187. Molluscicidal Saponins from *Cornus florida* L.

by **Kurt Hostettmann, Maryse Hostettmann-Kaldas** and **Koji Nakanishi**

Department of Chemistry, Columbia University, New York, N.Y. 10027, USA

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Summary

The molluscicidal principle of *Cornus florida* L. *(Cornaceae)* has been isolated from the methanolic bark extract by droplet counter-current chromatography (DCC). The structure of the active compounds has been determined as sarsapogenin-*O*-*β*-D-xylopyranosyl-(1→2)-*β*-D-galactopyranoside (**2**) and sarsapogenin-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-galactopyranoside (**3**).

Introduction. – *Cornus florida* L. *(Cornaceae)*, commonly called dogwood, is a small forest tree very common in the southern United States, as well as in Mexico. The Delaware Indians boiled the bark in water, employing the decoction to reduce fevers [1]. During the last century, dogwood was substituted for cinchona bark, an important source of quinine, as an efficacious remedy for malarial fevers. The iridoid glucosides cornin [2] and dihydrocornin [3], which are contained within the bark, are presently recognized for their astringent properties, but the antipyretic factor is still unknown.

In connection with our systematic isolation and structural studies on biologically active compounds from medicinal plants [4], we noticed that the crude methanol extract of *Cornus florida* L. possesses strong molluscicidal properties. Plants showing this activity are currently receiving considerable attention as potential agents for the control of bilharzia. In the present paper, we report the isolation and structure determination of the active principle.

Results. – The dried bark (200 g) of *Cornus florida* L., collected near Monterrey, Mexico, was extracted with solvents of increasing polarity (hexane, ether, MeOH). The crude extracts obtained were submitted to bioassays for the detection of the active components. The methanol extract, at a concentration of 100 ppm, killed the snail *Biomphalaria glabratus*, which is currently being used in our laboratory, within 24 h. Since the hexane and ether extracts showed no molluscicidal activity they were not further examined in the present studies. The MeOH extract was separated into 10 fractions by Sephadex LH20 column chromatography (4 × 50 cm) with MeOH and each fraction was bioassayed. The biologically active third fraction showed 2 major spots by TLC. Since an efficient preparative scale separation could be achieved neither by open-column chromatography nor TLC, the

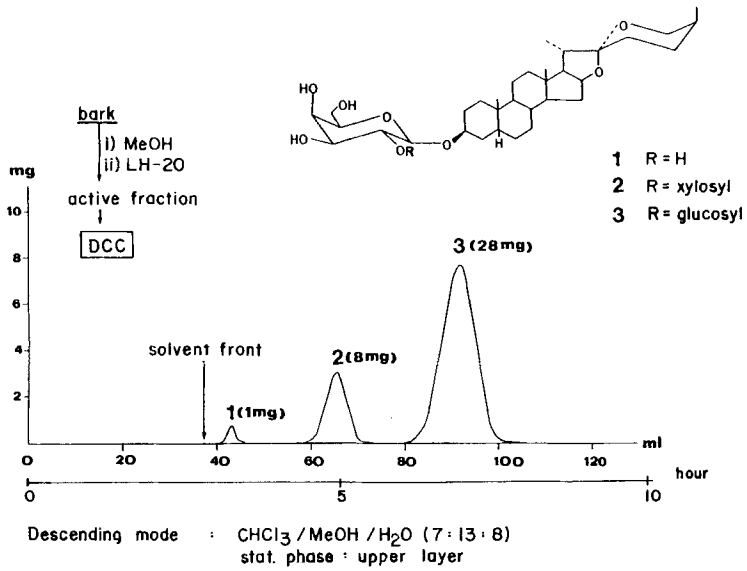

Fig. 1. *DCC. separation of* Cornus florida *saponins*

Table 1. *FD.-MS. data of compounds* **1–3**. Relative percentage and assignment of peaks are shown in brackets. The mass units which are lost correspond to the fragments as follows: 132: xylose; 162: galactose or glucose; 294: (xylose + galactose); 324: (glucose + galactose).

