Pancratium canariense as an Important Source of Amaryllidaceae Alkaloids

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Four new alkaloids (**1**−**4**) have been isolated from a methanolic extract of bulbs of *Pancratium canariense*, together with 12 known alkaloids (**5**−**16**). The structures of the new alkaloids were determined by extensive 1D and 2D NMR spectroscopic studies and X-ray diffraction.

The Amaryllidaceae family, a group of monocotyledonous species, consists of about 1100 species in 85 genera, is widely distributed throughout the tropics and warm temperature regions of the world,[1] and is one of the 20 most important alkaloid-containing plant families.[2] Amaryllidaceae alkaloids, such as galanthamine and pancratistatin, have attracted considerable interest due to their pharmacological activities.[3−7] Galanthamine (commercially available as Reminyl) is a centrally acting reversible inhibitor of acetylcholinoesterase (AchE), which significantly enhances cognitive functions of Alzheimer's patients.[8] Pancratistatin[9] induces apoptosis in several cancer cell lines.[10] Biosynthesis of Amaryllidaceae alkaloids involves intramolecular oxidative coupling of norbelladines derived from L-phenylalanine and L-tyrosine and, in this sense, are considered to be members of the large group of isoquinoline alkaloids.[11]

According to a recent classification, the genus *Pancratium* is represented by 18 species.[12] In Europe the species are found in the Mediterranean area. In the Canary Islands there is an endemic species, *Pancratium canariense* Ker-Gawl (Amaryllidaceae), used as an ornamental plant because of its white flowers.[13] The important bioactivities of the Amaryllidaceae alkaloids, together with the absence of any previous phytochemical work on *P. canariense*, encouraged us to examine this species. In this paper we describe the isolation and structural elucidation of four new alkaloids (**1**−**4**), which were isolated together with 12 known alkaloids (**5**−**16**). The known alkaloids were identified by comparison with published spectroscopic and physical data as hippeastrine (**5**),[14] pancracine (**6**),[15,16] 6-*O*-methylhaemanthidine (**7**),[17] vittatine (**8**),[18] (+)-8-*O*-demethylmaritidine (**9**),[19] haemanthamine (**10**),[20] haemanthidine (**11**),[21,22] 11-hydroxyvittatine (**12**),[23] tazettine (**13**),[24,25] ungiminorine (**14**),[26] 1-*O*-acetyl-8-norpluviine (**15**),[27] and lycorine (**16**).[28]

Results and Discussion

Repeated chromatography of a methanol extract from bulbs of *P. canariense* on silica gel yielded four new compounds (**1**−**4**) and the known compounds **5**−**16**. Compound **1** was isolated as an amorphous, white solid with positive optical activity ($[\alpha]_D^{20}$ +40.0, *c* 0.35, MeOH) and molecular formula $C_{17}H_{17}NO_6$ as determined by HREIMS and ^{13}C-DEPT experiments. Preliminary spectroscopic data showed that **1** was related to hippeastrine **5**.[14] The ^{1}H NMR spectrum exhibited singlets at δ 7.36 and 7.29 assigned to aromatic protons H-7 and H-10, a broad singlet at δ 6.10 (2H) attributable to a methylendioxy group, and a singlet at δ 5.70 for the olefinic proton H-3. The ^{1}H NMR spectrum also displayed singlets at δ 4.48 and 4.25, corresponding to H-1 and H-2, and a singlet at δ 1.99 (3H) characteristic of an NMe group. The main differences of the ^{1}H NMR spectrum of **1** with respect to that of hippeastrine (**5**) were the absence of a doublet corresponding to H-10b and the presence of H-4a as a singlet at δ 2.86 (1H) instead of a doublet at δ 2.63 (J = 9.4 Hz). These data together with the molecular formula and the presence of a quaternary carbon at δ 67.5 in the ^{13}C NMR and DEPT spectra indicated the existence of an OH group at C-10b. This conclusion was corroborated by the HSQC and HMBC spectra; thus the C-10b-OH was confirmed by the three-bond correlations of H-10 and H-2 to C-10b. The relative configuration of **1** was determined by X-ray analysis from suitable crystals of **1**, obtained from a mixture of DCM−MeOH, 1:1 (Figure 1). The result showed that **1** had the same relative configuration of hippeastrine (**5**). However, the absolute configuration of **5** has not been reported previously, although circular dichroism[26,27] and X-ray[28] studies of homolycorine-type alkaloids have been reported. Since compound **5** was isolated in quantity, we decided to determine its absolute configuration and then correlate it to that of the new compound **1**. Riguera's method[29,30] was applied in order to establish the absolute configuration of **5**.

