

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, unguiminorine and unguiminorine 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<sup>1,2</sup>, some of them of pharmacological interest<sup>3,4</sup>. 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**), unguiminorine (**4**), unguiminorine 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**).

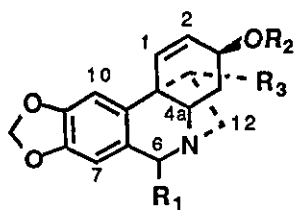
Crude basic material was extracted from dried bulbs of *P. maritimum* L. with methanol in a soxhlet apparatus. After acid-basic extraction with CH<sub>2</sub>Cl<sub>2</sub>-CHCl<sub>3</sub>, the organic extracts were subjected to column chromatography using silicagel-dichloromethane-methanol to obtain alkaloids **1-14**.

6-O-Methylhaemanthidine (**1**) eluted from the column with CH<sub>2</sub>Cl<sub>2</sub>-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<sup>-1</sup> and a methylenedioxy group at 930 cm<sup>-1</sup>, but no carbonyl absorption. The <sup>1</sup>H 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 <sup>1</sup>H nmr spectrum of **1** with those of haemanthidine (**7**) and haemathamine (**6**) showed the presence of an extra -OCH<sub>3</sub> at δ 3.56 and a singlet for one benzyl proton at δ 4.39, thus suggesting the structure of 6-methylhaemanthidine. This hypothesis was further supported by extensive 2D nmr and <sup>13</sup>C nmr analysis and was finally confirmed by the finding that acidic cleavage (50% AcOH) of the 6-methoxy group of (**1**) gave haemanthidine (**7**)<sup>5</sup> (whereas treatment of the latter with MeOH/p-TSA did not give **15**, proving that 6-O-methylhaemanthidine was not an artifact). The configuration at C-6 was found

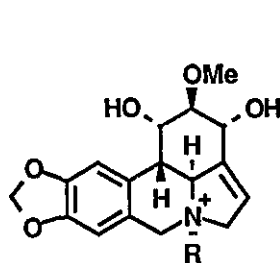
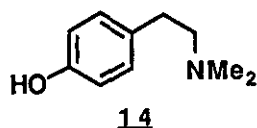
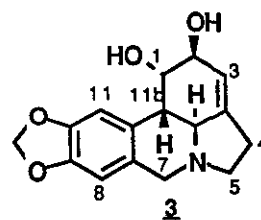
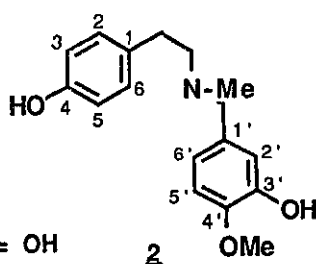
to be beta (the methoxyl group trans to the ethanamine bridge), for when H-6 was irradiated a nOe effect was observed on H-12.

The second component of interest in *P. maritimum* was O,N-dimethylnorbelladine (2), which eluted with CH<sub>2</sub>Cl<sub>2</sub>-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<sup>6</sup> and O-benzylisovainilline by formation of the corresponding imine, reduction, N-methylation, and deprotection.

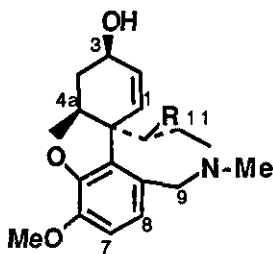
O,N-Dimethylnorbelladine (2) has been proposed as an intermediate in the biosynthesis of Amaryllidaceae alkaloids<sup>7</sup> but it has never before been isolated as a natural product. Our result proves unambiguously that 2 is being produced in the metabolism of the plant, and hence that it has a role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



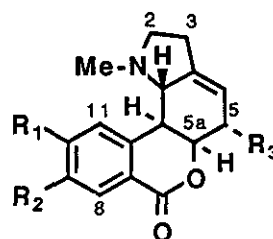
- 1, R<sub>1</sub> = OMe, R<sub>2</sub> = Me, R<sub>3</sub> = OH  
 6, R<sub>1</sub> = H, R<sub>2</sub> = Me, R<sub>3</sub> = OH  
 7, R<sub>1</sub> = OH, R<sub>2</sub> = Me, R<sub>3</sub> = OH  
 8, R<sub>1</sub> = H, R<sub>2</sub> = H, R<sub>3</sub> = H  
 9, R<sub>1</sub> = H, R<sub>2</sub> = H, R<sub>3</sub> = OH



- 4, free base  
 5, R = O



- 10, R = H  
 11, R = OH



- 12, R<sub>1</sub>+R<sub>2</sub> = -OCH<sub>2</sub>O-, R<sub>3</sub> = OH  
 13, R<sub>1</sub> = OMe, R<sub>2</sub> = OH, R<sub>3</sub> = H

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

been found. Habranthine and unginorine are important components of the alkaloidal mixture, and were identified by comparison of spectral data with those of the bibliography<sup>9</sup>.

