THE GRACILINES: A NOVEL SUBGROUP OF THE AMARYLLIDACEAE ALKALOIDS

Seda Noyan,[1] Gudrun Hopp Rentsch,[2] Mustafa Ali Önür,[1] Tekant Gözler,[1] Belkıs Gözler,[1] and Manfred Hesse[2*]

[1] Faculty of Pharmacy, Ege University, Bornova 35100, İzmir, Turkey

[2] Organisch-chemisches Institut, Universität Zürich, Winterthurerstrasse 190, 8057 Zürich, Switzerland

Abstract- A new subgroup of the Amaryllidaceae alkaloids having a 10b,4a-ethanoiminodibenzo[*b,d*]pyrane (2-benzopyrano[3,4-*d*]indole) nucleus is isolated from two different *Galanthus* species as three novel monomeric alkaloids, (+)-graciline (**1**), (+)-11-acetoxygraciline (**2**) and (+)-3,4-dihydro-3-hydroxygraciline (**3**). The fourth new compound, (-)-digracine (**4**) is a dimer of two such moieties.

The Amaryllidaceae alkaloids constitute an exclusive group of basic compounds elaborated almost solely by species of the specified plant family. These alkaloids, some of which display remarkable physiological and pharmacological activities,[1-5] have so far been classified on structural considerations into eight subgroups.[1] A thorough literature survey has revealed that the members of the

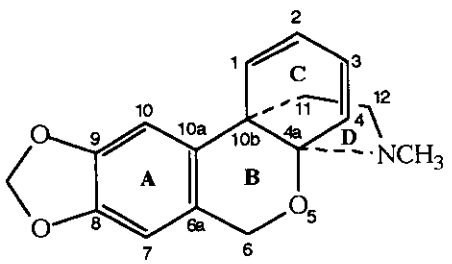
genus *Galanthus* have so far been reported to contain alkaloids from almost all of these subgroups only with the exception of simple phenanthridines.

During our ongoing phytochemical studies on *Galanthus* species of Turkey, we have isolated from *Galanthus gracilis* a strongly dextrorotatory basic compound, (+)-graciline (**1**). The ^{1}H NMR spectrum of **1** taken in $CDCl_3$ (300 K), accounting for seventeen hydrogens, displayed relevant signals for a basic *N*-methyl group, a methylenedioxy substituent, two aromatic protons appearing as singlets, an isolated deshielded methylene as two well-defined doublets along with four aliphatics in the δ 2.2-3.0 region. A striking finding, however, was the presence of four olefinic protons.

The ^{13}C NMR and DEPT spectra revealed the presence of seventeen carbons as one methyl, four methylene, six methine and six nonprotonated carbons. Among these, the noteworthy signals were of the quaternary carbons resonating at δ 47.2 and δ 92.6. Since these spectral findings conformed to none of the so far described subgroups and strongly suggested that we were indeed dealing with a so far unknown structure, extensive 2D NMR experiments were performed.

Using the information gathered from the ^{1}H, ^{1}H DQF COSY, HSQC and HMBC experiments, the construction of the skeleton was initiated from ring A which incorporated the methylenedioxy substitution. In the HMBC spectrum, H-7 (δ 6.45) showed a prominent three-bond correlation with the δ 64.2 carbon carrying the isolated geminal hydrogens (H-6), which in turn were connected to the quaternary carbon at δ 92.6 through a $^{3}J_{CH}$ coupling. The informative three-bond coupling of H-10 (δ 6.73) beyond ring A was with the quaternary carbon at δ 47.2.

In the ^{1}H NMR spectrum, the signals for two of the olefinic protons were almost superimposed on each other while the resonance of another was buried under the methylenedioxy signals. Therefore, it was



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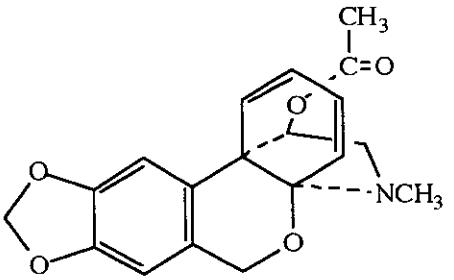

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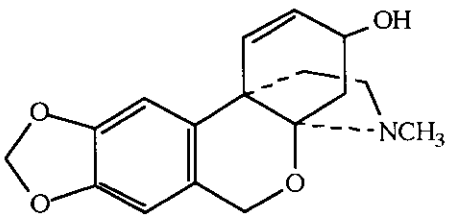

