Phytochemistry, Vol. 20, No. 7, pp. 1689–1691, 1981.
Printed in Great Britain.

0031-9422/81/071689-03 $02.00/0
Pergamon Press Ltd.

OLAXOSIDE, A SAPONIN FROM *OLAX ANDRONENSIS*, *OLAX GLABRIFLORA* AND *OLAX PSITTACORUM*

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(*Received* 6 *October* 1980)

Key Word Index—*Olax andronensis*; *O. glabriflora*; *O. psittacorum*; Olacaceae; olaxoside; (3β)-28-(β-D-glucopyranosyloxy)-28-oxoolean-12-en-3-yl-(1 → 4)-*O*-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranosiduronic acid.

Abstract—A new saponin, olaxoside, has been isolated from the methanol extract of the leaves, roots and barks of *Olax andronensis*, *O. glabriflora* and *O. psittacorum*. Structure determination was performed by spectroscopic and chemical methods. The anti-inflammatory and laxative activities are discussed briefly.

INTRODUCTION

Several saponins [1–6] have been obtained from species of the genus *Olax* [7]. We have now examined *Olax andronensis* Bak [8], *O. glabriflora* P. Danguy [8] and *O. psittacorum* (Lamk) Vahl [9]. All parts of these plants contain the same saponin, which we have called olaxoside.

RESULTS AND DISCUSSION

Olaxoside (**1**), $C_{48}H_{76}O_{18}$, gave a copious lather when it was shaken with water, it haemolyzed red blood cells, and it responded to colour reactions characteristic of saponins. It was hydrolysed with 2N H_2SO_4 and the sapogenin which precipitated was separated from the sugar moiety by filtration and purified by crystallization. The variations of olaxoside content in the different parts of the three *Olax* species examined are summarized in Table 1.

Acid hydrolysis of olaxoside gave oleanolic acid, D-glucuronic acid, D-glucose and L-rhamnose. The genin and sugar contents were shown to be 48.5 and 51.5% respectively. On methylation with CH_2N_2, the saponin furnished a methyl ester $C_{49}H_{78}O_{18}$ (**2**) which, on hydrolysis with 2N H_2SO_4, yielded oleanolic acid, indicating that the CO_2H group of the sapogenin was esterified with a carbohydrate moiety and that of the D-glucuronic acid of olaxoside was not esterified in the saponin. The saponin was hydrolysed with a solution of $Ba(OH)_2$ for 2 hr at 60°. Acetylation of the sugar moiety gave the peracetylated levoglucosan which was characterized by comparison of IR, ^{1}H NMR and mass spectra with an authentic sample. It was concluded that olaxoside contained one molecule of D-glucose present in ester combination with the 28-COOH group of the sapogenin. It is known that an *O*-β-D-glucoside hydrolysed with NaOH gives levoglucosan [10].

Table 1. Variations in olaxoside content in the different *Olax* species (g/kg)

Species	Leaves	Leaves and stem	Stem roots	Stem barks
O. andronensis	90	45	3	11
O. glabriflora	30	—	3	6
O. psittacorum	10	—	1	3

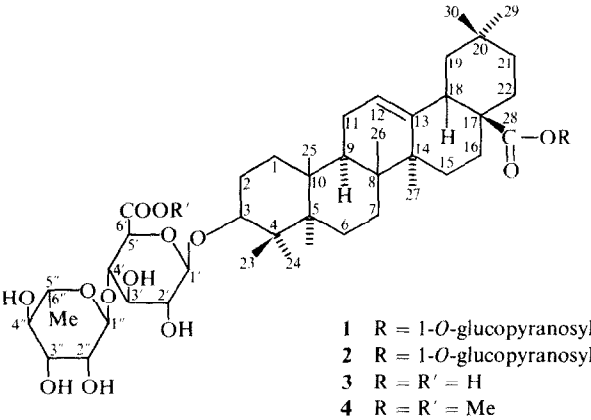
The saponin (**3**), $C_{42}H_{66}O_{13}$, was methylated with CH_2N_2 to give the dimethyl ester saponin (**4**). The positions of the C atoms were determined from the ^{13}C NMR spectrum (Table 2) in the light of recent studies on the ^{13}C NMR spectrum of oleanolic acid [11, 12].

From a comparison of the ^{13}C NMR chemical shifts of the sugar carbon atoms (free OH and acetylated OH), it was concluded that C-4′ of the glucuronic acid was substituted by rhamnose. The C-1′ (β), anomeric carbon, underwent displacement (2.6 ppm towards high field) following the acetylation of the hydroxyl group which was on the neighbouring C-2′ carbon. This excludes glycosidic linkage to C-2′. There was also a slight displacement (82.5 ppm → 80.8 ppm) after acetylation by the C-4′ carbon carrying rhamnose. This is in agreement with the presence of a vicinal hydroxyl group and excludes position C-3′. Finally the β-glycosidation at C-4′ was reflected by the displacement of the signals for the C-3′ and C-5′ carbons.

