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Computer-assisted Structural Elucidation. Alkaloids with a Novel Diaza-adamantane Skeleton from the Seeds of *Acosmium panamense* (Fabaceae).

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Abstract: Three alkaloids, acosmine **1**, acosmine acetate **2** and panacosmine **3** with a novel diaza-adamantane skeleton and an unusual N-acetyl enamine moiety, have been isolated from the seeds of *Acosmium panamense* (Benth.). The structures were assembled on the basis of COSY, HMQC and HMBC data with the assistance of the LSD structure elucidation program. Proton-nitrogen HMBC experiments support the proposed structures. Other isolated compounds include 4α-angeloyloxy-3β-hydroxy-13β-methoxylupanine **4**, a new compound, and the known alkaloids lupanine and multiflorine.

Acosmium panamense (Benth.) Yakovlev (Fabaceae)[1], known as *Sweetia panamense*[2], is a tall tree originating in central America, from Mexico to Venezuela. In central America, the bitter bark has been used in traditional medicine as a remedy for diseases such as syphilis and malaria[3]. The hard wood of *A. panamense* is used as building material. *A. panamense* was introduced into Africa in the Congo (Kinshasa) under the erroneous name *A. brachystachya*[4]. Chemical investigation of the root bark resulted in the isolation of the alkaloids sweetenine[3] and 4α-hydroxysparteine[5]. More recently methoxylated quinolizidine alkaloids have been described[6]. This article describes the results of a chemical investigation of the seeds of *A. panamense*.

The ground seeds of *A. panamense* were first defatted and then submitted to alkaloid separation by means of acid-base extraction[7]. Purification of the alkaloid mixtures by column chromatography on silica gel led to the isolation of six compounds. The known alkaloids lupanine and multiflorine were identified by comparison of their spectra with literature data[8]. The structure elucidation of the four new compounds is described below.

The EI mass spectrum of acosmine **1** reveals an $[M]^+$ ion at *m/z* 359. The regular and J-modulated ^{13}C NMR spectra show 21 carbons (1 methyl, 10 methylenes, 8 methines and 2 non-protonated carbons) bearing a total of 31 hydrogen atoms. A proton at δ 7.4 ppm (NH) is not bound to a carbon atom as indicated by the ^{1}H-^{13}C HSQC spectrum. The odd mass of the molecular ion imposes an odd number of hydrogen and nitrogen

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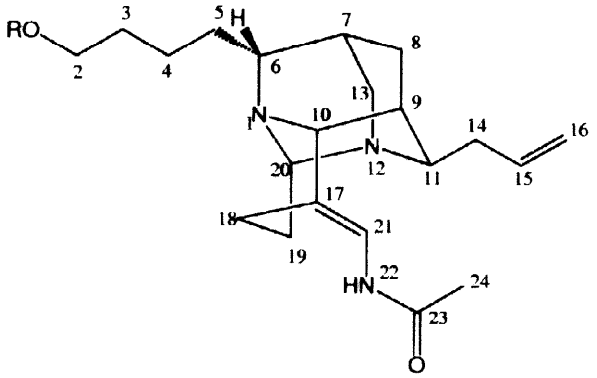
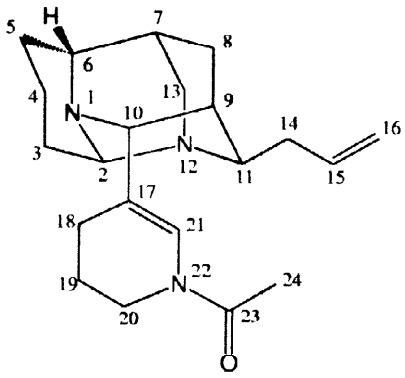
atoms. A molecular formula $C_{21}H_{33}N_3O_2$ is consistent with these observations. This was confirmed by the high resolution mass spectrum of acosmine acetate **2**.



