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TRITERPENOID DIGLUCOSIDE OF *ENTEROSPERMUM PRUINOSUM* *

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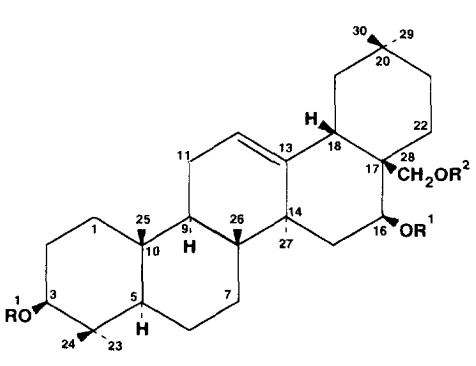
Key Word Index—*Enterospermum pruinosum*; Rubiaceae; saponins; longispinogenin.

Abstract—From the leaves of *Enterospermum pruinosum* longispinogenin (= olean-12-ene-3β,16β,28-triol) 3,16-di-*O*-β-D-glucopyranoside was isolated and identified by chemical and spectroscopic methods.

INTRODUCTION

Enterospermum pruinosum Baillon ex Dubard (= *Ixora pruinosa*) is a Mahagasy plant whose leaves are used in traditional medicine as an antidiarrhoic and an antihelminthic. By a preliminary chromatographic survey the presence of flavonoids, iridoids and terpenoids was shown as in other Rubiaceae. The most abundant saponin **1**, (0.09% of the dried leaves) isolated by counter-current distribution (CCD) corresponded to the formula $C_{42}H_{70}O_{13}$ ([M − H]$^-$ at *m/z* 781]). On hydrolysis in dioxane-HCl it gave D-glucose and a pentacyclic triterpene, $C_{30}H_{50}O_3$, identified from ^{13}C NMR data [2] as longispinogenin, (**2**) (= olean-12-ene-3β,16β,28-triol). It had been obtained previously from the saponins of *Lemaireocereus longispinus* [3] and the fatty acid esters of *L. chichipe* [4] and *Dolichothele longimamma* (Cactaceae) [5], and the saponins of *Bupleurum falcatum* (Umbelliferae) [6] and was isolated from the flowers of *Calendula officinalis* [7].



	R¹	R²
1	*O*-β-D-glucose	H
2	H	H
3	tetraacetyl-*O*-β-D-glucose	Ac
4	Ac	Ac

The ^1H NMR spectrum of the diglucoside **1**, besides the well distinguished signals of seven methyl groups, showed the signals of the hydroxymethylene (δ4.35, *d*, and 4.37, *d*, J_{gem} = 11.0 Hz) and of the two secondary alcoholic groups at C-3 and C-16 (δ3.87 and 3.89, *dd*, *J* = 5.1 and 12.0 Hz). In longispinogenin (where a six-membered ring hydrogen bond is between the hydroxy groups at C-16 and C-28) the corresponding signals are remarkably different, i.e. H-a and H-b of C-28 at δ3.15 and 4.10, H-16 at δ4.28 and H-3 at δ3.19.

The ^{13}C NMR spectrum of **1** showed practically identical signals for the two glucose moieties (Table 1) which ruled out any disaccharide linkage. The downfield shifts of the two secondary alcoholic carbons (C-3, δ90.7, and C-16, δ75.6) in comparison with longispinogenin (78.9 and 67.8, respectively), observed also for the nonacetyl derivative of **1**, **3**, in comparison with triacetyllongispinogenin, **4** [2], accounted for the corresponding position of the two glucose units in **1**.

The chemical shifts of the two anomeric carbons of **1**, (δ102.9 and 102.4) were consistent with their β-configuration assignable by the coupling constants of the anomeric hydrogens in **3** (δ4.48 and 4.49, 2*d*, *J* = 7.5 Hz). The structure of **1** was thus fully established. The only glycoside so far described for longispinogenin is corchorusin A (= longispinogenin 3-*O*-β-D-galactopyranoside) isolated from *Corchorus acutangulus* (Tiliaceae) [8].

EXPERIMENTAL

A Craig Post apparatus (200 stages, 10:10 ml, upper and lower phase) was used for CCD. The purification

*Part 35 in the series 'Research on African Medicinal Plants'. For part 34 see ref. [1].

