# Alkaloids from *Eucharis amazonica* (Amaryllidaceae)

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Received September 3, 2002; accepted November 25, 2002

**Thirteen alkaloids have been isolated from dried bulbs and leaves of flowering *Eucharis amazonica* (Amaryllidaceae). The alkaloids, 7-methoxyoxoassoanine, 6-*O*-methylpretazettine and apohaemanthamine, are reported for the first time from a natural source.**

**Key words** *Eucharis*; Amaryllidaceae; alkaloid; 7-methoxyoxoassoanine, 6-*O*-methylpretazettine; apohaemanthamine

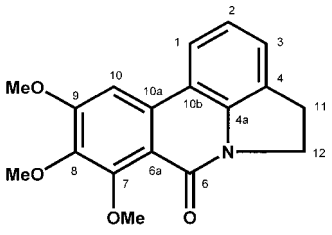
The genus *Eucharis*, also known as Amazon lily, comprises 17 species of bulbous, rain forest geophyte plants adapted to the low light conditions of the forest understory. The major center of distribution for *Eucharis* is the western Amazon basin and the adjoining lower slopes of the eastern Andean cordillera. Large-scale deforestation has proven catastrophic for these plants, and several species are probably extinct. *Eucharis amazonica* Linden ex Planchon is widely known in horticulture, although it is often confused with the sterile hybrid taxon *E. x grandiflora* Planchon & Linden.[1)] From the ethnobotanical point of view, mashed bulbs of *Eucharis* species have been used by lowland native people as poultices applied to sores and tumors. Lewis[2)] describes the use of mucilage from *Eucharis* bulbs by the Jivaro indians of Perú for treating facial blemishes and acne.

The present investigation deals with the isolation and characterization of thirteen alkaloids, from which 7-methoxyoxoassoanine, 6-*O*-methylpretazettine and apohaemanthamine are reported for the first time from a natural source.

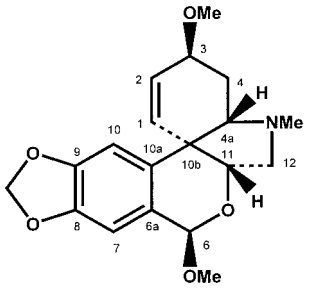
## Results and Discussion

Compound **1** (7-methoxyoxoassoanine), $C_{18}H_{17}NO_4$, is a pyrrolo[d,e]phenanthridone alkaloid belonging to the lycorine type series.[3)] The IR spectrum showed an important band at 1647 $cm^{-1}$, corresponding to the lactam. $^1$H- and $^{13}$C-NMR spectra (Table 1) were very close to those of the alkaloid oxoassoanine, isolated previously from several *Narcissus* species,[4)] and only the substitution of the aromatic proton at position 7 by a methoxyl group was noteworthy. The nuclear Overhauser effect (NOE) contour between H-1 and H-10 confirmed the substitution proposed in the aromatic ring. In the $^{13}$C-NMR spectrum, the shielding effect on the carbon C-10 in relation to other *para* unsubstituted alkaloids, such as oxoassoanine,[4)] is due to the effect of the methoxyl groups. Furthermore, the methoxyl groups flanked by two *ortho* substituents have higher $\delta$ $^{13}$C-NMR values (62.1, 61.4 ppm) than those with one *ortho* substituent (56.0 ppm). These measurements indicated that the methoxyl groups with two *ortho* substituents acquire the out-of-plane conformation, while those without this kind of substituents or with only one *ortho* substituent exist in the planar conformation.[5)]

Compound **2** (6-*O*-methylpretazettine), $C_{19}H_{23}NO_5$, showed a mass spectrum similar to that of tazettine and its fragmentation pattern was congruent with a $\beta$ configuration of the methoxyl group at the C-3 position.[6)] The $^1$H-NMR spectum (Table 2) showed: (i) three singlets at $\delta$ 6.75, 6.73, 5.56, for the two aromatic protons *para* oriented H-7 and H-10, and for the benzilic proton H-6, respectively; the assignment of the two signals belonging to the aromatic ring was carried out by two dimensional rotating frame Overhauser enhancement spectroscopy (2D ROESY) experiment (Table 3), (ii) four intensive signals at $\delta$ 5.89, 3.53, 3.41 and 2.47 corresponding to the methylenedioxy, the two methoxyl and the *N*-methyl groups, respectively, (iii) two doublet of doublets and




**1**

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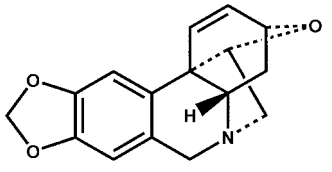


