PHAEANTHINE-2'α-N-OXIDE AND PYCMANILLINE, NEW BISBENZYLISOQUINOLINE ALKALOIDS FROM PYCNARRHENA MANILLENSIS

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Abstract - Chromatography of an ethanolic extract of Pycrarrhena manillensis over silica gel afforded phaeanthine-2'α-N-oxide (1) and pycmanilline (2) which were characterized as new bisbenzylisoquinoline alkaloids by a consideration of physicochemical data and the utilization of simple chemical reactions. Phaeathine-2'α-N-oxide (1) was reduced to phaeanthine (3) while pycmanilline (2), a secobisbenzyliso-quinoline alkaloid, was prepared via oxidation of phaeanthine (3).

Pycnarrhena manillensis Vidal (Menispermaceae) is a medicinal plant indigenous to the Philippines.[1] Decoctions of the roots of this plant have been used in the therapy of stomachache and other gastric disturbances.[1] The genus Pycnarrhena is a rich source of bisbenzylisoquinoline alkaloids, with sixteen different compounds of this type having been isolated from among five different species.[2-4] There have been, however, only two reports concerning the alkaloids of P. manillensis. In 1935, the presence of an incompletely characterized alkaloid designated ambalinine ($C_{18}H_{21}O_3N$, mp 203-204°C) was reported.[5] Some twenty five years later the second report appeared and detailed the isolation of the bisbenzylisoquinoline alkaloids berbamine, pycnamine, isotetrandrine and phaeanthine from the roots of this species.[6] This paper is to report the isolation and identification of two new benzylisoquinoline-derived dimeric alkaloids, phaeanthine-2'α-N-oxide (1) and pycmanilline (2), as well as the reisolation of phaeanthine (3), isotetrandrine (4), pycnamine and berbamine.

Extraction of the roots and stems (2 kg) with ethanol and systematic partitioning in a classical manner[7] afforded nonquaternary phenolic (7.2 g) and nonphenolic (13.9 g) alkaloid fractions.

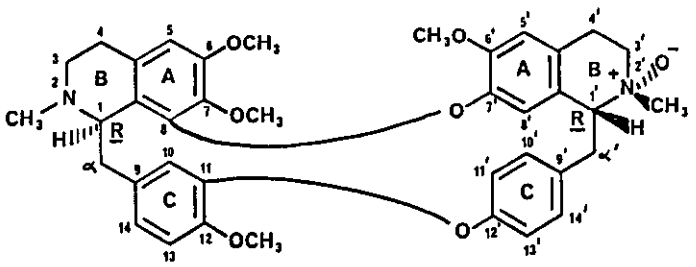
Chromatography of the nonquaternary nonphenolic alkaloid fraction over silicic acid in petrol-$CHCl_3$ (4:1) and elution with a petrol-$CHCl_3$ gradient failed to elute any alkaloids. However, elution with a $CHCl_3$-MeOH gradient (49:1 to 4:1) successively afforded phaeanthine (3)(286 mg), isotetrandrine (4)(137 mg), pycnamine (113 mg) and berbamine (76 mg). Greater quantities of pycnamine and berbamine were found in the phenolic alkaloid fractions, as anticipated. Elution of the column with $CHCl_3$-MeOH (7:3) gave a mixture of two alkaloids which separated on rechromatography over silica gel in $CHCl_3$-MeOH-NH_4OH (8:2:0.1) to yield phaeanthine-2'α-N-oxide (1)(9 mg) and pycmanilline (2)(4 mg).