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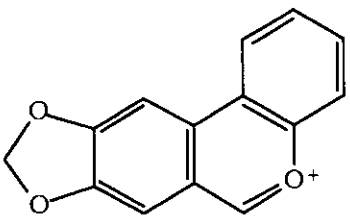
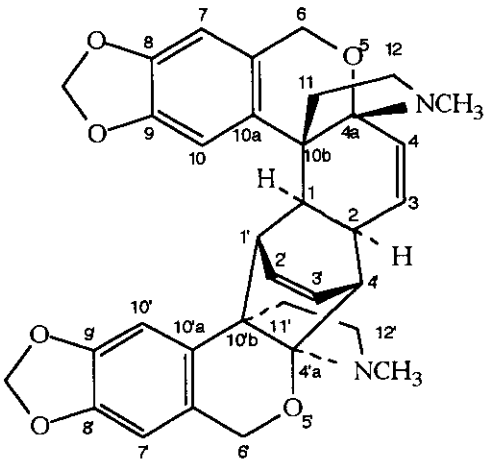


m/z 225
(from **1**, **2** and **3**)



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practically impossible to calculate the coupling constants for individual signals. A ^{1}H NMR spectrum taken in benzene-d_6 furnished even more complicated results where three of the olefinic protons had almost identical chemical shifts. However, the data obtained from the $^{1}H, ^{1}H$ COSY and TOCSY experiments using $CDCl_3$ as solvent clearly pointed out that these four olefinic protons were members of a conjugated system. The relative positions of these hydrogens on ring C could be concisely assigned by the information obtained from the HMBC spectrum, particularly by the three-bond connectivities from δ 5.90 and 5.78 protons to the carbon resonating at δ 47.2, and from δ 6.10 and 6.09 signals to δ 92.6 quaternary carbon, finally allowing us to construct a tentative dibenzo[*b*,*d*]pyrane nucleus partially

saturated in ring C.

$^1H,^1H$ DQF COSY and TOCSY spectra indicated that the remaining four aliphatic protons were members of a four-spin system. In the HMBC spectrum, the more deshielded carbon (δ 49.4) of this ethylene chain showed a three-bond correlation with the *N*-methyl protons, the latter also being connected to the centrally located carbon at δ92.6 through a $^3J_{CH}$ coupling, thus establishing the fusion of one end of the ethanoimino bridge. The shielded methylene protons (δ 2.34 and 2.30) of this bridge displayed all of the expected three-bond correlations with C-1 (δ 136.9), C-10a (δ 133.5) and C-4a (δ 92.6), consequently establishing the final linkage of the ethanoimino bridge between 10b and 4a of the dibenzo [*b,d*]pyrane skeleton. The foregoing results, therefore, add a new tetracyclic ring system, a 2-benzopyrano[3,4-*d*]indole, to the rich structural diversity found among the Amaryllidaceae subgroups.

An extremely simple EI MS showed a low abundance molecular ion peak at m/z 283 (4 %), verifiying the molecular formula as $C_{17}H_{17}NO_3$. A facile rupture of the ethanoimino bridge by the expulsion of $CH_3CH_2NCH_3$ from the molecular ion furnished the stable aromatic cation as the base peak at m/z 225, which was practically the only sizable peak in the spectrum.

In the NOESY experiment (mixing time: 1.2 s) , the strong spatial relationships of H-1 (δ 6.09), H-10 (δ 6.73) and H-11β (δ 2.30) were clearly observed. Moreover, the *N*-methyl protons dislayed expected cross signals with the methylene hydrogens at C-12 and with H-4 (δ 5.90). However, a more remarkable interaction of H-4 was with the β-oriented hydrogen of the isolated geminal pair at C-6 (δ 4.67). This information applied on the Dreiding models favored the projection of ring C in front of the mean plane of the A/B rings. Therefore, the relative stereochemistry of the ethanoimino bridge was concluded to be α. The CD curve of **1** resembled closely those of the (+)-crinine bases with α-oriented ethanoimino

bridges.[6-8] Therefore, it is likely that (+)-graciline (**1**) may have 4a*R* and 10b*R* absolute configuration.

The $CDCl_3$ ^1H NMR spectrum of the new base (**2**), isolated from *G. plicatus* subspecies *byzantinus* revealed noticeable similarities with that of **1**. Once again, signals for two aromatic *p*-positioned protons, a methylenedioxy group, a deshielded isolated methylene and an *N*-methyl singlet were present. Of particular interest were the resonances for four olefinic protons, indicating that **1** and **2** were indeed analogous bases. In the case of **2**, however, a three-spin system with a very deshielded methine at δ 5.42 and a three-proton singlet at δ 2.13 replaced the four-spin system of **1**, suggesting an acetoxy substituent on the ethanoimino bridge. The presence of the ester carbonyl was unambiguously verified by the 1730 cm^{-1} absorption in the IR spectrum as well as by the signal at δ 171.0 in the ^{13}C NMR spectrum.

