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^{13}C-N.M.R. CHARACTERIZATIONS OF THE SARSASAPOGENIN DISACCHARIDES, THE FILIFERINS A AND B: 2-*O*-(β-D-XYLOPYRANOSYL)- AND 2-*O*-(β-D-GLUCOPYRANOSYL)-β-D-GALACTOPYRANOSIDES*

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ABSTRACT

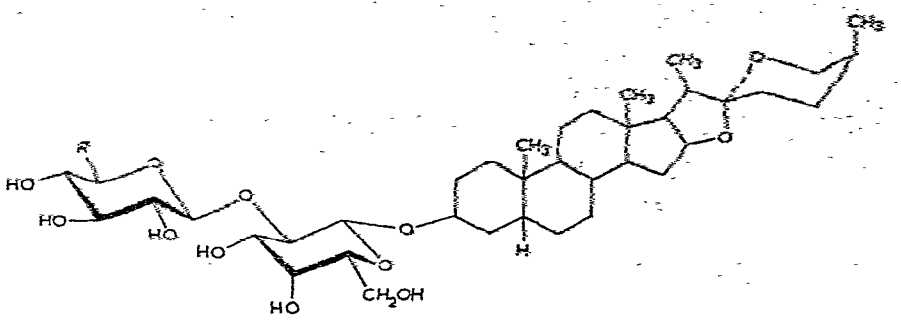
Filiferin B is identical to timosaponin A-III, which had previously been shown to be 3-*O*-[2-*O*-(β-D-glucopyranosyl)-β-D-galactopyranosyl]sarsasapogenin. A larger-scale isolation of filiferin B from the seeds of *Yucca filifera* led to the isolation of filiferin A, now shown to be 3-*O*-[2-*O*-(β-D-xylopyranosyl)-β-D-galactopyranosyl]-sarsasapogenin. The presence of the xylose residue was established by way of hydrolysis. 8-Methoxycarbonyloctyl 2-*O*-(β-D-glucopyranosyl)-β-D-galactopyranoside was synthesized to serve as a model for interpretation of the ^{13}C-n.m.r. spectrum of filiferin B. The information thus gained, together with the ^{13}C-n.m.r. spectra of other, simple model-compounds, permitted assignment of the structure for filiferin A. 8-Methoxycarbonyloctyl 2-*O*-(α-D-glucopyranosyl)-β-D-galactopyranoside was also synthesized.

INTRODUCTION

In 1963, Kawasaki and Yamauchi[1] isolated a steroidal glycoside (**1**) termed timosaponin A-III from dried rhizomes of *Anemarrhena asphodeloides*. Recently, compound **1** (herein also termed filiferin B) was isolated by extraction of the seeds of *Yucca filifera* with alcohol[2]. The extraction provided a second sarsasapogenin glycoside that contained a pentose and was termed[2] filiferin A. The main purpose of this communication is to provide evidence that filiferin A is 3-*O*-[2-*O*-(β-D-xylopyranosyl)-β-D-galactopyranosyl]sarsasapogenin (**2**).

*Dedicated to the memory of Professor J. K. N. Jones, F.R.S.

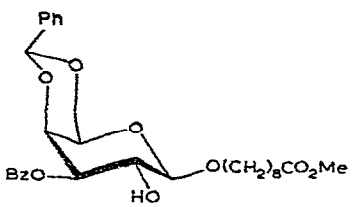
†University of Alberta Postdoctoral Fellow, 1975–1976.



1 R = CH_2OH (filiferin B = timosaponin A-III)
2 R = H (filiferin A)

RESULTS AND DISCUSSION

Lemieux, Bundle, and Baker[3] introduced the use of 8-methoxycarbonyloctyl glycosides for the preparation of artificial antigens and reported[4,5] the corresponding β-D-galactopyranoside. A study was made of structure–activity relationships for the combination of the β-D-galactopyranosyl group and antibodies raised to antigens prepared from this hapten[4,5]. In an extension of this research to the preparation of the terminal trisaccharide unit of the antigenic determinant for the human B blood



