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A NEW OLEANOLIC GLYCOSIDE FROM *POLYSCIAS SCUTELLARIA*

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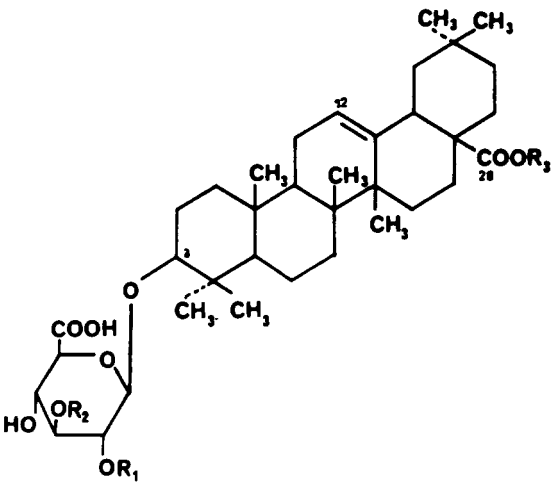
ABSTRACT.—A new triterpenoid saponin, named polysciasaponin P_1 [**8**], has been isolated from *Polyscias scutellaria* leaves and identified as 3-*O*-[β-D-glucopyranosyl(1→4)-β-D-glucopyranosyl(1→2)-β-D-glucuronopyranosyl] oleanolic acid 28-*O*-β-D-glucopyranoside. The structure was established by chemical and spectroscopic means (fabms, ^{13}C nmr, gc-ms).

In previous work, we reported the isolation and structure determination of polysciasaponins P_7 [**2**], P_6 [**3**], P_4 [**5**] (1), P_5 [**4**], and P_2 [**7**] (2) from the leaves of *Polyscias scutellaria* (Burm.f.) Fosb (Araliaceae) used in the islands of the Western Pacific Ocean, especially in Vanuatu, as an anti-inflammatory. In the present paper we report the isolation and structure elucidation of polysciasaponins P_3 [**6**] and P_1 [**8**]; P_1 is a new oleanolic glycoside.

An EtOH extract of dried leaves was fractionated. The *n*-BuOH fraction yielded a crude mixture of saponins. Separation of the *n*-BuOH extract by cc and preparative tlc on Si gel afforded pure saponins P_1 and P_3.

On acid hydrolysis, polysciasaponins P_1 and P_3 afforded oleanolic acid [**1**] as the aglycone, identified by comparison with a standard sample (tlc, ms, and gc-ms). The sugars obtained from the hydrolysates were identified by tlc and gc



1 OH at C-3, R_3=H
2 R_1=R_2=R_3=H
3 R_1=R_3=H, R_2=Glc
4 R_2=R_3=H, R_1=Glc
5 R_1=H, R_2=R_3=Glc
6 R_2=H, R_1=R_3=Glc
7 R_2=R_3=H, R_1=Glc(1→4)Glc
8 R_2=H, R_1=Glc(1→4)Glc, R_3=Glc

Glc=β-D-glucopyranosyl

as glucuronic acid and glucose for P_1 (1:3), glucuronic acid and glucose for P_3 (1:2).

The mol wt and the sugar sequence of P_1 and P_3 were established by fabms in negative ion mode. The following signals were observed: *m/z* 1117 for P_1, *m/z* 955 for P_3 corresponding to depronated molecular ion $[M-H]^-$; *m/z* 955 for P_1 and *m/z* 793 for P_3 resulting from the loss of a glucosyl moiety $[M-H-162]^-$.

On mild acid hydrolysis, P_1 initially gave P_2, P_3, P_5, then P_7; polysciasaponin P_3 initially gave P_5 and P_7.

Basic hydrolysis of polysciasaponin P_1 yielded P_2, 3-*O*-[β-D-glucopyranosyl(1↦4)-β-D-glucopyranosyl(1↦2)-β-D-glucuronopyranosyl] oleanolic acid; basic hydrolysis of P_3 afforded P_5, 3-*O*-[β-D-glucopyranosyl(1↦2)-β-D-glucuronopyranosyl] oleanolic acid. These results indicated that polysciasaponins P_1 and P_3 are ester glycosides of P_2 and P_5, respectively. The structure of polysciasaponin P_2 was established as 3-*O*-[β-D-glucopyranosyl(1↦4)-β-D-glucuronopyranosyl] oleanolic acid (1), and polysciasaponin P_5 as 3-*O*-[β-D-glucopyranosyl(1↦2)-β-D-glucuronopyranosyl] oleanolic acid (4). Upon comparison of the ^{13}C-nmr spectrum of P_1 with that of P_2 (Table 1), all of the carbon signals of the

TABLE 1. ^{13}C nmr Spectral Data of Polysciasaponins P_1 [8] and P_2 [7] in C_5D_5N (δ ppm/TMS).

