^{1}H and ^{13}C NMR studies of 6,11-dimethyl-6*H*-indolo[2,3-*b*]quinoline and some of its derivatives

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The ^{1}H and ^{13}C NMR spectra of the parent 6,11-dimethyl-6*H*-indolo[2,3-*b*]quinoline and of its nine 2- and 9- methyl-, methoxy- and fluoro-substituted derivatives have been measured and analysed by the use of COSY, HETCOR, SPT INEPT and selective decoupling experiments. The INADEQUATE experiment was applied to yield the one-bond ^{13}C–^{13}C coupling constants for most of the compounds studied. Proton–proton coupling constants including long-range ones have also been determined. Strong concentration effects on the spectra have been observed for all the compounds studied. In particular, large upfield shifts upon the increase of concentration have been observed for some proton resonances. This has been explained in terms of self-association of the compounds.

Introduction

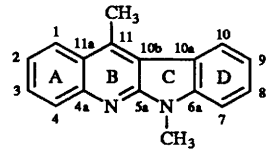
The compounds whose skeleton contains the quinoline fragment have been the subject of much interest, since many of them reveal strong cytotoxic and/or antimicrobial properties combined with relatively low toxicity. The strength of their activity depends on the number of benzene rings attached to the main fragment of a molecule, and on the nature and position of substituents.[1,2] Ellipticine, *i.e.* 5,11-dimethyl-6*H*-pyrido[4,3-*b*]carbazole, which is administered as an anticancer drug, represents the best known example. A number of papers devoted to analysis of its ^{13}C NMR spectra have been published.[3–5] Quite recently the interaction of ellipticine derivatives with nucleic acids has been studied by the use of ^{1}H NMR spectroscopy by Behravan *et al.*,[6] and the effect of the size of the heterocyclic ring system has been discussed by these authors.

Two series of ellipticine related compounds, *i.e.* derivatives of 5,11-dimethyl-5*H*-indolo[2,3-*b*]quinoline and 6,11-dimethyl-6*H*-indolo[2,3-*b*]quinoline have been synthesized in our laboratory.[1] It has been found that some of them reveal very high cytotoxic activity.[2] Therefore it seemed of great importance to obtain an insight into the electronic structure of this group of compounds. In our previous paper the ^{13}C and ^{1}H NMR data obtained for a series of derivatives of 5,11-dimethyl-5*H*-indolo[2,3-*b*]quinoline was published.[7] In the present paper the ^{1}H and ^{13}C NMR spectra obtained for their analogues, *i.e.* for 6,11-dimethyl-6*H*-indolo[2,3-*b*]quinoline and some of its derivatives are presented and analysed.

Results

The compounds studied in the present work are the parent 6-methyl-6*H*-indolo[2,3-*b*]quinoline **1** and its derivatives: 2-methyl **2**, 9-methyl **3**, 2,9-dimethyl **4**, 2-methoxy **5**, 9-methoxy, **6**, 2-methyl-9-methoxy **7**, 2-methoxy-9-methyl **8**, 2,9-dimethoxy **9** and 2-fluoro **10**.

For all these compounds the ^{1}H and ^{13}C NMR spectra were recorded and analysed (Tables 1–7). The most intriguing feature of the spectra measured was their selective dependence on the concentration of the solution which was observed for all compounds and for both the ^{1}H and ^{13}C NMR spectra. A detailed study was performed for the parent compound (see Tables 1 and 3). The changes in the proton part of the NMR spectrum are very irregular. Upon passing from dilute to concentrated solutions some resonances shift towards higher field quite significantly (by *ca.* 0.3 ppm or more); the others either do not change their positions or move only slight, by *ca.* 0.1 ppm. As a result, some resonances interchange their positions and the spectra recorded at higher concentrations do not resemble those recorded for diluted solutions (see Table 1 where the spectra of the compound **1** measured at various concentrations are presented).



In the case of ^{13}C NMR spectra an upfield shift of all signals also takes place upon increase of concentration (see Table 3 where the concentration dependent data for compound **1** are displayed). The $\Delta\delta_{^{13}C}$ values vary from 0.43 ppm for C4 up to *ca.* 1 ppm for most of the remaining carbons when the spectra of 0.02 and 1.2 mol dm^{-3} solutions are compared. Similar patterns were observed for all the remaining compounds. The described phenomenon, if neglected, could lead to serious errors in the assignments of the spectra and, in consequence, to incorrect conclusions. Therefore, special care has been paid in the present work to record all spectra in carefully standardized conditions. This concerns both concentration and temperature. Thus, all ^{1}H NMR data presented in Table 2 were recorded for 0.08 mol dm^{-3} solutions where the changes upon dilution are already rather small. Another set of ^{1}H NMR spectra was recorded for 0.2 mol dm^{-3} solutions and these results were used for the assignments of the ^{13}C NMR spectra, which were also recorded at the same, *i.e.* 0.2 mol dm^{-3}, concentration. As follows from the data of Table 3 further dilution only slightly influences the ^{13}C NMR spectrum.

It is worthwhile to mention at this point that such an approach combined with application of modern (see below) NMR techniques allowed us unambiguous assignments of all, even very closely spaced, signals in the ^{13}C NMR spectra of the compounds studied. The $\delta_{^{13}C}$ differences (see Tables 3 and 4) are sometimes smaller than 0.1 ppm.

