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STRUCTURE ELUCIDATION OF ROEMERIDINE BY X-RAY CRYSTALLOGRAPHY

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Key Word Index—*Roemeria hybrida*; Papaveraceae, alkaloid roemeridine; X-ray crystallography.

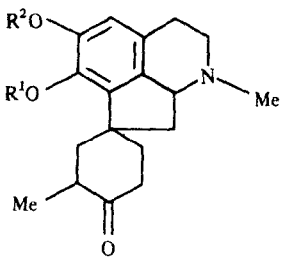
Abstract—The structure of roemeridine, the major alkaloid from *Roemeria hybrida* was established by X-ray crystallography. Structure-significant features of the ^{1}H and ^{13}C NMR spectra and mass spectral fragmentations are reported.

INTRODUCTION

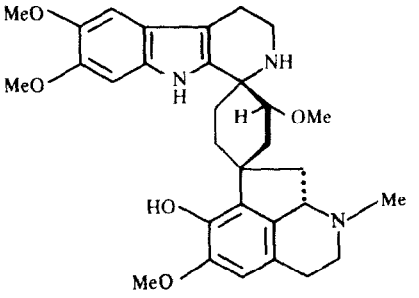
Roemeridine was isolated for the first time from *Roemeria hybrida* by Platonova and co-workers more than thirty years ago [1]. It was also found to occur in *Papaver pavonium* [1]. Slavík and co-workers [2] succeeded in isolating roemeridine as the main component of the basic fraction of the aerial parts of the latter plant; a particularly high content was found in the roots. The same authors [2] confirmed the elemental composition of roemeridine ($C_{31}H_{39}N_3O_5$) and used IR, UV, ^{1}H NMR and mass spectral data to discuss the type and number of functional groups present in the molecule. Based on these earlier findings a partial structure of roemeridine was proposed containing the β-carboline and tetrahydroisoquinoline ring systems [3]. The present paper reports the complete structure elucidation of roemeridine by X-ray crystallography aided by NMR and mass spectral measurements.

RESULTS AND DISCUSSION

High-field (9.4 T) ^{1}H and ^{13}C NMR spectra disclosed the substitution patterns of the heterocyclic moieties. The aromatic ring of the tetrahydroisoquinoline system was found to carry a carbon substituent, a hydroxy group and a methoxyl in a vicinal arrangement. The β-carboline system was found to contain two *ortho*-positioned aromatic methoxy groups each flanked by an aromatic



1 R^1, R^2 = Me, H



methine. Furthermore, the attached-proton-test (APT) [4] and proton-coupled ^{13}C NMR spectra revealed the presence of two sp^3 quaternary carbon atoms, one located α to N-2 of the β-carboline system, and the other attached to three methylene groups and one aromatic carbon atom. The structure information and bond connectivity deduced from the NMR spectra pointed to a unique spiro [4.5] decane unit connecting the heterocyclic moieties, with its five-membered ring being condensed to the 1- and 8-positions of the tetrahydroisoquinoline system, and the six-membered carbocyclic ring being anchored by another spiro-coupling at the 1-position of the β-carboline sub-unit

Biogenetically, this corresponds to a condensation with tryptamine of a proaporphine unit containing a carbonyl group to form the β-carboline ring with the spirocyclic link at C-1. This concept was supported by both the known reactivity of tryptamine towards carbonyl compounds, and synthesis of model β-carboline derivatives containing a spiro-coupling at C-1 [5, Trojánek, J and Koblicová, Z, unpublished results] With roemeridine we conjectured that the condensation could involve roehybrine (1) as the proaporphine unit, since 1 occurs in the same plant material [2]. Nevertheless, a complete structure of roemeridine, namely, the positions of substituents at the alicyclic rings and the configuration at the quarternary carbon atoms, could not be fully established from the spectral data and were finally solved by X-ray crystallography

A perspective view of the molecule with atom numbering is depicted in Fig 1 In the crystal the molecules are linked by hydrogen bonds which also involve molecules of water and methanol as the solvents As suggested from the previous spectral analysis the molecule contains a proaporphine unit (rings A, B, C and D) and a β-carboline unit (rings E, F and G) which share one spiro-atom (C-13) According to the Cahn–Ingold–Prelog convention, the relative configurations at the asymmetric centres are denoted as 7*R**, 10*S**, 12*S** and 13*S** Unfortunately, the quality of the crystals was insufficient to allow the determination of the absolute configuration by anomalous dispersion The molecule exists in an essentially strain-free conformation with normal values of bond lengths and angles. The non-aromatic rings are puckered as follows ring B–distorted half-chair with

