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PACHYOVATAMINE, A BISBENZYLISOQUINOLINE ALKALOID, AND OTHER ALKALOIDS FROM *PACHYGONE OVATA*

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Key Word Index—*Pachygone ovata*; Menispermaceae; leaves and stems; dibenzo-*p*-dioxinbiphenyl bisbenzylisoquinoline alkaloids; pachyovatamine.

Abstract—A new dibenzo-*p*-dioxin biphenyl bisbenzylisoquinoline alkaloid, pachyovatamine, has been isolated from an extract of the leaves and stems of *Pachygone ovata* from Sri Lanka. The alkaloid was characterized by a consideration of its physicochemical data and conversion to *O*-acetyltiliacorinine. Pachygonamine, *N*-methylpachygonamine and tiliamosine were also isolated from the same extract.

INTRODUCTION

Pachygone ovata Miers ex Hook. f. and Thoms., a woody climber indigenous to the sandy seashores of the Coromandel Coast of India, has been used as a rodenticide, fish poison and insect repellent for many years [1–3]. In the last 5 years, a total of 14 different benzylisoquinoline-derived alkaloids have been isolated from extracts of this Indian plant. These alkaloids include the proaporphine *N*-methylcrotsparine from the leaves [4]; the aporphines magnoflorine [5, 6], *O,O*-dimethylmagnoflorine [5] and isoboldine [7] from the roots; the oxoaporphine liriodenine from the stems [4] and the roots [6]; the benzylisoquinolines reticuline and reticuline-*N*-oxide from the leaves [4], plus coclaurine [4, 6] and norjuziphine [6] from the roots; the erythrinane pachygonine from the roots [5]; the tetrahydroprotoberberines stepholidine and coreximine from the roots [6]; and the bisbenzylisoquinolines trilobine [4, 6] and nortrilobine [6] from the roots. Most recently, the isolation of tiliamosine (**1**) and the isolation and structural elucidation of the new dibenzo-*p*-dioxin biphenyl bisbenzylisoquinoline alkaloids pachygonamine (**2**) and *N*-methylpachygonamine (**3**) from *P. ovata* of Sri Lankan origin were reported by our laboratories [7]. This paper gives the details of the isolation procedures used in that study [7] and reports the isolation and identification of pachyovatamine (**4**), another new dibenzo-*p*-dioxin biphenyl bisbenzylisoquinoline alkaloid from extracts of the leaves and stems of Sri Lankan *P. ovata*.

RESULTS AND DISCUSSION

Leaves and stems of *P. ovata* from Sri Lanka were dried, ground and extracted with methanol. The concentrated methanolic extract was treated with citric acid solution and filtered. The filtrate was partitioned with chloroform then basified with ammonium hydroxide and extracted with ether. The ether extract was treated in the usual manner to separate phenolic and non-phenolic alkaloids [8]. Chromatography of the non-phenolic alkaloid fraction over silica gel in petrol–chloroform (1:1) and elution with chloroform–methanol (49:1) afforded tiliamosine (**1**) whose identification was the subject of an earlier paper [7]. As further confirmation, we report here for the first time the oxidation of *N,O*-dimethyltiliamosine (**5**) with ceric ammonium nitrate [9] to the diamine **6** and 2,2'-dimethoxy-5,5'-hydroxymethylbiphenyl (**7**), the latter characterized as its dicarbomethoxy ester **8**.