Finally, 9-O-demethylhomolycorine (compound **13**, mp 128-130°C) was isolated by elution with CH<sub>2</sub>Cl<sub>2</sub>-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<sub>11</sub>-H only. The spectroscopic data are coincident to those reported by Jeffs<sup>10</sup> (who isolated this alkaloid, mp 138-140°C, from *Crinum defixum*), so that the compound with mp 213-214°C reported by S. Uyeo<sup>11</sup> may reflect polymorphism<sup>12</sup>.

## 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<sub>4</sub>Si as internal standard) on a Bruker WP-250 operating at 250 MHz (for <sup>1</sup>H) or 62.85 MHz (for <sup>13</sup>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<sub>254</sub>) or aluminium oxide (Merck Aluminiumoxid GF<sub>254</sub> type E).

Isolation of alkaloids from *Pancratium maritimum* 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<sub>2</sub>CO<sub>3</sub> and extracted with CH<sub>2</sub>Cl<sub>2</sub>/CHCl<sub>3</sub>. Removal of organic solvent gave 9 g of a syrup which was chromatographed on silica gel using a CH<sub>2</sub>Cl<sub>2</sub>-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); unginorine (**4**), (1.1g); O,N-dimethylnorbelladine (**2**), (0.01g); vitattine (**8**), (0.01g); 11-hydroxyvitattine (**9**), (0.02g); unginorine 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<sub>3</sub>); uv λ<sup>EtOH</sup> nm: 205, 245, 293; ir ν<sup>NaCl</sup> cm<sup>-1</sup>: 3400, 2900, 1610, 1500, 1475, 1330, 1190, 980, 930, 860; ms m/z

(rel. int.) 331 (M<sup>+</sup>, 50), 316 (39), 300 (55), 284 (100), 268 (99), 227 (55), 225 (95), 224 (99); <sup>1</sup>H nmr (CDCl<sub>3</sub>) δ 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<sub>2</sub>O-), 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<sub>6</sub>-OMe), 3.34 (3H, s, C<sub>3</sub>-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); <sup>13</sup>C nmr (CDCl<sub>3</sub>) δ 147.8, 146.3, 136.5, 127.2, 109.6, 102.8 (arom.), 132.5, 126.6 (C-1, C-2), 101.0 (OCH<sub>2</sub>O), 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.<sup>7</sup> 133-135°C); uv λ<sup>MeOH</sup> nm: 212, 225, 282; uv λ<sup>MeOH+NaOH</sup> nm: 216, 244, 294; ms m/z 181, 137, 107; <sup>1</sup>H nmr (CDCl<sub>3</sub>) δ 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<sub>2</sub>-Ar), 2.80-2.71 (2H, m, -CH<sub>2</sub>CH<sub>2</sub>N), 2.62-2.55 (2H, m, -CH<sub>2</sub>CH<sub>2</sub>N), 2.26 (3H, s, NMe); <sup>13</sup>C nmr (CDCl<sub>3</sub> + CD<sub>3</sub>OD) δ 154.9, 146.5, 145.6, 130.6, 129.6, 121.2, 116.2, 115.4, 110.7 (arom.), 61.0 (N-CH<sub>2</sub>-Ar), 58.6 (N-CH<sub>2</sub>-CH<sub>2</sub>), 55.7 (OMe), 41.2 (NMe), 31.8 (N-CH<sub>2</sub>-CH<sub>2</sub>).

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

**Ungimnorine (4).**- mp 204-206°C (lit.<sup>8,13</sup> 206-208°C).

**Ungimnorine N-oxide (5).**- mp 182-184°C, (lit.<sup>8</sup> 182-184°C)

**Haemanthamine (6).**- mp 201-203°C (descomp., acetone); [α]<sub>D</sub> +17° (c=0.9, MeOH), (lit.<sup>14</sup> +19.7°).