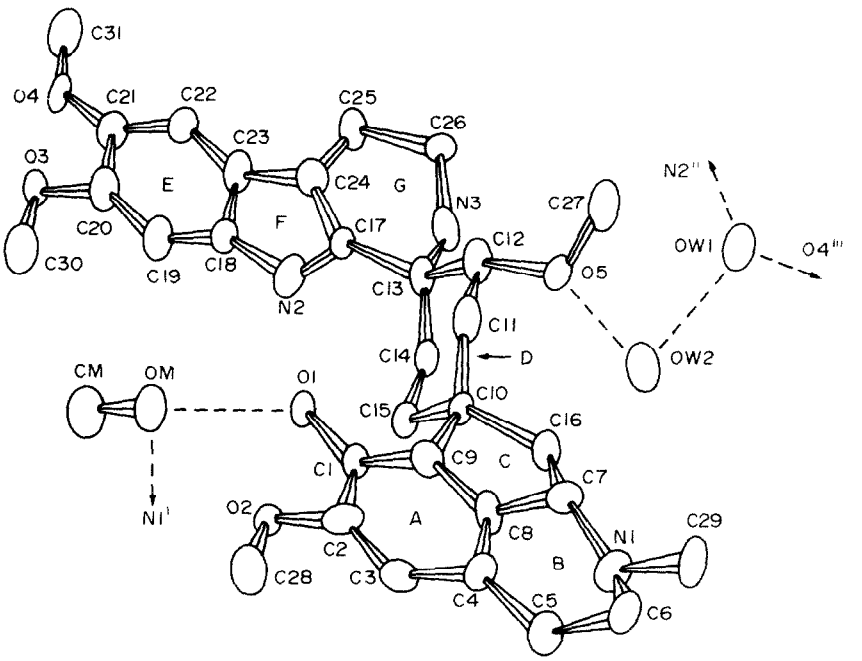


Fig 1. Perspective view of the molecule with (arbitrary) atom numbering Thermal ellipsoids are at 50% probability level Dashed lines indicate hydrogen bonding Symmetry code i, 1−x, 1/2+y, 1/2−z, ii, x−1/2, 1/2−y, 1−z; iii, x, 1−y, z

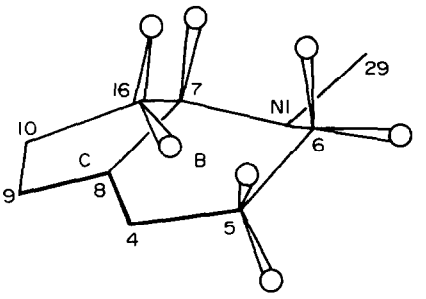


Fig 2 Perspective view of hydrogens on rings B and C.

ΔC_2(C4, C8) = −19.7° [6]; ring C–distorted half-chair with $\Delta = 6.1°$, $\varphi_m = 22.5°$ [7]; ring D–nearly ideal chair; ring G—slightly distorted half-chair with ΔC_2 (C17, C24) = 5.2°. The detailed arrangement of the hydrogen atoms at rings B and C is apparent from Fig. 2. The geometry of the β-carboline moiety in roemeridine is very similar to that in other β-carboline alkaloids, as found in the literature [8]. The endocyclic torsion angles in the present structure lie within 1 e.s.d. of the mean values for 61 structures containing the β-carboline ring system. For the proaporphine part, there is only one known structure for comparison [9], which is characterized by a similar conformation but differs in its ring C possessing an envelope conformation.

Roemeridine represents a novel alkaloid structural type consisting of a proaporphine and a β-carboline unit which share one spiro-atom. The structure is depicted by the classical formula **2**.

From the taxonomic point of view it should be emphasized that *Roemeria hybrida* belongs to the few species of the family Papaveraceae that produce alkaloids of the β-carboline type. Model synthetic experiments with the condensation of cyclic ketones with tryptamine indicate that proaporphines containing a carbonyl function and substituted tryptamines may play the role of intermediates in the biogenesis of alkaloids of this group.