In the ^{1}H NMR spectrum of the parent compound **1** only the signal of the protons of the NCH_3 group, which appears at 3.96 ppm and that of the CH_3 group attached at C11 appearing at 3.16 ppm (measured at 0.08 mol dm^{-3}) could be easily

Table 1 Concentration dependence of $\delta_{^1H}$ chemical shifts for compound **1** measured at 303 K; all values are given in ppm *vs.* Me_4Si

	c/mol dm^{-3}													
H	1.18	1.00	0.80	0.60	0.42	0.40	0.21	0.20	0.10	0.08	0.04	0.02	0.003	Δ_δ
H1	8.00	8.02	8.06	8.11	8.13	8.14	8.19	8.19	8.23	8.23	8.24	8.25	8.26	0.26
H2	7.43	7.44[a]	7.46[a]	7.45	7.46	7.46	7.47	7.47	7.48	7.48	7.49	7.49	7.49	0.06
H3	7.72	7.72	7.72	7.72	7.72	7.72	7.72	7.72	7.72	7.72	7.72	7.72	7.72	0.00
H4	8.14	8.13	8.13	8.11	8.13	8.14	8.13	8.13	8.13	8.13	8.13	8.13	8.13	0.01
H7	7.11	7.16	7.19	7.25[a]	7.28	7.28[a]	7.36	7.36	7.40	7.40	7.42	7.43	7.44	0.33
H8	7.43	7.44[a]	7.46[a]	7.50	7.52	7.53	7.55	7.55	7.57	7.57	7.58	7.58	7.59	0.16
H9	7.20	7.22	7.23	7.25[a]	7.27	7.28[a]	7.30	7.30	7.32	7.31	7.32	7.32	7.33	0.13
H10	7.97	8.02	8.05	8.11	8.15	8.14	8.22	8.22	8.27	8.27	8.28	8.29	8.31	0.34
11-Me	2.77	2.83	2.88	2.94	3.01	3.01	3.11	3.10	3.17	3.16	3.19	3.20	3.22	0.45
6-NMe	3.69	3.72	3.75	3.80	3.85	3.85	3.92	3.92	3.96	3.96	3.98	3.98	4.00	0.31

[a] Overlapping signals.

Table 2 ^1H NMR chemical shifts for 6,11-dimethyl-6*H*-indolo[2,3-*b*]quinoline and its derivatives (in ppm); all spectra were recorded in 0.08 mol dm^{-3} $CDCl_3$ solutions

H	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
H1	8.23	7.97	8.20	7.95	7.39	8.20	7.94	7.43	7.38	7.79
H2	7.48	—	7.47	—	—	7.46	—	—	—	—
H3	7.72	7.57	7.70	7.53	7.40	7.70	7.53	7.39	7.40	7.47
H4	8.13	8.03	8.12	8.01	8.04	8.10	8.00	8.03	8.01	8.08
H7	7.40	7.39	7.29	7.27	7.37	7.28	7.27	7.27	7.26	7.40
H8	7.57	7.56	7.38	7.36	7.55	7.18	7.17	7.37	7.17	7.58
H9	7.31	7.30	—	—	7.29	—	—	—	—	7.31
H10	8.27	8.26	8.06	8.04	8.25	7.79	7.78	8.05	7.78	8.25
11-CH_3	3.16	3.14	3.16	3.12	3.09	3.12	3.10	3.09	3.06	3.09
6-NCH_3	3.96	3.95	3.93	3.91	3.92	3.91	3.90	3.90	3.89	3.93
2-CH_3	—	2.61	—	2.60	—	—	2.59	—	—	—
9-CH_3	—	—	2.58	2.57	—	—	—	2.57	—	—
2-OCH_3	—	—	—	—	3.98	—	—	3.98	3.97	—
9-OCH_3	—	—	—	—	—	3.95	3.94	—	3.95	—

identified. In the case of the aromatic part of the spectrum which consists of two four proton sets the decision as to which set of the signals should be attributed to ring A and which to ring D could not be made without additional information. This was provided by the NOE experiment. A strong enhancement observed for the signal at 7.40 ppm (c = 0.08 mol dm^{-3}), upon irradiation of the *N*-methyl group protons allowed us to assign the former to H7. A similar strong effect was observed for two signals at 8.23 and 8.27 ppm when the methyl group at C11 was irradiated. A discrimination between them and assignment of the remaining protons in the molecule followed from an analysis of the COSY spectrum with H7 being the starting point. The results obtained for compound **1** were further used in an analysis of the spectra of the remaining compounds. Especially the knowledge of the precise $^3J(^1H,^1H)$ and $^4J(^1H,^1H)$ values, which were found to be concentration independent, was a great help. Additionally, all the remaining spectra are considerably simplified due to substituent effects which made the task much easier. The only problem was connected with the aliphatic portion of the ^1H NMR spectra of compounds **4–9** where the CH_3 resonances appear within 0.08 ppm or less. Nevertheless, analysis of the trends observed and the HETCOR spectra (Fig. 1) recorded for somewhat higher concentrations (in order to save the experiment time) allowed us to assign also these signals with a high degree of confidence.