Olaxoside had a laxative action when given orally to mice at concentrations of 25–50 mg/kg, but it possessed little toxicity (LD_{50} *ca* 2 g/kg). It had anti-inflammatory properties and decreased oedema induced by carrageenin at doses of 50 mg/kg. The saponin lost its activity following methylation of the acid group of glucuronic acid or by removing the glucose from position C-28. It was inactive against leukemia L 1210 at 200 mg/kg (subcutaneous), the LD_{50} by this route being 750 mg/kg.

EXPERIMENTAL

UV spectra were run in EtOH and IR spectra as KCl discs. ^{1}H NMR spectra were determined at 90 MHz in $CDCl_3$ using TMS as internal standard. ^{13}C NMR analyses were carried out in $CDCl_3$ at 22.63 MHz. TLC was generally performed on Si gel, using EtOAc–MeOH–H_2O (65:23:12) for development and



1 R = 1-*O*-glucopyranosyl, R′ = H
2 R = 1-*O*-glucopyranosyl, R′ = Me
3 R = R′ = H
4 R = R′ = Me

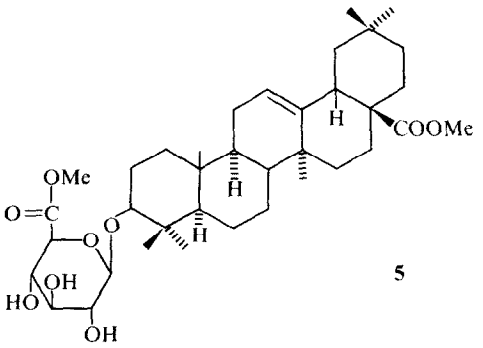


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Table 2. ^{13}C NMR spectral data of compound **4**

	Carbon	ppm	Carbon	ppm	Carbon	ppm
	1	38.4	11	23	21	33.8
	2	25.9	12	122.3	22	32.4
	3	90.6	13	143.7	23	27.7
	4	38.7	14	41.6	24	16.3
Oleanolic	5	55.5	15	27.7	25	15.2
acid	6	18.2	16	23.6	26	16.8
	7	32.6	17	46.7	27	25.4
	8	39.2	18	41.2	28	178.2
	9	47.6	19	45.8	29	33.1
	10	36.5	20	30.7	30	23.4
					CO_2Me	51.4
	1′	105.5	Glucuronic		1′	102.9
Glucuronic	4′	82.5			2′	70.8
methyl ester	5′	72.5	methyl ester		3′	71.4
	6′	169.9	acetylated		4′	80.8
	Me ester	52.7	derivative		5′	72.6
					6′	170
					Me ester	52.8
	1″	101.2			1″	99.3
	6″	17.4			2″	69.9
Rhamnose			Rhamnose		3″	67.4
			acetylated		4″	70.6
			derivative		5″	68.7
					6″	17.2

anisaldehyde as the spray reagent. Mps were determined in a Büchi apparatus (technique of Dr. Tottoli). MS were obtained at 70 eV. Analytical GC was carried out with a stainless steel capillary column (2 m × 3 mm) packed with 5% OV-17 on 100–120 mesh Chromosorb. Operating conditions were: 172° isothermal for 10 min, temp. programmed to 220° at 4°/min with N_2 at 15 ml/min. Injection temp. was held at 220° and FID detector at 240°.

Extraction. The defatted powdered plant (1 kg) was exhaustively extracted with MeOH. The MeOH extract was concd under red. pres. to dryness (242 g), redissolved in 1 l. H_2O and washed with EtOAc. The aq. soln was acidified to pH 1 with conc. 1.18 M HCl and kept in the refrigerator overnight. Under these conditions no hydrolysis was observed by TLC comparison of the MeOH extract and olaxoside. Colourless crystals (86 g) of olaxoside (**1**) were obtained on crystallization from EtOH–H_2O (1:1), mp 216–218°. The purity of **1** was checked by TLC, EtOAc–MeOH–H_2O (65:23:12), spray conc. H_2SO_4 + Ac_2O and heating at 110°, red spot. R_f 0.40. (Found: C, 60.34, H, 8.31. $C_{48}H_{76}O_{18}\cdot H_2O$ requires: C, 60.11; H, 8.20%).

Isolation and study of sapogenin from olaxoside. Olaxoside (10 g) was hydrolysed by refluxing with 2 N H_2SO_4 (200 ml) for 2 hr. The reaction mixture was extracted with $CHCl_3$ to yield the sapogenin (5.1 g). It was crystallized from MeOH–$CHCl_3$ (1:1) to yield colourless crystals (4.8 g), mp 308–310°, $[\alpha]_D^{20}$ +78° ($CHCl_3$; *c* 1). It was compared (TLC, ^{1}H NMR, and MS) with authentic samples of oleanolic acid and its acetyl and methyl ester derivatives.

