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PROTOLIMONOIDS AND QUASSINOIDS FROM *PICROLEMMA GRANATENSIS*

EDSON RODRIGUES FO., JOÃO B. FERNANDES,* PAULO C. VIEIRA, M. FÁTIMA DAS G. F. DA SILVA, J. ZUKERMAN-SCHPECTOR, ROSÁLIA M. O. CORRÊA DE LIMA,† SILENE C. NASCIMENTO† and WAIT THOMAS‡

Departamento de Química, Universidade Federal de São Carlos, CP 676, CEP 13565-905 São Carlos, SP, Brazil; †Departamento de Antibióticos, Universidade Federal do Pernambuco, Recife, PE, CEP 50.670-901, Brazil; ‡New York Botanic Garden, NY, U.S.A.

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Key Word Index—*Picrolemma granatensis*; Simaroubaceae; stem bark; protolimonoids; quassinoids; X-ray analysis; antineoplastic effect.

Abstract—From the stem bark of *Picrolemma granatensis* three novel triterpenoids were isolated: 7α,24,25-trihydroxy-3-oxo-apotirucalla-14,20(22)-dien-21,23-olide and 7α,24,25-trihydroxy-3-oxo-apotirucalla-1,14,20(22)-trien-21,23-olide, 21,23-epoxy-7α,20,21,24,25-pentahydroxyapotirucalla-14-en-3-one which were identified on the basis of spectroscopic and chemical methods and X-ray diffraction. The known quassinoids glaucarubolone, $\Delta^{13,18}$-dehydroglaucarubolone, glaucarubinone, glaucarubol, 2′-acetylglaucarubin, excelsin, ailanthinone and holocanthone were also identified. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

In two previous papers, we reported the isolation and structure elucidation of five canthin-6-one alkaloids and two tetranortriterpenoid γ-hydroxybutenolides from *Picrolemma granatensis* [1, 2]. The alkaloids are typical of the Simaroubaceae family [3], while butenolides are known to occur in allied families of the order Rutales (Meliaceae, Rutaceae and Cneoraceae). During our continued investigation of this species, three new apotirucallane derivatives along with eight known quassinoids have been isolated. The first compound (**1**) is a putative precursor of the two butenolides we have reported before.

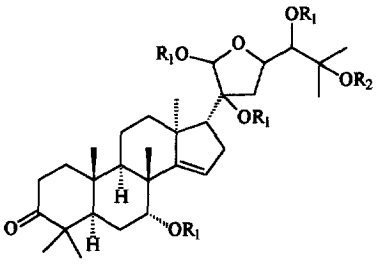
RESULTS AND DISCUSSION

Further examination of the stem bark of *P. granatensis* led to the isolation of three new triterpenoids (**1–3**), in addition to eight known quassinoids which were identified by comparison of their spectroscopic data (IR, MS, ^{1}H NMR, ^{13}C NMR) with those reported previously in the literature: glaucarubolone [4, 5], $\Delta^{13,18}$-dehydroglaucarubolone [6–8], glaucarubinone (**4**) [7, 9], 2′-acetylglaucarubin [9], excelsin [9], ailanthinone [7, 10], glaucarubol [11, 12] and holacanthone [4, 7]. The last was identified as an acetate derivative and the ^{13}C NMR data of $\Delta^{13,18}$-dehydroglaucarubolone and holacanthonetriacetate are reported for the first time (Table 1).