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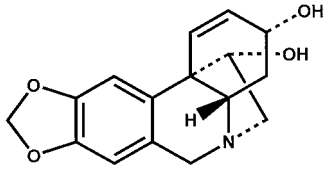


**4**

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one triplet at $\delta$ 4.23, 2.64 and 2.98 respectively, for the protons of the pyrrolidine ring, and (iv) six chemical shifts corresponding to the protons of C ring. The broad doublet at $\delta$ 5.86 and the double triplet at $\delta$ 5.51 were assigned to the olefinic protons H-2 and H-1, respectively. The multiplicity of H-4a (br s) and H-3 (m), together with the coupling constants of H-4$\beta$ ($J_{4\beta/4\alpha}$=13.5, $J_{4\beta/3}$=9.5, $J_{4\beta/4a}$=2.0 Hz) allowed us to confirm the $\beta$ configuration (pseudoequatorial) of the methoxyl group at C-3.

The $^{13}$C-NMR signals of compound **2** were assigned considering the connectivities from $^{1}$H-detected heteronuclear multiple quantum coherence (HMQC) and heteronuclear multiple bond connectivity (HMBC) spectra (Table 2). The most characteristic signals were (i) chemical shifts at $\delta$ 129.1 and 128.6 corresponding to the olefinic carbons, (ii) chemical shifts at $\delta$ 108.0 and 104.8 assignable to the C-7 and C-10 carbons of the aromatic ring, (iii) a doublet at $\delta$ 100.4 corresponding to C-6 and (iv) a singlet at $\delta$ 46.0 assignable to the spiro position C-10b.

Compound **3** (apohaemanthamine), $C_{16}H_{15}NO_3$, was recently obtained by semisynthesis from crinamine[7] and all spectroscopic data closely matches those of the natural compound.

Table 1. $^{1}$H-NMR, HMQC and HMBC Data for Compound **1**[a]

| Proton | $\delta_H$ | Correlated C-atom | |
|---|---|---|---|
| | | HMQC | HMBC |
| 1 | 7.81 d (8.0) | 119.6 d | C-4 |
| 2 | 7.18 t (8.0) | 122.9 d | C-4a, C-10b |
| 3 | 7.31 d (7.5) | 123.2 d | |
| | | 124.3 s (C-4) | |
| | | 132.3 s (C-4a) | |
| | | 156.3 s (C-6) | |
| | | 130.8 s (C-6a) | |
| | | 109.0 s (C-7) | |
| | | 150.0 s (C-8) | |
| | | 152.0 s (C-9) | |
| 10 | 7.44 s | 99.7 d | C-6, C-10b |
| | | 122.5 s (C-10a) | |
| | | 115.9 s (C-10b) | |
| 11 (2H) | 3.40 t (8.0) | 27.2 t | C-4a |
| 12 (2H) | 4.45 t (8.0) | 46.5 t | C-4a, C-11 |
| 7-OMe | 4.06 s | 62.1 q | C-7 |
| 8-OMe | 4.02 s | 61.4 q | C-8 |
| 9-OMe | 3.97 s | 56.0 q | C-9 |

*a*) Chemical shifts in ppm relationated to TMS. Coupling constants (*J*) in Hz. C-multiplicities were determined by distortionless enhancement by DEPT data.

## Experimental

**General Experimental Procedures** Melting points were uncorrected. Optical rotations were measured in a Perkin-Elmer 241 Polarimeter. Circular Dicroisms were performed in a Jasco J-700 Spectropolarimeter. IR spectra were recorded in a Perkin-Elmer 1600 FTIR series Spectrometer in dry film. Mass Spectra were measured in a Hewlett Packard 5989A Mass Spectrometer at 70 eV. $^{1}$H-, $^{13}$C-NMR, distortionless enhancement by polarization transfer (DEPT), correlation spectroscopy (COSY), HMQC, HMBC and

Table 3. COSY and ROESY Data for Compound **2**

| Proton | COSY | ROESY |
|---|---|---|
| 1 | H-2, H-3, H-4a | H-2 |
| 2 | H-1, H-3 | H-1, 3-OMe |
| 3 | H-1, H-2, H-4$\alpha$, H-4$\beta$ | H-4$\alpha$, 3-OMe |
| 4$\alpha$ | H-3, H-4$\beta$, H-4a | H-3, H-4$\beta$, H-4a |
| 4$\beta$ | H-3, H-4$\alpha$, H-4a | H-4$\alpha$, H-4a, H-10 |
| 4a | H-1, H-4$\alpha$, H-4$\beta$ | H-4$\alpha$, H-4$\beta$, H-11 |
| 6 | H-7 | H-7, 6-OMe |
| 7 | H-6 | H-6 |
| 10 | | H-4$\beta$ |
| 11 | H-12$\alpha$, H-12$\beta$ | H-4a, H-12$\alpha$, H-12$\beta$ |
| 12$\alpha$ | H-11, H-12$\beta$ | H-11, H-12$\beta$ |
| 12$\beta$ | H-11, H-12$\alpha$ | H-11, H-12$\alpha$, NMe |
| $OCH_2O$ | | |
| 3-OMe | | H-2, H-3 |
| 6-OMe | | H-6 |
| NMe | | H-12$\beta$ |

