Indole Alkaloids of *Rauwolfia reflexa*. Carbon-13 Nuclear Magnetic Resonance Structural Analysis of the Bis(indole) Alkaloid Flexicorine[1]

Asima Chatterjee and Ashim Kumar Ghosh

Department of Pure Chemistry, University College of Science, Calcutta 700009, India

Edward W. Hagaman*

Chemistry Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830

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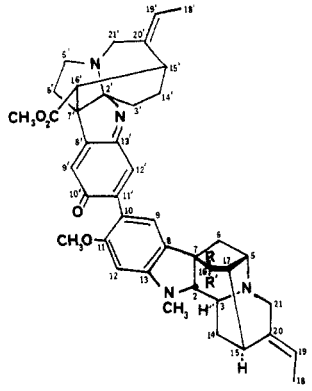
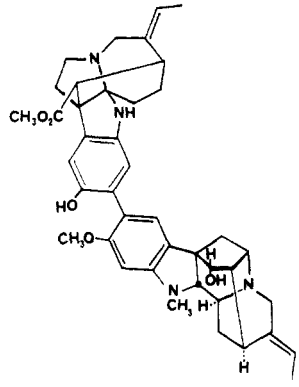
The ^{13}C NMR spectral analyses of the new bis(indole) alkaloid flexicorine and of its chemically modified derivatives were used to determine the structure of the natural base.

As part of our continuing structural analysis of the alkaloidal components of *Rauwolfia* species, we report here the structure of an unusual bis(indole) alkaloid, flexicorine (1), a congener of rauflexine (2) and reflexine (3) isolated

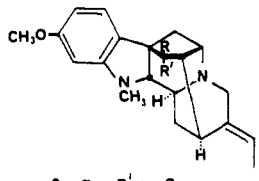




1, R = R' = O

5, R = H, R' = OH

4



2, R = R' = O

3, R = H, R' = OH

from the leaves of *R. reflexa* Teijsm and Binn.[2] Flexicorine is a difficultly purified red amorphous solid with a molecular formula, $C_{41}H_{44}N_4O_5$, determined by ^{13}C NMR spectroscopy. Flexicorine was isolated from the benzene-soluble basic fraction of the ethanolic extract of the leaves of *R. reflexa*. Repeated preparative thin-layer chromatography of a fraction, migrating from a column of alumina with 5% methanolic chloroform, afforded flexicorine as an amorphous red solid: mp >360 °C; $[\alpha]^{25}_D$ −519.5° ($CHCl_3$). Homogeneity of the compound was indicated by thin-layer chromatography with different solvent systems. The high-resolution mass spectrum of flexicorine displays an apparent M^+ of m/e 674.3413 corresponding to the molecular formula $C_{41}H_{46}N_4O_5$. This parent ion likely arises from a dihydro contaminant of flexicorine discussed in the NMR analysis below.

The chromophore of flexicorine is a substituted iminoquinone whose reduced form prepared by $NaBH_4$ reduction in methanol is a colorless 10′-hydroxyindoline moiety. The latter quickly reoxidizes to the iminoquinone upon exposure to air. Borohydride reduction also converts a saturated ketone in the molecule to its corresponding alcohol. The ^{13}C NMR chemical shift assignments of flexicorine (1), its borohydride reduced derivative maintained in an inert atmosphere (4), and the air-oxidized form of the latter (5) are listed in Table I. The use of methanol as the solvent for the NMR measurement of 4 required that key monomer alkaloid ^{13}C NMR data (Table II) also be obtained in this solvent for direct comparisons.