1	2	3	
		779 (18.2, M + K) 763 (100, M + Na) 741 (60.2, M + H) 740 (53.7, M)	glu-gal-sarsapogenin
	749 (31.5, M + K) 733 (100, M + Na) 711 (53.1, M + H) 710 (38.5, M)		xyl-gal-sarsapogenin
601 (69.2, M + Na) 579 (100, M + H) 578 (44, M)	601 (14.5, [M + Na]-132) 579 (16.6, [M + H]-132) 578 (14.5, M-132)	601 (8.9, [M + Na]-162) 579 (11.8, [M + H]-162) 578 (12.9, M-162)	gal-sarsapogenin
417 (15.4, [M + H]-162) 416 (10.1, M-162)	417 (13.8, [M + H]-294) 416 (20.8, M-294)	417 (10.7, [M + H]-324) 416 (12, M-324)	sarsapogenin

bioactive fraction was submitted to droplet counter-current chromatography (DDC.) [5] *(Fig. 1)* and afforded **1**, **2** and **3**.

Acid hydrolysis of **1–3** afforded the same aglycone, identified as sarsapogenin [6] [7]. The sugars obtained from the saponin hydrolysates were galactose in **1**, xylose and galactose in **2** and glucose and galactose in **3** (TLC.). The results of field-desorption mass spectroscopy (FD./MS.) [8] carried out on the underivatized saponins are shown in *Table 1* and *Figure 2*. The sequence of the sugar residues in **2**

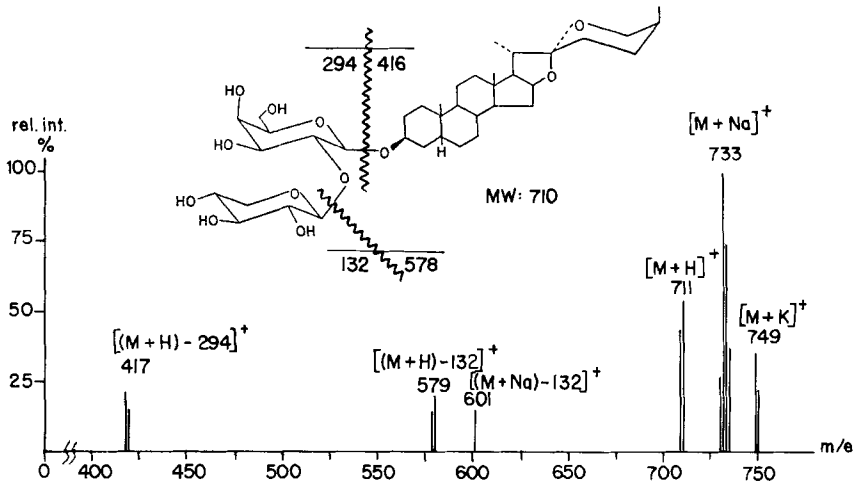

Fig. 2. *FD.-MS. of compound* **2**. (Emitter current 21 mA)

Table 2. *^{13}C-NMR. shifts of sugar carbon atoms.* The peaks due to the aglycone sarsapogenin (see [7]) have been deleted. Pertinent shifts discussed in the text are shown in italics

Sugar carbon atoms C′ and C″	**2**		**3**		Methyl-β-D-galacto-pyranoside [16]
	xyl	gal	gluc	gal	
1	106.7	*101.6*	106.0	*102.4*	*104.9*
2	72.3	*81.4*	75.4	*81.3*	*71.8*
3	74.6	*76.8*	77.9	*76.8*	*73.9*
4	71.1	69.7	71.6	69.9	69.8
5	66.3	76.5	78.2	76.4	76.2
6	–	62.2	62.9	62.1	62.1

becomes immediately clear from the FD./MS. fragmentation pattern. Thus, the fragments with *m/e* 601, 579, and 578 in the spectrum of **2** *(Fig. 2)* are also present in **1** which is sarsapogenin galactoside; hence the sugar sequence in **2** is xylose-galactose-sarsapogenin. In the case of **3** the disaccharide sequence cannot be determined solely by MS. since glucose and galactose both have the same molecular formulas. However, the sequence glucose-galactose-sarsapogenin was determined by mild hydrolysis of **3** to the monogalactoside **1**.