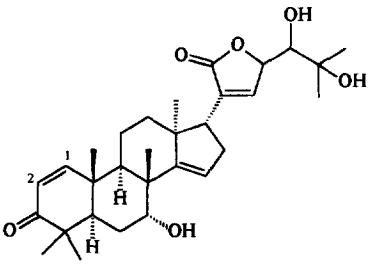
The triterpenoid **1** was obtained in a mixture with holacanthone, which was first acetylated and the corresponding acetates chromatographed on a silica gel flash column to yield holacanthone triacetate [7], **1a** and **1b** (3:1 respectively). Compound **1a** exhibited similar spectral data to dihydrobruceajavanina (**5**) [13]. The ^{1}H NMR spectrum (Table 2) showed signals for a side-chain relative to that of melianodiol 21,24-diacetate obtained from melianodiol [14,15]. The ^{13}C NMR spectral data (Table 3), including HMBC and HMQC, showed that, apart for the signals of the tetracyclic skeleton and its substituents, **1a** has carbons at δ 89.3, 98.8 (C-21), 31.6 (C-22), 76.5 (C-23), 76.5 (C-24), 72.3 (C-25), 25.8 (C-26) and 26.5 (C-27). The multiplicities and chemical shifts of H-21 (δ 6.33, *s*), H-22 α (δ 2.26, *dd*) and H-22β (δ 2.28, *dd*), led unequivocally to the assignment of C-20 to the singlet at δ 89.3 in the ^{13}C NMR spectrum, so placing an acetoxyl at C-20. H-21 shows multiple bond correlation with an acetoxy carbonyl ester; C-20 (δ 89.3, *s*); C-23; C-17 and C-22. The configuration of the 21-acetoxy group was inferred as α since H-21-β shows a significant NOE with H-17-β in NOESY experiments (Table 4).

The only difference between **1a** and **1b** is the presence of one more acetoxyl group in the side chain of the latter. The downfield shift of the signals of the Me-26 and Me-27, compared with **1a**, clearly suggested the presence of an acetoxyl group at C-25. These data led to the structure of the natural triterpenoid **1** as 21,23-

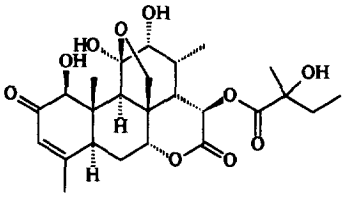
*Author to whom correspondence should be addressed.



1: $R_1 = R_2 = H$
1a: $R_1 = Ac$; $R_2 = H$
1b: $R_1 = R_2 = Ac$



2 1,2-dihydro
3



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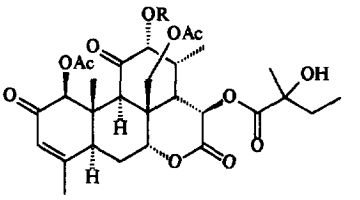
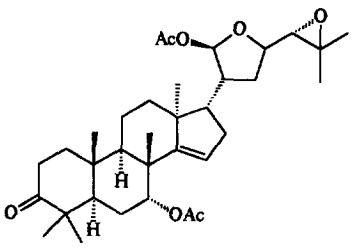
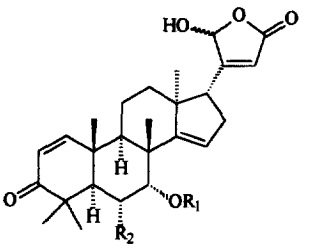


4a R = Ac
4b R = H



5 dihydrobruceajavanin



6 $R_1=R_2=H$
7 R_1=Ac, R_2=OH, 1,2-dihydro

epoxy-7α,20,21,24,25-pentahydroxy-appotirucalla-14-en-3-one.

Compound **2** also showed the spectral characteristics of a 7α-hydroxyapotirucalla-14-en-3-one. In addition, the ^{1}H NMR spectrum showed signals for a secondary hydroxyl (δ 3.52, H-24) and α,β-unsaturated γ-lactone (δ 7.14, H-22; δ 5.19, H-23). The ^{13}C NMR spectrum revealed resonances for C-1 to C-17 in close agreement with those for the 17-γ-hydroxybutenolide apotirucallane derivative isolated before [2], while signals for an α,β-unsaturated γ-lactone (δ 133.8, C-20; 173.6, C-21; 149.4, C-22 and 80.9, C-23), two methyl groups (δ 27.1 and 26.9) and two oxygen-bearing carbons, one secondary and one quaternary (δ 76.2 and 72.3), confirmed the presence of the 24,25-dihydroxy-20(22)-en-21,23-olide side chain at C-17. Final confirmation of the structure of **2** as 7α,24α,25-trihydroxy-3-oxo-apotirucalla-14,20(22)-dien-21,23-olide (or its enantiomer) came from an X-ray diffraction analysis. A stereoscopic view of the structure is given in Fig. 1.

