NEW ALKALOIDS FROM *PANCRATIUM MARITIMUM L.*

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Abstract- Two new alkaloids, 6-O-methylhaemanthidine (1) and O,N-dimethylnorbelladine (2), were isolated from *Pancratium maritimum L.*, together with lycorine, hippeastrine, galanthamine, haemanthamine, haemanthidine, vittatine, 11-hydroxyvittatine, hordenine and 9-O-demethylhomolycorine (all previously isolated from this plant), and habranthine, ungiminorine and ungiminorine N-oxide.

Pancratium maritimum L. (Amaryllidaceae) is typical of sandy coastal habitats. The alkaloid constituents of *P. maritimum* have been studied intensively, and to date more than 10 alkaloids have been found[1,2], some of them of pharmacological interest[3,4]. In this paper we describe the isolation from bulbs of *P. maritimum* of two new alkaloids, 6-O-methylhaemanthidine (1) and O,N-dimethylnorbelladine (2), together with the previously reported natural products lycorine (3), ungiminorine (4), ungiminorine N-oxide (5), haemanthamine (6), haemanthidine (7), vittatine (8), 11-hydroxyvittatine (9), galanthamine (10), habranthine (11), hippeastrine (12), 9-O-demethylhomolycorine (13) and hordenine (14).

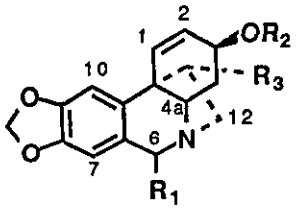
Crude basic material was extracted from dried bulbs of *P. maritimum L.* with methanol in a sohxlet apparatus. After acid-basic extraction with CH_2Cl_2-$CHCl_3$, the organic extracts were subjected to column chromatography using silicagel-dichloromethane-methanol to obtain alkaloids 1-14.

6-O-Methylhaemanthidine (1) eluted from the column with CH_2Cl_2-MeOH (95:5) and was purified by preparative tlc (mp: 110-112°C). Its ir spectrum showed absorption due to a hydroxyl group at 3400 cm^{-1} and a methylenedioxy group at 930 cm^{-1}, but no carbonyl absorption. The ^1H nmr spectrum showed the presence of two olefinic protons, one para-oriented aromatic proton and a methylenedioxy group, but no signal due to an N-methyl group. These findings suggested that base 1 had a 5,10b-ethanophenanthridine skeleton. Comparison of the ^1H nmr spectrum of 1 with those of haemanthidine (7) and haemathamine (6) showed the presence of an extra $-OCH_3$ at ∂ 3.56 and a singlet for one benzyl proton at ∂ 4.39, thus suggesting the structure of 6-methylhaemanthidine. This hypothesis was further supported by extensive 2D nmr and ^{13}C nmr analysis and was finally confirmed by the finding that acidic cleavage (50% AcOH) of the 6-methoxyl group of (1) gave haemanthidine (7)[5] (whereas treatment of the latter with MeOH/p-TSA did not give 1[5], proving that 6-O-methylhaemanthidine was not an artifact). The configuration at C-6 was found

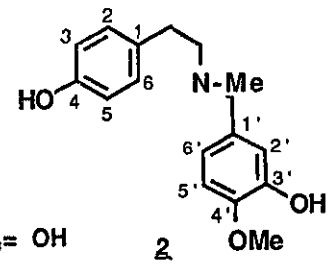
to be beta (the methoxyl group trans to the ethanamine bridge), for when H-6 was irradiated a nOe effect was observed on H-12.

The second component of interest in *P. maritimum* was O,N-dimethylnorbelladine (2), which eluted with CH_2Cl_2-MeOH (90:10) and was identified by comparison of its physical and spectroscopic data with those of a synthetic sample prepared from 4-O-benzylphenylethylamine[6] and O-benzylisovainilline by formation of the corresponding imine, reduction, N-methylation, and deprotection.

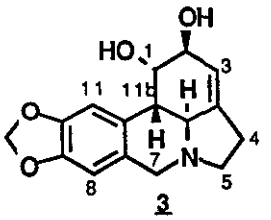
O,N-Dimethylnorbelladine (2) has been proposed as an intermediate in the biosynthesis of Amaryllidaceae alkaloids[7] but it has never before been isolated as a natural product. Our result proves unambiguously that 2 is being produced in the metabolism of the plant, and hence that it has a role in the biosynthesis of the Amaryllidaceae alkaloids.