Table 1. ^{13}C NMR spectral data assignments of compounds **1** and **3**

C	**1** (CD_3OD)	**3** ($CDCl_3$)
Aglucone moiety		
1	40.1	38.5
2	24.6	23.5
3	90.7	90.4
4	38.0	38.8
5	56.9	55.5
6	19.7	18.0
7	35.2[a]	33.7[a]
8	40.0[b]	39.9[b]
9	48.0	46.7
10	35.5	36.5
11	24.6	23.5
12	123.8	123.5
13	144.0	141.4
14	42.9	43.1
15	35.1[a]	32.5[a]
16	75.6	76.6
17	41.1[b]	40.8[b]
18	44.7	42.8
19	47.9	46.3
20	31.7	30.7
21	33.7	34.2
22	27.0	25.7
23	27.5	27.6
24	16.9[c]	16.6[c]
25	16.1[c]	16.3[c]
26	17.4	15.4
27	28.5	27.0
28	66.8	65.9
29	33.5	33.0
30	24.2	23.5
Sugar moiety		
1′	106.1, 106.7	102.4, 102.9
2′	77.6	71.4, 71.5
3′	78.2	72.7, 72.8
4′	71.7	68.5, 68.7
5′	78.1	71.5
6′	62.8	62.1, 62.4
MeCO		20.5
MeCO		169.1, 169.4, 170.3, 170.5, 171.0

[a–c]These assignments may be interchanged within the same column.

was monitored by TLC (silica gel F_{254}, solvent *n*-BuOH–HOAc–H_2O, 4:1:1, spray reagent anisaldehyde–sulphuric acid). ^1H and ^{13}C NMR: Bruker 500 MHz (TMS as int. ref.). FAB-MS: Kratos MS9/50TC.

Plant material, extraction and separation. Leaves of *E. pruinosum* were collected in southern Madagascar. Dried leaves (500 g) were extracted with MeOH and the residue (41 g) partitioned between H_2O–EtOH–cyclohexane–EtOAc (5:2:5:2). The upper phase, containing mainly chlorophylls, was discarded and the residue of the lower phase was partitioned between H_2O and *n*-BuOH. The *n*-BuOH extract (12 g) was subjected to CCD with the biphase system H_2O–EtOH–EtOAc–cyclohexane (10:4:13:1). The middle fraction was further purified by CCD with the system H_2O–EtOH–CH_2Cl_2 (4:6:5). The less mobile fraction ($K_r = 0.67$) was chromatographically pure and amounted to 0.45 *g* (0.09% of the starting material).

Longispinogenin 3,16-di-O-β-D-glucopyranoside (**1**). Mp 157–159° from aq. EtOH. $[\alpha]_D^{20} + 4.6$ (MeOH; *c* 0.4). FAB-MS (negative ion mode) *m/z*: 781 $[M - H]^-$, $C_{42}H_{70}O_{13}$; (positive ion mode) *m/z* 805 $[M + Na]^+$, 783 $[M + H]^+$, 765 $[M + H - H_2O]^+$. ^1H NMR (CD_3OD): δ0.90, 0.95, 0.97, 1.02, 1.06, 1.10, 1.29 (*s*, 7 Me), 3.7 (*m*, $2H_2$-6, Gl), 3.87 and 3.89 (*dd*, *J* = 5.1 and 12.0 Hz, H-3 and H-16), 4.35 and 4.37 (2*d*, J_{gem} = 11.0 Hz, H_2-28), 5.29 (*t*, *J* = 4 Hz, H-12).

Hydrolysis of compound **1** *to give longispinogenin* (**2**). The soln of **1** (60 mg) in 4 M HCl (4 ml) and dioxane (4 ml) was kept in a boiling water bath for 1 hr. The soln was diluted with water and extracted with CH_2Cl_2. The residue of the organic phase was purified by CCD with H_2O–Me_2CO–EtOH–cyclohexane 2:2:3:5) and was identified as longispinogenin (**2**). ^1H NMR ($CDCl_3$): δ0.77, 0.89 (×2), 0.91, 0.97 (×2), 1.20 (*s*, 7 Me), 3.15 (*d*, J_{gem} = 11.0 Hz, H_a-28), 3.19 (*dd*, *J* = 4.4 and 11.4 Hz, H-3), 4.10 (*d*, H_b-28), 4.28 (*dd*, *J* = 4.8 and 12.0 Hz, H-16), 5.17 (*t*, *J* = 4 Hz, H-12). The aq. soln was percolated through a column of a slightly alkaline anion-exchanger and in the residue glucose was identified by TLC and through its β-pentaacetate.

Nonaacetate of compound **1** (**3**). Compound **1** was acetylated with a 1:1 mixture of pyridine and Ac_2O. After 2 days the reagents were evapd *in vacuo* and the residue purified by CCD with H_2O–Me_2CO–EtOH–EtOAc–cyclohexane (10:8:9:1:20). Mp 121–123° from *n*-hexane, $[\alpha]_D^{20} + 16.2$ ($CHCl_3$; *c* 0.5). ^1H NMR ($CDCl_3$): δ0.71, 0.83 (×4), 0.95, 1.15 (*s*, 7 Me), 1.96–2.03 (*s*, 9 Ac), 3.61 (*m*, 2H-5, Gl), 3.66 (H-3 and H-16, overlapped), 3.9–4.2 (*m*, $2H_2$-6, Gl), 4.48 and 4.49 (2 *d*, *J* = 7.5 Hz, 2H-1, Gl), 5.13 and 5.16 (2 *d*, J_{gem} = 11.0 Hz, H_2-28), 5.19 (*t*, *J* = 4 Hz, H-12).

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