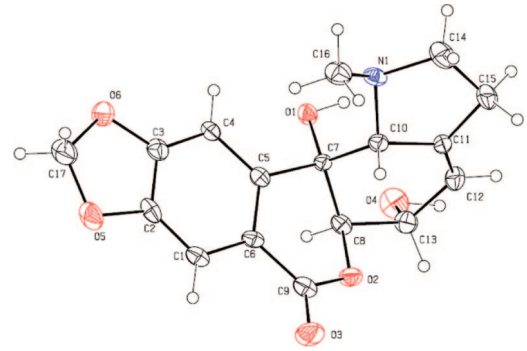

Figure 1. View of the structure of one of the two independent molecules of **1**. Ellipsoids are drawn at the 30% probability level, and H atoms are shown as spheres of arbitrary radii.

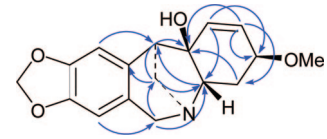

Figure 2. Selected HMBC correlations for compound **2**.

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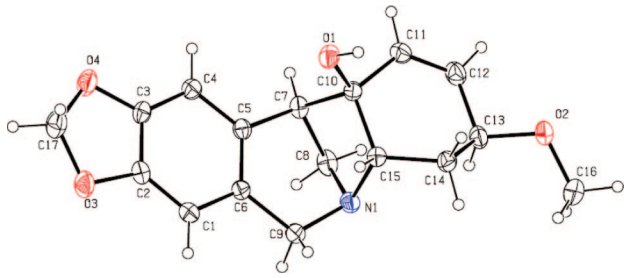


Figure 3. View of the molecular structure of **2**. Ellipsoids are drawn at the 30% probability level, and H atoms are shown as spheres of arbitrary radii.

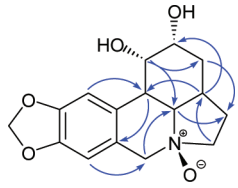


Figure 4. Selected HMBC correlations for compound **4**.

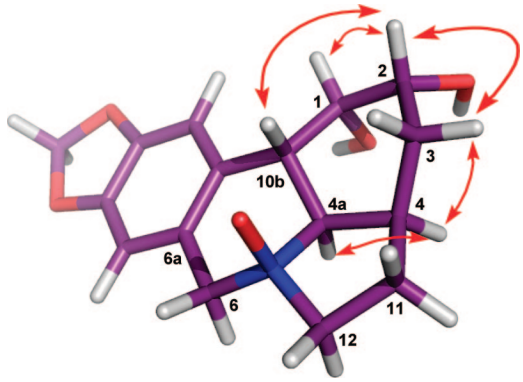


Figure 5. Observed NOE effects for compound **4**.

Esterification of the secondary OH of **5** with (*R*)-(−)-phenylmethoxyacetic acid led to the corresponding ester in 44% yield. The ^{1}H NMR (CD_3CN) spectrum of the esterified compound showed signals for H-1 at δ 4.21 and for H-3 at δ 5.53. After addition of 100 mg of $Ba(ClO_4)_2$ to the NMR tube, the resulting ^{1}H NMR spectrum showed a downfield shift for H-3 (δ 5.58), while H-1 appeared upfield (δ 4.18). Application of the corresponding rules for Riguera's method[29,30] indicated that these shifts were consistent with a 2*S* configuration. Thus, the absolute configuration of **5** is 1*S*,2*S*,4a*S*,10b*S*-hippeastrine.

Taking into account the above data and the fact that **1** is biogenetically related to **5**, the structure of **1** was assigned as (1*R*,2*S*,4a*R*,10b*R*)-10b-hydroxyhippeastrine, which we named pancratinine A.