An assignment of the signals in the ^{13}C NMR spectra was made in the following way. The DEPT experiment allowed us an easy discrimination between the carbons bearing protons and the quaternary ones. The HETCOR experiment adjusted for one-bond carbon–proton coupling constants of *ca.* 160 Hz yielded an unambiguous assignment of the signals due to the carbons bearing hydrogens. The COLOC and SPT INEPT

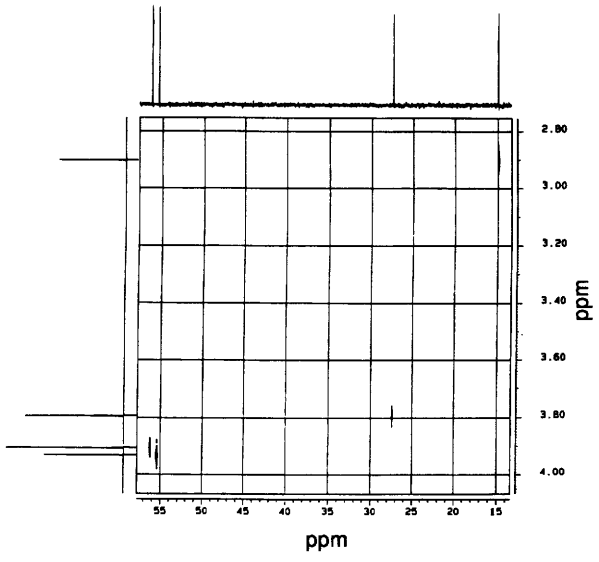


Fig. 1 HETCOR spectrum (aliphatic part) of compound **9** in 0.2 mol dm^{-3} solution in $CDCl_3$; from high-to-low field in the ^{13}C axis the methyl groups are assigned as follows: 11-CH_3, 6-NCH_3, 2-OCH_3 and 9-OCH_3

experiments adjusted for three-bond CH couplings of *ca.* 6 Hz, combined with analysis of fully proton-coupled and selectively decoupled spectra yielded full and unequivocal assignment of all quaternary carbons. An additional proof for the validity of the assignments made was obtained from the INADEQUATE

Table 3 Concentration dependence of $\delta_{^{13}C}$ chemical shifts for compound **1** measured at 303 K; all values are given in ppm *vs.* Me_4Si

c/mol dm^{-3}	C1	C2	C3	C4	C4a	C5a	C6a	C7	C8	C9	C10	C10a	C10b	C11	C11a	11-Me	6-NMe
1.18	123.43	121.77	127.74	127.66	145.99	151.18	141.78	107.56	126.41	118.98	122.67	120.55	115.27	137.76	123.37	14.05	26.60
1.00	123.72	122.11	128.12	127.81	146.21	151.61	142.14	107.96	126.79	119.35	123.04	120.88	115.71	138.28	123.65	14.50	27.02
0.42	123.87	122.34	128.33	127.97	146.43	151.98	142.47	108.23	127.05	119.59	123.30	121.17	116.06	138.57	123.87	14.80	27.29
0.20	123.98	122.45	128.45	128.01	146.50	152.13	142.59	108.36	127.18	119.71	123.42	121.28	116.23	138.75	123.95	14.96	27.43
0.10	123.99	122.52	128.51	128.04	146.55	152.24	142.68	108.44	127.26	119.78	123.50	121.35	116.33	138.83	124.04	15.05	27.52
0.02	124.01	122.56	128.54	128.09	146.61	152.32	142.76	108.49	127.30	119.83	123.54	121.42	116.41	138.87	124.09	15.10	27.56
$\Delta\delta^a$	0.58	0.79	0.80	0.43	0.62	1.14	0.98	0.93	0.89	0.85	0.87	0.87	1.14	1.11	0.72	1.05	0.96

[a] The difference between spectra measured at 0.02 and 1.18 mol dm^{-3}.

Table 4 ^{13}C NMR chemical shifts for 6,11-dimethyl-6*H*-indolo[2,3-*b*]quinoline and its derivatives (in ppm); all spectra were recorded in 0.2 mol dm^{-3} $CDCl_3$ solutions if not otherwise stated

