Triterpenoids Isolated from *Parahancornia amapa*

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Do extrato diclorometânico da casca e do látex de um espécime de *Parahancornia amapa*, família Apocynaceae, foram isolados e identificados os triterpenos pentacíclicos lupeol (**1**), β-amirina (**6**) e α-amirina (**8**), seus derivados acetilados **2**, **7** e **9**, ácidos alifáticos e outros ésteres 3-O-acil-lupeol (**3-5**). As estruturas destas substâncias naturais foram determinadas através de análise de dados espectrométricos, destacando-se os resultados fornecidos por técnicas modernas de RMN (DEPT, ^1H x ^1H-HOMOCOSY e ^1H x ^{13}C-HETECOSY).

From the dichloromethane extract of the bark and latex of *Parahancornia amapa*, family Apocynaceae, were isolated and identified the pentacyclic triterpenes lupeol (**1**), β-amyrin (**6**) and α-amyrin (**8**), their acetyl derivatives **2**, **7** and **9**, aliphatic acids and other 3-O-acyl-lupeol esters (**3-5**). The structures of these natural products have been established by spectroscopic data, including NMR (DEPT ^1H x ^1H-HOMOCOSY and ^1H x ^{13}C-HETECOSY).

Key words: Parahancornia amapa, *Apocynaceae, pentacyclic triterpenes.*

Introduction

Parahancornia amapa (Huber) Ducke, family Apocynaceae is a tree which occurs in Amapá State in the Brazilian Amazonian region, where it as commonly called "amapá" and the bark and latex are known to natives as a tonic and anti-syphilis[1]. The specimen examined in the present report was collected near Macapá.

In this paper we report the structures of three new pentacyclic triterpenes 3-O-acyl lupeol esters (**3-5**), along with seven others (**1, 2, 6-10**) previously reported, isolated from the bark and latex of a specimen of this plant.

Experimental

General Experimental Procedures. Melting points were determined using a Kofler hot stage instrument and are uncorrected. IR spectra were recorded as KBr discs on a Perkin-Elmer 1420 spectrometer. ^1H and ^{13}C NMR spectra were measured in $CDCl_3$, using TMS as internal standard, employing a Bruker AC-200 (^1H: 200 MHz; ^{13}C: 50.3 MHz) spectrometer. Low resolution mass spectra were obtained on a Hewllet Packard-5890/5988A GC/MS instrument operating at 70 eV. Silica gel was used for TLC (0.1 mm thick) and CC.

Plant material: Bark and latex of *Parahancornia amapa* were collected and identified by botanist Benedito Vitor Rabello in Amapá State, Brazil, and a voucher specimen (nº 07231) was deposited in the herbarium (Herbário Amapaense, HAMAB) of the Divisão de Botânica, Museu Ângelo Moreira da Costa Lima, Macapá-AP, Brasil.

Extraction and isolation of constituents. The pulverized, air-dried bark material (2.47 kg) was percolated with CH_2Cl_2 at room temperature and the extract was concentrated *in vacuo* to afford 86 g of residue. This material was successively recrystallized from benzene-acetone to yield crystalline fractions A (450 mg), B (850 mg), C (644 mg), D (180 mg), E (80 mg) and F (3.6 g). Fraction A was methylated with CH_2N_2 (ether), furnishing a mixture of methyl esters mainly composed of compound **11a**. Fraction B was examined by TLC (hexane -EtOAc, 9:1) and revealed the presence of **11**, along with compounds **4** and **10**. Fraction C was chromatographed on a silica gel column (120 g) and eluted with hexane gradually enriched with EtOAc to furnish 166 fractions of 10 ml each; fractions 2-30, 47-48, 59-83 [after prep-TLC (hexane-EtOAc, 8:2)] and 144-166 furnished **3** (50 mg), **4** (40 mg), a mixture of **1** + **6** + **8** (80 mg) and **5** (200 mg), respectively. TLC (hexane-EtOAc, 9:1) of fraction D showed the presence of compounds **4** and **10**. Fraction E was recrystallized from benzene to afford **10** (50 mg). Fraction F (1.0 g) was chromatographed on a silica gel column (50 g) and eluted with benzene gradually enriched with EtOAc to provide 40 fractions of 10 ml each, yielding additional quantities of compounds **3** (560 mg) and **4** (375 mg) from fractions 2-16 and 25-40, respectively.