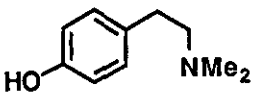
1, R_1= OMe, R_2= Me, R_3= OH
6, R_1= H, R_2= Me, R_3= OH
7, R_1= OH, R_2= Me, R_3= OH
8, R_1= H, R_2= H, R_3= H
9, R_1=H, R_2= H, R_3=OH



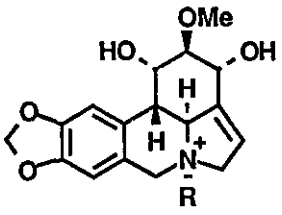
2



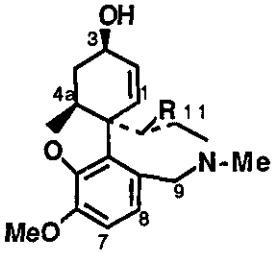
3



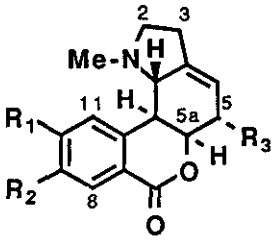
14



4, free base
5, R= O^-



10, R= H
11, R= OH



12, R_1+R_2= -OCH_2O-, R_3= OH
13, R_1=OMe, R_2= OH, R_3= H

Lycorine (3), hippeastrine (12), galanthamine (10), haemanthamine (6) haemanthidine (7), vittatine (8), 11-hydroxyvittatine (9) and hordenine (14) have already been reported as present in this plant[1,2], but this is the first time that habranthine (11), ungiminorine (4), and ungiminorine N-oxide (5)[8] have

been found. Habranthine and ungiminorine are important components of the alkaloidal mixture, and were identified by comparison of spectral data with those of the bibliography[9].

Finally, 9-O-demethylhomolycorine (compound 13, mp 128-130°C) was isolated by elution with CH_2Cl_2-MeOH (93:7). The O-methyl group was located by means of nOe experiments: when both the N-methyl and O-methyl groups were irradiated, nOe was observed on C_{11}-H only. The spectroscopic data are coincident to those reported by Jeffs[10] (who isolated this alkaloid ,mp 138-140°C, from *Crinum defixum*), so that the compound with mp 213-214°C reported by S. Uyeo[11] may reflect polymorphism[12].

EXPERIMENTAL

Melting points were determined using a Kofler apparatus and are uncorrected. Ir spectra were recorded with a *Pye Unicam 1100 spectrophotometer, uv spectra with a Pye-Unicam 1700 spectrophotometer,* nmr spectra (in the solvent specified and with Me_4Si as internal standard) on a Bruker WP-250 operating at 250 MHz (for ^1H) or 62.85 MHz (for ^{13}C), and mass spectra on a Kratos MS-25 apparatus. The absorbent used for column chromatography was silica gel (Merck 70-230 mesh ASTM) and the plates used for ptlc were coated with silica gel (Merck Kieselgel 60 HF_{254}) or aluminium oxide (Merck Aluminiumoxid GF_{254} type E).

Isolation of alkaloids from *Pancratium maritimun* L.- Bulbs of *Pancratium maritimum L.*, collected in the northwest of Spain (Barrañan and Corrubedo, La Coruña), were dried (to 5.3 kg), powdered and extracted in a soxhlet apparatus with MeOH. The extracts were concentrated and the resulting syrup was dissolved in 5% HCl and extracted with hexane. The aqueous layer was brought to pH 9 with Na_2CO_3 and extracted with $CH_2Cl_2/CHCl_3$. Removal of organic solvent gave 9 g of a syrup which was chromatographed on silica gel using a CH_2Cl_2-MeOH step gradient (0-100% MeOH) to give compounds 1-14 (yield in parenthesis) in the *following order of elution: 6-O-methylhaemanthidine* (1),(0.06g); habranthine (11),(0.10g); hippeastrine (12),(0.06g); 9-O-demethylhomolycorine (13),(0.40g); lycorine (3),(3.22g); galanthamine (10),(0.90g); haemanthamine (6),(0.12g): haemanthidine (7),(0.30); ungiminorine (4),(1.1g); O,N-dimethylnorbelladine (2),(0.01g); vitattine (8),(0.01g); 11-hydroxyvitattine (9),(0.02g); ungiminorine N-oxide (5),(0.03g); and hordenine (14),(0.05g). Further purification by preparative tlc of the fractions obtained afforded pure alkaloids.

