14
	-	-	-	3-CH_3	2.07	2.09	-0.02
5-OCH_3	4.02	3.94	0.08	2'-OCH_3	3.77	3.70	0.07
6	6.94	6.95	-0.01	3'	6.99	7.00	-0.01
7	7.32	7.36	-0.04	4'	7.39	7.43	-0.04
8	6.78	6.88	-0.10	5'	6.85	7.08	-0.23
9-CH_2	2.82	2.80	0.02	6'	7.11	7.38	-0.27
10-CH_2	2.72	2.67	0.05				

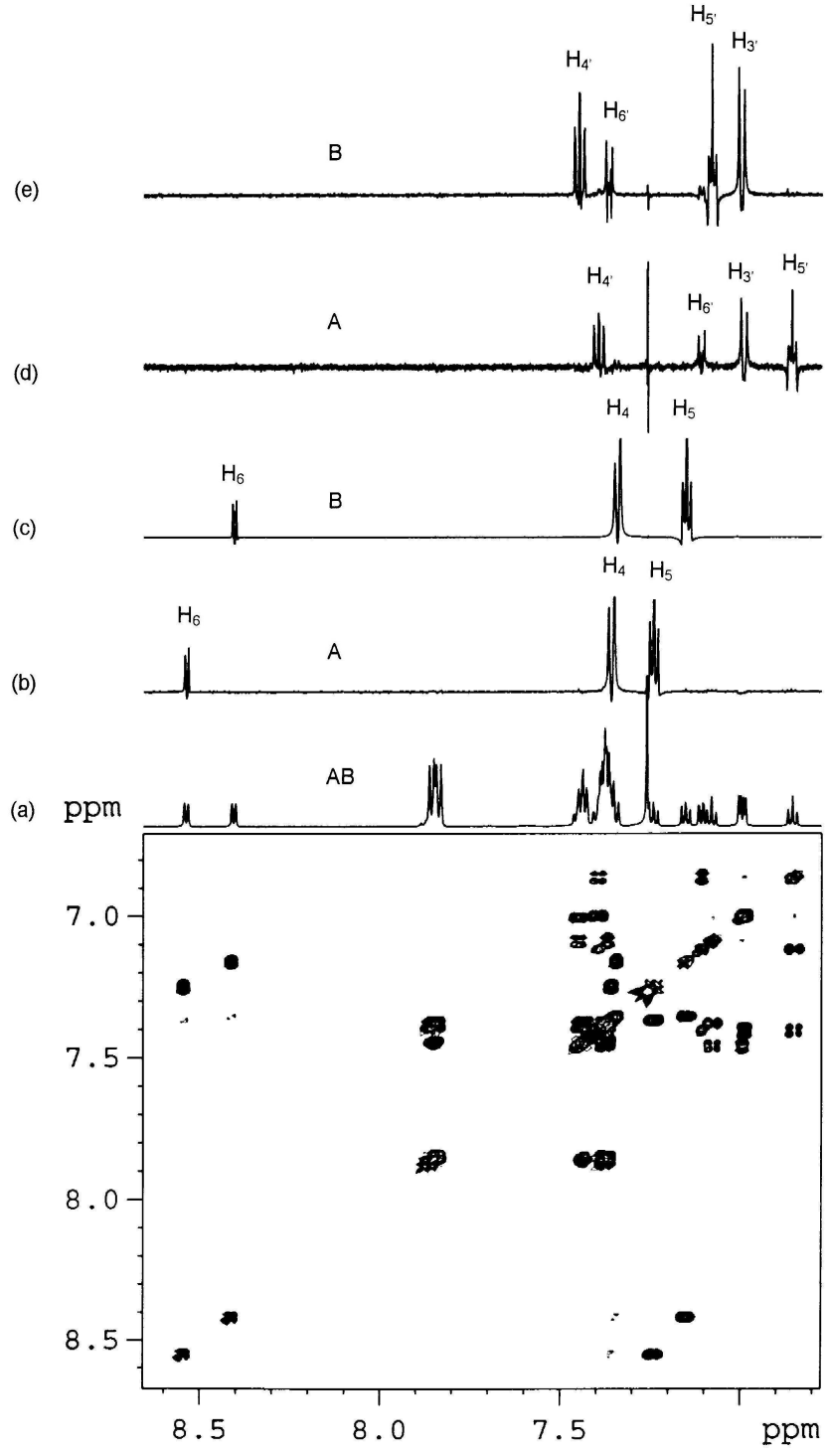

Figure 1. The COSY and 1D gradient selected z-filtered TOCSY patterns of the aromatic region for **5** with CSA **7**. (a) The proton spectrum of the solvate. (b) and (c) Separated enantiomer spectra of the pyridine fragment of atropisomer "A" and "B", respectively. (d) and (e) Separated enantiomer spectra of the phenyl fragment of atropisomer "A" and "B" respectively. The absolute configuration of the atropisomers "A" and "B" is unknown.