Compound **2** was isolated as an amorphous, white solid with positive optical activity ($[\alpha]_D^{20}$ +1.9, *c* 0.7, MeOH), and its molecular formula was established by HREIMS as $C_{17}H_{19}NO_4$. The IR spectrum showed signals for OH (3100 cm^{-1}) and aromatic groups (1601 cm^{-1}). The ^{1}H NMR spectrum exhibited signals characteristic of alkaloids with a montanine skeleton. It displayed resonances for two aromatic protons as singlets (δ 6.63 and 6.55) assignable to H-10 and H-7, a singlet at δ 5.94 (2H) corresponding to a methylendioxy group, and the AB system typical for this class of alkaloids corresponding to H-12 [δ 3.02 d (1H, J = 11.8 Hz), 2.88 d (1H, J = 11.8 Hz)]. The main differences with respect to the also isolated pancracine (**6**) were the presence of an additional AB system attributable to two olefinic protons [δ 5.77 d (1H, J = 10.4 Hz, H-1), 6.15 d (1H, J = 10.4 Hz, H-2)] and the existence of a methoxy group at δ 3.43 (s, 3H). The ^{13}C NMR and DEPT spectra indicated the presence of a tertiary OH group with the existence of a quaternary carbon signal at δ 81.8. The positions of these groups were established by the ^{1}H−^{13}C long-range correlations detected in the HMBC spectrum (Figure 2). The β-orientation for the methoxy group was established on the basis of the NOE effect

Table 2. ^{13}C NMR[a] Data (δ) of Compounds **1**−**4** at 75 MHz

C	1	2	3	4
1	82.9 d	134.6 d	135.0 d	68.1 d
2	67.2 d	130.3 d	130.8 d	67.5 d
3	118.2 d	71.4 d	62.0 d	27.3 t
4	143.8 s	29.3 t	32.6 t	34.5 d
4a	69.7 d	67.7 d	67.0 d	79.9 d
4b		81.8 s	80.9 s	
6	164.1 s	60.3 t	60.4 t	68.7 t
6a	116.1 s	124.0 s	123.1 s	123.6 s
7	108.1 d	106.9 d	105.8 d	107.7 d
8	147.8 s	146.2 s	145.9 s	146.2 s
9	152.5 s	147.6 s	147.0 s	147.9 s
10	104.8 d	109.7 d	109.5 d	104.8 d
10a	141.0 s	127.7 s	129.4 s	130.3 s
10b	67.5 s			38.5 d
11	27.5 t	48.8 d	48.7 d	28.3 t
12	55.5 t	53.7 t	54.1 t	73.9 t
OCH_2O	102.2 t	100.9 t	100.5 t	100.8 t
NMe	42.6 q			
OMe		56.1 q		

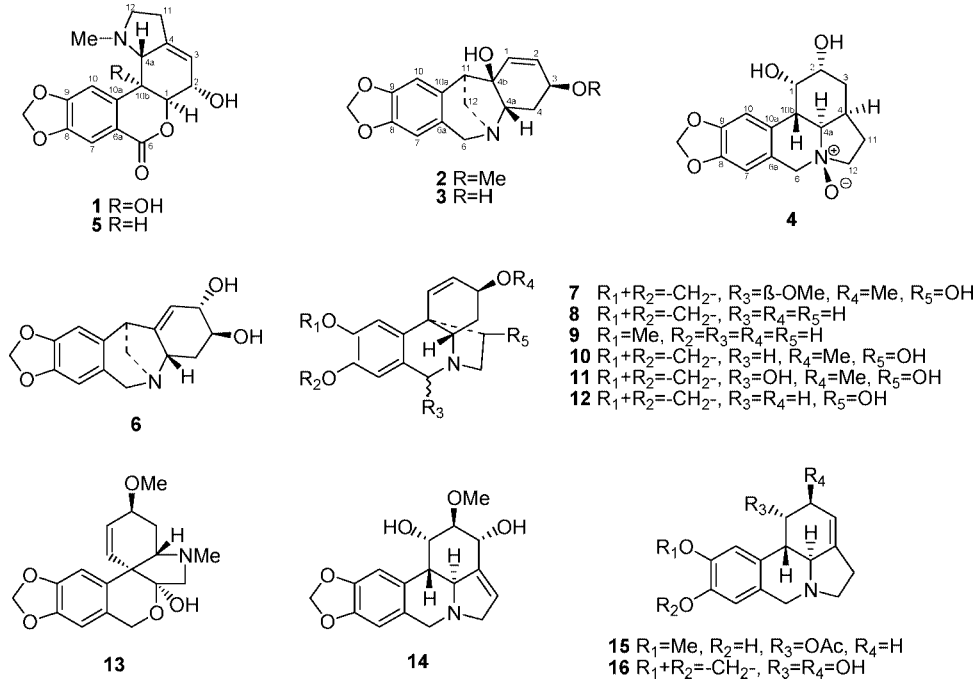
[a] Spectra recorded in MeOD except for **2** ($CDCl_3$).

