3β-(Stearyloxy)olean-12-ene from *Austroplenckia populnea:* Structure Elucidation by 2D-NMR and Quantitative ^{13}C-NMR Spectroscopy

by **S. A. Vieira Filho**[a])[b]), **L. P. Duarte**[b]), **G. D. F. Silva***[b]), **O. W. Howarth**[c]), and **I. S. Lula**[b])

[a]) DEFAR, Escola de Farmácia, Universidade Federal de Ouro Preto, Rua Costa Sena 171, CEP 35400-000, Ouro Preto, Minas Gerais, Brazil

[b]) NEPLAM, Departamento de Química, ICEx,Universidade Federal de Minas Gerais, Av. Antônio Carlos 6627, Pampulha, CEP 31270-901, Belo Horizonte, Minas Gerais, Brazil (e-mail: gdfsqui@dedalus.lcc.ufmg.br)

[c]) Department of Chemistry, University of Warwick, Coventry, CV4 7AL, UK

3β-(Stearyloxy)olean-12-ene was isolated from a hexane extract of *Austroplenckia populnea* Reiss (Celastraceae) leaves. The structure was solved by means of quantitative ^{13}C-NMR, HMBC, HMQC, COSY, NOESY, and NOE difference spectra. The mass spectrum showed an $[M+1]^+$ ion peak at *m/z* 693, and the molecular formula $C_{48}H_{84}O_2$ was confirmed by combustion analysis.

Introduction. – We are interested in pentacyclic triterpenes of '*Mangabarana*', '*Mangabeira-brava*' or '*Marmelinho-do-campo*', popular names of *Austroplenckia populnea* Reiss (Celastraceae) in Minas Gerais, Brazil. This plant is used in Brazilian folk medicine as an antidysenteric [1] and antirheumatic [2] agent. We have already reported the structure determination of 3,4-*seco*-friedelan-3-oic acid, 3,4-*seco*-28-hydroxyfriedelan-3-oic acid [3], 3β-hydroxyolean-12-en-29,22α-olide [4], and a polyester sesquiterpene, 4-hydroxy-1,2,6,15-tetraacetyl-9-benzoylagarofuran [5]. Phytochemical studies regarding the constituents of this plant yielded a further pentacyclic triterpenoid: 3β-(stearyloxy)olean-12-ene (**1**).

The present paper corroborates the structure and configuration of this compound by 1D NMR and 2D shift-correlated NMR experiments, *i.e.*, HMQC [^1J(C,H)], HMBC [^nJ(C,H); $n=2$ and 3]. 2D ^{1}H-NMR, ^{1}H-NOESY, and COSY were used for complete ^{1}H- and ^{13}C-chemical-shift assignments. The mass spectrum of **1** showed the $[M+1]^+$ peak at *m/z* 693. By quantitative ^{13}C-NMR, the molecular formula was established as $C_{48}H_{84}O_2$ and confirmed by combustion analysis.

Results and Discussion. – A positive *Liebermann–Burchard* test and IR absorptions at 2920, 2850, 1450, and 1720 cm^{-1} suggested that the compound is a pentacyclic triterpene with a C=O group. The presence of a single methine H at δ(H) 5.19 (t, $J=3.5$ Hz), signals at δ(C) 121.77 (CH) and 145.21 (C) (see *Table*), plus a DEPT experiment together with the occurrence of a diagnostically important MS peak at *m/z* 218 (50%) arising from a retro-*Diels–Alder* fragmentation, indicated an oleanane compound of Δ^{12}-β-amyrin type [6].

Signals at δ(C) 80.62 and δ(H) 4.52 suggested an ester group. 2D Connectivity and ^{1}H/^{1}H coupling were consistent with an assignment to C(3), with H–C(3) being axial. Intense methylene signals at δ(C) 29.72 and δ(H) 1.26, together with signals at δ(C)

Table. *^{1}H- and ^{13}C-NMR Spectral Data of 3β-(Stearyloxy)olean-12-ene* (**1**) *in $CDCl_3$/(D_5)pyridine* (T = 300 K)