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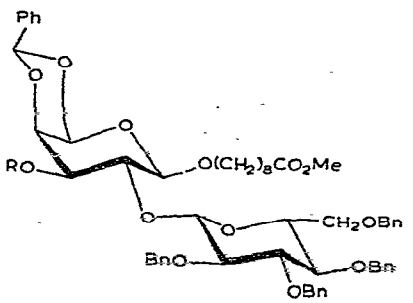
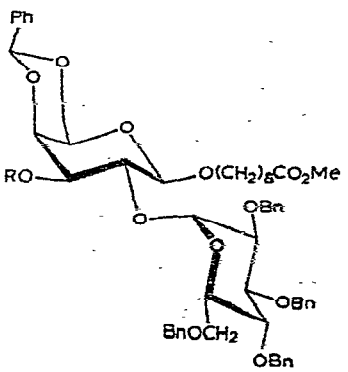
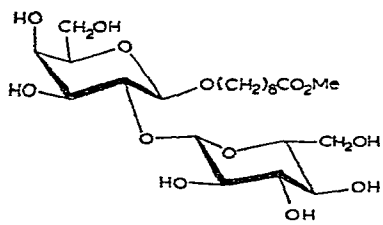


4 R = Bz
6 R = H



5 R = Bz
7 R = H

group, 8-methoxycarbonyloctyl 4,6-*O*-benzylidene-3-*O*-benzoyl-β-D-galactopyranoside (3) was prepared[6] following the procedures described by Chittenden[7] for the preparation of the corresponding methyl glycosides. It was considered of interest to use 3 for the preparation of a model structure for examining the structures of the filiferins A and B. Thus, condensation of 3 with tetra-*O*-benzyl-α-D-glucopyranosyl chloride in the presence of silver trifluoromethanesulfonate–*sym*-collidine (1:1 complex) gave a 68% yield of an ~5:3 mixture of the α- and β-glucopyranosides, respectively. The products were separated by column chromatography and, after deprotection, afforded crystalline preparations of the α- (9) and β- (8) anomers, having specific rotations of 58° and −3.8°, respectively. Interestingly, the disaccharide component of 9 is the terminal unit of an antigenic determinant related to antibody-mediated glomerulonephritis[8]. Immunochemical studies involving compounds 8 and 9 as precursors to artificial antigens are planned.



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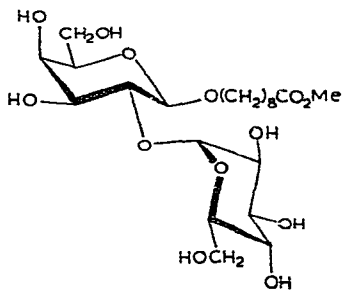

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As may be seen from Table I, the ^{13}C-n.m.r. spectra for compounds 8 and 9 are in accord with the assigned structures, as the chemical shifts of the twelve signals assignable to the two sugar residues, except those for the intersugar, anomeric center (C-1′) and the intersugar, aglyconic carbon atom (C-2) correspond well with those anticipated from the ^{13}C-n.m.r. spectra of 8-methoxycarbonyloctyl β-D-galactopyranoside and the methyl α- and β-D-glucopyranosides. The spectra were measured in dimethyl sulfoxide-d_6, as this solvent could be used to measure the spectra of the filiferins A and B.

The spectrum for filiferin B was in accord for a structure containing 39 carbon atoms. All of the signals below 56 p.p.m. are assignable to the steroidal aglycon. Of the seventeen signals above 56 p.p.m., five signals at 108.8, 80.4, 73.2, 64.3, and 62.0 p.p.m. are assigned to the sarsasapogenin residue because these five signals were also present in the spectrum of filiferin A and four are observed in the spectrum of sarsasapogenin itself. For the latter compound, the signal at 67.1 p.p.m. is shifted downfield to 73.2 and 73.3 for the filiferins A and B, respectively, and may be assigned to C-3 of the aglycon. The twelve remaining signals in the spectrum of filiferin B are assigned in Table I, and the close correspondence of the chemical shifts with those found for compound 8 confirmed the structure (1) and provided the basis for assignments of the structure of filiferin A based on ^{13}C-n.m.r. spectroscopy.

TABLE I

^{13}C NUCLEAR MAGNETIC RESONANCE SPECTRA (δ P.P.M.) OF SUGAR COMPONENTS OF SYNTHETIC DISACCHARIDES, PILIFERINS A AND B, AND MODEL COMPOUNDS, IN Me_2SO-d_6