^1H NMR, IR, and UV spectra established the presence of one carbomethoxyl, one indoline *N*-methyl, one aromatic methoxyl, two ethylidene functionalities, and a conjugated ketone in 1. The proton-noise-decoupled ^{13}C NMR spectrum of this substance exhibits 39 unique resonances and a double intensity signal at 50.0 ppm representing two protonated carbons. One-bond ^1H–^{13}C coupling patterns observed in SFORD spectra indicate that 44 hydrogens are bonded directly to carbon. The presence of chemical shifts in 1 indicating saturated ketone, ethylidene, and *N*-methyl functional groups suggested comparison of the spectrum of 1 with that of its congener rauflexine (2).[3] This comparison reveals that all nonaromatic resonances of 2 are reproduced in the spectrum of 1, identifying rauflexine as half of the bisalkaloid and indicating it to be attached to the other half of the molecule through its aromatic ring.

Included among the remaining nonaromatic signals of 1 are resonances which reveal the presence of a second ethylidine unit, a carbomethoxy substituent, two aminomethylene carbons, and two nonprotonated carbons. The chemical shift of one of the latter signals, 103.6 ppm, indicates a carbon attached directly to two heteroatoms. This functional group distribution is reminiscent of that of vincorine (6).[4] Spectral comparison of 6 with the unassigned nonaromatic resonances of 1 reveals a one-to-one chemical shift and multiplicity identity for all but two signals. Resonances of C(2), 97.9 ppm, and C(6), 20.4 ppm, of vincorine correspond to signals of like multiplicity in the spectrum of flexicorine at 103.6 and 26.5 ppm, respectively. The disparity in the C(2′) resonances reflects predominantly the difference in ring A′ oxidation state (vide infra). Ring A′ modification cannot account for the C(6′) shift differences. The latter may be accommodated by removal of the γ effect at C(6′) from the C(16′) carbomethoxy substituent and implies that the configuration of this group is opposite that in 6. The C(16′)-epivincorine-like residue of 1 is attached to the rauflexine moiety through its A′ ring.

(1) Research sponsored in part by the Division of Chemical Sciences, U.S. Department of Energy, under Contract W-7405-eng-26 with the Union Carbide Corp. and in part by the University Grants Commission, New Dehli, India.

(2) Ghosh, A. K. Ph.D. Thesis, University of Calcutta.

(3) Chatterjee, A.; Chakrabarty, M.; Ghosh, A. K.; Hagaman, E. W.; Wenkert, E. *Tetrahedron Lett.* **1978**, 3879.

(4) Das, B. C.; Coisson, J. P.; Lukacs, G.; Potier, P. *Tetrahedron Lett.* **1974**, 4299.

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Table I. Carbon Shifts of 1, 4, and 5[a]

C atom	compd 1[b]	1[c]	4[c]	5[b]	C atom	compd 1[b]	1[c]	4[c]	5[b]
2′	103.6	104.5	95.0	103.7	2	78.1	79.2	77.8	75.9
3′	40.7	41.6	41.7	40.9	3	50.0[d]	50.8[d]	51.3[d]	50.7[d]
5′	53.5	54.0	54.8	53.7	5	52.8[d]	54.0[d]	56.9[d]	56.4[d]
6′	26.5[d]	26.9[d]	26.9[d]	26.5[d]	6	35.0	35.2	36.3	35.5
7′	56.5	57.7	58.5	56.6	7	57.5	58.6	53.8	53.3
8′	144.4	146.1	137.9	145.2	8	120.6	121.5	119.3[e]	119.1
9′	122.9	124.3	113.1	123.1	9	124.1	125.0	123.8	122.1
10′	186.6	188.0	147.8	186.6	10	115.5	116.3	125.4[e]	114.6
11′	157.5	159.3	126.9	157.7	11	157.5	159.3	155.6	157.7
12′	130.5	130.8	113.5	129.9	12	94.4	95.3	95.8	94.6
13′	164.0	165.7	142.4	163.9	13	155.7	157.2	157.5	155.9
14′	27.5[d]	28.3[d]	28.2[d]	27.3[d]	14	31.3	31.8	30.6	29.7
15′	35.4	36.5	36.3	35.5	15	28.3	29.2	26.9	27.3
16′	49.8	50.7	51.3	49.8	16	212.7	213.7	72.4	71.6
17′	172.2	173.5	174.7	172.5	17	50.0[d]	50.8[d]	43.1	41.9
18′	13.6	13.8	13.6	13.7	18	12.8	12.7	12.7	12.8
19′	122.7	124.1	124.1	122.9	19	115.7	117.0	115.0	115.4
20′	138.8	139.6	139.5	138.9	20	136.8	137.1	139.8	137.1
21′	58.8	59.2	58.2	58.9	21	55.4	55.6	55.8	55.1
OCH_3′	51.6	52.0	51.8	51.8	NCH_3	33.9	33.8	34.9	34.4
					OCH_3	55.7	56.0	56.5	55.9

