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STRUCTURAL STUDIES ON SOME SAPONINS FROM *LECANIODISCUS CUPANIOIDES*

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Key Word Index—*Lecaniodiscus cupanioides*; Sapindaceae; structural determination; hederagenin glycosides.

Abstract—Four triterpenoid saponins isolated from the stem bark of *Lecaniodiscus cupanioides* and denoted S-2, S-3, S-4 and S-5, were identified as follows. S-2:3-*O*-[α-L-arabinopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl]-hederagenin; S-3:3-*O*-[β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl]-hederagenin; S-4:3-*O*-[α-L-arabinofuranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl]-hederagenin; S-5:3-*O*-[α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl]-hederagenin. Of these, S-2 and S-4 are new substances.

INTRODUCTION

In an inventory of medicinal plants of equatorial Africa the bark of *Lecaniodiscus cupanioides* Planch. ex Benth. (Sapindaceae) was attributed magical properties such as ensuring good luck in hunting [1]. In a subsequent pharmacological screening a crude extract of the bark stimulated muscle relaxation, enophthalmus and lacrimation and it had a minimal lethal dose of 300 mg/kg i.p. with death within 4 hr [2]. The stem bark is also reported as a remedy against fever [3].

Delaude *et al.* [4] reported that a group of plants of the Sapindaceae is characterized by the presence of saponins with hederagenin as the aglycone. They also demonstrated that the mixture of glycosides obtained from the root bark of *Lecaniodiscus cupanioides* upon hydrolysis yielded hederagenin and the sugars arabinose, glucose, rhamnose and xylose [5]. Studies of pharmacologically active compounds from the stem bark of *L. cupanioides* have now resulted in the isolation of a group of saponoins with hederagenin as the aglycone. This paper describes the isolation and structure determination of four saponins.

RESULTS AND DISCUSSION

The saponins were isolated from an ethanolic extract of the stem bark, which was then extracted with chloroform–ethanol, followed by chromatography on a silica gel column and preparative thin layer chromatography. Four saponins were obtained as the main components and were given the designations S-2, S-3, S-4 and S-5 (numbering based upon increasing chromatographic mobility).

All the saponins on acid hydrolysis yielded hederagenin, indistinguishable from an authentic sample (TLC, ^{13}C NMR). The monosaccharides simultaneously released were identified by GC/MS of their alditol acetates [6, 7], and their absolute configurations determined by GLC after reaction with (+)-2-octanol and acetylation [8]. L-Arabinose, D-xylose and L-rhamnose were the only sugars detected and their proportions are given in Table 1.

The linkages by which the sugars are connected were determined by methylation analysis. The samples were methylated according to the Hakomori procedure [9], hydrolysed by acid, and the released, partially methylated monosaccharides analysed as their alditol acetates [10]. The different methylated sugars obtained are listed in Table 2. By using sodium borodeuteride as a reducing agent, 2- and 4-linked pentopyranosyl residues could be distinguished by MS.

The methylation analyses showed that S-5 contained a terminal rhamnopyranosyl group and a 2-linked arabinopyranosyl residue, while both these sugars were chain residues in S-2, S-3 and S-4. In the three latter compounds, the rhamnopyranosyl residue was 3-linked. The terminal group was arabinopyranosyl, xylopyranosyl and arabinofuranosyl in S-2, S-3 and S-4, respectively. As only one terminal glycosyl group was obtained from each saponin, the sugars were present as a linear oligosaccharide linked to one of the hydroxyl groups of hederagenin.

It has recently been shown that the sugar sequence of the oligosaccharides linked to *O*-3 and to the carboxyl group of hederagenin can be determined by field

Table 1. Sugar content in the different saponins of *Lecaniodiscus cupanioides*

	Residue/mol			
	S-2	S-3	S-4	S-5
L-Arabinose	2	1	2	1
D-Xylose	—	1	—	—
L-Rhamnose	1	1	1	1

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Table 2. Methylation analysis of the saponins of *L. cupanioides*

Methylated sugar*	T†	Residue/mol S-2	S-3	S-4	S-5
2,3,5-Ara	0.41			1	
2,3,4-Ara	0.54	1			
2,3,4-Xyl	0.54		1		
2,3,4-Rha	0.35				1
2,4-Rha	0.94	1	1	1	
3,4-Ara	1.13	1	1	1	1

*2,3,5-Ara = 2,3,5-tri-*O*-methyl-L-arabinose, etc.

