CONSTITUENTS OF WEST AFRICAN MEDICINAL PLANTS. XXVIII.[1] ADDITIONAL ALKALOIDS OF *TRICLISIA GILLETTI*

P. D. Owusu, D. J. Slatkin, J. E. Knapp and P. L. Schiff, Jr.*

Department of Pharmacognosy, School of Pharmacy, University of Pittsburgh, Pittsburgh, PA 15261

Abstract.—*Triclisia gilletii* (DeWild.) Staner is a woody climber indigenous to Ghana and other parts of West Africa. Extracts of the plant have been used medicinally and as an arrow poison. Chromatography of an extract of the leaves afforded the new bisbenzylisoquinoline dibenzo-*p*-dioxin alkaloids gilletine and isogilletine-*N*-oxide as well as two other bisbenzylisoquinoline alkaloids, obamegine and stebisimine.

Triclisia gilletii (DeWild.) Staner is a woody climber indigenous to Ghana and other parts of West Africa (1). Extracts of this plant have been used in the treatment of diarrhea, pyorrhea and as an arrow poison (1). Earlier reports from our laboratories have detailed the isolation and identification of the bisbenzylisoquinoline alkaloids stebisimine (2) and gilletine (3) from the leaves, isotetrandrine (2) and trigilletimine (4) from the stems and roots, and cocsuline (2, 5) from the stems of this species. In addition, Huls *et al.* have also isolated cocsuline (6) and phaeanthine (7) from the stems of *T. gilletii* as well as the oxoaporphine alkaloid *O*-methylmoschatoline (7) and an unusual indeno[1,2,3-ij]isoquinoline (8).

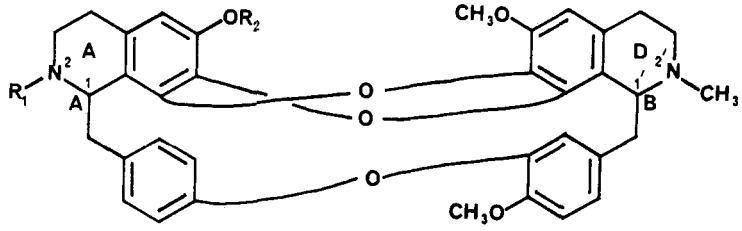
It was decided to extend our previous studies of the alkaloids of the leaves of this species in order to seek a source of novel or previously uncharacterized alkaloids of potential pharmacological or phytochemical importance. This paper is to report the isolation and identification of the new bisbenzylisoquinoline dibenzodioxin alkaloids gilletine (**1**) and isogilletine-*N*-oxide (**2**), the bisbenzylisoquinoline alkaloid obamegine (**3**), and the reisolation of the bisbenzylisoquinoline base stebisimine (**4**).

The dried, powdered leaves were percolated with methanol and the solvent evaporated to afford a syrupy residue. The dried marc from the extraction was treated with dilute hydrochloric acid and filtered, and the filtrate was added to the original syrupy residue. The resulting suspension was filtered; the filtrate was basified to pH 8–10 with ammonium hydroxide and extracted with chloroform. Chromatography of the chloroform extract over silicic acid (column A) in hexane, hexane-chloroform mixtures, chloroform, and chloroform-methanol mixtures afforded a mixture of five alkaloids in the chloroform-methanol fractions. Combination of these fractions and chromatography over silicic acid (column B) in hexane-chloroform then chloroform and chloroform-methanol (98:2) afforded a mixture of stebisimine (**4**) and an incompletely characterized alkaloid designated TGLM-IV in the chloroform and chloroform-methanol elutes. These bases were eventually separated by repeated rechromatography over both silicic acid and silica gel. Stebisimine, which was identified by a direct comparison (ur, ir, nmr, mp) with an authentic reference sample, was first isolated from *Stephania japonica* (Menispermaceae) (9, 10) and later from *Anisocycla grandidieri* (Menispermaceae) (11). This alkaloid was previously isolated from the leaves of *Triclisia gilletii* in our laboratories some seven years ago (4).

[1]Previous paper: *J. Nat. Prod.*, **43**, 123 (1980).