The usefulness of CSAs for our purposes was first tested on compound (**3**) and the experiments indicated **7** to be the most effective one, because the signals in the aromatic region of both **3** and **7** did not overlap each other and, additionally, the separations of diastereotopic signals turned out to be, in general, larger than those obtained with **8** and **9**. (Actually, they were even larger than those reported in the literature for aliphatic chiral compounds.[23]) (Table 3, Figure 1.) Therefore, experiments with other orellanine analogues (**2**), (**4**) and (**5**) were carried out with the use of **7** only. (Tables 4 and 5.)

The differences between the chemical shift of H_6 ($H_{6'}$) as well as of 3-OCH_3 (3'-OCH_3), revealed by the atropisomers A and B ($\Delta\delta_{A\text{-}B}$), are identical for the compounds (**2**) and (**3**) (Tables 3 and 4), suggesting a similar mechanism of solvation in both cases.

The 1D gradient selected z-filtered TOCSY experiments ran for the symmetric bipyridine derivatives (**2**) and (**3**) led to obtaining simple spectra for both enantiomers showing the superimposed patterns of both rings with the chemical shift values displayed in the Tables. Yet a point of particular interest turned out to be the spectra of unsymmetrical molecules of phenylpyridine derivatives, whose spectra, obtained by TOCSY technique, required to find the correlation between the separated pyridine patterns with those obtained for the phenyl fragment of the molecule. Such a correlation was achieved by NOESY experiment for compounds (**4**) and (**5**). The use of adopted techniques makes it possible to see, by NMR spectrum, a particular enantiomer (being in a racemic mixture) in the case of more complicated molecules. It is also worthwhile to point out that by using the techniques mentioned above, we were able to observe the influence of the CSA **7** on the chemical shift of all atoms in the molecule, not only on those being in the close vicinity of the most polar *N*-oxide function.[27]

It follows from the obtained data that the stability of the atropisomers of tetramethylorellanine (and of most of orellanine analogues being investigated) should be enough to prevent their fast interconversion at room temperature. Based on these results, further studies on the sterical structure of naturally occurring orellanine are in progress.

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24. To avoid an overlapping of signals in the aromatic region, our preliminary experiments with the compounds (**1**-**6**) were based on aliphatic CSAs like: (*1S*)-(-)-camphanic acid and some other monoterpene derivatives, but no magnetic nonequivalence was observed. Reports on the synthesis and application in stereochemistry of BINOL (**8**) and TADDOL (**9**) can be exemplified by those of F. Toda, K. Mori, Z. Stein, and I.Goldberg, *Tetrahedron Lett.*, 1989, **30**, 1841, and of C. v.d. Bussche-Hünnefeld, A. K. Beck, U. Lengweiler, and D. Seebach, *Helv. Chim. Acta*, 1992, **75**, 438, respectively.
25. In the case of orellanine, our results, obtained by modern NMR spectral technigues (HMQC and HMBC at 600 MHz), confirmed the assignment of the NMR spectral signals to the carbons as done by S. Rapior and A. Fruchier a number of years ago. (*An. Quim.*, 1989, **85C**, 69).
26. On the basis of HMBC experiments, some of the carbon chemical shift values of compounds (**4**) and (**5**) collected in Table 2 are revised as compared with those reported earlier in ref. 22 and 10, respectively.
27. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Mercury 300 MHz spectrometer, operating at 300.07 MHz and 75.461 MHz, respectively. A Bruker DRX-600 spectrometer, operating at 600.055 MHz (^{1}H) and 150.898 MHz (^{13}C), was used for 2D spectra. Measurements were carried out at temperature 25°C using 1% $CDCl_3$ solutions of a mixture containing CSA and the investigated compound in a molar ratio 1 to 0.8, respectively. All spectra were acquired with Bruker 5 mm inverse triple resonance gradient probe (TBI), using standard Bruker pulse sequences for respective experiments. Chemical shifts were reported in ppm downfield from TMS.