Fluorescence and Structure of Methylated Acridin-9-ylthioureas

[a]P. KRISTIAN, [a]E. BALENTOVÁ, [a]J. BERNÁT, [a]J. IMRICH, [a]E. SEDLÁK, [a]I. DANIHEL, [b]S. BÖHM, [c]N. PRÓNAYOVÁ, [d]K. D. KLIKA, [d]K. PIHLAJA, and [a]J. BARANOVÁ

[a]*Institute of Chemistry, Faculty of Natural Sciences, P. J. Šafárik University, SK-041 67 Košice*
e-mail: bernat@kosice.upjs.sk, balentova@kosice.upjs.sk, jimrich@kosice.upjs.sk, kristian@kosice.upjs.sk, sedlak.er@saske.sk, idanihel@kosice.upjs.sk

[b]*Department of Organic Chemistry, Institute of Chemistry, University of Chemical Technology, CZ-166 28 Prague*
e-mail:bohm@vscht.cz

[c]*Central Laboratories, Faculty of Chemical and Food Technology, Slovak University of Technology, SK-812 37 Bratislava*
e-mail: nada@cvt.stuba.sk

[d]*Department of Chemistry, University of Turku, FIN-20014 Turku, Finland*
e-mail: karkli@utu.fi, kpihlaja@utu.fi

Received 28 February 2003

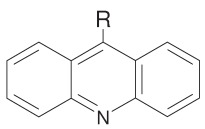
Fluorescence and structure of acridin-9-ylthioureas with primary amino rest and their *S*-methylated products have been studied. The synthesized 1-(acridin-9-yl)-3-alkyl-*S*-methylisothiuronium iodides exhibit about one order increased fluorescence in comparison with corresponding thioureas. The structure of products obtained by methylation reactions was confirmed by NMR techniques including PDQF-COSY, selective INEPT, NOE difference experiments, and quantum-chemical calculations (AM1, *ab initio*). A free rotation of 9-substituents in relation to acridine skeleton has been found. The conformers of acridinylisothioureas are in *E*-configuration relative to the C═N bond with more favourable imino (C-9═N) tautomeric structure.

Acridine derivatives are characterized by interesting biological activity [1], intercalation effect [2], and intensive fluorescence [3]. In continuation with the previous results regarding fluorescence of acridinyl derivatives, namely 9-isothiocyanatoacridine *I,* thioureas *II,* and iminothiocarbonates *III* [4, 5], it was of interest to study quaternary acridinium salts as a new prospective class of fluorogenes.

In the present work, methylation reactions, structure and spectral properties of acridin-9-ylthioureas have been studied to obtain simple acridinium derivatives as compounds with expected fluorescence properties which have not been described as yet.

The starting thioureas *IVa—IVf* have been obtained *via* the reaction of primary amines with 9-acridinylisothiocyanate [6]. Thioureas reacted readily with methyl iodide to produce *S*-methylisothiuronium iodides *Va—Vf* which were deprotonated by K_2CO_3 in acetone to yield corresponding free isothiourea bases *VIa—VIf* (Scheme 1).

The NMR data of synthesized compounds are shown in Experimental. The assignments have been based on ^{1}H and ^{13}C NMR spectra of model compounds *Va, Vc, VIa, VIc* applying PDQF-COSY [7], selective INEPT [8] technique, and NOE difference experiments. The equivalence of corresponding proton

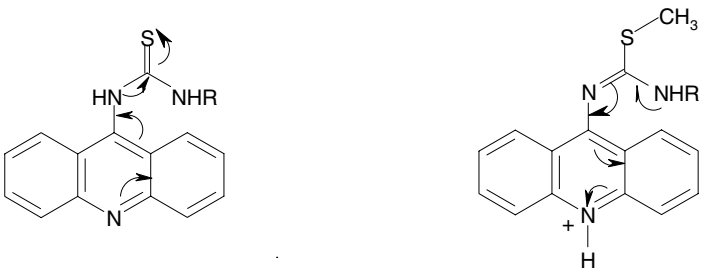


	R
I	NCS
II	$NHCSNR^1R^2$
III	$NCSR^3OMe$

Formulae I—III

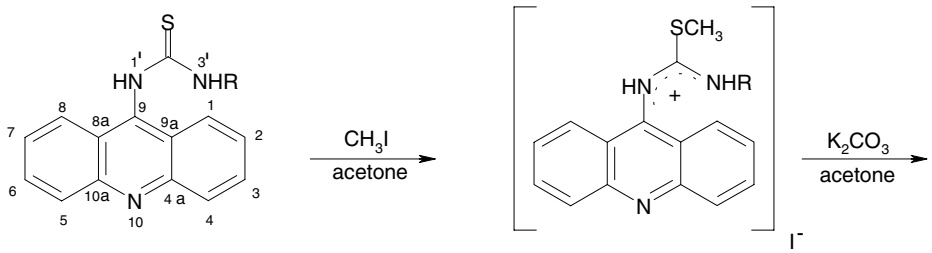
and carbon chemical shifts of both acridine side rings evidenced a free rotation of 9-substituent in relation to the acridine skeleton. Quantum-chemical optimization (AM1) confirmed unscrewed orientation of acridine and thiourea (isothiourea) planes.