Table 2. $^{1}$H-NMR, HMQC and HMBC Data for Compound **2**[a]

| Proton | $\delta_H$ | Correlated C-atom | |
|---|---|---|---|
| | | HMQC | HMBC |
| 1 | 5.51 dt (10.0, 2.0) | 129.1 d | |
| 2 | 5.86 br d (10.5) | 128.6 d | C-4, C-10b |
| 3 | 4.14 m | 73.0 d | |
| 4$\alpha$ | 2.49 m | 30.1 t | |
| 4$\beta$ | 1.74 ddd (13.5, 9.5, 2.0) | 30.1 t | C-3 |
| 4a | 2.91 br s | 64.0 d | |
| 6 | 5.56 s | 100.4 d | C-6a, C-7, C-10a, C-11, 6-OMe |
| | | 126.6 s (C-6a) | |
| 7 | 6.75 s | 108.0 d | C-6, C-8, C-9, C-10, C-10a |
| | | 145.0 s (C-8) | |
| | | 147.0 s (C-9) | |
| 10 | 6.73 s | 104.8 d | C-6a, C-7, C-8, C-9, C-10b |
| | | 135.1 s (C-10a) | |
| | | 46.0 s (C-10b) | |
| 11 | 4.23 dd (11.0, 7.5) | 73.5 d | C-2, C-6, C-10a, C-12 |
| 12$\alpha$ | 2.98 t (10.5) | 53.9 t | C-11, NMe |
| 12$\beta$ | 2.64 dd (10.0, 7.5) | 53.9 t | C-4a, C-10b, C-11, NMe |
| $OCH_2O$ | 5.89, 5.88 2d (1.5) | 101.1 t | C-8, C-9 |
| 3-OMe | 3.41 s | 56.0 q | C-3 |
| 6-OMe | 3.53 s | 55.6 q | C-6 |
| NMe | 2.47 s | 43.3 q | C-4a, C-12 |

*a*) Chemical shifts in ppm relationated to TMS. Coupling constants (*J*) in Hz. C-multiplicities were determined by DEPT data.

ROESY spectra were recorded in a Varian VXR 500 or a Gemini 200 spectrometer, in $CDCl_3$ or $CD_3OD$. Chemical shifts are reported in units of $\delta$ (ppm) relative to the tetramethylsilane (TMS) signal and coupling constants (*J*) in Hz. Silica gel SDS 60 A CC (6—35 microns) was used for VLC. Sephadex LH-20 Pharmacia was used for gel filtration, and silica gel Alugram SIL G/$UV_{254}$ (Macherey–Nagel) for analytical (0.25 mm) and pre coated plates SIL G-25 $UV_{254}$ (Macherey–Nagel) for prepreparative (0.25 mm) TLC. Spots on chromatograms were detected under UV light (254 nm) and by Dragendorff's reagent.

**Plant Material** Bulbs and leaves of *Eucharis amazonica* were collected during the flowering period (July 1999) in Tambito Natural Reserve, El Tambo, Cauca, Colombia, at 1450 m above sea level. Samples were authenticated by Prof. Alan W. Meerow (Missouri Botanical Garden, U.S.A.) and a voucher specimen (AC-18) has been deposited in the Herbarium of the Museo de Historia Natural, Universidad del Cauca, Popayán, Colombia.

**Extraction and Isolation** Fresh leaves and bulbs of flowering *E. amazonica* plants (8.78 kg) were dried and powdered, obtaining 1.2 kg of plant material which was extracted following the usual work-up procedure[8] giving 17.3 g of brown gum. After VLC (Vacuum Liquid Chromatography) on silica gel, using *n*-hexane; *n*-hexane–AcOEt; AcOEt and AcOEt–MeOH up to 20% in MeOH, five fractions were obtained. Fraction I yielded lycorine (64 mg) as precipitate, ismine (26 mg), trisphaeridine (21 mg) and tazettine (156 mg). Fraction II by PTLC using *n*-hexane–AcOEt (70 : 30) saturated with $NH_3$ atmosphere, yielded 3-epimacronine (24 mg) and haemanthamine (16 mg). Fraction III subjected to PTLC, eluting with *n*-hexane–AcOEt (2 : 1) saturated with $NH_3$ atmosphere yielded, after final purification on Sephadex LH-20, galanthamine (81 mg) and **2** (16 mg). Fraction IV subjected to PTLC, eluting with AcOEt–MeOH (1 : 1) yielded **3** (49 mg). Finally, fraction V by PTLC, eluting twice with AcOEt–MeOH (95 : 5) saturated with $NH_3$ vapour, yielded 3-*O*-methylgalanthamine (41 mg), vittatine (42 mg), 8-*O*-demethylmaritidine (47 mg) and **1** (12 mg).

