Polish J. Chem., **74**, 1599–1608 (2000)

^{1}H and ^{13}C NMR Studies of 5,6,11-Trimethyl-6*H*-indolo[2,3-*b*]quinolinium Methylsulfate and Some of Its Derivatives

by L. Kania[1], K. Kamieńska-Trela[1*] and Ł. Kaczmarek[2]

[1]*Institute of Organic Chemistry, Polish Academy of Sciences, Kasprzaka 44, 01-224 Warsaw, Poland*
[2]*Pharmaceutical Research Institute, Rydygiera 8, 01-793 Warsaw, Poland*

(Received May 30th, 2000; revised manuscript July 20th, 2000)

^{1}H and ^{13}C NMR spectra of the parent 5,6,11-trimethyl-6*H*-indolo[2,3-*b*]quinolinium methylsulfate and of its nine 2- and 9-methyl-, methoxy- and fluoro-substituted derivatives were measured and assigned from COSY ^{1}H-^{1}H, HETCOR ^{1}H-^{13}C and SPT INEPT experiments. Proton and carbon-13 chemical shifts and long range spin-spin coupling constants ($^{n}J_{HH}$, n = 3, 4) were considered in terms of the electron density distribution in the indoloquinolinium moiety and compared with the corresponding data obtained earlier for 5,11-dimethyl-5*H*-and 6,11-dimethyl-6*H*-indolo[2,3-*b*]quinoline derivatives. The sensitivity of the proton chemical shifts to the changes in concentration was found for all the compounds studied.

Key words: 5,6,11-trimethyl-6*H*-indolo[2,3-*b*]quinolinium derivatives, ^{1}H and ^{13}C NMR spectra, spin-spin coupling constants, concentration dependence of $\delta(^{1}H)$

Tetracyclic planar indolo[2,3-*b*]quinolines, related in structure to indole alkaloid ellipticine, have recently been investigated in terms of their cytotoxic activity and interaction with DNA. Two series of compounds: 5*H*- and 6*H*-indolo[2,3-*b*]quinoline derivatives were synthesized and the relationship between their structure and biological activity was studied [1,2]. Of the compounds investigated, only derivatives belonging to 5*H*-series have shown the DNA binding properties and cytotoxic activity and might be expected to be a new class of cytotoxic DNA topoisomerase II inhibitors. None of the 6*H*-indolo[2,3-*b*]quinolines was found to display cytotoxic activity. The results of the preliminary evaluation of indoloquinolines for their cytotoxic and DNA binding properties have confirmed the significance of both the shape of the molecule and the position and character of the substituents for biological activity of the compounds studied. These observations clearly indicate that the electron density distribution throughout the indolo[2,3-*b*]quinoline molecules is of deciding importance for specifity of their action. In view of the foregoing, the ^{1}H and ^{13}C NMR spectroscopy constitutes a suitable means of investigating the electronic structure of indoloquinoline system as well as the nature of solute-solute and solute-solvent interactions in the solutions of the compounds studied.

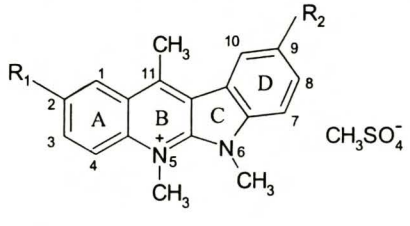
* To whom the correspondence should be addressed.

The biological significance of the indolo[2,3-*b*]quinoline system has prompted us to measure the ^{1}H and ^{13}C NMR spectra of the compounds belonging to both 5*H*- and 6*H*-series of indoloquinolines [3,4]. An analysis of the experimental NMR parameters: $\delta(^{1}H)$, $\delta(^{13}C)$ and spin-spin coupling constants, in particular one-bond carbon-carbon couplings, has provided valuable information on the electron density distribution within the indolo[2,3-*b*]quinoline framework.

The potential application of the indoloquinolines in chemotherapy has been considerably limited by their poor water solubility. Structure-activity relationship studies have shown [1,2], that compounds of the 5*H*-series are partially protonated under physiological conditions (pH 7.4) and at low pH values occur in the form of salts, which made them better soluble in water. In an attempt to avoid the limitations concerning poor solubility in water, a novel class of indolo[2,3-*b*]quinoline derivatives with additional methyl substitution of nitrogen atom N_5 has been synthesized [5]. The 5,6,11-trimethyl-6*H*-indolo[2,3-*b*]quinolinium salts have shown good solubility in water and met all the requirements for cytotoxic and antimicrobial activity. The structure-activity relationship studies have proved strong antimicrobial and cytotoxic properties of the indoloquinolinium salts [5]. At this point a question arises as to whether the additional methyl substitution of the N_5 atom in the indoloquinoline moiety, leading to indoloquinolinium charged system, can affect the redistribution of the electron density in a molecule. In order to throw light on this problem, we decided to perform an analysis of the ^{1}H and ^{13}C NMR spectra of the 5,6,11-trimethyl-6*H*-indolo[2,3-*b*]quinolinium methylsulfate and some of its derivatives. The sensitivity of the proton chemical shifts to the changes in concentration is also discussed.

