# ALKALOIDS FROM THE ROOTS OF *Papaver pseudo-orientale* (FEDDE) MEDW.*

František VĚŽNÍK[a], Eva TÁBORSKÁ[a], Petr SEDMERA[b], Ladislav DOLEJŠ[c] and Jiří SLAVÍK[a]

[a] *Department of Medical Chemistry, Purkyně University, 662 43 Brno,*
[b] *Institute of Microbiology, Czechoslovak Academy of Sciences, 142 20 Prague 4 and*
[c] *Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 166 10 Prague 6*

Received August 12th, 1985

*Dedicated to Prof. J. Tomko, on the occassion of his 65th birthday.*

Two new alkaloids, pseudorine (*Ia*) (quaternary benzyltetrahydroisoquinoline type) and pseudoronine (*II*) (benzil type), were isolated from the roots of *Papaver pseudo-orientale* (FEDDE) MEDW. This is a first occurence of this type of alkaloids in *Papaveraceae*. The main component of the tertiary alkaloid fraction was isothebaine accompanied by smaller amounts of orientalidine, mecambridine, bracteoline, nuciferine, protopine, and allocryptopine. The presence of salutaridine and papaverrubine D was established. Traces of coptisine and palmatine were also found in some samples. Strongly polar portion of the extract was converted to iodides. N-Methylisothebainium iodide (*IIIa*) (major component), pseudorine iodide (*Ia*), and corytuberine hydriodide (*IV*) (significant amounts), a minor alkaloids such as alborine iodide (alkaloid PO-5), pseudoronine (*II*), and magnoflorine iodide (*IIIb*) were isolated from this source. Structure of pseudorine was determined by UV, IR, $^1$H, and $^{13}$C NMR spectroscopy and using the correlation with laudanosine and codamine. Proposed structure of pseudoronine is based on the mass spectra comparison.

*Papaver pseudo-orientale* (FEDDE) MEDW. is a perennial herb of the *Macrantha* ELKAN (*Oxytona* BERNH.) section belonging to the family *Papaveraceae*. It is abundant in Armenian – Iran region; in our country it is cultivated as a decorative plant. Ambiguous classification and unaccurate description of species put by Fedde in the section *Macrantha* (*Oxytona*)[1] often caused their mutual interchange. The situation was resolved by Goldblatt in 1974, who had characterized three species only in the Macrantha section on the basis of morphological, caryological, and biochemical properties[2]: *P. bracteatum* LINDL. (diploid, $2n = 14$) with thebaine as the major alkaloid, *P. orientale* L. (tetraploid, $2n = 28$) with prevailing oripavine, and *P. pseudo-orientale* (FEDDE) MEDW. (hexaploid, $2n = 42$) in which isothebaine is the dominant

* Part LXXXIII in the series Alkaloids of the *Papaveraceae*; Part LXXXII: This Journal *51*, 1743 (1986).

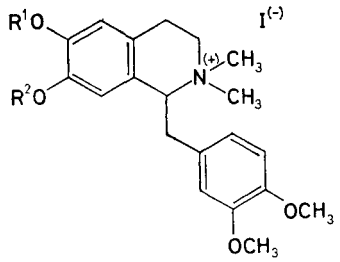
alkaloid component. The Goldblatt's conclusions were also confirmed by Novák[3].

Several geographic chemotypes of *P. pseudo-orientale* are given in the newer literature. Most characteristic from the chemotaxonomic point of view is isothebaine[2-4]. As the dominant alkaloid, it is replaced by salutaridine or macrantaline in some Turkish chemotypes[5-10]. Orientalidine, mecambridine, bracteoline, and some other alkaloids are often reported as the minor components. Also the alkaloids reported in older papers (before the Goldblatt's revision of the section Oxytona) in taxons designated as *P. bracteatum* or *P. orientale*[11-18] originated really from the species[2] *P. pseudo-orientale**. Despite the enormous attention paid to the study of alkaloids of this species, little is known about the occurrence of quaternary alkaloids. In our earlier work, we made a preliminary investigation of alkaloids from smaller amount of capsules and roots of cultivated *P. pseudo-orientale*[19]. Isothebaine was found as a major alkaloid in the non-quaternary fraction in both cases. Other alkaloids were orientalidine, mecambridine, salutaridine, and bracteoline. The presence of palmatine was proved chromatographically. Alborine and a new alkaloid N-methylisothebainium hydroxide (*IIIa*) were isolated as iodides from the quaternary fraction.

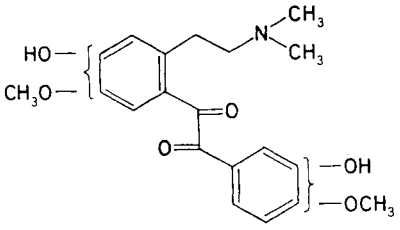
This work is devoted to the study of quaternary alkaloids from the roots of *P. pseudo-orientale* obtained from larger amount of plant material. Total 0·62% of nonquaternary alkaloids were isolated by usual manner[20]. Main alkaloid of this fraction was isothebaine (0·28% of dry weight). The other alkaloids were orientalidine, bracteoline, mecambridine, and small amounts of nuciferine, protopine, allocryptopine (so far not reported in *P. pseudo-orientale*), and an unidentified base PS 1. Salutaridine and papaverrubine D were detected chromatographically. Strongly polar alkaloids were converted to iodides and extracted with chloroform[21]. Main components isolated from this fraction were N-methylisothebainium iodide (0·077%), an iodide of new dextrorotatory quaternary alkaloid, m.p. 204–206°C (0·032%), that was named pseudorine, alborine iodide, small amount of magnoflorine iodide (*IIIb*) (not found in *P. pseudo-orientale* yet), and two non-identified alkaloids. We have isolated two tertiary bases from the strongly polar fraction of *P. pseudo-orientale* alkaloid extract: corytuberine (*IV*) (as hydriodide, a dominant alkaloid, first occurrence in this source) and smaller amount of a new alkaloid, m.p. 249–252°C which we have named pseudoronine.