NMR has also been successfully applied to recognize localization of two NH protons at three possible basic sites of iodides *V*. In *Vc*, their signals were observed at δ = 13.81 and 9.40. NOE irradiation of NH protons proved not to be very useful, because NOE enhancements have been spread over the neighbourhood of other NH group due to a fast chemical exchange. For example, irradiation of NH proton at δ = 13.88 caused 28.4 % enhancement of H-4, H-5 signal at δ = 7.98, while irradiation of NH at δ = 9.40 enhanced the same signal by 20.1 %. However, this indicated the acridine nitrogen as a target of protonation. Really, we have found significant differences between po-

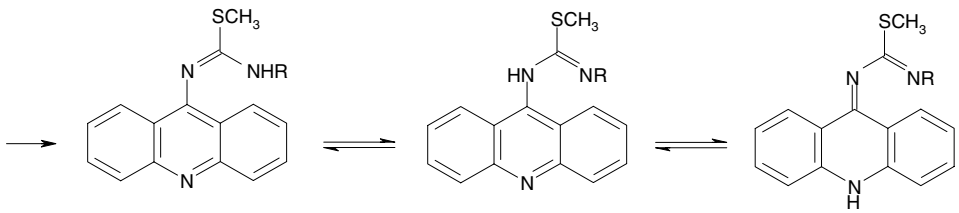


IV *V*

Formulae IV, V



IVa –IV f *Va – Vf*



VIa – VIf

IV—VI	*a*	*b*	*c*	*d*	*e*	*f*
R	ethyl	propyl	isopropyl	cyclohexyl	phenyl	4-tolyl

Scheme 1

sitions of carbon signals C-4, C-5 and C-4a, C-10a in iodides *V* and corresponding free bases *VI*. By dehydroiodination of *Va* (*Vc*) to free base *VIa* (*VIc*), the resonance signals of carbons C-4a, C-10a adjacent to acridine NH-10 were shifted markedly downfield from $\delta = 140.28$ (139.83) to $\delta = 149.58$ (148.97). The same tendency was observed for those of C-4, C-5 being shifted from $\delta = 119.64$ (118.83) in *Va* (*Vc*) to $\delta = 129.32$ (128.88) in *VIa* (*VIc*). The downfield shift of bz about $\delta = 3$ due to dehydroiodination was recorded for chemical shifts of C-8a, C-9a together with upfield shift by about $\delta = 4$ of C-9. All these facts point to the acridine N-10 nitrogen as a main target of protonation by HI released during methylation. The other homonuclear NOE of *Vc, VIc* listed in Tables 1 and 2 confirmed assignments done by INEPT and correlation methods. The irradiation of the NH of the free base *VIc* at $\delta = 6.98$ manifested the intensity enhancement of SCH_3 protons by about 14.6 %, of CH by 5.0 %, and of 2 × CH_3 by 9.4 %, but did not influence protons of acridine skeleton.

To explain the methylation of thioureas *IVa*—*IVf* we have also studied their tautomeric structures (Scheme 2) by theoretical methods. As the thiol tautomeric structure can be excluded [9], reaction took course according to Scheme 1 [10]. This fact is also in accordance with quantum-chemical calculation of energy. For *IVa*, the energy of a thiol structure *VII* is by about 50.71 kJ mol^{-1} more advantageous than that of the thiol structure *IX* obtained by B3LYP/6-311 G (*d, p*) calculation [11—13]. For comparison, the AM1 calculations [14] give energetic differences from 6.91

Table 1. NOE Enhancements in *S*-Methyl-1′-(acridin-9-yl)-3′-isopropylisothiuronium Iodide (*Vc*)

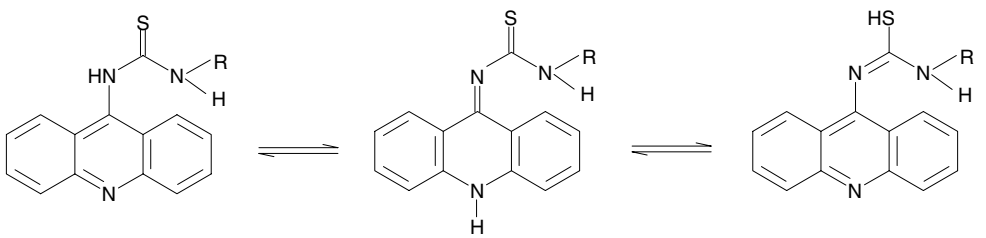
	^{1}H Chemical shifts δ and NOE enhancements/%[a]						
Irradiated signal	2.25 SCH_3	7.98 H-4,5	7.66 H-2,7	8.16 H-1,8	9.40 NH	13.88 H-10	4.10 CH
H-1,8	1.2		9.1				1.9
H-2,7				8.6			
H-3,6		7.2	3.7				
H-4,5					20.1	28.4	
SCH_3				1.0			
2 × CH_3				1.2			6.1
CH				1.0	1.9		
NH-10		1.4					
NH-3′		0.7					

a) Relative to the irradiated spin (set to – 100 %).

Table 2. NOE Enhancements in *S*-Methyl-1′-(acridin-9-yl)-3′-isopropylisothiourea (*VIc*)

	^{1}H Chemical shifts δ and NOE enhancements/%[a]				
Irradiated signal	2.25 SCH_3	6.98 NH	7.46 H-2,7	7.95 H-1,8	8.01 H-4,5
H-1,8	0.9		15.1		
H-2,7				12.8	
H-3,6			11.3		10.8
CH		5.0			
2 × CH_3		9.4			
SCH_3		14.6			
NH	0.8				

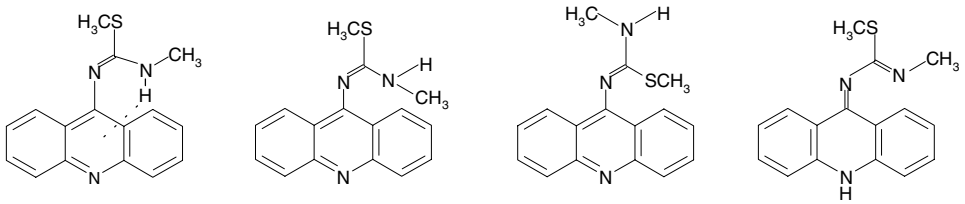
a) Relative to the irradiated spin (set to – 100 %).