6-O-Methylhaemanthidine (1)- mp 110-112°C (acetone); [α] -43° (c= 0.46,$CHCl_3$); uv λ^{EtOH} nm: 205, 245, 293; ir ν^{NaCl} cm^{-1}: 3400, 2900, 1610, 1500, 1475, 1330, 1190, 980, 930, 860; ms m/z

(rel. int.) 331 (M^+, 50), 316 (39), 300 (55), 284 (100), 268 (99), 227 (55), 225 (95), 224 (99); ^1H nmr ($CDCl_3$) ∂ 6.74 (1H, s, H-10), 6.72 (1H, s, H-7), 6.34 and 6.32 (2 x 1H, 2s, H-1, H-2), 5.89 (2 x 1H, 2d, J= 1.4 Hz, -OCH_2O-), 4.39 (1H, s, H-6), 3.85 (2H, m, H-11, H-3), 3.58 (1H, dd, J(4a,4 eq.)= 5 Hz, J(4a,4 ax.)= 12.6 Hz, H-4a), 3.56 (3H, s, C_6-OMe), 3.34 (3H, s, C_3-OMe), 3.21 (2H, m, H-12), 2.12 (1H, td, J(gem.)= J(4a,4)= 13.7 Hz, J(4,3)= 4.3 Hz, H-4), 1.89 (1H, ddd, J(gem.)= 13.7 Hz, J(4a,4)= 5 Hz, J(3,4)= 1.6 Hz, H-4); ^{13}C nmr ($CDCl_3$) ∂ 147.8, 146.3, 136.5, 127.2, 109.6, 102.8 (arom.), 132.5, 126.8 (C-1, C-2), 101.0 (OCH_2O), 96.2 (C-6), 78.6, 72.6 (C-3, C-11), 58.6 (C-12), 56.6 (2 x OMe), 56.3 (C-4a), 50.1 (C-10b), 28.0 (C-4).

O,N-Dimethylnorbelladine (2).- mp 130-132°C (lit.[7] 133-135°C); uv λ^{MeOH} nm: 212, 225, 282; uv $\lambda^{MeOH+NaOH}$ nm: 216, 244, 294; ms m/z 181, 137, 107; ^1H nmr ($CDCl_3$) ∂ 7.02 (2H, d, J(ortho)= 8.5 Hz, H-3, H-5), 6.88 (1H, broad s, H-2'), 6.78 (2H, broad s, H-3', H-6'), 6.71 (2H, d, J(ortho)= 8.5 Hz, H-2, H-6), 3.87 (3H, s, OMe), 3.48 (2H, s, N-CH_2-Ar), 2.80-2.71 (2H, m, -$CH_2C\underline{H_2}N$), 2.62-2.55 (2H, m, -$C\underline{H_2}CH_2N$), 2.26 (3H, s, NMe); ^{13}C nmr ($CDCl_3$ + CD_3OD) ∂ 154.9, 146.5, 145.6, 130.6, 129.6, 121.2, 116.2, 115.4, 110.7 (arom.), 61.0 (N-CH_2-Ar), 58.6 (N-$\underline{C}H_2$-CH_2), 55.7 (OMe), 41.2 (NMe), 31.8 (N-CH_2-$\underline{C}H_2$).

Lycorine (3).- mp 257-259°C (lit.[4] 250-283°C); ir ν^{KBr} cm^{-1}: 3340 (OH), 3060-2700, 1500, 1040, 940; ms m/z (rel. int.) 287 (M^+), 268, 250, 227, 226 (100).

Ungiminorine (4).- mp 204-206°C (lit.[8,13] 206-208°C).