Phaeanthine-2'α-N-oxide crystallized as colorless needles from acetone, mp 193-195°C; $[\alpha]_D^{22}$-253° (c 0.17, $CHCl_3$); uv, λ_{max}^{MeOH} 282nm (logϵ 3.95); ir, ν_{max}^{KBr} cm^{-1} 2930, 2850, 1603, 1580, 1503, 1460, 1445, 1435, 1410, 1350, 1340, 1322, 1310, 1265, 1231, 1215, 1185, 1162, 1120, 1101, 1060, 1020, 995, 962, 912, 860, 852, 835, 819 and 745. The ms of the alkaloid showed the M^+ at m/z 638 and other significant fragment ions at m/z 622(M-16), 396, 395, 381 and 198. These spectral data were characteristic of a bisbenzylisoquinoline mono-N-oxide alkaloid[2-4] which was confirmed by reduction of the alkaloid with sulfurous acid[8] to afford phaeanthine (3), thereby establishing the skeletal structure, oxygenation pattern and stereochemistry of the N-oxide. Only the placement of the oxide-nitrogen atom at N-2 or N-2' and the spatial relationship of that oxygen atom to its neighboring H-1 or H-1' proton remained to be determined. The ^{1}H-nmr spectrum (300MH_z, $CDCl_3$, TMS, δ in ppm) of the N-oxide indicated the presence of one N-methyl group at 2.36(3H,s), one N-oxide methyl group at 3.42(3H,s); and three aromatic methoxy groups as three singlets at 3.21(3H)(C-7), 3.76(3H)(C-6) and 3.93(3H)(C-12), with a fourth aromatic methoxy group overlapping the N-oxide methyl group and being found at 3.42(3H)(C-6'). Since it has been established that the chemical shift of the 2-N-methyl group of phaeanthine is at 2.33 while that of the 2'-N-methyl group is at 2.62,[2-4,9] the presence of a three proton singlet at 2.36 in the N-oxide plus the absence of a signal at about 2.6 fixes the N-oxide at the N-2' position. It has also been established that the 2-N-methyl (or 2'-N-methyl) signal of a bisbenzylisoquinoline N-oxide of the same 8-7',11-12' family (subgroup C)[9] as phaeanthine (1R,1'R) undergoes a downfield shift of 0.3-0.4 ppm when H-1 (or H-1') is on the same side as the N-oxide oxygen atom, with a larger downfield shift (0.7-0.9 ppm) being observed when the H-1 (or H-1') proton is on the side opposite to the N-oxide oxygen atom[9-11]. Since the chemical shift of the 2'-N-methyl signal of phaeanthine is at 2.62 while that of the naturally occurring 2'-N-oxide at 3.42, the downfield shift of 0.80 ppm suggests an α-N-oxide configuration in which the H-1' proton and N-oxide oxygen atom bear an anti-relationship to one another. There have been at least thirteen other bisbenzylisoquinoline-mono-N-oxide alkaloids isolated previously.[2-4,10,11] Of these, ten alkaloids have been isolated from eight different

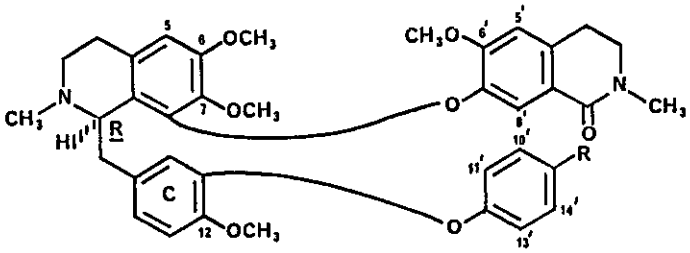
genera of the family Menispermaceae while the remaining three alkaloids were isolated from two species of the genus Berberis of the family Berberidaceae. There are six alkaloids that possess the same linkage (8-7',11-12' family-subgroup C)[9](type VIII)[2-4] as phaeanthine-2' -N-oxide within this group.[2,3,10,11] Of these six alkaloids, five are from Menispermaceous species and three of the five have identical stereochemistry (R, R).

