THE FLUORESCENCE PROPERTIES OF PROTOBERBERINE AND TETRAHYDROPROTOBERBERINE ALKALOIDS

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Ultraviolet absorption spectra and fluorescence spectra of 19 protoberberine, pseudoprotoberberine and tetrahydroprotoberberine alkaloids in ethanol have been determined. All the fluorescence spectra were represented by a single, structureless emission band. The maxima of the fluorescence bands of protoberberine and pseudoprotoberberine alkaloids were in a wave-length region of 540 to 570 nm. All the tetrahydroprotoberberine alkaloids were fluorescent too. The maxima of their fluorescence bands occurred between 320 and 330 nm.

In a spectrophotometric and fluorometric study of the interactions of horse liver alcohol dehydrogenase with berberine and a number of related protoberberine alkaloids it was first necessary to determine the absorption and fluorescence properties of these compounds[1]. As some of these compounds were not accessible we considered it worth while to complete this series and include, in our study, some compounds of related structures, whose spectra might find analytical use. Determination of the absorption and fluorescence spectra of a series of protoberberine, pseudoprotoberberine and tetrahydroprotoberberine alkaloids is the subject of the present paper. We have also attempted to correlate the fluorescence parameters with the structures of the compounds under study.

Whereas the ultraviolet absorption spectra of protoberberine and tetrahydroprotoberberine alkaloids are described in detail in several papers[3-8], only one comprehensive work[2] has been devoted to the fluorescence spectra of these compounds. However, a criticizable experimental approach and lack in exactitude reduce its value. The absorption spectra of the studied alkaloids pertain to the azanaphthalene type[4]; their pattern is affected not only by aromaticity of the heterocyclic nucleus, but also by the nature and position of oxygenous electron-donating substituents (methylenedioxy, *ortho*-dimethoxy or hydroxy groups) on the aromatic rings A and D of their berberine skeleton[4-6]. The pattern of the ultraviolet absorption spectra

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of quaternary salts of protoberberine and pseudoprotoberberine bases is determined by auxochromic groups, bound to carbon atoms $C_{(9),(10)}$ and $C_{(10),(11)}$ of the isoquinoline benzene ring D[4,6,7]. The other substituents on the berbine skeleton just insignificantly affect the wave-length maxima and intensities of the absorption bands, while the number of absorption bands and the main features of the spectra are essentially unchanged[8]. Quaternary salts of protoberberine alkaloids in polar media exhibit four well-defined absorption bands, designated in the order of decreasing wave lengths of their maxima as *A*, *B*, *C* and *D*. Bands *B* and *D* are each probably composed of at least two absorption bands. A quaternary berberinium structure is characterized[4] by the absorption band *A*. On the basis of the solvent polarity effect and the effect of electronegativity of the anion on the spectral pattern of berberine salts, band *C* has been assigned to a π-π^* electron transition[7]. Ultraviolet absorption spectra of pseudoprotoberberine alkaloids are markedly different from those of protoberberine alkaloids. (The only difference between pseudoberberines and berberines is in the position of substituents on the aromatic ring D; in pseudoberberines the auxochromic groups are bound to carbons $C_{(10),(11)}$, whereas in berberines to $C_{(9),(10)}$.) According to Preininger and coworkers[7] it is the oxygenous electron-donating substituent on $C_{(11)}$ that has the decisive effect on polarisation of a pseudoprotoberberine molecule in excited state. The ultraviolet spectra of tetrahydroprotoberberine bases contain two absorption bands: a narrow, low-intensity ($\varepsilon_{max} \approx 6000$) band *A*, maximum at 280–290 nm, and an inflexion band *B*, wave-length range from c. 230 to 240 nm. (The intense absorption bands below 210 nm is usually not evaluated.) The positions of these bands depend on the nature of the auxochromic groups bound to carbon atoms $C_{(2),(3)}$ whereas substituents on $C_{(9),(10)}$ or $C_{(10),(11)}$ have little effect on the wave lengths of the absorption maxima. The spectral pattern (intensity ratio of the bands *A* and *B*) depends on the number and position of methylenedioxy groups in respect to *ortho*-dimethoxy groups[4,5].

EXPERIMENTAL

The alkaloids are given in Table I. Their purity was tested by their melting points and by thin-layer chromatography. Berberrubine chloride was obtained by pyrolysis of berberine chloride at 190°C in an atmosphere of carbon dioxide[8]. The compounds were used in the form of quaternary salts — chlorides or iodides (type *a*-berberines and pseudoberberines); the tetrahydro derivatives were in the form of tertiary bases (type *b*-berbines).