RESULTS AND DISCUSSION

The compounds studied in this work are the parent 5,6,11-trimethyl-6*H*-indolo[2,3-*b*]quinolinium methylsulfate (**1**) and the following 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**).



The complete ^{1}H and ^{13}C assignments for a series of 6*H*-indolo[2,3-*b*]quinolinium derivatives were carried out by 2D proton-proton and carbon-proton correlated experiments in addition to the standard DEPT, SPT INEPT and NOE experiments. The $^{n}J_{CF}$ coupling constants observed in ^{13}C spectrum of 2-fluoro derivative (compound

10) were also of significant diagnostic importance in the assignment of carbon resonances. Due to the low solubility of the compounds studied in DMSO-d_6, INADEQUATE experiments could be performed.

The ^1H and ^{13}C chemical shift assignments are given in Tables 1 and 2. The vicinal $^3J_{HnHm}$ and long-range $^4J_{HnHm}$ coupling constants are collected in Tables 3 and 4.

Table 1. ^1H NMR chemical shifts for 5,6,11-trimethyl-6*H*-indolo[2,3-*b*]quinolinium derivatives (in ppm)[a].

H_n	1	2	3	4	5	6	7	8	9[b]	10
H_1	8.56	8.42	8.59	8.36	7.86	8.63	8.39	7.79	7.87	8.47
H_2	7.86	–	7.82	–	–	7.84	–	–	–	–
H_3	8.14	7.96	8.10	7.93	7.76	8.12	7.93	7.71	7.76	8.06
H_4	8.41	8.31	8.36	8.27	8.35	8.37	8.26	8.30	8.32	8.49
H_7	7.92	7.89	7.75	7.75	7.88	7.82	7.80	7.72	7.81	7.91
H_8	7.76	7.75	7.50	7.52	7.75	7.32	7.32	7.51	7.37	7.78
H_9	7.56	7.55	–	–	7.54	–	–	–	–	7.57
H_{10}	8.36	8.45	8.17	8.20	8.46	7.85	7.81	8.18	7.88	8.47
11-Me	3.31	3.28	3.24	3.24	3.29	3.28	3.24	3.22	3.28	3.28
5-N$^+$Me	4.66	4.63	4.60	4.59	4.64	4.61	4.58	4.59	4.60	4.66
6-NMe	4.31	4.29	4.25	4.25	4.28	4.27	4.24	4.22	4.24	4.30
2-Me	–	2.60	–	2.57	–	–	2.58	–	–	–
9-Me	–	–	2.46	2.49	–	–	–	2.48	–	–
2-OMe	–	–	–	–	4.01	–	–	3.98	4.02	–
9-OMe	–	–	–	–	–	3.90	3.89	–	3.93	–

[a]spectra recorded in 0.08 mol dm^{-3} DMSO-d_6 solutions if not otherwise stated
[b]saturated solution in DMSO-d_6 (0.036 mol dm^{-3}).

Table 2. ^{13}C NMR chemical shifts for 5,6,11-trimethyl-6*H*-indolo[2,3-*b*]quinolinium derivatives (in ppm)[a].