Pycmanilline (2) was isolated as an amorphous solid, $[\alpha]_D^{22}$ + 50° (c 0.1, $CHCl_3$); uv, λ_{max}^{MeOH} nm (log ε) 306(sh)(3.77), 295(sh)(3.92), 285(sh)(4.08), 272(sh)(4.25), 261(sh)(4.41), 250(sh)(4.52) and 225(sh)(4.79): ir, ν_{max}^{KBr} cm^{-1} 2930, 1710(ArCOOH), 1650($ArCONR_2$), 1605(Ar) and 1505(Ar). The ei-ms failed to display the parent ion but showed significant fragment ions at m/z 411(100%)(5), 396(7)(5-CH_3), 365(15)(5-CH_3-OCH_3) and 257(13)(6). The ci-ms (isobutane) displayed the M^++1 ion at m/z 669(100%), M^+ at m/z 668 (16%) and other important fragment ions at m/z 623(10%)(M-COOH) and 411(81)(5). The ci-ms(NH_3) likewise displayed the M^++1 ion at m/z 669 (100%), with M^+ m/z 668 (24) and 411(35)(5). These spectral data were characteristic of a secobisbenzylisoquinoline alkaloid derived from phaeanthine (3) or isotetrandrine (4), in which the benzylic carbon atom in the right hand ring has undergone an in vivo catabolic oxidation to a carboxylic acid.[4,10,11] The ^{1}H-nmr spectrum (300 MHz, $CDCl_3$, TMS, δ in ppm) indicated the presence of one N-methyl group at 2.52(3H,s); one conjugated tertiary lactam N-methyl group at 3.03(3H,s); and four methoxy groups as four singlets (3H each) at 3.62(C-7), 3.69(C-6), 3.79(C-6') and 3.86(C-12). The aromatic protons in the isoquinoline-portion of the dimer were observed as one proton singlets at 6.50(H-5), 6.62(H-5') and 7.16(H-8') while those of the benzyl-portion were found as an A_2B_2 system centered at 6.83(2H,d)(H-11' and H-13') and 7.83(2H,d)(H-10' and H-14'), J_o=8.1 Hz, plus a three proton multiplet from 6.75-7.05 for the protons of ring C. Pycmanilline (2) was prepared via oxidation of phaeanthine (3) with $KMnO_4$. Briefly, to a stirred solution of phaeanthine (3)(500 mg) in acetone (100 ml) at room temperature was added a solution of $KMnO_4$ (250 mg) in acetone (200 ml) in a dropwise fashion over 45 min. The resulting solution was stirred for an additional 6 h and after standing for 15 h filtered and evaporated. The residue was chromatographed over silica gel (55g) in $CHCl_3$-MeOH-NH_4OH (8:2:0.05) to afford pycmanilline (2)(6 mg), R_f 0.05 on silica gel tlc (Sigel 60 F_{254}, pre-coated sheets on Al support, EM Science) in $CHCl_3$-MeOH-NH_4OH (9:1:0.05), mp 254-255°C, $[\alpha]_D^{22}$ + 33° (c 0.61, $CHCl_3$), identical by direct comparison (^{1}H-nmr, ms, optical activity, tlc) with the naturally occurring alkaloid. A most important factor in affecting the sign of rotation in either monomeric or dimeric benzyltetrahydroisoquinoline alkaloids is the location of the lower pendant aromatic ring. Alkaloids of this type which are of the R configuration and which have their lower rings preferentially anti- to the nitrogen atom will be levorotatory.[2-4,13,14] However, alkaloids of identical absolute configuration in which the lower rings lie syn-to the nitrogen atom will be dextrorotatory.[13,14] The latter is true for pycmanilline, as the