The latex (90 g) was treated with MeOH (200 ml) to yield 67 g of insoluble material. This material (33 g) was chromatographed on silical gel (255 g) and eluted with $CHCl_3$ gradually enriched with MeOH to afford 216 fractions of 100 ml each: fraction 7 (605 mg) was rechromatographed on a silica gel column, using hexane $CHCl_3$ (1:1) as eluent, to furnish **3** (60 mg) and a mixture of **2** + **7** + **9** (300 mg); the fractions 9-11 was recrystallized from benzene and acetone, affording a mixture of **1** + **6** + **8** (747 mg); the fraction 91-96 furnished **5** (648 mg), after recrystallization from acetone.

*Lupeol (**1**), β-amyrin (**6**) and α-amyrin (**8**).* Mp 150-152°C (benzene acetone). The presence of these three triterpenes as a mixture was revealed by spectral data, mainly ^{13}C NMR (proton noise decoupled and DEPT) through chemical shifts and multiplicity of the signals of carbon atoms and comparison with those of the corre-

Table 1. ^{13} *CNMR spectral data a of compounds 1-5*

C	1	2	3	4	4A	5	5A	5B
1	38.67	38.56	38.34	38.37	37.94	38.26	38.32	38.34
2	27.37	23.61	23.72	23.74	23.59	23.65	23.67	23.70
3	78.97	80.68	80.81	81.33	81.24	81.40	81.49	80.97
4	38.83	37.68	37.79	37.78	38.27	37.70	38.01	38.00
5	55.26	55.29	55.35	55.36	55.31	55.26	55.36	55.38
6	18.29	17.95	18.18	18.21	18.12	18.12	18.17	18.16
7	34.25	34.17	34.19	34.20	34.11	34.12	34.18	34.16
8	41.07	40.77	40.79	40.82	40.73	40.74	40.83	40.79
9	50.41	50.26	50.29	50.33	50.23	50.23	50.31	50.27
10	37.13	36.98	37.03	37.69	36.98	36.97	37.03	37.04
11	20.90	20.61	20.98	20.97	20.87	20.88	20.90	20.91
12	25.09	26.54	25.06	25.09	25.00	24.99	25.08	24.68
13	38.01	37.96	38.01	38.04	37.67	37.95	37.86	37.98
14	42.81	42.73	42.79	42.81	42.73	42.73	42.80	42.95
15	27.34	27.38	27.41	27.43	27.35	27.36	27.85	27.39
16	35.55	35.50	35.55	35.57	35.49	35.48	35.55	35.52
17	42.97	42.88	42.95	42.86	42.73	42.88	42.98	42.95
18	48.25	48.21	48.25	48.23	48.19	48.19	48.25	48.23
19	47.96	47.89	47.86	47.97	47.92	47.89	47.98	47.96
20	150.93	150.39	150.79	150.71	150.77	150.63	150.60	150.89
21	29.43	29.75	29.79	29.80	29.30	29.63	29.37	29.34
22	39.98	39.94	39.98	39.99	39.92	39.91	39.98	39.95
23	27.95	28.00	27.94	28.00	27.79	27.91	27.40	27.88
24	15.34	15.94	15.94	15.97	15.90	15.89	15.97	15.93
25	15.93	16.14	16.15	16.15	16.08	16.06	16.14	16.15
26	16.08	16.46	16.54	16.58	16.45	16.51	16.51	16.57
27	14.51	14.46	14.48	14.52	14.41	14.43	14.50	14.48
28	17.96	17.49	17.96	18.00	17.90	17.91	17.99	17.97
29	109.29	109.27	109.53	109.42	109.32	109.33	109.35	109.35
30	19.27	19.24	19.27	19.31	19.21	19.22	19.27	19.30
1'	-	-	173.68	172.73	170.21	172.07	170.08	170.72
2'	-	-	34.79	41.69	39.47	42.14	39.17	42.25
3'	-	-	25.15	68.18	70.75	69.10	68.15	66.03
4'	-	-	29.70-29.15	36.62	33.82	42.27	37.86	36.54
5'	-	-	29.70-29.15	25.31	25.00	72.05	70.99	68.75
6'	-	-	29.70-29.15	29.70-29.37	29.73-29.30	37.79	34.18	36.34
7'	-	-	29.70-29.15	29.70-29.37	29.73-29.30	25.31	25.08	25.04
8'-17'	-	-	29.70-29.15[b]	29.70-29.37[b]	29.73-29.30	29.63-29.29[b]	29.70-29.48	29.66-29.55
18'	-	-	31.91	31.93	31.88	31.85	31.91	31.95
19'	-	-	22.69	22.69	22.63	22.61	22.69	22.67
20'	-	-	14.12	14.12	14.05	14.05	14.11	14.12
OAc	-	170.43	-	-	170.12	-	169.81(2)	-
	-	21.17	-	-	21.09	-	21.21,21.15	-
CMe_2	-	-	-	-	-	-		98.59
	-	-	-	-	-	-	-	30.12
	-	-	-	-	-	-	-	19.79

[a] Spectra were run in $CDCl_3$ and chemical shifts are given downfield from TMS. Assignments were made with the aid of the DEPT (**1–5**) and 2D-shift-correlated [^{1}H x ^{13}C-HETECOSY, optimized for one-bond couplings (^{1}JCH)] spectra of **4, 5** and **5B**.