1: R = H

2: R = Ac



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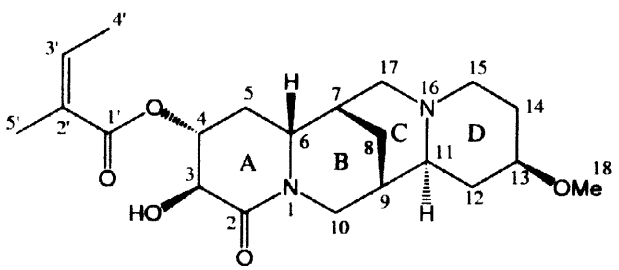

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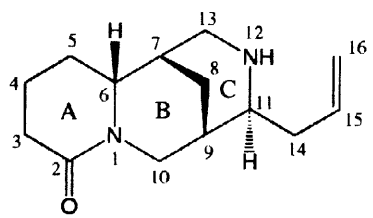

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Acosmine contains a carbonyl group, a vinyl group and a trisubstituted double bond. The vinyl group C-15/C-16 was readily identified by the H-15/H-16 correlations in the ^{1}H-^{1}H COSY spectrum (see the Experimental Section for the ^{1}H chemical shifts). The remaining sp^2 carbon atoms C-17 and C-21 must be bound together. This was confirmed by an H-21/C-17 HMBC correlation. The methyl group at δ 2.0 ppm (H-24) belongs to an acetamido group as shown by HMBC correlations from H-24 to C-23 and H-22 to C-24. The amide group presents an IR absorption at 1663 cm^{-1}. The coupling between H-22 and H-21 (11 Hz) must be vicinal and leads to the bond N-22/C-21. Hence there is an N-acetyl enamine in the molecule. This unusual chromophore has a UV absorption maximum at 242 nm.

Other structural fragments can be derived from COSY and HSQC spectra, with confirmation from HMBC correlations. These fragments are: C-2/C-3/C-4/C-5/C-6, C-13/C-7/C-8/C-9/C-10, C-14/C-15, and C-19/C-20. The chemical shift of the methylene carbon C-2 (62 ppm) is consistent with that of a primary alcohol. The remaining bonds were deduced using HSQC and HMBC correlations as input to the LSD program[9]. Carbons C-18, C-24, C-4, C-7, C-19, C-5, C-3, C-8, C-9, and C-14 have chemical shifts ranging from 19.8 ppm to 35.5 ppm and are constrained to be bound exclusively to carbon atoms. A single solution **1** for acosmine was found by the LSD program, in less than 0.1 s. The structure of acosmine **1** is closely related to the lupine alkaloids. Carbon atom C-20 bridges the two nitrogens of a bicyclo-[3.3.1] ring system to form a diaza-adamantane skeleton. This is the first example of this structural moiety in natural products.

The C-14/C-15/C-16 side chain is a structural element already observed in angustifoline **5**, that may originate from the degradation of ring D in lupanine.

Table 1. ^{13}C NMR data (δ; mult) for acosmine (**1**), acosmine acetate (**2**), panacosmine (**3**) and 4α-angeloyloxy-3β-hydroxy-13β-methoxylupanine (**4**) in $CDCl_3$.

C	1	2	3	4
2	62.1; t	66.2; t	69.8; d	170.9; s
3	32.3; t	30.5; t	28.4; t	70.7; d
4	23.0; t	25.3; t	17.9; t	70.3; d
5	30.4; t	32.3; t	28.4; t	30.1; t
6	64.9; d	66.8; d	58.7; d	57.2; d
7	26.2; d	28.0; d	30.5; d	31.9; d
8	31.4; t	33.1; t	33.0; t	26.4; t
9	35.2; d	37.0; d	25.2; d	33.6; d
10	64.8; d	66.6; d	68.1; d	48.2; t
11	52.4; d	54.6; d	59.9; d	61.0; d
12				36.2; t
13	46.4; t	48.3; t	44.1; t	77.4; d
14	35.5; t	37.1; t	36.3; t	29.1; t
15	135.4; d	136.8; d	135.5; d	52.4; t
16	116.3; t	118.8; t	117.1; t	
17	120.8; s	122.2; s	117.9; s	50.3; t
18	19.8; t	21.7; t	23.3; t	55.3; q
19	26.4; t	28.2; t	21.5; t	
20	68.0; d	70.6; d	40.1; t	
21	117.0; d	119.2; d	122.6; d	
23	167.4; s	168.5; s	168.0; s	
24	23.1; q	25.1; q	21.5; q	
25		172.0; s		
26		22.9; q		
1'				167.1; s
2'				127.4; s
3'				138.7; d
4'				15.7; q
5'				20.4; q