**Haemanthidine (7).**- mp 198-199°C (lit.<sup>14</sup> 195°C), [α]<sub>D</sub> -23° (c=1, CHCl<sub>3</sub>), (lit.<sup>15</sup> -41°).

**Vittatine (8).**- mp 205-206°C (Amorphous powder), (lit.<sup>1</sup> 209-210°C). Ir ν<sup>NaCl</sup> cm<sup>-1</sup>: 3350, 2900, 1500, 1030, 930; uv λ<sup>EtOH</sup> nm: 206, 240sh, 293; ms m/z (rel.int.) 271 (M<sup>+</sup>, 100), 254 (9), 228 (25), 216 (19), 199 (70), 187 (70), 173 (23); <sup>1</sup>H nmr (CDCl<sub>3</sub>) δ 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<sub>2</sub>O), 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'); <sup>13</sup>C nmr (CDCl<sub>3</sub>) 146.4, 146.0, 137.9, 131.6, 127.8, 125.3, 107.0, 102.9 (arom., C-1, C-2), 100.9 (OCH<sub>2</sub>O), 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.<sup>2</sup> mp 248-250°C); uv λ<sup>EtOH</sup> nm: 205, 242sh, 295; ir ν<sup>NaCl</sup> cm<sup>-1</sup>: 3360, 2900, 1500, 1480, 1030, 930, 850; ms m/z (rel. int.)

287 (M<sup>+</sup>, 58), 269 (30), 243 (56), 227 (74), 225 (54), 224 (46), 209 (40), 181 (100); <sup>1</sup>H nmr (CDCl<sub>3</sub> + CD<sub>3</sub>OD) δ 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<sub>2</sub>O), 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<sub>W</sub>(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); <sup>13</sup>C nmr (CDCl<sub>3</sub> + CD<sub>3</sub>OD) δ 146.6, 146.2, 135.3, 133.2, 126.2, 125.8, 106.7, 103.2 (arom., C-1, C-2), 100.8 (OCH<sub>2</sub>O), 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.<sup>14</sup> 127-129°C), [α]<sub>D</sub> -97° (c= 0.37, EtOH), (lit.<sup>15</sup> -107°C).

**Habranthine (11).**- mp 203-205°C (Cl<sub>2</sub>CH<sub>2</sub>-MeOH), (lit.<sup>9</sup> 198-199°C); [α]<sub>D</sub> -107° (c= 0.3, EtOH), (lit.<sup>9</sup> -320°); ir ν<sup>KBr</sup> cm<sup>-1</sup>: 3460, 3400 (OH), 3010, 2920, 1620, 1510, 1280, 970; uv λ<sup>EtOH</sup> nm: 232, 285; ms m/z (rel. int.) 303 (M<sup>+</sup>, 14), 286 (5), 230 (54), 213 (27), 115 (100); <sup>1</sup>H nmr (CDCl<sub>3</sub>) δ 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<sub>W</sub>(2,4)= 1.4 Hz, H-2), 5.78 (1H, dt, J(1,2)= 10.2 Hz, J(1,3)= J<sub>W</sub>(1,4a)= 1 Hz, H-1), 5.33 (1H, ddd, J(4a,4)= 4 Hz, J(4a,4')= 2 Hz, J<sub>W</sub>(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<sub>W</sub>(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<sub>W</sub>(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.); <sup>13</sup>C nmr (CDCl<sub>3</sub>) δ 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.<sup>17</sup> 214-215°C); [α]<sub>D</sub> +138° (c= 0.47, CHCl<sub>3</sub>), (lit.<sup>17</sup> +160°); ms m/z (rel. int.) 257 (5), 125 (100), 96 (42).

**9-O-Demethylhomolycorine (13).**- mp 128-130°C (acetone-MeOH) (lit.<sup>10</sup> 138-140°C); [α]<sub>D</sub> +53° (c=0.75, CHCl<sub>3</sub>); ms m/z (rel. int.) 302 (M<sup>+</sup>+1), 192, 164, 110, 109 (100), 108, 94, 82, 81.

**Hordeanine (14).**- <sup>1</sup>H Nmr (CDCl<sub>3</sub>) δ 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<sub>2</sub>CH<sub>2</sub>-), 2.43 (6H, s, NMe<sub>2</sub>).

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