Elution of column B with chloroform-methanol [(95:5), (9:1) and (8:2)] afforded a mixture of two alkaloids which, upon rechromatography over silica gel in chloroform and elution with chloroform-methanol (17:3), gave gilletine (**1**). The structure of gilletine was the subject of a previous paper (3). Gilletine is not only the first example of a menisarine-type alkaloid to be isolated from *Triclisia* species but is also the first example of an alkaloid of this type to be found outside the genus *Cocculus*. The other biscoclaurine bases of this type include menisarine from *Cocculus sarmentosus* (Menispermaceae) (12–14), normenisarine (a partially characterized *O*-demethylmenisarine) from *Cocculus trilobus* (12), and cocsulinine from *Cocculus pendulus* (15).

Elution of column B with chloroform-methanol mixtures [(7:3), (3:2), (1:1)] and methanol afforded a mixture of two alkaloids which was rechromatographed

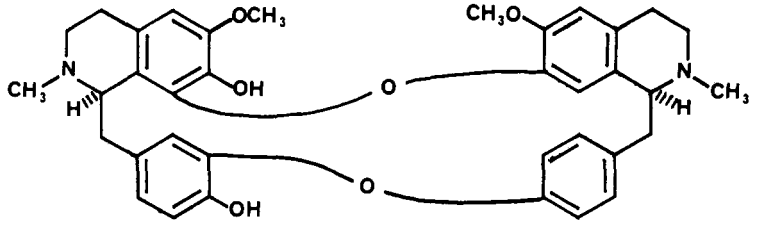


1 $R_1 = R_2 = H$ A=B=S

2 $R_1 = R_2 = H$, N-2′ OXIDE

5 $R_1 = CH_3$, $R_2 = H$ A=B=S

6 $R_1 = R_2 = CH_3$ A=B=S



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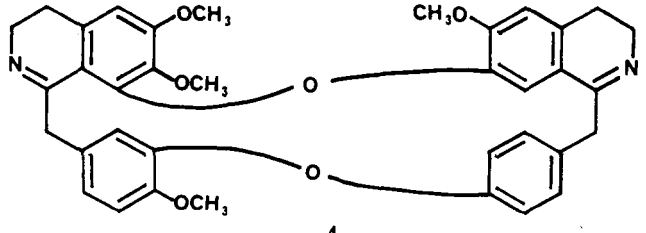

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over silica gel in chloroform. Elution of the column with chloroform-methanol (9:1) gave obamegine (**3**), which was identified by a direct comparison (uv, ir, nmr, ms, sp rotn, mp) with an authentic sample and by methylation to isotetrandrine (*O,O*-dimethylobamegine). Obamegine was first isolated from *Berberis tschonoskyana* (Berberidaceae) (16) and later from *Stephania japonica* (Menispermaceae) (17), *Thalictrum rugosum* (Ranunculaceae) (18), *Thalictrum lucidum* (Ranunculaceae) (19), and *Xanthorrhiza simplicissima* (Ranunculaceae) (20). Continued elution of the column with chloroform-methanol (9:1) afforded isogilletine-*N*-oxide (**2**), mp 218–220° dec (Et_2O–MeOH); $[\alpha]^{29}D$ +216° (c 0.94, $CHCl_3$–MeOH [9:1]); uv, λ max (MeOH) 223nm(sh) (log ε 4.49), 229(sh) (4.54), 240(4.55), 289(3.80) and 296(sh)(3.75); ir, ν max (KBr) 3380 cm^{-1} (br) 1585, 1500 and 1270. The nmr spectrum indicated the presence of one low field *N*-methyl group at δ2.85 (s, 3H), two *O*-methyl groups at 3.83 (s, 3H) and 3.85 (s, 3H) and nine aromatic protons from 6.05–7.65 with one broad proton singlet at δ4.30 for a N–H function and δ5.1 for a phenolic hydroxy group. The mass spectrum showed the molecular ion at *m/e* 594 (2%) and other significant fragment ions at *m/e* 578 (10%), 352 (7), 351 (29), 176 (4) and 57 (100). Finally, the base afforded a blue color on treatment with a mixture (1:1) of concentrated sulfuric and nitric acids. The low field *N*-methyl signal (δ2.85) in the nmr spectrum (3, 21, 22), the M–16 ion (*m/e* 578) in the mass spectrum (21, 22), and the color reaction (3, 23), collectively considered with the rest of the spectral data, were indicative of a dibenzo-1,4-dioxin, monophenolic, bisbenzylisoquinoline-*N*-oxide containing one secondary amino group (24–27).