The cage structure fixes the relative configurations at C-7 and C-9. Their absolute configurations are choosen to match those of lupanine, also a constituent of the plant. In order to close the ring that is external to the adamantane ring system, C-17 and C-19 must be axial on the six-membered ring N-1/C-10/C-9/C-11/N-12/C-20. This is confirmed by a H-13/H-20 ROESY correlation. The proximity of H-6 and H-10 is also apparent in the ROESY spectrum. Their 1-3 diaxial relationship leads to the *S* absolute configuration at C-6. A ROESY correlation between H-11 and one of the H-19 protons helps deducing the 11-*S* absolute configuration. The *E* geometry of the C-17/C-21 double bond is proposed from the observation of H-21/H-9 and H-21/H-10 ROESY correlations. Disconnection of C-20 from N-1 and N-12 leads to a molecule whose asymmetric centers have the same absolute configurations as the corresponding centres in lupanine and angustifoline **5**.

The molecular formula $C_{23}H_{35}N_3O_3$ of acosmine acetate **2** was determined from the HREIMS analysis of its $[M]^+$ ion (m/z 401.2669, calc. 401.2678). It corresponds to acosmine with 42 more amu. The 1D and 2D NMR spectra are very similar to those of **1**. The ^1H NMR spectrum shows a supplementary methyl signal at δ 2.0 ppm. Two new ^{13}C signals at δ 22.9 and 172 ppm confirm the presence of an acetate group. The HMBC spectrum indicates its attachment to C-2. A thorough analysis of the 2D NMR spectra of **2** confirmed the identity of the carbon skeleton in **1** and **2**. The ^{13}C data for both compounds are reported in Table 1.

Further structure confirmation was obtained by means of the ^1H-^{15}N HMBC spectrum of **2**. Such a technique has already been successfully used for the structural study of alkaloids[10]. The spectrum in Figure 1 shows the presence of three nitrogen atoms, whose chemical shift is reported using CH_3NO_2 as reference. Atoms N-1 and N-22 are readily identified by their respective correlations with H-10 and H-21. Both N-1 and N-12 correlate with H-20, proving thus their proximity to C-20. The only protons of the angustifoline skeleton that could otherwise correlate with both nitrogen atoms are H-7 and H-9, whose chemical shifts (1.2 and 1.3 ppm) are clearly different from the one of H-20 (4.45 ppm).

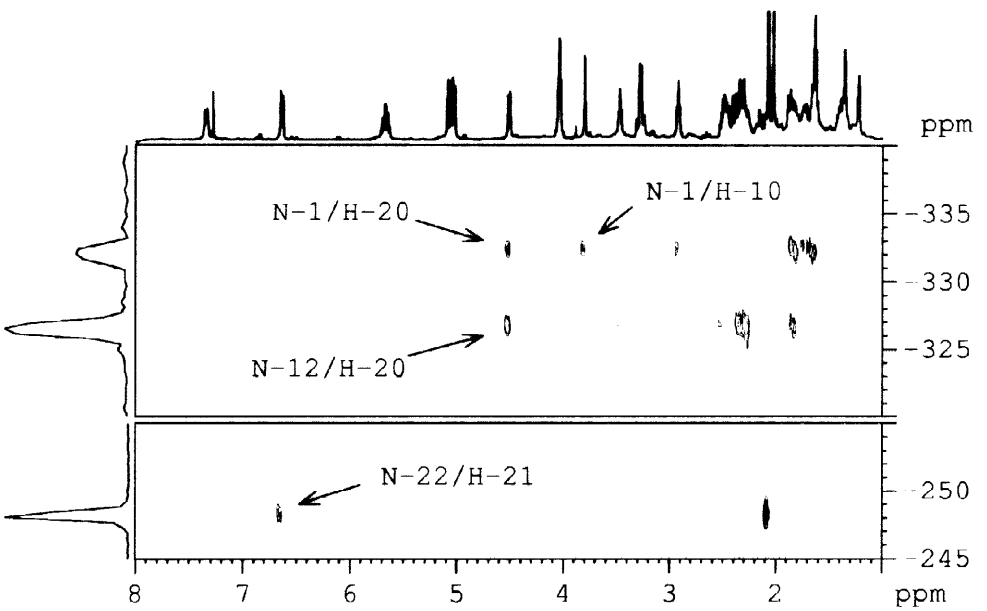

Figure 1. The ^1H-^{15}N HMBC correlation spectrum of acosmine acetate **2**.

