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MOLLUSCICIDAL SAPONINS FROM *CUSSONIA SPICATA*

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Key Word Index—*Cussonia spicata*; Araliaceae; saponins; oleanolic acid glycosides; molluscicidal activity; schistosomiasis.

Abstract—Two saponins are responsible for the molluscicidal activity of an extract of the stem bark of *Cussonia spicata*. Their structures have been established as [α-L-arabinofuranosyl-(1 → 4)-β-D-glucuronopyranosyl-(1 → 3)]-3β-hydroxyolean-12-en-28-oic acid and [(α-L-arabinofuranosyl-(1 → 4)-β-D-galactopyranosyl-(1 → 2))-β-D-glucuronopyranosyl-(1 → 3)]-3β-hydroxyolean-12-en-28-oic acid.

INTRODUCTION

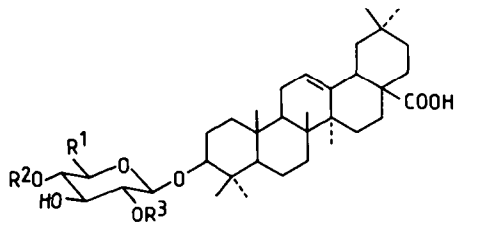
In the course of our systematic screening studies of medicinal plants for molluscicidal activity [1], we observed that the water extract of *Cussonia spicata* Thunb. (Araliaceae) stem bark showed an activity of 400 ppm within 24 hr against *Biomphalaria glabrata* snails, the intermediate host of *Schistosoma mansoni*. This activity was strong enough for us to undertake a phytochemical investigation of this plant.

The bark of *Cussonia spicata* and other species of *Cussonia* is used in African traditional medicine against malaria [2]. An infusion of the roots of *C. spicata* prevents skin irritation and is antifebrile [2]. To our knowledge, no phytochemical investigation of *C. spicata* and related species has yet been reported. In this paper, we report the isolation and structure elucidation of two saponins responsible for the molluscicidal activity of the crude extract.

RESULTS AND DISCUSSION

The water extract of the stem bark was partitioned with *n*-BuOH. A part of this extract was separated on silica gel and the fractions were further purified on a reversed-phase (RP-8) support using medium pressure liquid chromatography (MPLC) [3], affording pure saponins **1** and **2**.

Compounds **1** and **2** were not affected by alkaline treatment, indicating that they were monodesmosidic saponins. On acidic hydrolysis, **1** and **2** gave the same aglycone, identified as oleanolic acid by comparison with an authentic sample (TLC, ^{13}C NMR, mp). The sugars obtained from the hydrolysates were identified by TLC and GC, as arabinose and glucuronic acid for **1** and as arabinose, galactose and glucuronic acid for **2**. The sugar sequence was established by fast atom bombardment (FABMS [4]) in the negative ion mode. The spectrum of **1** showed a quasi molecular ion at m/z 763 $[M-H]^-$ and signals at m/z 631 $[(M-H)-132]^-$ and 455 $[(M-H)-308]^-$, corresponding to the subsequent loss of an arabinosyl moiety and a glucuronic acid moiety, indicating clearly that arabinose was the terminal sugar.



	R^1	R^2	R^3
1	COOH	Ara(f)	H
2	COOH	Ara(f)	Gal
2a	COOMe	H	Gal

The mass spectrum of **2** showed a quasi molecular ion at m/z 925 $[M-H]^-$ and signals at m/z 793 $[(M-H)-132]^-$, 763 $[(M-H)-162]^-$, 631 $[(M-H)-294]^-$ and 455 $[(M-H)-370]^-$, corresponding to a subsequent loss of the two terminal sugars, an arabinosyl moiety and a galactosyl moiety, attached to a glucuronic acid.

The interglycoside linkages as well as the positions of attachment of the sugars were established by GC and GC/MS analysis of the methylated alditol acetates obtained from **1**, **2** and **2a**, the partially hydrolysed product from **2** [5]. Permethylations were carried out by the method described for monosaccharides [6]. This procedure adapted for saponins is easier to perform and requires shorter reaction times than the usual method [5, 7]. The permethylated compounds were obtained quantitatively (verification by D/CIMS and ^1H NMR). $LiAlH_4$ reduction of the permethylated compounds resulted in the conversion of COOMe groups to CH_2OH. Each product was hydrolysed and the resulting methylated monosaccharides reduced with $NaBH_4$, followed by acetylation. The GC/MS analysis of the methylated alditol acetates indicated the formation of 1,4-di-*O*-acetyl-2,3,5-tri-*O*-methylhexitol and 1,4,5,6-tetra-*O*-acetyl-2,3-