N-Methylisothebainium iodide was identified in our previous work[19] by direct comparison with the authentic sample prepared from isothebaine. Its mass spectrum (see ref.[22]), $^1$H and $^{13}$C NMR spectra (see ref.[23]) agree well with the aporphine structure of N-methylisothebainium iodide.

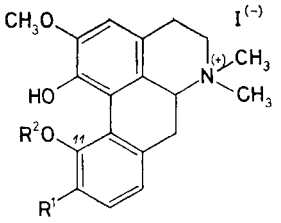
* We have also obtained from the seeds designated as "*P. orientale*" (see refs[11,12]) kindly donated to us several years ago by the late Dr V. Preininger (Olomouc, Czechoslovakia) the plants identical with *P. pseudo-orientale*.



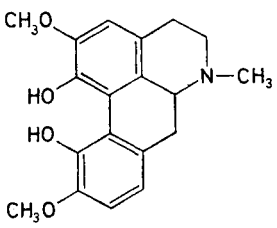
*Ia*, $R^1 = H$; $R^2 = CH_3$
*Ib*, $R^1 = R^2 = CH_3$
*Ic*, $R^1 = CH_3$; $R^2 = H$



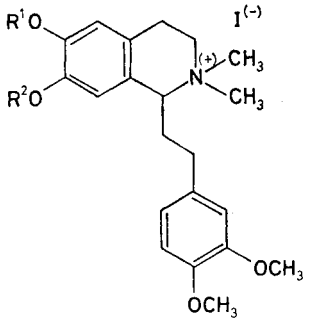
*II*



*IIIa*, $R^1 = H$; $R^2 = CH_3$
*IIIb*, $R^1 = CH_3O$; $R^2 = H$



*IV*



*Va*, $R^1 = H$; $R^2 = CH_3$
*Vb*, $R^1 = CH_3$; $R^2 = H$
*Vc*, $R^1 = R^2 = CH_3$

Peak $m/z$ 357 ($C_{21}H_{27}NO_4$) in the mass spectrum of pseudorine iodide could be assigned either to a molecular ion of a quaternary benzyltetrahydroisoquinoline alkaloid (*Ia* or *Ib*) or to a tertiary base formed from the quaternary phenylethyltetra-

hydroisoquinoline derivative *Va* or *Vb* by splitting off methyl iodide in the ion source of the mass spectrometer. Comparison with the mass spectroscopic fragmentation of an analogous benzylisoquinoline alkaloid[24] and with the mass spectra of N,N--dimethyl-7-hydroxy-6,3',4'-trimethoxyphenylethyl-1,2,3,4-tetrahydroisoquinolinium iodide (*Vb*) and N,N-dimethyl-6,7,3',4'-tetramethoxyphenylethyl-1,2,3,4-tetrahydroisoquinolinium iodide (*Vc*) prepared from an authentic tertiary derivative[25] supported the benzyltetrahydroisoquinoline structure of pseudorine. Derivatives *Vb* and *Vc* displayed the mass spectra very similar to those of pseudorine iodide (*Ia*) and its O-methylderivative *Ic*. However, they exhibited molecular ions of methine and tertiary base (roughly in 1 : 1 ratio). Moreover, compounds *Vb* and *Vc* differred from *Ia* and *Ic* in their IR spectra and TLC behaviour. Labelling with [O-$^2$H]ethanol caused one mass unit shift of ions *m/z* 357, 342, 326, 192, and 177. That suggests a presence of one hydroxyl group at the isoquinoline moiety. The presence of a hydroxyl group is also supported by characteristic infrared absorption at 3 200 $cm^{-1}$, by bathochromic shift in the UV spectrum upon alkalization and by formation of O-methylpseudorine. $^1$H NMR spectrum of pseudorine contains signals of two N-methyl and three O-methyl groups. Aliphatic protons form an AA'BB' system (3·10 and 3·68 ppm) and an ABX system (2·87, 3·57, and 4·77 ppm, $J_{AB}$ = 14 Hz, $J_{AX}$ = 9 Hz, $J_{BX}$ = 4 Hz). Signals at 3·68 and 4·77 ppm can be assigned to a methylene and methine groups adjacent to a positively charged nitrogen atom. Singlets of two aromatic protons at 5·95 and 6·79 ppm suggest their mutual *para*-position. Coupling pattern of the remaining three protons forming an ABC system (6·65, 6·67, and 6·85 ppm, $J_{AB} = J_{ortho}$ = 8·4 Hz, $J_{AC} = J_{para} = 0$, $J_{BC} = J_{meta}$ = = 2 Hz) is an evidence for a 1,3,4-trisubstituted benzene ring. $^{13}$C NMR spectrum contains signals of five methyls, three methylenes, six methines, and seven quaternary carbons. The multiplicities of carbon signals at 54·5 (triplet) and 71·2 ppm (doublet) confirm the presence of methylene and methine groups in the neighbourhood of nitrogen atom. All quaternary carbon atoms are $sp^2$-hybridized. Four of them resonate in the lowest field and therefore carry oxygen atoms. From their chemical shifts it follows that they form two vicinal pairs. The remaining three quaternary carbons are attached to $sp^3$-carbons. Therefore, the molecule is composed from two aromatic rings (one 1,2,4,5-tetrasubstituted and one 1,3,4-trisubstituted), a moiety —$CH_2CH_2N^{(+)}(CH_3)_2CHCH_2$—, three methoxyl groups and one phenolic hydroxyl. The requirement of two *ortho*-oriented $OCH_3$ groups means that the they must occupy the positions 3 and 4 in the trisubstituted ring. NMR data correspond to the benzyltetrahydroisoquinoline structure *Ia*. O-Methylpseudorine iodide has identical UV, IR, mass, $^1$H, and $^{13}$C NMR spectra with those of N-methyllaudanosinium iodide (*Ic*). High value of the similarity index (S = 0·9941) calculated on the basis of $^{13}$C NMR spectra[26] confirms their identity. That leaves for the hydroxyl group the position 6 or 7. Mass spectra of pseudorine iodide and N-methylcodaminium iodide (*Ib*) obtained by methylation of codamine[27] with

methyl iodide are identical. However, their IR and $^1$H NMR spectra are different. O-Methylation of N-methylcodaminium iodide provides also *Ic*. From all the above results it follows that pseudorine iodide is an isomer of N-methylcodaminium iodide having a hydroxyl group at the position 6 (*Ia*).*