Analysis of the ^{13}C NMR spectrum of the third new alkaloid, panacosmine **3**, indicated the molecular formula $C_{21}H_{31}N_3O$. This was supported by an $(M+1)^+$ ion at m/z 342 in the FAB mass spectrum. It was apparent in the NMR spectra of **3** that there were two components present in a ratio of 80:20. Efforts to separate the compounds by silica gel or liquid-liquid partition chromatography[11] were unavailing and we were led to the conclusion that we were dealing with an equilibrium mixture. The relative proportions of the two compounds did not change between 20 °C and 60 °C. The major compound was used to carry out the structural elucidation since its signals were readily identifiable in the NMR spectra of the mixture. The principal difficulty in carrying out the analysis was the superimposition of several signals e.g H-6 and H-11,

H-13a and H-10 and C-3 and C-5. Moreover the protons attached to C-4, C-19, C-18 and C-3 had similar chemical shifts and appeared as a complex group of unresolved signals.

The COSY spectrum readily revealed the vinyl group H-15/2H-16. The other double bond had to be C-17/C-21 and involves N-22. The acetyl group C-23/C-24 had to be part of an acetamido system, as indicated by the C-23/H-21 HMBC correlation. The corresponding chromophore shows UV absorptions at 245 and 318 nm. The carbons with chemical shifts less than 40 ppm were all considered to be attached only to other carbons. Table 2 lists the thiry-one pertinent correlations extracted from the HMBC spectrum. Of these, eight can be interpreted in two ways because of the superimposition of resonances. Other correlations present in the HMBC spectrum are not mentioned, since they do not provide any additional information. For example, both C-6/H-4 and C-4/(H-6 or H-11) correlations are visible. Clearly, if the a structure takes into account the former then the latter is necessarily taken into account as well, thus proving its lack of pertinence. A straightforward analysis of the problem by LSD provides 32 solution structures. A biogenetic hypothesis was introduced in eliminating those solutions missing the B-C ring system of angustifoline. This substructure fits only in 2 structures: **3** and the result of the permutation of C-6 with C-13. The structure **3** was preferred since it preserves the A-B-C ring system of angustifoline. The adamantane ring system results from a bond formation between C-2 and N-12.

C	H
4	2; 3 or 5
3 or 5	2
6	2; 3 or 5; 4; 8; 13
7	3 or 5; 8; 13
9	6 or 11; 8; 10 or 13; 14
10	6 or 11; 8; 21
11	10 or 13; 14
13	2; 8
15	6 or 11; 14; 16
18	20; 21
21	20
23	20; 21

Table 2. Pertinent HMBC correlations of panacosmine (**3**).

It is apparent from structure **3** that the explanation for the presence of an equilibrium mixture of two compounds lies in the restricted rotation about the amide bond C-23/N-22. The more stable form is represented in **3**. The vinyl proton of the minor rotamer is strongly deshielded ($\Delta\delta = 0.6$ ppm) by the carbonyl group. The relative configurations of C-7 and C-9 are fixed by the bicyclic system and are drawn the same way as in angustifoline. This also determines the configurations of C-2 and C-6. Correlations in the ROESY spectrum between H-2 and H-21 and between H-2 and H-11 suggest that the configurations at C-10 and C-11 are *R* and *S* respectively. The configuration at C-11 is the same as in angustifoline. The presence of a substituent at C-10 is novel in the lupanine related quinolizidine alkaloids.