[b] Theses signals included those corresponding to other esters present in the mixture (see experimental). The same observation is valid for signals of 18' to 20' (the last 3C in the side chain $-CH_2-CH_2CH_3$).

sponding compounds recorded in the literature[2,3]. A sample of this mixture (50 mg) was dissolved in pyridine (1.0 ml) and Ac_2O (1.0 ml) and the solution allowed to stand for 24 h at room temperature. The usual work-up produced a mixture of monoacetates **2 + 7 + 9** (41,6 mg) crystallized from MeOH, identified by comparison with the mixture which was also isolated as natural products (*vide infra*).

Acetates of lupeol (**2**, *β-amyrin and* α-amyrin (**9**). Mp. 154–156°C (MeOH). The existence of these acetates in the mixture was inferred from spectral data, mainly ^{13}C NMR (proton noise decoupled and DEPT) through analysis of chemical shifts and multiplicity of the signals of carbon atoms and comparison with the values described in the literature[2,3] for the corresponding compounds.

Mixture of triterpenoids **3**. Mp. 50–68°C (benzene); ν_{max} (KBr)cm^{-1}: 1730, 1240, 1220, 1190, 1040 (ester carbonyl), *1640, 890 (C=CH$_2$)*; ^{13}C NMR see Table 1; ^{1}H NMR see Table 2; EIMS *m/z* (rel. int.) M$^{\cdot+}$ (absent), 409 (5), 393 (6), 366 (3), 340 (3), 297 (6), 271 (2), 225 (3), 229 (7), 218 (49), 203 (25), 189 (31), 175 (14), 161 (15), 149 (23), 135 (34), 133 (23), 123 (30), 121 (36), 119 (26), 109

Table 2. *^1H NMR spectral data of compounds* **3,4, 4A, 5, 5A** and **5B** [*($CDCl_3$, 200 (^1H) and 50.3 MHz (^{13}C)*][a]

Position	3	4[b]	4A	5[b]	5A	5B[b]
3	4.32(dd,J=6,9.7)	4.50(dd,J=6,9.7)	4.49(dd,J=6,9.1)	4.6-4.4(m)	4.45(dd,J=6,9.7)	4.53(dd,j=6,9.2)
19	2.6-2.2(m)	2.6-2.2(m)	2.6-2.2(m)	2.6-2.2(m)	2.6-2.2(m)	2.5-2.3
23	0.87(s)	0.86(s)	0.86(s)	0.85(s)	0.84(s)	0.81(s)
24	1.07(s)	1.05(s)	1.02(s)	1.03(s)	0.99(s)	1.03(s)
25	0.86(s)	0.88(s)	0.85(s)	0.88(s)	0.79(s)	0.83(s)
26	0.86(s)	0.88(s)	0.85(s)	0.88(s)	0.79(s)	0.83(s)
27	0.98(s)	0.98(s)	0.94(s)	0.95(s)	0.90(s)	0.94(s)
28	0.80(s)	0.78(s)	0.78(s)	0.79(s)	0.75(s)	0.78(s)
29	4.72(br s)	4.70(br s)	4.69(br s)	4.68(br s)	4.66(br s)	4.69(br s)
	4.52(br s)	4.58(br s)	4.56(br s)	4.57(br s)	4.54(br s)	4.56(br s)
30	1.72(br s)	1.70(br s)	1.68(br s)	1.68(br s)	1.65(br s)	1.68(br s)
2'	2.32(t,J=7.4)	2.6-2.2(m)	2.60(d,J=6.4)	2.48(d,J=6)	2.56(d,J=6)	2.5-2.3(m)
3'	1.27(br s)	4.00(m)	5.19(qu,J=6.4)	4.26(qu,J=6)	5.19(qu,J=6)	4.4-4.1(m)
4'	1.27(br s)	1.5-1.3(m)	1.5-1.3(m)	1.7-1.5(m)	1.7-1.5(m)	1.5-1.3(m)
5'	1.27(br s)	1.25(br s)	1.25(br s)	3.84(br s)	4.89(qu,J=6)	3.83 (m)
$(CH_2)n$	1.27(br s)	1.25(br s)	1.25(br s)	1.25(br s)	1.22(br s)	1.25(br s)
CH_3	0.91(t,J=7)	0.90(t,J=7)	0.87(t,J=7)	0.91(t,J=7)	0.84(t,J=7)	0.88(t,J=7)
CMe_2	–	–	–	–	–	1.36(s)
	–	–	–	–	–	1.44(s)
OAc	–	–	2.01(s)	–	2.01(s)	–
					1.97(s)	–