Compound	*β-D-Galactopyranosyl residue*						*β-D-Glucopyranosyl residue*					
	C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*C-1'*	*C-2'*	*C-3'*	*C-4'*	*C-5'*	*C-6'*
c	103.5	70.6	73.6	68.2	75.1	60.4						
b							103.9	73.3	76.7	70.1	76.7	61.0
8	101.7	80.1	74.8	67.6	74.8	60.7	104.2	72.9	76.9	69.9	76.9	60.3
1	100.8	79.1	74.7	67.8	75.0	61.2	103.8	73.3	76.9	70.1	76.2	60.2
							α-D-Glucopyranosyl residue					
c							99.7	72.5	73.4	70.1	71.9	61.0
9	103.0	76.0	71.8	68.2	74.8	60.7	97.9	71.8	73.5	70.1	72.2	60.3
							D-Xylopyranosyl residue					
2	99.9	80.4	74.5	67.8	74.5	60.3	105.4	72.7	75.9	69.6	65.9	
d							104.7	73.2	76.5	69.6	65.6	
e							100.2	72.0	73.4	70.0	61.7	

[a]8-Methoxycarbonyloctyl β-D-galactopyranoside. [b]Methyl β-D-glucopyranoside. [c]Methyl α-D-glucopyranoside. [d]Methyl β-D-xylopyranoside. [e]Methyl α-D-xylopyranoside.

As expected, the ^{13}C-n.m.r. spectrum of **1** gave signals characteristic for the two hydroxymethyl groups of the sugar, at 61.2 and 60.2 p.p.m. (see Table I). However, the spectrum of filiferin A (**2**) showed only one such group, with the signal assignable to only one carbon atom occurring at 60.7 p.p.m. Furthermore, the spectrum contained only eleven signals not attributable to the steroidal aglycon. Thus, the presence of a pentose unit was confirmed. That the pentose was xylose became evident by examination of the acid hydrolyzate of **2** both by paper and gas–liquid chromatography. The chromatograms indicated that the hydrolysis of **2** yielded an equimolar mixture of galactose and xylose, as required by the ^{13}C-n.m.r. data. That these were both the D forms was evident from the similar molecular rotations of filiferins A and B, $-359°$ and $-259°$, respectively. The molecular rotation of D-xylopyranoside is expected to be about 50° less dextrorotatory than that of a D-glucopyranoside having the same aglycon.

That the disaccharide group of filiferin A is 2-*O*-(β-D-xylopyranosyl)-β-D-galactopyranosyl was evident from a comparison of its ^{13}C-n.m.r. spectrum and those of filiferin B, and methyl β-D- and α-D-xylopyranoside. As may be seen from Table I, comparison of the spectra for the filiferins A and B allows assignment, to the β-D-galactopyranosyl portion, of six of the eleven signals attributable to the disaccharide residues. Of the remaining five signals in the spectrum for filiferin A, the chemical shifts assigned to C-2′, C-3′, C-4′, and C-5′ are in good accord with expectations based on the spectrum of methyl β-xylopyranoside, but not from that of its α-anomer. Therefore, it is concluded that filiferin A is 3-*O*-[2-*O*-(β-D-xylopyranosyl)-β-D-galactopyranosyl]sarsasapogenin.

EXPERIMENTAL

Isolation and characterization of filiferins A and B. — Dried, finely ground seeds of *Yucca filifera* (552 g) were extracted with hexane in a Soxhlet apparatus to remove an oil (130 g) that had the usual characteristics of a vegetable oil. The solvent was then changed to ethanol and, after extraction for 60 h, removal of the solvent gave 84.7 g (15% yield) of a white solid. A 10-g portion of this crude saponin was suspended in 20 ml of ethanol and 10 g of silica gel (Grace, grade 922) was added. The mixture was dried under vacuum in a rotatory evaporator to leave a powder that was applied to the top of a 3-m column loaded with 5.9 kg of the silica gel. The chromatogram was developed with 16:4:1 (v/v) ethyl acetate–methanol–water.

The first six liters of eluent afforded 75 mg of free sarsasapogenin, m.p. 204°, identified by comparison with an authentic sample. The following two liters of eluent contained 1.21 g of crude filiferin A, m.p. 280°. Recrystallization from methanol provided the analytical sample, m.p. 292°, $[\alpha]_D^{25}$ $-50.5°$ (*c* 1, dimethyl sulfoxide).

Anal. Calc. for $C_{38}H_{62}O_{12}\cdot 0.5H_2O$: C, 63.40 H, 8.81 O, 27.78. Found: C, 63.51, H, 8.82; O, 27.58.

After several 50-ml fractions which, on evaporation, left no residue, filiferin B appeared. The fraction containing this material gave 3.2 g of crude material, m.p. 310–

312°. The analytical sample was obtained by recrystallization from methanol; m.p. 319–321°, $[\alpha]_D^{25}$ −35° (*c* 1.2, dimethyl sulfoxide) (lit.[2] m.p. 317–322°, $[\alpha]_D^{25}$ −41.6°).