The EIMS spectrum of the fourth new compound, 4α-angeloyloxy-3β-hydroxy-13β-methoxylupanine **4**, showed an $[M]^+$ ion at *m/z* 392. The ^{13}C and HMQC spectra indicate the presence of 21 carbons and at least

31 protons. A molecular formula $C_{21}H_{32}N_2O_5$ was assumed. Characteristic signals in the ^1H NMR spectrum (δ 1.9, 2.0, and 6.1), in the ^{13}C NMR spectrum (δ 15.7, 20.4, 127.4, 138.7, 167.1), and in the correlation spectra indicate the presence of an angelate group. From analysis of the 2D maps it was possible to trace the entire skeleton of lupanine, but with oxidised positions at C-3, C-4, and C-13. The HMBC spectrum enabled the placement of the angeloyloxy group at C-4, and the methoxy group (δ_H 3.35; δ_C 55.3) at C-13. Such an oxidation pattern is already described in the literature. The proton H-3 (δ 4.15, d, J = 10 Hz) and its coupling partner H-4 (δ 5.05, J = 4, 10, 12 Hz) are both axial. The ROESY spectrum shows that H-6 is β, on the same side as the bridging C-8. Therefore, with ring A in a normal chair conformation, the substituents at C-3 and C-4 are β and α respectively. The axial position of H-11 (δ 1.95) is deduced from its coupling pattern (J = 10 Hz), measured in the corresponding row in the HMQC spectrum. Similarly H-13 (δ 3.15, J = 4, 10, 12 Hz) is axial and hence the methoxy group has a β orientation.

In conclusion, the seeds of *Acosnium panamense* contain rearranged lupanine alkaloids in which the nitrogen atoms are both bonded to the same carbon atom. The resulting molecules display a novel diaza-adamantane ring system. Moreover, the lupanine skeleton is functionalised at C-10 by a fragment bearing an N-acetyl enamine chromophore. The structural analysis was assisted by means of the LSD program and supported by 2D ^1H-^{15}N HMBC spectra.

EXPERIMENTAL

Spectroscopy: ^1H NMR (500 MHz) and ^{13}C NMR (125 MHz) were recorded at room temperature with a Bruker DRX spectrometer with an inverse multinuclear 5 mm probehead equipped with a shielded gradient coil. The spectra were recorded in $CDCl_3$, and the solvent signals (7.27 and 77.0 ppm, respectively) were used as reference. The chemical shifts (δ) are given in ppm, and the coupling constant (J) in Hz. COSY, HMQC and HMBC experiments were recorded with gradient enhancements using sine shaped gradient pulses. For the 2D heteronuclear correlation spectroscopy the delays of coupling evolution were optimised for $^1J_{CH}$ = 145 Hz and $^nJ_{CH}$ = 7 Hz. In ROESY measurements the spin-lock duration was set to 250 ms. Raw NMR data were acquired using standard pulse programs. Processing was performed by the xwinnmr 2.0 software. Mass (EIMS: 70 eV), IR, and UV spectra were recorded on VG-Micromass ZAB2-SEQ, Beckman AccuLab 4, and Philips PU 8720 spectrometers, respectively. Optical rotations were measured with a Perkin-Elmer 241 polarimeter.

Plant material: *Acosnium panamense* was collected in Lower Congo and a voucher specimen, H. Breyne 4148, has been deposited in the National Botanic Garden in Brussels (Belgium).

Extraction and isolation: Ground seeds (0.85 kg) were defatted by 18 L of petroleum ether. The remaining solid material was dried, macerated in 420 mL of aq. NH_3 (12 M) and lixiviated by 24 L of AcOEt. The basic compounds were extracted with aq. H_2SO_4 (0.33 M). The resulting solution was basified with aq. NH_3 (12 M) and the alkaloids extracted with $CHCl_3$. The organic solution was dried over Na_2SO_4. Distillation of the solvent left a crude extract (6.97g, yield 8.2 g/kg). The alkaloid mixture was separated by chromatography on silica gel columns. TLC analyses were made on Merck Kieselgel 60 F_{254} plates. Compounds were eluted with $CHCl_3$ followed by $CHCl_3$-MeOH mixtures of increasing polarity. Fractions (100 mL) were collected,

analysed by TLC, and grouped according to their composition. The elution order was **4** (fractions 7-10, 1.5 %), lupanine (f. 16-21, 5.5 %), multiflorine and **3** (f. 25-41, 2 and 29 %), **2** (f. 48-55, 9%), **1** (f. 80-94, 1.5 %). Multiflorine and **3** were separated from the collected fractions using the same chromatographic conditions.