Table 1. ^{1}H NMR[a] Data of Compounds **1**−**4** at 300 MHz

H	1	2	3	4
1	4.48, s	5.77, d (10.4)	5.74, d (10.3)	4.47, s
2	4.25, s	6.15, d (10.4)	6.01, d (10.3)	3.87, dd (3.8; 11.3)
3	5.70, s	3.97, m	4.30, m	2.16, m
				1.94, m
4		2.40, d (9.5)	2.32, d br (13.0)	3.12, m
		1.57, dt (4.4; 12.6)	1.62, dt (4.3; 11.0)	
4a	2.86, s	2.96, s	3.12, s	3.44, dd (7.1; 12.1)
6		4.32, d (16.6)	4.33, d (16.2)	4.53, d (14.1)
		3.86, d (16.6)	3.98, d (16.2)	4.41, d (14.1)
7	7.36, s	6.55, s	6.58, s	6.82, s
10	7.29, s	6.63, s	6.62, s	7.06, s
10b				2.78, d (12.1)
11	2.53, m	2.67, s	2.75, d (2.3)	2.16, m
12	3.19, t (8.5)	3.02, d (11.8)	3.06, dd (2.3; 11.5)	4.08, m
	2.40, dd (8.5; 16.4)	2.88, d (11.8)	2.95, d (11.5)	3.68, dd (11.1; 19.0)
OCH_2O	6.10, bs	5.94, s	5.89, s	5.95, bs
NMe	1.99, s			
OMe		3.43, s		

[a] Spectra recorded in MeOD except for **2** ($CDCl_3$).

Chart 1



observed between H-3 and H-12. In order to verify the relative configuration of compound **2**, crystals were obtained from a 1:1 mixture of DCM−MeOH and an X-ray analysis was performed. Figure 3 shows the final structure for compound **2**. Compound **2** is biogenetically related to pancracine (**6**), an alkaloid with known absolute configuration.[31] Assuming the same configuration at C-3, C-4a, and C-11, and using X-ray data for C-4b, **2** has the configuration *S* at C-3 and C-4a and *R* at C-11 and C-4b. This compound was named pancratinine B.

Pancratinine C (**3**) had the molecular formula $C_{16}H_{17}NO_4$ as determined by HRMS. Its ^{1}H NMR and ^{13}C NMR spectra were very similar to those of pancratinine B (**2**). The main differences were the absence of the signal corresponding to the methoxy group and the shift of H-3, which appeared 0.33 ppm downfield from those in compound **2**. The data pointed to the presence of an OH group at C-3, which presents a β-disposition on the basis of the NOE effect detected between H-3 and H-12. It is assumed, by biogenetic considerations, that **3** has the same absolute configuration as **2**.

