ON ALKALOIDS FROM *Roemeria hybrida* (L.) DC.*

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From *Roemeria hybrida* (L.) DC. the alkaloids roemeridine, (—)-isocorypalmine, new alkaloids roehybridine, roehybrine and alkaloid RH 5 were isolated and traces of coptisine proved. From the data of spectral analyses the structure of roehybrine was deduced.

Roemeria hybrida (L.) DC. (synonymum: *R. violacea* MEDIK.) is an annual occurring in the whole Mediterranean area and reaching as far as Central Asia. The alkaloids of this plant were investigated by Platonova and coworkers[1] who isolated protopine from it as the main alkaloid (0·04%), a new alkaloid roemeridine (0·02%), and a small amount of an unidentified alkaloid of m.p. 230°C. Roemeridine was also found in *Papaver pavoninum* FISCH. *et* MEY[1]. (0·002%; *cf.*[2]).

We started our own investigation of this plant some ten years ago[2]. The material from two collections was worked up and a mixture of alkaloids isolated in a 0·16% yield. In contrast to the Soviet authors[1] we were not able to detect the presence of protopine in our material. We isolated as the main component of the basic fraction of the above-ground part of the plant and its root an alkaloid which according to all its properties was identical with roemeridine[1,2]. The structure of this alkaloid is not known so far. The composition $C_{31}H_{39}N_3O_5$, given by the Soviet authors[1], was confirmed both by elemental analysis and high resolution mass spectral measurements. Evidently the alkaloid is of a type unique in *Papaveraceae*. The base is of phenolic character; in its IR spectrum there are bands at 1565—1608 cm^{-1} (aromatic ring) and 3290 and 3350 cm^{-1} (OH, or also NH). It does not contain a keto group. In the NMR spectrum a multiplet at 1·50 to 3·40 p.p.m. (protons of the CH, CH_2 and similar types), singlets (3 H each) at 2·35 and 3·10 p.p.m. due to two N-methyl groups (confirmed by the shifts of these signals to 2·76 and 3·24 p.p.m. after addition of CD_3COOH), further a three-proton singlet at 3·88 p.p.m. and a six-proton singlet at 3·90 p.p.m. (totally three OCH_3 groups bound to the aromatic ring), one-proton singlets at 6·57, 6·90 and 7·26 (3 aromatic H), and a broad exchangeable singlet at 9·13 p.p.m. (NH or phenolic OH) were present. From the

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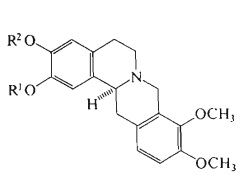
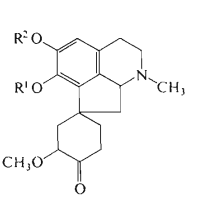
nuclear Overhauser effect it can be deduced that one of the aromatic protons is in *ortho* position to one methoxyl group, and the second probably *ortho* to two methoxyl groups. The mass spectrum of roemeridine contains intense peaks at *m/e* 257 ($C_{15}H_{17}N_2O_2$), 244 (b.p., $C_{14}H_{16}N_2O_2$), 230 ($C_{14}H_{16}NO_2$) and 229. The metastable transitions at 123·9 and 111·7 indicate that the first two fragments are formed by direct decomposition of the ionised molecule. The lack of other more pronounced fragmentation indicates that the roemeridine molecule is composed of two parts of which especially that containing two nitrogen atoms very effectively stabilises the positive charge. In the mass spectrum of roemeridine labelled in the inlet probe of the mass spectrometer with deuterioethanol the molecular ion and the fragments of *m/e* 257 and 244 are shifted by two mass units to higher masses.

From these findings it follows that roemeridine contains three methoxyl groups (in accordance with the literature[1]), two NCH_3 groups and at least one phenolic hydroxyl.

