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TRITERPENIC GLYCOSIDES FROM *POLYSCIAS SCUTELLARIA*

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Key Word Index—*Polyscias scutellaria*; Araliaceae; triterpenoid saponins; oleanolic glycosides.

Abstract—The leaves of *Polyscias scutellaria* afforded a new triterpenic glycoside: saponin C in addition to saponins A (=calenduloside E) and B which were previously isolated from *Calendula officinalis* and *Spinacia oleracea*. The structures of the saponins were established by chemical and spectroscopic means (FABMS, ^{13}C NMR, GC/MS).

INTRODUCTION

Polyscias scutellaria (Burm. f.) Fosb. is a bush which grows in Pacific countries, especially in Vanuatu. It is reported for its use as an anti-inflammatory drug. Until now no chemical studies had been carried out on this plant. From the leaves of this plant, we have isolated seven triterpenic glycosides. In this paper we report the isolation procedure and structure elucidation of saponins: A, B and C.

RESULTS AND DISCUSSION

An ethanolic extract of the leaves was fractionated with *n*-butanol which led to a crude mixture of saponins. Further separations using column chromatography and preparative thin-layer chromatography on silica gel gave pure saponins A–C.

Acid hydrolysis of saponins A, B and C gave the same aglycone moiety identified as oleanolic acid (**1**) by comparison with a standard sample (TLC, MS and GC/MS). The sugars obtained from the hydrolysates were identified by TLC and GC as glucuronic acid for saponin A, glucose and glucuronic acid for saponins B and C. Compounds A and B were not modified by alkaline treatment indicating that they were monodesmoside saponins; conversely C gave B, indicating that C is an ester glycoside of B.

The sugar sequence was established by fast atom bombardment mass spectrometry (FABMS) in negative ion mode [1]. The FAB spectrum of A showed a deprotonated molecular ion $[M-H]^-$ at *m/z* 631 ($C_{36}H_{55}O_9$) and an ion at *m/z* 455 (oleanolic acid) resulting from the loss of glucuronic acid $[M-H-176]^-$.

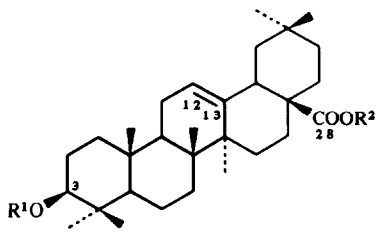
The FAB spectrum of B showed a deprotonated molecular ion $[M-H]^-$ at *m/z* 793 ($C_{42}H_{65}O_{14}$) and fragment ions at *m/z* 631 $[M-H-162]^-$ and at *m/z* 455 $[M-H-162-176]^-$ corresponding to a subsequent loss of a glucosyl moiety and a glucuronic acid moiety, clearly indicating that glucose is the terminal sugar.

A deprotonated molecular ion $[M-H]^-$ at *m/z* 955 ($C_{48}H_{75}O_{19}$) was characterized in the FAB spectrum saponin C, corresponding to an oleanolic acid linked with two molecules of glucose and one molecule of glucuronic acid. The fragment at *m/z* 793 $[M-H-162]^-$ clearly indicated that glucose is the terminal sugar.

The β-D-pyranosyl structure configuration of glucuronic acid of saponin A was deduced from ^{13}C NMR data and enzymic hydrolysis with β-glucuronidase. The structure of A was established as 3-*O*-β-D-glucuronopyranosyl oleanolic acid (**2**) and confirmed with an authentic sample calenduloside E.

Under Smith's degradation (periodate oxidation, $NaBH_4$ reduction, and partial hydrolysis) saponins B and C gave the same compound: A, i.e. calenduloside E. This result only matches with a 3 substituted glucuronic acid, leading to the following sugar sequence: glucose 1→3 glucuronic acid 1→3 oleanolic acid.