The triterpenoid **3** was obtained in a mixture with **2**, which by recycling HPLC on a normal phase column afforded **2** and a mixture of a small amount of **2** contaminating **3**, that could not be separated. In addition to the data described earlier, for compound **2**, the ^{1}H NMR spectrum of this mixture revealed a pair of doublets (δ 5.79, H-2 and 7.09, H-1) that together with the signals at δ 206.1 (C-3), 158.3 (C-1) and 125.2 (C-2) in the ^{13}C NMR indicated an enone in ring A as observed for 7α,21-dihydroxy-3-oxo-24,25,26,27-tetranorapotirucalla-1,14,20(22)-trien-21,23-olide isolated from a less polar fraction [2]. Thus, the structure of the new triterpenoid was characterized as 7α,24,25-trihydroxy-3-oxo-apotirucalla-1,14,20(22) -trien-21,23-olide (**3**).

Compound **4** was acetylated with acetic anhydride in pyridine to yield a mixture (4:1) of the 1,12,30-triacetate **4a** [16] and the 1,30-diacetate **4b**, which were purified by TLC. Compounds **4a** and **4b** correspond to hemiketal ring opening followed by acetylation of the resultant alcohols [17]. Upon acetylation of the hydroxyl at C-12 an appreciable upfield shift of C-11 (**4a** = δ 203.0: **4b** = δ 210.9) was observed, indicating the position of the hydroxy group.

Compound **1** may represent an intermediate stage in the formation of the butenolides **6** and **7** previously

Table 1. ^{13}C NMR data of $\Delta^{13,18}$-dehydroglaucarubolone, holocanthonetriacetate and **4b**

C	$\Delta^{13,18}$-Dehydroglaucarubolone	Holacanthonetriacetate	**4b**
1	84.3 *d*	83.1 *d*	83.8 *d*
2	197.4 *s*	190.6 *s*	191.4 *s*
3	126.2 *d*	125.9 *d*	126.9 *d*
4	162.4 *d*	160.2 *s*	160.3 *s*
5	42.6 *d*	43.0 *d*	40.8 *d*
6	26.1 *t*	27.5 *t*	25.2 *t*
7	78.6 *d*	78.9 *d*	77.0 *d*
8	47.5 *s*	45.2 *s*	41.8 *s*
9	46.1 *d*	41.9 *d*	47.9 *d*
10	45.7 *s*	45.8 *s*	44.8 *s*
11	110.1 *s*	70.7 *d*	210.9 *s*
12	80.8 *d*	82.9 *d*	82.7 *d*
13	143.4 *s*	79.6 *s*	38.2 *d*
14	55.8 *d*	52.7 *d*	47.2 *d*
15	68.0 *d*	66.7 *d*	70.8 *d*
16	173.4 *s*	167.0 *s*	167.6 *s*
18	121.5 *t*	22.3 *q*	16.0 *q*
19	10.4 *q*	11.9 *q*	12.3 *q*
29	22.9 *q*	22.3 *q*	22.5 *q*
30	72.5 *t*	72.2 *t*	61.4 *t*
1′			176.8 *s*
2′			75.2 *s*
3′			33.4 *t*
4′			7.8 *q*
5′			25.3 *q*
OAc		170.2/20.8	
OAc		169.6/20.7	
OAc		169.3/20.5	

$CDCl_3$, 100 MHz. Multiplicity obtained by DEPT or J-MOD experiments.

isolated from this plant [2]. γ-Hydroxybutenolides usually arise by singlet oxygen oxidation of furans.

As reported in a previous paper [2], limonoids, commonly associated with the Meliaceae, Rutaceae and Cneoraceae, had not been reported from the Simaroubaceae until recently [3]. If limonoids can be converted enzymically to the γ-hydroxybutenolides **6** and **7**, further reports of the co-occurrence of quassinoids and limonoids in the Simaroubaceae may be expected.