Treatment of isogilletine-*N*-oxide with sulfurous acid afforded a base whose spectral properties (uv, ir, nmr) and mp were identical to those of gilletine (**1**). Thus, it remained only to be established with which nitrogen atom in the parent alkaloid the oxide was associated and the stereochemistry at C–1 and C–1'. The nmr chemical shift values for the *N*-methyl groups of *N*-methylgilletine (**5**), *N,O*-dimethylgilletine (**6**) and related bases have been postulated as δ2.55–2.61 for N–2 (Ring A) and δ2.41–2.43 for N–2' (Ring D) (3). Since gilletine has only one *N*-methyl group (δ2.42 [3]) to begin with and since the *N*-methyl group of isogilletine -*N*-oxide was already shifted downfield, the return of this downfield signal to δ2.45 in the sulfurous acid reduction product whose spectral properties and mp were identical with those of gilletine, confirms the position of the oxide at N–2' in ring D. Since the specific rotation of the reduced product $[\alpha]^{28}D$ +210° (c 0.21, MeOH) differed significantly from naturally occurring gilletine $[\alpha]^{28}D$ +294° (c 0.56, MeOH), it appeared the naturally occurring *N*-oxide was a diastereoisomer of gilletine and was, therefore, named isogilletine-*N*-oxide. The determination of the stereochemistry of the two asymmetric centers at C–1 and C–1' will have to await the isolation of further quantities of the alkaloid so that reductive cleavage to the respective monomers followed by cd studies may be utilized (28). It is unlikely that isogilletine-*N*-oxide is an artifact formed during the isolation procedure since it can be detected by thin-layer chromatography in fresh extracts of *T. gilletii*. To our knowledge, this is the first reported isolation of a menisarine-type alkaloid *N*-oxide from nature. Alkaloid *N*-oxides have been previously reviewed (21, 29), and it was suggested that many alkaloids might occur naturally in the form of their *N*-oxides (21, 29). Furthermore, numerous plant families contain alkaloid-*N*-oxides, including Gramineae, Polygonaceae, Rubiaceae, Solanaceae, Monimiaceae, Ranunculaceae, Boraginaceae, Compositae, Orchidaceae, and Menispermaceae (22). These *N*-oxides have been reported for many dif-

fering alkaloid classes such as monomeric and dimeric indole, pyrrolizidine, quinolizidine, aporphine, tropane, pyridine-pyrrolidine, piperidine and bisbenzylisoquinoline (22).

EXPERIMENTAL[3]

PLANT MATERIAL.—The plant material used in this study was collected by Mr. Philip D. Owusu in the eastern rain forest region near Oda in Ghana from January to May, 1976. A voucher specimen is on deposit at the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Science and Technology, Kumasi, Ghana. The whole plant was dried and ground to coarse powder.

EXTRACTION, FRACTIONATION AND CHROMATOGRAPHY.—Powdered leaves (12 kg) were percolated with methanol (50 liters) and the methanol evaporated to a syrupy residue (400 g). The dried marc from the extraction was treated with dilute hydrochloric acid (5%) (7 liters) and filtered. The filtrate was added to the original syrupy residue, and the resulting mixture was diluted to 10 liters with dilute hydrochloric acid (5%). The resulting suspension was filtered; the filtrate was basified to pH 8–10 with ammonium hydroxide and extracted with chloroform (10 liters) (4X). The resulting chloroform extract (62.3 g) was dissolved in chloroform-methanol (4:1) (150 ml), adsorbed onto silicic acid (150 g), and chromatographed over a column of silicic acid (column A) (1.5 kg) in hexane. After elution with hexane (2.5 liters), hexane-chloroform (4:1) (8 liters), hexane-chloroform (1:4) (6 liters), and chloroform (10 liters), elution with chloroform-methanol mixtures [(99:1) (4 liters), (49:1) (3 liters), (24:1) (5 liters), (9:1) (3 liters), (8:2) (4 liters) and (1:1) (4 liters)] afforded a combined fraction (23.6 g) of an alkaloid mixture. This mixture was dissolved in hexane-chloroform (7:3) (25 ml) and rechromagraphed over a column of silicic acid (column B) (600 g) in hexane-chloroform (7:3). After elution with hexane-chloroform mixtures [(7:3) (3 liters) and (1:1) (3 liters)], the column was eluted with chloroform (3 liters) and chloroform-methanol mixtures [(99:1) (1.5 liters) and (98:2) (1.5 liters)].