The multiplicities of the nineteen carbons accounted for in the ^{13}C NMR spectrum were determined by a DEPT experiment. The telling features were the resonances at δ 21.2 for the methyl carbon of the acetoxy group and at δ 82.0 for the methine carbon incorporating the acetoxy substituent. The ^1H, ^1H DQF COSY, HSQC and HMBC experiments allowed explicit assignments for most of the proton and carbon chemical shifts, with the exception of those of the olefinic protons, two of which were completely obscured by the methylenedioxy signals. Therefore, some of the NMR experiments were repeated in benzene-d_6 (See Experimental) which resulted in better resolution specifically of the olefinic signals. The easily calculable coupling constants provided firm proof to the assignments of olefinic signals with respect to their relative positions on ring C. Although H-1 (δ 6.10) was seemingly a doublet with $J_{1,2}$ 9.7 Hz, a small coupling of about 1 Hz with H-3 (δ 5.86) indicated the presence of a long-range interaction. $J_{2,3}$ (5.3 Hz) and $J_{3,4}$ (9.4 Hz) coupling constants provided sound proof to the sequence of the olefinic hydrogens on ring C, which were also unambiguously verified by the spatial interactions

depicted in the NOESY spectrum (mixing time: 1.3 s). Thus, the foregoing data furnished conclusive evidence to assign an 11-acetoxygraciline structure to compound (**2**).

In the EI MS of **2**, the base peak at m/z 43 resulted from the acetyl cation. The other remarkable peak at m/z 225 (53 %) belonged to $[M - CH_3N(CH_2)_2OCOCH_3]^+$ ion. The molecular ion was observed at m/z 341 but in a very low abundance. However, a CI MS did indeed furnish the same molecular ion, in accord with the proposed molecular formula of $C_{19}H_{19}NO_5$. In the EI MS, peaks at m/z 298 $[M- 43]^+$ and 281 $[M- 60]^+$ were also observed.

The readily noticeable resemblance of the CD spectrum of **2** with that of **1** suggested that **2** may also incorporate the same 4a*R* and 10b*R* stereochemistry. The NOESY spectrum taken in $CDCl_3$ provided information about the conformational preference of the molecule, and also about the relative configuration at the C-11 chiral center. The conformation which satisfied the almost equal magnitude cross signals from H-10 (δ 7.04) to H-1 (δ 6.06) and H-11β (δ 5.42) favored the α orientation of the acetoxy group at C-11, and also effectively accounted for the spatial proximity of the olefinic H-4 (*ca.* δ 5.94-5.88) to both the *N*-methyl group and H-6β (δ 4.61).

The ^1H and ^{13}C NMR spectra of our third new base (**3**) isolated again from *G. gracilis* was recorded in MeOH-d_4 due to its polar nature. The ^{13}C NMR spectrum, accounting for seventeen carbons, displayed the characteristic quaternary carbon chemical shifts at δ 48.3 and 97.7, pointing once again to the presence of a 10b,4a-ethanoiminodibenzo[*b,d*]pyrane nucleus. In the ^1H NMR spectrum of **3** at 300 K, although the presence of signals for two aromatic protons, a methylenedioxy group, an isolated high-frequency methylene and an *N*-methyl were the immediately noticeable similarities when compared to the spectra of **1** and **2**, the aliphatic region, accounting for nine protons, looked remarkably different.

All signals were relatively unresolved, suggesting a conformational flexibility in solution. Even a more conspicious feature, however, was the presence of two broadened doublets for only two olefinic protons. Once again, detailed 2D NMR experiments at 280 K were performed. Even at this temperature, most of the signals in the ^{1}H NMR spectrum were broad and the multiplets were not well resolved.

The ^{1}H, ^{1}H DQF COSY showed that two pairs of geminal protons resonating at δ 3.83 and 3.40 and at δ 2.35 and 2.44 were members of a four-spin system. The relevant HMBC correlations once again established the ethanoimino bridge connecting C-4a and C-10b of the dibenzo[*b,d*]pyrane skeleton.