†Retention time of the corresponding alditol acetate relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol on an OV-225 column at 170°.

desorption (FD) mass spectroscopy [11], but when the carboxyl group was free, no fragmentation was observed on FD. However, when the carboxyl group of S-3 was esterified with diazomethane, the fragments *m/z* 787, 641 and 509 were obtained, in addition to the molecular ion ($[M + Na]^+$, *m/z* 919), showing consecutive losses of a pentose, a deoxyhexose and a pentose. This, in conjunction with the methylation analysis, gave the structure D-xylopyranosyl-(1 → 3)-L-rhamnopyranosyl-(1 → 2)-L-arabinopyranoside for the trisaccharide chain of S-3.

^{13}C NMR spectra of the different saponins supported the results from the sugar and methylation analyses. The signals of the carbohydrate carbons have their chemical shifts in the δ 60–110 region, well separated from the other signals. Only the signals from C-3 and C-23 of the hederagenin residue are in the same region. The signals were assigned by comparison with spectra from reference substances, by using substitution rules [12, 13] and by measuring chemical shift differences obtained by replacing the hydroxyl protons with deuterium atoms. The ^{13}C NMR spectra of different methyl glycosides in water [14] have been reported earlier but in the present investigation pyridine was used as solvent. The deuterium-induced isotope shifts have previously been determined for carbohydrates in DMSO [15] and in aqueous solutions [16]. A sample of S-2 in pyridine was measured before and after treatment with deuterium oxide. The chemical shift differences are given in Table 3. Significant differences were obtained for substituted and non-substituted carbons, thus facilitating the assignments.

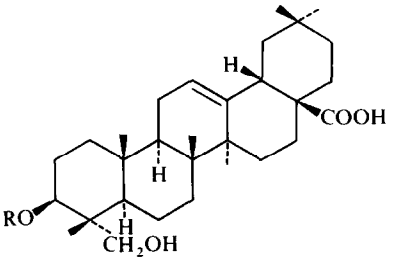
The ^{13}C NMR spectra of S-2, S-3 and S-4 were similar except for five signals in each spectrum. These could be assigned to the terminal glycosyl groups and corresponded to the signals given by methyl α-L-arabinopyranoside, methyl β-D-xylopyranoside and methyl α-L-arabinofuranoside (Table 3). This result indicates that they all contain the same rhamnopyranosyl–arabinopyranosyl residue linked through *O*-3 in rhamnose.

The signals given by the rhamnopyranosyl and the 2-linked arabinopyranosyl residues of S-2, S-3, S-4 and S-5 showed clear similarities, except for the expected shifts caused by the substitution of *O*-3 of the rhamnopyranosyl unit in S-2, S-3 and S-4. The close correspondence strongly suggests the same sequence for the rhamnopyranosyl and the 2-linked arabinopyranosyl residues in all the investigated saponins. The small downfield shift (~3.5 ppm) of C-2 of the 2-linked arabinopyranosyl residue by substitution with a rhamnopyranosyl residue, compared to ~10 ppm obtained by *O*-methylation of C-2, is difficult to explain, but small downfield shifts (~4 ppm) on substitution with α-L-rhamnopyranosyl groups have been observed previously [17].

The anomeric configurations of the sugar residues were determined by comparison with the reference methyl glycosides showing that the xylopyranosyl unit was β-linked and the other sugars were α-linked.

The position to which the oligosaccharide chain was linked was also evident from the ^{13}C NMR spectra of the saponins and reference substances. Comparison of signals for C-3 and C-23 of hederagenin, the mono-*O*-methyl-hederagenin obtained in the methylation analysis of S-2, and the different saponins showed that *O*-3 was substituted with the oligosaccharide. Similar chemical shifts for C-3 and C-23 in closely related *O*-3 substituted saponins have been observed [18]. Some low-field signals of the hederagenin residue are given in Table 4.