Atom	δ_C [ppm][a])	δ_H [ppm][b])	HMBC	Atom	δ_C [ppm][c])	δ_H	HMBC
1	38.38 (38.60)	1.07ax, 1.61eq		1′	173.54	–	
2	23.69 (26.95)	1.64	3	2′	34.87 (34.20)	2.30 (*t*, *J* = 7.4)	1′,3′,4′
3	80.62 (79.05)	4.52 (*dd*, *J* = 8.5, 7.5)	1′, 1, 2, 4, 23, 24	3′	25.21 (24.75)	1.63	
4	37.83 (38.80)	–		4′	29.21 (28.98)	1.29	
5	55.40 (55.18)	0.80		5′	29.28 (29.20)	1.29	
6	18.35 (18.39)	1.43ax, 1.56eq		6′	29.37 (29.35)	1.26	
7	32.71 (32.66)	1.36ax, 1.52eq		7′	29.61 (29.65)	1.26	
8	39.92 (39.80)	–		8′	29.68 (29.76)	1.26	
9	47.67 (47.65)	1.60		9′	29.70 (29.78)	1.28	
10	36.94 (36.96)	–		10′	29.72 (29.79)	1.28	
11	23.61 (23.55)	1.86	12	11′	29.72 (29.80)	1.26	
12	121.77 (121.74)	5.19 (*t*, *J* = 3.5)	9, 11, 14, 18	12′	29.72 (29.80)	1.26	
13	145.21 (145.22)	–		13′	29.72 (29.80)	1.26	
14	41.82 (41.73)	–		14′	29.66 (29.77)	1.26	
15	26.24 (26.17)	0.96eq, 1.78ax		15′	29.49 (29.47)	1.26	
16	27.04 (27.25)	2.00ax, 0.74eq	28	16′	31.95 (32.04)	1.26	
17	32.54 (32.51)	–		17′	22.70 (22.80)	1.26	
18	47.36 (47.24)	1.94	12	18′	14.09 (14.21)	0.88	17′, 16′
19	46.90 (46.84)	1.03eq, 1.76ax	–				
20	31.10 (31.11)	–	–				
21	34.83 (34.75)	1.10ax, 1.39eq	–				
22	37.24 (37.15)	1.22eq, 1.46ax	–				
23	28.13 (28.11)	0.88	3, 4, 5, 24				
24	16.82 (15.60)	0.88	3, 4, 5, 23				
25	15.57 (15.52)	0.96	1, 5, 9, 10				
26	16.90 (16.82)	0.97	7, 8, 9, 14				
27	26.00 (26.01)	1.14	8, 13, 14, 15				
28	28.43 (28.42)	0.84	16, 17, 18, 22				
29	33.35 (33.36)	0.88	19, 20, 21, 30				
30	23.75 (23.71)	0.88	19, 20, 21, 29				

[a]) In parentheses, the literature data [6][7] for β-amyrin are given. [b]) The terms *ax* and *eq* denote *axial vs. equatorial* positions (see the *Figure*). [c]) In parentheses, the ^{13}C-NMR literature data for a linear carbon chain are given [8][9][10].

173.54 (CO) and δ(H) 2.30 (CH_2), suggested the presence of an alkyl-chain ester group.

Initial analysis of the HMBC data confirmed that the signal at δ_H 4.52 for H−C(3) is commonly found in oleananes [6]. It correlates with the C=O signal at δ(C) 173.54, and with the two Me groups at δ(C) 28.13 (C(23)) and 16.82 (C(24)). The C=O signal was also coupled with a signal at δ(H) 2.30. All these observations confirmed the presence of a β-amyrin moiety having C(3) attached to a long-chain ester.

A ^{13}C-NMR spectrum was obtained with a pre-pulse delay of 10 s to permit full relaxation of all C-atoms. Integration and DEPT data revealed the presence of 48 C-atoms: nine Me, 26 CH_2, five CH, and eight quaternary C-atoms. This supported the proposed structure of 3β-(stearyloxy)olean-12-ene (3β-stearyloxy-β-amyrin).

HMQC Experiments confirmed the assignments of C(3) (δ(C) 80.62, (δ(H) 4.52), C(2)/(δ(C) 34.87, δ(H) 2.30), and C(12) (δ(C) 121.77, δ(H) 5.19). Then, HMBC correlations were used for a full ^{13}C assignment.

H–C(12) (δ(H) 5.12) correlated with δ(C) 47.67 (C(9)), 47.36 (C(18)), 41.82 (C(14)), and 23.61 (C(11)). The signal at δ(C) 41.82 (C(14)) correlated with those at δ(H) 1.14 and 0.97 (H–C(27) and/or H–C(26)). The signal at δ(H) 1.14 correlated with those at δ(C) 39.92 (C(8)), 26.24 (C(15)), and 145.21 (C(13)), and so, this H signal must arise from Me(27) (δ(C) 26.00).