In the NOESY spectrum (mixing time: 400 ms), strong spatial interactions detected between H-10 (δ 6.96), H-11β (δ 2.44) and δ 6.35 olefinic signal established the gross conformation of the molecule and also confirmed the position of the latter as H-1. In the ^{1}H, ^{1}H DQF COSY spectrum, this H-1 furnished a cross signal only with the other at δ 6.02 (H-2), while the latter displayed an additional correlation with the deshielded methine at δ 4.40 (H-3), which in turn correlated to the methylene protons at δ 2.30 and 2.24 (H-4), thus establishing their sequence on ring C. The high frequency resonance of H-3 suggested the presence of a hydroxyl substituent at C-3, further evidenced by the strong absorption at 3370 cm^{-1} in the IR spectrum.

At this point, the NOESY spectrum provided complementary information on the probable β orientation of the hydroxyl group at C-3. The cross signals between H-4β (δ 2.30) and H-6β (δ 4.93), and between H-4α (δ 2.24) and the *N*-methyl (δ 2.93) signals completed the stereochemical picture, where ring C seemed to adopt a quasi-chair conformation. The relatively deshielded chemical shift of the *N*-methyl protons could be attributed to the deshielding effect of the nearby oxygen in ring B.

The molecular ion in the EI MS was at m/z 301 (54 %), which was in accordance with the proposed

molecular formula as $C_{17}H_{19}NO_4$. Three fragmentation signals at m/z 283 $[M-H_2O]^+$, m/z 243 [m/z 225 + H_2O] and at m/z 225 confirmed the finding that the hydroxyl group was located at a different bridge than the nitrogen atom.

Turning now to the last novel compound, (-)-digracine (**4**), isolated from *G. gracilis*, it was immediately recognized that the chemical shifts and the multiplicities in both ^1H and ^{13}C NMR spectra pointed to the presence of two 10b,4a-ethanoiminodibenzo[*b,d*]pyrane structures. For example, the key resonances in the ^{13}C NMR were duplicated as singlets at δ 44.2, 46.8 and 91.1, 99.3 for C-10b and C-4a, respectively. Interestingly, there were only four signals for olefinic carbons.

In the ^1H NMR spectrum, the signals for four aromatic protons, two methylenedioxy groups, two *N*-methyls and two pairs of isolated high-frequency methylenes were easily noticeable duplications of some characteristic features. With the data obtained from ^1H, ^1H DQF COSY and TOCSY experiments, it was possible to sort the proton chemical shifts of the two moieties. It could also be firmly established that both moieties had the ethanoimino bridge between C-10b and C-4a, and that ring C of each contained only one double bond and two relatively low frequency methines.

An HSQC experiment allowed the assignment of corresponding protonated carbon chemical shifts. However, the key experiment was the HMBC, which aside from providing a complete map of carbon chemical shifts, displayed unambiguous $^2J_{CH}$ and $^3J_{CH}$ correlations interrelating the two parts, thus ruling out the possibility of an epimeric mixture and confirming that **4** was indeed a dimeric compound. Some of the diagnostic correlations interlocking the two monomeric moieties were from H-1 (δ 2.82) to C-1' (δ 44.2), C-2' (δ 134.2) and C-10'b (δ 52.3), from H-2 (δ 2.61) to C-4' (δ 45.0), from H-3 (δ 5.69) to C-4' (δ 45.0), from H-1' (δ 3.25) to C-1 (δ 41.2) and C-10b (δ 46.8), and from H-4' (δ 2.80) to C-1 (δ

41.2). These findings provided unambiguous proof to the fact that **4** was formed by a fusion of two 10b, 4a-ethanoiminodibenzo[*b,d*]pyrane structures *via* two bonds between C-1:C-1' and C-2:C-4' .