VII *VIII* *IX*

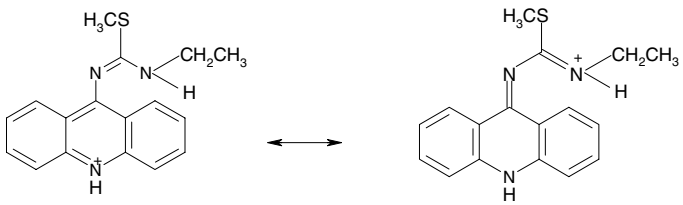
Scheme 2

to 37.15 kJ mol^{-1} for the possible tautomeric structures (*VII*, *IX*). According to calculations of thiones *VII* and *VIII*, the structure *VII* with amino nitrogen N-1′ is more favourable than the imino structure *VIII* for 5.58 kJ mol^{-1}. Among possible structural isomers, those with hydrogen on N-3′ are the most stable. The reason is the stabilization of these isomers by intramolecular interaction of the N-3′ proton with a π-orbital of the aromatic ring [9, 15] for *IVa* (AM1: ΔH_f = 16.00 kJ mol^{-1}, B3LYP/6-311 G (*d, p*): ΔE = 34.21 kJ mol^{-1}). The results of mentioned calculations for acridine isothioureas *X*—*XIII* show the conformers with *E*-configuration of a C═N bond (*X, XI*) as the most stable isomers compared to those with *Z*-configuration (*XII*). Moreover, for these molecules a structure *X* with 9-acridinylimino rest is more energetically preferred in comparison with the 9-iminoacridine structure *XIII* by 7.91 kJ mol^{-1} (AM1). Due to the above-mentioned interaction of hydrogen on N-3′ with the acridine skeleton the structure *X* is more preferred than structure *XI* by 4.44 kJ mol^{-1} (AM1), or by 7.24 kJ mol^{-1} (B3LYP/6-311 G (*d, p*)). Isothiourea iodides *Va*—*Vf* exhibit similar structural features as previously mentioned acridine derivatives *IV* and *VIa*—*VIf*. Among possible tautomeric structures, the most stable are structures *XIV*, *XV* with a low energetical difference (*ca.* 12.31 kJ mol^{-1} – AM1) (Scheme 3).



X *XI* *XII* *XIII*

Formulae X—XIII



XIV *XV*

Scheme 3

Table 3. UV VIS and Fluorescence Properties of Synthetized Acridine Derivatives *IVa*, *IVc—IVe*, *Va—Vf*

Comp.	Absorbance λ_{max}/nm ε/(dm^3 mmol^{-1} cm^{-1})	$\varepsilon_{410\,nm}$ ε/(dm^3 mmol^{-1} cm^{-1})	Fluorescence λ_{max}/nm F/a.u. (F/F_o)	Sensitivity $F_{max} \cdot \varepsilon_{410\,nm}$	Efficiency $F_{max}/\varepsilon_{410\,nm}$
IVa	420.0	0.92	465.6	0.34	0.40
	1.01		0.37 (0.06)		
IVc	420.3	3.50	463.2	2.56	0.21
	3.82		0.73 (0.12)		
IVd	419.0	0.56	462.4	0.34	1.07
	0.59		0.60 (0.10)		
IVe	423.0	0.63	458.0	0.43	1.09
	0.65		0.68 (0.11)		
Va	422.2	9.55	468.0	204.52	2.24
	10.62		21.42 (3.47)		
Vb	420.8	8.85	467.2	312.26	3.99
	9.25		35.3 (5.71)		
Vc	423.2	9.74	469.2	282.40	2.98
	11.45		29.0 (4.69)		
Vd	423.4	9.85	467.2	301.90	3.11
	11.20		30.65 (4.69)		
Ve	422.8	9.90	463.0	4.95	0.05
	11.01		0.50 (0.08)		
Vf	425.7	8.74	459.6	10.75	0.14
	10.34		1.23 (0.20)		

F_o refers to maximum intensity fluorescence of 9-isothiocyanatoacridine at λ_{em} = 446 nm at 1.0 × 10^{-6} mol dm^{-3} in acetonitrile.

Electronic absorption spectra of acridine thioureas *IVa, IVc—IVe* and *S*-methylisothiuronium iodides *Va—Vf* measured in acetonitrile exhibit absorption maxima near 420 nm. Extinction coefficients of acridinium iodides *Va—Vf* are considerably higher than those of acridinethioureas *IVa, IVc—IVe* (Table 3, Fig. 1). Analogously, the higher intensity of fluorescence is revealed by the *S*-methyl-9-acridinylisothiuronium iodides *Va—Vf*, with more than 2 orders of magnitude greater values of fluorescence compared to the starting derivatives *IVa—IVf* (Table 3, Fig. 2). This is probably caused by an enhanced conjugation of their NHR amino rests with the acridinium skeleton [16]. On the other hand, the reversed polarization oriented from acridine skeleton to C═S group of thioureas *IV* diminished the electron density on a heteroatom N-10, and decreased the intensity of fluorescence (Formulae *IV, V*). Unlike the previous acridine derivatives, the