[a] In parts per million downfield from Me_4Si; $\sigma(Me_4Si) = \sigma(CDCl_3) + 76.9$ ppm $= \sigma(CD_3OH) + 48.6$ ppm. [b] $CDCl_3$ solution. [c] CD_3OH solution. [d,e] Signals in any column may be reversed.

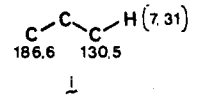
Table II. Carbon Chemical Shifts of 2 and 3[a]

C atom	2[b,e]	2[c]	3[b,d]	3[c,d]
2	78.4	79.3	76.9	77.3
3	50.1[f]	50.9[f]	50.1[f]	g
5	53.1[f]	54.2[f]	55.3[f]	57.2
6	35.3	35.0	36.0	36.0
7	57.8	58.6	53.2	g
8	121.6	122.1	124.1	g
9	122.5	123.5	119.9	121.8
10	103.8	105.1	102.9	104.3
11	160.1	161.7	160.1	g
12	97.5	98.1	97.2	97.8
13	155.1	156.3	155.6	g
14	31.5	31.6	30.3	30.2
15	28.5	29.1	27.5	27.8
16	214.0	214.2	72.8	72.3
17	50.3[f]	50.7[f]	42.1	43.0
18	12.9	12.6	12.8	g
19	115.7	117.6	113.6	115.7
20	137.3	136.5	140.2	g
21	55.7	55.5	55.3	55.4
NCH_3	34.2	34.1	34.5	34.6
OCH_3	55.3	55.5	55.3	55.4

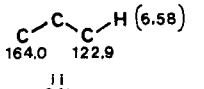
[a] In parts per million downfield from Me_4Si; $\sigma(Me_4Si) = \sigma(CDCl_3) + 76.9$ ppm $= \sigma(CD_3OH) + 48.6$ ppm. [b] $CDCl_3$ solution. [c] CD_3OH solution. [d] Prepared by $NaBH_4$ reduction of 2. [e] From ref 3. [f] Signals in any column may be reversed. [g] Small sample size precluded observation of these signals.

The $NaBH_4$ reduction of 1 in methanol (inert atmosphere) reduces the keto group in the rauflexine part of the alkaloid to the 16α-hydroxy derivative (cf. with reflexine) and discharges the deep red color of the starting material. The latter change is reflected in major chemical shift alterations of ca. half of the aromatic carbon resonances of 1. Upon exposure of the methanolic solution of this material to the air, the red color is restored. The aromatic portion of the spectrum of the air-oxidized derivative (in $CDCl_3$) is identical with that of 1. This unusual observation in conjunction with the following NMR data establishes the nature of the A rings of the alkaloid components and their linkage.