Acosmine (1) was obtained as a yellow amorphous solid (0.2 g). $[\alpha]_D$ -10.8 ($CHCl_3$, *c* 1.29); UV λ_{max} (MeOH) 241.6 and 290 (sh) nm; IR ν_{max} (film from $CHCl_3$) 3445, 3312, 3019, 2934, 1663, 1499, 1371, 1256, 1215, 762 cm^{-1}; ^1H NMR: δ_H = 7.4 (1H, d, J = 11 Hz, H-22), 6.6 (1H, d, J = 11 Hz, H-21), 5.7 (1H, m, H-15), 5.05 (2H, m, H-16), 4.4 (1H, d, J = 8 Hz, H-20), 3.75 (1H, s, H-10), 3.55 (2H, t, J = 6.5 Hz, H-2), 3.35 (1H, t, J = 7 Hz, H-11), 3.2 (2H, AB, J = 14.5 Hz, H-13), 2.9 (1H, t, J = 6.5 Hz, H-6), 2.4 (2H, m, H-14 and H-18), 2.35 (1H, m, H-8), 2.3 (2H, m, H-14 and H-18), 2.25 (1H, m, H-19), 2.0 (3H, s, H-24), 1.85 (1H, m, H-8), 1.75 (1H, m, H-5), 1.7 (1H, m, H-19), 1.6 (1H, m, H-5), 1.55 (2H, m, H-3), 1.4 (2H, m, H-4), 1.25 (1H, s, H-9), 1.15 (1H, s, H-7); ^{13}C NMR: listed in Table 1; EIMS *m/z* (rel. int.): 359 ($[M]^+$, 20), 318 (100), 151 (25), 109 (30); HRMS: calcd for $C_{21}H_{33}N_3O_2$ (M+) 359.2573 found 359.2596.

Acosmine acetate (2) was obtained as a yellow amorphous solid (0.4 g). $[\alpha]_D$ -15.7 ($CHCl_3$, *c* 0.86); UV λ_{max} (MeOH) 241.9 and 290 (sh) nm; IR ν_{max} (film from $CHCl_3$) 3391, 2924, 2855, 2633, 1734, 1651, 1521, 1454, 1369, 1254, 1034, 918 cm^{-1}; ^1H NMR: δ_H = 7.4 (1H, d, J = 10 Hz, H-22), 6.6 (1H, d, J=10 Hz, H-21), 5.65 (1H, m, H-15), 5.0 (2H, m, H-16), 4.45 (1H, d, J = 8 Hz, H-20), 4.0 (2H, m, H-2), 3.8 (1H, s, H-10), 3.45 (1H, t, J = 7 Hz, H-11), 3.25 (2H, AB, J = 14.5 Hz, H-13), 2.9 (1H, t, J = 7 Hz, H-6), 2.4 (2H, m, H-14 and H-18), 2.35 (1H, m, H-8), 2.3 (2H, m, H-14 and H-18), 2.25 (1H, m, H-19), 2.05 (3H, s, H-24), 2.0 (3H, s, H-26), 1.85 (1H, d, J = 14 Hz, H-8), 1.75 (1H, m, H-19), 1.65 (1H, m, H-5), 1.6 (3H, m, H-5, H-3), 1.35 (2H, m, H-4), 1.3 (1H, s, H-9), 1.2 (1H, s, H-7); ^{13}C NMR: listed in Table 1; ^{15}N NMR: δ_N = -248 (N-22), -327 (N-12), -332 (N-1); EIMS *m/z* (rel. int.): 401 ($[M]^+$, 22), 360 (100), 342 (6), 300 (12), 151 (8), 109 (13); HRMS: calcd for $C_{23}H_{35}N_3O_3$ (M+) 401.2678 found 401.2669.