Methods. Before spectrophotometric measurements the compounds were dried for 2 h at 90°C / / 0·1 Torr. Samples of these, weighed with a precision of $\pm 0{\cdot}005$ mg, were dissolved in 96% ethanol and the solutions were brought to a concentration of $2 \cdot 10^{-4}$M. The absorption spectra in the UV and in the visible regions were recorded with a spectrophotometer Specord UV-VIS (Zeiss, Jena). The fluorescence spectra were recorded with a spectrofluorometer constructed by ourselves[9]; it consisted of two monochromators SPM 2 (Zeiss, Jena,) a 500-W xenone discharge tube XBO 500 (Narva, G.D.R.) as a source of excitation energy, and a photomultiplier EMI 9558 QB as a detector of the emitted radiation.

TABLE I

Structures of the Compounds Studied

Number	Type[a]	Name	Substituents					
			$C_{(2)}$	$C_{(3)}$	$C_{(9)}$	$C_{(10)}$	$C_{(11)}$	$C_{(13)}$
I	a	protoberberine	H	H	H	H	H	H
II	a	coptisine	O—CH_2—O		O—CH_2—O		H	H
III	a	berberine	O—CH_2—O		OCH_3	OCH_3	H	H
IV	a	palmatine	OCH_3	OCH_3	OCH_3	OCH_3	H	H
V	a	berberrubine	O—CH_2—O		OH	OCH_3	H	H
VI	a	jatrorrhizine	OCH_3	OH	OCH_3	OCH_3	H	H
VII	a	pseudocoptisine	O—CH_2—O		H	O—CH_2—O		H
VIII	a	pseudoepiberberine	OCH_3	OCH_3	H	O—CH_2—O		H
IX	a	pseudopalmatine	OCH_3	OCH_3	H	OCH_3	OCH_3	H
X	a	corysamine	O—CH_2—O		O—CH_2—O		H	CH_3
XI	b	stylopine (tetrahydrocoptisine)	O—CH_2—O		O—CH_2—O		H	H
XII	b	canadine (tetrahydroberberine)	O—CH_2—O		OCH_3	OCH_3	H	H
XIII	b	sinactine (tetrahydroepiberberine)	OCH_3	OCH_3	O—CH_2—O		H	H
XIV	b	tetrahydropalmatine	OCH_3	OCH_3	OCH_3	OCH_3	H	H
XV	b	tetrahydropseudoepiberberine	OCH_3	OCH_3	H	O—CH_2—O		H
XVI	b	tetrahydrocorysamine	O—CH_2—O		O—CH_2—O		H	CH_3
XVII	b	thalictricavine (13-methyltetrahydroberberine)	O—CH_2—O		OCH_3	OCH_3	H	CH_3
XVIII	b	corydaline (13-methyltetrahydropalmatine)	OCH_3	OCH_3	OCH_3	OCH_3	H	CH_3
XIX[b]	b	coralydine (8-methyltetrahydropseudopalmatine)	OCH_3	OCH_3	H	OCH_3	OCH_3	H

[a] Type a berberines and pseudoberberines, type b berbines. [b] Methyl-substituted at $C_{(8)}$.

The results of the spectrophotometric measurements are given in Table II. All the fluorometric data are corrected for the spectral characteristics of the apparatus. The following parameters are given for each compound: the wave lengths of the absorption maxima and minima, the molar absorptivities and their differences from two long-wave (last) absorption bands ($\Delta\varepsilon_{B-A}$), wave lengths and wave numbers of fluorescence maxima, half-widths of the fluorescence bands, Stokes shifts ($\tilde{\nu}_{abs} - \tilde{\nu}_{f1}$) and relative intensities of fluorescence.