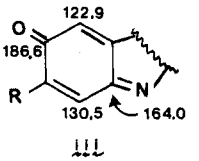
The ^1H NMR spectrum of 1 exhibits four one-proton singlets in the aromatic region, indicating a C(10),C(11) and C(10′),C(11′) substitution pattern in the A rings of the bases. The ^{13}C NMR spectrum of 1 contains a resonance at 186.6 ppm, a field position 20 ppm to lower field than any known heterosubstituted dihydroindole resonance. It suggests, inter alia, a quinone carbonyl resonance.[5] It and another nonprotonated carbon signal at 164.0 ppm are destroyed by borohydride reduction. The same resonances each reveal a single long-range ^1H–^{13}C coupling constant of 8.5 Hz, typical of $^3J_{CH}$ transmitted through a trans-trigonal path. A ^1H–^{13}C cross-correlation experiment established that the δ 7.31 proton exhibits one- and three-bond coupling to the carbon resonances at 130.5 and 186.6 ppm, respectively, and the δ 6.58 proton shows one- and three-bond coupling to the carbon resonances at 122.9 and 164.0 ppm, respectively. Since the 130.5- and 122.9-ppm methines are shifted strongly by the borohydride reduction, four of the six carbon resonances of the A ring of the alkaloid that is altered in the reduction may be grouped into the two geminally related pairs i and ii. These fragments, together with the constraint of the proton substitution pattern are compatible only with the iminoquinone partial structure iii.



i



ii



iii

Since the reduction also causes a 9-ppm upfield shift of the nonprotonated C(2′) resonance of the epivincorine-like base [104.5 ppm in 1 (CD_3OH); 95.0 ppm in 4 (CD_3OH)], iii comprises the A′ ring of this base in 1. The C(2′) shift

(5) The carbonyl resonances of 2,6-di-*tert*-butylquinone are at 187.2 and 188.2 ppm, with the former exhibiting $^3J_{CH} = 9.5$ Hz and the latter no resolved coupling. The C(2) resonances of indolenines[6] appear between 180 and 190 ppm but are excluded here by long-range ^1H–^{13}C coupling data.

(6) Wenkert, E.; Hagaman, E. W.; Wang, N.-Y.; Kunesch, N. *Heterocycles* **1979**, *12*, 1439.

in 4 is similar to the 97.9-ppm C(2) resonance of vincorine,[4] the 3-ppm lower field value in the latter reflecting N_a-CH_3 substitution. Partial structure iii also is supported by the aromatic resonances of C(8')–C(13') observed in 4 which, with account taken for the C(11') substitution in 4, are in good agreement with the resonances of 10-methoxyindoline moieties.[3] We attribute the observed high-resolution mass spectral parent ion to 4, presumably a minor impurity in 1.

The two remaining methines which suffer minimal perturbation between 1 and 4 must belong to the A ring of the rauflexine residue. One of these shifts, 94.4 ppm, is diagnostic for a C(11) oxygen substituent[3] and establishes C(10) as the linkage site in this base. This is confirmed by the field position of the remaining aromatic methine, 124.1 ppm, which cannot be situated ortho to the oxygen-bearing carbon.

From these data, we conclude that the structure of flexicorine is as represented in formula 1 and that of its borohydride-reduced derivative is as shown in 4. To our knowledge, flexicorine is the first 10'-hydroxy-N_a'-unsubstituted indoline which preferentially exists in the oxidized iminoquinone form.

Experimental Section

^{13}C NMR spectra were recorded on a Varian XL-100-15 spectrometer operating at a ^{13}C radio frequency of 25.2 MHz in the Fourier transform mode. Deuteriochloroform or deuteriomethanol solutions of the substrates (0.005–0.2 M) were spun in 12-mm-o.d. tubes at 30 °C. The σ values of all compounds are referenced to the Me_4Si scale.

The preparation of 4 from flexicorine (1) was accomplished as follows. To 50 mg of 1 in CD_3OD contained in an NMR tube under an argon atmosphere was added excess $NaBH_4$ (15 mg) at 0 °C. The solution was warmed to room temperature and allowed to stand for 2 h. Two drops of concentrated HCl were added to complete the decomposition of excess $NaBH_4$. The argon-purged tube was capped, and the ^{13}C NMR spectra of 4 was recorded immediately. When the tube was opened 4 was converted to 5 rapidly.

Registry No. 1, 80765-85-1; 2, 70522-05-3; 3, 61091-18-7; 4, 80780-62-7; 5, 80765-86-2.