The interglycoside linkages as well as the positions of attachment of the sugars were confirmed by GC/MS analysis of the methylated alditol acetates obtained from saponins B and C. Permethylations were carried out by Hakomori's method [2]. The permethylated saponins were reduced using $LiAlD_4$ (whereby the uronic acid



1	R^1 =	R^2 = H		oleanolic acid
2	R^1 =	GlcA-,	R^2 = H	saponin A
3	R^1 =	Glc$^{1\text{-}3}$GlcA-,	R^2 = H	saponin B
4	R^1 =	Glc$^{1\text{-}3}$GlcA-,	R^2 = Glc	saponin C

GlcA = β-D-glucuronopyranosyl
Glc = β-D-glucopyranosyl

moiety was converted to a deuterated hexose). After a new permethylation, each product was hydrolysed and the resulting methylated monosaccharides reduced with $NaBD_4$, followed by acetylation. The GC/MS analysis of the methylated alditol acetates indicated the formation of 1,5-di-*O*-acetyl 2,3,4,6-tetra-*O*-methyl glucitol and 1,3,5-tri-*O*-acetyl 2,4,6-tri-*O*-methyl glucitol, identified by comparing with the authentic samples. These are characteristic of a terminal glucose and a 1,3-substituted glucuronic acid.

The second molecule of glucose in saponin C might be linked either with ^{28}COOH of oleanolic acid or $^{6'}$COOH of glucuronic acid. Methylated derivative of saponin C obtained with diazomethane was successively hydrolysed with β-glucosidase and β-glucuronidase. Oleanolic acid, and no 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 saponin C was established as 3-β-*O*-[β-D-glucopyranosyl (1→3)-β-D-glucuronopyranosyl] oleanolic acid 28-*O*-β-D-glucopyranoside (**4**) and the structure of saponin B as 3-β-*O*-[β-D-glucopyranosyl(1→3)-β-D-glucuronopyranosyl] oleanolic acid (**3**).

Saponin A which showed a molluscicidal activity was found previously in *Calendula officinalis* [3], *Lonicera nigra* [4] and *Atriplex nummularia* [5]. Saponin B was earlier only reported from *Spinacia oleracea* [6]. Saponin C is a new natural compound.

EXPERIMENTAL

FABMS spectra were performed on a VG Micromass ZAB-HF mass spectrometer in the negative ion mode. Analytical GC was carried out on a glass column (3 mm × 3 m) packed with 5% SE 52 on Gas Chrom Q (80–100 mesh). Operating conditions were: temp. programmed from 130 to 180° at 2°/min; N_2: 40 ml/min; inj. temp. was held at 200° and detector at 250°. GC/MS was performed on a quartz capillary (25 m × 0.21 mm) coated with SE 54. Operating conditions were: temp. programmed from 270° to 285° at 4°/min and isothermal at 285°, 20 min; N_2: 0.5 ml/min; inj. temp.: 250°. ^{13}C NMR spectra were recorded in C_5D_5N. The chemical shifts are given in ppm. TMS was used as int. standard. TLC was carried out on silica gel Merck G 60 for saponins: TLC system employed *n*-BuOH–AcOH–H_2O (4:1:1) (system 1); EtOAc–MeOH–H_2O (20:5:2) (system 2); $CHCl_3$–MeOH–H_2O (30:15:2) (system 3); for sapogenins $CHCl_3$–MeOH (97:3) (system 4); C_6H_6–EtOAc (3:1) (system 5). Spray reagents for saponins were conc. H_2SO_4 and *p*-anisaldehyde–H_2SO_4, and for sugars aniline hydrogen phthalate was used.

Plant materials. Polyscias scutellaria (leaves) was collected by P. Cabalion (ORSTOM) in the Vanuatu. Voucher specimen have been deposited at the Herbarium of Botany and Pharmacognosy (Lyon).