RESULTS AND DISCUSSION

The ultraviolet absorption spectra (positions of the absorption maxima and minima and molar absorptivities) of the alkaloids under study (Table II, Fig. 1) well accord with the reported data[4-6]. The fluorometric measurements (Table II, Fig. 2) show that

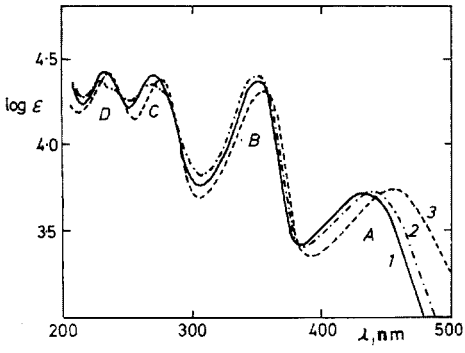


FIG. 1

Ultraviolet Absorption Spectra of Protoberberine Alkaloids in Ethanol

1 Berberine chloride (*III*); *2* jatrorrhizine chloride (*IV*); *3* berberrubine chloride (*V*).

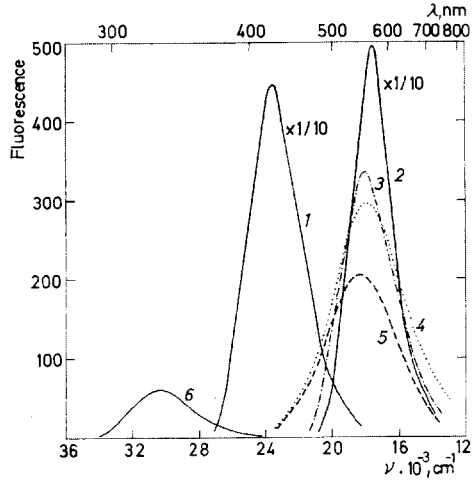
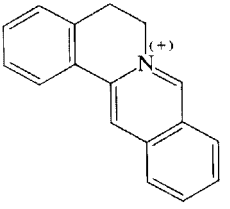


FIG. 2

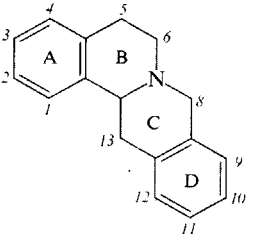
Fluorescence Spectra of Protoberberine, Pseudoprotoberberine and Tetrahydroprotoberberine Alkaloids in Ethanol

$2 \cdot 10^{-4}$M solutions, excitation under standard conditions at the wave lengths of the last absorption maxima of the solutes. The fluorescence intensities of protoberberine and coptisine are plotted in a ten-times reduced scale; with protoberberine the intensity of fluorescence is an approximate value. The relative intensities of fluorescence are plotted in chosen units. *1* Protoberberine chloride (*I*), *2* coptisine chloride (*II*), *3* palmatine chloride (*IV*), *4* pseudocoptisine chloride (*VII*), *5* pseudopalmatine chloride (*IX*), *6* stylopine (*XI*).

the energetic difference between the excited singlet state and the ground state of the studied molecules, determining the positions of the fluorescence bands, is influenced not only by the aromacity degree of a heterocyclic nucleus but also by the nature and positions of oxygenous electron-donating substituents (methylenedioxy, *ortho*-dimethoxy or methoxy and hydroxy groups) on the aromatic rings A and D of their berbine skeleton. The transition from the tetrahydroisoquinoline tertiary nitrogen in tetrahydroprotoberberine alkaloids to the quaternary nitrogen in the salts of protoberberine and pseudoprotoberberine bases manifests itself in the fluorescence spectra of these compounds (like in the absorption spectra) by a marked bathochromic shift of the emission bands and by a considerable increase in the intensity of fluorescence.



a



b

Thus in the transition from stylopine (*XI*) (tetrahydrocoptisine) to coptisine (*II*) the shift is approx. 12600 cm^{-1}, in the transition from tetrahydropalmatine (*XIV*) to palmatine (*IV*) it is about 13000 cm^{-1}, which corresponds to energy differences of 36·0 kcal mol^{-1} and 37·2 kcal mol^{-1}, respectively. The oxygenous electron-donating groups in the studied alkaloids appreciably reduce the energy of electron transfers between the ground and the excited singlet states of the molecules, and moreover influence their absorbances and fluorescence intensities. The significant bathochromic shifts of the absorption and the fluorescence bands on the introduction of these auxochromic groups into a protoberberine or tetrahydroprotoberberine molecule are due to the prolonged conjugated system of these molecules, as a result of interaction of free electron pairs of oxygen with the conjugated π-electron system of the aromatic rings. Methylenedioxy groups invariably bring about a greater bathochromic shift of the absorption and fluorescence bands than *ortho*-dimethoxy groups if introduced to the same positions (Table II, *cf.*[2-6]). This may be due to a difference between the electron-donating properties of these groups[1,6], as well as to different steric effects.