C_n	1	2	3	4	5	6	7	8	9[b]	10[c]
C_1	126.6	125.7	126.6	125.7	106.3	126.7	125.7	108.2	106.5	111.3
C_2	125.9	135.7	125.8	135.6	156.7	125.7	135.5	156.6	156.7	159.2
C_3	133.3	134.9	133.2	134.8	123.6	133.4	134.9	123.4	123.5	121.8
C_4	117.6	117.4	117.5	117.3	119.3	117.5	117.3	119.1	119.1	120.5
C_{4a}	137.0	135.3	136.9	135.2	131.9	137.1	135.3	131.8	132.0	133.9
C_{5a}	148.2	147.8	147.9	147.5	147.2	148.0	147.5	146.9	147.2	148.3
C_{6a}	142.6	142.6	140.6	140.6	142.7	136.6	136.6	140.7	136.9	142.9
C_7	111.8	111.7	111.4	111.3	111.6	112.6	112.5	111.2	112.4	111.8
C_8	129.4	129.4	130.2	130.2	129.5	116.2	116.2	130.2	116.3	129.9
C_9	124.0	123.9	133.4	133.3	123.7	156.3	156.2	133.1	156.1	124.1
C_{10}	124.0	124.0	124.0	124.0	124.1	108.5	108.3	124.0	108.6	124.2
C_{10a}	119.9	119.9	119.9	119.9	119.8	120.6	120.4	119.7	120.5	119.6
C_{10b}	120.6	120.5	120.5	120.4	120.8	120.6	120.6	120.7	120.9	121.7
C_{11}	148.5	148.1	148.3	147.9	147.4	148.8	147.3	147.1	147.7	147.8
C_{11a}	123.2	123.3	123.1	123.1	124.7	123.0	123.0	124.5	124.4	124.7
11-Me	16.1	16.1	16.1	16.1	16.3	16.1	16.1	16.3	16.3	16.3
5-N$^+$Me	39.6	39.5	39.6	39.4	39.7	39.5	39.5	39.5	39.4	40.0
6-NMe	35.3	35.3	35.3	35.3	35.3	35.4	35.3	35.3	35.3	35.4
2-Me	–	20.6	–	20.5	–	–	20.5	–	–	–
9-Me	–	–	20.9	20.9	–	–	–	20.8	–	–
2-OMe	–	–	–	–	56.1	–	–	56.0	56.1	–
9-OMe	–	–	–	–	–	55.9	55.9	–	56.0	–

[a]spectra recorded in 0.08 mol dm^{-3} DMSO-d_6 solutions if not otherwise stated,
[b]saturated solution in DMSO-d_6 (0.036 mol dm^{-3}),
[c]carbon-fluorine couplings: $^1J_{C2F2}$ = 245.2 Hz, $^2J_{C1F2}$ = 23.9 Hz, $^2J_{C3F2}$ = 25.1 Hz, $^3J_{C4F2}$ = 9.0 Hz, $^3J_{C11aF2}$ = 9.2 Hz, $^4J_{C4aF2}$ = 0 Hz, $^4J_{C11F2}$ = 4.1 Hz.

Table 3. Vicinal coupling constants, $^3J_{HnHm}$, in 5,6,11-trimethyl-6*H*-indolo[2,3-*b*]quinolinium derivatives (in Hz)[a].

$^3J_{HnHm}$	1	2	3	4	5	6	7	8	9[b]	10[c]
$^3J_{H1H2}$	8.2	–	8.4	–	–	8.5	–	–	–	–
$^3J_{H2H3}$	7.0	–	7.0	–	–	7.0	–	–	–	–
$^3J_{H3H4}$	8.8	8.9	8.9	8.9	9.5	8.8	8.9	9.5	9.5	9.6
$^3J_{H7H8}$	8.3	8.3	8.3	8.3	8.3	8.9	8.9	8.4	8.9	8.3
$^3J_{H8H9}$	7.4	7.3	–	-	7.4	–	–	–	–	7.4
$^3J_{H9H10}$	7.9	7.9	–	-	8.1	–	–	–	–	7.9

[a]spectra recorded in 0.08 mol dm^{-3} DMSO-d_6 solutions if not otherwise stated
[b]saturated solution in DMSO-d_6 (0.036 mol dm^{-3}),
[c] $^3J_{H3,2F}$ = 7.64 Hz.

Table 4. Long-range proton-proton coupling constants, $^4J_{HnHm}$, in 5,6,11-trimethyl-6*H*-indolo[2,3-*b*]quinolinium derivatives (in Hz)[a].

$^4J_{HnHm}$	1[b]	2	3[c]	4	5	6	7	8	9[d]	10
$^4J_{H1H3}$	1.4	2.0	1.4	2.0	2.8	1.4	2.0	2.9	2.7	e
$^4J_{H2H4}$	1.0	–	e	–	–	1.0	–	-	-	–
$^4J_{H7H9}$	1.0	–	–	-	1.0	-	–	–	-	1.0
$^4J_{H8H10}$	1.2	e	1.6	1.6	1.0	2.5	2.5	1.6	2.4	1.2
$^4J_{H3,2Me}$	–	0.6	–	0.6	–	–	0.6	-	-	–
$^4J_{H9Me}$	–	–	0.6	0.7	–	–	-	0.7	–	–

[a]spectra recorded in 0.08 mol dm^{-3} DMSO-d_6 solutions if not otherwise stated,
[b] $^5J_{H1H4}$ = 0.39 Hz,
[c] $^5J_{H1H4}$ = 0.44 Hz
[d]saturated solution in DMSO-d_6 (0.036 mol dm^{-3}),
[e]not found.