[a] Chemical shifts in δ, coupling constants (J) in Hz and TMS as internal standard. The ^1H x ^1H and ^1H x ^{13}C-2D-shift-correlated NMR spectra of compounds 4,5 and 5B aided in these assignments;

[b] The signals of the protons at C-1, C-2, C-15 (1H and C-18 were observed betwen β 1.8-1.5; at C-5 and C-15 (1H) betwen β 1.0-0.7; at C-6, C-7, C-9, C-11, C-12, C-13, C-16, C-21 and C-22 betwen β 1.6-1.0.

Tabela 3. *GC/MS data of methyl esters* **3a'–3d'** *and* **4a'–4b'***a and lactones* **5a'–5b'**.

Compound	GC		Ms($M^{\cdot+}$) or $\lvert M-18\rvert^+$	
	Retention time (min)	Relative abundance (%)	*m/z*	rel.int.(%)
3a'	9.735	57	354	10
3b'	10.503	25	382	13
3c'	11.241	13	410	8
3d'	12.133	5	438	7
4a'	9.694	12	324[b]	1
4b'	10.668	72	352[b]	1
4c'	11.340	11	380[b]	1
4d'	12.243	5	408[b]	1
5a'	27.160	90	308	1
5b'	30.203	10	336	absent

[a] CG/MS analysis was performed with a Hewlett Packard 5890/5988A instrument fitted with a HP-1 capillary column (12 m x 0.02 mm i.d.). Temp. programmed 100-300°C at 20°C/min. Carrier gas He. Flow rate 20 ml/min. The chromatograph was coupled to a mass selective detector at 70 eV.

[b] These highest peaks in the EIMS of these compouds were interpreted as $[M-H_2O]^{+\cdot}$.

(43), 107 (33), 105 (26), 98 (27), 97 (32), 95 (53), 93 (38), 91 (17), 85 (28), 83 (39), 81 (73), 79 (23), 73 (46), 71 (49), 69 (100), 67 (33).

Acidic methanolysis of **3**. Compound **3** (100 mg) was refluxed for 6 h with MeOH (5 ml) and HCl (1 ml). After cooling, the solution was extracted with $CHCl_3$. The $CHCl_3$ extract was washed with H_2O, dried with anihydrous Na_2SO_4 and concentrated under vacuum, furnishing lupeol (**1**) and a mixture of methyl esters (**3a'– 3d'**), after chromatography on silica gel column. Analysis of methyl esters mixture by GC/MS technique see Table 3. EIMS *m/z* (rel.int): **3a'** 354 ($M^{\cdot+}$, 10), 311 (5), 225 (4), 213 (1), 199 (5), 143 (18), 87 (63), 75 (36), 74 (100); **3b'** 382 ($M^{\cdot+}$, 13), 339 (6), 283 (4), 241 (2), 199 (5), 143 (26), 87 (69), 75 (48), 74 (100); **3c'**410 ($M^{\cdot+}$, 8), 367 (3), 311 (2), 199 (4), 143 (16), 87 (64), 75 (47), 74 (100); **3d'** 438 ($M^{\cdot+}$, 7), 395 (3), 199 (4), 143 (16), 87 (66), 75 (57), 74 (100).

Mixture of triterpenoids **4**. Mp 73-75°C (benzene-EtOAc); ν_{max} (KBr) cm^{-1}; 3500 (OH), 1740, 1260, 1170, 1150 (ester), 1640, 880 (C=CH_2) ^{13}C NMR see Table 1; ^1H NMR see Table 2; EIMS *m/z* (rel. int.): $M^{\cdot+}$ (absent), 408 (5), 393 (2), 364 (3), 297 (5), 218 (15), 203 (16), 189 (32), 175 (11), 161 (11), 149 (10), 147 (16), 135 (29), 133 (14), 123 (21), 121 (30), 119 (16), 109 (40), 107 (28), 105 (11), 97 (32), 96 (41), 95 (62), 85 (17), 83 (43), 82 (65), 81 (52), 71 (36), 69 (66), 68 (42), 67 (44), 57 (100), 55 (75).

Acetate **4A.** Mp 140-141°C, ^{13}C NMR see Table 1; ^{1}H NMR see Table 2.