An especially marked shift of the absorption and fluorescence spectra is observed on the introduction of a hydroxy group into a protoberberine alkaloid (berberrubine (*V*), jatrorrhizine (*VI*)). Replacement of methoxyl by hydroxyl on carbon $C_{(3)}$ of the

TABLE II

Absorption and Fluorescence Parameters of Protoberberine, Pseudoprotoberberine and Tetrahydroprotoberberine Alkaloids in Ethanol

The value of relative fluorescence intensities are given for $2 . 10^{-4}$M solutions and are expressed in chosen units. The solutes were excited at the wave lengths of their last absorption maxima.

Compound	λ^{abs}_{max} nm	ε_{max}	λ^{abs}_{min} nm	ε_{min}	$\Delta\varepsilon_{B-A}$ [a]	λ^{fl}_{max} nm	$\tilde{\nu}^{fl}_{max}$ cm^{-1}	$\tilde{\nu}_{1/2}$ [b]	$\tilde{\nu}_{abs} - \tilde{\nu}_{fl}$ [c]	I_{fl}
Protoberberine chloride	211	(21 500)	217	(16 800)	6 200	422	(23 700)	3 700	3 600	(223)[d]
	224	(20 500) s	236	(12 500)						
	228	(21 500)	288	(7 700)						
	261	(34 300) s	308	(9 900)						
	265	(35 300)	331	(2 000)						
	303	(10 100)								
	315	(12 000)[e]								
	366	(5 800)[f]								
Protoberberine iodide	222	(35 300)	217	(32 000)	7 000	422	(23 700)	3 700	3 700	(223)[d]
	260	(37 500) s	239	(16 700)						
	265	(38 300)	288	(8 700)						
	303	(11 300)	308	(11 100)						
	315	(13 500)[e]	330	(3 000)						
	365	(6 500)[f]								
Coptisine chloride	229	(23 700)	237	(22 700)	17 800	568	(17 600)	2 900	3 800	4 920
	241	(23 200)	255	(18 500)						
	268	(21 000)	309	(4 250)						
	354	(22 200) s	397	(1 500)						
	363	(22 800)[e]								
	467	(5 000)[f]								
Berberine chloride	230	(26 700)	215	(17 500)	18 200	548	(18 250)	3 900	4 900	422
	267	(25 500)	251	(16 800)						

	344	(22 800) s	305	(5 750)	(5 750)					
	352	(23 200)[e]	382	(2 600)	(2 600)					
	432	(5 000)[f]								
Berberine iodide	226	(38 600)	212	(29 400)	20 900	548	(18 250)	3 900	4 850	440
	267	(28 900)	251	(18 800)						
	344	(26 200) s	305	(6 250)						
	353	(26 600)[e]	383	(2 600)						
	433	(5 700)[f]								
Palmatine chloride	228	(24 400)	215	(18 600)	19 900	552	(18 100)	3 950	5 000	336
	240	(21 000) s	252	(16 800)						
	268	(24 100)	305	(6 600)						
	276	(24 000) s	381	(2 400)						
	343	(24 400) s								
	350	(25 000)[e]								
	433	(5 100)[f]								
Berberrubine chloride	234	(26 200)	213	(15 200)	14 800	563	(17 750)	3 700	4 250	133
	275	(23 700)	255	(14 000)						
	353–8	(20 200)[e]	306	(5 000)						
	455	(5 400)[f]	394	(2 250)						
Jatrorrhizine chloride	228	(23 400)	216	(18 000)	20 000	538	(18 600)	3 600	4 150	27
	241	(20 800) s	253	(17 600)						
	267	(22 600)	306	(6 600)						
	275	(21 000) s	387	(2 500)						
	352	(25 200)[e]								
	440	(5 200)[f]								
Pseudocoptisine chloride	220	(34 400) s	250	(18 100)	25 850	556	(18 000)	5 000	8 350	296
	231	(29 000) s	274	(22 000)						

TABLE II
(*Continued*)