The new technique of plasma-desorption MS. (PD./MS.) [9] also successfully gave the molecular weights of underivatized compounds **2** and **3**. Thus **2** gave peaks at 733 ($M^+ + Na$) and 756 ($M^+ + 2Na - H$) and **3** *(Fig. 3)* gave peaks at 763 ($M^+ + Na$), 780 ($M^+ + K$) and 786 ($M^+ + 2Na - H$).

The interglycosidic linkages in **2** and **3** have been determined by ^{13}C-NMR. spectroscopy *(Table 2)*. Assignment of the sugar carbon atoms of **2** and **3** have been made by comparing their spectra[1]) with those of β-D-glucopyranoside, β-D-xylopyranoside and β-D-galactopyranoside [10]. Whereas the anomeric carbon atoms appear in the region of 104.5–106.5 ppm in these glycosides, they appear

[1]) Measured in pyridine-d_5.

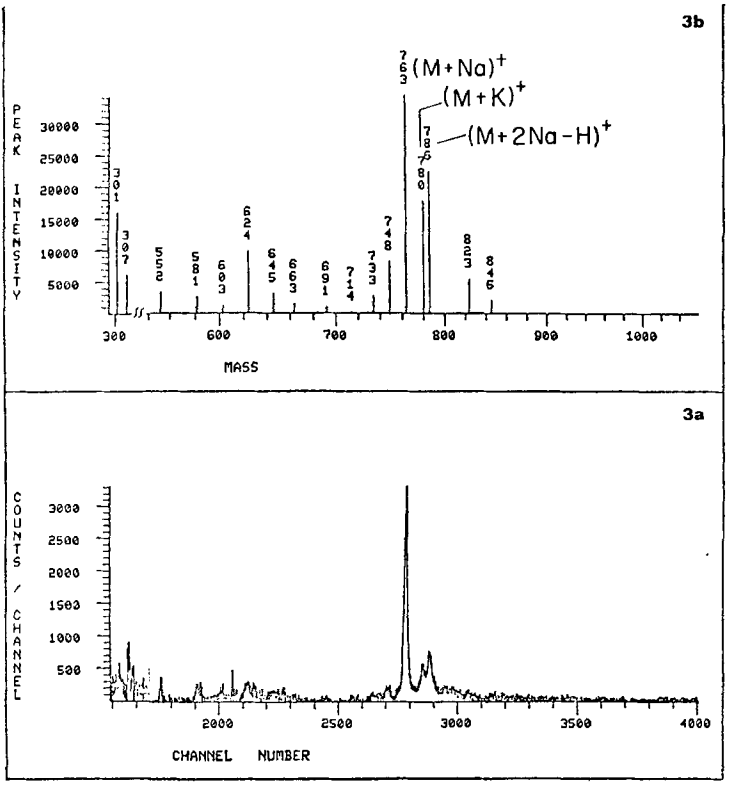

Fig. 3. *Plasma desorption MS. (PD./MS.) of positive ions of* **3** *in the range 300–1000.* 3a) Original data; 3b) Original data converted into conventional MS. format ('reduced form').

at 101.6 and 102.4 ppm in the galactose moiety of **2** and **3**, respectively. Appearance of the anomeric carbon atom signal at this relatively high field can be explained in terms of a substitution effect by another glycosyl linkage at its C(2′) position. This high-field shift of the anomeric carbon atom in saponins, as noticed recently by *Konishi et al.* [11], suggests that the HO–C(2′) is the point of linkage for another sugar residue. This shift implies that the C(1′)/O/C(1″) bonds adopt a gauche conformation, the shift to high fields being due to the well-known γ-gauche effect [12]. The C(2′)-glycosidic linkage is further evidenced by the fact that the C(2′) carbons in **2** and **3** are shifted downfield by about 9 ppm (β-effect) to 81.4 and 81.3 ppm, respectively; the C(3′) carbon atoms are also downfield shifted by 3 ppm, whereas the other signals of the galactose moiety remain almost unaffected. The C(3) carbon atom signal of sarsapogenin is shifted from 66.2 ppm (free sapogenin) to 75.4 and 75.2 ppm in saponins **2** and **3** respectively. An analogous glycosidation shift has been reported by *Tori et al.* [13] for smilagenin glucoside.