Acidic methanolysis of **4.** Compound **4** (100 mg) was refluxed with stirring in 10% MeOH-HCl (20 ml) for 6 h. After cooling, the reaction mixture was submitted to the same work-up as above, affording lupeol (**1**) and a mixture of methyl esters **4a' – 4d'**. Analysis of methyl es - ter mixture by GC/MS see Table 3. EIMS *m/z* (rel.int.): **4a'** 324 (1, M-H_2O), 103 (100; **4b'** 352 (1, M-H_2O), 103 (100) **4c'** 380 (1, M-H_2O), 103 (100): **4d'** 408 (1, M-H_2O), 103 (100).

Mixture of triterpenoids **5.** Mp 88-90°C (benzene - EtOAc); ν_{max} (KBr) cm^{-1}: 3480 (OH), 1730, 1260, 1200 (ester), 1640, 870 (C=CH_2); ^{13}C NMR see Table 1; ^{1}H NMR see Table 2; EIMS *m/z* (rel.int.) $M^{\cdot+}$ (absent), 426 (3), 302 (2), 274 (6), 273 (11), 246 (9), 232 (7), 231 (9), 218 (9), 217 (7), 205 (13), 203 (5), 189 (9), 187 (5), 163 (18), 161 (16), 147 (13), 145 (9), 137 (21), 136 (10), 135 (23), 134 (10), 133 (20), 125 (23), 124 (14), 123 (46), 121 (41), 119 (24), 109 (66), 107 (47), 95 (81), 93 (49), 91 (38), 81 (80), 79 (46), 69 (98), 67 (75), 55 (100), 43 (36).

Acetate **5A.** Oil. ^{13}C NMR see Table 1; ^{1}H NMR see Table 2.

Acetonide **5B.** The natural product **5** (54 mg) in dry Me_2CO (5 ml) and *p*-toluenesulfonic acid (5.0 mg) was stirred at room temperaure for 24 h. Filtration and removal of solvent gave the acetonide **5B (59 mg), mp 53-55°C**; ^{13}C NMR see Table 1; ^{1}H NMR see Table 2.

Acidic methanolysis of **5.** Compound **5** (105 mg) was refluxed with 10% MeOH-HCl (15 ml) for 10 h. After cooling, the reaction mixture was submitted to the same work-up as above, yielding lupeol (**1**, 23 mg) and **5a + 5b** (8.9 mg) after separation by prep-TLC (silica gel PF 254), mp 53-54°C, ^{1}H NMR (200 MHz $CDCl_3$) δ 6.84 (*m*, H-3'), 5.99 (*br d*, J=9.8 Hz, H-2'), 4.39 (*m*, H-5'), 2.35-2.27 (*m*,CH_2-4'), 1.57 (*m*, CH_2-6'), 1.23 (*br s*, CH_2-7' to CH_2-19' and CH_2-21'), 0.85 (*t*, J=6.3 Hz, CH_3-20' and CH_3-22'); ^{13}C NMR (50.3 MHz, $CDCl_3$) δ 165.00 (C-1'), 145.01 (C-3'), 121.45 (C-2'), 78.03 (C-5'), 34.89 (C-6'), 31.91 (C-18'), 29.69-29.37 (C-4' and C-7' to C-17'and C-19'), 22.69 (C-19'and C-21'), 14.11 (C-20' and C-22'); GC/MS: see Table 3; EIMS *m/z* (rel.int.): **5a** 308 ($M^{\cdot+}$, 1), 248 (3, M-60), 97 (100), 96 (12), 95 (15), 94 (11), 86 (14), 83 (16), 82 (12), 81 (18), 69 (28), 68 (46), 67 (14), 57 (17), 55 (24), 43 (22); **5b** 336 ($M^{\cdot+}$, absent), 276 (4, M-60), 97 (100), 96 (16), 95 (19), 94 (13), 86 (15), 83 (23), 82 (13), 81 (23), 69 (38), 68 (46), 67 (20), 57 (32), 55 (36), 43 (43).

Friedelin (**10**). Mp 256-258°C (benzene-acetone); ^{1}H and ^{13}C NMR spectral data in agreement with reported values in the literature[4,5] along with IR (ν_{CO} 1710) and EIMS ($M^{\cdot+}$ 426). The confirmation of this triterpene was obtained by comparison with an authentic sample of friedelin.

Results and discussion

The dichlorometane extract of the bark and of the methanol insoluble latex material in MeOH of *Parahancornia amapa* afforded the triterpenoids **1-10**. Among these triterpenes, **1, 2, 6-10** have been previously reported in other plants and were identified from their spectral properties (see experimental)[3-6].