Elution of the column with chloroform–methanol (9:1) yielded a fraction which on rechromatography over silica gel in chloroform–methanol (19:1) afforded pachyovatamine (**4**) as an amorphous residue, mp 182–185°, $[\alpha]_D^{25}$ + 269° ($CHCl_3$; *c* 0.29), with UV maxima at 291 (log ε 3.63), 234 (4.35) and 225 nm (4.34). The ^{1}H NMR spectrum indicated the presence of two aromatic methoxy groups at δ 3.83 (3H, *s*) and 3.96 (3H, *s*), and nine aromatic protons at δ 6.25 (1H, *s*), 6.60 (1H, *s*), 6.8–7.6 (6H, *m*) and 8.07 (1H, *s*) with no signals attributable to *N*-methyl groups [10]. The mass spectrum showed a $[M]^+$ at *m/z* 548 with other significant fragment ions at *m/z* 322, 321 (100%) and 161 attributable to a double benzylic cleavage of a bisbenzylisoquinoline alkaloid [11]. These spectral data are indicative of a dibenzo-*p*-dioxin biphenyl bisbenzylisoquinoline alkaloid of the tiliacorinine (**9**) type containing two secondary amine groups and one methoxy 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 [10–18]. Treatment of pachyovatamine with formaldehyde and formic acid afforded *N,N*-dimethylpachyovatamine (**9**), as substantiated by its ^{1}H NMR spectrum which showed the addition of two *N*-methyl groups at δ 2.29 (3H, *s*) and 2.63 (3H, *s*) and by its mass spectrum which showed a $[M]^+$ at *m/z* 576 and other important fragment ions at *m/z* 350, 349 (100), 335 and 175. Acetylation of *N,N*-dimethylpachyovatamine with acetic

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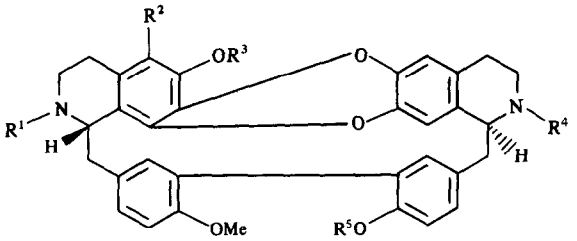


1 $R^1 = R^3 = Me$; $R^2 = OMe$; $R^4 = R^5 = H$

2 $R^1 = R^3 = R^4 = R^5 = H$; $R^2 = OMe$

3 $R^1 = Me$, $R^2 = OMe$, $R^3 = R^4 = R^5 = H$

4 $R^1 = R^2 = R^4 = R^5 = H$, $R^3 = Me$

5 $R^1 = R^3 = R^4 = R^5 = Me$, $R^2 = OMe$

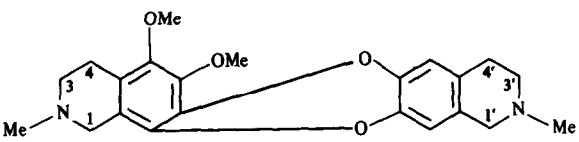
9 $R^1 = R^3 = R^4 = Me$, $R^2 = R^5 = H$ (S,S)

10 $R^1 = R^3 = R^4 = Me$, $R^2 = H$, $R^5 = Ac$

11 **9** (R,S)

12 $R^1 = R^3 = Me$, $R^2 = R^4 = R^5 = H$

14 $R^1 = R^3 = R^4 = Me$, $R^2 = OMe$, $R^5 = H$



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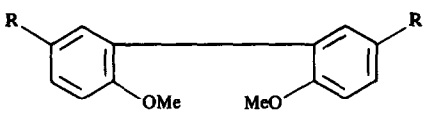
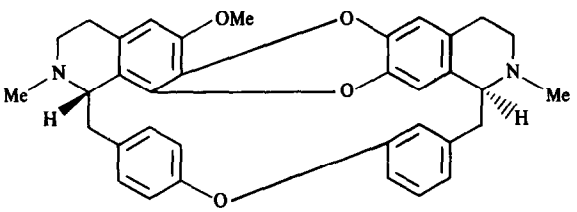