The resonance at δ(H) 0.97 correlated with the signals at δ(C) 47.67 (C(9)), 41.82 (C(14)), 39.93 (C(8)), and 32.71 (C(7)). These correlations demonstrated that the H-atom at δ(H) 0.97 corresponds to Me(26) (δ(C) (16.90).

C(8) (δ(C) 39.92) also coupled with H–C(25) (δ(C) 15.97, δ(H) 0.96), and the latter with C(1) (δ(C) 38.38), C(5) (δ(C) 55.40), C(9) (δ(C) 38.38), and C(10) (δ(C) 36.94).

C(18) (δ(C) 47.36) correlated with H–C(28) (δ(C) 28.43, δ(H) 0.84), and the latter with C(17) (δ(C) 32.54), C(16) (δ(C) 27.04), and C(22) (δ(C) 37.24).

H–C(3) (δ(H) 4.52) coupled with C(24) (δ(C) 16.82), C(2) (δ(C) 23.69), C(23) (δ(C) 28.13), C(4) (δ(C) 37.83), C(1) (δ(C) 38.38), and C(1′) (δ(C) 173.54). These data suggested that the ester group was attached to C(3).

H–C(2′) (δ(H) 2.30) coupled, as expected, with C(3′) (δ(C) 25.21), C(4′) (δ(C) 29.28), and C(1′) (δ(C) 173.54).

The COSY spectrum confirmed the assignment of H–C(2′) to δ(H) 1.63 (δ(C) 25.21).

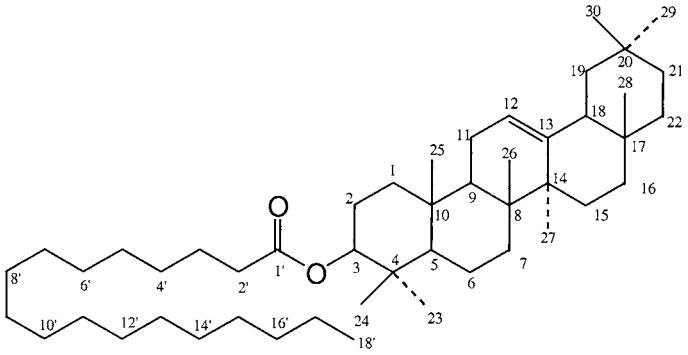
HMQC Experiments showed that the complex peak cluster at δ(H) 0.88 arose from five Me groups, namely Me(18′), Me(23), Me(24), Me(29), and Me(30).

Comparison with literature data [6][7] for β-amyrin also confirmed the following assignments: C(6) (δ(C) 18.35), C(19) (δ(C) 46.90), C(20) (δ(C) 31.10), C(21) (δ(C) 34.83), C(29) (δ(C) 33.35), and C(30) (δ(C) 23.75).

The NOESY spectra showed NOEs between H–C(18) and H–C(22), H–C(28), and H–C(30); between H–C(3) and equatorial H–C(2), axial H–C(1), H–C(23), and H–C(5); between equatorial H–C(11) and equatorial H–C(1) and H–C(25); between H–C(12) and H–C(11) and H–C(13); and also between H–C(23) and axial H–C(15), axial H–C(22), and equatorial H–C(22). Thus, these data confirmed the configuration of the molecule. Selected NOESY correlations are shown in the *Figure*.

In the NOE difference spectra, the intensity enhancement of both H–C(26) (δ(H) 0.97) and equatorial H–C(16) (δ(H) 0.80) was observed upon irradiation of axial H–C(15) (δ(H) 1.78). Moreover, on irradiation of axial H–C(16) (δ(H) 2.00) and H–C(18) (δ(H) 1.94), NOE enhancement was observed for H–C(27), equatorial H–C(16), H–C(30), H–C(28), and H–C(12). By irradiation of H–C(28) (δ(H) 0.84) together with H–C(5) (δ(H) 0.80), it was possible to observe NOEs for H–C(3) and H–C(18). Irradiation of H–C(3) (δ(H) 4.52) showed NOEs for equatorial H–C(2), H–C(23), axial H–C(1), and H–C(5).

HMBC Experiments showed that H–C(18′) is coupled with C(17′) (δ(C) 22.70) and C(16′) (δ(C) 31.95). The remaining C-atoms of the aliphatic side chain were assigned according to literature data [8][9][10] and using the ACD software [11]. Extrapolations were made for C(5′), C(10′), and C(11′).



