Ophiorrhizine, a New Quaternary Indole Alkaloid Related to Cinchonamine, from *Ophiorrhiza major* Ridl.

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Extraction of the aerial parts of *Ophiorrhiza major* Ridl. has yielded a new C_{19} pentacyclic quaternary indole alkaloid, ophiorrhizine. The structure was established as 1 on the grounds of its spectral properties (optical rotation) and its X-ray molecular structure. Its biosynthetic relationship to the *Cinchona* alkaloids is discussed.

In continuation of our phytochemical survey of West Sumatra[1] we have examined an extract of the fresh aerial parts of *Ophiorrhiza major* Ridl. (Rubiaceae), which gave a positive field test for alkaloids using the Culvenor–Fitzgerald method.[2] *O. major* is a small shrub, *ca.* 20 cm high, which although rare, is used by traditional healers in West Sumatra as a component of a poultice used for skin disorders, especially eczema.

After a small amount of tertiary alkaloids had been removed from the extract, the remaining aqueous phase was extracted with butanol. After chromatography, a low yield of a quaternary chloride was obtained which crystallized from methanol–ethyl acetate. We suggest the trivial name ophiorrhizine for this new alkaloid. The FABMS exhibited a molecular ion at m/z 297 which in conjunction with the ^{13}C and ^{1}H NMR spectra allowed the molecular formula $C_{19}H_{25}N_{2}O$ to be advanced for the cation. The electronic spectrum of ophiorrhizine was typical of an indole chromophore.[3] The ^{13}C (75.5 MHz) and the ^{1}H (500 MHz) NMR spectra were determined in deuteriomethanol (see Table 1) and were analysed by the help of proton–proton decoupling, and by the double quantum filtered COSY, HETCOR proton–carbon correlation, TOCSY[4] and DEPT techniques. These spectra, particularly the ^{13}C spectrum,[5] allowed the identification of the eight carbon atoms of an 11-hydroxyindole system[6] and the connections between C-5 and C-6; C-18, C-19, C-20 and C-21; C-3 and C-14 and also between C-17, C-16 and C-15 (see structure 1). Long-range coupling was observed between 3-H and the protons attached to C-6. Owing to the overlap of key proton resonances and the lack of strong evidence for coupling between 15-H and 20-H the structure of ophiorrhizine 1 could not be advanced with certainty from the available spectroscopic data. Recourse was therefore taken to the X-ray method which gave structure 1 (see Fig. 1) and defined the relative stereochemistry at C-3, C-15 and C-20.

Both dihydrocorynantheol 3, readily available from dihydrocorynantheine 4, and dihydrocinchonamine 5, readily available

Table 1 ^{13}C (75.5 MHz) and ^{1}H (500 MHz) NMR data for ophiorrhizine 1 in deuteriomethanol

Carbon no. δ_C		δ_H, multiplicity, coupling constants (Hz)
2	127.72	
3	62.48	Obscured by OH (4.90,* br t, $J_{3.14\alpha} = J_{3.14\beta} = 9$)
5	61.29	3.54, ddd, $J_{5\alpha.5\beta}$ 12.5, $J_{5\alpha.6\beta}$ 12, $J_{5\alpha.6\alpha}$ 5
		3.58, dd, $J_{5\beta.5\alpha}$ 12.5, $J_{5\beta.6\beta}$ 6.5
6	18.25	2.96, br d, $J_{6\alpha.6\beta}$ 18
		3.10, dddd, $j_{6\beta.6\alpha}$ 18, $J_{6\beta.5\alpha}$ 12, $J_{6\beta.5\beta}$ 6.5
		$J_{6\beta.3\alpha}$ 2.5
7	105.37	
8	120.71	
9	119.72	7.28, d, $J_{9.10}$ 8.4
10	111.05	6.64, dd, $J_{10.9}$ 8.4, $J_{10.12}$ 2.1
11	155.22	
12	97.96	6.79, d, $J_{12.10}$ 2.1
13	139.98	
14	26.82	~1.9, overlaps with 16-H
		(1.79,* dd, $J_{14.14}$ 13.5, $J_{14.3}$ 9)
		2.71, ddd, $J_{14.14}$ 13.5, $J_{14.3}$ 9, $J_{14.15}$ 4
15	25.12	2.24, m
16	26.26	1.90–1.98, m
17	49.17	3.28, m
		3.67, m
18	12.00	1.04, t, $J_{18.19}$ 7.5
19	27.97	1.70, AB of $ABM_{3}X$
20	38.12	2.15, m
21	65.23	3.25, m
		3.83, dd, $J_{21.21}$ 12.5, $J_{21.20}$ 10.5
NH		9.21 *
OH		10.98 *

* These values determined at 300 MHz in deuteriodimethyl sulfoxide.

from cinchonamine 6, and of known absolute configuration,[7] undergo *O*-tosylation on treatment with tosyl chloride and pyridine. The resultant tosylates when boiled in *N,N*-dimethylformamide yield the quaternary tosylate 2. Dihydroantirhine 7 also yields the tosyl salt 2 on similar treatment.[8] The sign and magnitude of the specific rotation of ophiorrhizine $\{[\alpha]_D^{20} -60$ (*c* 0.001, MeOH)$\}$† are similar to those of the tosylate 2 $\{[\alpha]_D -67$ (90% MeOH)$\}$ so that it is likely that both possess the same absolute configuration.