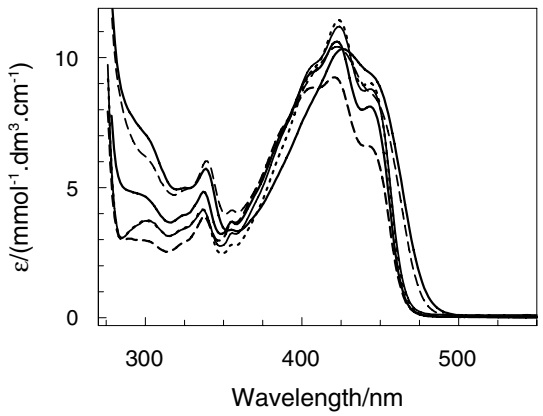


Fig. 1. Electronic absorption spectra of acridine derivatives *Va* (solid thick line), *Vb* (dashed thick), *Vc* (dotted thick), *Vd* (solid thin), *Ve* (dashed thin), *Vf* (solid intermediate thick).

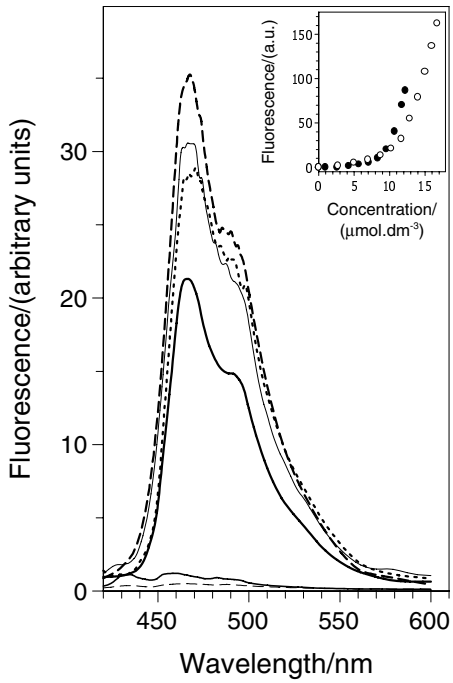


Fig. 2. Fluorescence emission spectra of acridine derivatives *Va* (solid thick line), *Vb* (dashed thick), *Vc* (dotted thick), *Vd* (solid thin), *Ve* (dashed thin), *Vf* (solid intermediate thick). Inset: Concentration dependence of fluorescence of the derivatives *Va* (solid circle) and *Vb* (empty circle).

acridine isothioureas *VIa*—*VIf* show no fluorescence. It is interesting that in the case of ethyl and propyl derivatives *Va*, *Vb*, the fluorescence exponentially increases at low concentrations (≈ 10 μmol dm^{-3}) (the relation should be linear) and sharply decreases at high concentration (>100 μmol dm^{-3}) due to inner filter effect. Nonlinear concentration dependence of fluorescence most likely indicates a change in oligomeric state of a fluorogen. Most common oligomeric form of fluorogens – J-aggregates and excimers – would manifest itself by sharpening of absorbance spectra and broad red-shift fluorescence, respectively. There is observed only slight red shift at high concentration of fluorogens without any perturbation in their absorbance spectra (data not shown). This most likely indicates the formation of excimer-like structures. However, one may speculate that the observed nonlinear dependence of fluorescence is not due to interaction of fluorogens in excited states but in the ground state as a result of hydrophobic effect between hydrophobic side chains of the acridine derivatives. More exact explanation of this phenomenon would require a deeper study of the problem, which is out of the frame of this work.

In conclusion, we can summarize that among synthesized compounds *IV*—*VI* only acridine isothiuronium iodides *Va*—*Vf* exhibit remarkable fluorescence. The results obtained from the spectral measurements and quantum-chemical calculations allowed us to characterize the spectral features and energetically favourable stereoisomers of compounds under study.

EXPERIMENTAL

Melting points were determined on a Kofler hot-stage apparatus. IR spectra were measured on a Specord 75 IR spectrophotometer (Zeiss) in chloroform or KBr discs. NMR spectra were obtained on a Tesla BS 5287 A (80.018 MHz) (^{1}H, *IVa*—*IVf*, *Va*—*Vf*, *VIa*—*VIf*), Varian VXR (300.131 MHz) (^{1}H, ^{13}C, *Va*, *Ve*, *VIa*, *VIe*) at room temperature in deuterochloroform or a mixture of deuterochloroform and hexadeuterodimethyl sulfoxide. NMR spectra of the compounds *Vc* and *VIc* were acquired at 25 °C on a Jeol JNR-L-400 spectrometer operating at 399.78 MHz for ^{1}H (internal standard tetramethylsilane, $\delta(\mathrm{TMS_{int}}) = 0$) and 100.54 MHz for ^{13}C ($\delta(\mathrm{TMS_{int}}) = 0$) in hexadeuterodimethyl sulfoxide. Spin analysis was performed using PERCH software [17] for the extraction of chemical shifts and coupling constants. The spectral width of two-dimensional spectra was optimized from one-dimensional spectra. All spectra were produced by using standard pulse sequences. Quantum-chemical calculations of totally optimized structures were carried out by both semiempirical (AM1) and nonempirical (RHF using the 6-311G (*d, p*) basis sets) methods and density functional theory (B3LYP/6-311G (*d, p*)). An elemental analysis was done on a Perkin—Elmer analyzer CHN 2400. Absorption spectra of 9-acridinyl derivatives were obtained using UV-3000 Shimadzu spectrophotometer at concentrations 1.5—2.0 $\times$ 10^{-5} mol dm^{-3} in acetonitrile. Extinction coefficients are expressed in dm^3 mmol^{-1} cm^{-1} (Fig. 1, Table 3). Fluorescence measurements were performed on a Shimadzu RF-5000 spectrofluorometer. Emission spectra were recorded in the region λ = 420—600 nm at the excitation wavelength. Obtained fluorescence spectra are averages of 3—6 subsequent scans at the excita-