Carbon	Polysciasaponin P_1	Polysciasaponin P_2
Oleanolic acid		
C-3	89.52	89
C-12	122.10	122.2
C-13	144.12	144.52
C-28	176.28 (−3.6 ppm)	179.9
Glucuronic acid		
C-1′	104.40	104.6[g]
C-2′	83.26	82.9
C-3′	76.43[c]	76.4[h]
C-4′	71.85[c]	71.15
C-5′	76.46[c]	76.1[h]
C-6′	174.35	174.4
Glucose		
C-1″	104.71[a]	104.4[g]
C-2″	73.91[b]	74.5
C-3″	78.10[d]	77.20[i]
C-4″	80.97	80.5
C-5″	77.63[d]	78.07[i]
C-6″	62.40[f]	62.2
Glucose		
C-1‴	104.67[a]	104.3[g]
C-2‴	74.71[b]	74.5
C-3‴	77.63[d]	77.25[i]
C-4‴	71.34[e]	71.16
C-5‴	77.58[d]	77.85[i]
C-6‴	62.31[f]	61.9[i]
Glucose		
C-1⁗	95.54	
C-2⁗	73.74[b]	
C-3⁗	78.73[d]	
C-4⁗	71.90[e]	
C-5⁗	78.47[d]	
C-6⁗	62.97[f]	

[a–i]Signals with the same superscript are interchangeable.

aglycone and glucosyl moieties appeared at almost the same positions, demonstrating that P_1 is an ester glycoside of P_2. The chemical shift of C-28 of the aglycone moiety of P_1 was displaced downfield by 3.6 (176.3 ppm for P_1; 179.9 ppm for P_2), indicating that the carboxy group is esterified with a glucosyl moiety.

The second molecule of glucose in polysciasaponin P_3 might be linked either with COOH-28 of oleanolic acid or COOH-6′ of glucuronic acid. Methylated derivative of P_3 obtained with CH_2N_2 was successively hydrolyzed with β-glucosidase and β-glucuronidase. Oleanolic acid, and not an aglycone methylester, was obtained, clearly indicating that one β-D-glucose is linked to the C-28 at the carboxylic acid of oleanolic acid.

In conclusion, the structure of polysciasaponin P_1 was established as 3-*O*-[β-D-glucopyranosyl (1↦4)-β-D-glucopyranosyl (1↦2)-β-D-glucuronopyranosyl] oleanolic acid-28-*O*-β-D-glucopyranoside [**8**] and the structure of polysciasaponin P_3 as 3-*O*-[β-D-glucopyranosyl(1↦2)-β-D-glucuronopyranosyl] oleanolic acid 28-*O*-β-D-glucopyranoside [**6**]. Polysciasaponin P_3 is identical with chikusetsusaponin V, isolated from *Panax* species (3–9).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Gc-ms was performed on a SE54 coated quartz capillary column with a Hewlett-Packard in electronic impact mode. Fabms were obtained on a VG Micromass ZAB-HF spectrometer in the negative ion mode; samples were suspended in polyethylene glycol; ^{13}C-nmr spectra were recorded on a Brucker WP 200 in C_5D_5N. The chemical shifts are given in ppm. TMS was used as internal standard. Tlc was carried out on Si gel 60 (Merck). The tlc systems employed were: for saponins *n*-BuOH–HOAc–H_2O (4:1:1) (system 1), EtOAc-MeOH-H_2O (20:5:2) (system 2), and $CHCl_3$-MeOH-H_2O (30:15:2) (system 3); and for aglycones $CHCl_3$-MeOH (97:3) (system 4) and C_6H_6-EtOAc (3:1) (system 5).

PLANT MATERIAL.—The leaves of *P. scutellaria* were collected from Vanuatu by P. Cabalion (ORSTOM). A voucher specimen is deposited in the Herbarium of Botany and Pharmacognosy, Faculté de Pharmacie, Lyon.