Pseudoronine, $C_{20}H_{23}NO_6$ ($M^+$ 373·1499), is a yellow compound sparingly soluble in common solvents. Its structure is defined by complementary benzil fragments $C_{12}H_{16}NO_3$ (*m/z* 222) and $C_8H_7O_3$ (*m/z* 151). The former fragment loses a molecule of dimethylamine giving the ion $C_{10}H_9O_3$ (*m/z* 177). Labelling with [O-$^2$H]ethanol causes a shift of the $M^+$ ion in the mass spectrum of two mass units; the ions *m/z* 177 and 151 are shifted of one mass unit. That indicates a presence of two hydroxyl groups in the molecule. Mass spectrum of pseudoronine is analogous to that of cryptopleurospermine isolated from the species *Cryptocarya pleurosperma* WHITE *et* FRANCIS (family *Lauraceae*)[28] and to that of saxoguattine found in *Guatteria discolor* R. E. FRIES (family *Annonaceae*)[29]. Mass spectra of these alkaloids exhibit also characteristic ions $M^+ - H_2O$, $M^+ - 61$, and *m/z* 58 (base peak), similarly to the mass spectra of structuraly related secophtalideisoquinoline alkaloids bicuculinine and bicuculinidine[30]. There are absorption bands of C=O groups at 1 625 and 1 650 $cm^{-1}$ in the IR spectrum of pseudoronine. The structure *II* is proposed for pseudoronine on the basis of UV, IR, and mass spectra interpretation and comparison with the published data[28,29]. Small amount of material available and its low solubility prevented the determination of the localization of two hydroxyl and two methoxyl groups. Pseudoronine is the third known representative of the benzil type alkaloids. This is the first occurrence of this structural type in the family *Papaveraceae*.

The presence of coptisine and palmatine was proved in other samples of *P. pseudo-orientale*. We were unable to detect the oxoaporphine alkaloid PO-3 (refs[11,13]) in the total alkaloid fraction. However, its presence was demonstrated after some time in the mother liquors from crystallization of isothebaine. This finding supports the opinion that alkaloid PO-3 is an artifact formed from isothebaine by oxidation with air oxygen.

The alkaloid composition in the studied population indicates its classification as an isothebaine chemotype of *P. pseudo-orientale* known from Iran[4]. Quaternary alkaloids of the roots of *P. pseudo-orientale* are represented by N-methylisothebainium hydroxide, alborine, and magnoflorine that are derivatives of mostly dominating tertiary bases isothebaine, mecambridine and corytuberine, respectively, and by a new benzyltetrahydroisoquinoline alkaloid pseudorine whose corresponding tertiary base was not found. Our results are further confirmation of nearly general

* After receiving this work in press we ascertained, that benzylisoquinoline alkaloid with the same constitution — N-methyl-pseudolaudanine — was isolated from bark Fagara mayu (Assem E. M., Benages I. A., Albonico S. M.: Planta Med. *48*, 7780 (1983)). But in contrast to dextrorotatory pseudorine it deals with (±) form.

occurrence of the complementary pair of aporphine alkaloids corytuberine (*IV*) and magnoflorine (*IIIb*).

## EXPERIMENTAL

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. UV spectra were measured in methanol using a Pye Unicam SP 1 800 spectrophotometer. IR spectra were recorded in nujol mulls on a Specord IR-75 instrument (Zeiss, Jena). Mass spectra were measured on an AEI MS-902 spectrometer. Jeol FX-60 (59·797 MHz for $^1$H, 15·036 MHz for $^{13}$C, FT mode) and IEF 400 (400 MHz for $^1$H, FT mode, prototype at Institute of Electronic Fundamentals, Orsay, France) NMR spectrometers were used for NMR studies. When not given otherwise, the reported NMR spectra were measured in dimethyl sulfoxide at 25°C. They are referenced to internal tetramethylsilane. Column chromatography was carried out on silica gel L (particle size 80—110 μ, Lachema, Brno) in benzene or chloroform. As eluents were used: benzene, systems benzene–methanol (99 : 1 to 4 : 1), chloroform or systems chloroform–methanol (99 : 1 to 4 : 1). Aluminium oxide in benzene (activity III according to Brockmann, Merck, Darmstadt) was used for preparative chromatography. Benzene–chloroform systems were used as eluents in those cases. Thin-layer chromatography (TLC) was performed on silica gel LS (5—40 μ, Lachema, Brno) in the systems cyclohexane–diethylamine 9 : 1 ($S_1$), cyclohexane–chloroform–diethylamine 7 : 2 : 1 ($S_2$), benzene–diethylamine 19 : 1 ($S_3$), benzene–acetone–methanol 7 : 2 : 1 ($S_4$), methanol–water–25% ammonia 15 : 3 : 1 ($S_5$), methanol–25% ammonia 200 : 1 ($S_6$), methanol–water–36% hydrochloric acid 15 : 3 : 1 ($S_7$), 1-propanol–water–85% formic acid 7 : 2 : 1 ($S_8$) or on Silufol (Kavalier, Votice) in the system methanol–diethylamine 4 : 1 ($S_9$). Descending paper chromatography (PC) was made on Whatman No 1 paper in the systems 1-butanol–98% acetic acid–water 10 : 1 : 3 ($S_{10}$) and ethanol–water 3 : 2 ($S_{11}$). Fluorescent alkaloids were detected under UV light (365 and 254 nm), papaverrubines were detected by hydrogen chloride vapours (purple spots), other alkaloids were detected by spraying with potassium hexaiodoplatinate (TLC) or with Dragendorff's reagent (PC).

### Extraction and Isolation of Alkaloids

Plant material used in this study was cultivated at the Centre for Cultivation of Medicinal Plants of Medical Faculty, Purkyně University, Brno, from the seeds marked as "*P. bracteatum*" or "*P. orientale*" obtained from various botanical gardens. Roots of plants several years old were harvested at the end of vegetation season (19 Sept 1978). The plant material was air dried at room temperature and then ground. Dried roots (5 kg) were extracted with methanol in a Soxhlet apparatus. Methanol was distilled off, extract dissolved in the cold acetic acid (3·50 l, 0·2 mol $l^{-1}$). Alkaloid fractions A, B, and I were obtained by usual way[20,21].