Thus the structure of **2** is established as sarsapogenin-*O*-β-D-xylopyranosyl-(1→2)-β-D-galactopyranoside and that of **3** as sarsapogenin-*O*-β-D-glucopyranosyl-(1→2)-β-D-galactopyranoside. Compound **2** is a new natural product

whereas saponin **3** has already been described [14]. Compound **1** (sarsapogenin galactoside) was obtained by partial hydrolysis of **3** [14] and could be an artefact in the present study.

Discussion. – Droplet counter-current chromatography (DCC.) has proven to be an extremely efficient method for the separation of the saponins described above; indeed, we could not separate them on a semi-preparative scale by other methods. However, a base-line separation is achieved by DCC. as depicted in *Figure 1*; the approximate theoretical plates number obtained with 300 tubes was *ca.* 625.[2])

The isolated saponins **2** and **3** are quite strong molluscicides. *Biomphalaria glabratus* snails are killed within 24 h by a 6 ppm solution of **2** and within 24 h by a 12 ppm solution of **3** (see exper. part).

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Experimental Part

Instrumental. – ^{13}C-NMR. spectra were recorded at 25.02 MHz on a *Jeol* PS-100 instrument using pyridine as solvent, TMS internal standard; due to scarcity of samples, measurements were carried out employing a micro-probe. FD./MS. spectra were obtained on a *Varian-Mat* Model 731 mass spectrometer using carbon dendrite emitters; resolution 1,000; emitter current 21 mA. For PD./MS., see [9]. Droplet countercurrent [5] separation was made on a DCC.-A apparatus manufactured by *Tokyo Rikakikai*, Nishikawa Bldg., Toyama-cho, Kanda, Chiyoda, Tokyo (Japan). The apparatus basically consists of a number of glass tubes (length 400 mm, ID 2 mm) interconnected in series by capillary Teflon tubings. In the present studies, 300 tubes were used. The solvent system was $CHCl_3$/MeOH/H_2O 7:13:8 in which the stationary phase consisted of the upper layer; hence the apparatus was used in the descending mode. About 45 mg of the crude active fraction (see below) was dissolved in 4 ml of a 1:1 mixture of both upper and lower phases and then injected into the DCC. apparatus. The flow rate was 10–15 ml/h. The elutants were collected in 1 ml fractions and monitored by TLC. on silica precoated aluminium sheets *(Merck)* with $CHCl_3$/MeOH/H_2O 65:35:10 (lower layer); detection with ceric sulfate/H_2SO_4. After elution of compound **3**, the stationary phase containing very polar inactive substances was recovered from the apparatus.

Isolation and Identification. – The dried bark was treated as indicated under Results (see above). The third fraction of column chromatography, checked by TLC. on silica gel $CHCl_3$/MeOH/H_2O 65:35:10 (lower layer), was submitted to DCC. and afforded 1 mg of compound **1** (Rf 0.80), 8 mg of **2** (Rf 0.64), m.p. 286–288° (EtOH) and 28 mg of **3** (Rf 0.60), m.p. 320–322° (lit. [14] m.p. 317–322°). Acid hydrolysis (HCl 4N, 6 h) of **1**–**3** gave the same aglycone ($M^+ = 416$, $(C_{27}H_{44}O_3)$ identified as sarsapogenin [6] by IR., ^{1}H-NMR. and ^{13}C-NMR. [7]. TLC. (silica gel; AcOEt/H_2O/MeOH/AcOH 65:15:15:20; detection with *p*-anisidine phthalate) of the sugars obtained from the

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[2]) Applications of DCC. to other classes of compounds will be published separately.

saponin hydrolysates showed the presence of galactose in **1**, xylose and galactose in **2** and glucose and galactose in **3**. Mild hydrolysis of **3** (HCl 0.5N, 2 h) afforded a mixture of sarsapogenin, **1** and **3**. *Bioassays* were made with *Biomphalaria glabratus*. Snails of uniform sizes were used (average diameter of the shell 9 mm). The test is carried out by placing two snails in a deionized water solution of known concentration. At several time intervals, the snails are placed on a *Petri* dish, light is shone from the bottom, and the heart-beat is checked by a microscope [15].

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