7 $R = CH_2OH$

8 $R = COOMe$



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anhydride and pyridine gave *N,N*-dimethyl-*O*-acetylpachyovatamine (**10**), identical to *O*-acetyltiliacorinine (**10**) by direct comparison (IR, ^{1}H NMR, mass spectrum), thus establishing the skeletal structure, the position of the methoxy group in the biphenyl portion, and the bis-secondary nature of pachyovatamine. Although the literature value for the specific rotation of *O*-acetyltiliacorinine ($[\alpha]_D$ +363.8° (pyridine; *c* 0.25) [15]) is higher than that determined for *N,N*-dimethyl-*O*-acetylpachyovatamine (**10**) ($[\alpha]_D^{27}$ +269° (pyridine; *c* 0.42)), when the measurement of an authentic sample of *O*-acetyltiliacorinine was repeated in our laboratory we found a value ($[\alpha]_D^{27}$ +290° (pyridine; 0.41)) more in agreement with that determined for *N,N*-dimethyl-*O*-acetylpachyovatamine. Thus, it is probable that pachyovatamine possesses the same stereochemistry at its asymmetric centres as tiliacorinine (*S,S*) [19] which is consistent with the empirical observation that tiliacorine (**11**) (*R, S*)-like alkaloids are characterized by small positive specific rotations (+20° to +120°) while tiliacorinine (**9**) (*S,S*)-like alkaloids possess much higher positive values (+250° to +600°) [20]. Finally, the CD curve of pachyovatamine ($[\theta]_{309}$ +6300, $[\theta]_{271}$ +13 700 and $[\theta]_{238}$ +134 900) showed a distinct similarity to that of nortiliacorinine A (**12**) ($[\theta]_{311}$ +3200, $[\theta]_{274}$ +8600 and $[\theta]_{238}$ +68 100) lending further credence to the assignment of *S,S* stereochemistry for pachyovatamine (**4**). The presence of the cryptophenolic alkaloids tiliamosine (**1**) and pachyovatamine (**4**) in the non-phenolic fraction is not unexpected due to the hindered nature of the phenolic group in a biphenyl system of this type [12, 21].

Flash column chromatography of the phenolic fraction over silica gel in chloroform–methanol (19:1) afforded *N*-methylpachygonamine (**3**) from the earlier fractions and pachygonamine (**2**) from subsequent fractions. The structural elucidation of these alkaloids was the subject of an earlier paper [7]. It is interesting to note that all of the dimeric alkaloids isolated from *P. ovata* to date are dibenzo-*p*-dioxin bisbenzylisoquinolines, with those isolated from the Indian *P. ovata* or the Asian *P. pubescens* (isotrilobine) being of the isotrilobine (**13**)-type (biphenyl ether) while those isolated from the Sri Lankan *P. ovata* are of the tiliacorinine (**9**)-type (biphenyl). To our knowledge, dibenzo-*p*-dioxin bisbenzylisoquinoline alkaloids with a biphenyl lower ring system are of restricted distribution to the genera *Tiliacora* and *Pachygone* of the family Menispermaceae while these same alkaloids with a biphenyl ether lower ring system are found in the genera *Anisocyclea, Cocculus, Pachygone, Synclisia* and *Triclisia* of the family Menispermaceae and the genera *Daphnandra* and *Doryphora* of the family Monimiaceae [17, 18].

EXPERIMENTAL

General. Mps are uncorr. UV spectra were obtained in MeOH and IR spectra in KBr pellets. ^{1}H NMR spectra were recorded 60 MHz or 600.6 MHz in $CDCl_3$ with TMS as internal standard, chemical shifts are reported in δ (ppm) units. Low-resolution MS were recorded on a quadrapole instrument and high-resolution MS on a magnetic sector spectrometer. CD curves were measured in MeOH. Silicic acid (100 mesh) (Mallinckrodt) and silica gel (Merck or Baker) were used for CC while silica gel (HF_{254}, Merck) was used for TLC. Alkaloids were visualized by spraying with Dragendorff reagent [22]. Dry Na_2SO_4 was routinely used for drying solvents and all solvents were evapd under red pres. at 40°.

Plant material. Plant material used in this study was collected in the vicinity of Peradeniya, Sri Lanka in 1979. A herbarium specimen has been deposited at the Department of Chemistry, University of Peradeniya, Peradeniya, Sri Lanka.

Extraction and fractionation. Powdered, dried leaves and stems of *P. ovata* (2.75 kg) were extracted by percolation with MeOH (100 l.) and the solvent was evapd to leave a residue (367 g). The residue was stirred with citric acid (2%) (5 l.) and filtered. The filtrate was extracted × 5 with $CHCl_3$ (1 l) (fraction A) and then made alkaline with NH_4OH to pH 8–9 was extracted × 10 with Et_2O (1 l.) (fraction B). The Et_2O extract (fraction B) was concd

to 100 ml and extracted × 5 with NaOH (5%) (100 ml). The alkaline soln was treated with solid NH_4Cl to pH 8–9 and extracted × 5 with Et_2O (300 ml). The Et_2O was evapd to an oily residue (3 g) (fraction C) of phenolic non-quaternary alkaloids. The Et_2O extract (fraction B) remaining from partitioning with NaOH was extracted with H_2O and evapd to a residue (7 g) (fraction D) of non-phenolic non-quaternary alkaloids.