The ^{13}C-chemical shifts for signals near the same positions and considered possibly to arise from similar carbon atoms in sarsasapogenin, filiferin A, and filiferin B are listed consecutively in that order, in the following list. These shifts are given for spectra measured in $CDCl_3$ for sarsasapogenin and in Me_2SO-d_6 for the filiferins, with tetramethylsilane as the internal standard:

109.8, 108.7, 108.8; —, 105.4, 103.8; —, 98.8, 100.8; 81.1, 80.4, 80.4; —, 80.4, 79.1; —, —, 76.9; —, 75.9, 76.2; —, 74.7, 75.0; —, 74.7, 74.7; —, 73.2, 73.9; —, 72.7, 73.3; —, 69.6, 70.1; —, 67.8, 67.8; 67.1, —, —; —, 65.9, —; 65.2, 64.3, 64.3; 62.3, 62.0, 62.0; —, —, 61.2; —, 60.3, 60.2; 56.6, 55.8, 55.8; 42.2, 41.6, 41.6; 40.7, 39.6, 40.2; 40.4, 39.6, 40.2; 40.0, 39.6, 40.2; 36.6, 36.8, 35.9; 35.4(2), 34.9(2), 35.0(2); 33.7, 34.5, 34.5; 31.8, 31.4, 31.5; 30.1, 29.9, 30.0; 27.9, 29.9, 30.0; 26.7(3), 29.9(3), 30.0(3); 26.1, 26.5, 26.5; 25.9, 23.4, 23.5; 24.0, 23.4, 23.5; 21.0, 20.5, 20.5; 16.5, 16.1, 16.1; 16.1, 15.9, 15.9; 14.4, 14.3, 14.3.

The numbers of carbon atoms present in each of the three compounds are accounted for on the foregoing basis. The assignments of the signals in Table I are based on the assignments for the various corresponding methyl glycosides reported in the book by Stothers[9].

Hydrolysis of Filiferin A. — Compound **2** (0.30 g) was boiled for 4 h in 11% aqueous hydrochloric acid (50 ml) under reflux. The cooled mixture was filtered to remove a solid identified as sarsasapogenin, m.p. 204°, by its mixed melting-point with an authentic sample and by comparison of the i.r. spectra. Evaporation of the filtrate left a water-soluble, yellow syrup (yield 0.117 g, 82%), which was applied to a small column of 1:1 Celite–charcoal (2 g). Elution with water gave a colorless syrup which, on examination by descending paper-chromatography on Whatman No. 1 paper and a 4:1 (v/v) 2-propanol–water as developing phase, showed two spots, visualized by spraying with *p*-anisidine pthalate solution[10], having R_{Glc} values of 0.90 and 1.18. These values were those found by using authentic samples of D-galactose and D-xylose. The syrup was trimethylsilylated[11] and applied to a 6-ft (¼-in. o.d.) column packed with 80–100 mesh AW-DMCS Chromosorb W (Johns–Manville Products Corp.) coated (1%) with XE-60 (Wilkens Instrument and Research Inc.) for g.l.c.[12] at 143°. Six peaks were observed, and these had the same retention times as the peaks observed by using an equimolar mixture of authentic, equilibrated (in water) samples of D-xylose and D-galactose. Furthermore, co-injection of the sample prepared from the hydrolyzate and the sample prepared from the equilibrated mixture of D-xylose and D-galactose produced no change in the recorded peaks.

8-Methoxycarbonyloctyl 3-O-benzoyl-(tetra-O-benzyl-β-D-glucopyranosyl)-4,6-O-benzylidene-β-D-galactopyranoside (**4**) *and the corresponding α-D-glucopyranosyl*

