PACHYGONAMINE AND N-METHYLPACHYGONAMINE, NEW BISBENZYLISOQUINOLINE ALKALOIDS FROM PACHYGONE OVATA

M. Uvais S. Sultanbawa[1], Subramaniam Sotheeswaran

and Sinnathamby Balasubramaniam

Department of Chemistry

University of Peradeniya

SRI LANKA

and

Moustafa Abd El-Kawi, David J. Slatkin and

Paul L. Schiff, Jr.*

Department of Pharmacognosy

School of Pharmacy

University of Pittsburgh

Pittsburgh, Pennsylvania 15261 U.S.A.

Abstract - Chromatography of methanolic extract of Pachygone ovata over silica gel afforded pachygonamine (1) and N-methylpachygonamine (2) which were characterized as new dibenzo-p-dioxin biphenyl bisbenzylisoquinoline alkaloids by a consideration of physicochemical data and conversion to N-methyltiliamosine (7). In addition, tiliamosine (3) was isolated for the first time from nature as the free base instead of the previously isolated N-acetylamide (4).

Pachygone ovata (Poir.) Miers ex Hook. (Menispermaceae) is a woody climber indigenous to the Indo-Malaysian region of southeast Asia. The dried fruit of the plant has been used as a rodenticide and to stupeify or poison fish.[2-4] In 1979, Dasgupta and Ray published the first account of the constituents of P. ovata and reported the presence of the alkaloids reticuline, reticuline-N-oxide, N-methylcrotsparine, coclaurine, liriodenine, and trilobine and the cyclitol

quercitol.[5] Shortly thereafter, Bhat et al. reported the isolation and characterization of a new quaternary erythrinan alkaloid, pachygonine, and the isolation of magnoflorine and O,O-dimethylmagnoflorine from extracts of P. ovata roots.[6]

This paper is to report the isolation and identification of two new dibenzo-p-dioxin biphenyl bisbenzylisoquinoline alkaloids, pachygonamine (1) and N-methylpachygoname (2), as well as the isolation of tiliamosine (3) which was first isolated by Guha et al. in 1976 as its N-acetyl amide (4).[7]

Leaves and stems of Pachygone ovata from Sri Lanka were dried, ground and extracted with methanol. The concentrated methanolic extract was treated with citric acid and filtered. The filtrate was made alkaline with ammonium hydroxide, extracted with chloroform and the chloroform extract treated in the usual manner to separate nonphenolic and phenolic alkaloids.[8] Chromatography of the nonphenolic alkaloid fraction over silica gel in chloroform and elution with $CHCl_3$-MeOH (98:2) gave tiliamosine (3) (700 mg) as an amorphous solid, mp 167-170°C ($CHCl_3$-MeOH), $[\alpha]_D^{25}$ + 267° (c 0.48, $CHCl_3$), uv λ_{max}^{MeOH} nm (log ε) 290(3.98) and 235(sh)(4.70) with no bathochromic shift upon addition of either 0.1N HCl or 0.1N KOH: ir ν_{max}^{KBr} cm^{-1} 2940, 2840, 2800, 1590, 1505, 1480, 1460, 1435, 1415, 1365, 1330, 1305, 1280, 1240, 1205, 1180, 1140, 1120, 1110, 1090, 1055, 1030, 1020, 990, 970, 955, 940, 925, 900, 865, 835, 820 and 755. The ^{1}H-nmr spectrum (600.6 MHz,$CDCl_3$, TMS, δ in ppm) indicated the presence of one N-methyl group at 2.32(3H,s), three aromatic methoxy groups at 3.83 (3H,s), 3.94(3H,s) and 3.98(3H,s) and eight aromatic protons at 6.66(1H,s), 6.97 (1H,d,J=8.5Hz), 7.00(1H,d,J=8.5Hz), 7.32(1H,dd,J=2.2,8.1Hz), 7.35(1H,dd,J=2.2,8.5Hz), 7.62 (1H,d,J=1.8Hz), 7.69 (1H,d,J=2.2Hz) and 8.13(1H,s). The ms showed M^+ at m/z 592 and other significant fragment ions at m/z 591, 366, 365, 351, 211 and 183(100%). Treatment of tiliamosine with acetic anhydride and pyridine afforded N,O-diacetyltiliamosine (5), mp 182-184°C ($CHCl_3$), $[\alpha]_D^{25}$ + 423° (c 0.35,$CHCl_3$); uv λ_{max}^{MeOH} nm (log ε) 291(3.79) and 236(sh)(4.59); ir ν_{max}^{KBr} cm^{-1} 1765 (phenolic acetate) and 1645 (tertiary amide). The ^{1}H-nmr spectrum (60 MHz,$CDCl_3$,TMS,δ in ppm) indicated the presence of one O-acetyl group at 2.13(3H,s), one N-methyl group and one N-acetyl group at 2.24 (6H,s), three aromatic methoxy groups at 3.81(6H,s) and 3.84(3H,s) and eight aromatic protons at 6.61(1H,s), 6.79-7.95(6H,m) and 8.05(1H,s). The ms showed M^+ at m/z 676 and other significant fragment ions at m/z 634, 408, 393, 366, 365, 211 and 183(100%). Finally, hydrolysis of N,O-diacetyltiliamosine (5) with methanolic potassium carbonate afforded N-acetyltiliamosine (4), mp 265-267°C (MeOH); $[\alpha]_D^{25}$ + 338° (c 0.24, $CHCl_3$) identical by direct comparison (uv, ir,

