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TRITERPENOID SAPONINS FROM *POLYSCIAS SCUTELLARIA*

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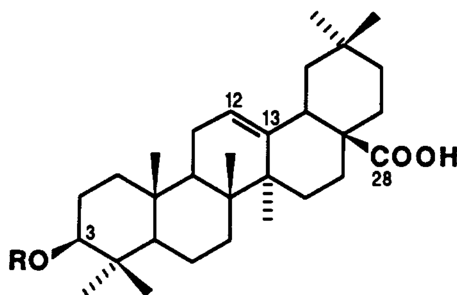
ABSTRACT.—The leaves of *Polyscias scutellaria* afforded a new oleanolic glycoside, polysciasaponin P_2 [3], in addition to polysciasaponin P_5 [2], previously reported from *Panax zingiberensis*. The structures were established by chemical and spectroscopic means (fabms, ^{13}C nmr, gc-ms).

Polyscias scutellaria (Burm.f.) Fosb. (Araliaceae) is a bush used in the Pacific countries, especially in Vanuatu, as an anti-inflammatory (1). In previous work, we reported the isolation and structure determination of polysciasaponins P_7, P_6, and P_4 (2). In the present paper, we report the isolation and structure elucidation of polysciasaponins P_5 [2] and P_2 [3]. Compound **3** is a new triterpene glycoside. An EtOH extract of dried leaves was treated with several solvents (see Experimental); the *n*-BuOH fraction yielded a crude mixture of saponins. Separation of the *n*-BuOH extract by different chromatographic techniques afforded saponins P_7, P_6, P_5, P_4, and P_2.

On acid hydrolysis polysciasaponins P_5 [2] and P_2 [3] afforded oleanolic acid [1] as the aglycone, identified by comparison with an authentic sample (tlc, ms, and gc-ms). The sugars obtained from the hydrolysates were identified by tlc and gc as glucuronic acid and glucose for **2** (1:1); glucuronic acid and glucose for **3** (1:2). Compounds **2** and **3** were not affected by alkaline treatment, indicating that they were monodesmosidic saponins. Mild acid hydrolysis of **2** gave 3-*O*-β-D-glucuronopyranosyl oleanolic acid, while mild acid hydrolysis of **3** gave **2** followed by 3-*O*-β-D-glucuronopyranosyl oleanolic acid.

Saponins **2** and **3** were submitted to fabms (negative ion mode) in order to establish the sugar sequence (3). The fabms spectrum of **2** showed a deprotonated molecular ion $[M-H]^-$ at *m/z* 793 ($C_{42}H_{65}O_{14}$) and fragments at *m/z* 631 $[M-H-162]^-$ and *m/z* 455 $[M-H-162-176]^-$ corresponding to a subsequent loss of a glucosyl moiety and a glucuronic acid moiety, clearly indicating that the glucose was the terminal sugar. A deprotonated molecular ion $[M-H]^-$ at *m/z* 955 ($C_{48}H_{76}O_{19}$) was character-



1 R=H
2 R=Glc(1→2) GlcA-
3 R=Glc(1→4) Glc(1→2) GlcA-
GlcA = β-D-glucuronopyranosyl
Glc = β-D-glucopyranosyl

ized in the fabms of **3**, corresponding to an oleanolic acid linked with two molecules of glucose and one of glucuronic acid. The fragments at *m/z* 793 $[M - H - 162]^-$ and *m/z* 455 $[M - H - 162 - 162 - 176]^-$ corresponding to a sequential loss of two glucosyl moieties and one glucuronic acid moiety, clearly indicated that the glucose molecules were the terminal sugars. The β-D-pyranosyl configurations of glucose and glucuronic acid in saponins **2** and **3** were derived from ^{13}C-nmr spectra (Table 1).

TABLE 1. ^{13}C-nmr Spectral Data of Polysciasaponin P_2 [**3**] in C_5D_5N (δ ppm/TMS).