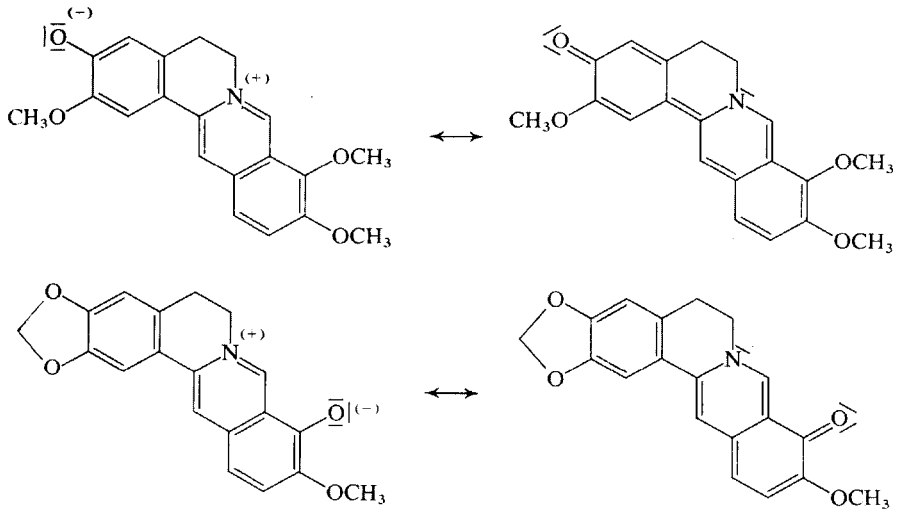
Compound	λ^{abs}_{max} nm	ε_{max}	λ^{abs}_{min} nm	ε_{min}	$\Delta\varepsilon_{B-A}$ [a]	λ^{fl}_{max} nm	$\tilde{\nu}^{fl}_{max}$ cm^{-1}	$\tilde{\nu}_{1/2}$ [b]	$\tilde{\nu}_{abs} - \tilde{\nu}_{fl}$ [c]	I_{fl}
	266	(24 800)	308	(19 300)						
	289	(31 600)[e]	336	(13 000)						
	317	(20 500)								
	346	(13 700)								
	380	(5 750) s[f]								
Pseudoepiberberine iodide	220	(29 000)	237	(22 300)	34 800	550	(18 200)	5 000	8 150	290
	240	(22 700)	249	(16 000)						
	264	(21 700)	272	(20 000)						
	290	(41 600)[e]	335	(17 800)						
	310	(30 000)								
	341	(18 200)								
	380	(6 800) s[f]								
Pseudopalmatine chloride	242	(17 000)	250	(12 200)	31 500	546	(18 300)	5 000	8 050	205
	265	(17 700)	271	(17 500)						
	288	(37 500)[e]	335	(15 700)						
	310	(26 000) s								
	342	(16 000)								
	380	(6 000) s[f]								
Corysamine chloride	230	(25 800)	255	(16 700)	14 100	548	(18 250)	3 600	3 750	2 020
	240	(22 100) s	306	(5 000)						
	268	(23 200)	390	(1 650)						
	344	(19 200) s								
	352	(19 300)[e]								
	455	(5 200)[f]								

Stylopine	238 290	(8 500)[e] (8 200)[f]	233 257	(8 450) (900)	300	328	(30 450)	4 300	4 050	58
Canadine	229 288	(13 500) s[e] (5 600)[f]	253	(700)	7 900	327	(30 550)	4 250	4 200	60
Sinactine	232 287	(13 600) s[e] (7 200)[f]	257	(1 500)	6 400	322	(31 000)	4 300	3 850	50
Tetrahydropalmatine	227 282	(19 000) s[e] (5 600)[f]	251	(700)	13 400	318	(31 400)	4 350	4 100	23
Tetrahydropseudoepiberberine	233 290	(8 700)[e] (6 700)[f]	227 257	(8 650) (1 000)	2 000	328	(30 450)	4 200	4 000	53
Tetrahydrocorysamine	238 291	(9 000)[e] (7 000)[f]	260	(1 000)	2 000	327	(30 550)	3 900	3 850	102
Thalictricavine	227 291	(15 200) s[e] (5 600)[f]	253	(750)	9 600	327	(30 550)	4 300	3 850	37
Corydaline	227 282	(20 200) s[e] (5 600)[f]	251	(700)	14 600	318	(31 400)	4 300	4 100	21
Coralydine	227 287	(16 500) s[e] (7 500)[f]	255	(900)	9 000	320	(31 250)	4 300	3 600	25

[a] Difference of molar absorptivities of bands B and A; [b] half-width of the fluorescence band; [c] wave number difference between the last absorption band and the fluorescence band (Stokes shift); [d] 10^{-5} M solution (strong fluorescence of ethanolic solutions of protoberberine prevented use of the standard, higher concentration at the same sensitivity of the apparatus); [e] maximum of the absorption band B; [f] maximum of the longest-wave band A.