By direct crystallisation two further phenolic alkaloids were isolated in a negligible amount. One of them corresponded according to its melting point to the unidentified alkaloid of m.p. 230°C mentioned in the paper by the Soviet authors[1]. From the mass spectrum with the peaks at masses 341 (M^+), 178, 176, 164 and 149 the possible identity with corypalmine (*Ia*) or isocorypalmine (*Ib*) was deduced. Direct comparison with authentic samples demonstrated that the alkaloid from *R. hybrida* is identical with (−)-isocorypalmine (*Ib*). The second of them, undescribed in the literature so far, was named roehybridine. Its UV spectrum was very similar to that of roemeridine, but it differed from it, for example, by its melting point, and the R_F value. Its mass spectrum was identical with that of roemeridine, from which it may be deduced that the substances are very closely related.



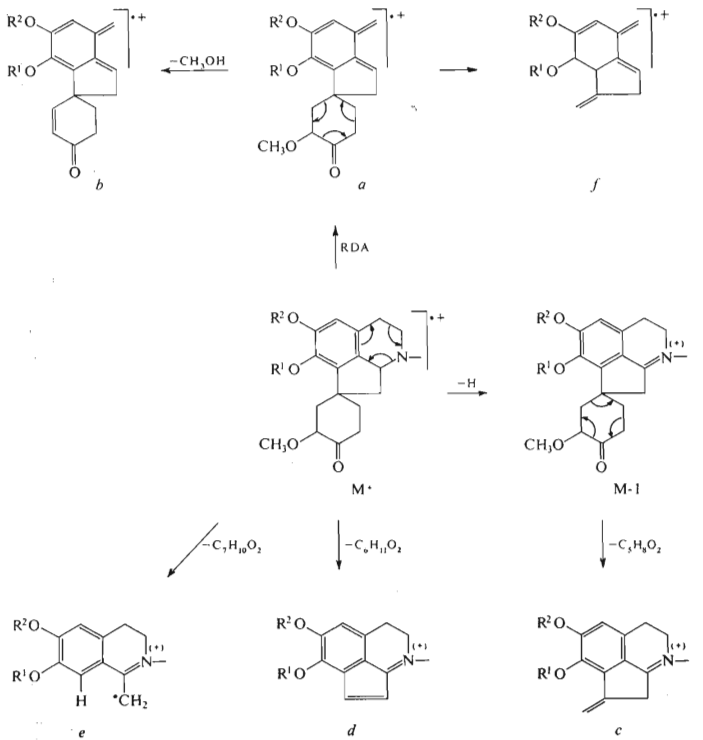
Ia, $R^1 = CH_3$, $R^2 = H$
Ib, $R^1 = H$, $R^2 = CH_3$



IIa, $R^1 = H$, $R^2 = CH_3$
IIb, $R^1 = CH_3$, $R^2 = H$

An appreciable part of the bases from *R. hybrida* remained amorphous and according to thin-layer chromatography it was composed of a larger number of substances. From this mixture of bases we isolated the perchlorate of another new alkaloid which we named roehybrine. Its base was also obtained crystalline and it had

a phenolic character. According to its IR spectrum it contains a keto and a hydroxy group (1725 and 3520 cm^{-1}, resp.). The most intense peaks of the mass spectrum (Fig. 1) are the molecular ion and the fragment M-1 (b.p.). The composition of the molecular ion, $C_{19}H_{25}NO_4$, corresponding to a molecule with eight unsaturations, together with the presence of a carbonyl group detected by IR spectroscopy, enable the exclusion from the number of alkaloid types occurring in *Papaveraceae* the structures with two aromatic rings and leaves as most probable the proaporphine and



