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3β-*O*-PALMITYL LONGISPINOGENIN FROM *TRICHOCEREUS CHILENSIS*

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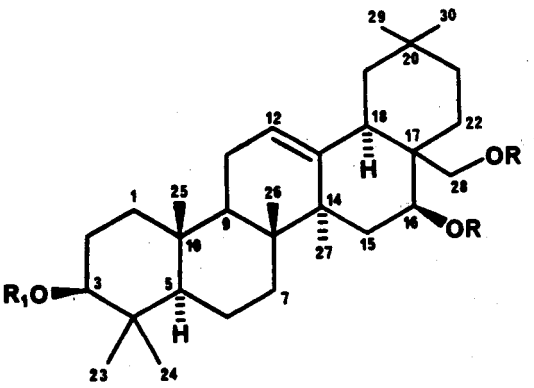
ABSTRACT.—From the aerial parts of *Trichocereus chilensis* a new triterpenetriol fatty acid ester was isolated. Its structure was shown to be 3β-*O*-palmityl longispinogenin (olean-12-ene-3β,16β,28-triol-3-palmitate) [**1**] by chemical and spectroscopic methods. Compound **1** is inactive in brine shrimp lethality and cytotoxicity bioassays.

The plant family Cactaceae is widely distributed in the north of Chile. In this communication, we report the isolation and structure determination of a new triterpenoid from *Trichocereus chilensis* (Colla) Br. and R., known in Chile as "buisco." The quaternary alkaloid, candicine, has been previously found in the plant (1).



1′ 16′

1 R_1=CO$(CH_2)_{14}CH_3$, R=H
2 R_1=CO$(CH_2)_{14}CH_3$, R=Ac
3 R_1=R=H

After repeated flash and low pressure cc on Si gel, the concentrated MeOH-Et_2O-petroleum ether (1:1:1) extract of *T. chilensis* afforded the new compound **1** (1.0% yield). Compound **1** was obtained as a colorless gum that solidified into a glass upon standing. The ir spectrum of **1** showed absorptions at 3400–3150 cm^{-1} (OH), 1730 and 1250 cm^{-1} (ester carbonyl), and 1680 cm^{-1} (C=C).

The ^{1}H-nmr spectrum of **1** displayed an ethylenic proton at δ 5.20 (t, J = 3.4 Hz), and accounted for seven tertiary methyl groups between δ 0.88–1.21, suggesting a triterpenoid structure. The fully decoupled and DEPT ^{13}C-nmr spectra of **1** (Table 1) showed 38 signals between δ 13.9 and 173.6: seven Me, eighteen CH_2, six CH groups, and seven quaternary carbon atoms. The presence of six sp^3 and one sp^2 quaternary carbon atoms and the resonances at δ 122.5 (C-12) and 142.8 (C-13) were characteristic of an olean-12-ene skeleton. Acetylation of **1** yielded **2**, which showed in its ^{1}H-nmr spectrum two singlets at δ 2.01 (3H) and 2.05 (3H), indicating that **1** is an ester diol.

The eims of **1** showed ions at *m/z* 232 (14%), $[232 - CH_2OH]^+$ 201 (base),

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TABLE 1. ^{13}C-Nmr Spectral Data (ppm) of Compounds **1**, **2**, and **3** ($CDCl_3$).

Carbon	Compound			Carbon	Compound		
	1	**2**	**3**		**1**	**2**	**3**
1	38.4 t	38.2	28.3	21	33.6 t	33.6	33.5
2	26.1 t	26.8	26.9	22	26.1 t	23.5	26.0
3	80.6 d	80.4	78.9	23	29.0 q	28.0	28.1
4	37.7 s	37.7	38.6	24	16.7 q	16.8	16.8
5	55.4 d	55.2	55.2	25	15.6 q	15.5	15.6
6	18.3 t	18.1	18.4	26	16.7 q	16.8	16.8
7	32.6 t	32.5	32.6	27	26.8 q	26.9	27.2
8	39.9 s	39.6	39.8	28	71.9 t	66.4	71.3
9	46.8 d	46.6	46.6	29	33.1 q	32.9	33.1
10	36.8 s	36.7	36.9	30	23.9 q	23.5	23.9
11	23.6 t	23.5	23.5	1′	173.6	173.6	
12	122.5 d	123.5	122.6	2′	34.6	34.8	
13	142.8 s	141.7	142.7	3′	25.2	25.1	
14	43.2 s	43.3	43.7	4′–13′	29.3–29.6	29.2–29.6	
15	36.0 t	31.6	36.0	14′	31.9	31.9	
16	67.8 d	68.9	67.9	15′	22.6	22.7	
17	40.3 s	39.9	40.2	16′	13.9	14.1	
18	44.8 d	43.1	44.7	Ac		21.1	
19	46.7 t	46.1	46.8			170.4	
20	30.8 s	30.6	30.8			171.1	

and 189 (20%), while in **2** these ions appeared at *m/z* 274 (16%), $[274 - CH_2OAc]^+$ 201 (base), and 189 (28%); these are the typical retro-Diels-Alder fragments of a triterpenoid of the olean-12-ene series with two substituents in the D or E rings, one of them being a CH_2OH group (2,3).