ISOLATION OF STEBISIMINE (4).—The chloroform and chloroform-methanol fractions were pooled to afford a residue (8.4 g) which was rechromatographed over silica gel G (50 g) in chloroform. After elution with chloroform (1 liter) and chloroform-methanol mixtures [(99:1) (1 liter), (98:2) (1 liter)], elution with chloroform-methanol (95:5) (1 liter) afforded fine white needles of stebisimine (4) (100 mg), mp 227–229°; $[\alpha]^{25}D$ 0° (c 0.27, $CHCl_3$); identical by direct comparison (uv, ir, nmr, mp) with an authentic sample available in our laboratory.

ISOLATION OF TGLM-IV.—The mother liquors from the crystallization of stebisimine afforded a white amorphous residue of TGLM-IV (8 mg), mp 139–142°, which awaits the future isolation of additional quantities for final structural analysis.

ISOLATION OF GILLETINE (1).—Elution of column B with chloroform-methanol mixtures [(99:5) (4.5 liters), (90:10) (4 liters) and (80:20) (4 liters)] afforded a combined alkaloid fraction (4.6 g). Chromatography of this fraction over silicic acid (35 g) in chloroform (1 liter) and chloroform-methanol mixtures [(19:1) (1 liter), (9:1) (1 liter), (4:1) (1 liter)] afforded a residue (3.2 g) which was rechromatographed over a column of silica gel G (50 g) in chloroform and chloroform-methanol mixtures. Elution with chloroform-methanol (17:3) (1.5 liters) yielded a residue (2.1 g) which, on treatment with ether, afforded fine white needles of gilletine (1) (1.6 g), mp 174–176 $[\alpha]^{28}D$ +294° (c 0.56, MeOH); uv, λ max (MeOH) 237 nm (log ϵ 4.34), 274 (sh) (3.33), 290 (3.41) and 301 (sh) (3.36); ir, ν max (KBr) 3520 cm^{-1} (br) and 1505; nmr, δ2.42 (s, 3H, NCH_3), 3.91 (s, 3H, OCH_3), 3.95 (s, 3H, OCH_3), 4.35 (br, s, 1H, NH), 5.16 (s, 1H, OH, D_2O exchanged), 6.11 (s, 1H, ArH), 6.53 (s, 2H, ArH), 6.82 (s, 2H, ArH), 6.90–7.07 (m, 2H, ArH) and 7.59–7.68 (m, 2H, ArH); ms, M^+ *m/e* 578 (33%) for $C_{35}H_{34}N_2O_6$, 352 (21), 351 (100), 337 (2) and 176 (27) whose structure has been previously described (3).

ISOLATION OF OBAMEGINE (3).—Elution of column B with chloroform-methanol mixtures [(7:3) (1 liter), (6:4) (1 liter), (5.5) (2 liters)] and methanol (2 liters) afforded a residue (5.3 g) which was rechromatographed over silica gel G (90 g) (column C) in chloroform and chloroform-

[3]Melting points were taken on a Fisher-Johns Apparatus and are uncorrected. The uv spectra were obtained on a Perkin-Elmer model 202 recording spectrophotometer in methanol, and the ir spectra were determined on a Perkin-Elmer model 257 recording spectrophotometer in potassium bromide pellets. The nmr spectra were recorded in deuterated chloroform on a Hitachi Perkin-Elmer model R-24 high resolution spectrometer with tetromethylsilane as internal standard and chemical shifts recorded in δ(pmm) units. The optical rotations were measured on a Perkin-Elmer model 241 polarimeter. Silicic acid (100 mesh) (Mallinckrodt) and silica gel G (Camag) were used for column chromatography, while silica gel G (Camag) was used for thin-layer chromatography. All solvents were evaporated under reduced pressure at 40°.

