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ON THE SAPONINS OF THE STARFISH *ASTERIAS VULGARIS*

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ABSTRACT.—The isolation and characterization of three major saponins from *Asterias vulgaris* is described. All three possess identical thornasterol A aglycones and have C-3 sulfate and C-6 oligosaccharide features. In two cases, the oligosaccharide side-chain features only quinovose and fucose (3:2 and 3:1) and the third has quinovose and an unidentified sugar (3:1).

The toxicity of starfishes has been widely known for more than a century and is attributed to their saponin (asterosaponin) content. The toxicity of various echinoderms to fish, mollusks, arthropods, vertebrates, and annelids has been demonstrated; however, asterosaponins of one species of starfish are probably not toxic to others (1-4). Asterosaponins have been found to possess a wide range of interesting pharmacological activities. For example, the saponin from *Asterias forbesi*, a close relative of *Asterias vulgaris* Verrill, was reported to possess antiinflammatory, analgesic, and hypotensive activity (5), while other saponins have been shown to possess cytotoxic, hemolytic, antiviral, antifungal, and antineoplastic activity (6).

We have recently established (7) the structure **4** for a partial hydrolysis product of the saponin fraction from *A. vulgaris*. This finding has prompted us to pursue the separation and characterization of the major saponins from this starfish because little is known about their properties or structures despite the abundance of this species in the Bay of Fundy and elsewhere.

Rapid silica gel column chromatography of the saponin fraction from *A. vulgaris* gave five distinct fractions which were each further purified by preparative tlc. Subsequent examination of these fractions on reverse-phase silica gel plates indicated that each contained one major compound contaminated with several minor components. The three major saponins isolated in this way were then individually subjected to final purification by hplc on a reverse-phase column providing asterosaponins AV1, AV2, and AV3 in the approximate ratio (by weight) 4:3.5:1.

The ir spectra (KBr) of all three are very similar and show absorption bands at 3450 (hydroxyl), 1060 (glycosidic linkage), and 1730 (broad, ketone) cm^{-1}. Examination of the high field ^{1}H-nmr spectra (see Table 1) of AV1, AV2, and AV3 reveals that each possesses the thornasterol A (8) aglycone with a sulfate moiety at C-3 and a C-6 oligosaccharide side-chain; thus the 400 MHz ^{1}H-nmr spectrum; (D_2O) of AV1 shows singlets at δ 0.78 and δ 0.98 for the C-13 and C-10 methyls, respectively, doublets at δ 0.90 and δ 0.92 for the isopropyl methyls, and a singlet at δ 1.34 for the tertiary C-20 methyl. Doublets at δ 1.23 (3H), δ 1.27 (6H), δ 1.37 (3H), and δ 1.38 (3H) are attributed to the five methyls associated with the 6-deoxy features of the oligosaccharide moiety. The C-22 methylene α to the C-23 ketone gives rise to doublets (J=14.5 Hz), one centred at δ 2.53 and the other at δ 2.63, while the C-24 methylene signal is a doublet at δ 2.37 (J=7 Hz). The downfield region depicts a multiplet centred at δ 4.21 for the C-6 methine, doublets (1H each) at δ 4.30 (J=7 Hz), δ 4.43 (J=9.5 Hz), δ 4.55 (J=7 Hz), and δ 4.57 (J=8 Hz) for four anomeric protons, while the fifth at δ 4.89 is partially obscured by the signal due to solvent. The large coupling constant for these signals is indicative of β-configurations at all anomeric sites. A broad multiplet at δ 5.34 is typical for the C-11 olefinic proton. A multiplet arising from the sulfate methine at C-3 is largely obscured by the strong signal due to solvent (δ 4.85 - δ 4.95).

These assignments and others contained in Table 1 are consistent with literature data (8) for thornasterol A and related compounds.

TABLE 1. ^{1}H-nmr Parameters of Saponins[a]