Chromatography of fraction D. Fraction D was dissolved in $CHCl_3$ (10 ml), adsorbed onto silica gel (10 g) and chromatographed over silica gel (250 g) (column A) in petrol–$CHCl_3$ (1:1). Elution with petrol–$CHCl_3$ mixtures followed by $CHCl_3$ and $CHCl_3$–MeOH mixtures afforded various fractions which were collected (50 ml each) and combined according to TLC analysis [C_6H_6–Me_2CO–NH_4OH (4:8:0.1) (system A), $CHCl_3$–MeOH–NH_4OH (8.5:1.5:0.1) (system B) and $CHCl_3$–MeOH–NH_4OH (9:1:0.1) (system C)].

Tiliamosine (**1**). Elution of column A with $CHCl_3$–MeOH (49:1) (3 l.) and combination of fractions 110–160 afforded tiliamosine (**1**) (700 mg) as an amorphous solid, mp 167–170° ($CHCl_3$–MeOH), R_f 0.42 (system A) and 0.63 (system B), whose spectral properties have been reported elsewhere [7].

Preparation of N-*methyltiliamosine* (**14**). To tiliamosine (**1**) (200 mg) in MeOH (10 ml) was added HCO_2H (88%) (2 ml) and HCHO (37%) (1 ml) and the resulting mixture refluxed for 24 hr. The soln was cooled, made alkaline with NH_4OH to pH 8–9 and extracted × 5 with Et_2O (25 ml). The Et_2O extracts were combined, dried, filtered and the filtrate was evapd to afford a white residue (180 mg). Treatment of this residue with $CHCl_3$–MeOH afforded *N*-methyltiliamosine (**14**) as pale-yellow prisms, mp 142–145°; R_f 0.71 (system B); $[\alpha]_D^{26}$ +281° ($CHCl_3$; *c* 0.89); UV λ_{max}^{MeOH} nm (log ε): 288 (3.61) and 235 (sh) (4.06); IR ν_{max}^{KBr} cm^{-1}: 2930, 1585, 1500, 1474, 1458, 1428, 1417, 1412, 1407, 1360, 1322, 1312, 1270, 1238, 1222, 1198, 1115, 1092, 1062, 1048, 1010, 980, 962, 892, 868, 842, 820 and 750; ^1H NMR (60 MHz, $CDCl_3$, TMS): δ2.30 (3H, *s*, NMe), 2.66 (3H, *s*, NMe), 3.85 (3H, *s*, OMe), 3.95 (3H, *s*, OMe), 3.99 (3H, *s*, OMe), 6.63 (1H, *s*, ArH), 6.88–7.78 (6H, *m*, ArH) and 8.08 (1H, *s*, ArH); EIMS (probe) 70 eV *m/z* (rel. int.): 606 $[M]^+$ (32), 605 (10), 591 (2), 380 (22), 379 (100), 365 (27), 211 (2), 190 (69), 182 (26), 175 (12) and 173 (15); CD (MeOH; *c* 0.45) $[\theta]_{310}$ + 4700, $[\theta]_{268}$ + 22 200 and $[\theta]_{240}$ + 196 600.