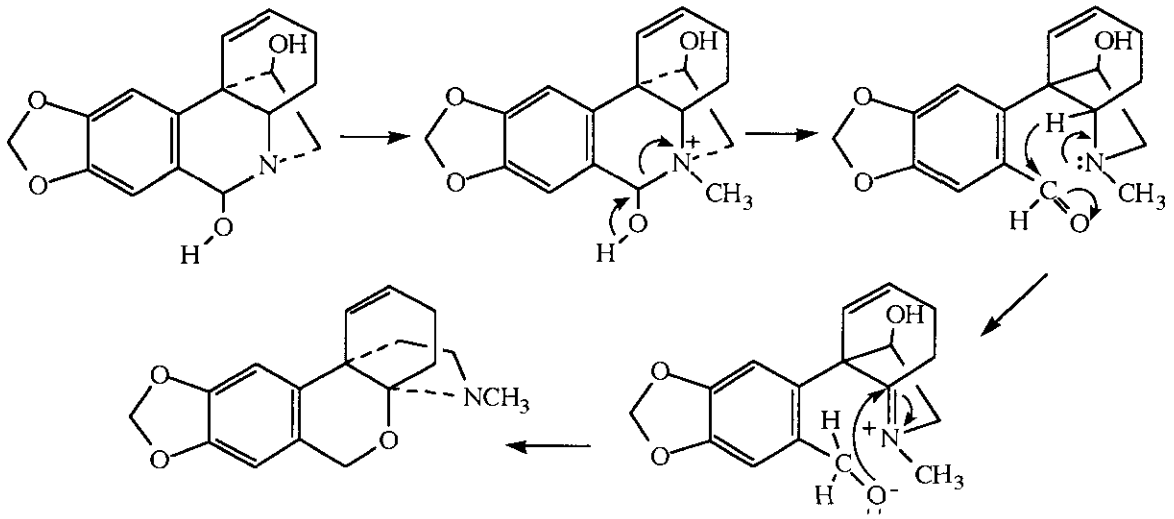
In order to establish the relative configuration at the newly created chiral centers C-1, 2, 1' and 4', as well as the conformational preference of the molecule, a NOESY experiment (mixing time: 850 ms) was undertaken. Once again, the key correlations were the ones indicating the spatial proximities of hydrogens from the two monomeric moieties. For example, H-1' (δ 3.25) showed cross signals of equal intensities with H-2' (δ 5.89) and H-10' (δ 6.89) from its parent monomer and H-12b (δ 2.67), H-1 (δ 2.82) and H-10 (δ 7.01) from the opposite half of the molecule, while the strongest cross signal was with H-11b (δ 2.11) also from the latter moiety. Another rather centrally located hydrogen, H-1 (δ 2.82), displayed equally strong cross signals with H-2 (δ 2.61), H-10 (δ 7.01), H-11'a (δ 2.12), H-12'a (δ 2.95) and H-1' (δ 3.25), the latter three hydrogens being members of the opposite half of the dimer. Studies using Dreiding models showed that the NOESY spectrum can be interpreted only when the junctions between the two halves are as is given in structure (**4**).

The ESI MS spectrum of **4** furnished 567 ($M+H^+$) which is in agreement with molecular weight calculated for the proposed molecular formula of $C_{34}H_{34}N_2O_6$. A lower abundance ion at 284 accounts for the cleavage into two identical monomeric moieties, $C_{17}H_{17}NO_3$.

Unlike the previously mentioned analogous monomers (**1-3**), compound (**4**) is levorotatory. It is also interesting to note that the CD curve of **4**, although not exactly a mirror image of those of **1** and **2**, has reversed positive and negative maxima.

The fact that compounds of this novel subgroup, called gracilines after the prototypic compound (**1**), are detected in two different *Galanthus* species suggests that they may very well be common elements in the

alkaloidal profile of the Amaryllidaceae plants. The possible biogenetic pathway leading to this newly established skeleton may originate from the frequently encountered (+)-6-hydroxycrinine species and proceed as suggested in Scheme 1.



Scheme 1

EXPERIMENTAL

Optical rotations: Perkin-Elmer 241 Polarimeter; UV: Perkin-Elmer 555 Spectrophotometer; IR: Perkin-Elmer 297 Infrared Spectrophotometer; ^{1}H NMR of **2** and ^{13}C DEPT of **1-3**: Bruker ARX 300; other ^{1}H NMR, ^{13}C NMR, ^{13}C DEPT and 2D NMR [gs-HSQC, gs-HMBC, TOCSY (mixing time: 100 ms), NOESY, DQF-COSY] : Bruker AMX 600 Spectrometer; EI MS: Finnigan MAT SSQ 700; CI MS: Finnigan MAT 90; ESI MS: Finnigan MAT TSQ 700; CD: Jasco J-715 Spectropolarimeter.

Plant Material

Galanthus gracilis Celak was collected from Mount Nif near Kemalpaşa, İzmir, on March 26, 1995 at an

altitude of 900-1500 m. *G. plicatus* Bieb. subsp. *byzantinus* (Baker) D. A. Webb was collected in the vicinity around Lake Abant, Bolu, on April 4, 1995. Voucher samples are deposited, No's 1192 and 1194, in the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Ege University.