The ^{1}H NMR spectra of indoloquinolinium salts revealed the characteristic feature observed for the whole family of indolo[2,3-*b*]quinolines, *i.e.* the aromatic region consisted of two overlapping sets of the signals of protons attached to rings A and D, a discrimination of which was not a straightforward task. An analysis of the ^{1}H spectrum of the compound **9** can serve as an example. As the first step the NOE experiment, which involved an irradiation of the signal of the methyl group attached to C_{11} (easily distinguished singlet at 3.28 ppm), was performed. This caused a strong enhancement of the signal at 7.87 ppm, which allowed us to ascribe it to H_1. This result, in turn, was used as a starting point in the COSY experiment, leading to unambiguous assignment of the protons to particular positions in rings A and D, respectively.

Special attention had to be paid to the reliable differentiation between the methyl groups attached to the nitrogen atoms N_5 and N_6. This was realized by the NOE experiment and confirmed by the HETCOR pulse sequence. Thus, for the saturated solution of the compound **9**, an irradiation of the singlet at 4.60 ppm resulted in a strong enhancement of the H_4-signal at 8.32 ppm; this enabled us to attribute the former to the N_5-methyl protons. Further support for such assignment was provided by the response of the H_7-signal (7.81 ppm) upon irradiation of the N_6-CH_3 group (4.24 ppm). The NOE experiments were also helpful in unequivocal assignment of the closely positioned signals of two methyl as well as two methoxy substituents attached to the carbon atoms C_2 and C_9 of the indoloquinolinium framework (compounds **4** and **9**,

respectively). Further assistance in proton assignment followed from an analysis of the vicinal $^{3}J(H_nH_m)$ and long-range $^{4}J(H_nH_m)$ proton-proton coupling constants.

The unambiguous assignment of the proton signals proved to be useful for the interpretation of the chemical shifts in the ^{13}C domain of the ^{13}C-^{1}H heterocorrelated spectra (Table 2). Thus, tertiary and quaternary carbon resonances were distinguished and ascribed by correlating the proton assignments with the ^{13}C signals in the HETCOR experiments. Further support for these assignments was provided by the Overhauser effect, the signals of the tertiary carbon nuclei being more intense than the quaternary carbon atoms. DEPT experiment performed for the compound **9** confirmed the conclusions drawn from the HETCOR spectra and Overhauser effect. Of the three quaternary bridge carbon atoms in the close vicinity of N_5 and N_6, two of them: C_{5a} and C_{6a} were discriminated owing to the very strong deshielding effect of the nitrogen atom N_6. The signal of the third one, *i.e.* C_{4a}, appeared in the same range as that measured for derivatives of the 5*H*-series; it experienced upfield shift relative to the corresponding values obtained for the 6*H*-derivatives. Three closely spaced signals in the region of 119.7 to 124.7 ppm were attributed to the remaining bridge carbon atoms C_{10a}, C_{10b} and C_{11a}. To support the assignment of the closely positioned quaternary carbon nuclei: C_{5a} towards both C_{6a} and C_{11}, as well as C_{10a} towards C_{10b}, SPT INEPT experiments were carried out for all the compounds studied. Moreover, the substituent effects of fluorine as well as methyl and methoxy groups additionally confirmed the assignment of the carbon atoms C_2 and C_9.

There is a considerable amount of evidence, derived from measurements on various classes of compounds as well as the correlation equations obtained on the basis of the semiempirical and *ab initio* calculations, to suggest that ^{13}C chemical shifts are related to the total electron densities at carbon nuclei, *i.e.* an increase in the electron density at a carbon nucleus causes an increase in the shielding [6,7].

Therefore, $\delta(^{13}C)$ is a valuable means of insight into the electron density distribution within the indoloquinolinium framework.

As in the case of the 5*H*- and 6*H*-compounds [3,4], the nitrogen atoms N_5 and N_6 exert a significant deshielding effect on the carbon nuclei in their close vicinity; the signals in order of the increasing chemical shifts: C_{4a}, C_{6a} and C_{5a}. The three remaining quaternary carbon nuclei (C_{10a}, C_{10b} and C_{11a}) experience the significant shielding effect. The largest shielding, however, is observed for the tertiary carbon atoms in the proximity of N_5-CH_3 and N_6-CH_3 (C_4 and C_7, respectively). The analysis of the $\delta(^{13}C)$ data allows the conclusions concerning the charge distribution within the indoloquinolinium framework. Thus, the highest electron density charge is expected to be located symmetrically at the positions C_4 and C_7 of the benzene rings of the quinoline (ring A) and indole (ring D) moietes. The negative charge can also be attributed to the carbon atoms C_{10a}, C_{10b} and C_{11a} as well as C_9 and C_{10}. On the other hand, the large positive charges are supposed to be concentrated on the carbon nuclei C_{4a}, C_{5a} and C_{6a}.