C	1	2	3	4	5	6	7	8[a]	9	10[b]
C1	123.98	122.95	123.93	122.95	102.41	123.94	122.92	102.18	102.36	107.33[c]
C2	122.45	131.81	122.27	131.63	154.98	122.24	131.57	155.00	154.78	158.32[d]
C3	128.45	130.65	128.35	130.54	120.58	128.50	130.68	120.43	120.53	118.20[e]
C4	128.01	127.61	127.84	127.58	128.93	127.76	127.50	129.26	129.10	129.89[f]
C4a	146.50	144.79	146.35	144.78	141.68	146.39	144.83	142.23	142.19	143.31[g]
C5a	152.13	151.72	152.21	151.93	150.65	152.20	151.92	151.35	151.20	151.78
C6a	142.59	142.49	140.65	140.65	142.35	137.18	137.20	140.78	137.19	142.70
C7	108.36	108.27	108.00	107.92	108.25	108.63	108.45	107.98	108.39	108.41
C8	127.18	127.02	128.12	127.99	127.08	114.13	114.04	128.14	114.10	127.56
C9	119.71	119.53	128.93	128.74	119.48	153.81	153.71	128.69	153.56	119.79
C10	123.42	123.35	123.71	123.69	123.34	108.54	108.58	123.75	108.52	123.57
C10a	121.28	121.28	121.27	121.35	120.91	121.59	121.65	121.19	121.31	120.80
C10b	116.23	116.13	116.18	116.12	116.15	116.16	116.09	116.29	116.08	116.69
C11	138.75	138.11	138.61	137.95	137.44	138.89	138.18	137.10	137.19	137.74[h]
C11a	123.95	123.83	123.84	123.75	124.21	123.57	123.48	124.33	123.92	124.21[i]
C11-CH_3	14.96	14.94	14.97	14.96	15.10	14.81	14.79	15.16	14.91	15.06
C6-NCH_3	27.43	27.44	27.45	27.45	27.47	27.47	27.46	27.47	27.39	27.40
C2-CH_3	—	21.76	—	21.75	—	—	21.72	—	—	—
C9-CH_3	—	—	21.51	21.51	—	—	—	21.52	—	—
C2-OCH_3	—	—	—	—	55.41	—	—	55.50	55.38	—
C9-OCH_3	—	—	—	—	—	56.17	56.17	—	56.17	—

[a] Saturated solution (0.15 mol dm^{-3}). [b] Saturated solution (0.11 mol dm^{-3}). [c] ^2J(C1–2F) = 22.5 Hz. [d] ^1J(C2–2F) = 241.7 Hz. [e] ^2J(C3–2F) = 25.4 Hz. [f] ^3J(C4–2F) = 8.8 Hz. [g] ^4J(C4a–2F) = 0. [h] ^4J(C11–2F) = 5.5 Hz. [i] ^3J(C11a–2F) = 8.9 Hz.

Table 5 Vicinal coupling constants ^3J(H,H) in 6,11-dimethyl-6*H*-indolo[2,3-*b*]quinoline and its derivatives

	^3J(H,H)/Hz									
H–H	1	2	3	4	5	6	7	8	9	10
1–2	8.45	—	8.45	—	—	8.41	—	—	—	*a*
2–3	6.73	—	6.73	—	—	6.66	—	—	—	*b*
3–4	8.45	8.53	8.46	8.52	9.10	8.41	8.57	9.04	9.09	9.20
7–8	8.10	8.05	8.14	8.07	8.07	8.58	8.73	8.14	8.67	8.14
8–9	7.31	7.32	—	—	7.32	—	—	—	—	7.30
9–10	7.79	7.79	—	—	7.79	—	—	—	—	7.77

[a] Coupling with fluorine: ^3J(1–2F) = 10.7 Hz. [b] Coupling with fluorine: ^2J(3–2F) = 7.86 Hz.

Table 6 Long-range proton–proton coupling constants in 6,11-dimethyl-6*H*-indolo[2,3-*b*]quinoline and its derivatives

	^4J(H,H)/Hz									
H–H	1	2	3	4	5	6	7	8	9	10
1–3	1.44	1.97	1.44	1.96	2.79	1.42	1.97	2.75	2.77	2.84
2–4	1.32	—	1.33	—	—	1.30	—	—	—	*a*
7–9	1.05	1.03	—	—	1.04	—	—	—	—	1.03
8–10	1.17	1.17	1.66	1.68	1.16	2.48	2.49	1.66	2.46	1.17
1–2Me	—	1.03	—	1.03	—	—	*b*	—	—	—
2Me–3	—	0.52	—	0.55	—	—	0.52	—	—	—
8–9Me	—	—	0.77	0.70	—	—	—	0.70	—	—
9Me–10	—	—	*b*	1.39	—	—	—	*b*	—	—
	^5J(H,H)/Hz									
1–4	0.63	*b*	0.63	*b*	*b*	0.61	*b*	0.53	*b*	0.46
7–10	*b*	*b*	*b*	*b*	*b*	*b*	0.54	*b*	*b*	*b*

[a] Coupling with fluorine: ^4J(4–2F) = 5.63 Hz. [b] Not found.

spectra. This technique has been widely used for two purposes: (*i*) to determine one-bond CC couplings and (*ii*) to establish the connectivity of the skeleton carbon, which is actually equivalent to the proper assignment of the signals to the corresponding carbon atoms. However, due to its low inherent sensitivity the INADEQUATE experiment can be applied to rather concentrated solutions only. For this reason the one-bond CC couplings could be measured for concentrated, in most cases, saturated solutions.

Discussion

The concentration dependent changes observed in the ^1H NMR (Table 1) and ^{13}C NMR spectra (Table 3) of **1** allow one to assume that some association $\rightleftarrows$ dissociation processes take place upon dissolution of the compound in [^2H]chloroform. The presence of two processes should be taken into account. One of them involves the formation of the solute–solvent aggregates. They consist of chloroform, which is a weak donor

of protons, and the molecules of 6,11-dimethyl-6*H*-indolo-[2,3-*b*]quinoline which is a weak base. Since, however, a large excess of the solvent is already present even in the most concentrated solutions (the solute/solvent ratio is *ca.* 1:1000 in 1 mol dm^{-3} solution) it seems justified to assume that further addition of the solvent should not influence the spectra significantly and only small or no changes at all will be observed upon dilution.