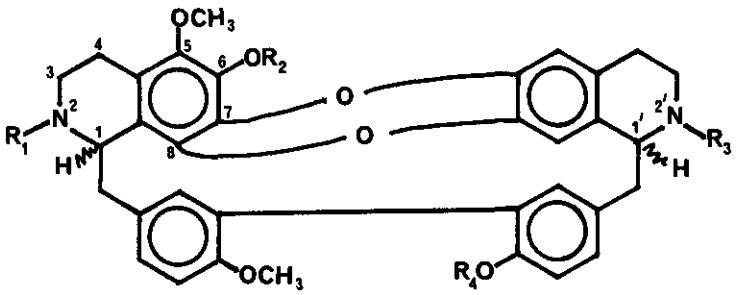
^{1}H-nmr, ms) with an authentic reference sample (mp 276-277°C (lit)).[7] This is the first reported isolation of tiliamosine from nature and the first report of its spectral properties, although Guha et al. reported the isolation of N-acetyltiliamosine (4) from Tiliacora racemosa (Menispermaceae) in 1976.[7] The presence of this cryptophenolic alkaloid in the nonphenolic fraction is not unexpected due to the hindered nature of the phenolic group in a biphenyl system of this type.[9,10] Chromatography of the phenolic fraction over silica gel and elution with $CHCl_3$-MeOH (95:5) afforded two alkaloids, pachygonamine (1) and N-methylpachygonamine (2). Pachygonamine (1), obtained by preparative tlc, was an amorphous mass (30 mg), mp 225-227°C (dec)($CHCl_3$), $[\alpha]_D^{25}$ + 257° (c 0.28, MeOH); uv λ_{max}^{MeOH} nm (log ε) 291(4.05) and 234(sh)(4.71); ir ν_{max}^{KBr} cm^{-1} 3400, 2930, 1595, 1505, 1462, 1435, 1370, 1335, 1305, 1285, 1240, 1200, 1140, 1110, 1040, 995, 970, 945, 885, 860, 810 and 750. The ^{1}H-nmr spectrum (60 MHz,$CDCl_3$,TMS,δ ppm) indicated the presence of two aromatic methoxy groups at 3.89(3H,s) and 3.95(3H,s) and eight aromatic protons at 6.58(1H,s), 6.90-7.65 (6H,m) and 8.12(1H,s). The ms showed M^+ at m/z 564 and other significant fragment ions at m/z 338, 337(100%) 323, 211 and 169. These spectral data are indicative of a dibenzo-p-dioxin biphenyl bisbenzylisoquinoline alkaloid of the tiliamosine (3) type containing two secondary amine groups, one methoxy group and one phenolic hydroxy group in the dibenzo-p-dioxin portion (top half) of the alkaloid plus one methoxy group and one hydroxy group in the biphenyl portion (bottom half) of the alkaloid.[7,10-16] Treatment of pachygonamine (1) with formalin and formic acid afforded N,N-dimethylpachygonamine (6), mp 199-202°C (dec)($CHCl_3$-MeOH), $[\alpha]_D^{25}$ + 327° (c 0.43, $CHCl_3$); uv λ_{max}^{MeOH} nm (log ε) 290(3.26) and 235(sh)(3.95); ir ν_{max}^{KBr} cm^{-1} 3400, 2930, 1600, 1505, 1465, 1370, 1270, 1235, 1040, 1005, 985, 870 and 810; ^{1}H-nmr (60 MHz,$CDCl_3$,TMS,δ in ppm) 2.28 (3H,s,NCH_3), 2.63(3H,s,NCH_3), 3.97(3H,s,OCH_3), 4.01(3H,s,OCH_3), 6.62(1H,s,ArH), 6.6-7.75 (6H,m, ArH) and 8.09 (1H,s,ArH); ms M^+ m/z 592, 577, 366, 365, 351, 211, 183 and 175(100%). Treatment of N,N-dimethylpachygonamine (6) with ethereal diazomethane at 0°C for three days afforded N,N,O-trimethylpachygonamine (7) mp 142-145°C ($CHCl_3$), $[\alpha]_D^{26}$ + 264° (c 0.44, $CHCl_3$); uv λ_{max}^{MeOH} nm (log ε) 288(3.61) and 235(sh)(4.06). The ^{1}H-nmr spectrum (60 MHz,$CDCl_3$, TMS,δ in ppm) indicated the presence of two N-methyl groups at 2.30(3H,s) and 2.66(3H,s), three aromatic methoxy groups at 3.85(3H,s), 3.95(3H,s) and 3.99(3H,s) and eight aromatic protons at 6.63(1H,s), 6.88-7.78 (6H,m) and 8.08 (1H,s). The ms showed M^+ at m/z 606 and other significant fragment ions at 605, 591, 380, 379(100%), 365, 211 and 190. A direct comparison of N,N,O-trimethylpachygonamine (7) with N-methyltiliamosine (prepared by the treatment of tiliamosine with formalin and formic acid)