Comparison of the ^{13}C-nmr (Table 1) and ^{1}H-nmr (see Experimental) data of **1** with those of **2** suggested that the original ester unit in **1** was linked to the C-3, while at C-16 and C-28 were attached the OH groups in **1** and the OAc groups in **2**. Thus, for example, the signals at δ 80.6 in **1** and at δ 80.4 in **2** corresponded to the site of esterification at C-3 of the A ring without substitutions at C-23 or C-24 (4). The signals at δ 4.46 (t, $J = 7.8$ Hz in **1** and $J = 8.3$ Hz in **2**) were indicative of a 3β orientation of the ester residue. The signals at δ 4.32 (m) in **1**, which shifted to δ 5.51 (dd, $J = 11.7$ and 4.8 Hz) in **2**, indicated a 16β orientation of the OH group (5). Finally, the CH_2OH must then be involved at C-28.

Basic hydrolysis of the ester **1** yielded longispinogenin [**3**] (6) as the alcoholic portion and palmitic acid as the acidic portion. On the basis of these spectral and chemical data, the structure of this new triterpenoid ester **1** was established as 3-β-*O*-palmityl longispinogenin (olean-12-ene-3β,16β,28-triol-3-palmitate). This structure for **1** required a molecular formula of $C_{46}H_{80}O_4$ and a mol wt of 696. No peak at *m/z* 696 in the eims was observed, but a small peak appeared at *m/z* $[MH]^+$ 697 (0.5%) in cims (isobutane). In the cims of **2**, the molecular peak increased to *m/z* $[MH]^+$ 781 (0.3%). The highest peak in the eims of **1** appeared at *m/z* 678 (1%) and was interpreted as $[M - H_2O]^+$. The exact mass of the peak at *m/z* 650 (649.5641) indicated the formula $C_{44}H_{74}O_3$, and it was interpreted as $[M - Me - CH_2OH]^+$.

The base peak (*m/z* 201) in the eims of **1** can be rationalized by retro-Diels-Alder fragmentation of the ion at *m/z* 422 $[C_{30}H_{46}O]^+$, generated by successive loss of palmitic acid and H_2O from the molecular ion to give *m/z* 232 (14%)

and *m/z* 189 (20%). The loss of CH_2OH from the ion at *m/z* 232 thus explained the base peak of the ms at *m/z* 201 $(C_{15}H_{21})^+$ (7).

In human tumor cell cytotoxicity screening, compound **1** showed no activity ($ED_{50}>10$ ppm) against A-549 (lung), MCF-7 (breast), and HT-29 (colon). Also, it was nontoxic to brine shrimp ($LC_{50}>1000$ ppm) (8). Longispinogenin [**3**] has been found as an aglycone in many natural products (9). It was first isolated from the acidic hydrolysis products of the Guatemalan cactus *Lemaireocereus longispinus* (10). This is apparently the first report of its 3-O-palmityl ester. The high yield of **1** (1.0% of the dry material) is especially noted.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—^{13}C- and ^1H-nmr spectra were recorded in $CDCl_3$ on various instruments. Cims (isobutane) and eims spectra were run on a Finnigan 4000. Exact masses were determined on a Kratos MS50. Cell culture bioassays were performed at the Purdue Cell Culture Laboratory. Brine shrimp lethalities were determined as usual (8).

PLANT MATERIAL.—*T. chilensis* was collected in November 1986 and identified by Dra. Adriana Hoffman. Voucher specimens are kept at the Herbarium of the Laboratorio de Productos Naturales de la Universidad de Antofagasta, Chile.

EXTRACTION AND ISOLATION.—After removal of the spines, the fresh cactus was cut into small pieces, dried at 80° (48 h), and powdered, and the dry material (348 g) was extracted with MeOH-Et_2O-petroleum ether (1:1:1) (1.5 liters × 3) for a week at room temperature. The evaporation of solvent under reduced pressure led to a viscous residue (20.5 g). This extract was then subjected to flash cc on Si gel using petroleum ether-EtOAc (10:0, 4:1, 3:1, 1:1, 0:10).

