

Alkaloids from *Eucharis amazonica* (Amaryllidaceae)

Fabio CABEZAS,^a Arnaldo RAMÍREZ,^b Francesc VILADOMAT,^c Carles CODINA,^c and Jaume BASTIDA^{*,c}

^aDepartamento de Química, Grupo de Estudios Ambientales, Universidad del Cauca; Popayán, Colombia:

^bDepartamento de Química, Universidad del Valle; Cali, Colombia: and ^cDepartamento de Productos Naturales, Facultad de Farmacia, Universidad de Barcelona; Avda. Diagonal 643, E-08028 Barcelona, Spain.

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-methoxyoxoasoanine, 6-*O*-methylpretazettine and apohaemanthamine, are reported for the first time from a natural source.

Key words *Eucharis*; Amaryllidaceae; alkaloid; 7-methoxyoxoasoanine, 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. Lewis²⁾ describes the use of mucilage from *Eucharis* bulbs by the Jivaro indians of Perú for treating facial blemishes and acne.

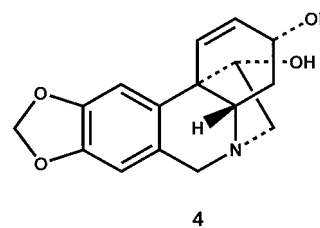
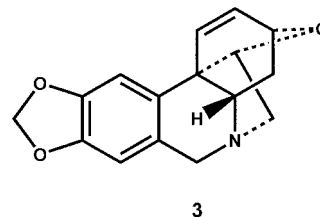
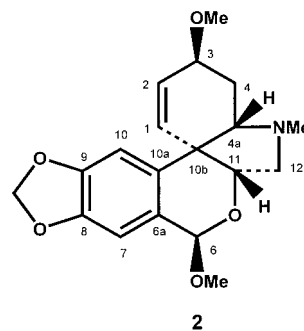
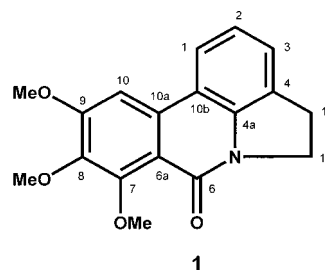
The present investigation deals with the isolation and characterization of thirteen alkaloids, from which 7-methoxyoxoasoanine, 6-*O*-methylpretazettine and apohaemanthamine are reported for the first time from a natural source.

Results and Discussion

Compound **1** (7-methoxyoxoasoanine), C₁₈H₁₇NO₄, 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. ¹H- and ¹³C-NMR spectra (Table 1) were very close to those of the alkaloid oxoasoanine, 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 ¹³C-NMR spectrum, the shielding effect on the carbon C-10 in relation to other *para* unsubstituted alkaloids, such as oxoasoanine,⁴⁾ is due to the effect of the methoxyl groups. Furthermore, the methoxyl groups flanked by two *ortho* substituents have higher δ ¹³C-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), C₁₉H₂₃NO₅, showed a mass spectrum similar to that of tazettine and its fragmentation pattern was congruent with a β configuration of the methoxyl group at the C-3 position.⁶⁾ The ¹H-NMR spectrum (Table 2) showed: (i) three singlets at δ 6.75, 6.73, 5.56, for

the two aromatic protons *para* oriented H-7 and H-10, and for the benzylic 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 δ 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



* To whom correspondence should be addressed. e-mail: bastida@farmacia.far.ub.es

one triplet at δ 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 δ 5.86 and the double triplet at δ 5.51 were assigned to the olefinic protons H-2 and H-1, respectively. The multiplicity of H-4 α (br s) and H-3 (m), together with the coupling constants of H-4 β ($J_{4\beta/4\alpha}=13.5$, $J_{4\beta/3}=9.5$, $J_{4\beta/4a}=2.0$ Hz) allowed us to confirm the β configuration (pseudoequatorial) of the methoxyl group at C-3.

The ^{13}C -NMR signals of compound **2** were assigned considering the connectivities from ^1H -detected heteronuclear multiple quantum coherence (HMQC) and heteronuclear multiple bond connectivity (HMBC) spectra (Table 2). The

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

Proton	δ_{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 related to TMS. Coupling constants (J) in Hz. C-multiplicities were determined by distortionless enhancement by DEPT data.

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

Proton	δ_{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 α	2.49 m	30.1 t	
4 β	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 α	2.98 t (10.5)	53.9 t	C-11, NMe
12 β	2.64 dd (10.0, 7.5)	53.9 t	C-4a, C-10b, C-11, NMe
OCH ₂ O	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 related to TMS. Coupling constants (J) in Hz. C-multiplicities were determined by DEPT data.

most characteristic signals were (i) chemical shifts at δ 129.1 and 128.6 corresponding to the olefinic carbons, (ii) chemical shifts at δ 108.0 and 104.8 assignable to the C-7 and C-10 carbons of the aromatic ring, (iii) a doublet at δ 100.4 corresponding to C-6 and (iv) a singlet at δ 46.0 assignable to the spiro position C-10b.

Compound **3** (apohaemanthamine), $\text{C}_{16}\text{H}_{15}\text{NO}_3$, was recently obtained by semisynthesis from crinamine⁷⁾ and all spectroscopic data closely matches those of the natural compound.