The presence of lupeol moiety in compounds **3, 4** and **5** was recognized by chemical shifts and multiplicity (number of bound protons) of all ^{13}C NMR signals, deduced by the comparative analysis of the proton noise decoupled and DEPT spectra (Table 1), and of the ^{1}H NMR spectrum that displayed singlet for seven methyl groups (six bound to sp^3 carbon atoms between δ 1.05-.078 and one bound to tertrary sp^2 between δ 1.70-1.68) and two for ethylenic protons in a 1,1-disubstituted double bond (δ 4.70-4.57) and one multiplet at δ 4.6-4.4 for one carbinolic proton (Table 2). The ^{1}H and ^{13}C NMR assignments of this moiety were greatly facilitated by the homonuclear (^{1}H x ^{1}H-HOMOCOSY) and heteronuclear (^{1}H x ^{13}C-HETECOSY) 2D shift-correlated[7] NMR spectra as well as comparison with compounds **1** and **2** (Tables 1 and 2), along with the application of the usual shift parameters, comparison with literature data[3-6] and the observed multiplicity of signals in the DEPT ^{13}C NMR. The chemical shifts of H-3 (δ 4.6-4.4) and carbon atoms C-3 [δ 80.81 (**3**), 81.33 (**4**) and 81.40 (**5**)], C-2 [δ 23.72 (**3**), 23.74 (**4**) and 23.65 (**5**)] and C-4 [(δ 37.79 (**3**), 37.78 (**4**) and 37.70 (**5**)] of these compounds **3, 4** and **5** compared with those of triterpenoids 3β-O-acetyllupeol (**2**) indicated the presence of an acyl group at the oxygen atom of C-3. Thus, the three new triterpenoids **3, 4** and **5** were classified as esters and the site of esterification was established at C-3 of the A ring of the basic skeleton of lupeol (**1**). The recognition of these presence of this basic skeleton in the natural esters (**3-5**) pointed to the esterification of the hydroxyl function at C-3 with different acyl groups.

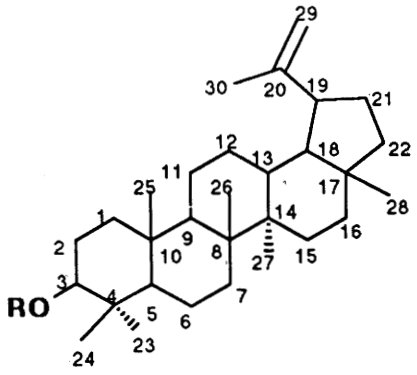
After confirming the existence of absorption corresponding to ester carbonyl at 1730 (**3**), 1740 (**4**) and 1730 cm^{-1} (**5**) and to a double bond [C=CH_2: ν_{max} 1640, 890 (**3**); 1640, 880 (**4**); 1640, 870 cm^{-1} (**5**)] of the basic skeleton of lupeol (**1**), the analysis of the IR spectra revealed as a major distinction between the three compounds the presence of a broad absorption in the hydroxyl group region in **4** (ν_{max} 3500 cm^{-1}) and **5** (ν_{max} 3480 cm^{-1}).

Further analysis of the ^{1}H NMR spectra (Table 2), including ^{1}H x ^{1}H-HOMOCOSY, showed additional signals for methylene [δ 1.25 (*br s*, relatively intense absorption)] and methyl [δ 0.90-0.91 (t)] groups in the three compounds as well as for one [δ 4.00 (*m*, H-3')] and two [δ 4.26 (*qu*, J=6, H-3') and 3.84 (*br s*, H-5')] oxymethine protons in **4** and **5**, respectively, which were shifted to lower field upon acetylation [**4A**: δ 5.19 (H-3'), Δδ=1.19 ppm; **5A**: δ 5.19 (H-3'), Δδ=0.93 and 4.89 (H–5'), Δδ=1.05 ppm]. These data indicated the presence of a secondary hydroxyl group in **4** and two of these groups in **5** (Table 2). The multiplicity (*d*, J=6 Hz) of the signal at δ 2.48, a chemical shift compatible with methylenic protons α to a carbonyl function, suggested the localization of one hydroxyl group at C-3' of compound **5**. The ^{1}H x ^{1}H-HOMOCOSY spectrum, clearly showed the spin-spin interaction between the protons at C-2' (δ 2.48) and C-3' (δ 4.26) and the coupling of the oxymethine protons at C-3' and C-5' with the same methylene group (δ 1.7-1.5, CH_2-4'), allowing us to localize the remaining hydroxyl function of this compound at C-5'. Analogously, the ^{1}H x ^{1}H-HOMOCOSY [correlation of the absorptions at δ 2.6-2.2 (m, CH_2 -2') and 4.0 (m, H-3')] spectrum of compound **4** was also used to localize its hydroxyl group at C-3'. The base peak at *m/z* 103 (**13**) in the EIMS of methyl esters (**4a' - 4d'**) obtained by transesterification of **4** by reaction with MeOH/HCl (see experimental), furnished additional support for this deduction.