Ungiminorine N-oxide (5).- mp 182-184°C,(lit.[8] 182-184°C)

Haemanthamine (6).- mp 201-203°C (descomp., acetone); $[\alpha]_D$ +17° (c=0.9,MeOH),(lit.[14] +19.7°).

Haemanthidine (7).- mp 198-199°C (lit.[14] 195°C), $[\alpha]_D$ -23° (c=1, $CHCl_3$), (lit.[15] -41°).

Vittatine (8).- mp 205-206°C (Amorphous powder), (lit.[1] 209-210°C). Ir ν^{NaCl} cm^{-1}: 3350, 2900, 1500, 1030, 930; uv λ^{EtOH} nm: 206, 240sh, 293; ms m/z (rel.int.) 271 (M^+, 100), 254 (9), 228 (25), 216 (19), 199 (70), 187 (70), 173 (23); ^1H nmr ($CDCl_3$) ∂ 6.84 (1H, s, H-10), 6.56 (1H, d, J(1,2)= 10 Hz, H-1), 6.47 (1H, s, H-7), 5.96 (1H, ddd, J(1,2)= 10 Hz, J(2,3)= 5.3 Hz, J(2,4 "W")= 1.1 Hz, H-2), 5.90 (2 x 1H, 2d, J= 1.4 Hz, OCH_2O), 4.39 (1H, d, J(gem)= 16.8 Hz, H-6eq.), 4.35 (1H, m, H-3), 3.77 (1H, d, J= 16.9 Hz, H-6ax.), 3.43 (2H, m, H-12, H-4a), 2.89 (1H, m, H-12'), 2.18 (2H, ddd, J(gem)= 13 Hz, J(11,12')= 9 Hz, J(11',12')= 4 Hz, H-11, -OH), 2.05 (1H, m, H-4), 1.97 (1H, m, H-11'), 1.73 (1H, ddd, J(gem)= J(4',4a transdiaxial)= 13.5 Hz, J(4',3)= 4.1 Hz, H-4'); ^{13}C nmr ($CDCl_3$) 146.4, 146.0, 137.9, 131.6, 127.8, 125.3, 107.0, 102.9 (arom., C-1, C-2), 100.9 (OCH_2O), 63.7 (C-3), 63.0 (C-4a), 61.9 (C-6), 53.3 (C-12), 44.3 (C-10b), 43.7 (C-11), and 32.3 (C-4).

11-Hydroxyvittatine (9).- mp 245-247°C,(Amorphous powder) (lit.[2] mp 248-250°C); uv λ^{EtOH} nm: 205, 242sh, 295; ir ν^{NaCl} cm^{-1}: 3360, 2900, 1500, 1480, 1030, 930, 850; ms m/z (rel. int.)

287 (M^+, 58), 269 (30), 243 (56), 227 (74), 225 (54), 224 (46), 209 (40), 181 (100); ^1H nmr ($CDCl_3$ + CD_3OD) ∂ 6.84 (1H, s, H-10), 6.45 (1H, s, H-7), 6.37 (1H, d, J(1,2)= 10.1 Hz, H-1), 6.27 (1H, dd, J(1,2)= 10.1 Hz, J(2,3)= 4.7 Hz, H-2), 5.90 (2H, s, OCH_2O), 4.34 (1H, td, J(3,2)= J(3,4)= 4.5 Hz, J(3,4')= 1.8 Hz, H-3), 4.27 (1H, d, J(gem)= 16.8 Hz, H-6), 3.98 (1H, td, J(11,12)= 3.2 Hz , J(11,12')= 6.6 Hz, J_W(11,4a)= 1 Hz, H-11), 3.66 (1H, d, J(gem)= 16.9 Hz, H-6), 3.37 (2H, m, H-12, H-4a), 3.18 (3H, dd, J(gem)= 14 Hz, J(11,12)= 3.2 Hz, H-12', 2 x OH), 2.21 (1H, td, J(gem)= J(4a,4)= 13.6 Hz, J(4,3) = 4.4 Hz, H-4α), 1.84 (1H, br dd, J(gem)= 13.4 Hz, J(4a,4)= 4.7 Hz, H-4β) ; ^{13}C nmr ($CDCl_3$ + CD_3OD) ∂ 146.6, 146.2, 135.3, 133.2, 126.2, 125.8, 106.7, 103.2 (arom., C-1, C-2), 100.8 (OCH_2O), 79.6 (C-11), 63.4, 62.2 (C-3, C-4a), 62.9 (C-6), 60.7 (C-12), 49.0 (C-10b), and 31.7 (C-4).