Preparation of N,O-*dimethyltiliamosine* (**5**). To *N*-methyltiliamosine (**14**) (120 mg) in Et_2O–MeOH (1:1) (15 ml) was added CH_2N_2–Et_2O [23]. After standing 7 days in the dark, a second portion of CH_2N_2–Et_2O (60 ml) was added, which was followed by a third portion (60 ml) after another 7 days in the dark. At the end of a total of 17 days, the soln was evapd to dryness and the resulting residue dissolved in HCl (1%) (20 ml) and extracted with Et_2O (100 ml). The acidic soln was made alkaline with NH_4OH to pH 8–9 and extracted × 5 with Et_2O (20 ml). The Et_2O extracts were combined, dried, filtered and evapd to leave a residue (117 mg). The residue was dissolved in $CHCl_3$ (2 ml) and chromatographed over silicic acid (10 g). Elution with $CHCl_3$–MeOH (19:1) afforded *N,O*-dimethyltiliamosine (**5**) as a colourless, amorphous residue (100 mg), mp 157–160°, R_f 0.75 (system B); $[\alpha]_D^{24}$ +230° ($CHCl_3$; *c* 0.59); UV λ_{max}^{MeOH} nm (log ε): 290 (3.73) and 235 (4.08); IR ν_{max}^{KBr} cm^{-1}: 1590, 1510, 1464, 1425, 1410, 1365, 1320, 1310, 1274, 1245, 1130, 1100, 1070, 1055, 1010, 985, 970, 925, 900, 875, 815, 754 and 660; ^1H NMR (60 MHz, $CDCl_3$, TMS). δ2.30 (3H, *s*, NMe), 2.60 (3H, *s*, NMe), 3.79 (3H, *s*, OMe), 3.83 (3H, *s*, OMe), 3.88 (3H, *s*, OMe), 3.93 (3H, *s*, OMe), 6.62 (1H, *s*, ArH), 6.82–7.57 (6H, *s*, ArH) and 7.98 (1H, *s*, ArH), EIMS (probe) 70 eV *m/z* (rel. int.): 620 $[M]^+$ (18), 380 (19), 379 (100), 365 (26), 191 (11), 190 (77), 189 (11), 182 (30), 173 (15), 168 (10), 159 (12) and 153 (10); CD(MeOH; *c* 0.73), $[\theta]_{309}$ + 6800, $[\theta]_{272}$ + 11 900 and $[\theta]_{240}$ + 88 400.

Oxidation of N,O-*dimethyltiliamosine* (**5**). To a soln of *N,O*-dimethyltiliamosine (**5**) (109 mg, 0.175 mmol) in HOAc (50%) (25 ml), a soln of ceric ammonium nitrate (0.938 g, 1.71 mmol) and NaOAc (85.9 mg, 1.05 mmol) in HOAc (50%) (25 ml) was added dropwise over a period of 5 min with gentle warming at 100°. After the addition was complete, the mixture was evapd to a syrup, treated with H_2SO_4 (10%) (10 ml) and warmed gently. The resulting suspension (suspension A) was extracted with $CHCl_3$ (15 ml) and the $CHCl_3$ layer separated and evapd to afford a yellow oil. This oil was redissolved in MeOH (10 ml) and treated portion-wise with $NaBH_4$ (100 mg) with stirring. The soln was stirred at room temp. for 30 min, diluted with H_2O (10 ml), and heated at 100° for an additional 30 min. After being cooled to room temp., the mixture was extracted × 3 with $CHCl_3$ (20 ml) and the $CHCl_3$ extracts were pooled and evapd to a pale yellow oil (15 mg). This oil was dissolved in Me_2CO (10 ml) and $KMnO_4$ (1%) in Me_2CO (3 ml) was added dropwise, with stirring, at room temp. over a period of 1 hr. After stirring overnight, the precipitated MnO_2 was dissolved by SO_2 and the resulting soln evapd. The residue was dissolved in MeOH–H_2O (1:1) (15 ml) and extracted × 5 with EtOAc (15 ml). The EtOAc was evapd with the residue dissolved in MeOH. CH_2N_2–Et_2O (30 ml) [23] was added portion-wise until effervescence ceased. The soln was evapd and the residue dissolved in $CHCl_3$ (1 ml) and chromatographed over silica gel (5 g) in $CHCl_3$. Elution with $CHCl_3$–MeOH (19:1) (100 ml) afforded a colourless residue (13 mg) which was crystallized from MeOH to afford 2,2'-dimethoxy-5,5'-dicarbomethoxybiphenyl (**8**) as white needles, mp 155–157°; UV λ_{max}^{MeOH} nm (log ε): 255 (3.29), 233 (3.35) and 218 (sh) (3.33); IR ν_{max}^{KBr} cm^{-1}: 2960, 2850, 1718, 1604, 1510, 1495, 1440, 1320, 1298, 1278, 1265, 1255, 1222, 1182, 1157, 1145, 1045, 1025, 970, 938, 915, 892, 848, 838, 775 and 770; ^1H NMR (600.6 MHz, $CDCl_3$, TMS): δ3.81 (6H, *s*, 2COOMe), 3.87 (6H, *s*, 2OMe), 6.98 (2H, *d*, J = 8.8 Hz, ArH), 7.93 (2H, *d*, J = 2.2 Hz, ArH) and 8.06 (2H, *dd*, J = 8.5, 2.2 Hz, ArH); EIMS (probe) 70 eV *m/z* (rel. int.): 330 $[M]^+$ (9), 299 (10), 285 (2), 269 (2), 241 (6), 212 (8), 197 (10), 182 (10), 165 (6), 149 (19), 134 (100), 126 (17), 113 (24) and 106 (10) identical by direct comparison (UV, IR, MS) with an authentic sample [21].