EXPERIMENTAL

Characteristics of roemeridine. The sample of roemeridine [2] was purified by recrystallization from MeOH–Me_2CO; needles of mp 241–243° as described [2] ^{13}C NMR (100 MHz, $CDCl_3$, TMS, 30°): δ23.01 (*t*), 27 41 (*t*), 31 49 (*t*), 32.24 (*t*), 34.02 (*t*), 39.02 (*t*), 43 55 (*q*, N-Me), 47.08 (*s*), 54 97 (*t*, N-CH_2), 55.90 (*t*, N-CH_2), 55.89 (*s*), 56 47 (*q*, O-Me), 56.51 (*q*, 2C, 2 × OMe), 57 31 (*q*, O-Me), 65.28 (*d*, CH-N), 80.28 (*d*, CH–O–), 95 13 (*d*), 100.46 (*d*), 109.73 (*s*), 119 99 (*s*), 122.97 (*s*), 129 57 (*s*), 132 26 (*s*), 134 85 (*s*), 136.76 (*s*), 139.43 (*s*), 144.76 (*s*), 146 52 (*s*), 146 95 (*s*) ^{1}H NMR (400 MHz, $CDCl_3$, TMS, 30°): δ1.654 (1H, *t*, *J* = 11 2 Hz), 1.918 (2H, AA'BB', CH_2), 1.925 (2H, AA'BB', CH_2), 2.093 (1H, *dd*, *J* = 14.0 Hz, *J* = 3.7 Hz), 2 195 (1H, *dd*, *J* = 14.0 Hz, *J* = 7 4 Hz), 2.383 (3H, *s*, N-Me), 2 449 (1H, *ddd*, *J* = 11.7 Hz, *J* = 11.6 Hz, *J* = 5.2 Hz), 3 460 (3H, *s*, OMe), 3.760 (1H, *dd*, *J* = 7.4 Hz, *J* = 3.7 Hz), 3 917 (3H, *s*, OMe), 3.928 (6H, *s*, 2 × OMe), 6 558 (1H, *s*), 6 909 (1H, *s*), 6.947 (1H, *s*) EIMS (probe) 80 eV, 240°, *m/z* (elemental composition, rel. int; only ions with rel. int. higher than 5% are reported, isotopic satellites omitted): 533 $[M]^+$, $[C_{31}H_{39}N_3O_5]^+$ (10), 518 $[M-Me]^+$ (6), 257 $[C_{15}H_{17}N_2O_2]^+$ (45), 244 $[C_{14}H_{16}N_2O_2]^+$ (100), 230 $[C_{14}H_{16}NO_2]^+$ (19), 229 $[C_{13}H_{13}N_2O_2]^+$ and $[C_{14}H_{15}NO_2]^+$ (18), 216 $[C_{13}H_{14}NO_2]^+$ (5), 215 $[C_{13}H_{13}NO_2]^+$ (5); metastable transitions: $533^+ \rightarrow 257^+ + 276$, $533^+ \rightarrow 244^+ + 289$; $533^+ \rightarrow 230^+ + 303$; $244^+ \rightarrow 229^+ + 15$; $230^+ \rightarrow 215^+ + 15$.

Spectral methods. ^{1}H and ^{13}C NMR spectra were measured on Varian XL-200 and VXR-400 spectrometers in $CDCl_3$ and DMSO-d_6. The values of the chemical shifts in the ^{13}C NMR spectra were found to depend on both the solvent used and the concentration of the sample The proton multiplicities of the ^{13}C signals were established by the APT pulse sequence [4] and from proton-coupled spectra. The quaternary carbons were distinguished by using 3.8 msec delay in the APT sequence which effectively suppressed the signals of the protonated sp^3 carbons. The ^{1}H spin systems were identified by the COSY and delayed COSY experiments. Electron-impact mass spectra: 75 eV, 3 keV. Accurate *m/z* values were measured by the peak matching technique using perfluorokerosene as int standard. The metastable parent ion spectra were detected by scanning the accelerating voltage

X-Ray crystallography. After numerous trials, single crystals were finally obtained by layering 1 ml of a cold satd MeOH soln onto 1 ml H_2O placed in a long test tube of 5 mm int. diameter. Slow diffusion at 5° invariably afforded a mixture of single crystals of the solvate, $C_{31}H_{39}N_3O_5 \cdot MeOH\ 2H_2O$, M_r 601.75, together with a microcrystalline unsolvated material; the two solid phases were sepd by flotation with H_2O. The selected crystal with approx. dimensions of $0.30 \times 0.10 \times 0.15$ mm^3 was found to be orthorhombic, space group $P2_12_12_1$, Z = 4, *a* = 7.173(1), *b* = 15.289(8), *c* = 28.213(8) Å, $\rho_m = 1.262$, $\rho_c = 1\ 291$ g cm^3 Measurement of lattice constants and integrated intensities was carried out on a Syntex $P2_1$ diffractometer. Intensity data were collected with monochromated CuK_α radiation ($\lambda = 1\ 54056$ Å) in the $\Theta-2\Theta$ scan mode, $0 < h \leqslant 8$, $0 < k \leqslant 17$, $0 < l \leqslant 32$; 2422 reflections were used [$I > 1.96\ \sigma(I)$] from 2882 total. The phase problem was solved by direct methods and then full-matrix least squares refinement in two blocks was applied. All hydrogens except those of the OH groups were located in a difference map and then fixed in calculated positions, refinement converged at $R = 0\ 076$, $wR = 0.080$ with maximal residual electron density of 0.55 e Å^{-3}. Atomic coordinates, bond lengths and angles of the structure have been deposited at Cambridge Crystallographic Data Centre, Cambridge, U.K.

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