Panacosmine (3) was obtained as a yellow amorphous solid (1.2 g). $[\alpha]_D$ +45 ($CHCl_3$, *c* 1); IR ν_{max} (film from $CHCl_3$) 3293, 3074, 2941, 1661, 1408, 1300, 1194, 987, 752 cm^{-1}; UV λ_{max} (MeOH) 245, 318 nm; ^1H NMR: δ_H = 6.6 (1H, s, H-21), 5.75 (1H, dddd, J = 15.3, 9.8, 8.1, 7.3 Hz, H-15), 5.1 (1H, bd, J = 15.3 Hz, H-16a), 5.05 (1H, bd, J = 9.8 Hz, H-16b), 4.15 (1H, d, J = 6.8 Hz, H-2), 3.78 (1H, ddd, J = 12.8, 6.9, 3.8 Hz, H-20a), 3.67 (2H, m, H-13a, H-10), 3.48 (1H, ddd , J = 12.8, 8.8, 3.6 Hz, H-20b), 3.15 (2H, m, H-6, H-11), 3.0 (1H, dd, J = 14.4, 1.2 Hz, H-13b), 2.58 (1H, ddd, J = 13.5, 10.0, 9.1 Hz, H-14a), 2.42 (1H, dt, J = 9.9, 3.3 Hz, H-8a), 2.32 (1H, dddt, J = 13.5, 6.0, 4.5, 1.5 Hz, H-14b), 2.20 (1H, m, H-5a), 2.12 (3H, s, H-24), 2.0 (3H, m, H-3a, H-4a, H-18a), 1.85 (4H, m, H-3b, H-8b, 18b, H-19a), 1.75 (3H, m, H-4b, H-5b, H-19b), 1.65 (1H, bs, H-9), 1.30 (1H, bs, H-7); ^{13}C NMR: listed in Table 1; ^{15}N NMR: δ_N = -242 (N-22, major), -246 (N-22, minor), –325 (N-12), -334 (N-1); FAB glycerol *m/z* (rel. int.): 342 ($[M]^+$+1, 100), 300 (47), 217 (8), 190 (7), 164 (6), 146 (13), 134 (12), 122 (20), 108 (12), 96 (18), 82 (28); HRMS: calcd for $C_{21}H_{31}N_3O$ (M+) 341.2467 found 341.2425.

4α-Angeloyloxy-3β-hydroxy-13β-methoxylupanine (4) was obtained as a yellow amorphous solid (0.1 g). $[\alpha]_D$ -72 ($CHCl_3$, *c* 0.57); IR ν_{max} (film from $CHCl_3$) 3422, 2924, 2853, 2816, 2770, 1714, 1643,

1452, 1228 cm^{-1}. ^1H NMR: δ_H = 6.1 (1H, qq, J = 7.3, 1.5 Hz, H-3'), 5.05 (1H, ddd, J = 4, 10, 12 Hz, H-4), 4.35 (1H, dt, J = 13.5, 2.2 Hz, H-10), 4.15 (1H, d, J = 10 Hz, H-3), 3.5 (1H, ddd, J = 11.5, 5, 2 Hz, H-6), 3.35 (3H, s, H-18), 3.15 (1H, tt, J = 11, 4.5 Hz, H-13), 2.95 (1H, dd, J = 12, 8 Hz, H-17), 2.75 (1H, m, H-15), 2.7 (1H, m, H-10), 2.2 (1H, m, H-5), 2.1 (2H, m, H-8 and H-15), 2.0 (1H, m, H-17), 2.0 (3H, dq, J = 1, 7.3 Hz, H-4'), 1.95 (1H, m, H-11), 1.9 (1H, m, H-7), 1.9 (3H, d, J = 1 Hz, H-5'), 1.8 (1H, m, H-14), 1.75 (2H, m, H-12 and H-5), 1.7 (1H, m, H-9), 1.4 (1H, m, H-14), 1.35 (1H, m, H-12), 1.3 (1H, m, H-8); ^{13}C NMR: listed in Table 1; EIMS *m/z* (rel. int.): 392 ($[M]^+$, 65), 377 (15), 361 (30), 293 (100), 275 (95), 261 (50), 243 (20), 233 (15), 221 (10), 207 (10), 191 (5), 148 (40), 134 (40), 114 (45); HRMS: calcd for $C_{21}H_{32}N_2O_5$ (M+) 392.2311 found 392.2324.

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