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TERTIARY DIHYDROPROTOBERBERINE ALKALOIDS OF *BERBERIS LYCIUM*

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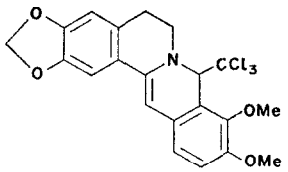
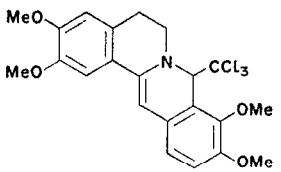
Key Word Index—*Berberis lycium;* Berberidaceae; alkaloids; oxyberberine; artefacts from chloroform addition.

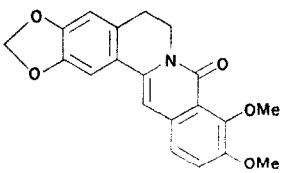
We have already reported the isolation and characterization of berberine chloride,[1] palmatine iodide[2] and berbamine[3] from the roots of *Berberis lycium* Royle. In this communication, the isolation and characterization of two artefact alkaloids, berberine–$CHCl_3$ (I) and palmatine–$CHCl_3$ (II), and of oxyberberine (III), is described.



(I)



(II)



(III)

On usual work up, three alkaloids were obtained. The first, assuming that it was tertiary dihydroprotoberberine, had the empirical formula $C_{21}H_{18}NO_{10}$. A search of the literature showed that only the alkaloid umbellatine[4] has this composition. However, umbellatine, from its UV spectrum had been identified by Govindachari *et al.*[5] and also by us[1] as berberine. Finally, the clue as to the true composition of the alkaloid was given by the NMR spectrum, which showed the presence of two methoxyl groups and one methylenedioxy group and no hydroxyl protons. Thus, the alkaloid must contain an element other than, C,H,N and O, and this was found to be chlorine (23·59%). Hence, the correct molecular formula is $C_{21}H_{18}NO_4Cl_3$ and assuming that the alkaloid is an artefact related to berberine, its structure must be I. The UV spectrum is characteristic of dihydroprotoberberines[6] and the NMR spectrum showed a singlet at 4·34 τ (IH), which can be assigned to C-8 proton. As expected, such a peak is absent in the spectra of berberine and oxyberberine. This alkaloid is therefore berberinechloroform, already reported by Gaze.[7] In conformity with the formation of berberinechloroform is the ready preparation of berberine–acetone[8] from berberine and acetone in the presence of a base. A second alkaloid was obtained from the mother liquors

[1] MIANA, G. A. and IKRAM, M. (1970) *Pak. J. Sci. Ind. Res.* **13,** 49.
[2] MIANA, G. A., IKRAM, M. and HOLUBEK, J. (1969) *Pak. J. Sci. Ind. Res.* **12,** 309.
[3] MIANA, G. A., IKRAM, M. and WARSI, S. A. (1969) *Pak. J. Sci. Ind. Res.* **12,** 159.
[4] CHATTERJEE, R. (1941) *J. Am. Pharm. Assoc.* **30,** 249.
[5] GOVINDACHARI, T. R., PAI, B. R., RAJADURAI, S. and RAO, U. R. (1958) *Proc. Indian Acad. Sci.* **47(A),** 41.
[6] SHAMMA, M., HILLMAN, M. J. and JONES, C. D. (1969) *Chem. Rev.* **69,** 779.
[7] GAZE, R. (1890) *Arch. Pharm.* **228,** 625.
[8] GAZE, R. (1890) *Arch. Pharm.* **228,** 607.

after removal of I, and also from the latter benzene fractions. It analyzed for $C_{22}H_{22}NO_4Cl_3$ and its NMR spectrum showed the presence of four methoxyl groups at 6·3, 6·4, 6·6 and 6·7 τ and a singlet for C-8 proton at 4·36 τ. Its UV spectrum was also characteristic of dihydroprotoberberines, so that it is the alkaloid palmatinechloroform (II). This is the first report of the isolation of this alkaloid artefact.