The other process which should be considered involves the formation of the solute–solute aggregates which are formed due to π–π interaction. These are expected to dissociate upon dilution. Numerous examples of such aggregates have already been reported in the literature and the nature of forces responsible for their formation studied.[8–14] Thus, for example, interactions between aromatic rings play an especially important role in stabilizing protein structures.[12]

π–π-Interactions are highly probable also in the compounds studied by us and may be responsible for their specificity and activity as drugs. A strong argument in their favour follows from the fact that selective up-field ^{1}H NMR shifts occur upon increase of concentration: ^{1}H1 (0.26 ppm), ^{1}H7 (0.33 ppm), ^{1}H10 (0.34 ppm), ^{1}H11-Me (0.45 ppm), ^{1}H-6-Me (0.31 ppm), ^{1}H2 (0.06 ppm), ^{1}H8 (0.16 ppm) and ^{1}H9 (0.13 ppm). The positions of the ^{1}H3 and ^{1}H4 resonances remain constant < 0.01 ppm (see Table 1 and also Experimental) which excludes the drift of the reference signal as the possible reason for the changes observed.

Although the data available do not allow us to make a decisive conclusion concerning the form and shape of associates present, it is judicious to assume that a dimer–monomer equilibrium prevails at the concentration range studied.

Based on this assumption the dimerization constant was calculated using ^{1}H NMR data collected in Table 1 for resonances ^{1}H1, ^{1}H7, ^{1}H10, 11-CH_3 and 6-NCH_3. Consistent results were obtained for all these protons, the k_{dim} average constant being of 0.16 dm^3 mol^{-1} (Table 8). In view of excellent fits obtained the possible contribution of higher aggrgates seems to be small (see Fig. 2).

An analysis of the NMR data collected for the compounds studied provides interesting information on the electron distribution within the molecule framework and on the interactions occurring in it.

In the ^{13}C NMR spectrum of the parent compound **1**, the lowest-field signals represent, as expected, the quaternary bridge carbons C4a (146.50 ppm), C5a (152.13 ppm) and C6a (142.59 ppm). This is caused by a very strong deshielding effect of both nitrogen atoms. The three remaining bridge carbon atoms are strongly shielded and appear at 121.28 ppm (C10a), 116.23 ppm (C10b) and 123.95 ppm (C11a). Out of the eight tertiary carbons present in this molecule, C3, C4 and C8 appear at 128.45, 128.01 and 127.18 ppm, respectively. The remaining proton bearing carbons resonate at much higher field strength, falling in the region of 108–124 ppm. Carbon C7 ($\delta_{^{13}C7}$ = 108.36 ppm) appears at the highest field. These results indicate that the highest electron density charge in the 6,11-dimethyl-6*H*-indolo[2,3-*b*]quinoline is concentrated at C7, C10b and C9. Negative charge can be also attributed to C1, C2, C10, C10a and C11a. Large positive charges are expected to be located on C4a, C5a and C6a.

The above observations are in a rough agreement with the INDO total charge density calculations performed for some representatives of the compounds studied (see Table 9).

It has been suggested by Hamilton and co-workers[12] that

Table 7 One-bond CC coupling constants in 6,11-dimethyl-6*H*-indolo[2,3-*b*]quinoline and its derivatives

	^1J(CC)/Hz						
CC	1	2	3	4	5	6	9
C1C2	60.6	62.4	60.1	62.4	73.1	60.1	73.1
C2C3	52.8	53.4	52.9	*a*	64.5	53.0	64.6
C3C4	*a*	59.9	*a*	*a*	61.3	*a*	62.5
C4C4a	64.4	64.5	64.5	64.7	64.7	64.4	64.6
C4aC11a	55.4	55.5	55.6	55.7	*a*	55.4	56.2
C5aC10b	55.8	56.3	55.7	56.0	56.3	55.9	56.5
C6aC10a	56.3	55.3	55.3	55.4	55.8	56.2	56.5
C6aC7	67.2	66.9	67.1	67.4	66.5	68.0	67.8
C7C8	58.9	58.8	59.4	58.8	57.0	61.2	61.3
C8C9	*a*	57.5	57.5	59[b]	*a*	69.5	69.0
C9C10	57.9	57.6	59[b]	59.4	57.8	69.7	70.0
C10C10a	63.7	63.8	64.5	64.4	64[b]	66.6	67.0
C10aC10b	59.1	59.0	59.0	59.0	*a*	58.9	59.0
C10bC11	67.2	67.1	67.9	67.4	67.4	67.1	67.6
C11C11a	55.7	55.8	55.7	55.2	56.6	55.8	56.2
C1C11a	*a*	58.6	*a*	58.5	60.5	*a*	60.5
C11CH_3	43.0	43.1	43.1	43.4	43.4	43.4	43.3
C2CH_3	—	44.8	—	45.0	—	—	—
C9CH_3	—	—	45.6	45.0	—	—	—

[a] Could not be determined. [b] Approximate values.