The antineoplasic effect of compound **2** was tested in KB, P388, L1210, PC3 and MDA-MB 231 cells *in vitro* [18–25] and also solid experimental tumours sarcoma 180 and Ehrlich Carcinoma in Swiss albino mice were

Table 2. ^1H NMR data of compounds **1a** and **1b***

H	**1a**	**1b**
2α	2.53 (*ddd*, 7/10/16)	2.50 (*ddd*, 7/10/16)
2β	2.41 (*ddd*, 4/7/16)	2.38 (*ddd*, 4/7/16)
7	5.21 (*bt*, 2)	5.20 (*t*, 2)
15	5.32 (*bt*, 3)	5.30 (*bt*, 3)
17	2.96 (*bt*, 9)	3.00 (*bt*, 9)
18	1.09 (*s*)	1.09 (*s*)
19	1.02 (*s*)	1.02 (*s*)
21	6.33 (*s*)	6.30 (*s*)
22α	2.36 (*dd*, 5/14)	2.29 (*dd*, 5/15)
22β	2.28 (*dd*, 8/14)	2.19 (*dd*, 8/15)
23	4.74 (*ddd*, 3/5/8)	4.51 (*ddd*, 4/5/8)
24	4.88 (*d*, 3)	5.48 (*d*, 4)
26	1.28 or 1.20 (*s*)	1.50 or 1.45 (*s*)
27	1.20 or 1.28 (*s*)	1.45 or 1.50 (*s*)
28	1.03 (*s*)	1.03 (*s*)
29	1.01 (*s*)	1.01 (*s*)
30	1.12 (*s*)	1.11 (*s*)
Ac(Me)	2.10; 2.04; 2.03 and 1.95	2.08; 2.04; 2.03; 1.97; 1.94

*$CDCl_3$, 400 MHz, *J* (Hz).

Table 3. ^{13}C NMR data of compound **1a–3***

C	1a	1b	2	3
1	38.7 *d*	38.8 *t*	38.4 *t*	158.3 *d*
2	33.8 *d*	33.9 *t*	33.8 *t*	125.2 *d*
3	216.7 *s*	216.7 *s*	217.4 *s*	205.1 *s*
4	46.8 *s*	46.9 *s*	46.8 *s*	44.8 *s*
5	48.5 *d*	48.7 *d*	46.4 *d*	44.3 *d*
6	24.1 *t*	23.5 *t*	24.6 *t*	24.1 *t*
7	74.9 *d*	74.4 *d*	71.9 *t*	71.4 *d*
8	48.5 *s*	48.7 *s*	44.0 *s*	44.1 *s*
9	42.0 *d*	42.1 *d*	40.9 *d*	40.8 *d*
10	36.7 *s*	36.9 *s*	37.0 *s*	40.0 *s*
11	16.2 *t*	16.3 *t*	16.1 *t*	16.1 *t*
12	33.3 *t*	31.7 *t*	32.2 *t*	32.2 *t*
13	48.1 *s*	48.2 *s*	47.5 *s*	47.4 *s*
14	157.6 *s*	157.5 *s*	160.7 *s*	160.6 *s*
15	118.6 *d*	118.7 *d*	119.4 *d*	119.5 *d*
16	31.9 *s*	29.7 *t*	33.5 *t*	33.5 *t*
17	56.3 *d*	56.3 *d*	50.6 *d*	50.6 *d*
18	22.1 *q*	22.7 *q*	20.3 *q*	18.8 *q*
19	15.0 *q*	15.1 *q*	14.8 *q*	14.8 *q*
20	89.3 *s*	89.6 *s*	133.8 *s*	133.7 *s*
21	98.8 *d*	98.9 *d*	173.6 *s*	173.5 *s*
22	36.2 *t*	36.6 *t*	149.4 *d*	149.5 *d*
23	76.5 *d*	75.0 *d*	80.9 *d*	80.9 *d*
24	76.5 *d*	75.8 *d*	76.2 *d*	76.2 *d*
25	72.3 *s*	82.7 *s*	72.3 *s*	72.3 *s*
26	27.4 *q*	25.8 *q*	27.1 *q*	27.1 *q*
27	26.8 *q*	21.0 *q*	26.9 *q*	26.4 *q*
28	21.8 *q*	20.8 *q*	26.0 *q*	25.9 *q*
29	26.5 *q*	25.8 *q*	25.8 *q*	25.8 *q*
30	27.3 *q*	27.1 *q*	21.0 *q*	21.4 *q*

*$CDCl_3$, 100 MHz. Multiplicity obtained by DEPT or J-MOD experiments.