The biosynthesis of the *Cinchona* alkaloids involves cleavage of ring *C* of corynantheal 8 to produce cinchonaminal 9,[9] and further transformations. Cinchonamine 6 is, therefore, likely to be derived from cinchonaminal 9, so that ophiorrhizine 1 would be derived from a hydroxylated strictosidine by a similar pathway.
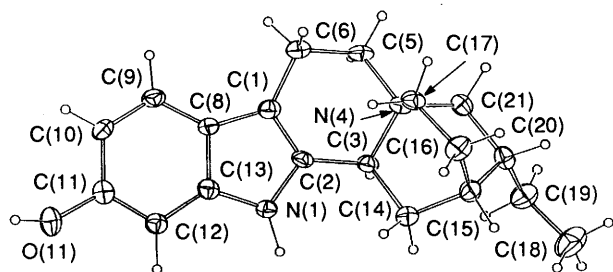

Fig. 1 Projection of a single cation of 1. 20% Thermal ellipsoids are shown for the non-hydrogen atoms; hydrogen atoms have arbitrary radii of 0.1 Å.

† $[\alpha]_D$ Values are recorded in 10^{-1} deg cm^2 g^{-1}.

1; R = OH, X = Cl
2; R = H, X = Ts

3

4

5; R = Et
6; R = CH=CH₂

7

8

9
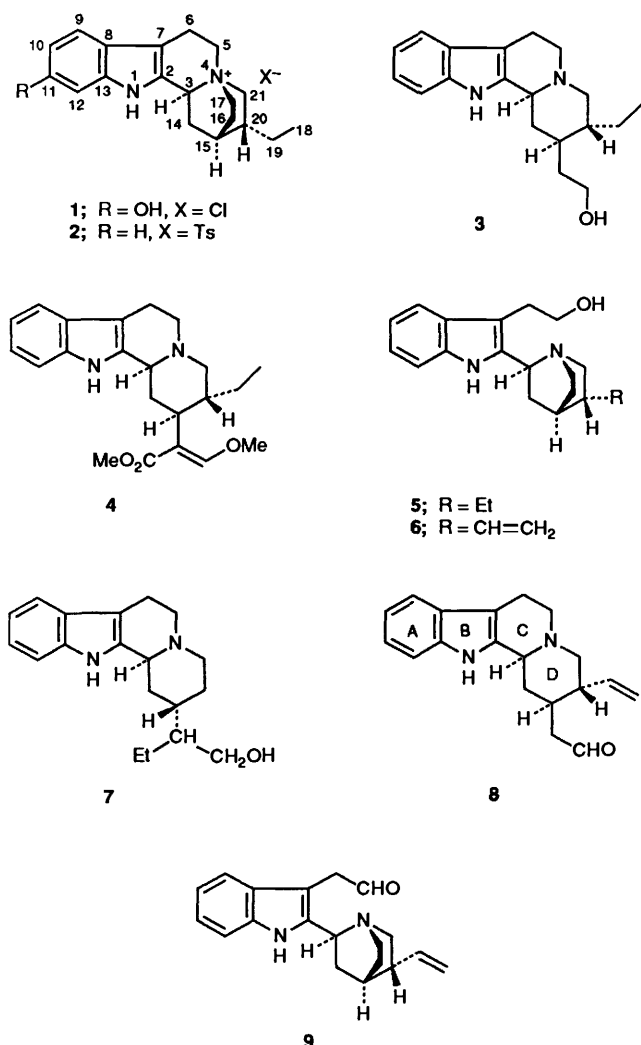
Experimental

General directions have been given previously.[10]

Extraction of Ophiorrhiza major.—The botanical material was collected in November 1989 in Anai Reserved Forest, West Sumatra. Herbarium specimens (DA840) are deposited in Herbarium Bogoriense (BO) and Herbarium Biology, Universitas Andalas (AND). The chopped fresh aerial parts of the plant (3 kg) were covered with methanol (10 dm³) and allowed to stand for 2 d and then filtered. The process was repeated twice more and the combined filtrates were concentrated under reduced pressure. The concentrate (400 cm³) was diluted with sulfuric acid (10%; 50 cm³) and set aside for 12 h. The acidic solution was decanted and extracted with chloroform (3 × 100 cm³) and next basified with ammonia and extracted with chloroform (4 × 500 cm³). Work-up of the extract yielded a small amount of crude tertiary alkaloids which was reserved for further study. The aqueous phase, which gave a strong Mayer's test, was extracted with butanol (4 × 500 cm³) and the extract was evaporated to dryness under diminished pressure to afford a dark brown gum (12.5 g). This was chromatographed over silica gel with increasing proportions of methanol in butanol as eluent. Those fractions which gave a positive Dragendorff test were combined and evaporated and the residue was crystallized from methanol–ethyl acetate to give ophiorrhizine 1 as plates (45 mg), m.p. 162–164 °C (decomp.); $[\alpha]_D^{20}$ −60 (c 0.001, MeOH); λ_{max}/nm 220, 260sh, 270 and 295 (ε 18,000, 4000, 4100 and 5700 respectively).