The $CHCl_3$-extracted suspension A from above was filtered and the filtrate made alkaline with NH_4OH to pH 9. MeOH (20 ml) was added and the mixture treated with $NaBH_4$ (100 mg) portion-wise while stirring and cooling. After 30 min at room temp., the mixture was boiled for an additional 30 min, cooled to room temp., acidified with H_2SO_4 (20%) and filtered. The filtrate was washed with $CHCl_3$ (30 ml), made alkaline with NH_4OH and then extracted × 4 with $CHCl_3$ (30 ml). The pooled $CHCl_3$ extract was evapd to yield the diamine **6** (25 mg) as an amorphous residue, mp 238–240° (MeI salt); R_f 0.55 ($CHCl_3$–MeOH–NH_4OH, 95:5:1); UV λ_{max}^{MeOH} nm (log ε): 296 (3.13) and 233 (3 86); IR ν_{max}^{KBr} cm^{-1}: 2970, 2940, 2840, 2770, 1598, 1518, 1490, 1430, 1380, 1355, 1330, 1300, 1280, 1260, 1220, 1210, 1195, 1160, 1130, 1080, 1050, 1000, 985, 965, 940, 925, 905 and 867; ^1H NMR (60 MHz, $CDCl_3$, TMS): δ2.40 (3H, *s*, NMe), 2.45 (3H, *s*, NMe), 2 68 (*m*, 8H, H-3, H-3', H-4, H-4'), 3.45 (*br s*, 4H, H-1, H-1'), 3.78 (3H, *s*, OMe), 3.90 (3H, *s*, OMe), 6.52 (1H, *s*, ArH) and 6.68 (1H, *s*, ArH); EIMS (probe) 70 eV *m/z* (rel. int.): 382 $[M]^+$ (23), 381 (7), 352 (8), 339 (26), 324 (10), 308 (3), 296 (6), 281 (4), 190 (9), 175 (5), 161 (14), 148 (5), 141 (21), 140 (27), 85 (16) and 42 (100) identified by comparison with a similar diamine prepared by identical oxidation of *O*-methylmicranthine [9].

Pachyovatamine (**4**). Elution of column A with $CHCl_3$–MeOH (9:1) (2 l.) and combination of fractions 280–300 afforded a fraction which on rechromatography over silica gel (10 g) and elution with $CHCl_3$–MeOH (19:1) yielded pachyovatamine (**4**) (70 mg) as an amorphous residue, mp 182–185°; R_f 0.30 (system

C); $[\alpha]_D^{25}$ +259° ($CHCl_3$; c 0.29); UV λ_{max}^{MeOH} nm (log ε): 291 (3 68) and 230 (4.34); IR ν_{max}^{KBr} cm^{-1}: 2930, 2840, 1625, 1590, 1505, 1450, 1435, 1415, 1360, 1290, 1277, 1240, 1225, 1130, 895, 865 and 825, ^1H NMR (60 MHz, $CDCl_3$, TMS): δ3.83 (3H, s, OMe), 3 96 (3H, s, OMe), 6.25 (1H, s, ArH), 6.60 (1H, s, ArH), 6.8–7.6 (6H, m, ArH) and 8.07 (1H, s, ArH); EIMS (probe) 70 eV m/z (rel. int.): 548 $[M]^+$ (3), 335 (15), 322 (16), 321 (100), 211 (6) and 161 (30); CD (MeOH; c 0.26); $[\theta]_{309}$ +6300, $[\theta]_{271}$ +13 700 and $[\theta]_{238}$ +134 900.