Experimental

General Experimental Procedures Melting points were uncorrected. Optical rotations were measured in a Perkin-Elmer 241 Polarimeter. Circular Dichroisms 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. ^1H -, ^{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 α , H-4 β	H-4 α , 3-OMe
4 α	H-3, H-4 β , H-4a	H-3, H-4 β , H-4a
4 β	H-3, H-4 α , H-4a	H-4 α , H-4a, H-10
4a	H-1, H-4 α , H-4 β	H-4 α , H-4 β , H-11
6	H-7	H-7, 6-OMe
7	H-6	H-6
10		H-4 β
11	H-12 α , H-12 β	H-4a, H-12 α , H-12 β
12 α	H-11, H-12 β	H-11, H-12 β
12 β	H-11, H-12 α	H-11, H-12 α , NMe
OCH ₂ O		
3-OMe		H-2, H-3
6-OMe		H-6
NMe		H-12 β

ROESY spectra were recorded in a Varian VXR 500 or a Gemini 200 spectrometer, in CDCl₃ or CD₃OD. Chemical shifts are reported in units of δ (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/UV₂₅₄ (Macherey–Nagel) for analytical (0.25 mm) and pre coated plates SIL G-25 UV₂₅₄ (Macherey–Nagel) for preparative (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 procedure⁸⁾ 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 NH₃ atmosphere, yielded 3-epimacronine (24 mg) and haemanthamine (16 mg). Fraction III subjected to PTLC, eluting with *n*-hexane–AcOEt (2:1) saturated with NH₃ 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 NH₃ vapour, yielded 3-*O*-methylgalanthamine (41 mg), vittatine (42 mg), 8-*O*-demethylmaritidine (47 mg) and **1** (12 mg).

7-Methoxyoxoasoanine (**1**): White amorphous solid. High resolution (HR)-MS m/z 311, 1163 (Calcd C₁₈H₁₇NO₄ for 311, 1157). mp 245–247 °C. IR ν_{\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). ¹H-NMR (500 MHz, CD₃OD) and ¹³C-NMR (50 MHz, CD₃OD), see Table 1.

6-*O*-Methylpretazettine (**2**): Colourless amorphous solid. HR-MS m/z 345, 1584 (Calcd C₁₉H₂₃NO₅ for 345, 1576). mp 199–201 °C. CD [θ]₂₄₄ –4398, [θ]₂₉₆ +4524. IR ν_{\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). ¹H-NMR (500 MHz, CD₃OD) and ¹³C-NMR (50 MHz, CD₃OD), see Tables 2 and 3.

Apoaemanthamine (**3**): Colourless amorphous solid. HR-MS m/z 269, 1056 (Calcd C₁₈H₁₇NO₄ for 269, 1052). mp 145–147 °C. [α]_D²² +198° ($c=0.63$; CHCl₃). CD [θ]₂₄₈ –1605, [θ]₂₉₇ +2122. IR ν_{\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. ¹H-NMR (500 MHz, CD₃OD) and ¹³C-NMR (50 MHz, CD₃OD) in agreement 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, [α]_D, circular dichroism (CD), IR, MS, ¹H- and ¹³C-NMR) with those of authentic samples obtained from other plant sources.

Conversion of Compound 3 in 4 Apoaemanthamine (**3**) (10 mg) was dissolved in 2 N H₂SO₄ (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 CHCl₃ (3×25 ml). The organic phase was basified with 2 N NaOH (2×10 ml) and finally anhydrous Na₂SO₄ 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⁾

Acknowledgements The authors are very much indebted to the Comissió 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.

References

- 1) Meerow A. W., *Ann. Missouri Bot. Gard.*, **76**, 136–220 (1989).
- 2) Lewis W. H., *Discovery*, **17**, 2–6 (1986).
- 3) Bastida J., Viladomat F., Codina C., "Studies in Natural Product Chemistry. Narcissus Alkaloids," Vol. 20, ed. by Atta-ur-Rahman, Elsevier Science, Amsterdam, 1998, pp. 323–405.
- 4) Llabrés J. M., Viladomat F., Bastida J., Codina C., Rubiralta M., *Phytochemistry*, **25**, 2637–2638 (1986).
- 5) Knittel J. J., Makriyannis A., *J. Med. Chem.*, **24**, 906–909 (1981).
- 6) Duffield A. M., Aplin R. T., Budzikiewicz H., Djerassi C., Murphy C. F., Wildman W. C., *J. Am. Chem. Soc.*, **87**, 4902–4912 (1965).
- 7) Campbell W. E., Nair J. J., Gammon D. W., Codina C., Bastida J., Viladomat F., Smith P. J., Albrecht C. F., *Phytochemistry*, **53**, 587–591 (2000).
- 8) Nair J. J., Machocho A. K., Campbell W. E., Brun R., Viladomat F., Codina C., Bastida J., *Phytochemistry*, **54**, 945–950 (2000).
- 9) Likhitvitayawuid K., Angerhofer C. K., Chai H., Pezzuto J. M., Cordell G. A., Ruangrunsi N., *J. Nat. Prod.*, **56**, 1331–1338 (1993).
- 10) Suau R., Gómez A. I., Rico R., *Phytochemistry*, **29**, 1710–1712 (1990).
- 11) Pham L. H., Gründemann E., Wagner J., Bartozek M., Döpke W., *Phytochemistry*, **51**, 327–332 (1999).
- 12) Herrera M. R., Machocho A. K., Nair J. J., Campbell W. E., Brun R., Viladomat F., Codina C., Bastida J., *Fitoterapia*, **72**, 444–448 (2001).
- 13) Kreh M., Matusch R., *Phytochemistry*, **38**, 1533–1535 (1995).
- 14) Viladomat F., Bastida J., Codina C., Campbell W. E., Mathee S., *Phytochemistry*, **35**, 809–812 (1994).