Isothebaine (14·00 g) was obtained from the fraction A (30·96 g, 0·62% of dry weight) by crystallization from ether or ether–methanol 4 : 1, respectively. Bases from mother liquors were dissolved in chloroform and extracted with acetic acid (0·4 mol $l^{-1}$). Residue from the chloroform layer was recrystallized from methanol and provided orientalidine (115·0 mg). Saturated potassium chloride was added to the acidic aqueous layer and the mixture was separated to hydrochlorides of bases extractable with chloroform (AC) and hydrochlorides of bases insoluble in chloroform (AD)[20]. Bracteoline (61·7 mg) was separated from the AC fraction by crystallization from methanol and methanol–ether. Both fractions AC and AD were further divided into phenolic bases ACa and $AD_1$ and nonphenolic bases ACb and $AD_2$[20,31]. Crystallization of fraction ACa (0·53 g) from ether provided mecambridine (62·8 mg) and isothebaine (24·5 mg).

Remaining amorphous bases of the ACa fraction were subjected to column chromatography on silica gel (10·5 g, benzene). The elution was carried out with benzene and benzene–methanol mixtures (99·5 : 0·5, 99 : 1, 97 : 3, 95 : 5, and 9 : 1). Recrystallization of fractions obtained in this way gave nuciferine (4·1 mg), fractions containing protopine plus allocryptopine and a mixed fraction containing besides isothebaine the alkaloid PS 1 ($R_F$ 0·27 in $S_2$, yellow spot upon detection with potassium hexaiodoplatinate). Preparative TLC in $S_2$ afforded isothebaine (30·1 mg) and alkaloid PS 1 (4·4 mg, amorphous, UV spectrum in methanol: $\lambda_{max}$ 228, 287 nm, $\lambda_{min}$ 256 nm). Two compounds were detected chromatographically in the following fractions: papaverrubine D and an alkaloid having $R_F$ 0·30 ($S_2$) that we failed to characterize. Mother liquors contained in addition to the above mentioned alkaloids mecambridine, orientalidine, traces of bracteoline and some other bases. Fractional crystallization the portion ACb (3·00 g) from ether yielded isothebaine (0·11 g). The rest of the ACb fraction remained amorphous and therefore was separated by column chromatography on silica gel (60 g, chloroform); eluents were chloroform and its mixtures with ethanol (99·8 : 0·2, 99·5 : 0·5, 99 : 1, 97 : 3, 95 : 5, 9 : 1, and 4 : 1). The obtained fractions were combined according to TLC analyses. Their recrystallization from methanol allowed separation of isothebaine (0·17 g). Rest of fractions remained amorphous. They contained besides isothebaine some salutaridine, traces of bracteoline and further unidentified bases in addition to substantial amount of non-alkaloid oily substances. Portion $AD_1$ (0·11 g) was subjected to silica gel column chromatography (3·0 g, benzene, elution similar to fraction ACa). TLC analysis showed the presence of protopine and allocryptopine. Chromatographic separation on aluminum oxide column (benzene, elution with benzene–chloroform 98 : 2 and 97 : 3) gave protopine (4·1 mg) and allocryptopine (3·4 mg). Mother liquors contained besides these two alkaloids traces of some other bases. Portion $AD_2$ was subjected to column chromatography (silica gel, 4 g, chloroform) after the purification step. The same eluents as for ACb were used. Crystallization of chromatographic fractions from methanol or chloroform, respectively, provided bracteoline (25·2 mg). Amorphous bases from mother liquors contained besides bracteoline large amount of non-alkaloid components and also traces of isothebaine, salutaridine, alkaloid PS 1 and some not further identified bases.

Neither TLC on Silufol ($S_9$) nor PC ($S_{10}$ and $S_{11}$) proved the presence of quaternary protoberberine alkaloids in the fraction B (7·3 mg). Very small amount of coptisine and palmatine was found by TLC and PC in some other sample of plant material.

Crystallization of the fraction I (14·07 g, 0·28%) from methanol provided N-methylisothebainium iodide (3·84 g), corytuberine hydriodide (1·23 g), alborine iodide (0·29 g), and magnoflorine iodide (3·1 mg). Crystallization from ethanol afforded pseudorine iodide (1·58 g). Alkaloids of the mother liquors were separated on a silica gel column (elution: chloroform with increasing proportion of methanol). Crystallization of chromatographic fractions separated pseudoronine (9·9 mg) and mixture fractions containing besides the above mentioned alkaloids two non-identified alkaloids with $R_F$ 0·10 and 0·48 ($S_5$).

## Characterization of Isolated Alkaloids

Alkaloids were identified using melting points or mixed melting points, respectively, optical rotation, UV, IR, whenever possible also mass, $^1H$ and $^{13}C$ NMR spectra and by comparison of chromatographic $R_F$ values with those of authentic specimens. Yields of individual alkaloids are given in parentheses.

*Isothebaine* (0·28%): prisms m.p. 204–205°C (methanol), no depression with an authentic specimen, $[\alpha]_D^{20}$ +289 ± 4° (*c* 0·3, methanol). UV spectrum (methanol), $\lambda_{max}$ (log $\varepsilon$): 228 (4·12), 271 (4·07), 294 nm sh (3·81); $\lambda_{min}$ (log $\varepsilon$): 247 nm (3·70). IR spectrum: 3 250 $cm^{-1}$ (OH).