EXTRACTION AND ISOLATION OF THE SAPONINS.—Leaves (750 g) were extracted with hot EtOH. The EtOH extract was concentrated, diluted with H_2O, and extracted with $CHCl_3$ to remove lipid material. The H_2O solution was extracted with EtOAc, then *n*-BuOH. The *n*-BuOH layer was evaporated to dryness to give a crude saponin fraction (21 g). The separation was carried out on a column packed with Si gel 60 (Merck) using a solvent gradient of $CHCl_3$-MeOH-H_2O (70:2:2 to 70:40:10). Six fractions were collected. Fraction 4 gave polysciasaponin P_3 (30 mg); fraction 6 gave polysciasaponin P_1. Saponins P_1 and P_3 were further separated on tlc (Si gel) with *n*-BuOH–HOAc–H_2O (4:1:1) (detection with iodine) to give pure P_1 and P_3.

METHYLATION BY CH_2N_2.—To an Et_2O solution of saponin (15 mg), freshly prepared CH_2N_2 in Et_2O was added in excess. The mixture was kept at 37° for 1 h. The reaction product obtained on workup formed a yellow product.

ACID HYDROLYSIS.—The saponin [5 mg in MeOH-H_2O (2:1) (5 ml)] was refluxed in 0.1 M HCl (5 ml) for 6 h. The aglycone was extracted with $CHCl_3$ and identified with an authentic sample by tlc on Si gel and gc-ms after silylation. The aqueous layer was neutralized with *N,N*-octyl-methylamine and evaporated to dryness. The sugars were identified by gc after silylation and by tlc on Si gel using *n*-BuOH–iPrOH–H_2O (5:3:1), or on cellulose using *n*-BuOH–C_5H_5N–HCl (5:3:2); compounds were visualized by spraying with aniline hydrogen phthalate and then heating (110°, 10 min).

PARTIAL ACID HYDROLYSIS.—The saponin (5 mg) in MeOH (0.2 ml) and 1 M HCl (0.2 ml) was kept at 70°; after 80 min the mixture was separated by tlc on Si gel with *n*-BuOH–HOAc–H_2O (4:1:1) to obtain the partially hydrolyzed products.

ENZYMATIC HYDROLYSIS.—The enzymatic hydrolysis was carried out with 1 ml of saponin solution (0.5 ml, pH 6) and 5 mg of β-glucosidase (Extrasynthèse EC 3.2.1.21 300 U/mg) or 0.3 ml of β-glucuronidase (Merck Art. 4114, 12 U/ml, from *Helix pomatia*) during 24 h at 37°. After extraction with $CHCl_3$ (sapogenin), glucose or glucuronic acid was identified in the aqueous phase and oleanolic acid in the organic layer.

ALKALINE HYDROLYSIS.—Alkaline hydrolysis was performed at 80° for 3 h with 5 mg of saponin in 1 M NaOH (5 ml). After acidification by 1 M HCl (pH 5), monodesmoside was extracted with *n*-BuOH.

POLYSCIASAPONIN P_1 [**8**].—White powder: mp 263°; tlc R_f 0.22 (system 1), 0.03 (system 2), 0.02 (system 3); fabms *m/z* 1117 $[M-H]^-$, 955

$[M-H-162]^-$; ^{13}C nmr (C_5D_5N) δ 89.5 (C-3), 122.10 (C-12), 144.1 (C-13), 176.3 (C-28), 104.4 (C-1′), 104.7 (C-1″), 104.6 (C-1‴), 95.5 (C-1⁗).

POLYSCIASAPONIN P_3 [**6**].—Amorphous powder: mp 260°; tlc R_f 0.38 (system 1), 0.09 (system 2), 0.06 (system 3); fabms *m/z* 955 $[M-H]^-$, 793 $[M-H-162]^-$. Enzymatic hydrolysis of the methylate derivative of P_3 afforded oleanolic acid, identified by comparison with an authentic sample.

ACID HYDROLYSIS.—Polysciasaponins P_1 and P_3 gave oleanolic acid identified by tlc [R_f 0.71 (system 4), 0.56 (system 5)] and by gc-ms after silylation: ms *m/z* $[M]^+$ 600, $[M-COOSiMe_3]^+$ 483, $[M-280]^+$ 320, $[M-321]^+$ 279, $[M-397]^+$ 203, $[M-411]^+$ 189. The sugars were identified by tlc and by gc after silylation. The sugar components were Glc-GlcA (3:1) for saponin P_1, Glc-GlcA (2:1) for saponin P_3.

PARTIAL ACID HYDROLYSIS.—P_1 gave P_2, P_3, P_5, and P_7; P_3 yielded P_5 followed by P_7.

ALKALINE HYDROLYSIS.—P_1 afforded P_2; P_3 gave P_5.

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