Table 4. ^1H NMR data of compound **1a**

H	δ (m, *J* (Hz))	NOESY	HMBC
2α	2.53 (*ddd*, 7/10/16)	—	—
2β	2.41 (*ddd*, 4/7/16)	—	—
7	5.21 (*t*, 2)	H-6α, H-6β, H-15, H-30	C-8, C-9
15	5.32 (*bt*, 3)	H-16β, H-30	C-13, C-15, C-16, C-17
17	2.96 (*bt*, 9)	H-21	—
18	1.09 (*s*)	—	C-13, C-14
19	1.02 (*s*)	H-16β	—
21	6.33 (*s*)	H-17, H16β	C-17, C-20, C-22, C-23, Ac
22α	2.36 (*dd*, 5/14)	H-23	—
22β	2.28 (*dd*, 8/14)	—	—
23	4.74 (*ddd*, 3/5/8)	H-22	C-23, C-24
26	1.28 or 1.20 (*s*)	H-23, H-24	C-24, C-25, C-25
27	1.20 or 1.28 (*s*)	H-23, H-24	C-24, C-25, C-27
28	1.03 (*s*)	—	C-3, C-4, C-28
29	1.01 (*s*)	—	C-3, C-4, C-29
30	1.12 (*s*)	H-7, H-15	C-7, C-9, C-14, C-30
Ac(Me)	2.10; 2.04; 2.03 and 1.95	—	

$CDCl_3$, 400 MHz.

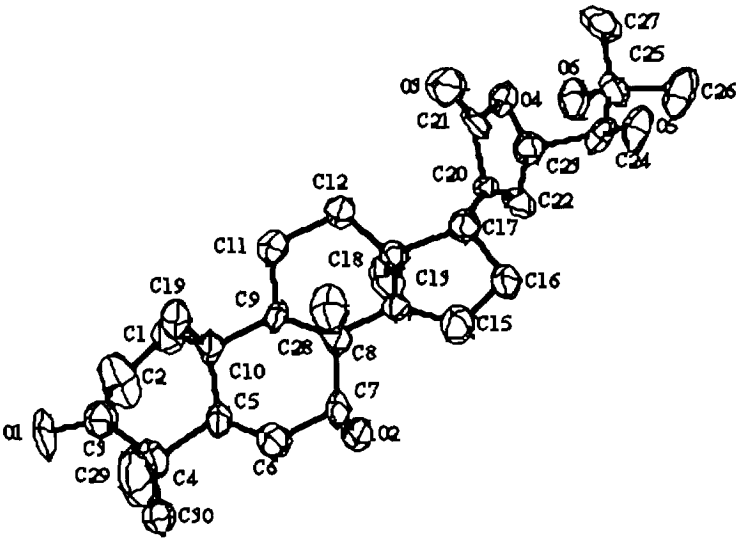


Fig. 1. Ortep view of **2**.

tested [26]. Compound **2** showed significant anticancer activity in these tests (unpublished results).

EXPERIMENTAL

General. IR (KBr, BOMEM – FT-IR); UV (Perkin Elmer); ^1H and ^{13}C NMR at 400 and 100 MHz respectively, TMS as standard, in a Bruker ARX; delay time for DCOSY = D6 = 400 ms; mixing time for NOESY = D8 = 600 ms. J for HMQC = 128 Hz and 9 Hz for HMBC; mp at Köffler on microscope; MS low resolution on a HP – 2576 instrument.

Plant material. Picrolemma granatensis was collected in the Colombian Amazon and a voucher specimen is deposited at the New York Botanical Garden (W. Thomas 5485). The extraction procedure was described previously [1,2].