methanol mixtures. Elution with chloroform-methanol (9.2) (800 ml) afforded a residue (250 mg) which, upon treatment with benzene, gave white crystals of obamegine (**3**) (180 mg), mp 171–173°; $[\alpha]^{22}D$ +143° (*c* 0.58, $CHCl_3$); uv, λ max (MeOH) 283 nm (log ε 4.22) ir, ν max (KBr) 3400 cm^{-1} (br), 2920, 1585, 1500, 1270, 1220 and 1110; nmr, δ2.28 (s, 3H, NCH_3), 2.47 (s, 3H, NCH_3), 3.75 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 5.65 (br, s, 2H, OH) exchanged with D_2O, 6.05–7.35 (m, 10H, ArH); ms, M^- *m/e* 594 (42%) for $C_{36}H_{38}N_2O_6$, 579(3), 403(8), 402(4), 382(21), 381(64), 367(23), 192(47), 191(100) and 174(24); identical by a direct comparison (uv, ir, nmr, mp) with an authentic sample available in our laboratory.

CONVERSION OF OBAMEGINE TO *O,O*-DIMETHYLOBAMEGINE (ISOTETRANDRINE).—To obamegine (**3**) (20 mg) in methanol (20 ml) was added ethereal diazo methane (20 ml) prepared by the addition of potassium hydroxide (0.4 g) in methanol (10 ml) to Diazald[4] (2.54 g) in ether (30 ml). The mixture was allowed to stand for 48 hours and evaporated; the resulting oil was chromatographed over a small column of silicic acid (2 g) in chloroform. Elution with chloroform-methanol (95:5) afforded *O-O*-dimethylobamegine (isotetrandrine) (17 mg) as a white residue, mp 179–181°; $[\alpha]^{24}D$ +122° (*c* 1.88, $CHCl_3$), identical by direct comparison (uv, ir, nmr, ms, sp rotn, mp) to a reference sample available in our laboratory.

ISOLATION OF ISOGILLETINE-*N*-OXIDE (**2**).—Continued elution of column C with chloroform-methanol (9:1) (850 ml) afforded a residue (110 mg) which, upon treatment with chloroform-methanol (9:1), afforded a white residue of isogilletine-*N*-oxide (**2**) (80 mg), mp 218–220° dec; $[\alpha]^{25}D$ +216° (*c* 0.94, $CHCl_3$-MeOH [9:1]); uv, λ max (MeOH) 223 nm (sh) (log ε 4.49), 229 (sh) (4.54), 240 (4.55), 289 (3.80) and 296 (sh) (3.75); ir, ν max (KBr) 3380 cm^{-1} (br), 2930, 1585, 1500, 1455, 1430, 1370, 1270, 1225, 1210, 1165, 1125, 1140, 990, 870, 830, and 750; nmr, δ2.85 (s, 3H, NCH_3), 3.83 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3) 4.30 (br s, 1H, NH), 5.10 (br s, 1H, OH), 6.05–7.65 (m, 9H, ArH); ms, M^- *m/e* 594 (2%) for $C_{36}H_{34}N_2O_7$, 578(10), 352(7), 351(29), 176(4) and 57(100).

REDUCTION OF ISOGILLETINE-*N*-OXIDE.—Isogilletine-*N*-oxide (**2**) (50 mg) was dissolved in sulfurous acid[5] (6%) (3 ml) and allowed to stand overnight. The resulting solution was basified with ammonium hydroxide to pH 8–9 and extracted with ether (20 ml) (2X) and chloroform (20 ml) (2X). The ether and chloroform solutions were pooled to afford a residue (45 mg) which was chromatographed over a small column of silicic acid (5 g) in chloroform. Elution with chloroform (20 ml) afforded gilletine as a white residue (35 mg), mp 168–170°; $[\alpha]^{25}D$ +210° (*c* 0.21, MeOH) identical to gilletine by direct comparison (uv, ir, nmr, mp) but differing in specific rotation.