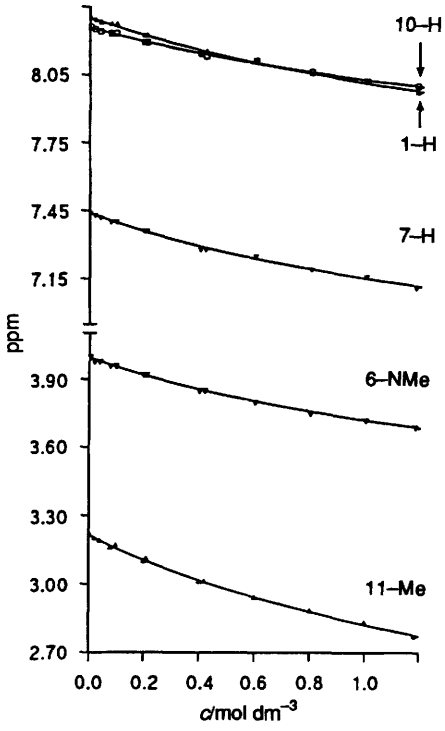


Fig. 2 Chemical shifts (δ) as a function of concentration (c/mol dm^{-3}) for H1, H7, H10, 11-Me and 6-NMe of compound **1** in $CDCl_3$ solutions

Table 8 Dimerization constant k and the calculated ^{1}H NMR chemical shifts (in ppm) of H1, H7, H10, 11-CH_3 and 6-NCH_3 for dimer and monomer of compound **1**; see also Experimental

	H1	H7	H10	11-CH_3	6-NCH_3
k/dm^3 mol^{-1}	0.15 ± 0.036	0.16 ± 0.037	0.15 ± 0.032	0.16 ± 0.026	0.17 ± 0.025
Dimer, δ	6.79 ± 0.24	6.04 ± 0.20	7.06 ± 0.17	1.25 ± 0.21	2.70 ± 0.12
Monomer, δ	8.30 ± 0.004	7.44 ± 0.004	8.26 ± 0.003	3.22 ± 0.004	4.00 ± 0.003

such uneven charge distribution across the π-system may give rise to the attractive electrostatic interaction between the molecules leading to their association. The compounds studied by these authors were, among others, 1-butylthymine and a diester macrocycle containing naphthalene fragment, which formed a strong π–π complex in $CDCl_3$. Selective upfield shifts in the thymine CH and CH_3 ^1H NMR resonances (0.17 and 0.16 ppm, respectively) have been invoked by these authors as evidence for the face-to-face geometry of this complex.

An analysis of the ^1H and ^{13}C NMR chemical shift data collected in Tables 2 and 4 clearly shows that an influence of substituents is limited strictly to that ring only to which a substituent is attached. No changes are observed in the part of the spectrum corresponding to ring A upon substitution in ring D and *vice versa*. As a result, the whole fragments of the spectra are transferable for the various molecules provided that the measurements are carried out under precisely the same conditions, *i.e.* at the same concentration and temperature. The conclusions drawn from the NMR data are in accord with those based on the total electron density calculations (see Table 9).

The further inspection shows that there is quite a remarkable difference between the electron distribution within the aromatic rings of the quinoline and indolo moieties. It manifests itself in the ^{13}C NMR chemical shift arrangement around the rings, in substituent β effects exerted on the ^{13}C chemical shifts of the *ortho* carbons, and in the magnitude of one-bond CC coupling constants and of three-bond ^1H–^1H couplings. Whereas the ^{13}C chemical shifts of ring D (the indolo part) alternate in a way typical for benzene derivatives, those of ring A (the quinoline part) do not (see Table 4). Thus, for example, the $\delta_{^{13}C}$ chemical shifts in ring D of compound **1** are 108.36 (C7), 127.18 (C8), 119.71 (C9) and 123.42 (C10), and 128.01 (C4), 128.45 (C3), 122.45 (C2) and 123.98 (C1) ppm in ring A. The same pattern holds for the remaining compounds if no substituent effects are involved (Table 4).

Quite remarkable differences are observed between the β substituent effects, A_{ij}, in rings A and D (Table 10). The β effects of the methoxy group exerted on C8 and C10 do not differ substantially from each other (−13.05 and −14.88 ppm, respectively) and are very similar to those observed in methoxybenzene (−14.4 ppm).[15] The situation is dramatically different in ring A, which belongs to the quinoline fragment. The β effect of the methoxy group on carbon C1 (compounds **5**, **8** and **9**) is as large as −21.72 ppm causing its very large shielding. The effect on C3 is, on the other hand, almost three times smaller, at −7.94 ppm only. The sum of these two effects is, however, very close to the sum of β-substituent effects in ring D, $\Sigma A_{2j} = -29.66$ and $\Sigma A_{9j} = -27.94$ ppm, respectively (−28.8 ppm in benzene). Similar non-equivalence of substituent effects occurs for the 2-fluoro and 2-methyl substituents, $A_{21}(F) = -16.65$ ppm, $A_{23}(F) = -10.25$ ppm, $A_{21}(CH_3) = -1.06$ ppm and $A_{23}(CH_3) = +2.17$ ppm. In both cases once again the sum ΣA_{2j} remains similar to that observed in benzene, ΣA_{2j} (F) = −26 ppm and $\Sigma A_{2j}(CH_3) = +1.4$ ppm.[15] Our observations are in accord with those reported by Zuika *et al.*,[16] who studied substituent effects in 2-methoxy, 6-methyl, 6-chloro and 6-bromo derivatives of quinoline, 8-mercaptoquinoline and methylthioquinoline (see Table 10 where the relevant A_{ij} values for derivatives of quinoline are included). The presence of a large negative charge on C5 in quinoline (C1 in indoloquinolines) was invoked by these authors in order to explain the differences observed between the $A_{65}(OCH_3)$ and $A_{67}(OCH_3)$ values in quinoline. This conclusion is confirmed by the semiempirical calculations performed in our work (see Table 9). Another interesting observation concerns the magnitude of spin–spin couplings in rings A and D (see Table 5 and 7).