The fully decoupled and DEPT ^{13}C NMR as well as ^{1}H x ^{13}C-HETECOSY (^{1}JCH) 2D shift-correlated spectra showed additional signals that allowed us to determine the partial molecular formula of the acyl groups in the three natural compounds:

3 → $CH_3CH_2CH_2(CH_2)_n$ CH_2CH_2COOR;

4 → $CH_3CH_2CH_2(CH_2)_n$ $CH_2CH_2CHOHCH_2COOR$;



1 R=H

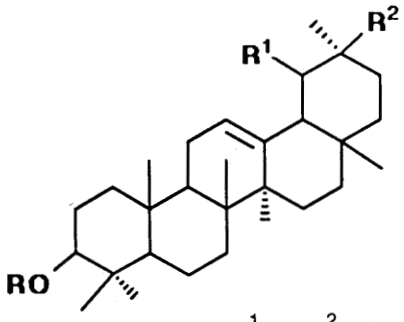
2 R=Ac

3 R= (n=20,22,24,26)(**3a-3d**)

4 R= (n=16,18,20,22)(**4a-4d**)

R^1=H

4A R^1=Ac

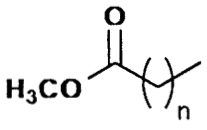


6 R=R^1=H, R^2=Me

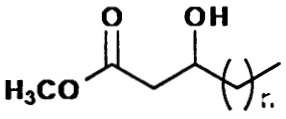
7 R=Ac, R^1=H, R^2=Me

8 R=R^2=H, R^1=Me

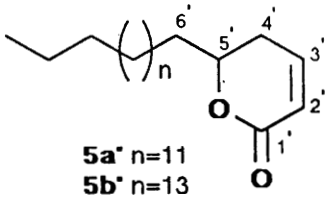
9 R=Ac, R^1=Me, R^2=H



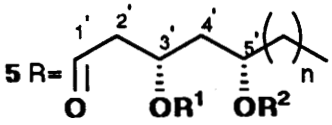
3a n=20
3b n=22
3c n=24
3d n=26



4a n=16
4b n=18
4c n=20
4d n=22



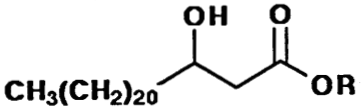
5a n=11
5b n=13



R^1=R^2=H (n=14,16)(**5a-5b**)

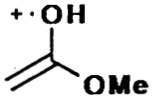
5A R^1=R^2=Ac

5B R^1,R^2=CMe_2

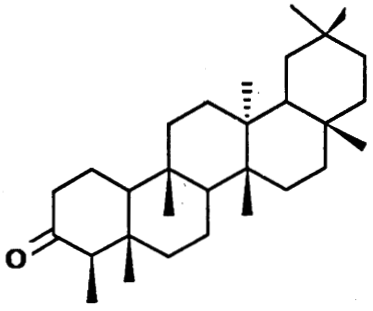


11 R=H

11a R=Me

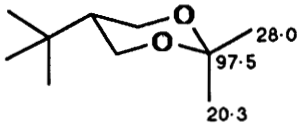


12 *m/z* 74(100%)

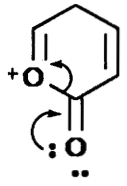


10

13 *m/z* 103(100%)



14



15

5 → $CH_3CH_2CH_2(CH_2)_nCH_2CH_2CHOHCH_2CHOHCH_2COOR$ (Table 1). These deductions were obtained on the basis of the chemical shifts and multiplicities observed for $CH_3CH_2CH_2$ [**3-5**: δ 14.12-14.05 (CH_3); 22.69-22.61 (CH_2 bound to CH_2 and CH_3); 31.85-31.93 (CH_2)], $(CH_2)_n$ (**3-5**: δ 29.80-29.37) and CH_2CH_2 COOR [**3**: δ 34.79 (α - CH_2), 25.15 (β - CH_2)] signals[9] and the expected modifications for these parameters by α, β and δ effects[8] with the presence of one [**4**: (δ) - CH_2(25.31)-CH_2(36.62) - CHOH (68.18) - CH_2(41.69) - COOR(172.73)] and two [**5**: (δ) - CH_2(25.31)-CH_2(37.79) - CHOH(72.05) - CH_2(42.27) - CHOH(69.10)-CH_2(42.14) - COOR(172.07)] hydroxyl groups (Table 1) deduced from the spectral comparison of **3** with **4** and **5**.