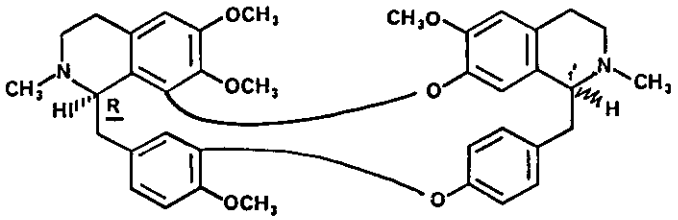
substitution at C-8 forces the lower pendant aromatic rings to lie on the same side as the amine nitrogen atom. In addition, the CD spectrum of pycmanilline ($[\theta]_{288}$ 0, $[\theta]_{282}$ - 3,960, $[\theta]_{273}$ 0, $[\theta]_{265}$ + 1,480, $[\theta]_{255}$ + 2,350, $[\theta]_{231}$ + 9,280, $[\theta]_{222}$ + 10,140, $[\theta]_{216}$ 0) displays a descending tail below 220 nm and is thus characteristic of the R configuration at C-1[13]. In order to contrast the reaction conditions necessary to prepare secophaeanthine (secophaeanthine aldehydolactam)(7) with pycmanilline and to further confirm the absence of an aldehyde function in pycmanilline, secophaeanthine (7) was prepared.[15] In brief, to a stirred solution of phaeanthine (3)(300 mg) (R_f 0.60 on silica gel tlc in $CHCl_3$-MeOH-NH_4OH (9:1:0.05)) in acetone (300 ml) at room temperature was added a solution of $KMnO_4$ (120 mg) in acetone (200 ml) in a dropwise manner over 45 min. The resulting solution was stirred for an additional 6 h, after standing another 15 h filtered and evaporated. The residue was chromatographed over silica gel (30 g) in $CHCl_3$-Me_2CO-MeOH-NH_4OH (20:2:1:0.05) to afford secophaeanthine (secophaeanthine aldehydolactam)(7)(11 mg), R_f 0.82 on silica gel tlc in $CHCl_3$-MeOH-NH_4OH (9:1:0.05), $[\alpha]_D^{22}$ + 8° (c 0.13, $CHCl_3$); uv, λ_{max}^{MeOH} nm (log ε) 291(sh)(4.14), 283(sh)(4.21), 272(sh)(4.29), 261(4.31) and 224(sh)(4.63); ir ν_{max}^{KBr} cm^{-1} 1695(ArCHO), 1650($ArCONR_2$), 1605(Ar); ^{1}H-nmr (300MH_z,$CDCl_3$,TMS, δ in ppm) 2.26(3H,s)(NCH_3), 3.04(3H,s)($ArCONCH_3$), 3.63(3H,s)(OCH_3), 3.73(3H,s)(OCH_3), 3.82(3H,s)(OCH_3), 3.83(3H,s)(OCH_3), 6.51(1H,s)(H-5 or H-5'), 6.58(1H,s)(H-5' or H-5), 6.93 and 7.78(4H,dd,J=8.7Hz)(H-11'+H-13' and H-10'+H-14'), 6.85-7.07(3H,m)(H-10,H-13,H-14), 7.23(H-8'), 9.90(1H,s)(ArCHO); ci-ms (Isobutane) M^++1 m/z 653(100%), M^+ 652(13), 411(73)(5) and 241(2)(8). Pycmanilline is no less than the fifteenth secobisbenzylisoquinoline alkaloid to have been isolated from a higher plant.[4] These alkaloids can be considered in vivo products of bisbenyzlisoquinoline catabolism in which oxidative cleavage has occurred at the benzylic C-1 to C-α bond of one of the two monomeric benzyltetrahydroisoquinoline moieties. This cleavage tends to occur preferentially at the benzyltetrahydroisoquinoline which is *unsubstituted at C-8 due to the lesser steric hindrance at the neighboring* C-1.[4,13,14] The resulting seco-alkaloids are usually lactam aldehydes (eleven reported), although reduction of the aldehyde to a primary alcohol (one reported) and to an aryl methyl group (one reported) are also known.[4] In addition, oxidation of the aldehyde to a carboxylic acid can occur. To our knowledge, pycmanilline is the first example of a lactam carboxylic acid secobisbenzylisoquinoline alkaloid, although gilgitine (Berberis lycium)[4,13] and talcamine (Berberis buxifolia)[4,16] are lactam carboxylic acid methyl ester derivatives.



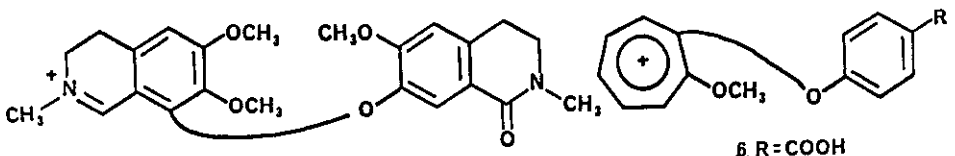
1



2 R= COOH
7 R=CHO



3 1′= R
4 1′= S



5

6 R=COOH
8 R=CHO

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