*N-Methylisothebainium iodide* (*IIIa*) (0·077%): needles m.p. 254—256°C (methanol), undepressed with an authentic sample, $[\alpha]_D^{20}$ +194 ± 3° (*c* 0·5, methanol). UV spectrum $\lambda_{max}$ (log ε): 223 (4·65), 273 (4·13), 300 nm (3·98); $\lambda_{min}$ (log ε): 252 (3·82), 287 nm (3·88). IR spectrum: 3 300 $cm^{-1}$ (OH). Mass spectrum *m/z* (intensity): 325 (2·3), 311 (9·4), 297 (6·3), 296 (4·7), 280 (5·6), 254 (9·4), 240 (20·0), 225 (9·4), 207 (5·9), 197 (5·6), 142 (100), 141 (17·5), 127 (26·3), 58 (87·5). $^1$H NMR spectrum (25°C): 3·37 s (6 H, $N(CH_3)_2$), 3·84 s (6 H, 2 × $OCH_3$), 4·49 dd (*J* = 13·4 and 3·6 Hz, 1 H), 6·88 s (1 H), 6·94—7·49 mt (3 H), 8·43 s (1 H, OH). $^{13}$C NMR spectrum: 22·9 t, 30·0 t, 42·9 q (2 C), 55·6 q (2 C), 60·2 t, 67·8 d, 109·7 d, 111·8 d, 118·1 s, 118·5 s, 120·1 d + s (2 C), 120·9 s, 128·7 d, 134·6 s, 143·2 s, 148·8 s, 155·7 s.

*Pseudorine iodide* (*Ia*) (0·032%): prisms m.p. 204—206°C (ethanol), $[\alpha]_D^{20}$ +107 ± 4° (*c* 0·5, methanol). UV spectrum (methanol), $\lambda_{max}$ (log ε): 228 (4·20), 284 nm (3·78); $\lambda_{min}$ (log ε): 256 nm (3·02). UV spectrum (methanol + NaOH): $\lambda_{max}$ (log ε): 229 (4·24), 254 sh (3·92), 288 (3·71), 304 nm (3·75); $\lambda_{min}$ (log ε): 274 (3·64), 293 nm (3·68). IR spectrum: 3 200 $cm^{-1}$ (OH). Mass spectrum, *m/z* (composition, intensity): 357 ($C_{21}H_{27}NO_4$, 1·1), 342 (0·4), 326 (0·2), 192 ($C_{11}H_{14}NO_2$, 100), 177 ($C_{10}H_{11}NO_2$, 15), 151 ($C_9H_{11}O_2$, 1·8), 149 ($C_9H_{11}NO$, 2·1), 148 ($C_9H_{10}NO$, 2·8), 142 (11·3), 127 (3·3), 58 (6·4). Ions *m/z* 357, 342, 326, 192, and 177 are shifted of one mass unit upon labelling with [O—$^2$H] ethanol. $^1$H NMR spectrum (400 MHz, deuteriochloroform + hexadeuteriodimethyl sulfoxide): 2·875 dd (*J* = 14 and 9 Hz, 1 H), 3·100 mt (2 H), 3·140 s (3 H), 3·392 s (3 H), 3·573 dd (*J* = 14 and 4 Hz, 1 H), 3·682 mt (2 H), 3·726 s (3 H), 3·802 s (3 H), 3·816 s (3 H), 4·774 dd (*J* = 9 and 4 Hz, 1 H), 5·950 s (1 H), 6·650 dd (*J* = 8·4 and 2 Hz, 1 H), 6·667 d (*J* = 2 Hz, 1 H), 6·792 s (1 H), 6·851 d (*J* = 8·4 Hz, 1 H), 8·449 s (1 H, exchangeable, OH). $^{13}$C NMR spectrum: 23·0 t, 38·9 t, 50·4 q, 51·1 q, 54·5 t, 55·5 q, 55·6 q (2 C), 71·2 d, 111·8 d (2 C), 113·5 d, 115·0 d, 119·2 s, 122·0 d, 122·9 s, 128·1 s, 144·6 s, 147·8 s, 147·9 s, 148·6 s.

*Corytuberine hydriodide* (*IV*) (0·025%): glistening leaves m.p. 212—214°C (methanol), $[\alpha]_D^{20}$ +181 ± 5° (*c* 0·2, methanol). UV spectrum (methanol), $\lambda_{max}$ (log ε): 223 (5·72), 268 (4·12), 303 nm (3·87); $\lambda_{min}$ (log ε): 249 (3·83), 289 nm (3·77). IR spectrum: broad absorption at 3 400 to 3 530 and 3 600 $cm^{-1}$ (OH). The identity was confirmed by TLC comparison with an authentic sample.

*Alborine iodide* (0·0058%): yellow needles (methanol), not melting up to 360°C. UV spectrum (methanol), $\lambda_{max}$ (log ε): 245 (4·34), 262 (4·32), 292 (4·50), 336 nm (4·20); $\lambda_{min}$ (log ε): 243 (4·30), 254 (4·28), 272 (4·25), 318 nm (4·20). IR spectrum: 3 300 $cm^{-1}$ (OH). The identity was proved by TLC comparison with an authentic specimen.

*Orientalidine* (0·0023%): needles m.p. 194—195°C (methanol), $[\alpha]_D^{20}$ −322 ± 7° (*c* 0·1, methanol), ref.[32] gives $[\alpha]_D^{20}$ −245°, other data agree well. UV spectrum (methanol), $\lambda_{max}$ (log ε): 233 (4·03), 287 nm (3·77); $\lambda_{min}$ (log ε): 256 nm (2·95). IR spectrum: 945 and 1 025 $cm^{-1}$ ($CH_2O_2$), Bohlman's bands at 2 715 $cm^{-1}$ (*trans*-junction of B/C rings).

*Bracteoline* (0·0017%): needles m.p. 225—229°C (methanol), quickly decomposing on air, $[\alpha]_D^{20}$ +51 ± 10° (*c* 0·05, methanol), ref.[14] gives $[\alpha]_D^{20}$ +35 ± 8°. UV spectrum (methanol), $\lambda_{max}$ (log ε): 231 (4·21), 270 sh (3·81), 280 (3·90), 307 nm (3·97); $\lambda_{min}$ (log ε): 256 (3·54), 289 nm (3·76). UV spectrum (methanol + NaOH), $\lambda_{max}$ (log ε): 236 (4·23), 280 sh (3·71), 330 nm (3·79); $\lambda_{min}$ (log ε): 294 nm (3·50). IR spectrum: 935 and 1 025 $cm^{-1}$ ($CH_2O_2$) and broad absorption at 3 100—3 350 $cm^{-1}$ (OH).