On the basis of the above results, the structures **1**–**4** are proposed for saponins S-2 to S-5, respectively. Several



1 R = α-L-Ara*p*-(1 → 3)-α-L-Rha*p*-(1 → 2)-α-L-Ara*p*-(1-
2 R = β-D-Xyl*p*-(1 → 3)-α-L-Rha*p*-(1 → 2)-α-L-Ara*p*-(1-
3 R = α-L-Ara*f*-(1 → 3)-α-L-Rha*p*-(1 → 2)-α-L-Ara*p*-(1-
4 R = α-L-Rha*p*-(1 → 2)-α-L-Ara*p*-(1-

saponins with hederagenin as the aglycone have been found in plants [19] and most of these saponins contain L-arabinose, D-glucose, L-rhamnose and D-xylose with the carbohydrate components glycosidically linked to *O*-3 of the aglycone. S-3 and S-5 have been found in other plants [19, 20] whereas S-2 and S-4 are new substances. A sample of saponin Pg from the pericarps of *Akebia quinata* [20], kindly supplied by Prof. Toshio Kawasaki, was identical with saponin S-3 isolated by us (TLC, ^{13}C NMR).

EXPERIMENTAL

Solvent evaporations were performed under red. pres. at below 40°. GLC was performed on a glass column (190 × 0.15 cm) containing 3% of OV-225 on Gas Chrom Q (100–120 mesh) and GC/MS was performed at an ionization potential of 70 eV. FDMS was performed with a JEOL JMS D-300 spectrometer. NMR spectra were recorded for solns in d_5-pyridine using TMS as internal reference. Spraying with 8% aq. H_2SO_4 and heating were used for visualization of spots on TLC.

Plant material. Stem bark of *Lecaniodiscus cupanioides* Planch. ex Benth. was collected by one of the authors (F.S.) in Sembe,

Table 3. ^{13}C NMR chemical shifts of the signals of the carbohydrate moieties of the saponins of *L. cupanioides**

	Carbon	Reference sub-stance†	S-2	S-3	S-4	S-5	S-2 (60°)	Δδ‡
	1	A 104.6	104.3	104.2	104.3	104.1	103.6	0.04
	2	81.9	74.8	75.2	75.3	75.5	75.2	0.01
	3	72.8	74.1	74.6	74.4	73.9	73.7	0.16
-2)-α-L-Arap-(1-	4	68.9	69.2	69.2	69.6	69.2	68.6	0.15
	5	65.9	65.8	65.9	65.6	65.5	64.7	0.06
	OMe	56.1	—	—				
	2-OMe	60.2	—	—				
	1	B 102.2	101.0	101.0	100.9	101.3	100.9	0.03
	2	71.8	71.6	71.9	71.5	72.1	71.3	0.13
	3	72.4	83.2	82.4	82.3	72.3	82.2	0.09
-3)-α-L-Rhap-(1-	4	73.5	72.7	73.2	72.2	74.5	72.4	0.11
	5	69.3	69.2	69.4	69.2	69.5	69.2	0.01
	6	18.4	18.1	18.2	18.3	18.2	—	—
	OMe	54.5						
	1	C 105.6	107.0				106.4	0.08
	2	71.9	72.7				72.6	0.13
	3	74.0	74.8				73.9	0.15
α-L-Arap-(1-	4	69.0	69.2				68.9	0.14
	5	66.4	66.7				66.4	0.07
	OMe	56.1	—					
	1	D 105.9		106.8				
	2	74.5		75.2				
	3	78.1		78.2				
β-D-Xylp-(1-	4	70.8		70.7				
	5	66.8		66.9				
	OMe	56.5		—				
	1	E 110.4			110.8			
	2	83.3			82.3			
	3	78.5			78.6			
α-L-Araf-(1-	4	85.3			86.8			
	5	62.7			62.7			
	OMe	55.1			—			

*Assignments of signals with very close chemical shifts may be interchanged.

†Reference substances: A, methyl 2-*O*-methyl-α-L-arabinopyranoside; B, methyl α-L-rhamnopyranoside; C, methyl α-L-arabinopyranoside; D, methyl β-D-xylopyranoside; E, methyl α-L-arabinofuranoside.

‡$\Delta\delta = \delta C_{OH} - \delta C_{OD}$, determined for S-2.

Table 4. ^{13}C NMR chemical shifts of some of the signals of the hederagenin part of the saponins of *L. cupanioides*

Carbon	Hederagenin	23-*O*-Me-hederagenin*	S-2†	S-3	S-4	S-5
3	73.8	71.6	80.8	81.0	81.2	80.8
12	122.3	122.4	122.2	122.6	122.6	122.4
13	144.5	143.6	144.4	144.7	144.6	144.4
23	68.5	76.7	63.7	63.8	63.9	63.7
28	179.5	177.4	180.8	179.8	—	180.5

*The methoxyl carbon gave a signal at δ 58.6.