Separation and identification of compounds. The CH_2Cl_2 extract (7.6 g) of the stem bark (1.13 kg) was chromatographed over silica gel using a CH_2Cl_2–EtOAc–MeOH gradient (column A). Elution of column A with CH_2Cl_2–EtOAc–MeOH (90:5:5) gave a mixt. of **2** and **3** (47 mg) which was crystallized in MeOH and analyzed by Recycling HPLC to give 20 mg of **2** pure and another mixt. of **2** and **3** (25 mg). Continued elution of the column A with CH_2Cl_2–EtOAc–MeOH (94:3:3) gave a fr. which was rechromatographed by a silica gel flash CC and then allowed to react as usual with Ac_2O in pyridine leading to **1a** (13 mg) and **1b** (21 mg). The acetates were purified by silica gel CC eluted with *n*-hexane–EtOAc–MeOH. The fr. eluted with CH_2Cl_2–EtOAc–MeOH (42:4:4) yielded a crude fr. which was rechromatographed by silica gel flash CC with CH_2Cl_2–EtOAc–MeOH (44:3:3). The frs (110 mg) obtained were crystallized from MeOH yielding the quassinoids described above. Glaucarubinone 1,30-diacetate (20 mg) and 1,12,30-triacetate (90 mg) were obtained after reaction with Ac_2O in pyridine.

21,23-*Epoxy*-7α,20,21,24-*tetraacetoxy*-25-*hydroxy*-*apotirucalla*-14-*en*-3-*one* (**1a**). Oil. $[\alpha]_D$ (25°; $CHCl_3$; c 0,067) = −12. IR ν_{max}^{KBr} cm^{-1}: 2960, 2940, 2880, 1731, 1726, 1703, 1641, 1530, 1470, 1370, 1230, 1023, 958, 837, 608, 541. MS m/z (rel. int.): 610 [M − 2 × AcOH]$^+$ (5), 551 (5), 488 (7), 377 (10), 338 (12), 324 (11), 301 (11), 281 (11), 257 (12), 244 (10), 233 (13), 207 (18), 198 (13), 195 (15), 186 (11), 176 (15), 163 (15), 157 (19), 143 (20), 132 (27), 96 (11), 83 (23), 69 (12), 59 (23), 43 (100). ^1H NMR (δ $CDCl_3$, 400 MHz): Table 2. ^{13}C NMR (δ $CDCl_3$, 100 MHz): Table 3.

21,23 - *Epoxy* - 7α,20,21,24,25 - *pentaacetoxy*-*apotirucalla*-14-*en*-3-*one* (**1b**). Oil. $[\alpha]_D$ (25°; $CHCl_3$; c 0,073) = −14. IR ν_{max}^{KBr} cm^{-1}: 3501, 2963, 2942, 2879, 1738, 1728, 1701, 1649, 1471, 1377, 1233, 1019, 957, 838, 605, 540. MS m/z (rel. int.): 568 [M − 2 × AcOH]$^+$ (0, 4), 508 (0, 5), 480 (0, 5), 371 (2), 310 (2), 279 (2), 258 (1), 185 (3), 171 (1), 165 (1), 153 (1), 143 (3), 137 (2), 127 (2), 111 (3), 109 (5), 105 (4), 97 (8), 83 (7), 71 (14), 69 (8), 59 (15), 55 (17), 43 (100). ^1H NMR (δ $CDCl_3$, 400 MHz): Table 2. ^{13}C NMR (δ $CDCl_3$, 50 MHz): Table 3.

7α,24,25-*Trihydroxy*-3-*oxo*-*apotirucalla*-14,20(22)-*dien*-21,23-*olide* (**2**). Pale yellow oil. IR ν_{max}^{KBr} cm^{-1}: 3571, 3527, 3387, 2970, 2935, 2898, 1746, 1705, 1698, 1456, 1387, 1256, 1387, 1251, 1184, 1088, 1051, 1017, 878, 808, 689, 570, 476. MS m/z (rel. int.): 482 [M − H_2O]$^+$ (45), 409 (4), 379 (4), 301 (8), 273 (12), 243 (12), 239 (20), 202 (32), 185 (12), 181 (36), 180 (32), 177 (32), 174 (28), 160 (12), 156 (32), 155 (28), 154 (32), 152 (28), 145 (28), 143 (24), 138 (36), 136 (32), 129 (12), 127 (32), 121 (12), 119 (12), 118 (32), 116 (28), 109 (28), 107 (24), 91 (40), 79 (24), 77 (24), 69 (20), 67 (28), 59 (44), 55 (16), 53 (24), 43 (100). ^1H NMR (δ $CDCl_3$, 400 MHz): 7.14 (t, J = 1.2 Hz,