The confirmation of a 3',5'-dihydroxy system in compound **5** was obtained by the preparation of acetonide **5B** after reaction of the natural product with acetone and *p*-toluenesulfonic acid. The chemical shift (δ 98.59) of the signal of the ketal carbon atom of this acetonide was consistent only with a hexacyclic 1,3-dioxane system (e.g. 14)[10]. In the pentacyclic 1,3-dioxane system the signal of this dialkoxy carbon appears at about δ108[11].

The acidic methanolysis of the ester **3** yielded lupeol (**1**) as the alcoholic portion and a mixture of the methyl esters (**3a'-ed'**) as the acid portion. The mixture of these methyl esters was recognized by GC/MS analysis (Table 3). The base peak at *m/z* 74 in the mass spectra of the four methyl esters (**3a'-3d'**) can be rationalized by McLafferty rearrangement of the molecular ion to produce fragment 12.

The application of an identical procedure for ester **4** furnished lupeol (**1**) and a mixture of methyl esters **4a'-4d'** (Table 3). The base peak at *m/z* 103 (**13**) was observed in the mass spectra of these four methyl esters (**4a'-4d'**). The highest peaks in the EIMS of these compounds ($M\cdot^+$ absent) were interpreted as [$M-H_2O$]$\cdot^+$ (Table 3).

The acid methanolysis of esters **5** afforded lupeol (**1**) and a mixtre of α,β-unsaturated δ-lactones **5a'** and **5b'**. The mixture of these lactones was revealed by GC/MS analysis (Table 3), along with ^1H [δ 6.84 (*m*, H-3'), 5.99 (*br d*, H-2'), 4.39 (*m*, H-5'), 2.35-2.27 (*m*, CH_2-4'), 1.57 (*m*, CH_2-6'), 1.23 (*br s*, CH_2-7' to CH_2-19' and CH_2-21') and 0.85 (*t*, J=6.3 HZ, CH_3-20' and CH_3-22')] and ^{13}C [δ 165.00 (C-1'), 145.01 (C-3'), 121.45 (C-2'), 29.69-29.37 (C-4' and C-7' to C-17' and C-19'), 34.89 (C-6'), 31.91 (C-18' and C-20'), 22.69 (C-19' and C-21') and 14.11 (C-20' and C-22')] NMR spectra. The base peak at *m/z* 97 (**15**) in the two EIMS is consistent with α,β-unsaturated δ-lactones[13]. The formation of these α,β-unsaturated δ-lactones can be rationalized by the attack of the hydroxyl group localized at C-5' on the carbonyl carbon atom of the protonated carboxyl group followed by acid catalysed dehydration.

The assignment of an equatorial-position for the acyloxy groups at C-3 was deduced from the chemical shifts of carbons 1 to 5, 23 and 24 (Table 1)[12] and by the appearance of a signal at δ 4.6-4.4 (H-3) as a double doublet (Jaa= 9.1- 9.7 HZ, Jae= 6 Hz)(Table 2).

The signals of carbon atoms C-3' (δ 69.10) and C-5' (δ 72,05) in the original ester **5** are shifted to δ 66.03 (C-3') and 68.75 (C-5') in derivative **5B** (acetonide obtained as usual by reaction of **5** with dry acetone in the presence of *p*-toluenesulfonic acid). These upfield shifts [Δδ= 3.07 (C-3') and Δδ=3.30 ppm (C-5')] indicated clearly a similar δ-effect of the axial methyl group of the 1,3-dioxane system at C-3' and C-5' and suggested the relative configuration (*erythro* type) of these carbons atoms as shown in **5**.

The assignments of the chemical shifts of these carbons and the corresponding hydrogens were supported by the homonuclear (^1H x ^1H-HOMOCOSY) and heteronuclear (^1H x ^{13}C-HETECOSY) 2D-shift-correlated experiments (Tables 1 and 2).

On the basis of the spectral and chemical data, the structures of the new triterpenoid esters present in mixture **3** were established as 3-β-O-behenyl- (**3a**), 3-β-O-lignoceryl- (**3b**), 3-β-O-hexacosanoyl- (**3c**) and 3-β-O-octacosanoyl lupeol (**3d**); in mixture **4**: 3-β-O-3'-hydroxyarachyl- (**4a**), 3-β-O-3'-hydroxybehenyl- (**4b**),3-β-O-3'-hydroxylignoceryl- (**4c**) and 3-β-O-3'-hydroxyhexacosanoyl lupeol (**4d**); in mixture **5**: 3-β-O-3' 5'-dihydroxyarachyl- (**5a**) and 3-β-O-3',5'- dihydroxybehenyl lupeol (**5b**).

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