7-Methoxyoxoassoanine (**1**): White amorphous solid. High resolution (HR)-MS *m/z* 311, 1163 (Calcd $C_{18}H_{17}NO_4$ for 311, 1157). mp 245—247 °C. IR $\nu_{max}$ (film on NaCl) $cm^{-1}$: 2926, 2360, 1647, 1594, 1469, 1328, 1262, 1126, 1069, 1027. Electron impact (EI)-MS 70 eV, *m/z* (rel. int.): 311 $[M]^+$ (44), 296 (100), 268 (30), 238 (19), 182 (20), 148 (46), 125 (22), 63 (27). $^1$H-NMR (500 MHz, $CD_3OD$) and $^{13}$C-NMR (50 MHz, $CD_3OD$), see Table 1.

6-*O*-Methylpretazettine (**2**): Colourless amorphous solid. HR-MS *m/z* 345, 1584 (Calcd $C_{19}H_{23}NO_5$ for 345, 1576). mp 199—201 °C. CD $[\theta]_{244}$ −4398, $[\theta]_{296}$ +4524. IR $\nu_{max}$ (film on NaCl) $cm^{-1}$: 2933, 1502, 1482, 1372, 1356, 1257, 1234, 1186, 1080, 1034, 990, 946, 900, 866, 824, 755. EI-MS 70 eV, *m/z* (rel. int.): 345 $[M]^+$ (21), 330 (17), 314 (11), 262 (17), 261 (100), 239 (23), 230 (13), 228 (23), 225 (18), 201 (19), 115 (14), 74 (13), 70 (21). $^1$H-NMR (500 MHz, $CD_3OD$) and $^{13}$C-NMR (50 MHz, $CD_3OD$), see Tables 2 and 3.

Apohaemanthamine (**3**): Colourless amorphous solid. HR-MS *m/z* 269, 1056 (Calcd $C_{18}H_{17}NO_4$ for 269, 1052). mp 145—147 °C. $[\alpha]_D^{22}$ +198° (*c*=0.63; $CHCl_3$). CD $[\theta]_{248}$ −1605, $[\theta]_{297}$ +2122. IR $\nu_{max}$ (film on NaCl) $cm^{-1}$: 2933, 1613, 1504, 1482, 1384, 1322, 1252, 1232, 1149, 1087, 1036, 989, 934, 933, 867, 820, 748, 721, 694. $^1$H-NMR (500 MHz, $CD_3OD$) and $^{13}$C-NMR (50 MHz, $CD_3OD$) in agrement with Campbell *et al.*[7]

Lycorine,[9] ismine,[10] trisphaeridine,[10] tazettine,[11] 3-epimacronine,[3] galanthamine,[3] 3-*O*-methylgalanthamine,[12] haemanthamine,[3] vittatine,[13] and 8-*O*-demethylmaritidine,[3] were identified by comparing their chromatographic and spectroscopic properties (TLC, $[\alpha]_D$, circular dichroism (CD), IR, MS, $^1$H- and $^{13}$C-NMR) with those of authentic samples obtained from other plant sources.

**Conversion of Compound 3 in 4** Apohaemanthamine (**3**) (10 mg) was dissolved in 2 N $H_2SO_4$ (2 ml) and heated with reflux at 105 °C for 25 h; the reaction was controlled by TLC, eluting with AcOEt–MeOH (1 : 1), and then cooled, filtered, and extracted with $CHCl_3$ (3×25 ml). The organic phase was basified with 2 N NaOH (2×10 ml) and finally anhydrous $Na_2SO_4$ was added. The dried extract was concentrated to give hamayne (**4**) (6 mg), which was identified by a comparison of their physical characteristics and spectroscopic data with an authentic sample.[14]

**Aknowledgements** The authors are very much indebted to the Comissionat per a Universitats i Recerca, Generalitat de Catalunya for giving financial support to this project. We also thank Prof. Alan W. Meerow for the authentication of the plant material.

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