Interesting conclusions can be drawn from the analysis of the substituent effects. Firstly, the influence of the substituents is closely confined to the ring to which the substituent is attached. The same phenomenon has been observed for the 5*H*- and 6*H*-derivatives [3,4]. For all the compounds studied, the α-effect of methyl groups is typical of aromatic and heteroaromatic compounds and causes deshielding of carbon atoms C_2 and/or C_9 by *ca* 9.5 to 10 ppm. Particularly interesting seems to be the effect of methoxy substitution of the C_2 and/or C_9 positions. The signals of the carbon atoms C_2 and/or C_9 experience the large downfield shifts: $\Delta\delta C_\alpha = 31–34$ ppm, which is a typical increment observed for methoxybenzene ($\Delta\delta C_\alpha = 31.4$ ppm) [6]. However, unexpectedly significant non-equivalence of β-effects is observed, depending on the location of the carbon atom (ring A or D) in which the substitution takes place. Thus, introduction of the methoxy group at C_9 (ring D in indole fragment) produces an upfield shift of both the C_8 and C_{10} resonances (13.2 and 13.5 ppm, respectively), which is in accord with the data found for methoxybenzene (14.4 ppm) [6]. On the other hand, methoxy substitution of the C_2 position (ring A in quinoline part) causes a particularly drastic shift of the C_1 signal towards higher field (β-effect of 20.3 ppm for **5**, 18.4 ppm for **8** and 20.1 ppm for **9**), whereas the C_3 resonances show much smaller upfield shifts (9.8 ppm on an average). The interesting point to note here is that the sum of the β-substituent effects in the ring A (29.4 ppm) and D (27.1 ppm) is close to that observed in benzene (28.8 ppm). The analogous differentiation of the β-substituent effects in ring A is also observed for 2-fluoro compound (**10**).

The replacement of the hydrogen atom H_2 by the methoxy group leads to deshielding of 1.5 ppm for the γ-carbon nucleus C_4. For the 9-methoxy substituent, the γ-effect observed on C_7 and C_{10a} is equal to 0.7 ppm. These values are very close to those observed in methoxybenzene (γ-effect: 1.0 ppm) [6]. The fluorine atom attached to C_2 exerts deshielding of 2.9 ppm on the carbon atom C_4; the γ-effect here is almost twice as large as that estimated for fluorobenzene (1.6 ppm) [6].

The substitution of the C_2 and C_9 carbon atoms of the indoloquinolinium molecule by the methoxy group or fluorine also results in appreciable upfield shifts of resonances attributed to the carbon nuclei in δ position to the place of substitution. Thus, both the 2-methoxy group and the 2-fluorine atom cause the shielding of the C_{4a} nucleus of 5.1 and 3.1 ppm, respectively. The carbon atom C_{6a} experiences upfield shift of 5.9 ppm upon substitution of C_9 position by the methoxy group. These results are slightly lower than the relevant values obtained for the benzene derivatives: 7.7 ppm for methoxybenzene and 4.4 ppm for fluorobenzene [6].

Another interesting point of the NMR spectra of the indoloquinolinium salts concerns the magnitude of the vicinal proton-proton coupling constants. From the data given in Table 3 we note that the largest $^3J_{HnHm}$ couplings observed are those across $C_3–C_4$ bond ($^3J_{H3H4} = 8.8$ Hz for **1**). The vicinal couplings across $C_1–C_2$ and $C_7–C_8$ bonds are slightly smaller (8.2 and 8.3 Hz respectively, for **1**). The smallest values of $^3J_{HnHm}$ couplings are those across $C_2–C_3$ and $C_8–C_9$ bonds; the diference between the largest ($^3J_{H3H4}$) and the smallest ($^3J_{H2H3}$) being 1.8 Hz. These observations indicate that there is a significant differentiation of bond orders in either of the two benzene

rings (A and D) in the indoloquinolinium framework. The reduced double bond character of the C_2–C_3 and C_8–C_9 bonds (as compared with C_1–C_2 and C_3–C_4 and C_7–C_8 and C_9–C_{10} in ring A and D, respectively) can be assumed.