Preparation of N,N-*dimethylpachyovatamine* (**9**). To pachyovatamine (**4**) (30 mg) in MeOH (20 ml) was added HCO_2H (88%) (1 ml) and HCHO (37%) (0.5 ml) and the resulting mixture refluxed for 24 hr. The soln was cooled, made alkaline with NH_4OH to pH 8–9 and extracted × 5 with Et_2O (20 ml). The Et_2O extracts were pooled, washed with H_2O, dried, filtered and the filtrate was evapd to afford *N,N*-dimethylpachyovatamine (**9**) (28 mg) as an amorphous residue, mp 135–138° (Me_2CO), R_f 0.78 (system B); $[\alpha]_D^{25}$ +287° ($CHCl_3$; c 0.37); UV λ_{max}^{MeOH} nm (log ε): 290 (3.95) and 234 (sh) (4.75), IR ν_{max}^{KBr} cm^{-1}. 2940, 2850, 2800, 1630, 1595, 1590, 1508, 1468, 1460, 1450, 1430, 1420, 1362, 1280, 1275, 1240, 1205, 1130, 1120, 1070, 1015, 990, 970, 952, 895, 875, 850, 825, 815, 780 and 755; ^1H NMR (60 MHz, $CDCl_3$, TMS): δ2.29 (3H, s, NMe), 2.63 (3H, s, NMe), 3.85 (3H, s, OMe), 3.98 (3H, s, OMe), 6.29 (1H, s, ArH), 6.63 (1H, s, ArH), 6 88–7.70 (6H, m, ArH) and 8.08 (1H, s, ArH); EIMS (probe) 70 eV m/z (rel. int.) 576 $[M]^+$ (10), 349 (100), 335 (30), 211 (1) and 175 (50).

Preparation of N,N-*dimethyl*-O-*acetylpachyovatamine* (O-*acetyltiliacorinine*) (**10**). To *N,N*-dimethylpachyovatamine (**9**) (25 mg) in pyridine (0.5 ml) was added Ac_2O (1 ml). After 36 hr the soln was chilled and cold H_2O (5 ml) added. The aq. soln was made alkaline with NH_4OH to pH 8–9 and extracted × 5 with Et_2O (15 ml). The Et_2O extracts were combined, dried, filtered and the filtrate was evapd to afford *N,N*-dimethyl-*O*-acetylpachyovatamine (**10**) as a white, amorphous residue, mp 170–174°; R_f 0.70 (system C); $[\alpha]_D^{27}$ +269° (pyridine, c 0.42): UV λ_{max}^{MeOH} nm (log ε): 288 (3.90) and 232 (sh) (4.72); IR ν_{max}^{KBr} cm^{-1}: 2940, 2860, 2800, 1770, 1630, 1590, 1510, 1470, 1450, 1440, 1430, 1420, 1380, 1365, 1300, 1282, 1270, 1240, 1220, 1210, 1200, 1120, 1070, 1045, 1030, 1010, 955, 915, 895, 880, 832, 810 and 750; ^1H NMR (60 MHz, $CDCl_3$, TMS): δ2.13 (3H, s, OCOMe); 2.32 (3H, s, NMe), 2 61 (3H, s, NMe), 3 82 (3H, s, OMe), 3.86 (3H, s, OMe), 6 30 (1H, s, ArH), 6 64 (1H, s, ArH), 6.83–7.68 (6H, m, ArH) and 8 00 (1H, s, ArH); EIMS (probe) 70 eV m/z (rel. int.) 618 $[M]^+$ (1), 617 (6), 350 (10), 349 (34), 335 (14) and 175 (100) identical to *O*-acetyltiliacorinine (**10**) by direct comparison (IR, ^1H NMR, MS, $[\alpha]_D$)

Chromatography of fraction C. Fraction C (3 g) was dissolved in $CHCl_3$–MeOH (19:1) (30 ml) and chromatographed over silica gel (150 g) (column B) in the same solvent via flash column chromatography [24] Fractions (50 ml) were collected and combined according to TLC analysis.

N-*Methylpachygonamine* (**3**). Combination of fractions 11–13 afforded *N*-methylpachygonamine (**3**) (120 mg) as an amorphous residue, mp 183–185° (dec) ($CHCl_3$–MeOH), R_f 0.60 (system B) and 0.45 (system C) whose spectral properties have been reported elsewhere [7].