The third alkaloid, $C_{22}H_{17}NO_5$, prominent bands in the IR spectrum at 3050, 2970, 1650 and 1600 cm^{-1}. The presence of a band at 1650 cm^{-1} suggested that the alkaloid contained an amide grouping. Its UV spectrum indicated that it was also related to dihydroprotoberberines.[6] The NMR spectrum of the base showed peaks at τ 7·12 (2H, methylene protons at C-5), 6·06 (OMe), 5·98 (OMe), 5·70 (2H, *t*, methylene protons at C-6), 4·01 (OCH_2O) and multiplets for aromatic protons at 3·3, 2·8 and 2·75 (4H). These data suggest that the alkaloid might be oxyberberine (berlambine)[9] (III). This was confirmed by comparison with an authentic sample (m.p., m.m.p. and IR).

EXPERIMENTAL

M.ps. are uncorrected.

Extraction. The ground roots (5 kg) were extracted 4–5× with 45 l. EtOH at room temp. The combined extracts were concentrated to a dark syrup (1 l.), which was further diluted with 5% HCl (2 l.), filtered and kept in refrigerator overnight. The yellow crystals of berberine chloride were filtered and the filtrate basified with NH_4OH (30%) (1 l.). There was immediate precipitation, which was allowed to settle and then filtered off. The filtrate was extracted with $CHCl_3$; the $CHCl_3$ extracts were combined, washed with H_2O, dried and concentrated *in vacuo*. The residue (20 g) was dissolved in hot C_6H_6 and the solution allowed to cool, whereby berbamine, probably in the form of berbamine–C_6H_6 complex, crystallized out (10 g). The mother liquor was chromatographed on a column of alumina (300 g; B.D.H.) prepared in C_6H_6. Fractions of 100 ml were collected; 15 fractions were eluted with C_6H_6, 10 by C_6H_6–$CHCl_3$ (9:1) and 10 fractions with $CHCl_3$. All these fractions were examined by TLC and were combined into 3 main groups: A (fractions 1–5, 200 mg), B (fractions 10–15, 110 mg) and C (fractions 17–24, 500 mg).

Characterization of Alkaloids. Berberinechloroform (I). Fraction A crystallized from C_6H_6, m.p. 178°. (Found: C, 56·78; H, 4·15; N, 3·12; Cl, 23·59. $C_{21}H_{18}NO_4Cl_3$ requires: C, 56·76; H, 4·06; N, 3·15; Cl, 23·43%.) UV spectrum (MeOH): λ_{max} 361 and 281 mn (log ϵ 4·1 and 3·8). NMR spectrum ($CDCl_3$): 7·14, 4·75, 6·1, 6·02 (2 OMe), 4·34 (1H, C-8), 4·02 (OCH_2O), 3·37, 3·28, 3·1, 3·02, 2·8 and 2·68 τ. *Palmatinechloroform* (II). Fraction B also crystallized from C_6H_6, m.p. 184°. (Found: C, 56·02; H, 4·63; N, 2·93; Cl, 22·71. $C_{22}H_{22}NO_4Cl_3$ requires: C, 56·11; H, 4·67; N, 2·97; Cl, 22·62%.) UV spectrum (MeOH); λ_{max} 362 and 280 nm (log ϵ 4·1 and 4·0). NMR spectrum ($CDCl_3$): 7·20, 6·60, 6·14, 6·12, 6·08, 6·06 (4 OMe), 4·38 (1H, C-8), 3·4, 3·1, 3·06 and 2·8 τ. *Oxyberberine* (III). Fraction C was concentrated and the residue crystallized from acetone to give reddish brown rods, m.p. 198–200°. (Found: C, 68·33; H, 4·85; N, 3·98. $C_{20}H_{17}NO_5$ requires: C, 68·37; H, 4·84; N, 3·99%.) UV spectrum ($CHCl_3$): λ_{max} 287, 315 and 369 nm (log ϵ 4·5, 4·1 and 3·9). NMR spectrum ($CDCl_3$): 7·12 (2H, *t*, CH_2), 6·06 (3H, *s*, OMe), 5·98 (3H, *s*, OMe), 5·70 (2H, *t*, CH_2), 4·01 (2H, *s*, OCH_2O), 3·3, 2·8 and 2·75 τ. IR spectrum ($CHCl_3$): 3020, 2990, 2930, 2890, 2840, 1650, 1620, 1600, 1500, 1480, 1360, 1280 and 1260 cm^{-1}.

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[9] TOMITA, M. and KUGO, T. (1959) *J. Pharm. Soc. (Japan)* **79**, 317.