H-22), 5.52 (*d*, $J = 3$ Hz, H-15), 5.19 (*dd*, $J = 1.2$, 1.6 Hz, H-23), 3.96 (*t*, $J = 2$ Hz, H-7), 3.52 (*d*, $J =$ 1.2 Hz, H-24), 2.83 (*ddd*, $J = 10, 7, 1.2$ Hz, H-17), 2.66 (*dd*, $J = 15$, 10.4 Hz, H-16α), 2.50 (*ddd*, $J =$ 16, 11, 8 Hz, H-2α), 2.28–2.41 (*m*, 2H, H-2β and H-16β), 1.39, 1.30, 1.12, 1.07, 1.02, 0.99 and 0.85 (Me). ^{13}C NMR (δ $CDCl_3$, 50 MHz): Table 3.

7α,24,25 - *Trihydroxy* - 3 - *oxo* - *apotirucalla* - 1,14,20(22) - *trien* - 21,23 - *olide* (**3**). IR ν_{max}^{KBr} cm^1: 1697. UV λ_{max}^{EtOH} nm (log ε): 230 (3.51). ^{1}H NMR (δ $CDCl_3$, 400 MHz): 7.16 (*t*, $J = 1.2$ Hz, H-22), 7.09 (*d*, $J =$ 9 Hz, H-1), 5.79 (*d*, $J = 9$ Hz, H-2), 5.55 (*d*, $J = 3$ Hz, H-15), 5.19 (*dd*, $J = 1.2$, 1.6 Hz, H-23), 4.00 (*t*, $J =$ 2 Hz, H-7), 3.52 (*d*, $J = 1.2$ Hz, H-24), 2.83 (*ddd*, $J = 10, 7$, 1.2 Hz, H-17), 2.66 (*dd*, $J = 15$, 10.4 Hz, H-16α), 1.30, 1.12, 1.07, 1.02, 0.99 and 0.85 (Me). ^{13}C NMR (δ $CDCl_3$, 50 MHz): Table 3.

Glaucarubinone 1,30-*diacetate* (**4b**). Amorphous solid (MeOH), mp 230–233°. $[\alpha]_D$ (25°; EtOH; *c* 0,049) = +28. UV λ_{max}^{EtOH} nm (log ε): 239 (4.01). IR ν_{max}^{KBr} cm^{-1}): 3442, 2969, 2932, 2876, 1740, 1664, 1543, 1454, 1382, 1262, 1037, 986, 691. MS *m/z* (rel. int.): 460 $[M - H_2O]^+$ (0.5), 358 (3), 301 (5), 285 (4), 241 (4), 225 (6), 189 (5), 151 (12), 142 (8), 135 (12), 123 (17), 109 (12), 102 (8), 95 (27), 91 (19), 85 (28), 83 (12), 79 (15), 69 (21), 57 (100), 55 (24), 43 (52). ^{1}H NMR (δ $CDCl_3$, 200 MHz): 6.20 (*d*, $J = 9.3$ Hz, H-15), 6.03 (*m*, H-3), 5.28 (*s*, H-1), 4.90 (*sl*, H-7), 4.67 (*d*, $J = 13.5$ Hz, H-30a), 3.97 (*s*, H-9), 3.88 (*d*, $J =$ 8.5 Hz, H-12), 3.80 (*d*, $J = 13.5$ Hz, H-30b), 3.22 (*d*, $J = 12.8$ Hz, H-5), 2.53 (*dd*, $J = 9.3$, 4.8 Hz, H-14), 1.97 (*sl*, H-29), 1.44 (*s*, H-19, H-5'), 1.22 (*d*, H-18), 0.95 (*t*, H-4'). ^{13}C NMR (δ $CDCl_3$, 50 MHz): Table 1.

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