Galanthamine (10).- mp 125-126°C (acetone-ether),(lit.[14] 127-129°C),$[\alpha]_D$ -97° (c= 0.37, EtOH), (lit.[15] -107°C).

Habranthine (11).- mp 203-205°C (Cl_2CH_2-MeOH),(lit.[9] 198-199°C); $[\alpha]_D$ -107° (c= 0.3, EtOH), (lit.[9] -320°); ir ν^{KBr} cm^{-1}: 3460, 3400 (OH), 3010, 2920, 1620, 1510, 1280, 970; uv λ^{EtOH} nm: 232, 285; ms m/z (rel. int.) 303 (M^+, 14), 286 (5), 230 (54), 213 (27), 115 (100); ^1H nmr ($CDCl_3$) ∂ 6.67 (1H, d, J(ortho)= 8.3 Hz, H-7), 6.59 (1H, d, J(ortho)= 8.2 Hz, H-8), 6.07 (1H, ddd, J(1,2)= 10.4 Hz, J(2,3)= 5.2 Hz, J_W(2,4)= 1.4 Hz, H-2), 5.78 (1H, dt, J(1,2)= 10.2 Hz, J(1,3)= J_W(1,4a)= 1 Hz, H-1), 5.33 (1H, ddd, J(4a,4)= 4 Hz, J(4a,4')= 2 Hz, J_W(4a,1)= 1.5 Hz, H-4a), 4.12 (1H, td, J(2,3)= J(3,4)= 5 Hz, J(3,4')= 0.8 Hz, H-3), 3.83 (3H, s, OMe), 3.76 (1H, d, J(gem)= 14.7 Hz, H-9β ax.), 3.59 (1H, dd, J_W(9α,11α)= 1.3 Hz, J(gem)= 14.7 Hz, H-9α eq.), 3.47 (1H, dd, J(11,12)= 1.6 Hz, J(12,11')= 4.5 Hz, H-12β eq.), 3.08 (2H, m, J(11,11')= 13.3 Hz, J(11,12)= 4.5 Hz, J(11',12)= J(11,9)= 1.5 Hz, H-11), 2.70 (1H, dddd, J(gem)=16.2 Hz, J(3,4)= 3.4 Hz, J(4a,4)= J_W (2,4)= 1.6 Hz, H-4α eq.), 2.57 (3H, s, N-Me), 2.05 (1H, ddd, J(gem)= 16 Hz, J= 5 Hz, J= 2.4 Hz, H-4β ax.); ^{13}C nmr ($CDCl_3$) ∂ 147.0, 143.9, 129.7, 129.3, 128.4, 125.4, 120.9, 111.4 (arom., C-1, C-2), 83.2 (C-4a), 67.1 (C-12), 62.7 (C-11), 61.5 (C-3), 60.8 (C-9), 55.7 (OMe), 53.8 (C-12a), 49.1 (NMe), and 29.4 (C-4).

Hippeastrine (12).- mp 212-213°C (acetone), (lit.[17] 214-215°C); $[\alpha]_D$ +138° (c= 0.47,$CHCl_3$), (lit.[17]+160°); ms m/z (rel. int.) 257 (5), 125 (100), 96 (42).

9-O-Demethylhomolycorine (13).- mp 128-130°C (acetone-MeOH) (lit.[10] 138-140°C); $[\alpha]_D$ +53° (c=0.75,$CHCl_3$); ms m/z (rel. int.) 302 (M^++1), 192, 164, 110, 109 (100), 108, 94, 82, 81.

Hordenine (14).- ^1H Nmr ($CDCl_3$) ∂ 7.01 (1H, d, J(ortho)= 8.4 Hz, ArH), 6.71 (1H, d, J(ortho)= 8.5 Hz, ArH), 2.74 (4H, m, -CH_2CH_2-), 2.43 (6H, s, NMe_2).

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