di-*O*-methylhexitol for **1**. Thus a terminal arabinose, present as a furanose (confirmed by ^{13}C NMR: C-1 Ara(f) δ108.7), and a 4-substituted glucuronic acid constituted the sugar chain. For saponin **2**, the analysis revealed a terminal arabinofuranosyl moiety. In addition, 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylhexitol and 1,2,4,5,6-penta-*O*-acetyl-3-*O*-methylhexitol were obtained, indicating a terminal galactopyranose and a 2,4-substituted glucuronic acid. For compound **2a**, GC/MS analysis showed the presence of a terminal galactopyranosyl moiety and a 2-substituted glucuronic acid (1,2,5,6-tetra-*O*-acetyl-3,4-di-*O*-methylhexitol formed). To distinguish between 3- and 4-*O*-methylhexose (compound **2**), the reduction of the esters was made by $LiAlD_4$.

The results have been confirmed by ^{13}C NMR spectroscopy, on using the glucosylation rules previously established by Konishi *et al.* [8]. Thus the structures are established as [α-L-arabinofuranosyl-(1 → 4)-β-D-glucuronopyranosyl-(1 → 3)]-3β-hydroxyolean-12-en-28-oic acid for **1** and [(α-L-arabinofuranosyl-(1 → 4))-(β-D-galactopyranosyl-(1 → 2))-β-D-glucuronopyranosyl-(1 → 3)]-3β-hydroxyolean-12-en-28-oic acid for **2**.

Compound **1** has been found previously in *Anemone narcissiflora* (Ranunculaceae) [9] and **2** was isolated from *Tetrapanax papyriferum* (Araliaceae) [10]. However, neither ^{13}C-data nor biological activities have been reported for these two compounds. In this context, we have found **1** and **2** to exhibit molluscicidal activity. Compound **1** was toxic to *Biomphalaria glabrata* snails at 12.5 mg l^{-1} and compound **2** at 100 mg l^{-1}. The activities of the isolated saponins are lower than other molluscicidal saponins such as [β-D-xylopyranosyl-(1 → 3)-β-D-glucuronopyranosyl-(1 → 3)]-3β-hydroxyolean-12-en-28-oic acid from *Talinum tenuissimum* [11] which has an LC_{100} value of 1.5 mg l^{-1}. Furthermore, a preliminary screening for spermicidal activity against human spermatozoids showed an activity at a concentration of 1 mg l^{-1} for **1** and 3 mg l^{-1} for **2** within 3 min [12].

EXPERIMENTAL

General. Mps are uncorr. FABMS were obtained on a ZAB 1S spectrometer in the negative ion mode. ^1H and ^{13}C NMR spectra were recorded on a Bruker WP-200 (200 and 50.29 MHz) in $CDCl_3$ and C_5D_5N using TMS as an internal standard. GC/MS were obtained on a Danni 6500 apparatus coupled with a Nermag R 3010 spectrometer. GC conditions: fused silica column 0.22 mm × 25 m packed with SE 54, injection temp. 210°, column temp. 150°/3 min and 5°/min up to 250°. TLC: silica gel precoated Al sheets (Merck) with $CHCl_3$–MeOH–H_2O (6:4:1) for the saponins. Detection was with Godin reagent [13].

Plant material. Cussonia spicata was collected at the Zomba Plateau, Malawi. Voucher specimens have been deposited at the Herbarium, Chancellor College, Zomba, Malawi.

Extraction and isolation. Stem bark (123.4 g) was extracted with H_2O to give 33 g of extract. A part of the crude extract (20 g) was suspended in H_2O (500 ml) and partitioned with *n*-BuOH (3 × 400 ml). The *n*-BuOH layer was evaporated to dryness to give a crude saponin fraction (4.3 g). A part of this fraction (2.3 g) was separated by MPLC using a Büchi B-681 system. The separation was carried out on a 36 mm × 92 cm column packed with silica gel (Merck 9385). Solvent: $CHCl_3$–MeOH–H_2O (6:4:1). Five fractions were collected. Fractions 3 (395 mg) and 5 (620 mg) were further purified by MPLC [3] using a reversed-phase 29 mm × 46 cm column packed with RP-8 (Merck 9324). Solvent: MeOH–H_2O (2:1) for fraction 3 and MeOH–H_2O (7:3) for fraction 5, affording **1** (140 mg) and **2** (370 mg).

Acidic hydrolysis. The saponin (5 mg) in MeOH (2 ml) was refluxed in 4 N HCl (5 ml) for 2 hr. The aglycone was extracted with Et_2O and identified by TLC on silica gel with (*iso*-$PrO)_2O$–Me_2CO (3:1) and mmp. The aq. layer was adjusted to pH 6 with $NaHCO_3$. After evaporation to dryness, the residue was extracted with pyridine. The sugars were identified by TLC on silica gel using EtOAc–MeOH–HOAc–H_2O (13:3:4:3) as eluent (detection with naphthoresorcinol) and by the alditol acetate derivatization.