The interesting spin-spin interactions are those across four bonds, transmitted *via* H_m–C_{sp2}–C_{sp2}–C_{sp2}–H_n path, $^4J_{HnHm}$. The coupling between meta protons in six-membered aromatic rings is generally between 0 and 3 Hz [8,9]. The values of $^4J_{HmHn}$ couplings obtained for indoloquinolinium derivatives (Table 4) are related to the presence of a substituent attached to ring A and/or D. They can be divided into three groups as follows: (a) the couplings between meta protons in nonsubstituted benzene ring A ($^4J_{H1H3}$, $^4J_{H2H4}$) or D ($^4J_{H7H9}$, $^4J_{H8H10}$) are within the range of 1.0–1.4 Hz, (b) the meta couplings in 2- and/or 9-methyl substituted benzene rings A ($^4J_{H1H3}$), and D ($^4J_{H8H10}$) vary from 1.6 to 2.0 Hz, and (c) the ^4J values in 2- and/or 9-methoxy substituted benzene rings A ($^4J_{H1H3}$) and D ($^4J_{H8H10}$) are within the range of 2.4–2.9 Hz. Much higher $^4J_{HmHn}$ spin-spin couplings for the methoxy substituted indoloquinolinium derivatives are attributed to the electronegativity of the substituent. Our observations are in agreement with those of Castellano and Sun [10], who performed the systematic investigations on the influence of electronegativity on the $^4J_{HH}$ couplings in a series of monosubstituted benzenes and showed the pronounced increase of these couplings with the increase of electronegativity of the substituents.

The spin-spin couplings transmitted *via* H_m–C_{sp3}–C_{sp2}–C_{sp2}–H_n path ($^4J_{H3Me}$ and $^4J_{H8Me}$) are within the range 0.6 to 0.7 Hz.

An important aspect of this work was to check whether the NMR spectra of the indoloquinolinium derivatives were sensitive to the changes in concentration. As we reported previously [4], the concentration dependence of chemical shifts in ^1H and ^{13}C NMR spectra of the 6*H*-indolo[2,3-*b*]quinolines indicated that some association-dissociation process took place upon dissolution of the compounds in chloroform. Two types of molecular interactions were assumed to be involved in this process: (1) the formation of solute-solvent aggregates due to intermolecular interactions between molecules of indoloquinoline derivative and the solvent and (2) the self-association of indoloquinoline molecules caused by π-π interactions between the aromatic rings. The dimer-monomer equilibrium was suggested to prevail in the solutions at the concentration range applied.The observed phenomenon is of particular importance as the specific interactions between the aromatic unit of the quinoline derivatives [11] (known for their DNA binding properties) and nucleotide bases are responsible for DNA complex formation.

Because of the limited solubility of the compounds studied, the NMR measurements could be performed in a very narrow range of concentrations: from 0.08 M to 0.01 M (for derivative **9**: 0.036 M to 0.009 M). The data collected in Table 5 show that for all the compounds studied the selective upfield shifts of the proton resonances occur upon increase of the concentration. The largest upfield shifts due to the increase in concentration are observed for resonances of the protons H_{10} (0.16 ppm in **1**, 0.14 ppm in **3**, 0.12 ppm in **8** and 0.10 ppm in **4**) and H_1 (0.12 ppm in **1**, 0.10 ppm in **4** and 0.009 ppm in **8**). On the other hand, the smallest shielding sensitivity to the changes in con-

centration (0.02 ppm to 0.05 ppm) is found for signals of the protons H_3 and H_4. It is interesting that the proton resonances of the methyl groups attached to the carbon atom C_{11} as well as to the nitrogen atom N_6 experience much smaller dependence on the concentration than those observed for the 6,11-dimethyl-6*H*-indolo[2,3-*b*]quinoline derivatives [4].

Table 5. The upfield shifts ($\Delta\delta_{11}$, in ppm) upon the increase of concentration for proton resonances measured for 5,6,11-trimethyl-6*H*-indolo[2,3-*b*]quinolinium derivatives.

H_n	1	2	3	4	5	6	7	8	9	10
H_1	0.12	0.05	0.07	0.10	0.06	0.04	0.07	0.09	0.03	0.04
H_2	0.03	–	0.05	–	–	0.03	–	–	–	–
H_3	0.02	0.03	0.04	0.05	0.03	0.02	0.05	0.05	0.02	0.02
H_4	0.02	0.02	0.04	0.03	0.03	0.02	0.03	0.03	0.02	0.02
H_7	0.02	0.03	0.06	0.05	0.03	0.04	0.04	0.05	0.02	0.03
H_8	0.05	0.04	0.11	0.08	0.04	0.09	0.07	0.08	0.03	0.04
H_9	0.05	0.04	–	–	0.04	–	–	–	–	0.04
H_{10}	0.16	0.05	0.14	0.10	0.06	0.08	0.10	0.12	0.04	0.04
11-Me	0.05	0.06	0.10	0.08	0.05	0.06	0.07	0.09	0.01	0.04
5-N^+Me	0.0	0.01	0.03	0.03	0.01	0.01	0.02	0.02	0.01	0.0
6-NMe	0.01	0.01	0.03	0.02	0.01	0.01	0.02	0.02	0.01	0.0
2-Me	–	0.03	–	0.03	–	–	0.04	–	–	–
9-Me	–	–	0.10	0.03	–	–	–	0.07	–	–
2-OMe	–	–	–	–	0.03	–	–	0.05	0.01	–
9-OMe	–	–	–	–	–	0.05	0.05	–	0.01	–

The limited set of the NMR data obtained for the indoloquinolinium methylsulfates does not allow to draw a decisive conclusion concerning the structure of the associates present in a solution. However, the similar character of the relationship between the concentration and chemical shifts observed for 6*H*-indolo[2,3-*b*]quinolines and 6*H*-indoloquinolinium methylsulfates justifies the suggestion that association-dissociation process takes place upon dissolution of indoloquinolinium derivatives in dimethylsulfoxide.