Extraction and isolation of the saponins. Leaves (750 g) were extracted with boiling EtOH. The EtOH extract was concd, diluted with H_2O, extracted with $CHCl_3$ to remove lipid material. The H_2O soln was extracted with EtOAc, then *n*-BuOH. The *n*-BuOH layer was evapd to dryness to give a crude saponin fraction (21 g). The separation was carried out on column packed with silica gel (Merck 60). Solvent: $CHCl_3$–MeOH–H_2O (35:1:1) to (7:4:1). Six fractions were collected. Fraction 2 gave a pure saponin A (200 mg); fractions 3 and 4 were further purified by TLC on silica gel with *n*-BuOH–AcOH–H_2O (4:1:1) (detection with iodine). Two saponins were obtained saponin B (60 mg) and saponin C (50 mg).

Acid hydrolysis. 5 mg of saponin in MeOH–H_2O (2:1) (5 ml) were refluxed in 0.1 M HCl (5 ml) for 4 hr. The aglycone was extracted with $CHCl_3$ and identified by TLC on silica gel with R_f 0.71 (system 4), 0.55 (system 5); the aq. layer was neutralised with *N,N*-octyl-methylamine and evapd to dryness. The sugars were identified by TLC on silica gel using *n*-BuOH–*iso*-PrOH–H_2O (5:3:1), on cellulose using *n*-BuOH–pyridine–HCl (5:2:2); compounds were visualized by spraying with aniline phthalate and heated (110°, 10 mn).

Partial hydrolysis. The saponin soln (5 mg) in MeOH (0.2 ml) and 1 M HCl (0.2 ml) were kept at 70°. After 20 min and 1 hr the mixture was analysed by silica gel TLC with system 1 to obtain the partially hydrolysed products.

Enzymic hydrolysis was carried out with 1 ml of saponin soln (0.5 ml) (pH 6) and 5 mg of β-glucosidase (300 U/mg) (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 hr to 37°. After extraction with $CHCl_3$ (sapogenin), glucose or glucuronic acid were identified in the aq. phase and oleanolic acid in organic layer.

Alkakine hydrolysis was performed at 80° for 3 hr 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 $NaIO_4$ for 48 hr. The soln was treated with ethyleneglycol (2 ml), then with $NaBH_4$ (200 mg) for 15 hr at 4°. The mixture was extracted with *n*-BuOH and evapd. The dry residue was hydrolysed with 0.5 M HCl for 7 hr at 37°, then extracted by $CHCl_3$ and analysed by TLC on silica gel using system 1 and system 4.

Methylated alditol acetates analysis. 10 mg of saponin B and C were permethylated according to [2]. After reduction and methylation of COOH of the uronic acid, the permethylated products were hydrolysed to the individual sugars (HCO_2H–H_2SO_4), reduced to alditols ($NaDB_4$), acetylated (Ac_2O) and the permethylated alditol acetates thus formed analysed by combined GC/MS.

Saponin A (**2**). White powder, mp 245°. TLC: R_f 0.62 (system 1), 0.35 (system 2), 0.22 (system 3). FABMS *m/z*: 631 $[M-H]^-$, 455 $[M-H-176]^-$. ^{13}C NMR: δ of oleanolic acid: 89.18 (C-3), 123.02 (C-12), 144.79 (C-13); δ of sugar moiety: 106.5 (C-1'), 75.26 (C-2'), 78.18 (C-3'), 73.54 (C-4'), 77.0 (C-5').

Saponin B (**3**). White powder, mp 255°. TLC: R_f 0.50 (system 1), 0.22 (system 2), 0.13 (system 3). FABMS *m/z*: 793 $[M-H]^-$, 631 $[M-H-167]^-$, 455 $[M-H-162-176]^-$.

Saponin C (**4**). White powder, mp 260°. TLC: R_f 0.42 (system 1), 0.12 (system 2), 0.08 (system 3). FABMS *m/z*: 955 $[M-H]^-$, 793 $[M-H-162]^-$, 455 $[M-H-500]^-$.

Acid hydrolysis of saponins A, B, C gave oleanolic acid identified by TLC: R_f 0.71 (system 4), 0.55 (system 5) and by EIMS after methylation (CH_2N_2): *m/z* 470 $M^{+\cdot}$, 262 $[M-207]^+$, 207 $[M-267]^+$, 203 $[(M-207)-COOMe]^+$, 189 $[(M-267)-H_2O]^+$. The sugars were identified by TLC and by GC after silylation. The sugars components were glucuronic acid for saponin A, Glc-GlcA (1:1) for saponin B, Glc-GlcA (2:1) for saponin C.