Extraction and Isolation

Dried and powdered total plant material of *G. gracilis* (5.25 kg) was extracted with EtOH (100 L) for 40 h at rt to furnish the crude extract (681 g), which was dissolved in 2% HCl and filtered. The acidic solution was basified with 10% NH_4OH and then extracted with $CHCl_3$. The organic solvent was evaporated to furnish the crude basic extract (9.58 g). During the preliminary separation through a silica gel (Merck, 70-230 Mesh) column, the first alkaloid-containing fraction (1.57 g) eluted with $CHCl_3$ was dissolved in MeOH, from which 0.152 g of **3** was precipitated. The mother liquor was evaporated to dryness and further fractionated by preparative CC on silica gel H (Merck, Type 60), using n-C_6H_{14}-EtOAc-Me_2CO (80:15:5). Fraction 21-32 (0.867 g) was further purified by successive preparative TLC on silica gel plates, using n-C_6H_{14}-Me_2CO (7:3) and $CHCl_3$-Me_2CO (9:1) as solvent systems to yield pure **1** (0.109 g) and **4** (0.069 g).

Dried and powdered total plant material of *G. plicatus* (3.7 kg) was also extracted with EtOH (75 L) for 30 h at rt to furnish the crude extract (425 g). This extract was dissolved in 2% HCl and filtered; the acidic solution basified with 10% NH_4OH and then extracted with $CHCl_3$. The evaporation of the organic solvent supplied the crude basic extract (10.28 g). During the preliminary separation through a silica gel column (Merck, 70-230 Mesh), elution with $CHCl_3$ afforded 0.799 g fraction, which was subjected to preparative CC on silica gel H (Merck, Type 60) using n-C_6H_{14}-C_6H_6-Me_2CO (50:45:5).

Fraction 11-16 was further purified by preparative TLC using the same solvent system to yield 0.0025 g of **2**.

(+)-Graciline (**1**): white amorphous solid; $[\alpha]_D$ + 521° (c 0.175, MeOH); CD (MeOH) nm (log ε) 335 (0), 275 (+38.41), 238 (0), 232 (-3.64), 225 (0), 219 (+3.45), 214 (0), negative tail beyond 214 nm; UV (MeOH) λ_{max} nm (log ε) 248 sh (3.89), 290 (3.76); IR ($CHCl_3$)ν_{max} cm^{-1} 2990, 2960, 2940, 2890, 2850, 1500, 1485, 1450, 1410, 1380, 1365, 1350, 1215, 1135, 1090, 1070, 1040, 980, 935, 860; ^1H NMR (600 MHz, $CDCl_3$, 300 K) δ (ppm) 6.73 (1H, s, H-10), 6.41 (1H, s, H-7), 6.10 (1H, dd, *J* 9.8, 5.5 Hz, H-3), 6.09 (1H, d, *J* 9.0 Hz, H-1), 5.90 (1H, obscured by the OCH_2O signals, H-4), 5.91 and 5.88 (each 1H, d, *J* 1.4 Hz, OCH_2O), 5.78 (1H, dd, *J* 9.3, 5.0 Hz, H-2), 4.67 (1H, d, J_{gem} 14.5 Hz, H-6β), 4.50 (1H, d, J_{gem} 14.5 Hz, H-6α), 2.92 (1H, dt, *J* 8.4, 6.8 Hz, H-12β), 2.86 (1H, m, H-12α), 2.54 (3H, s, NCH_3), 2.34 (1H, m, H-11α), 2.30 (1H, m, H-11β); ^{13}C NMR (150 MHz, $CDCl_3$, 300 K) δ (ppm) 33.3 (NCH_3), 40.1 (C-11), 47.2 (C-10b), 49.4 (C-12), 64.2 (C-6), 92.6 (C-4a), 100.8 (OCH_2O), 104.2 (C-7), 106.9 (C-10), 118.8 (C-2), 125.7 (C-6a), 126.8 (C-3), 128.2 (C-4), 133.5 (C-10a), 136.9 (C-1), 145.5 (C-8), 146.9 (C-9); EI MS m/z (%) 283 (M^+, 4), 282 (4), 264 (3), 254 (6), 240 (5), 227 (4), 226 (19), 225 (100), 139 (4).

(+)-11-Acetoxygraciline (**2**): white amorphous solid; $[\alpha]_D$ + 198° (c 0.175, MeOH); CD (MeOH) nm (Δε) 333 (0), 274 (+ 17.14), 249 sh (+ 5.59), 241 (0), 233 (- 4.15), 224 (0), 217 (+ 3.35), 211 (0), negative tail beyond 211 nm; UV (MeOH) λ_{max} nm (log ε) 252 sh (3.95), 288 (3.69); IR ($CHCl_3$)ν_{max} cm^{-1} 2950, 2920, 2850, 1730, 1580, 1500, 1485, 1430, 1410, 1380, 1370, 1350, 1215, 1135, 1040, 980, 935, 855; ^1H NMR (300 MHz, $CDCl_3$, 300 K) δ (ppm) 7.04 (1H, s, H-10), 6.41 (1H, s, H-7), 6.14 (1H, ddd, *J* 1.1, 5.1 and 9.8 Hz, H-3), 6.06 (1H, dd, *J* 11.2, 1.0 Hz, H-1), 5.92 and 5.90 (each 1H, d, *J* 1.2 Hz,

