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NEW TRITERPENES FROM THE LEAVES OF *TIMONIUS TIMON*[1]

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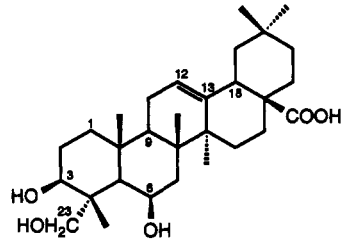
ABSTRACT.—Two new triterpenes, 3β,6β,23-trihydroxyolean-12-en-28-oic acid [**1**] and 3β,6β,19α,23-tetrahydroxyolean-12-en-28-oic acid [**2**], have been isolated from the leaves of *Timonius timon* (Rubiaceae) with other known compounds: 3β,6β,19,23-tetrahydroxyurs-12-en-28-oic acid, 3-*O*-[β-D-quinovopyranosyl]quinovic acid, 3-*O*-[α-L-arabinopyranosyl(1→2)β-D-quinovopyranosyl]quinovic acid, and loganin. Their structures were determined by spectroscopic methods (eims, hrfabms, ^{1}H nmr, ^{13}C nmr, ^{1}H-^{1}H COSY, and ^{1}H-^{13}C COSY).

Timonius timon (Speng.) Merr. (Rubiaceae) is a small tree growing in the highlands of the Central Province in Papua New Guinea. The leaves and bark of the genus *Timon* are used traditionally as a remedy against fever and as a contraceptive (1,2). In our continuing search for bioactive constituents from medicinal plants in Papua New Guinea, we collected the leaves for chemical and biological investigation. In preliminary biological screening, *T. timon* showed antibacterial activity against *Bacillus subtilis, Micrococcus luteus*, and *Escherichia coli* and molluscicidal activity against *Biomphalaria glabrata* snails (3). Here we report the isolation and structure elucidation of two new triterpenes.



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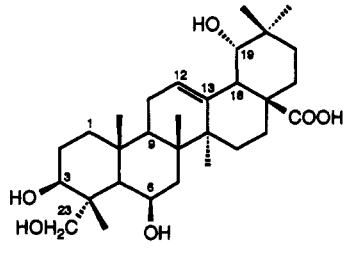

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RESULTS AND DISCUSSION

Compound **1** was assigned the molecular formula $C_{30}H_{48}O_5$ from hrfabms. The ^{13}C- and DEPT ^{13}C-nmr spectra showed six signals for Me carbons, nine methylene, four methine, and seven quaternary carbons together with a carboxyl group, a primary OH group, and two secondary OH groups. A total of 30 carbon resonances were observed, which showed its triterpenic nature. The chemical shifts of olefinic carbons at 123 ppm and 144 ppm were useful to distinguish between an urs-12-ene and an olean-12-ene analogue (4).

The ^{1}H-nmr spectrum of compound **1** confirmed the presence of an olefinic proton which resonated at δ 5.54 (1H, m, H-12), one primary OH group at δ 4.18 and 3.83 (AB system), and two secondary OH groups appearing at δ 4.04 and 4.85. There were also six singlets due to Me groups at quaternary carbons in the region δ 0.72–1.54.

The comparison of compound **1** with other known structurally related compounds (5–9) led to the conclusion that in compound **1** the C-23 Me group was

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hydroxylated (67.1 ppm, triplet) and the other two secondary OH groups should be positioned at C-3 (73.3 ppm, doublet) and C-6 (67.5 ppm, doublet).

The β-OH substitution at C-3 was evident from the chemical shift and *J* value of the axial proton at C-3 (δ 4.0, dd, *J*=11 and 4 Hz, H-3). The β configuration of the 6-OH was supported from the unresolved signal of the equatorial H-6 proton (δ 4.8, 1H, m, W½=5 Hz). From these data it was concluded that compound **1** is 3β,6β,23-trihydroxyolean-12-en-28-oic acid.

Compound **2** was assigned the molecular formula $C_{30}H_{48}O_6$ from hrfabms. The ^{13}C- and DEPT ^{13}C-nmr spectrum of compound **2** showed the presence of one more secondary OH group compared to compound **1**. In the ^1H-nmr of compound **2**, H-18 was shifted downfield (δ 3.55, compound **1** δ 3.14), and one more signal appeared at 3.44 ppm (br d). From the ^{13}C-^1H correlation spectrum, it was clear that the OH group should be at the C-19 position. The α-configuration of the 19-OH was deduced from the H-18 (δ 3.55, br d) and the ^{13}C shift of C-19. Thus, the structure of compound **2** was elucidated as 3β,6β,19α,23-tetrahydroxyolean-12-en-28-oic acid. Compounds **1** and **2** did not show any significant activity against *Ba. subtilis, M. luteus,* and *E. coli* nor molluscicidal activity against *Bi. glabrata* snails.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Reversed-phase low pressure liquid chromatography (lplc) was carried out using a Büchi 681 and a Labomatic mplc column (71.3 cm×1.85 cm i.d.). The column was packed with RP-18 material, particle size 40 μm (Bondesil, Analytichem International). The solvents used for lplc were hplc grade. Si gel 60 F_{254} plates from Merck (0.25 mm, Art. no. 5729) were used for tlc analysis. Ir spectra were recorded on a Perkin-Elmer 781 spectrophotometer, eims spectra were obtained on a ZAB 2-SEQ spectrometer, and hrfabms were recorded in a NBA matrix in the positive mode on a VG ZAB instrument. All nmr experiments were performed with a Bruker AMX-300 spectrometer operating at 300 (^1H) and 75.5 MHz (^{13}C). All samples prepared for nmr measurements were made as C_5D_5N solutions with TMS (δ=0) as the internal standard.