Pancratinine D (**4**) was isolated as an amorphous, white solid ($[\alpha]_D^{20}$ −2.3, *c* 1.0). It presented the molecular formula $C_{16}H_{19}NO_5$ in HRFABMS. Its ^{1}H NMR spectrum showed signals typical of a lycorine-type alkaloid:[26] two *para*-oriented protons, a methylendioxy group, an AB system [δ 4.53 d (J = 14.1 Hz, H-6α), δ 4.41 d (J = 14.1 Hz, H-6β)] due to the benzylic CH_2, and two protons geminal to two secondary OH groups [δ 4.47 s (H-1), δ 3.87 dd (J = 3.8, 11.3 Hz)]. The typical olefinic proton (H-3) was not observed. The ^{13}C NMR and DEPT spectra had signals for two CH_2 groups at δ 73.9 and 68.7 and for one CH at δ 79.9 assignable to C-12, C-6, and C-4a, respectively, but these signals appeared more downfield than those observed in other lycorine-type alkaloids such as 1-*O*-acetyl-8-norpluviine (**15**) or lycorine (**16**). These unusual shifts, together with the information obtained from the molecular formula and the ^{13}C NMR and DEPT spectra, suggested that **4** had an N-oxide function and that the usual C3−C4 double bond was absent. The locations of all functionalities present in **4** were confirmed by analysis of COSY, HSQC, and HMBC spectra. Figure 4 shows the key HMBC long-range correlations. Analysis of the coupling constants for H-1 and H-2 and the NOE effects (Figure 5) detected in the ROESY spectrum established the relative configuration for the OH groups at C-1 and C-2. A NOE effect between H-2 and H-10b was observed, suggesting α-disposition of the OH at C-2, and between H-1 and H-2, indicating that the OH group at C-1 also had an α-disposition. The α-disposition of H-4 was determined by the NOE effect between H-4 and H-4a. Figure 5 shows some NOE effects observed for **4**. These data allowed us to establish the structure of **4** as the N-oxide of zephyranthine, an alkaloid isolated previously form *Cyranthus elatus*.[35] We also suggested a β-disposition for the N-oxide on the basis of molecular mechanics calculations,[36] which determined that the β-N-oxide is 5.4 kilocalories per mole more stable than the α-N-oxide. There are only eight previously known Amaryllidaceae alkaloid N-oxides: hippeastrine *N*-oxide,[37] galanthamine *N*-oxide, lycoramine *N*-oxide, sanguinine *N*-oxide,[38] *O*-methyllycorenine *N*-oxide, homolycorine *N*-oxide, ungiminorine *N*-oxide,[39] and 9-*O*-demethylhomolycorine *N*-oxide.[40]

In conclusion, *P. canariense* is a rich source of Amaryllidaceae alkaloids. Alkaloids **1**−**4** had structures not described previously in the chemical literature, and alkaloids belonging to five of the seven main Amaryllidaceae skeletons have been isolated from this species. Three of the 16 alkaloids (hippeastrine, haemanthamine, and lycorine) were isolated in amounts that will allow preparation of new derivatives.

Experimental Section

General Experimental Procedures. Melting points were measured with a Büchi melting point B-540 apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer 241 automatic polarimeter. UV spectra were collected in absolute EtOH on a JASCO V-560 spectrophotometer. IR spectra were obtained using a Bruker IFS28/55 spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ or MeOD at 300 and 75 MHz, respectively, with TMS as the internal reference. 2D NMR experiments were conducted on a Bruker WP-400 SY NMR spectrometer at 400 MHz. High- and low-resolution mass spectra were obtained on a VG Autospec spectrometer. Macherey-Nagel polygram Sil G/UV254 and Analtech silica gel GF preparative layer with UV254 were used for TLC. Silica gel (0.2−0.63 mm) was used for column chromatography. Silica gel 60 (Merck) was used on a Harrison Research 7924T Chromatotron.

Plant Material. Fresh bulbs of *Pancratium canariense* were collected in the northeast of Tenerife (Igueste de San Andrés) in March 2007. A voucher specimen is on file TFC 48723 at the Herbarium of Botánica of the Departamento de Biología Vegetal, Universidad de La Laguna.

Extraction and Isolation. Fresh bulbs (8.5 kg) of *P. canariense* were chopped and macerated with MeOH (25 L) for two weeks at room temperature. The bulbs were filtered, dried, and powdered for a second extraction, using a Soxhlet apparatus with MeOH. Both extracts were collected, concentrated to a volume of 400 mL, dissolved in 10% HCl, and left overnight with stirring. The aqueous phase was extracted with CH_2Cl_2 and made alkaline (pH 10) by addition of 20% NH_4OH. After extraction with DCM and removal of the organic solvent, 17 g of a brown residue was obtained. Purification on silica gel, using a CH_2Cl_2−MeOH step gradient (0−100%), and further purification by preparative TLC or chromatotron, using CH_2Cl_2−MeOH or butanol−acetic acid−H_2O as eluants, afforded compounds **1**−**16**: pancratinine A (**1**; 4.5 mg), pancratinine B (**2**; 22 mg), pancratinine C (**3**; 6 mg), pancratinine D (**4**; 12 mg), hippeastrine (**5**; 1.35 g), pancracine (**6**; 17 mg), 6-*O*-methylhaemanthidine (**7**; 7 mg), vittatine (**8**; 8 mg), (+)-8-*O*-demethylmaritidine (**9**; 18 mg), haemanthamine (**10**; 2.01 g), haemanthidine (**11**; 36 mg), 11-hydroxyvittatine (**12**; 123 mg), tazettine (**13**; 3 mg), ungiminorine (**14**; 25 mg), 1-*O*-acetyl-8-norpluviine (**15**; 2 mg), and lycorine (**16**; 2.6 g).