showed them to be identical (uv, ir, ^{1}H-nmr, ms, mp, sp rotn) thereby establishing the skeletal structure, oxygenation pattern and position of the methoxy group in the biphenyl portion (bottom portion) of pachygonamine. The position of the remaining methoxy group, therefore, has to be at either C-5 or C-6. Through an examination of extensive spin decoupling and N.O.E. (nuclear Overhauser enhancement) data, Guha et al. determined the chemical shift of the C-6 methoxy group in both N-acetyltiliamosine (4) and N-acetylnortiliacorinine A (N-acetyl-5-demethoxytiliamosine) to be at δ3.84.[7] A consideration of the ^{1}H-nmr spectral data for five other alkaloids (tiliacorine, tiliacorinine, nortiliacorine A, nortiliacorinine A and dinklacorine) of this type reveals that the range for the chemical shift of the C-6 methoxy group is δ3.78-3.83.[14] The absence of any methoxy signal with a chemical shift at a higher field the δ3.89 in the ^{1}H-nmr spectra of either pachygonamine (1) or N-methylpachygonamine (2) and the appearance of a methoxy signal at δ3.83 in the ^{1}H-nmr spectrum of N,N,O-trimethylpachygonamine (7) is consistent with the assignment of the methoxy group in the dibenzo-p-dixin portion of pachygonamine to the C-5 position, thus establishing the structure of pachygonamine (1). Finally, prolonged treatment (17 days) of N,N-dimethylpachygonamine with ethereal dizaomethane at 27°C afforded N,N,O,O-tetramethylpachygonamine (8) which was identical to N,O-dimethyltiliamosine prepared in an identical manner from N-methyltiliamosine (7). It is well known that phenolic groups in biphenyl systems of alkaloids of this class are hindered and require a significantly longer time to methylate than these same groups when present in nonhindered positions.[9,10]

The second alkaloid from the phenolic fraction was N-methylpachygonamine (2) (30 mg), mp 183-185°C (dec) ($CHCl_3$-MeOH), $[\alpha]_D^{27}$ + 287° (c 0.23, MeOH); uv λ_{max}^{MeOH} nm (log ε) 291(3.59) and 235(sh)(4.19): ir ν_{max}^{KBr} cm^{-1} 3400, 2940, 1590, 1505, 1465, 1435, 1375, 1330, 1275, 1235, 1200, 1137, 1100, 1040, 1020, 1008, 980, 860, 810 and 750. The ^{1}H-nmr spectrum (60 MHz, $CDCl_3$+CD_3OD, TMS, δ in ppm) indicated the presence of one N-methyl group at 2.31(3H,s), two aromatic methoxy groups at 3.92(3H,s) and 3.99(3H,s) and eight aromatic protons at 6.60(1H,s), 6.90-7.65(6H,m) and 8.10(1H,s). The ms showed M^+ at m/z 578 with other significant fragment ions at 352(100%), 337, 211 and 176. These spectral data are indicative of a monosecondary tiliamosine-like alkaloid containing one N-methyl group, one methoxy group and one phenolic hydroxy group in the dibenzo-p-dioxin portion (top half) of the molecule and one methoxy group and one phenolic hydroxy group in the biphenyl portion (bottom half) of the molecule.[7,10-16] Treatment of N-methylpachygonamine with formalin and formic acid afforded N,N-dimethylpachygonamine (6). As the structure of the latter has been previously established, only the placement of the secondary amine group remained to be determined.

Since it has been previously established that the chemical shift for the N-2 methyl group in this type of alkaloid is found between δ2.22-2.32 while the chemical shift for the N-2' methyl group is found between δ2.60-2.65[7,14], the single N-methyl group in this alkaloid may be placed at the N-2 position thereby establishing the structure of N-methylpachygonamine (2).