tion wavelength. Fluorescence intensities expressed in arbitrary units (a.u.) (Fig. 2, Table 3) correspond to emission of compounds at concentration 1.0×10^{-6} mol dm^{-3} in acetonitrile. All measurements were performed at 25 °C. F_o refers to intensity of fluorescence maximum of 9-isothiocyanatoacridine at $\lambda_{em} = 446$ nm at the same conditions.

Substituted 1′-(Acridin-9-yl)-3′-alkyl/aryl-thioureas *IVa*—*IVf*

To a solution of *I* [18] (572 mg; 0.4 mmol) in a mixture of dichloromethane (5 cm^3) and diethyl ether (15 cm^3), the corresponding amine (0.4 mmol) diluted with a small amount of diethyl ether was added dropwise and the mixture was stirred for about 4 h. The reaction course was followed by TLC plates monitoring (eluent benzene—acetone, $\varphi_r = 5 : 2$), whereby the products remain on the start. The precipitated product was washed with diethyl ether, filtered off and dried.

IVa: Yield 67 %, m.p. = 193—194 °C. For $C_{16}H_{15}N_3S$ ($M_r = 281.4$) w_i(calc.): 68.30 % C, 5.37 % H, 14.93 % N; w_i(found): 68.38 % C, 5.15 % H, 14.78 % N. IR spectrum (KBr), $\tilde{\nu}/cm^{-1}$: 3195 (NH), 1620 (C═N), 1405 (NHCS). ^{1}H NMR spectrum, δ: 6.80—8.60 (m, $8H_{acridinyl}$), 3.16 (q, 2H, $J = 7.1$ Hz, NCH_2), 1.24 (t, 3H, $J = 7.1$ Hz, CH_3).

IVb: Yield 85 %, m.p. = 182—183 °C. For $C_{17}H_{17}N_3S$ ($M_r = 295.4$) w_i(calc.): 69.12 % C, 5.80 % H, 14.22 % N; w_i(found): 69.30 % C, 5.62 % H, 14.07 % N. IR spectrum (KBr), $\tilde{\nu}/cm^{-1}$: 3210 (NH), 1622 (C═N), 1405 (NHCS). ^{1}H NMR spectrum, δ: 8.68 (s, 1H, NH), 6.80—8.38 (m, $8H_{acridinyl}$), 3.25 (t, 2H, $J =$ 7 Hz, NCH_2), 1.49 (m, 2H, CH_2), 0.98 (t, 3H, $J = 7.5$ Hz, CH_3).

Thioureas *IVc*—*IVf* were described previously in the literature [6, 19].

IVc: Yield 73 %, m.p.= 195—196 °C (m.p. = 197—199 °C [6]).

IVd: Yield 79 %, m.p. = 196—198 °C (m.p. = 192—194 °C [6]).

IVe: Yield 86 %, m.p. = 182—184 °C (m.p. = 178—180 °C [19]).

IVf: Yield 96 %, m.p. = 180—182 °C (m.p. = 184—186 °C [6]).

Substituted *S*-Methyl-1′-(acridin-9-yl)-3′-alkyl/arylisothiuronium Iodides *Va*—*Vf*

To a stirred suspension of thioureas *IVa*—*IVf* (0.4 mmol) in acetone (15 cm^3) methyl iodide (3 cm^3) was added dropwise at room temperature during 3 h. The reaction mixture became clear, after a short time the precipitated product was filtered off, washed with diethyl ether and dried.

Va: Yield 77 %, m.p. = 163—166 °C. For $C_{17}H_{18}IN_3S$ ($M_r = 423.3$) w_i(calc.): 48.23 % C, 4.29 % H, 9.93 % N; w_i(found): 48.41 % C, 4.10 % H, 9.74 % N. IR spectrum (KBr), $\tilde{\nu}/cm^{-1}$: 3200 (NH), 1622 (C═N), 1350 (NCS). ^{1}H NMR spectrum, δ: 7.20—8.86 (m, 9H $NH_{acridinyl}$), 3.70 (q, 2H, $J = 7.1$ Hz, NCH_2), 2.36 (s, 3H, SCH_3), 1.48 (t, 3H, $J = 7.1$ Hz, CH_3). ^{13}C NMR spectrum, δ: 160.97 (C-9), 154.40 (N═C), 135.40 (C-3, C-6), 126.15 (C-1, C-8), 125.13 (C-2, C-7), 119.64 (C-4, C-5), 140.28 (C-4a, C-10a), 117.24 (C-8a, C-9a), 39.65 (NCH_2), 15.03, 14.65 (SCH_3, $CH_2\underline{CH}_3$).

Vb: Yield 93 %, m.p. = 160—164 °C. For $C_{18}H_{20}IN_3S$ ($M_r = 437.3$) w_i(calc.): 49.43 % C, 4.61 % H, 9.61 % N); w_i(found): 49.70 % C, 4.54 % H, 9.75 % N. IR spectrum (KBr), $\tilde{\nu}/cm^{-1}$: 3220 (NH), 1620 (C═N), 1360 (NCS). ^{1}H NMR spectrum, δ: 7.10—8.80 (m, 9H, $NH_{acridinyl}$), 3.59 (t, 2H, $J = 7.1$ Hz, NCH_2), 2.84 (m, 2H, CH_2), 2.30 (s, 3H, SCH_3), 1.05 (t, 3H, $J = 7.5$ Hz, CH_3).