OCH_2O), 5.94-5.88 (2H, m, H-2 and H-4), 5.42 (1H, dd, J_{vic} 2.8 and 6.7 Hz, H-11), 4.61 (1H, d, J_{gem} 14.6 Hz, H-6β), 4.50 (1H, d, J_{gem} 14.6 Hz, H-6α), 3.34 (1H, dd, J_{gem} 10.0, J_{vic} 6.7 Hz, H-12β), 2.73 (1H, dd, J_{gem} 10.0, J_{vic} 2.8 Hz, H-12α), 2.53 (3H, s, NCH_3), 2.13 (3H, s, CH_3CO); ^{1}H NMR (600 MHz, C_6D_6, 300 K) δ (ppm) 7.36 (1H, s, H-10), 6.10 (1H, s, H-7), 6.10 (1H, dd, *J* 9.7, 1.0 Hz, H-1), 5.86 (1H, ddd, *J* 9.7, 5.2, 1.0 Hz, H-3), 5.77 (1H, d, *J* 9.7 Hz, H-4), 5.69 (1H, dd, *J* 9.7, 5.2 Hz, H-2), 5.59 (1H, dd, *J* 7.1, 3.4 Hz, H-11β), 5.272 and 5.267 (each 1H, d, *J* 1.3 Hz, OCH_2O), 4.43 (1H, d, *J* 14.4 Hz, H-6β), 4.37 (1H, d, *J* 14.4 Hz, H-6α), 3.31 (1H, dd, *J* 9.7, 7.1 Hz, H-12β), 2.67 (1H, dd, *J* 9.6, 3.0 Hz, H-12α), 2.40 (3H, s, NCH_3), 1.65 (3H, s, CH_3CO); ^{13}C NMR (150 MHz, $CDCl_3$, 300 K) δ (ppm) 21.2 (CH_3CO), 32.8 (NCH_3), 51.5 (C-10b), 56.2 (C-12), 63.5 (C-6), 82.0 (C-11), 92.0 (C-4a), 100.9 (OCH_2O), 104.1 (C-7), 107.6 (C-10), 120.7 (C-4), 126.5 (C-2), 126.5 (C-6a), 127.0 (C-3), 130.5 (C-10a), 131.0 (C-1), 146.1 (C-8), 147.1 (C-9), 171.0 (CO); EI MS m/z (%) 341 (M^+, 1), 298 (1), 281 (6), 252 (3), 249 (2), 227 (2), 226 (13), 225 (53), 168 (3), 139 (12), 43 (100).

(+)-3,4-Dihydro-3-hydroxygraciline (**3**): white amorphous solid; $[\alpha]_D$ + 138° (c 0.113, MeOH); CD (MeOH) nm (Δ ε) 272 (+1.78), 254 (+1.36), 233 sh (+3.72), a positive tail beyond 220 nm; UV (MeOH) λ_{max} nm (log ε) 230 sh (4.08), 290 (3.71); IR (KBr) ν_{max} cm^{-1} 3370, 3260, 3010, 2980, 2880, 1610, 1500, 1480, 1450, 1430, 1415, 1380, 1360, 1340, 1320, 1255, 1235, 1210, 1200, 1175, 1150, 1140, 1110, 1090, 1060, 1025, 1010, 990, 955, 915, 895, 870, 830; ^{1}H NMR (600 MHz, CD_3OD, 280 K) δ (ppm) 6.96 (1H, br s, H-10), 6.63 (1H, s, H-7), 6.35 (1H, br d, *J* 9.7 Hz, H-1), 6.02 (1H, br m, H-2), 5.95 and 5.94 (each 1H, br s, OCH_2O), 4.97 (1H, br d, *J* 15.7 Hz, H-6α), 4.93 (1H, br d, *J* 15.6 Hz, H-6β), 4.40 (1H, br s, H-3α), 3.83 (1H, br m, H-12β), 3.40 (1H, br dd, *J* 10.3 and 16.4 Hz, H-12α), 2.93 (3H, br s, NCH_3), 2.44 (1H, m, H-11β), 2.35 (1H, br m, H-11 α), 2.30 (1H, br d, J_{gem} 15.5 Hz, H-4β), 2.24 (1H, br