Table 9 INDO net charges calculated for 6,11-dimethyl-6*H*-indolo[2,3-*b*]quinoline and some of its derivatives using Hyperchem software package (Autodesk Inc.)

C	**1**	**2**	**5**[a]	**6**[b]	**9**[a,b]
C1	−0.030	−0.047	−0.053	−0.029	−0.053
C2	−0.040	−0.010	+0.141	−0.040	+0.141
C3	−0.016	−0.025	−0.025	−0.015	−0.025
C4	−0.041	−0.039	−0.038	−0.041	−0.038
C4a	+0.163	+0.158	+0.155	+0.164	+0.155
C5a	+0.265	+0.263	+0.262	+0.266	+0.263
C6a	+0.104	+0.104	+0.104	+0.091	+0.092
C7	−0.053	−0.053	−0.053	−0.052	−0.053
C8	−0.022	−0.023	−0.022	−0.038	−0.038
C9	−0.043	−0.043	−0.043	+0.138	+0.138
C10	−0.030	−0.030	−0.030	−0.048	−0.048
C10a	−0.013	−0.013	−0.013	−0.010	−0.010
C10b	−0.032	−0.031	−0.030	−0.031	−0.029
C11	+0.047	+0.045	+0.045	+0.049	+0.045
C11a	−0.004	−0.003	−0.001	−0.004	−0.001

[a] Average for substituent OCH_3 *s-trans* and *s-cis* arranged with respect to bond C1C2. [b] Average for substituent OCH_3 *s-trans* and *s-cis* arranged with respect to bond C9C10.

Table 10 β-Substituent effects, A_{ij}, of substituent at carbon i on ^{13}C NMR chemical shifts, δ, of carbon j in substituted 6,11-dimethyl-6*H*-indolo[2,3*b*]quinolines

Compd.	$A_{21}(OCH_3)$	$A_{23}(OCH_3)$	Compd.	$A_{910}(OCH_3)$	$A_{98}(OCH_3)$
5	−21.57	−7.87	**6**	−14.88	−13.05
8	−21.80	−8.02	**7**	−14.84	−13.04
9	−21.80	−7.92	**9**	−14.90	−13.08
Average	−21.72	−7.94		−14.87	−13.06
Quinoline	−23.3[a]	−7.8[a]			
Compd.	$A_{21}(CH_3)$	$A_{23}(CH_3)$	Compd.	$A_{910}(CH_3)$	$A_{98}(CH_3)$
2	−1.05	+2.20	**3**	+0.29	+0.94
4	−1.05	+2.09	**4**	+0.27	+0.81
7	−1.07	+2.23	**8**	+0.33	+0.96
Average	−1.06	+2.17		+0.30	+0.90
Quinoline	−1.1[a]	+2.2[b]			
Compd.	$A_{21}(F)$	$A_{23}(F)$			
10	−16.65	−10.25			

[a] Corresponds to A_{65} in quinoline. [b] Corresponds to A_{67} in quinoline.

Whereas the values of vicinal ^3J(H8H9) and ^3J(H9H10) couplings, and one-bond ^1J(C8C9) and ^1J(C9C10) are close to each other within each pair mentioned, the corresponding couplings in ring A differ significantly from each other. The vicinal ^3J(H1H2) couplings are 1.7 Hz larger than those between H2H3 and one-bond C1C2 couplings are consistently larger than those across C2C3 bond. There is no doubt that the above results consistently indicate that there is quite a significant difference in bond order between C1C2 and C2C3 bonds, that of the latter being the lower one.

An especially interesting piece of information on an electron distribution within a given molecule can be extracted from one-bond CC coupling constants, ^1J(CC), whose magnitude is related to the Fermi contact term, *i.e.* to the density of s-electrons. A typical aromatic ring ^1J(CC) value is of 56 Hz (in benzene).[15] The ^1J(CC) couplings in pyridine are ^1J(C2C3) = 54.3 Hz and ^1J(C3C4) = 53.7 Hz.[15] It is also already well established that the ^1J(CC) coupling values increase significantly upon an increase in the electronegativity of substituent attached to a given bond.[17] Another characteristic feature of the one-bond CC couplings is their well defined stereospecificity towards the mutual orientation between the nitrogen lone pair and the carbon–carbon bond involved.[18,19] The ^1J(CC) values increase by *ca.* 9 Hz if the Csp^2Csp^2 bond is *cis* arranged with respect to the lone pair. This has been observed for various types of compounds such as oximes and imines and quinoline as well.[15,18,19]