aromatic ring A in a molecule of palmatine (*IV*) (jatrorrhizine (*VI*)) brings about only a small bathochromic shift of the absorption bands *A* and *B*, and has practically no effect on absorptivity. The same substitution on carbon $C_{(9)}$ of the aromatic ring D in a molecule of berberine (*III*) (berberrubine (*V*)) manifests itself by much more significant changes: all absorption bands shift bathochromically, the maximum of the longest-wave band *A* by as much as 23 nm, with simultaneous decreas in the difference of absorbances of bands *B* and *A* (Table II, Fig. 1). In the fluorescence spectrum the effect of the substitution was analogous – a marked bathochromic shift of the fluorescence maximum ($\Delta\lambda_{fl}$ = 14 nm) and a decrease in the relative intensity of fluorescence to about a third. By contrast, the same substitution on $C_{(3)}$ in the molecule of palmatine produced a hypsochromic shift of the fluorescence maximum ($\Delta\lambda_{fl}$ = = 14 nm) of thus derived jatrorrhizine and a marked decrease of the relative intensity of fluorescence to about a twelfth of the fluorescence intensity of palmatine. The different effects of the hydroxyl at $C_{(9)}$ of the aromatic D ring of berberrubine and the hydroxyl at $C_{(3)}$ of the aromatic A ring of jatrorrhizine on the absorption and fluorescence spectra of these compounds can be explained by unequal influence of these hydroxy groups on the chromophoric system. The free electron pairs of the hydroxyl oxygen on $C_{(9)}$ can directly participate in the conjugated π-electron chromophoric system of the isoquinoline nucleus (rings C and D), whereas the non-bonding electrons of the oxygen on $C_{(3)}$ can conjugate only with the π electrons of the benzene ring A. Consequently, the effect of the hydroxy group at position 3 on the location and intensity of the absorption bands should be much weaker, since its electron-donating effect is transferred *via* the benzene ring, functioning as part of an auxochromic group. In addition to this principal factor, the difference in ability of the phenolic hydroxyls on $C_{(3)}$ and $C_{(9)}$ to donate a proton is likely to manifest itself also. Dissociation of hydroxy groups of these phenolic protoberberine alkaloids gives rise to the corresponding zwitterions, capable of tautomerization to highly conjugated, electroneutral, quinoid structures (Scheme 1). The quinoid tautomeric form of jatrorrhizine can be expected to have a higher tension in the B ring, but the conversion of the bipolar tautomeric form into the quinoid one will not change the direction of the transition moment. By contrast, in the quinoid tautomeric form of berberrubine the direction of the transition moment will be different from that in the bipolar form, which will be well apparent in the absorption spectrum of the dissociated form of berberrubine[8].

The positions of the electron-donating substituents on the benzene ring D of the isoquinoline nucleus have a very conspicuous effect on the absorption and fluorescence spectra of the studied quaternary salts. Whereas the pattern of the absorption spectra of pseudoprotoberberine alkaloids is quite different from that of the protoberberine alkaloids, the fluorescence spectra of pseudoberberines and berberines are very similar (Table II, Fig. 2; refs[2,4,6]). The fluorescence spectra of all berberines (*II–IV, X*) and pseudoberberines (*VII–IX*) are represented by a single, structureless



SCHEME 1

emission band with a fluorescence maximum at c. 540–570 nm. The transition from berberines to pseudoberberines (shift of the auxochromic groups on benzene ring D from position 9, 10 to 10, 11) shows up in the fluorescence spectra by a mere widening of the bands and a moderate decrease in the fluorescence intensity (Table II, Fig. 2). The mutual replacement of the methylenedioxy and the *ortho*-dimethoxy groups in pseudoberberines affects the fluorescence and the absorption spectra much less than in berberines (*cf.*, *e.g.*, coptisine (*II*) and palmatine (*IV*) *vs* pseudocoptisine (*VII*) and pseudopalmitine (*IX*) in Fig. 2). In our opinion, the shift of the electron-donating substituents from positions 9, 10 (berberines) to positions 10, 11 (pseudoberberines) is associated with considerable alterations in electron distribution and molecular polarization of these compounds, in the ground and the excited states, which, in turn, are responsible for the marked changes in optical properties (absorption spectra) of the studied quaternary salts dissolved in a polar medium (ethanol). In agreement with other authors[6,7] we believe that the charge distribution and molecular polarization of pseudoberberines in the excited state are governed primarily by the oxygenous electron-donating substituent on $C_{(11)}$. The substituent with free electron pairs on the oxygen atom at position 11 extends the conjugated π-electron system of the isoquinoline nucleus of the pseudoprotoberberine alkaloid (rings C and D) in the direction of its longitudinal axis and will primarily influence the absorption bands corresponding to transitions polarized in this direction. The free electron pair on the oxygen atom of this substituent partially compensates for the positive charge of nitrogen in the molecule of the pseudoprotoberberine alkaloid in the ground state[6]. Consequently, the transition from berberines to pseudoberberines is accompanied