Vc: Yield 64 %, m.p. = 205—206 °C. For $C_{18}H_{20}IN_3S$ ($M_r = 437.3$) w_i(calc.): 49.43 % C, 4.61 % H, 9.61 % N; w_i(found): 49.30 % C, 4.78 % H, 9.72 % N. IR spectrum (KBr), $\tilde{\nu}/cm^{-1}$: 3185 (NH), 1620 (C═N), 1350 (NCS). ^{1}H NMR spectrum (DMSO-d_6), *δ:* 13.88 (br, 1H, NH-10), 8.16 (ddd, $^3J_{H\text{-}1\ H\text{-}2} = 8.5$ Hz, $^4J_{H\text{-}1\ H\text{-}3} = 1.4$ Hz, $^5J_{H\text{-}1\ H\text{-}4} = 0.6$ Hz, 2H, H-1 and H-8), 8.08 (ddd, $^3J_{H\text{-}3\ H\text{-}4} = 8.6$ Hz, $^3J_{H\text{-}3\ H\text{-}2} = 6.8$ Hz, $^4J_{H\text{-}3\ H\text{-}1} = 1.4$ Hz, 2H, H-3 and H-6), 7.98 (ddd, $^3J_{H\text{-}4\ H\text{-}3} = 8.6$ Hz, $^4J_{H\text{-}4\ H\text{-}2} = 1.1$ Hz, $^5J_{H\text{-}4\ H\text{-}1} = 0.6$ Hz, 2H, H-4 and H-5), 7.66 (ddd, $^3J_{H\text{-}2\ H\text{-}1} = 8.5$ Hz, $^3J_{H\text{-}2\ H\text{-}3} = 6.8$ Hz, $^4J_{H\text{-}2\ H\text{-}4} = 1.1$ Hz, 2H, H-2 and H-7), 9.40 (br, 1H, NH), 4.05 (br, 1H, CH), 2.25 (br, 3H, SCH_3), 1.3 (br, 6H, 2 × CH_3). ^{13}C NMR spectrum (DMSO-d_6), δ: 162.44 (C═N), 157.99 (C-9), 139.83 (C-4a, C-10a), 135.59 (C-3, C-6), 126.23 (C-1, C-8), 124.64 (C-2, C-7), 118.83 (C-4, C-5), 116.09 (C-8a, C-9a), 46.98 (d, CH), 21.63 (2 × CH_3), 14.23 (SCH_3).

Vd: Yield 81 %, m.p. = 168—171 °C. For $C_{21}H_{24}IN_3S$ ($M_r = 477.4$) w_i(calc.): 52.83 % C, 5.07 % H, 8.80 % N; w_i(found): 52.86 % C, 4.86 % H, 8.51 % N. IR spectrum (KBr), $\tilde{\nu}/cm^{-1}$: 3250 (NH), 1620 (C═N), 1420 (NCS). ^{1}H NMR spectrum, δ: 7.00—8.96 (m, $8H_{acridinyl}$), 3.39—4.22 (m, 1H, NCH), 2.19 (s, 3H, SCH_3), 1.00—1.98 (m, 10H, CH_2).

Ve: Yield 95 %, m.p. = 207—210 °C. For $C_{21}H_{18}IN_3S$ ($M_r = 471.4$) w_i(calc.): 53.51 % C, 3.85 % H, 8.92 % N; w_i(calc.): 53.38 % C, 3.76 % H, 9.01 % N. IR spectrum (KBr), $\tilde{\nu}/cm^{-1}$: 3190 (NH), 1610 (C═N), 1415 (NCS). ^{1}H NMR spectrum, *δ:* 6.76—8.39 (m, $13H_{acridinyl,\ ArH}$), 2.29 (s, 3H, SCH_3). ^{13}C NMR spectrum, δ: 159.46 (N═C), 158.70 (C-9), 139.20 (C-4a and C-10a), 134.81 (C-3, C-6), 125.29 (C-1, C-8), 124.36 (C-2, C-7), 121.48 (C_{ortho}); 136.84 (C_{ipso}), 128.03 (C_{meta}), 125.13 (C_{para}), 118.35 (C-1, C-4), 115.83 (C-8a, C-9a), 14.18 (SCH_3).

Vf: Yield 82 %, m.p. = 198—201 °C. For $C_{22}H_{20}IN_3S$ ($M_r = 485.4$) w_i(calc.): 54.44 % C, 4.15 % H, 8.66 % N; w_i(found): 54.32 % C, 4.28 % H, 8.57 % N. IR spectrum (KBr), $\tilde{\nu}/cm^{-1}$: 3210 (NH), 1623 (C═N), 1420 (NCS). ^{1}H NMR spectrum, δ: 6.76—

8.80 (m, $12H_{acridinyl,\,ArH}$), 2.30 (s, 3H, CH_3), 2.25 (s, 3H, SCH_3).

Substituted *S*-Methyl-1′-(acridin-9-yl)-3′-alkyl/arylisothioureas *VIa*—*VIf*

A suspension of thiourea *IVa*—*IVf* (0.6 mmol) and K_2CO_3 (0.5 g; 8 mmol) was treated at room temperature with excess of methyl iodide (0.5 cm^3, 8 mmol) in 10 cm^3 of acetone. The reaction course was followed by TLC plates monitoring (eluent benzene—acetone, 5 : 2). After 4 h stirring the reaction was finished, the product poured into water and three times extracted with 15 cm^3 of ethyl acetate. The extract was concentrated and the product precipitated by addition of diethyl ether to form crystals.