1

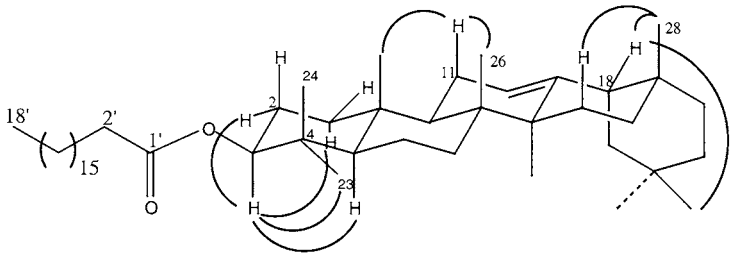


Figure. *Structure of 3β-(stearyloxy)olean-12-ene* (**1**) *and selected NOESY correlations*

The chemical shifts of C(2), C(3), C(4), and C(24) showed the expected small shift differences relative to β-amyrin, resulting from the esterification at C(3).

The chemical shifts for all C- and H-atoms of 3β-(stearyloxy)-β-amyrin, deduced experimentally and from literature data [6][7][8][9][10], and those for the linear alkyl chain attached to C(3) are given in the *Table*. Careful integration of the ^{13}C-NMR resonances revealed the presence of 48 C-atoms, corresponding to the molecular formula $C_{48}H_{84}O_2$, which was further confirmed by combustion analysis.

Experimental Part

General. M.p.: *Mettler FP-80-HT* (uncorrected). IR (KBr): *Shimadzu IR-408* spectrophotometer. CI-MS (2-methylpropane): *Finnigan MAT CGQ* spectrometer. Elemental anal.: *FISON EA1108 CHNS-0* instrument.

NMR Spectra. The NMR spectra were recorded on a *Bruker DRX-400* AVANCE spectrometer operating at 400 or 100 MHz at 300 K, equipped with a direct-detection 5-mm dual probe head (90° pulse widths of 14.3 and 9.3μs for ^{1}H and ^{13}C, resp.). The solvent was $CDCl_3$ with two drops of (D_5)pyridine. Approximately 5–10 mg of **1** was dissolved in 0.5 ml of this solvent and transferred to a 5-mm tube, with $SiMe_4$ as internal standard. One-dimensional (1D) ^{1}H- and ^{13}C-NMR spectra were acquired under standard conditions. ^{1}H-NMR spectra were obtained with a sweep width of 4,166.7 Hz over 32,768 data points and processed with a *Gaussian* function (LB = – 0.3, GB = 0.2). ^{13}C-NMR spectra were obtained using a sweep width of 31,847 Hz. Quantitative NMR experiments were carried out at 300 K, with the same direct-detection 5-mm dual probe, using a standard inverse-gated decoupling pulse program to effect NOE suppression. A total of 5,120 transients with 65,000 data points were collected at a spectral width of 32,000 Hz and a 10.0-s delay between two pulses to permit full

relaxation. The total experiment time was 16 h. The FID was processed with 32,000 data points and multiplied by an exponential factor corresponding to a 1-Hz line broadening prior to *Fourier* transformation. Two-dimensional (2D) inverse (proton-detected) heteronuclear shift-correlation spectra were obtained using the same direct probe (90° pulse widths of 14.3 and 7.0 μs for ^{1}H and ^{13}C, resp.). For HMQC (detecting ^1J (C,H)), 512 time increments were collected for each data set, with zero-filling to 2,048. Eight transients were collected for each time increment with a 2.0-s relaxation delay. The same conditions (but with 16 transients) were used for HMBC (detecting ^nJ (C,H); $n = 2$ and 3). Sine and squared sine-bell windows were used for processing the second and first dimensions, respectively. Standard pulse sequences were used for the 2D ^{1}H × ^{1}H-NOESY experiment, with a 400-ms mixing time. The F_1 dimension was 6,410.3 Hz with 2,048 data points. Sixteen transients were collected for each time increment and processed in each dimension with a sine-bell window. Selected 1D-NMR difference spectra were carried out to confirm the NOESY data. The same spectral width was used, a 70-dB power level for the NOE build-up, 50 cycles through each frequency list to determine overall irradiation time, and ten average cycles to improve the signal-to-noise rati. Two 2D ^{1}H × ^{1}H-COSYGR (using shim-coils-generated gradient pulses for selection) were carried out with different spectral widths to increase the resolution in the contour maps. In the full-sweep-width experiment, the spectral windows were 4,800 Hz with 1,000 data points. In the optimized experiment, the spectral width was reduced to 920 Hz (1,000 data points), resulting in a total experiment time of 2.5 h for either experiment. Sine bells were applied in both dimensions. Data processing was finally carried out on an *SGI* workstation with *Bruker DRX-400* microprograms.