not only by a change in charge distribution in the ground state, but also by a change in polarization of the electron transitions in their molecules, with the result of a changed pattern of the absorption spectra.

Attachment of another substituent to the molecule of a protoberberine alkaloid, *viz.* a methyl group to $C_{(13)}$ of coptisine, giving rise to corysamine (*X*), manifests itself, like in the absorption spectrum, by a significant hypsochromic shift of the fluorescence maximum ($\Delta\lambda_{f1} = 20$ nm), with a simultaneous decrease in the relative intensity of fluorescence (approximately to a half of the fluorescence of coptisine). The spectra of 13-methylberberine and 13-ethylberberine[10] also suggest that substitution of alkyls for hydrogen atoms at position 13 strongly affects the absorption and fluorescence spectra of protoberberine alkaloids in the same way.

Tetrahydroprotoberberine and tetrahydropseudoprotoberberine alkaloids and their methyl derivatives exhibit, in excitation in the region of the absorption maximum of the longer-wave band *A* (at approx. 290 nm), a single emission band with a fluorescence maximum at 320–330 nm. With berbines and pseudoberbines the position effect of oxygenous electron-donating substituents in the aromatic ring D on the pattern of their absorption and fluorescence spectra is negligible compared to that observed with the corresponding quaternary alkaloids (Table II, *cf.*[4,5]). This, of course, is hardly surprising, since these tertiary bases form a chromophoric system different from that of the corresponding quaternary bases: berbines and pseudoberbines can be regarded as 1-benzyl-1,2,3,4-tetrahydroisoquinoline derivatives with hindred rotation of the benzyl group, whereas berberines and pseudoberberines are, in fact, 3-phenylisoquinoline derivatives with hindred rotation of the phenyl group. Similarly, attachment of a methyl group to $C_{(13)}$ has no pronounced effect on the positions of the absorption and the fluorescence maxima of berbines, causing just a small decrease in the relative intensity of fluorescence (Table II).

What is also interesting are the effects of the nature and the position of electron-donating substituents in the berbine skeleton of the studied compounds on the Stokes shift, $\tilde{\nu}_{abs} - \tilde{\nu}_{f1}$, and half-width of the fluorescence bands, $\tilde{\nu}_{1/2}$. These two parameters reach extreme values in quaternary pseudoprotoberberine alkaloids (Table II). With molecules not reacting in the first excited singlet state the Stokes shift ranges between 2000 and 5000 cm^{-1} (ref.[11]). The increased Stokes shifts of pseudoberberines indicate that during the excited state their molecules participate in some other process. This process, according to Weller[12], may be: *1.* formation of complexes, polymerization (dimerization), or solvent–solute interactions; *2.* proton transfer (ionization); *3.* electron transfer. With pseudoberberines the most probable process is a strong interaction between excited molecules and the solvent, the interaction product being the source of the emission. The appreciable change in geometry of pseudoberberine molecules caused by their excitation shows up in their fluorescence spectra by separation of the last absorption and fluorescence bands (increase in Stokes shift) and by widening of the fluorescence band – it is probable that the emission occurs from

various, energetically different, singlet excited states (the Franck-Condon non-relaxed state and equilibrious excited state).

The determined fluorescence parameters (wave lengths of the fluorescence maxima, relative intensities of fluorescence and half-widths of the fluorescence bands) may be analytically useful for this group of alkaloids. Utilization of the fluorescence properties for qualitative analysis will be rewarding with compounds having very similar absorption spectra in both the UV and the visible regions (pseudoepiberberine–pseudopalmatine, palmatine–jatrorrhizine, *etc.*).

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