VIa: Yield 70 %, m.p. = 185—190 °C. For $C_{17}H_{17}N_3S$ ($M_r = 295.4$) w_i(calc.): 69.12 % C, 5.80 % H, 14.22 % N; w_i(found): 69.05 % C, 5.73 % H, 14.18 % N. IR spectrum (KBr), $\tilde{\nu}/cm^{-1}$: 3220 (NH), 1600 (C═N), 1402, 1068 (NCS). ^{1}H NMR spectrum, δ: 7.12—8.14 (m, $8H_{acridinyl}$), 3.58 (q, 2H, J = 7.1 Hz, NCH_2), 2.30 (s, 3H, SCH_3), 1.34 (t, 3H, J = 7.1 Hz, CH_3). ^{13}C NMR spectrum, δ: 155.17 (N═C), 153.28 (C-9), 149.58 (C-4a, C-10a), 130.22 (C-3, C-6), 129.32 (C-4, C-5), 124.62 (C-1, C-8), 124.10 (C-2, C-7), 119.23 (C-8a, C-9a), 38.42 (NCH_2), 15.22 (CH_3), 14.21 (SCH_3).

VIb: Yield 94 %, m.p. = 185—188 °C. For $C_{18}H_{19}N_3S$ ($M_r = 309.4$) w_i(calc.): 69.87 % C, 6.19 % H, 13.58 % N; w_i(found): 69.62 % C, 6.17 % H, 13.49 % N. IR spectrum (KBr), $\tilde{\nu}/cm^{-1}$: 3220 (NH), 1600 (C═N), 1410, 1080 (NCS). ^{1}H NMR spectrum, δ: 7.00—8.26 (m, $8H_{acridinyl}$), 3.38(t, 2H, J = 7.0 Hz, NCH_2), 2.38 (s, 3H, SCH_3), 1.60 (m, 2H, CH_2), 0.92 (t, 3H, J = 7.5 Hz, CH_3).

VIc: Yield 63 %, m.p. = 185—187 °C. For $C_{18}H_{19}N_3S$ ($M_r = 309.4$) w_i(calc.): 69.87 % C, 6.19 % H, 13.58 % N; w_i(found): 69.75 % C, 6.13 % H, 13.42 % N. IR spectrum (KBr), $\tilde{\nu}/cm^{-1}$: 3248 (NH), 1603 (C═N); 1418, 1070 (NCS). ^{1}H NMR spectrum (DMSO-d_6), δ: 8.01 (ddd, $^3J_{H\text{-}4\ H\text{-}3}$ = 8.7 Hz, $^4J_{H\text{-}4\ H\text{-}2}$ = 1.2 Hz, $^5J_{H\text{-}4\ H\text{-}1}$ = 0.5 Hz, 2H, H-4 and H-5), 7.95 (ddd, $^3J_{H\text{-}1\ H\text{-}2}$= 8.6 Hz, $^4J_{H\text{-}1\ H\text{-}3}$ = 1.5 Hz, $^5J_{H\text{-}1\ H\text{-}4}$ = 0.5 Hz, 2H, H-1 and H-8), 7.74 (ddd, $^3J_{H\text{-}3\ H\text{-}4}$ = 8.2 Hz, $^3J_{H\text{-}3\ H\text{-}2}$ = 6.5 Hz, $^4J_{H\text{-}3\ H\text{-}1}$ = 1.5 Hz, 2H, H-3 and H-6), 7.46 (ddd, $^3J_{H\text{-}2\ H\text{-}1}$ = 8.6 Hz, $^3J_{H\text{-}2\ H\text{-}3}$ = 6.5 Hz, $^5J_{H\text{-}2\ H\text{-}4}$ = 1.2 Hz, 2H, H-2 and H-7), 6.98 (d, $^3J_{NH\ CH}$ = 7.3 Hz, 1H, NH), 4.33 (dq, $^3J_{CH\ NH}$ = 7.3 Hz, $^3J_{CH\ CH_3}$ = 6.5 Hz, 1H, CH), 2.25 (s, 3H, SCH_3), 1.35 (d, $^3J_{CH_3\ CH}$ = 6.5 Hz, 6H, 2 × CH_3). ^{13}C NMR spectrum (DMSO-d_6), δ: 153.48 (C—SCH_3), 153.37 (C-9), 148.97 (C-4a, C-10a), 129.88 (C-3, C-6), 128.88 (C-4, C-5), 124.55 (C-1, C-8), 123.72 (C-2, C-7), 118.95 (C-8a, C-9a), 44.61 (CH), 22.03 (2 × CH_3), 13.85 (SCH_3).

VId: Yield 94 %, m.p. 183—186 °C. For $C_{21}H_{23}N_3S$ ($M_r = 349.5$) w_i(calc.) 72.17 % C, 6.63 % H, 12.02 % N; w_i(found): 72.21 % C, 6.58 % H, 12.04 % N. IR spectrum (KBr), $\tilde{\nu}/cm^{-1}$: 3190 (NH), 1610 (C═N), 1402, 1100 (NCS). ^{1}H NMR spectrum, δ: 7.12—8.29 (m, $8H_{acridinyl}$), 4.04—4.69 (m, 1H, NCH), 2.32 (s, 3H, SCH_3), 1.00—2.88 (m, 10H, CH_2).