SCHEME 1

morphinane types. The overall fragmentation pattern of roehybrine may indeed be explained satisfactorily by alternative proaporphine structures *IIa* and *IIb*. The ionised roehybrine molecule splits off by the retro-Diels–Alder mechanism a molecule of methyl-methyleneimine; the formed abundant fragment $C_{17}H_{20}O_4$ ("*a*") of mass 288 characterises the N-methyltetrahydroisoquinoline grouping of roehybrine (*cf.* for example[3–5]). In agreement with the presence of the aliphatic methoxyl group in the molecule of the substance the ion "*a*" splits off methanol yielding the fragment $C_{16}H_{16}O_3$ ("*b*") of mass 256. The fragmentation process is accompanied by the metastable transition at m^x 227·6. The spirocyclohexane system of roehybrine manifests itself by a series of fragmentation reactions in which, according to the high resolution data, the splitting off of its part or of the whole spirane cycle takes place. The fragments $M-C_5H_9O_2$, $M-C_6H_{11}O_2$ and $M-C_7H_{10}O_2$ are especially characteristic. The first fragment of mass 230 ("*c*") is probably formed from the ion M−1 by a retro-Diels–Alder mechanism (Scheme 1). The remaining two fragments of masses 216 ("*d*") and 205 ("*e*") are formed by the cleavage of two bonds protruding from the spirane carbon atom. The formation of both ions is evidently accompanied by the migration of hydrogen atoms. By a cyclic shift of the bonds in the spirocyclohexane ring, the ion "*a*" gives the fragment $C_{12}H_{12}O_2$ ("*f*"), approximately 66% of the doublet at mass 188, the composition of the departing particles being $C_5H_8O_2$. The second component of the doublet has the composition $C_{11}H_{10}NO_2$ and it probably represents the particle "*d*-C_2H_4". The satellite peak at mass 187 is a doublet (4 : 1) of the ions $C_{12}H_{11}O_2$ ("*f*-H") and $C_{12}H_{13}NO$ ("*d*-CHO" ?).

Hence, from the mentioned facts the structures *IIa* or *IIb* may be inferred for roehybrine, corresponding to the yet undescribed tetrahydro derivative of orientalinone[6] (*IIa*) or its positional isomer (*IIb*). Biogenetical reasons support rather the structure *IIa*.

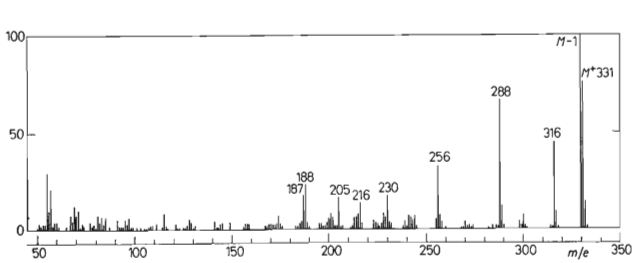


FIG. 1
Mass Spectrum of Roehybrine

From the remaining amorphous bases another crystalline alkaloid was isolated preliminarily called RH 5. However, its amount was so low that it could be characterised by its melting point and R_F value only. In the fraction of strong bases the presence of trace amounts of coptisine was also detected. In addition to the mentioned alkaloids a nitrogen-free substance of the composition $C_{11}H_{16}O_3$ was also isolated.

From the comparison of the alkaloids from both studied species of the genus *Roemeria, i.e. R. hybrida* and *R. refracta* (STEV.) DC. (see[5]) it is evident that both plants are biochemically appreciably different. The single common biochemical feature is the presence of proaporphine alkaloids which in *R. refracta* are represented by roemeramine, roemeronine and mecambrine. However, in both cases the alkaloids of this type occur as minor components of the basic fraction only. It is remarkable that *R. hybrida* contains practically exclusively alkaloids of phenolic character.

EXPERIMENTAL

The melting points (uncorrected) were determined in a capillary or on a Kofler block. The PMR spectrum was measured in a mixture of hexadeuteriodimethyl sulfoxide and deuteriochloroform on a Varian HA-100 apparatus (100 MHz). The mass spectra were measured with an AEI-MS 902 spectrometer, the IR spectra (in chloroform) on Infrascan Hilger and Watts, and the UV spectra (in methanol) on a Unicam SP 500 spectrophotometer. For thin-layer chromatography silica gel with gypsum (5 : 1) was used and for development a mixture of cyclohexane–chloroform–diethylamine in 6 : 3 : 1 (S_1) and 7 : 2 : 1 (S_2) ratios, and ethanol–water–25% ammonia 15 : 9 : 1 (S_3). The spots were detected with potassium iodoplatinate. The colour appearing is indicated in the brackets. Paper chromatography was carried out on paper Whatman No 1 (descending method) with the mixtures 1-butanol–acetic acid–water (10 : 1 : 3) (S_4) and ethanol–water 3 : 2 (S_5). Detection by fluorescence or Dragendorff reagent.