Pancratinine A (1): amorphous, white solid; mp 294−297 °C; $[\alpha]_D^{20}$ +40.0 (*c* 0.35, MeOH); UV (EtOH) λ_{max} (log ϵ) 307 (3.29), 268 (3.30), 226 (3.92) nm; IR (neat) ν_{max} 3410, 2923, 2854, 1721, 1615, 1555, 1503, 1470, 1411, 1275, 1119, 1034, 931, 883, 780, 730, 653 cm^{-1}; ^1H NMR (MeOD) see Table 1; ^{13}C NMR (MeOD) see Table 2; EIMS *m/z* 331 $[M]^+$ (2), 313 (64), 295 (100), 280 (9), 252 (2), 231 (5), 192 (7), 180 (9), 148 (16); HREIMS *m/z* 331.1044 (calcd for $C_{17}H_{17}NO_6$, 331.1056).

Pancratinine B (2): amorphous, white solid; mp 268−271 °C; $[\alpha]_D^{20}$ +1.9 (*c* 0.7, MeOH); UV (EtOH) λ_{max} (log ϵ) 291 (2.59), 238 (2.52) nm; IR (neat) ν_{max} 3101, 2925, 2873, 1568, 1481, 1334, 1232, 1203, 1090, 1035, 1006, 935, 848, 817, 739, 620 cm^{-1};^1H NMR ($CDCl_3$) see Table 1; ^{13}C NMR ($CDCl_3$) see Table 2; EIMS *m/z* 301 $[M]^+$ (100), 286 (26), 270 (18), 245 (4), 229 (6), 203 (36), 188 (21), 176 (47), 161 (27), 148 (21); HREIMS *m/z* 301.1316 (calcd for $C_{17}H_{19}NO_4$, 301.1314).

Pancratinine C (3): amorphous, white solid; $[\alpha]_D^{20}$ −1.8 (*c* 0.5, MeOH); UV (EtOH) λ_{max} (log ϵ) 292 (3.02), 239 (2.98) nm; IR (neat) ν_{max} 3439, 2955, 1645, 1558, 1483, 1415, 1236, 1095, 1035, 930, 731 cm^{-1}; ^1H NMR (MeOD) see Table 1; ^{13}C NMR (MeOD) see Table 2; EIMS *m/z* 287 $[M]^+$ (63), 270 (6), 244 (8), 226 (3), 203 (54), 188 (47), 176 (100), 161 (54), 148 (48); HREIMS *m/z* 287.1157 (calcd for $C_{16}H_{17}NO_4$, 287.1158).

Pancratinine D (4): amorphous, white solid; $[\alpha]_D^{20}$ −2.3 (*c* 1.0, MeOH); UV (EtOH) λ_{max} (log ϵ) 290 (2.60), 204 (3.45) nm; IR (neat) ν_{max} 3412, 2926, 1646, 1556, 1414, 1096, 930, 654 cm^{-1}; ^1H NMR (MeOD) see Table 1; ^{13}C NMR (MeOD) see Table 2; FABMS *m/z* 306 $[M + 1]^+$ (51), 305 (3), 288 (40), 285 (100), 270 (6), 242 (4), 226 (6), 201 (11), 176 (97), 154 (58); HRFABMS *m/z* 305.1277 (calcd for $C_{16}H_{19}NO_5$, 305.1263).