The fraction obtained on elution with petroleum ether-EtOAc (4:1) was evaporated to dryness, and repeated cc, using low pressure on Si gel, petroleum ether-EtOAc (10:1), gave compound **1** (3.5 g) as a colorless gum, which solidified on standing to a glass: mp 81°, $[\alpha]^{20}D$ +30 ($c=0.14$, $CHCl_3$), ir ν max KBr cm^{-1} 3400–3150; 3000–2850, 1730, 1680, 1450, 1250, 1000; ^1H nmr (470 MHz) ($CDCl_3$) δ 0.88 (6H, s), 0.91 (6H, s), 0.96 (3H, s), 1.01 (3H, s), 1.21 (3H, s) (altogether 7 Me), 2.29 (3H, m, 18-H and CH_2-COOR, H-2′), 2.51 (1H, br s, exchanged with D_2O, OH), 2.87 (1H, br s, exchanged with D_2O, OH), 3.16 (1H, d, $J=10.0$ Hz, H-28), 4.17 (1H, d, $J=10.0$ Hz, H-28′), 4.32 (1H, m, H-16), 4.46 (1H, t, $J=7.8$ Hz, H-3), 5.20 (1H, t-like, $J=3.4$ Hz, H-12); ^{13}C nmr see Table 1; eims *m/z* (% rel. int.) $[M-18]^+$ 678 (1), 650 $[M-Me-CH_2OH]^+$ (5), 635 (1), 632 (5), $[M-256]^+$ 440 (0.3), $[678-255]^+$ 423 (5), $[440-H_2O]^+$ 422 (1), $[423-H_2O]^+$ 405 (0.6), $[423-MeOH]^+$ 391 (5), 255 (12), 232 (14), 201 (100), 189 (20), 175 (12), 135 (18), 121 (24), 119 (23); cims (isobutane) *m/z* (% rel. int.) $[MH]^+$ 697 (0.5), 679 (10), 662 (6), 651 (19), 635 (5), $[M-255-H_2O]^+$ 423 (100), 229 (16), 201 (20), 191 (18); hrms *m/z* (composition) 649.5641 ($C_{44}H_{74}O_3$), 634.5360 ($C_{43}H_{71}O_3$), 621.5209 ($C_{42}H_{68}O_3$), 201.1649 ($C_{15}H_{21}$).

ACETYLATION OF **1**.—Compound **1** (50 mg) was dissolved in Ac_2O (2.0 ml) and pyridine (2.0 ml). After 48 h at room temperature, the reaction mixture was worked up as usual to yield the diacetate **2** (62 mg) as a colorless gum: $[\alpha]^{20}D$ +50 ($c=0.14$, $CHCl_3$); ir ν max (film) cm^{-1} 2980–2850, 1740, 1680, 1450, 1350, 1230, 1000; ^1H nmr ($CDCl_3$) 0.84 (9H, s), 0.88 (9H, s), 0.93 (3H, s), 0.98 (3H, s) (altogether 8 Me), 2.01 (3H, s, OAc), 2.05 (3H, s, OAc), 2.27 (2H, t, $J=10$ Hz, CH_2COOR, H-2′), 2.35 (1H, m, H-18), 4.00 (1H, d, $J=11.7$ Hz, H-28), 4.17 (1H, d, $J=11.7$, H-28′), 4.46 (1H, t, $J=8.3$ Hz, H-3), 5.25 (1H, br s, H-12), 5.51 (1H, dd, $J=11.7$, 4.8 Hz, H-16); ^{13}C nmr see Table 1; eims *m/z* (% rel. int.) $[M-120]^+$ 660 (0.6), 632 (2), 604 (1), $[M-255]^+$ 525 (0.5), $[525-60]^+$ 465 (4), 405 (7), 391 (5), 335 (0.2), $[335-HOAc-H]^+$ 274 (16), 255 (3), 215 (15), 214 (28), 201 (100), 191 (31), 190 (39), 189 (28); cims *m/z* (isobutane) $[MH]^+$ 781 (0.3), 721 (0.9), 689 (0.9), 661 (6), 539 (4), 525 (4), 465 (100), 405 (51), 274 (8), 215 (15), 201 (18), 191 (26), 190 (10), 189 (7).

SAPONIFICATION OF **1**.—Compound **1** (50 mg) was refluxed with 5% alcoholic KOH (10 ml) for 2 h. The reaction mixture was concentrated to one-half in vacuo, added to crushed ice, extracted with $CHCl_3$, and evaporated to dryness to yield a solid which crystallized from Me_2CO (23 mg): mp 239–245°; $[\alpha]^{20}D$ +53 ($c=0.96$, $CHCl_3$); **3** was identified as longispinogenin by comparison of its physical and spectroscopic properties with those reported in the literature (4–6, 9, 10).

The aqueous basic solution was acidified with aqueous HCl (1 N) and extracted with $CHCl_3$ to afford a solid. This was identified as palmitic acid by comparison (tlc, eims) with authentic samples as the free acid and as methyl ester derivatives prepared with CH_2N_2.

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