Linear Solvation Energy Relationships. 20. Intra- vs. Intermolecular Hydrogen Bonding by Some 2-Nitroaniline and 2-Nitrophenol Derivatives

Mortimer J. Kamlet* and R. W. Taft*

Naval Surface Weapons Center, White Oak Laboratory, Silver Spring, Maryland 20910 and Department of Chemistry, University of California, Irvine, California 92717

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The solvatochromic comparison method is used to unravel and quantify effects of solvent dipolarity/polarizability and intra- and intermolecular hydrogen bonding on the electronic absorption spectra of 2-nitro-*p*-toluidine (1), *N*-methyl-2-nitro-*p*-toluidine (2), and 2-nitrophenol (3). Evidence is presented which indicates that 2 remains intramolecularly hydrogen bonded in solvents as strongly basic as *N*-methylpyrrolidone, whereas 3 breaks its *intra*molecular hydrogen bond to nitro to form an *inter*molecular hydrogen bond in even so weakly basic a solvent as anisole.

In earlier reports we have shown that when hydrogen-bonding effects are excluded, as when neither solutes nor solvents are *inter*molecular hydrogen-bond donors, solvent effects on p→π* and π→π* electronic spectral transitions are well described by the solvatochromic equation:

$$\nu(i)_{max} = \nu(i)_0 + s\pi^* \quad (1)$$

where π^* is a measure of solvent dipolarity/polarizability[1] (on a scale which ranges from −0.08 for *n*-hexane and 0.00 for cyclohexane to 1.00 for Me_2SO).[2-4] When the spectra are also influenced by solute to solvent (type B)[5] hydrogen-bonding effects, the form of the solvatochromic equation becomes:

$$\nu(i)_{max} = \nu(i)_0 + s\pi^* + b\beta \quad (2)$$

where β is a measure of solvent HBA (hydrogen-bond acceptor) basicity (on a scale ranging from 0.00 for non-HBA solvents to 1.05 for hexamethylphosphoramide).[2,6,7]

In part 2 of this series,[7] we used the solvatochromic comparison method and eq 1 and 2 to unravel and evaluate the effects of solvent dipolarity/polarizability[1] and type-B hydrogen bonding[5] on the UV–visible spectra of some 3- and 4-substituted and 3,5-disubstituted aniline derivatives. In the present paper we carry out a similar analysis of solvatochromic shift data for 2-nitro-*p*-toluidine (1), *N*-methyl-2-nitro-*p*-toluidine (2), and 2-nitrophenol (3). We offer evidence that the amine proton of 2 remains *intra*molecularly hydrogen bonded to the neighboring nitro oxygen in even such strong HBA base solvents as *N*-methylpyrrolidone (β = 0.77),[2] whereas 3 breaks its *intra*molecular hydroxyl to nitro hydrogen bond to form *inter*molecular type-B hydrogen bonds[5] to even such weak HBA base solvents as anisole (β = 0.22).[2] Spectral data

(1) The term solvent dipolarity is intended as a more specific description than the often misused solvent polarity, which has frequently included as well the effects of hydrogen-bonding interactions in varying combinations with the dipole/dipole effects.

(2) Kamlet, M. J.; Abboud, J.-L. M.; Taft, R. W. *Prog. Phys. Org. Chem.* **1981**, *13*, 485.

(3) Kamlet, M. J.; Abboud, J.-L. M.; Taft, R. W. *J. Am. Chem. Soc.* **1977**, *99*, 6027.

(4) Kamlet, M. J.; Hall, T. N.; Boykin, J.; Taft, R. W. *J. Org. Chem.* **1979**, *44*, 2599.

(5) In type-A hydrogen bonding the solute acts as HBA base and the solvent as HBD acid. The converse applies in type-B hydrogen bonding.

(6) Kamlet, M. J.; Taft, R. W. *J. Am. Chem. Soc.* **1976**, *98*, 377.

(7) Kamlet, M. J.; Jones, M. E.; Taft, R. W.; Abboud, J.-L. M. *J. Chem. Soc., Perkin Trans. 2* **1979**, 342.