*Mecambridine* (0·0013%): needles m.p. 182—184°C (methanol), no depression with an authentic preparation. UV spectrum (methanol), $\lambda_{max}$ (log ε): 228 (4·15), 288 nm (3·74); $\lambda_{min}$ (log ε): 257 nm (2·80). IR spectrum: 3 200 $cm^{-1}$ (OH). The identity was also proved by TLC comparison with an authentic sample.

*Pseudoronine* (*II*) (0·0002%): yellow needles m.p. 249—252°C (methanol), sparingly soluble in chloroform, methanol, and ethanol. UV spectrum (methanol), $\lambda_{max}$ (log ε): 211, 233, 285, 315 nm; $\lambda_{min}$ (log ε): 220, 257, 303 nm. IR spectrum: 1 625 and 1 650 $cm^{-1}$ (C=O). Mass spectrum, *m/z* (composition, intensity): $M^+$ 373 ($C_{20}H_{23}NO_6$, 3·5), 355 (1·6), 328 ($C_{18}H_{16}O_6$, 1·6), 312 ($C_{18}H_{16}O_5$, 2·9, increases with time), 300 ($C_{17}H_{16}O_5$, 1·2), 222 ($C_{12}H_{16}O_3$, 12·8), 192 ($C_{11}H_{14}NO_2$, 2·3), 190 ($C_{11}H_{12}NO_2$, 1·6), 179 ($C_{10}H_{11}O_3$, 3·1), 177 ($C_{10}H_9O_3$, 8·2), 164 (1·9), 151 ($C_8H_7O_3$, 17·5), 136 ($C_8H_8O_2$, 3·5), 123 ($C_7H_7O_2$, 4·7), 58 (100). Labelling with [O—$^2$H]ethanol shifts $M^+$ ion of two mass units, ions *m/z* 222, 177, and 151 of one mass unit.

*Nuciferine* (0·00008%): prisms m.p. 165—167°C (methanol), mixed m.p. with an authentic sample without depression. UV spectrum (methanol), $\lambda_{max}$ (log ε): 216 (4·66), 231 sh (4·48), 273 nm (4·39); $\lambda_{min}$ (log ε): 249 nm (3·30). IR spectrum and TLC were identical with those of an authentic specimen.

*Protopine* (0·00008%): prisms m.p. 205—206°C (chloroform–ethanol), undepressed on admixture of an authentic preparation. UV spectrum (methanol), $\lambda_{max}$ (log ε): 230 (2·94), 290 nm (3·84); $\lambda_{min}$ (log ε): 263 nm (3·60). IR spectrum: 1 650 $cm^{-1}$ (C=O). The identity was proved by TLC comparison with an authentic sample.

*Allocryptopine* (0·00007%): prisms m.p. 162—163°C (ethanol), no depression with an authentic specimen. UV spectrum (methanol), $\lambda_{max}$ (log ε): 232 (3·88), 284 nm (3·78); $\lambda_{min}$ (log ε): 256 nm (3·56). IR spectrum: 1 665 $cm^{-1}$ (C=O). The identity was confirmed by TLC comparison with an authentic sample.

*Magnoflorine iodide* (*IIIb*): (0·00006%): prisms m.p. 261—262°C (methanol), without depression on admixture of an authentic preparation. UV spectrum (methanol), $\lambda_{max}$ (log ε): 225 (4·81), 270 (4·13), 311 nm (4·00); $\lambda_{min}$ (log ε): 258 (4·06), 292 nm (3·85). IR spectrum: 3 400 $cm^{-1}$ (OH). The identity was further confirmed by TLC comparison with an authentic sample.

O-Methylpseudorine Iodide (*Ic*)

Potassium hydroxide (0·2 g) and methyl iodide (0·2 ml) were added to the solution of pseudorine (39·7 mg) in methanol (2 ml). The mixture was refluxed 3 h on the water bath, diluted with water (15 ml), acidified to pH 6·5 by sulfuric acid and methanol was distilled off. After cooling, KI was added and the product was extracted with chloroform. Recrystallization from methanol afforded *Ic* (37·8 mg), m.p. 225—227°C; IR absorption of OH group disappeared. UV spectrum (methanol), $\lambda_{max}$ (log ε): 232 (4·20), 282 nm (3·68); $\lambda_{min}$ (log ε): 257 nm (3·07). Mass spectrum, *m/z* (intensity): 371 (2·3), 356 (0·4), 340 (0·5), 206 (100), 191 (10·4), 190 (16), 162 (6), 151 (3·3), 142 (23), 141 (4), 127 (5·6), 58 (18). $^1$H NMR spectrum (deuterio-chloroform): 2·84 dd ($J$ = 10·3 and 12·2 Hz, 1 H), 3·41 s (3 H), 3·54 s (3 H), 3·82 s (3 H), 3·85 s (3 H), 3·87 s (3 H), 3·92 s (3 H), 5·25 dd ($J$ = 10·3 and 3·4 Hz, 1 H), 5·76 s (1 H), 6·44 dd ($J$ = 7·8 and 2 Hz, 1 H), 6·64 s (1 H), 6·72 d ($J$ = 7·8 Hz, 1 H), 6·90 d ($J$ = 2 Hz, 1 H). $^{13}$C NMR spectrum (deuteriochloroform): 23·6 t, 38·0 t, 50·8 q, 52·9 q, 55·2 q, 55·5 q, 55·9 q (2 C), 56·4 q, 72·0 d, 110·5 d, 111·1 d, 111·5 d, 113·5 d, 119·2 s, 121·2 s, 122·7 d, 126·5 s, 147·2 s, 148·5 s, 149·4 s (2 C).

(±)-N,N-Dimethyl-7-hydroxy-6,3′,4′-trimethoxyphenylethyl-
-1,2,3,4-tetrahydroisoquinolinium Iodide (*Vb*)

Methyl iodide (0·5 ml) was added to the solution of (±)-N-methyl-7-hydroxy-6,3′,4′-trimethoxyphenylethyl-1,2,3,4-tetrahydroisoquinoline[25] (40 mg) in methanol (4 ml) and the mixture was refluxed 4 h on the water bath. Purification gave 38·2 mg of an amorphous product. Mass spectrum, *m/z*: 371, 357, 356, 206, 192, 177, 151, 142, 127, 58.