Structure Determination.—A unique diffractometer data set was measured at ca. 295 K within the limit $2\theta_{max} = 50°$ [monochromatic Mo-Kα radiation, $\lambda = 0.7107_3$ Å; 1839 independent reflections of which 1113 with $I > 3\sigma(I)$ were considered 'observed']. The structure was solved by direct methods and refined by full matrix least-squares with anisotropic thermal parameters for C,N,O; hydrogen atoms were included, constrained in (x, y, z, U_{iso}), the hydroxy hydrogen being located in a difference map. Conventional residuals R, R_w on $|F|$ at convergence were 0.057, 0.058 (both hands) [statistical weights, derivative of $\sigma^2(I) = \sigma^2(I_{diff}) + 0.0004\sigma^4(I_{diff})$]. The chirality adopted follows from the chemistry; neutral atom complex scattering factors were employed.[11] Computation used the XTAL 3.0 program system[12] implemented by S. R. Hall. Pertinent results are given in Fig. 1.*

Crystal Data.—$[C_{19}H_{25}N_2O]^+Cl^-\cdot H_2O$, $M = 350.8$. Orthorhombic, space group $P2_12_12_1$ (D_2^4, No. 19), $a = 15.610(10)$, $b = 15.079(6)$, $c = 7.689(3)$ Å, $U = 1810$ Å³. $D_c(Z = 4) = 1.29$ g cm⁻³. $F(000) = 752$. $\mu_{Mo} = 1.8$ cm⁻¹; specimen = 0.07 × 0.29 × 0.37 mm (no correction).

Molecular geometries are substantially as expected. Hydrogen-bonding interactions are found from the anion to the hydrogen atoms of independent water molecules [Cl ··· O ($\bar{x}$, $\frac{1}{2} + y, \frac{1}{2} - z; \frac{1}{2} + x, \frac{1}{2} - y, \bar{z}$), 3.073(7), 3.037(8) Å] and to the N(1) hydrogen of the cation [Cl ··· N(1), 3.177(6) Å]; a further hydrogen bond is found between the phenol moiety and the water oxygen atom [O ··· O(11) ($\frac{1}{2} + x, \frac{1}{2} - y, z$), 2.613(8) Å].

* Supplementary data: see section 5.6.3 of Instructions for Authors (1992), *J. Chem. Soc., Perkin Trans. 1*, 1992, Issue 1. Atom co-ordinates, thermal parameters, molecular non-H geometry tables have been deposited at the Cambridge Crystallographic Data Centre.

References

1 D. Arbain, R. Djamal, Wiryani and M. V. Sargent, *Aust. J. Chem.*, 1991, 44, 1013.
2 C. C. J. Culvenor and J. S. Fitzgerald, *J. Pharm. Sci.*, 1963, 52, 303.
3 A. I. Scott, *Interpretation of the Ultraviolet Spectra of Natural Products*, Pergamon, Oxford, 1964, p. 297.
4 L. Braunschweiler and R. R. Ernst, *J. Magn. Reson.*, 1983, 53, 521.
5 R. Verpoorte, T. A. van Beek, R. L. M. Riegman, P. J. Hylands and N. G. Bisset, *Org. Mag. Res.*, 1984, 22, 328.
6 This biogenetic numbering system is that of J. Le Men and W. I. Taylor, *Experientia*, 1965, 21, 508.
7 E. Wenkert and N. V. Bringi, *J. Am. Chem. Soc.*, 1958, 80, 3484; 1959, 81, 1474, 6535.
8 S. R. Johns, J. A. Lamberton and J. L. Occolowitz, *Aust. J. Chem.*, 1967, 20, 1463.
9 A. R. Battersby and R. J. Parry, *J. Chem. Soc., Chem. Commun.*, 1971, 31.
10 D. Arbain, A. A. Birkbeck, L. T. Byrne, M. V. Sargent, B. W. Skelton and A. H. White, *J. Chem. Soc., Perkin Trans. 1*, 1991, 1863.
11 J. A. Ibers and W. C. Hamilton, eds., *International Tables for X-ray Crystallography*, vol. 4, The Kynoch Press, Birmingham, 1974.
12 S. R. Hall and J. M. Stewart, eds., *The XTAL Users Manual – Version 3.0*, Universities of Western Australia and Maryland, 1990.