Although both the sign and magnitude of the specific rotations of pachygonamine (1), N-methylpachygonamine (2) and their derivatives are consistent with the assignment of S,S stereochemistry to the asymmetric centers at C-1 and C-1' in these alkaloids, solvent differences and potential intramolecular hydrogen bonding of phenolic alkaloids of this group may result in conformational changes and variations in sign and magnitude of the specific rotation,[18] thus making this type of prediction potentially inaccurate. Finally, this is the first reported isolation of dibenzo-p-dioxin biphenyl bisbenzylisoquinoline alkaloids outside of the genus Tiliacora[14] and the occurrence of these alkaloids appears to be restricted to the Family Menispermaceae to date.



1 $R_1=R_2=R_3=R_4=H$

2 $R_1=CH_3, R_2=R_3=R_4=H$

3 $R_1=R_2=CH_3, R_3=R_4=H$

4 $R_1=R_2=CH_3, R_3=COCH_3, R_4=H$

5 $R_1=R_2=CH_3, R_3=R_4=COCH_3$

6 $R_1=R_3=CH_3, R_2=R_4=H$

7 $R_1=R_2=R_3=CH_3, R_4=H$

8 $R_1=R_2=R_3=R_4=CH_3$

ACKNOWLEDGEMENTS

The authors are grateful to Dr. B. Mukherjee, Department of Pharmacology, B.C. Roy Postgraduate Institute of Basic Medical Sciences, Calcutta, India for the ir spectrum of N-acetyltiliamosine; Dr. Richard Stevens, NMR Facility for Biomedical Sciences, (Grant Number P41RR0292-16), Carnegie-Mellon University, Pittsburgh, PA for determining the 600.6 MHz ^{1}H-nmr spectra and Mr. Joseph Bender, School of Pharmacy, University of Pittsburgh for determining the mass spectra.

REFERENCES

1. Present address: Department of Chemistry, University of Maiduguri, Nigeria.
2. K.R. Kirtikar and B.D. Basu, 'Indian Medicinal Plants', L.M. Basu, Allahbad, 1975, p. 90.
3. J.D. Hooker, 'The Flora of British India', L. Reeve and Company, England, 1961, p. 105.
4. S.R.N. Chopra, R.L. Badhwar and S. Ghosh, 'Poisonous Plants of India', Government of India Press, Calcutta, 1949, pp. 152-153.
5. S. DasGupta, A.B. Ray, S.K. Bhattacharya and R. Bose, J. Nat. Prod., 1979, 42, 399.
6. S.V. Bhat, H. Dornauer and N.J. DeSouza, J. Nat. Prod., 1980, 43, 588.
7. K.P. Guha, P.C. Das, B. Mukherjee, R. Mukherjee, G.P. Juneau and N.S. Bhacca, Tetrahedron Lett., 1976, 4241.
8. W.-N. Wu, J.L. Beal and R.W. Doskotch, J. Nat. Prod., 1980, 43, 377.
9. A.N. Tackie, D. Dwuma-Badu, J.E. Knapp and P.L. Schiff, Jr., J. Nat. Prod., 1973, 36, 66.
10. D. Dwuma-Badu, J.S.K. Ayim, N.Y. Fiagbe, A.N. Tackie, J.E. Knapp, D.J. Slatkin and P.L. Schiff, Jr., J. Nat. Prod., 1976, 39, 213.
11. M. Tomita, T. Kikuchi, K. Fujitani, A. Kato, H. Furukawa, Y. Aoyagi, M. Kitano and T. Ibuka, Tetrahedron Lett., 1966, 857.
12. J. Baldas, Q.N. Porter, I.R.C. Bick and M.J. Vernengo, Tetrahedron Lett., 1966, 2059.
13. J. Baldas, I.R.C. Bick, T. Ibuka, R.S. Kapil and Q.N. Porter, J. Chem. Soc., Perkin I, 1972, 592.
14. K.P. Guha, B. Mukherjee and R. Mukherjee, J. Nat. Prod., 1979, 42, 56.
15. B. Anjaneyulu, T.R. Govindachari, S.S. Sathe, N. Viswanathan, K.W. Gopinath and B.R. Pai, Tetrahedron, 1969, 25, 3091.
16. A.N. Tackie, D. Dwuma-Badu, J.E. Knapp and P.L. Schiff, Jr., Phytochemistry, 1973, 12, 203.
17. B.K. Cassels and M. Shamma, Heterocycles, 1980, 14, 222.
18. B.K. Cassels and M. Shamma, Heterocycles, 1980, 14, 214.

Received, 13th June, 1983