(±)-N,N-Dimethyl-6,7,3′,4′-tetramethoxyphenylethyl-
-1,2,3,4-tetrahydroisoquinolinium iodide (*Vc*)

(±)-N,N-Dimethyl-6,7,3′,4′-tetramethoxyphenylethyl-1,2,3,4-tetrahydroisoquinolinium iodide (*Vb*, 20·6 mg) was dissolved in methanol (2 ml) and 3 h refluxed with KOH (0·2 g) and methyl iodide (0·2 ml). The reaction mixture was diluted with water (15 ml), acidified to pH 6·5, methanol distilled off, and after addition of KI extracted with chloroform. Recrystallization from methanol gave 17·6 mg of a product m.p. 162—164°C. UV spectrum (methanol), $\lambda_{max}$ (log $\varepsilon$): 226 (4·22), 281 nm (3·73); $\lambda_{min}$ (log $\varepsilon$): 256 nm (3·07). IR spectrum was different from that of O-methyl--pseudorine iodide. Mass spectrum, *m/z* (intensity): 385 (0·4), 371 (0·4), 370 (0·3), 356 (0·2), 206 (100), 191 (4·6), 190 (9·7), 162 (3·8), 151 (4·3), 142 (10·7), 127 (4·8), 58 (5·1).

(±)-N-Methyllaudanosinium Iodide (*Ic*)

Papaverine (90 mg) was dissolved in methanol (10 ml), methyl iodide (5 ml) was added and the mixture was refluxed 7 h on a water bath. Recrystallization of the crude product from methanol gave N-methylpapaverinium iodide (92 mg), m.p. 195—196°C, UV spectrum (methanol), $\lambda_{max}$ (log $\varepsilon$): 228 (4·73), 256 (4·83), 276—288 sh (4·00), 317 nm (4·17), $\lambda_{min}$ (log $\varepsilon$): 249 (4·61), 294 nm (3·95). Excess of $NaBH_4$ was stepwise added in small portions to the solution of N-methyl-papaverinium iodide (60 mg) in methanol (4 ml) at room temperature. The reaction mixture was diluted with water, neutralized by sulfuric acid, alkalized with ammonia and then extracted with ether. Recrystallization from methanol produced (±)-laudanosine (37 mg, m.p. 112—113°C). UV spectrum (methanol), $\lambda_{max}$ (log $\varepsilon$): 234 (4·05), 282 nm (3·77); $\lambda_{min}$ (log $\varepsilon$): 256 nm (3·04). Methyl iodide (0·7 ml) and ether (4 ml) was added to the solution of (±)-laudanosine (23 mg) in methanol (2 ml). The mixture was allowed to stand at room temperature. Crude product (25 mg) crystallized after 12 h. Recrystallization from methanol–ether yielded 20 mg of (±)-N--methyllaudanosinium iodide m.p. 215—216°C. Its UV, IR, mass, $^1$H and $^{13}$C NMR spectra were identical with those of O-methylpseudorine iodide.

(±)-N-Methylcodaminium Iodide (*Ib*)

(±)-Codamine[27] (24 mg) in methanol (3 ml) was refluxed 4 h on a water bath with methyl iodide (0·4 ml) and ether (3 ml). After cooling, 16 mg of the product crystallized. Its recrystallization from methanol–ether gave (±)-N-methylcodaminium iodide (14 mg), m.p. 228—229°C. UV spectrum (methanol), $\lambda_{max}$ (log $\varepsilon$): 231 (4·11), 284 nm (3·78); $\lambda_{min}$ (log $\varepsilon$): 257 nm (3·01); (methanol + NaOH): $\lambda_{max}$ (log $\varepsilon$): 231 (4·22), 254 sh (3·92), 288 (3·72), 304 nm (3·75); $\lambda_{min}$ (log $\varepsilon$): 274 (3·64), 294 nm (3·68). Mass spectrum, *m/z* (intensity): 357 (4·2), 192 (100), 177 (21·4), 142 (26·4), 127 (11·5), 58 (29·7). IR spectrum: 3 250 $cm^{-1}$ (OH). $^1$H NMR spectrum (deuteriochloroform + one drop of hexadeuteriodimethyl sulfoxide): 3·39 s, 3·65 s, 3·76 s, 3·84 s, 3·88 s, 6·47 s, 6·61 s.

(±)-N,O-Dimethylcodaminium Iodide (*Ic*)

Potassium hydroxide (0·8 g) and methyl iodide (0·6 ml) were added to the solution of *Ib* (9 mg) in methanol (2 ml). The mixture was refluxed 3 h on a water bath, after cooling diluted with water (15 ml), acidified by sulfuric acid to pH 6·5 and methanol removed by distillation. KI was added and the product extracted with chloroform. Recrystallization from methanol–ether provided 6 mg of (±)-N,O-dimethylcodaminium iodide, m.p. 216—217°C that gave no melting point depression with (±)-N-methyllaudanosinium iodide. UV, IR, and mass spectra were identical with those of O-methylpseudorine iodide and N-methyllaudanosine iodide.