Preparation of 2-Phenylmethoxyacetylhippeastrine. A 58 mg (0.184 mmol) amount of **5** dissolved in 5 mL of dry dichloromethane was treated with 62 mg (2 equiv) of (*R*)-(−)-phenylmethoxyacetic acid and 76 mg (2 equiv) of 1,3-dicyclohexylcarbodiimide. The reaction mixture was refluxed for 12 h. Then, the solvent was evaporated and the residue purified by Sephadex chromatography, to obtain 37 mg of the corresponding ester (44%): ^1H NMR (CD_3CN) δ 7.31 (6H, br s, H-7, H-4′, H-5′, H-6′), 6.87 (1H, s, H-10), 6.06 (1H, br s, OCH_2O), 6.04 (1H, br s, OCH_2O), 5.53 (1H, s, H-3), 5.33 (1H, s, H-2), 4.81 (1H, s, H-2′), 4.21 (1H, s, H-1), 3.34 (3H, s, OMe), 3.02 (1H, m, H-12), 2.48 (4H, m, H-4a, H-10b, H-11), 2.16 (4H, br s, H-12, NMe); ^1H NMR [CD_3CN + 100 mg $Ba(ClO_4)_2$] δ 7.34 (6H, br s, H-7, H-4′, H-5′, H-6′), 6.84 (1H, s, H-10), 6.08 (1H, br s, OCH_2O), 6.05 (1H, br s, OCH_2O), 5.58 (1H, s, H-3), 5.34 (1H, s, H-2), 4.96 (1H, s, H-2′), 4.18 (1H, s, H-1), 3.32 (3H, s, OMe), 3.03 (1H, m, H-12), 2.47 (4H, m, H-4a, H-10b, H-11), 2.23 (1H, br d, J = 9.2 Hz, H-12), 2.19 (3H, s, NMe).

X-ray Crystal Structure Analysis. Intensity data for both compounds were collected at room temperature on an Enraf-Nonius Kappa CCD diffractometer with Mo Kα radiation (λ = 0.71707 Å). Cell refinement and data reduction were performed with the programs COLLECT[41] and DENZO.[42] The structures were solved by direct methods.[43] Refinements were performed by full-matrix least-squares with anisotropic displacement parameters for all non-hydrogen atoms. The hydrogen atoms were placed at calculated positions and refined with a riding model. Calculations were mainly performed with WinGX programs.[44] Molecular graphics were computed with PLATON.[45]

X-ray Crystal Data of 1. $C_{17}H_{17}NO_6$, MW = 331.33, triclinic, space group $P1$, Z = 2, with two independent molecules in the asymmetric unit, a = 8.163(4) Å, b = 8.220(5) Å, c = 11.652(8) Å, α = 100.21(11)°, β = 101.26(9)°, γ = 91.22(7)°, V = 753.4(8) $Å^3$, μ(Mo Kα) = 0.11 mm^{-1}, ρ_{calc} = 1.46 g cm^{-3}; S = 1.03, final R indices: R_1 = 0.0707 and R_w = 0.1592 for 2649 observed reflections of 3298 (θ_{max} = 27.1°, $I > 2\sigma(I)$ criterion and 434 parameters); maximum and minimal residues are 0.72 and −0.24 e $Å^{-3}$, respectively.

X-ray Crystal Data of 2. $C_{17}H_{19}NO_4$, MW = 301.4, orthorhombic, space group $P2_12_12_1$, a = 10.074(5) Å, b = 10.771(3) Å, c = 13.494(7) Å, V = 1464.2(11) $Å^3$, Z = 4, μ(Mo Kα) = 0.098 mm^{-1}, ρ_{calc} = 1.38 g cm^{-3}, S = 1.09, final R indices: R_1 = 0.0440 and R_w = 0.114 for 1936 observed reflections of 2035 (θ_{max} = 28.3°, $I > 2\sigma(I)$ criterion and 200 parameters); maximum and minimal residues are 0.24 and −0.21 e $Å^{-3}$, respectively.

Crystallographic Data. Crystallographic data for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Center, as supplementary publications no. 688361 for **1** and 688362 for **2**. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge, CB2, 1EZ, UK (fax: +44-(0)1223-306033 or e-mail: deposit@ccdc.cam.ac.uk).

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Supporting Information Available: X-ray analysis data for compounds **1** and **2** in CIF format are available free of charge via the Internet at http://pubs.acs.org.

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