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LITERATURE CITED

1. F. R. Irvine, "Woody Plants of Ghana", Oxford University Press, London, 1961, pp. 36–37.
2. A. N. Tackie, D. Dwuma-Badu, T. U. Okarter, J. E. Knapp, D. J. Slatkin and P. L. Schiff, Jr., *Lloydia*, **37**, 1 (1974).
3. D. Dwuma-Badu, J. S. K. Ayim, A. N. Tackie, P. D. Owusu, J. E. Knapp, D. J. Slatkin and P. L. Schiff, Jr., *Heterocycles*, **9**, 995 (1978).
4. D. Dwuma-Badu, J. S. K. Ayim, A. N. Tackie, M. A. El-Sohly, J. E. Knapp, D. J. Slatkin and P. L. Schiff, Jr., *Experientia*, **31**, 1251 (1975).
5. A. N. Tackie, D. Dwuma-Badu, T. Okater, J. E. Knapp, D. J. Slatkin and P. L. Schiff, Jr., *Phytochemistry*, **12**, 2509 (1973).
6. R. Huls and C. Detry, *Bull. Soc. Roy. Sci. Liege*, **42**, 73 (1973); *Chem. Abstr.*, **79**, 32156 (1973).
7. R. Huls, *Bull. Soc. Roy. Sci. Liege*, **41**, 11 (1972); *Chem. Abstr.*, **79**, 15841 (1973).
8. R. Huls, J. Gaspers and R. Warin, *Bull. Soc. Roy. Sci. Liege*, **45**, 40 (1976); *Chem. Abstr.*, **85**, 160372 (1976).
9. D. H. R. Barton, G. W. Kirby and A. Wiechers, *Chem. Commun.*, 266 (1966).
10. D. H. R. Barton, G. W. Kirby and A. Wiechers, *J. Chem. Soc.* C, 2313 (1966).
11. E. Schlittler and N. Weber, *Helv. Chem. Acta*, **55**, 2061 (1972).
12. H. Kondo and M. Tomita, *J. Pharm. Soc. Japon*, **55**, 911 (1935); *Chem. Abstr.*, **30**, 726 (1936).
13. H. Kondo and M. Tomita, *J. Pharm. Soc. Japan*, **55**, 637 (1935); *Chem. Abstr.*, **33**, 626 (1939).
14. M. Tomita, S. Ueda and A. Taraoka, *Tetrahedron Lett.*, 635 (1962).

[4]Aldrich Chemical Company, Milwaukee, WI.
[5]Fisher Scientific Company, Pittsburgh, PA.

15. P. P. Joshi, D. S. Bhakuni and M. M. Dhar, *Indian J. Chem.*, **12**, 517 (1974).
16. M. Tomita and T. Kugo, *Yakugaku Zasshi*, **79**, 317 (1959); *Chem. Abstr.* **53**, 17161 (1959).
17. M. Tomita and T. Ibuka, *Yakugaku Zasshi*, **83**, 940 (1963); *Chem. Abstr.* **60**, 4202 (1964).
18. T. Tomimatsu and J. L. Beal, *J. Pharm. Sci.*, **55**, 208 (1965).
19. W.N. Wu, J. L. Beal, L. A. Mitscher, K. N. Salman and P. Patil, *J. Nat. Prod.*, **39**, 204 (1976).
20. J. E. Knapp, F. T. Hussein, J. L. Beal, R. W. Doskotch and T. Tomimatsu, *J. Pharm. Sci.*, **56**, 139 (1967).
21. J. D. Phillipson and S. S. Handa, *Lloydia*, **41**, 385 (1978).
22. D. Dwuma-Badu, T. U. Okarter, A. N. Tackie, J. A. Lopez, D. J. Slatkin, J. E. Knapp and P. L. Schiff, Jr., *J. Pharm. Sci.*, **66**, 1242 (1977).
23. I. R. C. Bick and A. R. Todd, *J. Chem. Soc.* 1606 (1950).
24. J. Baldas, I. R. C. Bick, T. Ibuka, R. S. Kapil and Q. N. Porter, *J. Chem. Soc.* (Perkin I), 592 (1972).
25. M. Shamma, "The Isoquinoline Alkaloids," Academic Press, New York, 1972, pp. 146–150.
26. K. Nakanishi, "Infrared Absorption Spectroscopy," Holden-Day, San Francisco, 1962, pp. 30–41.
27. A. I. Scott, "Ultraviolet Spectra of Natural Products," Pergamon Press, Oxford, 1964, pp. 91–97.
28. M. Shamma, "The Isoquinoline Alkaloids", Academic Press, New York, 1972, p. 121.
29. J. D. Phillipson, *Xenobiotica*, **1**, 419 (1971).