Pachygonamine (**2**). Combination of fractions 19–25 and prep. TLC in system B afforded pachygonamine (**2**) (30 mg) as an amorphous residue, mp 225–227° (dec.) ($CHCl_3$), R_f 0 46 (system B) and 0.20 (system C), whose spectral properties have been reported elsewhere [7]

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REFERENCES

1 Kirtikar, K. R. and Basu, B. D. (1975) *Indian Medicinal Plants*, p. 90. L. M Basu, Allahabad
2. Hooker, J. D. (1961) *The Flora of British India*, p. 105. L. Reeve, England.
3. Chopra, S. R. N., Badhwar, R L. and Ghosh, S. (1949) *Poisonous Plants of India*, pp. 152–153. Government of India Press, Calcutta.
4 DasGupta, S., Ray, A B., Bhattacharya, S K. and Bose, R. (1979) *J. Nat. Prod.* **42**, 399.
5. Bhat, S. V., Dornauer, H. and DeSouza, J. J (1980) *J. Nat. Prod.* **43**, 588.
6. Abd El-Kawi, M., Slatkin, D. J., Schiff, P. L, Jr., DasGupta, S, Chattopadhyay, S K. and Ray, A. B. (1984) *J. Nat Prod.* **47**, 459.
7. Sultanbawa, M. U. S., Sotheeswaran, S., Balasubramaniam, S, Abd El-Kawi, M. Slatkin, D. J. and Schiff, P. L., Jr. (1983) *Heterocycles* **20**, 1927.
8. Wu, W.-N., Beal, J L and Doskotch, R. W. (1980) *J. Nat. Prod.* **43**, 377.
9. Bick, I. R. C., Bremner, J. B., Cava, M. P. and Wiriyachitra, P. (1978) *Aust J. Chem.* **31**, 321.
10 Shamma, M. (1972) *The Isoquinoline Alkaloids*, p. 149. Academic Press, New York.
11. Baldas, J, Porter, Q N., Bick, I. R. C. and Vernengo, M. J. (1966) *Tetrahedron Letters* 2059
12 Dwuma-Badu, D., Ayim, J. S. K., Fiagbe, N. Y., Tackie, A. N, Knapp, J E., Slatkin, D. J. and Schiff, P L., Jr. (1976) *J. Nat. Prod.* **39**, 213.
13. Tomita, M, Kikuchi, T., Fujitani, K., Kato, A., Furukawa, H., Aoyagi, Y, Kitano, M. and Ibuka, T. (1966) *Tetrahedron Letters* 857
14. Baldas, J, Bick, I R C., Ibuka, T., Kapil, R S. and Porter, Q. N. (1972) *J. Chem. Soc., Perkin Trans.* 1, 592.
15 Anjaneyulu, B., Govindachari, T. R., Sathe, S. S., Viswanathan, N., Gopinath, K. W. and Pai, B R. (1969) *Tetrahedron* **25**, 3091.
16. Tackie, A. N., Dwuma-Badu, D., Knapp, J. E. and Schiff, P. L., Jr. (1973) *Phytochemistry* **12**, 203.
17 Guha, K. P, Mukherjee, B. and Mukherjee, R (1979) *J. Nat. Prod.* **42**, 56
18. Schiff, P. L., Jr (1983) *J. Nat Prod.* **46**, 1.
19. Bhakuni, D. S., Singh, A. N., Jain, S and Kapil, R. S. (1978) *J. Chem Soc Chem. Commun.* 226.
20. Cassels, B. K. and Shamma, M. (1980) *Heterocycles* **14**, 222.
21. Tackie, A. N., Dwuma-Badu, D., Knapp, J. E. and Schiff, P. L, Jr. (1973) *J. Nat. Prod.* **36**, 66.
22 Munier, R. and Macheboeuf, M. (1951) *Bull. Soc Chim. Biol.* **33**, 846.
23. Fieser, L. F. and Fieser, M. (1967) *Reagents for Organic Synthesis*, p. 191, part (b). John Wiley, New York.
24. Still, W. C. (1978) *J Org. Chem.* **43**, 2933.