Plant Material and Extraction. *Austroplenckia populnea* Reiss (Celastraceae) was collected in the Nova Lima Region, Minas Gerais State, Brazil. The plants were identified by Dr. *José Luiz Pedersoli*. A voucher specimen was deposited at the Herbarium of the Natural History Museum of Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil (Collection No. 10473). The cleaned leaves were dried at r.t. and ground to a powder (178.6 g), which was submitted to hexane extraction in a *Soxhlet* apparatus for 24 h. The weight of extract after solvent evaporation *in vacuo* was 26.8 g. The hexane extract was submitted to CC (*Merck* SiO_2, 200–300 Mesh, 1 kg), eluting with hexane, $CHCl_3$, AcOEt, and MeOH, pure or in mixtures of increasing polarity, yielding 275 fractions. Fractions 50–53 afforded a solid amorphous material. After recrystallization from $CHCl_3$/EtOH 4:1, **1** was obtained as a white solid (76.8 mg).

3β-(Stearyloxy)olean-12-ene (**1**). White powder. M.p. 47–49° (hexane). IR (KBr): 3950, 3850, 1730, 1470, 1380, 1360, 1260, 1250, 1190, 1100, 990, 810, 720, 650. ^{1}H- and ^{13}C-NMR data: see the *Table*. CI-MS: 693 (0.4, $[M+1]^+$), 426 (2.2, $[M - C_{17}H_{35}CO]^+$), 408 (38.5, $[M - C_{17}H_{35}COOH]^+$ (*McLafferty*), 249 (48.2), 235 (46.4), 218 (82.9), 217 (100.0), 203 (80.1). Anal. calc. for $C_{48}H_{84}O_2$ (693.19): C 83.17, H 12.21, O 4.62; found: C 83.61, H 12.47, O 3.92.

The authors thank *Conselho Nacional de Desenvolvimento Cientifico e Tecnológico* (*CNPq*) and *Coordenação de Aperfeiçoamento Pessoal de Ensino Superior* (*CAPES*) for financial support and *LARE-MAR-UFMG* (*Laboratório de Ressonância Magnética de Alta Resolução*) for recording NMR spectra.

REFERENCES

[1] M. P. Corrêa, 'Dicionário das Plantas Úteis do Brasil e das Plantas Exóticas Cultivadas', Imprensa Nacional, Rio de Janeiro, 1926–1978.
[2] A. G. Gonzalez, I. L. Bazzocchi, X. A. Domingues, A. G. Ravelo, J. G. Luis, G. Vasquez, G. Cano, *Rev. Latinoam. Quim*. **1987**, *18*, 83.
[3] S. A. Vieira Filho, L. P. Duarte, G. D. F. Silva, I. S. Lula, M. H. Santos, *Magn. Reson. Chem*. **2001**, *39*, 746.
[4] G. D. F. Silva, L. P. Duarte, H. C. S. Paes, J. R. Sousa, M. C. Nonato, P. J. Portezani, Y. P. Mascarenhas, *J. Braz. Chem. Soc*. **1998**, *9*, 461.
[5] S. A. Vieira Filho, L. P. Duarte, M. H. Santos, G. D. F. Silva, I. S. Lula, R. J. C. F. Afonso, *Magn. Reson. Chem*. **2000**, *38*, 1023.
[6] S. B. Mahato, A. P. Jundi, *Phytochemistry* **1994**, *37*, 1517.
[7] A. Patra, S. K. Chaudhuri, A. K. Acharyya, *Magn. Reson. Chem*. **1990**, *28*, 85.
[8] E. Bengsch, B. Perly, C. Deleuze, A. Valero, *J. Magn. Reson*. **1986**, *68*, 1.
[9] G. Bianchi, O. W. Howarth, C. J. Samuel, G. Vlahov, *J. Chem. Soc., Perkin Trans. 2* **1995**, 1427.
[10] O. W. Howarth, C. J. Samuel, G. Vlahov, *J. Chem. Soc., Perkin Trans. 2* **1995**, 2307.
[11] Advanced Chemistry Development, Inc., ACD/CNMR Version 1.1, 1995.

Received July 2, 2003