PLANT MATERIAL, EXTRACTION AND ISOLATION.—The plant material was collected on the road to Hisiu Beach, west of Lolorua, on the road from Port Moresby to Bereina, about 60 km north of Port Moresby, Central Province of Papua New Guinea, in September 1988. The voucher specimens are deposited at the Herbarium (ZT, 11804) ETH, Zurich, Switzerland, UPNG Herbarium, Port Moresby, and at the National Herbarium in Lae, Papua New Guinea. The air-dried leaves of *T. timon* (2 kg) were percolated with petroleum ether, EtOAc, and MeOH, which gave 40 g, 79 g, and 120 g of crude extracts, respectively. The EtOAc extract (15 g) was fractionated by a Si gel column (450 g) using EtOAc/MeOH with increasing polarity. Fractions 300–350 were combined and subjected to RP-18 column (300 g) using MeOH-H_2O (80:20) as an eluent, and fractions were collected after 500 ml eluent was passed. Fractions 12–18 contained compound **1** (30 mg), and 27–31 contained 40 mg of compound **2**.

BIOASSAYS.—The screening for antimicrobial and molluscicidal activities was carried out as described by Baumgartner (3).

Compound **1**.—Amorphous colorless powder: $[\alpha]^{20}D$ +40.3° (*c*=3.25, MeOH); ir (KBr) ν max cm^{-1} 3440, 1695, ^1H nmr (300 MHz, C_5D_5N) δ 5.44 (1H, m, H-12), 4.85 (1H, m, w½=5 Hz, H-6), 4.18 and 3.83 (each 1H, d, *J*=10.4 Hz, H-23), 4.04 (1H, dd, *J*=11.4 Hz, H-3), 3.14 (1H, dd, *J*=10.2 Hz, H-18), 1.51 (3H, s, H-24), 1.47 (3H, s, H-25), 1.42 (3H, s, H-26), 1.06 (3H, s, H-27), 0.80 (3H, s, H-29), 0.72 (3H, s, H-30); ^{13}C nmr see Table 1; eims *m/z* (%) $[M]^+$ 484 (5), 470 (4), 452 (8), 442 (15), 424 (35), 288 (40), 248 (100), 203 (60), 187 (35); hrfabms *m/z* $[M+Li]^+$ 495.3638 (calcd for $C_{30}H_{48}O_5Li$, 495.3662).

Compound **2**.—Amorphous powder: $[\alpha]^{20}D$ +20.5° (*c*=1.50, MeOH); ir (KBr) ν max cm^{-1} 3460, 1690, ^1H nmr (300 MHz, C_5D_5N) δ 5.44 (1H, m, H-12), 4.83 (1H, m, w½=5 Hz, H-6), 4.19 and 3.85 (each 1H, d, *J*=10.2 Hz, H-23), 4.0 (1H, dd, *J*=11.4 Hz, H-3), 3.55 (1H, dd, *J*=10.6 Hz, H-18), 3.44 (1H, d, w½=8 Hz, H-19), 1.53 (3H, s, H-24), 1.48 (3H, s, H-25) 1.46 (3H, s, H-26), 1.42 (3H, s, H-27), 0.99 (3H, s, H-29), 0.92 (3H, s, H-30); ^{13}C nmr see Table 1; eims *m/z* (%) $[M]^+$ 504 (4), 486 (5), 458 (10), 442 (22), 386 (6), 264 (28), 246 (57), 222 (78), 203 (40), 187 (36), 173 (38), 157 (17), 146 (100); hrfabms *m/z* $[M+Li]^+$ 511.3708 (calcd for $C_{30}H_{48}O_6Li$, 511.3611).

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TABLE 1. ^{13}C-nmr Data for Compounds **1** and **2** (75 MHz, pyridine-d_5, ppm).

Carbon	Compound	
	1	**2**
C-1	41.1 t	41.3 t
C-2	28.3 t	28.6 t
C-3	73.3 d	73.4 d
C-4	44.0 s	43.9 s
C-5	49.3 d	49.7 d
C-6	67.5 d	67.9 d
C-7	41.1 t	40.8 t
C-8	39.2 s	39.3 s
C-9	48.7 d	49.0 d
C-10	36.9 s	37.1 s
C-11	23.7 t	24.7 t
C-12	122.9 d	123.8 d
C-13	144.2 s	144.7 s
C-14	42.7 s	42.6 s
C-15	28.3 t	28.7 t
C-16	23.9 t	27.9 t
C-17	46.6 s	46.3 s
C-18	42.0 d	45.0 d
C-19	46.4 t	81.6 d
C-20	30.9 s	35.7 s
C-21	34.2 t	29.2 t
C-22	33.2 t	33.9 t
C-23	67.1 t	67.3 t
C-24	14.7 q	14.6 q
C-25	17.4 q	17.3 q
C-26	18.6 q	18.4 q
C-27	26.2 q	24.8 q
C-28	180.2 s	180.0 s
C-29	23.7 q	25.0 q
C-30	33.2 q	28.8 q

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