Partial hydrolysis. Saponin B was hydrolysed to A, and saponin C firstly gave B, then A.

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CYTOTOXIC COMPONENTS OF *DIOSPYROS MORRISIANA**

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Key Word Index—*Diospyros morrisiana*; Ebenaceae; isodiospyrin; β-amyrin; olean-12-en-3-one; β-amyrin acetate; X-ray crystal structure

Abstract—Two cytotoxic compounds, isodiospyrin and β-amyrin, in addition to an inactive triterpene, olean-12-en-3-one, have been isolated from *Diospyros morrisiana*. The cytotoxic activity of isodiospyrin, which has ED_{50} values of 4.9 and 0.59 μg/ml, respectively, against HCT-8 colon tumour and P-388 lymphocytic leukemia, was demonstrated for the first time. Results of single-crystal X-ray analyses of β-amyrin acetate and olean-12-en-3-one are also reported.

INTRODUCTION

Diospyros morrisiana Hance (Ebenaceae), known as 'Shan Hung Shih' in the herbal medicine of Taiwan, has been claimed to possess antibiotic activity [1]. Previous investigations of the roots of this plant led to the isolation of isodiospyrin [2, 3] and betulinic acid [3], as well as some natural naphthoquinone pigments [4]. In the course of our continuing search for novel potent plant antitumour agents, the hexane extract of the stem parts of this plant was found to show significant cytotoxicity against *in vitro* tissue culture cells of human KB and A-549 lung carcinoma, HCT-8 colon tumour and murine P-388 and L-1210 lymphocytic leukemia. Bioassay-directed fractionation of the foregoing extract led to the isolation and characterization of two cytotoxic components, isodiospyrin (**1**) and β-amyrin (**2**), as well as an inactive triterpene, olean-12-en-3-one (**3**). The binaphthoquinone, isodiospyrin (**1**), with ED_{50} values of 4.9 and 0.59 μg/ml, was shown for the first time to be cytotoxic in the HCT-8 and P-388 screens. That β-amyrin (**2**) was also moderately cytotoxic was indicated by its ED_{50} values of 3.5 and 4.6 μg/ml in KB and A-549 screens. X-Ray crystal structure analysis established the constitution of olean-12-en-3-one (**3**) while a corresponding study of β-amyrin acetate (**4**) confirmed the identity of β-amyrin (**2**). Views of the solid-state conformations of **3** and **4** are presented in Figs 1 and 2, respectively.

EXPERIMENTAL

Mps: uncorr. Optical rotations were taken in $CHCl_3$ sol. ^1H NMR spectra were run in $CDCl_3$ at 400 MHz with chemical shifts recorded in ppm, TMS as int. standard.

Plant material. The plant *D. morrisiana* used in this investigation was collected from Mt. Yang.-Ming, Taipei, Taiwan, in Feb. 1987. A voucher specimen is kept in the School of Agriculture, Chinese Culture University, Taipei, Taiwan, R.O.C.

Extraction and isolation. The dried stems of *D. morrisiana* (3 kg) were extracted with MeOH. Removal of solvent gave a syrup (125 g) which was then dissolved in MeOH–H_2O (3:1) and extracted exhaustively with hexane. The hexane extract (18 g) was chromatographed on silica gel (800 g) and eluted successively with $CHCl_3$ and then with $CHCl_3$–Me_2CO mixtures containing increasing amounts of Me_2CO to yield fractions A (3.6 g), B (2.4 g), C (1.4 g), and D (68 mg).

*Part 106 in the series 'Antitumor Agent' For part 105, see Y. H. Kuo, C. H. Chen, L. M. Yang Kuo, M. L. King, T. S. Wu, S. T. Lu, I. S. Chen, D. R. McPhail, A. T. McPhail and K. H. Lee (1989) *Heterocycles* (submitted).

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