$R_F$ Values

Systems $S_1$, $S_2$, and $S_4$: allocryptopine 0·18, 0·49, and 0·04; bracteoline 0·30, 0·10, and 0·45; codamine 0·11, 0·37, and 0·33; isothebaine 0·16, 0·45, and 0·53; laudanosine 0·35, 0·73, and 0·40; mecambridine 0·10, 0·30, and 0·60; N-methyl-7-hydroxy-6,3′,4′-trimethoxyphenylethyl-1,2,3,4-tetrahydroisoquinoline 0·10, 0·37, and 0·24; nuciferine 0·46, 0·65, and 0·53; orientalidine 0·22, 0·54, and 0·72; papaverine 0·11, 0·57, and 0·83; protopine 0·34, 0·54, and 0·36; alkaloid PS 1 0·05, 0·24, and 0·67; salutaridine 0·01, 0·25, and 0·20. Systems $S_1$, $S_2$, $S_3$, and $S_4$: papaverrubine D 0·04, 0·16, 0·20, and 0·46. Systems $S_5$, $S_6$, $S_7$, and $S_8$: alborine 0·10, 0·01, 0·60, and 0·55; corytuberine 0·78, 0·64, 0·70, and 0·64; N,O-dimethylcodaminium iodide 0·09, 0·01, 0·55, and 0·63; N,N-dimethyl-7-hydroxy-6,3′,4′-trimethoxyphenylethyl-1,2,3,4-tetrahydroisoquinolinium iodide 0·10, 0·02, 0·57, and 0·66; N,N-dimethyl-6,7,3′,4′-tetramethoxyphenylethyl-1,2,3,4-tetrahydroisoquinolinium iodide 0·09, 0·01, 0·55, and 0·63; N-methylcodaminium iodide 0·09, 0·1, 0·55, and 0·55; N-methylisothebainium iodide 0·03, 0·01, 0·40, and 0·38; N-methyllaudanosinium iodide 0·16, 0·01, 0·54, and 0·49; N-methylpapaverinium iodide 0·12, 0·01, 0·56, and 0·54; magnoflorine 0·31, 0·04, 0·40, and 0·47; O-methylpseudorine iodide 0·09, 0·01, 0·56, and 0·59; pseudorine 0·09, 0·01, 0·56, and 0·59; pseudoronine 0·10, 0·03, 0·70, and 0·72. Systems $S_9$, $S_{10}$, and $S_{11}$: coptisine 0·55, 0·43, and 0·07; palmatine 0·15, 0·53, and 0·20.

*It is a pleasure to express here our thanks to Prof. B. Umezawa (Science University of Tokyo, Japan) who kindly supplied the samples of N-methyl-7-hydroxy-6,3′,4′-trimethoxyphenylethyl-1,2,3,4-tetrahydroisoquinoline and codamine, and to Dr V. Preininger (Palacký University, Olomouc) who supplied us the samples of orientalidine and salutaridine. We are indebted to Dr V. Šimánek (Palacký University, Olomouc) for 400 MHz $^1$H NMR spectra measurement. Skillful technical assistence of Mrs A. Kuncová of our Department is also acknowledged.*

REFERENCES

1. Fedde F.: *Das Pflanzenreich — Regni vegetabilis conspectus* (A. Engler, Ed.), Part IV, Vol. 104, *Papaveraceae-Hypecoideae et Papaveraceae-Papaveroideae*. Leipzig 1909.
2. Goldblatt P.: Ann. Missouri Bot. Gard. *61*, 264 (1974).
3. Novák J.: Sb. Vys. Sk. Zemed. Praze, Fak. Agron., A *32*, 3 (1980).
4. Shaffie A., Lalezari P., Nasseri-Nouri P., Asgharian R.: J. Pharm. Sci. *64*, 1570 (1975).
5. Sariyar G.: Istanbul Univ. Eczacilik. Fac. Mecm. *12*, 171 (1976); Chem. Abstr. *86*, 185917 (1977).
6. Baytop T., Sariyar G.: Istanbul Univ. Eczacilik. Fac. Eczacilik. Fac. Mecm. *13*, 7 (1977); Chem. Abstr. *88*, 148949 (1978).
7. Sariyar G.: Istanbul Univ. Eczacilik. Fac. Mecm. *13*, 171 (1977); Chem. Abstr. *88*, 148950 (1978).
8. Sariyar G., Phillipson J. D.: Phytochemistry *16*, 2009 (1977).
9. Sariyar G., Baytop T.: Planta Med. *28*, 378 (1980).
10. Phillipson J. D., Scutt A., Baytop T., Özhatay N., Sariyar G.: Planta Med. *29*, 261 (1981).
11. Preininger V., Šantavý F.: Acta Univ. Palacki. Olomuc. *43*, 5 (1966).
12. Preininger V., Šantavý F.: Pharmazie *25*, 356 (1970).
13. Battersby A. R., Brown T. H.: Chem. Commun. *1966*, 170.
14. Pfeifer S.: Pharmazie *17*, 298 (1962).
15. Pfeifer S., Banerjee S. K.: Pharmazie *19*, 286 (1964).
16. Heydenreich K., Pfeifer S.: Pharmazie *20*, 521 (1965).
17. Heydenreich K., Pfeifer S.: Pharmazie *21*, 121 (1966).
18. Délenk-Heydenreich K., Pfeifer S.: Pharmazie *24*, 635 (1969).

19. Slavík J., Picka K., Slavíková L., Táborská E., Věžník F.: This Journal *45*, 914 (1980).
20. Slavík J., Slavíková L.: This Journal *26*, 1839 (1961).
21. Slavíková L., Slavík J.: This Journal *31*, 3362 (1966).
22. Dolejš L.: This Journal *49*, 2816 (1984).
23. Marsaioli A. J., Reis F. A. M., Magalhaes A. F., Rüveda E. A., Kuck A. M.: Phytochemistry *18*, 165 (1979).
24. Slavík J., Dolejš L., Slavíková L.: This Journal *50*, 2299 (1985).
25. Hoshino O., Toshioka T., Ohyama K., Umezawa B.: Chem. Pharm. Bull. *22*, 1307 (1974).
26. Bremser W., Klier M., Meyer E.: Org. Magn. Reson. *7*, 97 (1975).
27. Hoshino O., Toshioka T., Umezawa B.: Chem. Pharm. Bull. *22*, 1302 (1974).
28. Johns S. R., Lamberton J. A., Sioumis A. A., Willing R. I.: Aust. J. Chem. *23*, 253 (1970).
29. Hocquemiller R., Debitus C., Roblot F., Cavé A.: J. Nat. Prod. *47*, 353 (1984).
30. Kiryakov H. G., Mardirossian Z. H., Mac Lean D. B., Ruder J. P.: Phytochemistry *20*, 1721 (1981).
31. Slavíková L., Slavík J.: This Journal *24*, 559 (1959).
32. Holubek J., Štrouf O.: *Spectral Data and Physical Constants of Alkaloids*. Academia, Prague 1965.

Translated by the author (P.S.).

*Note added in proof:* In formula *Ib* for $R^1 = R^2 = CH_3$ should read $R^1 = CH_3$; $R^2 = H$ and in formula *Ic* for $R^1 = CH_3$; $R^2 = H$ should read $R^1 = R^2 = CH_3$.