Partial hydrolysis. Saponin **2** (60 mg) in MeOH (10 ml) and 0.1 N HCl (1 ml) was kept at room temp. for 48 hr. The solution was neutralized with $NaHCO_3$ and evaporated to dryness. The crude mixture was separated by MPLC on reversed phase (RP-8) with MeOH–H_2O (78:22) to obtain the partially hydrolysed product **2a** (25 mg).

Permethylation of saponins **1**, **2** *and* **2a**. To 5 mg of saponin was added 0.4 ml of dry DMSO, 40 mg of dry *t*-BuONa, 10 mg of finely powdered dry NaOH and 0.3 ml MeI. The mixture was stirred at room temp. for 0.5–1 hr. The soln was poured into ice water and extracted with Et_2O. The Et_2O layer was washed with a satd NaCl soln, dried and evaporated.

Reduction. Each permethylated saponin in dry Et_2O (5 ml) was treated with $LiAlH_4$ or $LiAlD_4$ (20 mg) at 12° for 2 hr. The mixture was acidified with aq. 5% HCl and extracted with Et_2O. The organic phase was washed with a satd NaCl soln, dried and evaporated.

Methylated alditol acetates. Each residue obtained after reduction was hydrolysed for 6 hr with a mixture of 2 N HCl and dioxan (1:1) under reflux. The soln was neutralized with Na_2CO_3 and evaporated to dryness. The partially methylated sugars were extracted successively with EtOAc (2 × 5 ml) and pyridine (2 × 5 ml) and the combined extracts were evaporated. The residue was treated with 0.8 ml of a 20% aq. soln of $NaBH_4$ or $NaBD_4$. The mixture was stirred for 1.5 hr at room temp. and acidified with HOAc, evaporated and subjected to azeotropic distillation with dry toluene (3 × 5 ml). The resulting methylated alditol mixture was acetylated with 2–3 ml of Ac_2O–pyridine (3:1) at room temp. overnight. The reaction mixture was evaporated and the methylated alditol acetates were analysed by GC and GC/MS. Spectra were compared with those of the literature [5].

[α-L-*Arabinofuranosyl*-(1 → 4)-β-D-*glucuronopyranosyl*-(1 → 3)]-3β-*hydroxyolean*-12-*en*-28-*oic acid* (**1**) [7]. Mp 240–260° (decomp.). FABMS *m/z*: 763 $[M-H]^-$, 631 $[(M-H)-132]^-$, 455 $[(M-H)-209]^-$. ^{13}C NMR: δ of sugar moiety: arabinofuranose: 108.6 (C-1), 78.6 (C-2), 76.3 (C-3), 87.4 (C-4), 62.8 (C-5); glucuronopyranose: 106.7 (C-1), 75.5 (C-2), 77.8 (C-3), 82.3 (C-4), 77.3 (C-5), 174.3 (C-6).

[(α-L-*Arabinofuranosyl*-(1 → 4)-β-D-*galactopyranosyl*-(1 → 2))-β-D-*glucuronopyranosyl*-(1 → 3)]-3β-*hydroxyolean*-12-*en*-28-*oic acid* (**2**) [8]. Mp 285–295°. FABMS *m/z*: 925 $[M-H]^-$, 793 $[(M-H)-132]^-$, 763 $[(M-H)-162]^-$, 631 $[(M-H)-294]^-$, 455 $[(M-H)-470]^-$. ^{13}C NMR: δ of sugar moiety: arabinofuranose: 108.7 (C-1), 78.0 (C-2), 76.0 (C-3), 87.0 (C-4), 62.8 (C-5); galactopyranose: 104.9 (C-1), 74.3 (C-2), 74.5 (C-3), 69.8 (C-4), 76.6 (C-5), 61.6 (C-6); glucuronopyranose: 106.5* (C-1), 83.1 (C-2), 77.8 (C-3), 82.4 (C-4), 77.3 (C-5), 174.0 (C-6).

[β-D-*Galactopyranosyl*-(1 → 2)-β-D-(6-*O*-*methyl*)-*glucuronopyranosyl*-(1 → 3)]-3β-*hydroxyolean*-12-*en*-28-*oic acid* (**2a**). Mp 257–264°. FABMS *m/z*: 807 $[M-H]$, 645 $[(M-H)-162]^-$, 455 $[(M-H)-352]^-$. ^{13}C NMR: δ of sugar moiety: galacto-

*Assignment may be reversed.

pyranose: 105.1* (C-1), 72.6 (C-2), 74.7 (C-3), 69.4 (C-4), 76.6 (C-5), 61.2 (C-6); glucuronopyranose: 106.8* (C-1), 83.4 (C-2), 77.3 (C-3), 74.4 (C-4), 76.7 (C-5), 170.2 (C-6).

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*Assignments may be reversed.