VIe: Yield 78 %, m.p. = 176—180 °C. For $C_{21}H_{17}N_3S$ ($M_r = 343.4$) w_i(calc.): 73.44 % C, 4.99 % H, 12.23 % N; w_i(found): 73.40 % C, 5.02 % H, 12.15 % N. IR spectrum (KBr), $\tilde{\nu}/cm^{-1}$: 3208 (NH), 1600 (C═N), 1405, 1090 (NCS). ^{1}H NMR spectrum, δ: 6.60—8.50 (m, $13H_{ArH,\,acridinyl}$), 2.32 (s, 3H, SCH_3).

VIf: Yield 77 %, m.p. = 178—180 °C. For $C_{22}H_{19}N_3S$ ($M_r = 357.5$) w_i(calc.): 73.92 % C, 5.36 % H, 11.75 % N; w_i(found): 73.79 % C, 5.23 % H, 11.67 % N. IR spectrum (KBr), $\tilde{\nu}/cm^{-1}$: 3165 (NH), 1615 (C═N), 1408, 1075 (NCS). ^{1}H NMR spectrum, δ: 6.76—8.38 (m, $12H_{ArH,\,acridinyl}$), 2.25 (s, 3H, CH_3), 2.23 (s, 3H, SCH_3).

Acknowledgements. This study was supported by the Grant Agency for Science of the Slovak Ministry of Education (Registry Number 1/9245/02).

REFERENCES

1. Sondhi, S. M., Johar, M., Singhal, N., Dastidar, G., Shukla, R., and Rakhubir, R., *Monatsh. Chem.. 131*, 511 (2000).
2. Chamantray, F., Duflos, A., Lhomme, J., and Demeunynck, M., *J. Chem. Soc., Perkin Trans. 1 22*, 2962 (2001).
3. Miyashita, O., Minagawa, M., Ueda, Y., Tada, Y., Hoshino, N., and Imanishi, T., *Tetrahedron 57*, 3361 (2001).
4. Kristian, P., Bernát, J., Imrich, J., Danihel, I., Suchár, G., Chomča, I., Hočová, S., Bušová, T., Guspanová, J., and Linden, A., *Molecules 1*, 181 (1996).
5. Kristian, P., Hočová, S., Imrich, J., Bernát, J., and Bušová, T., *Chem. Pap. 51*, 48 (1997).
6. Mazagová, D., Kristian, P., Suchár, G., Imrich, J., and Antalík, M., *Collect. Czech. Chem. Commun. 59*, 2632 (1994).
7. Eberstadt, M., Gemmecker, G., Mierke, D. F., and Kessler, H., *Angew. Chem., Int. Ed. Engl. 34,* 1671 (1995).
8. Bax, A., *J. Magn. Reson. 57*, 314 (1984).
9. Walter, W. and Ruess, K. P., *Justus Liebigs Ann. Chem. 743*, 167 (1971); *Justus Liebigs Ann. Chem. 746*, 54 (1971).
10. Duns, F., in *Comprehensive Organic Chemistry.* (Jones, D. N., Editor.) Vol. *3*, Part 11.17, p. 456. Pergamon Press, Oxford, 1979.
11. Frish, M. J., Head-Gordon, M., Trucks, G. W., Foresman, J. B., Schlegel, H. B., Raghavachari, K., Robb, M. A., Binkley, J. S., Gonzales, C., Defrees, D. J., Fox, D. J., Whiteside, R. A., Seeger, R., Melius, C. F., Baker, J., Martin, R. L., Kahn, L. R., Stewart, J. J. P., Topiol, S., and Pople, J. A., *Gaussian 90*, Gaussian Inc., Pittsburg, Pa, USA, 1991.
12. Frish, M. J., Trucks, G. W., Schlegel, H. B., Gill, P. M. W., Johnson, B. G., Robb, M. A., Cheeseman, J. R., Keith, T., Petersson, G. A., Montgomery, J. A., Raghavachari, K., Al-Laham, M. A., Zakrzewski, V. G., Ortiz, J. V., Foresman, J. B., Cioslowski, J., Stefanov,

B. B., Nanayakkara, A., Challacombe, M., Peng, C. Y., Ayala, P. Y., Chen, W., Wong, M. W., Andres, J. L., Replogle, E. S., Gomperts, R., Martin, R. L., Fox, D. J., Binkley, J. S., Defrees, D. J., Baker, J., Stewart, J. J. P., Head-Gordon, M., Gonzales, C., and Pople, J. A., *Gaussian 94*. Gaussian Inc., Pittsburg, Pa, USA, 1995.
13. Becke, A. D., *J. Chem. Phys. 98*, 5648 (1993).
14. Dewar, M. J. S., Zoebish, E. G., Healy, E. F., and Stewart, J. J. P., *J. Am. Chem. Soc. 107*, 3902 (1985).
15. Janovec, L., Suchár, G., Imrich, J., Kristian, P., Sasinková, V., Alföldi, J., and Sedlák, E., *Collect. Czech. Chem. Commun. 67*, 665 (2002).
16. Sato, N., *Tetrahedron Lett. 37*, 8519 (1996).
17. Laatikainen, R., Niemitz, M., Weber, U., Sundelin, J., Hassinen, T., and Vepsäläinen, J., *J. Magn. Reson., Ser. A 120*, 1 (1996).
18. Kristian, P., *Chem. Zvesti 15*, 641 (1961).
19. Sinsheimer, J. E., Jagodic, V., Polak, L. J., Hong, D. D., and Burckhalter, J. H., *J. Pharm. Sci. 64*, 925 (1975).