Carbon atom	
Oleanolic acid	
C-3	89
C-12	122.2
C-13	144.52
C-28	179.9
GlcA	
C-1′	104.6
C-2′	82.9
C-3′	76.4
C-4′	71.15
C-5′	76.1
C-6′	174.4
Glc	
C-1″	104.4
C-2″	74.5
C-3″	77.2
C-4″	80.5
C-5″	78.07
C-6″	62.2
Glc	
C-1‴	104.3
C-1‴	74.5
C-3‴	77.25
C-4‴	71.16
C-5‴	77.85
C-6‴	61.9

With Smith's degradation (periodate oxidation, $NaBH_4$ reduction, and partial hydrolysis), polysciasaponin P_5 [**2**] afforded oleanolic acid [**1**]. Thus, the molecule of glucose in **2** might be linked either with the 2′-OH or 4′-OH of glucuronic acid. In the ^{13}C-nmr spectrum of **2**, the chemical shifts at δ 82.9, 103.7, and 105.7 can be assigned to the C′-2 and the two anomeric carbon signals (C-1′, C-1″) of β-(1↦2) linked glucopyranose residues. The structure of polysciasaponin P_5 is established as 3-*O*-β-[β-D-glucopyranosyl(1↦2)-β-D-glucuronopyranosyl] oleanolic acid.

With Smith's degradation, polysciasaponin P_2 [**3**] afforded oleanolic acid [**1**]. This result is not possible with a second glucose on the glucuronic acid. The ^{13}C-nmr sugar signals (Table 1) indicated that the β-D-glucopyranosyl is the terminal unit and that this sugar is attached at the position 4″ of the inner glucosyl. Thus, the structure of polysciasaponin P_2 is established as 3-*O*-[β-D-glucopyranosyl (1↦4)-β-D-gluconopyranosyl (1↦2)-β-D-glucuropyranosyl] oleanolic acid.

Polysciasaponin P_2 [**3**] is a new natural compound. Polysciasaponin P_5 [**2**] was ear-

lier reported from *Panax zingiberensis* (4) and *Panax japonicus* var. *angustifolius* (5); **2** has molluscicidal (6) and hemolytic activities (7).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—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 for saponins were *n*-BuOH–HOAc–H_2O (4:1:1) (system 1), EtOAc-MeOH-H_2O (60:15:6) (system 2), and $CHCl_3$-MeOH-H_2O (60:30:4) (system 3). The systems employed for aglycones were $CHCl_3$-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 2 gave polysciasaponin P_2 [**3**] (80 mg); fraction 4 gave polysciasaponins P_5 [**2**] and P_6. Saponins P_5 and P_6 were further separated on tlc (Si gel) with *n*-BuOH–HOAc–H_2O (4:1:1) (detection with iodine) to give pure P_5 (90 mg) and P_6.

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 (100:60:20) and on cellulose using *n*-BuOH–pyridine–HCl (50:30:20); 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.

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.

SMITH'S DEGRADATION.—To 5 mg of saponin was added 50 ml of $NaIO_4$, and the reaction mixture was allowed to stand for 48 h. The solution was treated with ethylene glycol (2 ml), then with $NaBH_4$ (200 mg) for 15 h at 4°. The mixture was extracted with *n*-BuOH, then evaporated. The dry residue was treated with 0.5 M HCl for 7 h at 37°, then extracted with $CHCl_3$ and analyzed by tlc on Si gel using *n*-BuOH–HOAc–H_2O (4:1:1) and $CHCl_3$-MeOH (97:3).

POLYSCIASAPONIN P_5 [**2**].—Tlc R_f 0.46 (system 1), 0.21 (system 2), 0.12 (system 3); fabms *m/z* $[M-H]^-$ 793, $[M-H-162]^-$ 631, $[M-H-162-176]^-$ 455; ^{13}C nmr (C_5D_5N) δ 88.5 (C-3), 121.9 (C-12), 144.3 (C-13), 179.3 (C-28), 103.7 (C-1′), 105.7 (C-1″). Smith's degradation: **2** gave oleanolic acid [**1**].

POLYSCIASAPONIN P_2 [**3**].—Tlc R_f 0.31 (system 1), 0.09 (system 2), 0.05 (system 3); fabms *m/z* $[M-H]^-$ 955, $[M-H-162]^-$ 793, $[M-H-500]^-$ 455; ^{13}C nmr (C_5D_5N) see Table 1. Smith's degradation: **3** gave oleanolic acid [**1**].

ACID HYDROLYSIS.—Saponins **2** and **3** gave oleanolic acid [**1**] identified by tlc R_f 0.71 (system 4), R_f 0.56 (system 5) and by gc-ms after silylation: ms *m/z* $[M]^+$ 600, $[M-COOSi\ Me]^+$ 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 (1:1) for saponin **2** and Glc-GlcA (2:1) for saponin **3**.

MILD ACID HYDROLYSIS.—Compound **2** gave calenduloside E (3-*O*-β-D-glucuronopyranosyl oleanolic acid) (8). Compound **3** gave **2**, then calenduloside E.

ALKALINE HYDROLYSIS.—Compounds **2** and **3** were not hydrolyzed.

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