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NEW PEPTIDE ALKALOIDS FROM *HOVENIA DULCIS* AND *H. TOMENTELLA*

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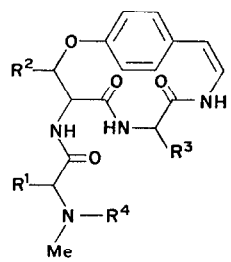
Key Word Index—*Hovenia dulcis*; *Hovenia tomentella*; Rhamnaceae; peptide alkaloids; frangulanine; des-*N*-methylfrangulanine.

Abstract—From the root bark of *Hovenia dulcis* Thunb. and *H. tommentella* (Makino) Nakai (Rhamnaceae), three peptide alkaloids, frangulanine, hovenins-*A* and -*B* have been isolated. Hovenin-*A* has been shown to be des-*N*-methylfrangulanine (II).

THREE peptide alkaloids, frangulanine and new compounds, hovenine-*A* and -*B*, have been isolated by preparative TLC from the methanolic extracts of the root bark of both *Hovenia dulcis* Thunb. and *H. tomentella* (Makino) Nakai.

The major alkaloid (yield, 0·05%) was identified by MS and NMR spectral analysis with frangulanine (I) which was first isolated by Tschesch *et al.*[1] from *Rhamnus frangula* L.

Hovenine-*A* (yield, 0·005%), m.p. 215°, was shown to have the formula $C_{27}H_{42}N_4O_4$ by accurate mass measurement (requires: 486·3206, found: 486·3216). Amino acid analysis of the hydrolysates of hovenine-*A* gave leucine, β-hydroxyleucine and glycine, while *N*-methylisoleucine was shown to be present by the reduced buffer flow rate.[2] Reductive methylation of hovenine-*A* afforded a dihydrofrangulanine which was identified by MS and IR spectra and TLC. Thus hovenine-*A* is des-*N*-methyl-frangulanine (II). The structure of hovenine-*B* is under investigation.



(I) R^4 = Me
(II) R^4 = H

EXPERIMENTAL

Isolation of peptide alkaloids. The root bark of *Hovenia dulcis* Thunb. (800 g) was extracted with MeOH The extracts were acidified with 0·4 N H_2SO_4 (200 ml) and extracted with Et_2O (500 ml × 3). The aqueous layer was then basified with NH_4OH (pH=9) and extracted with $CHCl_3$. Recrystallization of the crude

[1] TSCHESCHE, R., LAST, H. and FEHLHABER, H. W. (1967) *Chem. Ber.* **100**, 3937; WARNHOFF, E. W. (1970) *Fortschritte der Chemie Organischer Naturstoffe*, Bd. 28, S.192, Springer, Wien.
[2] BEVAN, K. (1969) Ph.D. Thesis, University College of Swansea.

alkaloids (*ca.* 1 g) from MeOH gave only frangulanine, and the mother liquor was developed preparatively on TLC (Kiesel gel GF_{254}) to isolate frangulanine (400 mg), hovenine-*A* (40 mg) and hovenine-*B* (4 mg).

Identification of the major alkaloid as frangulanine. The major alkaloid, colourless needles from MeOH, m.p. 275–277°, IR: (KBr) cm^{-1} 3275 (NH), 2784 (NMe), 1625 (CONH), 1235 (C–O–C). NMR: (C_5D_5N) δ 0·76 (*d*, *J* 6 Hz, isopropyl of R^3), 0.80 (3H, *t*, *J* 7 Hz, CH_3CH_2 - of R^1), 0·96 (3H, *d*, *J* 7 Hz, CH_3CH - R^1), 1·18 and 2.05 (3H, *d*, *J* 7 Hz, isopropyl of R^2), 2·44 (6H, *s*, $N(CH_3)_2$). MS: *m/e* 500 (M^+, 0·02%), 114 (100%), 85(4·33%). (Found: C, 66·91; H, 8·83; N, 11·16. Calc. for $C_{28}H_{44}N_4O_4$; C, 66·78; H, 8·96; N, 10·61%).

Amino acid analysis. Using in amino acid analyzer *threo* and *erythro*-β-Hydroxyleucine,[3] glycine and leucine were detected in the hydrolysate of the major alkaloid.

*Identification of hovenine-*A *as des-*N*-methyl-frangulanine.* Novenine-*A*, m.p. 215°. IR: ν_{KBr}^{max} cm^{-1} 3270 (NH), 1628 (CONH), 1237 (C–O–C). NMR: (C_5D_5N) 100 MHz δ 0·75 (6H, *d*, *J* 7 Hz, isopropyl of R^3), 0·85 (3H, *t*, *J* 7 Hz; CH_3CH_2 - of R^1), 0·98 (3H, *d*, *J* 7 Hz, CH_3CH - of R^1), 1·18 (6H, *d*, *J* 7 Hz, isopropyl of R^2), 2.38 (3H, *s*, NCH_3), 2·99 (1H, *d*, *J* 6 Hz, C-9 proton). MS: *m/e* 486 (M^+, 0·25%), 471 (M^+ — Me, 0·16%), 443 (0·29%), 387 (0·13%), 344 (4·48%), 303 (0·24%), 210 (0.53%), 190 (1·34%), 182 (1·16%), 135 (16·28%), 101 (6·98%), 100 (100%), 97 (3·98%). MW (by high resolution MS) 486·3216. $C_{27}H_{42}N_4O_4$ requires: 486·3206.

*Amino acid analysis of hovenine-*A. Hovenine-*A* (4 mg) was hydrolyzed with 6 N HCl at 110° for 12 hr in a sealed tube. The hydrolysate, after evaporation to dryness over KOH, was examined by the amino acid analyzer. *Threo*-β-hydroxyleucine, glycine, *N*-monomethylisoleucine,[4] *erythro*-β-hydroxyleucine and leucine were detected using a buffer flow rate reduced to 0·5 of the normal condition.

*Reductive methylation of hovenine-*A. A mixture of hovenine-*A* (5 mg), 37% formaldehyde (0·1 ml), and 10% Pd–C (10 mg) in 5 ml of aq. MeOH (1:1) was stirred vigorously in an atmosphere of H_2 at room temp. The suspension was filtered, and the filtrate was evaporated on a steam bath and re-evaporated after addition of a small amount of H_2O to remove formaldehyde polymers. The residue, reductive methylated hovenine-*A*, was shown to be identical with dihydrofrangulanine by TLC, IR and MS.

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[4] QUITT, P., HALLERBACH, J. and VOGLER, K. (1963) *Helv. Chim. Acta* **46**, 327.