A glance at the coupling ^1J(CC) values determined for all 6*H*-indolo[2,3-*b*]quinolines (see Table 7) shows that the coupling constants between the bridge carbon atoms, ^1J(C4aC11a), ^1J(C5aC10b) and ^1J(C6aC10a) have values very close to those observed in benzene, *i.e.* of *ca.* 56 Hz. All bonds in question are *trans* arranged with respect to the lone pair of nitrogens present in the molecules. On the other hand, a substantial increase in ^1J(CC) values across C4–C4a and C6a–C7 bonds, up to *ca.* 64 and 67 Hz, respectively, can be attributed to the influence of the lone pair arranged *cis* with respect to the bonds involved. Another interesting result concerns ^1J(CC) coupling across the C10b–C11 bond. Its value of *ca.* 67 Hz in all compounds is very close to that in unsubstituted ethylene (67.8 Hz) indicating that the C10b–C11 bond order is very high and close to that in a double bond. Rather unexpectedly large ^1J(CC) values of *ca.* 64 Hz are observed also across the C10–C10a bond. An effect of 2- and 9-methoxy groups on the ^1J(CC) couplings across the neighbour CC bonds, *i.e.* C1C2, C2C3, C8C9 and C9C10, is typical of this group (12 Hz in methoxybenzene) and varies from 11.5 Hz for ^1J(C8C9) to 12.5 Hz for ^1J(C1C2) causing an increase of these couplings by a comparison with unsubstituted compounds.

A few words should be devoted to ^nJ(CF) couplings, which are a very useful diagnostic tool in assignment of the carbons. Their values decrease monotonically upon increase of the number of separating bonds. This is also valid in the case of compound **10** studied in our work with one exception. Whereas the ^4J(CF) coupling of the expected value of *ca.* 5 Hz was found for C11, the four-bond coupling between fluorine and C4a was not observed. Since assignment of this carbon based on an SPT INEPT experiment is unequivocal we assume that in this particular case the ^4J(C4a–2F) coupling value, most probably due to influence of the nitrogen lone pair, is equal to zero. The long-range ^nJ(H,H) coupling constants (n = 4 and 5) determined for the compounds studied are presented in Table 6.

Conclusions

6,11-Dimethyl-6*H*-indolo[2,3-*b*]quinoline and its derivatives provide a new example of the compounds which in solution undergo self-association. The formation of the π–π stacking complexes may be suggested on the basis of the concentration dependent changes observed in the ^{1}H and ^{13}C spectra of the studied compounds. Full and unambiguous assignment of all ^{1}H and ^{13}C signals and the set of one-bond CC couplings, ^1J(CC), form a good starting point to further studies on the nature of interaction between indoloquinolines and biological molecules.

Experimental

The compounds studied were synthesized according to the procedures described earlier.[1]

All NMR experiments were carried out on a Bruker AM 500 instrument for samples dissolved in [^{2}H]chloroform, at 303 K. The solvent signal (calibrated against Me_4Si) was used as the reference for ^{1}H and ^{13}C spectra. The ^{1}H NMR measurements were performed at 500 MHz in purified and dried $CDCl_3$ for 0.08 mol dm^{-3} solutions. The digital resolution applied was 0.17 Hz/point. This gives an accuracy of ^{1}H NMR chemical shift values of 0.0002 ppm. However, taking into account all other possible sources of errors, such as for example small fluctuations of temperature, errors made during preparation of a sample, possible small drift of the reference signal *etc.*, we report the $\delta_{^1H}$ values up to 0.01 ppm. The ^{13}C NMR experiments were performed at 125.76 MHz for 0.2 mol dm^{-3} solutions; owing to the limited solubility saturated solutions for compounds **8** and **10** were used. Typical conditions were: acquisition time 1.1 s, relaxation delay 2 s, pulse width 3 μs (flip angle 45°). Two-dimensional experiments (COSY ^{1}H–^{1}H, HETCOR ^{1}H–^{13}C, COLOC ^{1}H–^{13}C) were performed for 0.2 mol dm^{-3} solutions. The HETCOR and COLOC experiments were adjusted for 160 and 6 Hz, respectively. One-dimensional ^{13}C INADEQUATE spectra adjusted for ^1J(CC) of 60 Hz were recorded overnight with the standard Bruker microprogram (32-phase Freeman cycle with automatic data storage).

The dimerization constant, k, was calculated according to the procedure described below. The concentration-dependent chemical shift changes are assumed to be governed by the equation comprising only the equilibrium dimer/monomer, thus

$$\delta_{obs}(c) = \delta_{mono} + (\delta_{dim} - \delta_{mono})\,(4kc + 1 - \sqrt{8kc + 1})/4kc$$

where c is the molar concentration of the compound and the dimerization constant is defined according to:

$$k = c_{dim}/c^2{}_{mono}$$

The equation was least-squares fitted to the experimental data of Table 1 with δ_{mono}, $(\delta_{dim} - \delta_{mono})$, and k treated as variable parameters. The best-fit values (95% of confidence) at these parameters and the standard errors thereof are given in Table 8. The best-fit curves for the protons H1, H7, H10, 11-CH_3 and 6-NCH_3 are displayed in Fig. 2.

The INDO net charges were calculated using Hyperchem software package (Autodesk Inc.).

Acknowledgements

This work was supported by KBN (Committee of Scientific Research) under grant No. 2 0622/91/01.

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Paper 5/01172H
Received 27*th February* 1995
Accepted 18*th April* 1995