It is also worth noting that 2,9-dimethoxy indoloquinolinium methylsulfate (**9**), proved to display the highest DNA binding constants and the strongest cytotoxic activity [5], reveals the weakest shielding sensitivity to the changes in concentration in the whole series of indoloquinolinium salts. These observations imply that – in addition to ionic or electrostatic interactions – the steric factor, *i.e.* the shape of the molecule and the character of the substituents contributes to DNA complex formation.

Finally, there is a question how the additional methyl substitution of the N_5 atom in the indoloquinoline moiety affects the magnitude of the carbon-13 chemical shifts. We measured the ^{13}C NMR spectra for the parent 6,11-dimethyl-6*H*-indolo[2,3-*b*]quinoline ($\mathbf{I_A}$), its 5,6,11-trimethyl-6*H*-indolo[2,3-*b*]quinolinium salt ($\mathbf{I_B}$), and their derivatives: 2,9-dimethyl ($\mathbf{II_A}$ and $\mathbf{II_B}$) and 2,9-dimethoxy ($\mathbf{III_A}$ and $\mathbf{III_B}$) in 0.045 mol dm^{-3} solutions in DMSO-d_6. The results are reported in Table 6.

The analysis of the $\delta(^{13}C)$ data allows the following conclusions. The most important observation is the significant influence of the positively charged nitrogen atom N_5 on the electron density distribution within the quinoline moiety of the indoloquinolinium molecule. Thus, the large shielding of the carbon nuclei C_4, C_{4a} and C_{5a}

(*ca* 10 ppm, 9 ppm and 3.5 ppm, respectively) in the close vicinity of the nitrogen atom N_5 is observed on passing from 6*H*-indolo[2,3-*b*]quinoline to its indoloquinolinium derivative. On the other hand, an involvement of the nitrogen N_5-lone pair of electrons in the bond formation with the methyl group, induces large deshielding of the carbon atom C_{11} in the para position (*ca* 10 ppm). The signals of the remaining carbon atoms of the quinoline fragment: C_1, C_2, C_3, C_{10b} and 11-Me experience small to medium downfield shifts: from 1.4 ppm to 5.4 ppm. Particularly interesting is the fact, that the electron density on the bridge carbon atom C_{11a} practically does not respond to the quaternization of the nitrogen atom N_5. The same phenomenon concerns the carbon atoms of the indole part of the indoloquinolinium molecule: C_{6a}, C_{10} and C_{10a}. Out of the remaining carbon atoms involved in the indole moiety, C_7, C_8 and C_9 undergo the deshielding effect in the range of 1.3 ppm to 4.7 ppm. As can be expected, the additional methyl substituent at N_5 exerts a remarkable deshielding effect on the methyl group at N_6 (*ca* 8 ppm).

Table 6. ^{13}C NMR chemical shifts for the parent 6,11-dimethyl-6*H*-indolo[2,3-*b*]quinoline $\mathbf{I_A}$, its 2,9-dimethyl $\mathbf{II_A}$ and 2,9-dimetoxy $\mathbf{III_A}$ derivatives, and for the indoloquinolinium salts: the parent 5,6,11-trimethyl-6*H*-indolo[2,3-*b*]quinolinium methyl-sulfate $\mathbf{I_B}$, 2,9-dimethyl $\mathbf{II_B}$ and 2,9-dimethoxy $\mathbf{III_B}$ derivatives (in ppm)[a].