Estimation of sugars in the saponin hydrolysate. The hydrolysate was neutralized with $BaCO_3$, filtered and concd to a syrup. A very small portion of the syrup was silylated with Trisyl (Varian) and analysed on a 2 m × 3 mm column packed with 5% OV 17 on Chromosorb WHP 100/120. Column temp. 172°, detector temp. 240°, N_2 flow-rate 15 ml/min. The remaining syrup was dissolved in H_2O and applied to Si gel plates (Merck F 254) using the solvent system EtOAc–MeOH–H_2O (65:23:12), spray anisaldehyde–conc. H_2SO_4–EtOH (5:5:90) and heating at 110°; R_f rhamnose: 0.58, glucose: 0.35 and glucuronic acid: 0.10.

Nature and position of glycosidic linkages in olaxoside. (1) Methylation of olaxoside followed by hydrolysis. Olaxoside (1 g) in MeOH was treated with an Et_2O soln of CH_2N_2 until a permanent yellow colour was obtained. The reaction mixture was concd and crystallized from $CHCl_3$–MeOH (2:1) to give an amorphous product (**2**), mp 215–220°, $[\alpha]_D^{20}$ −14° (EtOH; *c* 0.2). (Found: C, 59.01; H, 8.10. OMe, 3.45; $C_{49}H_{78}O_{18}\cdot 2H_2O$ requires: C, 59.27; H, 8.34; OMe, 3.23%). The olaxoside methyl ester (**2**) (1 g) was hydrolysed by refluxing with 2 N H_2SO_4 (100 ml) for 2 hr. The reaction mixture was extracted with $CHCl_3$ to yield the sapogenin. It was crystallized and identified as oleanolic acid.

(2) Alkaline hydrolysis. Olaxoside (5 g) was treated with a soln of $Ba(OH)_2$ (56 g/l.) for 2 hr at 60°. The soln was neutralized to pH 7 with H_2SO_4; $BaSO_4$ was removed by filtration and the soln was taken to dryness and treated with EtOH. The Ba salt of the saponin **3** was removed by filtration, the remaining EtOH soln concd to dryness and the residue then acetylated by Ac_2O–pyridine at 20° and poured into excess H_2O at 0°. A white solid was pptd which was crystallized from Et_2O to give the acetyl levoglucosan derivative (458 mg), mp 110°. This was compared with an authentic sample by TLC, ^{1}H NMR, MS and IR. The Ba salt of the saponin was redissolved in H_2O, acidified (HCl, pH 1) and then extracted with *n*-BuOH. The solvent was removed and the residue recrystallized from $CHCl_3$–EtOH (1:2) to yield an amorphous saponin (**3**) (free COOH), mp 235–240°, $[\alpha]_D^{20}$ −11° (MeOH; *c* 0.2). (C, 64.51; H, 8.70. $C_{42}H_{66}O_{13}$ requires: C, 64.75; H, 8.54%).

Dimethyl ester (**4**) *of the saponin* (**3**). The dimethyl ester **4** was prepared from the saponin **3** by the method described for the preparation of olaxoside methyl ester. The product obtained was crystallized from $CHCl_3$–Et_2O (1:1), mp 200°, $[\alpha]_D^{20}$ −10° (EtOH, *c* 0.2). (Found C, 63.90; H, 9.13; OMe 7.80. $C_{44}H_{70}O_{13}\cdot H_2O$ requires C, 64.05; H, 8.90; OMe, 7.53%). The saponin **3** was subjected to partial hydrolysis with 100 ml HOAc–H_2O (50:50) for 2 hr on a steam bath. The hydrolysate furnished oleanolic acid, rhamnose, glucuronic acid and the saponin **5**. Oleanolic acid 3-*O*-glucuronide (**5**) was isolated as the dimethyl ester derivative, mp 243°, $[\alpha]_D^{20}$ +12° (MeOH; *c* 1). [13, 14]. The enzymatic hydrolysis with β-glucuronidase (*Helix pomatia*) of the dimethyl ester saponin (**3**) according to [15] gave the sapogenin (oleanolic acid) and glucuronic acid, identified by comparison by TLC and GLC with the authentic samples.

Acknowledgements—We thank Mr. A. Broin, for collection and identification of plant materials *Olax andronensis*, No. 154, *O. glabriflora*, No. 161, Botanex, Tanarive, Madagascar, and Mr. M. Vuilhorgne, CNRS, Gif sur Yvette, France, for ^{13}C NMR spectra. We are grateful to Dr. P. Susplugas, Faculté de Pharmacie, Montpellier, France, for collection of *Olax psittacorum*, La Reunion Island; and Mrs. V. Antoine and Mr. R. Tiberghien for excellent technical assistance.

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