d, J_{gem} 15.0 Hz, H-4α); ^{13}C NMR (150 MHz, CD_3OD, 280 K) δ (ppm) 30.8 (C-4), 34.7 (NCH_3), 38.2 (C-11), 48.3 (C-10b), 53.7 (C-12), 63.5 (C-3), 66.3 (C-6), 97.7 (C-4a), 102.9 (OCH_2O), 105.6 (C-7), 107.5 (C-10), 124.1 (C-6a), 128.7 (C-2), 130.5 (C-10a), 134.0 (C-1), 148.5 (C-8), 149.3 (C-9); EI MS m/z (%) 301 (M^+, 54), 285 (6), 286 (34), 283 (12), 282 (8), 272 (7), 256 (6), 254 (9), 244 (20), 243 (21), 240 (10), 231 (10), 228 (6), 227 (7), 226 (22), 225 (100), 200 (6), 185 (5), 139 (5), 132 (5).

(-)-Digracine (**4**): white amorphous solid; $[\alpha]_D$ -74° (c 0.167, MeOH); CD (MeOH) nm (Δ ε) 312 (0), 298 (-6.21), 268 (0), 237 (+15.4), 214 (0), negative tail beyond 214 nm; UV (MeOH) λ_{max} nm (log ε) 232 sh (4.29), 293 (3.99); IR ($CHCl_3$) ν_{max} cm^{-1} 2990, 2960, 2930, 2880, 1500, 1485, 1450, 1365, 1355, 1215, 1075, 1040, 935, 910, 860; ^{1}H NMR (600 MHz, $CDCl_3$, 300 K) δ (ppm) 7.01 (1H, s, H-10), 6.89 (1H, s, H-10'), 6.46 (2H, s, H-7 and H-7'), 5.96 and 5.933 (each 1H, d, *J* 1.4 Hz, OCH_2O), 5.928 and 5.919 (each 1H, d, *J* 1.5 Hz, OCH_2O'), 5.92-5.88 (1H, m, H-3'), 5.90-5.86 (1H, m, H-2'), 5.77 (1H, dd, *J* 10.2, 2.4 Hz, H-4), 5.69 (1H, dd, *J* 10.2, 2.0 Hz, H-3), 4.65 (1H, d, *J* 14.7 Hz, H-6b), 4.59 (1H, d, *J* 14.7 Hz, H-6a), 4.49 (1H, d, *J* 13.8 Hz, H-6'a), 4.20 (1H, d, *J* 13.8 Hz, H-6'b), 3.25 (1H, d, *J* 5.9 Hz, H-1'), 3.08 (1H, td, *J* 8.4, 5.5 Hz, H-12'b), 2.95 (1H. dd, *J* 14.2, 8.0 Hz, H-12'a), 2.89 (1H, td, *J* 8.9, 5.7 Hz, H-12a), 2.82 (1H, d, *J* 8.4 Hz, H-1), 2.80 (1H, ddd, *J* 6.1, 3.1, 1.2 Hz, H-4'), 2.67 (1H, td, *J* 9.2, 4.0 Hz, H-12b), 2.61 (1H, m, H-2), 2.49 (3H, s, NCH_3'), 2.32 (3H, s, NCH_3), 2.12 (1H, m, H-11'a), 2.11 (1H, m, H-11b), 1.90 (1H, ddd, *J* 12.8, 9.5, 4.0 Hz, H-11a), 1.69 (1H, ddd, *J* 13.4, 8.4, 6.0 Hz, H-11'b); ^{13}C NMR (150 MHz, $CDCl_3$, 300 K) δ (ppm) 32.46 (NCH_3), 32.48 (NCH_3'), 33.0 (C-11), 37.2 (C-11'), 37.3 (C-2), 41.2 (C-1), 44.2 (C-1'), 45.0 (C-4'), 46.8 (C-10b), 49.6 (C-12), 52.3 (C-10'b), 53.3 (C-12'), 62.2 (C-6'), 63.1 (C-6), 91.1 (C-4a), 99.3 (C-4'a), 100.66 (OCH_2O'), 100.72 (OCH_2O), 104.4 (C-7), 104.9 (C-7'), 105.5 (C-10'), 105.9 (C-10), 123.5 (C-4), 126.1 (C-6a), 128.4 (C-6'a), 128.8 (C-3'), 134.2 (C-2'),

135.9 (C-3), 136.2 (C-10a), 137.6 (C-10'a), 144.6 (C-8'), 145.2 (C-8), 146.4 (C-9), 146.8 (C-9'); ESI MS m/z 567 ($M+H^+$).

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