C_n	$\mathbf{I_A}$	$\mathbf{I_B}$	$\mathbf{II_A}$	$\mathbf{II_B}$	$\mathbf{III_A}$	$\mathbf{III_B}$
C_1	124.5	126.7	123.3	125.7	102.9	106.5
C_2	122.5	125.9	131.4	135.6	154.6	156.7
C_3	128.6	133.4	130.6	134.8	120.8	123.6
C_4	127.6	117.7	127.3	117.3	128.9	119.2
C_{4a}	145.9	137.1	144.3	135.2	141.8	132.0
C_{5a}	151.4	148.4	151.3	147.7	150.7	147.2
C_{6a}	142.2	142.6	140.3	140.7	136.9	136.9
C_7	109.0	111.8	108.6	111.4	109.4	112.5
C_8	127.5	129.5	128.3	130.2	115.0	116.3
C_9	119.9	124.0	128.6	133.3	153.5	156.1
C_{10}	123.5	124.1	123.6	124.0	108.2	108.6
C_{10a}	120.4	120.0	120.5	120.0	120.6	120.5
C_{10b}	115.5	120.7	115.4	120.5	115.6	120.9
C_{11}	138.9	148.6	138.0	147.9	137.6	147.7
C_{11a}	123.5	123.3	123.4	123.2	123.7	124.4
11-Me	14.8	16.2	14.8	16.2	14.9	16.3
5-NMe[b]	–	39.6	–	39.4	–	39.5
6-NMe	27.4	35.4	27.4	35.3	27.4	35.4
2-Me	–	–	21.3	20.6	–	–
9-Me	–	–	21.1	20.9	–	–
2-OMe	–	–	–	–	55.4	56.1
9-OMe	–	–	–	–	55.9	56.0

[a]spectra recorded in 0.045 mol dm^{-3} solutions in DMSO-d_6,
[b]5-N^+Me in the case of indoloquinolinium derivatives.

EXPERIMENTAL

The compounds studied were prepared by the published procedure [5]. All NMR measurements were carried out in standard 5 mm tubes at 300 K using a Bruker AM 500 spectrometer system at a proton and a carbon resonance frequency 500.13 MHz and 125.76 MHz, respectively. Because of the limited solubility of indoloquinolinium salts in most of the organic solvents, all NMR spectra had to be carried out in

DMSO-d_6, in which the solubility of the compounds studied was the best. In consequence of the low concentrations of the solutions, INADEQUATE experiments could be performed.

The ^{1}H NMR spectra were recorded in DMSO-d_6 solutions at the concentrations 0.08 mol dm^{-3} and 0.01 mol dm^{-3}. The digital resolution applied was 0.17 Hz/point. The ^{13}C NMR measurements and two-dimensional experiments (COSY ^{1}H-^{1}H, HETCOR ^{1}H-^{13}C) were performed for 0.08 mol dm^{-3} solutions in DMSO-d_6; due to the limited solubility saturated solution for compound **9** (*ca* 0.036 mol dm^{-3}) was used. The solvent signal, calibrated against Me_4Si, was used as a reference for proton and carbon-13 spectra. The typical parameters were as follows: acquisition time 1.1 s, relaxation delay 2 s and pulse width 3 μs. The HETCOR experiments were adjusted for one-bond carbon-proton coupling constants of 160 Hz.

Acknowledgment

We gratefully acknowledge support from the Polish State Committee for Scientific Research, grant No 3 TO9A 033 10.

REFERENCES

1. Peczyńska-Czoch W., Pognan F., Kaczmarek Ł. and Boratyński J., *J. Med. Chem.*, **37**, 3503 (1994).
2. Kaczmarek Ł., Peczyńska-Czoch W., Osiadacz J., Mordarski M., Sokalski W.A., Boratyński J., Marcinkowska E., Glazman-Kuśnierczyk H. and Radzikowski C., *Bioorg. Med. Chem.*, **7**, 2457 (1999).
3. Kamieńska-Trela K., Kania L. and Kaczmarek Ł., *J. Mol. Struct.*, **347**, 467 (1995).
4. Kamieńska-Trela K., Kania L., Sitkowski J. and Kaczmarek Ł., *J. Chem. Soc., Perkin Trans.*, 2, 1617 (1995).
5. Kaczmarek Ł., Peczyńska-Czoch W., Opolski A., Wietrzyk J., Marcinkowska E., Boratyński J. and Osiadacz J., *Anticancer Res.*, **18**, 3133 (1998).
6. Kalinowski H.O., Berger S. and Braun S., *C-NMR Spektroskopie*, Thieme, Stuttgart, 1984.
7. (a) Pople J.A., *Discuss. Faraday Soc.*, **34**, 7 (1962); (b) Martin G.J., Martin M.L. and Odiot S., *Org. Magn. Reson.*, **7**, 2 (1975); (c) Fliszár S., Cardinal G. and Béraldin M.T., *J. Am. Chem. Soc.*, **104**, 5287 (1982); (d) Spiesecke H. and Schneider W.G., *Tetrahedron Lett.*, 468 (1961).
8. Barfield M. and Chakrabarti B., *Chem. Rev.*, **69**, 757 (1969).
9. Sternhell S., *Quat. Rev. Chem. Soc.*, **23**, 236 (1969).
10. Castellano S., and Sun C., *J. Am. Chem. Soc.*, **88**, 4741 (1966).
11. Davidson M.W., Griggs B.G., Boykin D.W. and Wilson W.D., *J. Med. Chem.*, **20**, 1117 (1977).