Extraction and Isolation of Alkaloids

The plants were cultivated both in a private garden in Olomouc (gathered on July 4th, 1958, sample 1) and in the Experimental Botanical Garden, Medical Faculty, Brno (gathered on June 27th, 1966, sample 2). They were gathered during the period of flowering and unripe fruits and the plants were dried at room temperature. The above-ground parts (1746 g; 0·074% of alkaloids) and the roots (158 g; 0·47% of alkaloids) of sample 1 were worked up separately, while the whole plant of sample 2 (3065 g; 0·19% of alkaloids) was used. Unless otherwise stated, the yields indicated in the text are calculated per total plant material. The dry ground plant (totally 4969 g) was extracted in a Soxhlet extractor with ethanol and alkaloid fractions *A*, *B*, *E* and *I* were obtained from the extract in the conventional manner[7,8]. The total yield of the crude alkaloid fraction (without fraction *I* which was predominantly non-alkaloidal) was 7·80 g (0·16%). The bases of fraction A were separated[7] to fractions AC_1, AC_2 and *AD*. Fraction AC_1 contained traces of alkaloids only. Crystallisation from an ether–hexane mixture gave a nitrogen-free substance (50 mg) of m.p. 144–146°C (Kofler block) and of the composition $C_{11}H_{16}O_3$ (according to high resolution mass spectrometry), evidently containing an aliphatic hydroxyl group. The bases of fraction AC_2 gave on crystallisation from ether (—)-isocorypalmine (6 mg; 0·0001,2%). The presence of other alkaloids was not detected. The rest of the fraction AC_2 contained a nitrogen-free substance (6·2 mg), identical with that from fraction AC_1. From fraction *AD* the bulk of

roemeridine was separated by crystallisation from acetone and a smaller amount by crystallisation of hydrochlorides from ethanol (total yield of roemeridine, including the fraction obtained by chromatography — see below — was 2·68 g; 0·054%). Crystallisation of the remaining bases of fraction *AD* from methanol afforded 4 mg of roehybridine (total yield, including the fraction obtained chromatographically, was 9 mg; 0·00018%). The amorphous residue of fraction *AD* and the amorphous fraction *E*, which according to thin-layer chromatography had the same composition, were combined. After transforming the bases to perchlorates and crystallisation from water 192 mg of roehybrine perchlorate were obtained (together with the fraction isolated by chromatography the total yield — calculated as base — was 180 mg; 0·0036%). The bases obtained from the mother liquors after roehybrine perchlorate crystallisation were further purified but gave an amorphous brown residue only (1·38 g). This fraction was further separated by chromatography on alumina (95 g; Reanal, according to Brockmann) activated by several hours' heating at 250°C. Chloroform (200 ml) and chloroform with an increasing concentration of methanol: 3% (400 ml), 6% (100 ml), 10% (150 ml), 20% (150 ml), and 40% (150 ml) served as eluent. Fractions of 25 ml each were collected and the composition of fractions was controlled by thin-layer chromatography (in S_1). Fractions 1 and 2 were not alkaloids (0·02 g). From fractions 3—6 (0·15 g) 3·7 mg of crude alkaloid RH 5 were obtained by crystallisation from acetone, which after recrystallisation had m.p. 264—265°C and R_F value in S_1 0·65 (rusty spot). The amorphous residue contained as the main component a base of R_F 0·39 (in S_1; yellow spot) and a small amount of a base of R_F 0·74 (brown-violet spot). Fractions 7—11 (0·20 g) afforded on crystallisation from acetone 32 mg of roemeridine; the amorphous residue contained roemeridine and roehybrine mainly. From fractions 12—15 (0·22 g) 5 mg of roehybridine were obtained by crystallisation from methanol and the remaining bases contained alkaloids of R_F (in S_1) 0·28 and 0·48 (white spots) as the main components. The amorphous fractions 25—34 contained bases of R_F (in S_1) 0·12, 0·28, and 0·46 (white spots), and the fractions 35—44 bases of R_F (in S_1) 0·07, 0·12, and 0·28 (white spots). The prevailing number of these amorphous bases rapidly darkened and decomposed when standing in air. The presence of protopine (R_F in S_1 was 0·83, brown-violet spot) could not be detected in any fraction. From the amorphous rests obtained from fractions 3—15, perchlorates were prepared which on crystallisation from methanol afforded 42·7 mg of roehybrine perchlorate. Fraction *B* (92·8 mg) contained according to thin-layer chromatography several bases of which one was identified as coptisine on the basis of its R_F values (0·42 in S_4 and 0·08 in S_5) and yellow-golden fluorescence. Fraction *I* (2·26 g) contained predominantly substances of a non-alkaloidal character of which a part was obtained in crystalline form but was not further investigated. In this fraction only a negligible amount of a substance of alkaloidal character was detected (R_F in S_3 was 0·80).