†The signals for C-3 and C-23 at 60° were at δ 80.9 and 63.9 with a chemical shift difference of 0.01 and 0.09 ppm, respectively.

northern Congo/Brazzaville in August 1963. Botanical identification of the plant was made by Dr. A. J. M. Leeuwenberg in Wageningen, Holland. A voucher specimen (Collection 1963–Sembe 48 F.S.) is kept at the Department of Pharmacognosy, University of Uppsala.

Extraction of plant material. Dried and ground stem bark (6 kg) was extracted twice with 95% EtOH (20 l.) with stirring for 24 hr. The combined extracts were taken to dryness yielding 581 g of crude material. Part of this material (150 g) was defatted by extraction in a Soxhlet apparatus with petrol followed by Et_2O.

Isolation of saponins. Crude acidic saponins (6.3 g) were obtained from the defatted extract (30 g) as described by Sandberg [21]. The crude material was adsorbed on Si gel (70–230 mesh, 50 g) and added to a Si gel column (3.8 × 110 cm). Elution was performed with $CHCl_3$–Me_2CO (1:4, 2.6 l.), $CHCl_3$–Me_2CO (1:9, 2.6 l.), $CHCl_3$–Me_2CO (200:1, 0.8 l.), Me_2CO (4 l.), Me_2CO–EtOH (200:1, 0.8 l.), Me_2CO–EtOH (99:1, 0.8 l.) and Me_2CO–EtOH (39:1, 4 l.). Saponins in major amounts were eluted by the more polar solvents. Further purification by prep. TLC using *n*-BuOH–H_2O (9:1) and $CHCl_3$–MeOH–H_2O (14:6:1) as solvents yielded the pure saponins S-2 (335 mg) $[\alpha]_D^{25}$ + 17°, S-3 (78 mg) $[\alpha]_D^{25}$ ±0°, S-4 (34 mg) $[\alpha]_D^{25}$ +12° and S-5 (25 mg) $[\alpha]_D^{25}$ +8° (*c* 0.5, EtOH).

Identification of the sapogenin. The saponin (9 mg) was hydrolysed with 0.5 M aq. H_2SO_4 (2 ml) in dioxane (1 ml) at 100° for 4 hr. The ppt. formed during the hydrolysis was filtered off and washed with H_2O to pH 7. The product was purified by prep. TLC using $CHCl_3$–MeOH (9:1) as solvent. The isolated sapogenin was indistinguishable from a sample of authentic hederagenin by TLC and ^{13}C NMR spectroscopy.

Sugar analysis. The soluble material obtained on hydrolysis of the saponin as described above was used for sugar analysis. The soln was neutralized with barium carbonate, filtered and half of the material used for analyses of the sugars as the alditol acetate derivatives by GC/MS [6, 7]. The other half was used to determine the absolute configuration of the sugars as described by Leontein *et al.* [8].

Methylation analysis. The saponin (1–2 mg) was methylated according to Hakomori [9]. The permethylated product was analysed as described by Jansson *et al.* [10] using sodium borodeuteride as the reducing agent. A sample of S-2 (8 mg) was methylated, hydrolysed as above and the partly methylated sapogenin was isolated by prep. TLC.

Methyl ester formation. A sample of S-3 (2 mg) in MeOH (2 ml) was treated with excess CH_2N_2 in Et_2O for 2 hr at 25° and then concd to dryness. This material was used for FDMS. The mass spectrum showed, *inter alia*, peaks at *m/z* (rel. int.): 919(60), 787(69), 641(100) and 509(55).

^{13}C NMR spectroscopy. Samples in d_5-pyridine were run in 5 mm or 1.5 mm tubes depending on the amounts of material available. For the deuterium-induced shift experiment, a sample of S-2 (50 mg) in pyridine (2 ml.) was twice treated with deuterium oxide (1 ml) and taken to dryness. The product was then dissolved in d_5-pyridine (0.4 ml.) and the spectrum was obtained at 60°. The chemical shifts were then compared with those obtained for the original sample.

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