anomer (**5**). — 8-Methoxycarbonyloctyl 3-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranoside (**3**), m.p. 96–97°, $[\alpha]_D^{25}$ +60.5° (*c* 1, chloroform), (0.90 g, 1.66 mmol)[6] was dissolved in 42:38 (v/v) benzene–nitromethane (40 ml) that contained *sym*-collidine (0.32 g, 2.64 mmol) and silver trifluoromethanesulfonate (0.68 g, 2.64 mmol). The solution was cooled to 0° and a solution of tetra-*O*-benzyl-α-D-glucopyranosyl chloride[13] (1.02 g, 1.82 mmol) in 2 ml of the benzene–nitromethane mixture was added dropwise with efficient stirring. The resulting, heterogeneous mixture was stirred for 1 h at 0°, at which time t.l.c. examination indicated that the glycosyl chloride was absent. The solids were removed and the mother liquor was evaporated *in vacuo* to leave a syrup (2.10 g) that was dissolved in dichloromethane (25 ml) for washing with 5% aqueous sodium hydrogencarbonate and water. The solution was dried (sodium sulfate) prior to removal of the solvent. The residual syrup (1.80 g) was applied to a column containing 50 g of silica gel (Merck silica gel H). Development with 9:1 v/v benzene–ethyl acetate eluted a fraction (yield 1.20 g, 68%) that proved to be a mixture of compounds **4** and **5**. Separation was achieved by column chromatography on the silica gel (175 g) by using a 4:7 (v/v) Skellysolve B–diethyl ether as the developing phase. The first component to be eluted was **4** (0.37 g), $[\alpha]_D^{25}$ +50.7° (*c* 1, chloroform). The α anomer **5** followed (0.60 g), $[\alpha]_D^{25}$ +82.5° (*c* 1.1, chloroform). The p.m.r. spectra were consistent with the assigned structures, but neither product could be induced to crystallize.

8-Methoxycarbonyloctyl 2-O-(β-D-glucopyranosyl)-β-D-galactopyranoside (**8**). — Compound **4**, just described, was conventionally *O*-debenzoylated with sodium methoxide in methanol to provide compound **6** in quantitative yield. The product was recrystallized from ethyl acetate–pentane, m.p. 115–115.5°, $[\alpha]_D^{25}$ +6.5° (*c* 1, chloroform).

Anal. Calc. for $C_{53}H_{63}O_{12}$: C, 71.23; H, 7.12. Found: C, 71.19; H, 6.98.

The benzyl and benzylidene groups in **6** (0.30 g) were simultaneously removed by hydrogenolysis in 1:1 ethyl acetate–ethanol (20 ml) over 5% palladium on charcoal (0.20 g) with hydrogen (100 lb. $in.^{-2}$) at room temperature. A syrup (0.155 g) was obtained that crystallized from a mixture of methanol, ethanol, and diethyl ether. The purified compound, m.p. 129–130°, $[\alpha]_D^{52}$ −3.8° (*c* 1, methanol) gave a ^{1}H-n.m.r. spectrum in CD_3OD in general accord with the assigned structure. The doublet signals at 4.60 and 4.32 p.p.m. having spacings of 7.0 and 6.5 Hz, respectively, were assigned to the protons at the two anomeric centers. The ^{13}C-n.m.r. spectrum confirmed the overall structure. The chemical shifts for the twelve carbon atoms of the disaccharide group are reported in Table I.

Anal. Calc. for $C_{22}H_{40}O_{13}$: C, 51.56 H, 7.86. Found: C, 51.43 H, 7.72.

8-Methoxycarbonyloctyl 2-O-(α-D-glucopyranosyl)-β-D-galactopyranoside (**9**). — Compound **5**, just described, was *O*-debenzoylated and purified as described for the preparation of **6**. The product, compound **7**, m.p. 124–126°, $[\alpha]_D^{25}$ +57.3° (*c* 1.1, chloroform), was obtained in near-quantitative yield. The ^{1}H-n.m.r. spectrum was in general accord with the assigned structure.

Anal. Calc. for $C_{53}H_{63}O_{12}$: C, 71.23; H, 7.12. Found: C, 71.32 H, 6.99.

Hydrogenolysis of **7** as already described for **6** produced the title compound **9** in near-quantitative yield, m.p. 151–153°, $[\alpha]_D^{25}$ +57.6° (*c* 1.6, methanol). The ^{1}H-n.m.r. spectrum in CD_3OD was in general accord with the assigned structure. The doublet signals at 5.31 and 4.43 p.p.m. having spacings of 3.4 and 7.0 Hz, respectively, were assigned to the anomeric hydrogen atoms of the α-D-glucopyranosyl and β-D-galactopyranosyl groups, respectively. The ^{13}C-n.m.r. spectrum confirmed the overall structure. The chemical shifts for the twelve carbon atoms of the disaccharide group are reported in Table I.

Anal. Calc. for $C_{22}H_{40}O_{13}$: C, 51.56; H, 7.86. Found: C, 51.36; H, 7.77.

ACKNOWLEDGMENTS

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