From the roots (158 g) of sample I 0·74 g of alkaloids (0·47%) were isolated in the same manner. From this mixture 0·37 g of roemeridine (0·23%) and 1·8 mg of roehybridine (0·0011%) were separated and trace amounts of coptisine detected. The remaining amorphous bases were worked up together with the corresponding fractions from the other two samples.

Characterisation of the Isolated Alkaloids

Roemeridine: from methanol-acetone needles of m.p. 236—237°C (capillary) or 241—243°C (Kofler block), easily soluble in cold methanol or ethanol, less so in chloroform, poorly soluble in acetone and ether, $[\alpha]_D^{21} -37° \pm 2°$ (*c* 0·30, chloroform). Literature[1] gives m.p. 228—230°C (acetone). For $C_{31}H_{39}N_3O_5$ (533·7) calculated: 69·77% C, 7·37% H, 7·88% N; found: 69·92% C, 7·41% H, 7·87% N (see[2]). Molecular weight, determined by high resolution mass spectrometry, is 533·2886 ($C_{31}H_{39}N_3O_5$ requires 533·2889). UV spectrum: λ (log ε) at 220 nm (4·63), λ_{max} 273 nm (3·96), 291 nm (4·04), λ_{min} 250 nm (3·69), 280 nm (3·93). R_F value in S_1 0·34 (white spot). With

conc. sulfuric acid it gives a lemon-yellow colour, with Erdmann reagent a violet one turning to yellow-green and then red-violet, with Fröhde reagent a blue-violet colour, with Marquis reagent a dark blue one, and with conc. nitric acid first an orange and later a yellow one.

(—)-*Isocorypalmine:* from methanol m.p. 228—229°C (capillary), undepressed in admixture with an authentic sample[9], $[\alpha]_D^{22} -300° \pm 30°$ (*c* 0·02, methanol). Molecular weight 341 (mass spectrometry), R_F value in S_2 0·31 (yellow spot), identical with an authentic sample, but different from the value of corypalmine (0·25, orange spot), in S_1 0·45.

Roehybridine: from methanol clusters, m.p. 210—211°C (Kofler block); in contrast to roemeridine poorly soluble in cold methanol. High resolution mass spectrometry indicated a molecular weight of 533·2901 units (for $C_{31}H_{39}N_3O_5$ calculated 533·2889). UV spectrum: λ (log ε) at 220 nm (4·55), shoulder at 272 nm (3·82), λ_{max} 294 nm (3·91), λ_{min} 252 nm (3·57). R_F value in S_1 0·08 (white spot). With concentrated sulfuric acid it gave a lemon-yellow colour, with Erdmann reagent a transient violet colour, changing to yellow-green, with conc. nitric acid an orange one, turning to yellow.

Roehybrine: from ether–hexane mixture needles of m.p. 191—192°C (Kofler block), easily soluble in methanol, well soluble in ether, almost insoluble in hexane, $[\alpha]_D^{24} -67° \pm 2°$ (*c* 0·29, methanol). Molecular weight 331 (mass spectrometry). UV spectrum: λ (log ε) shoulder at 230 nm (3·92), λ_{max} 286 nm (3·55), λ_{min} 257 nm (2·99). Perchlorate crystallises from water or methanol in long needles, m.p. 290—291°C (Kofler block), poorly soluble in water and methanol. R_F value (in S_1) 0·52 (violet spot). With conc. sulfuric acid no colour was obtained, with Erdmann reagent it gave an olive colour turning to brown-yellow, with Fröhde reagent a violet colour turning to olive, and with conc. nitric acid a yellow one.

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