Proton	AV1 (D_2O)	AV2 (C_5D_5N)	AV3 (C_5D_5N)
H_3	4.84 (m)[d]	d	d
H_6	4.21 (m)	d	4.32 (m)
H_{11}	5.34 (m)	5.19 (bs)	5.20 (bs)
H_{18}	0.78 (s)	0.93 (s)	0.96 (s)
H_{19}	0.98 (s)	0.98 (s)	0.99 (s)
H_{21}	1.34 (s)	1.54 (s)	1.55 (s)
H_{22_A}	2.53 (d, J=14.5)	2.58 (d, J=14.5)	2.57 (d, J=14.5)
H_{22_B}	2.63 (d, J=14.5)	2.77 (d, J=14.5)	2.77 (d, J=14.5)
$H_{24_{A,B}}$	2.37 (d, J=7)	2.45 (dd, J=17, J=7) 2.53 (dd, J=17, J=7)	2.36 (dd, J=17, J=7) 2.44 (dd, J=17, J=7)
H_{26}	0.90 (d, J=7)	0.85 (d, J=7)	0.85 (d, J=7)
H_{27}	0.92 (d, J=7)	0.86 (d, J=7)	0.86 (d, J=7)
H_1i	4.30 (d, J=7)[b]	4.78 (d, J=7)[b]	4.69 (d, J=7)[b]
H_1ii	4.43 (d, J=9.5)[b]	4.90 (d, J=7)[b]	4.78 (d, J=7)[b]
H_1iii	4.55 (d, J=7)[b]	4.96 (d)[b,d]	4.91 (d, J=7)[b]
H_1iv	4.57 (d, J=8)[b]	5.36 (d, J=7)[b]	5.27 (d, J=7)[b]
H_1v	4.89[d]	—	—
H_6i	1.23 (d, J=6)[c]	1.45 (d, J=6)[c]	1.45 (d, J=6)[c]
H_6ii	1.27 (d, J=7)[c]	1.55 (d, J=6)[c]	1.51 (d, J=6)[c]
H_6iii	1.27 (d, =7)[c]	1.73 (d, J=6)[c]	1.63 (d, J=6)[c]
H_6iv	1.37 (d, J=7)[c]	—	1.73 (d, J=6)[c]
H_6v	1.38 (d, J=7)[c]	—	—

[a]Chemical shifts are reported in ppm relative to internal TMS, couplings are in Hz.
[b]Values may be interchanged in columns.
[c]Values may be interchanged in columns.
[d]Partly obscured by OH signal (assignment tentative).

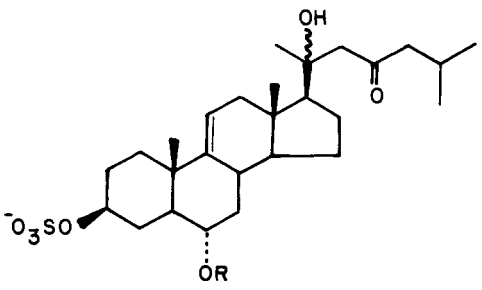
Hydrolysis of saponin AV1 with 2 N HCl provided a mixture of sugars that were analyzed as their trimethylsilyl (TMS) ether derivatives by gc and showed the presence of only quinovose (6-deoxyglucose) and fucose (6-deoxygalactose) in the ratio 3:2.

In view of the foregoing, we conclude that saponin AV1 possesses structure **1**, featuring five sugar units attached via C-6.

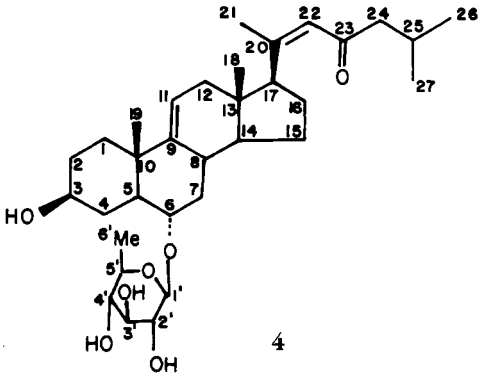
On the basis of comparison of parallel ^{1}H-nmr spectral data (Table 1) and gc analysis of hydrolysis products, we propose the partial structures **1** and **2** for saponins AV2 and AV3, respectively. We have established that the unidentified sugar X in AV2 is not fucose, xylose, glucose, galactose, nor mannose, all of which are known to occur in the oligosaccharide side-chain of other saponins. It is apparent that the unidentified sugar is not a 6-deoxy-aldohexose, inasmuch as only three secondary methyls are present in AV2 and are assigned to the three quinovose moieties.

We thus conclude that the major saponins of *A. vulgaris* possess the thornasterol A aglycone with a C-3 sulfate and a C-6 oligosaccharide side-chain and that the first sugar is probably quinovose in view of our isolation of **4** following partial hydrolysis of total saponin. Thus, the major saponins (AV1 and AV2) from *A. vulgaris* bear considerable resemblance to the major saponin, thornasteroside A, from the crown-of-thorns starfish, *Acanthaster planci* L., differing in the nature of the oligosaccharide side-chain, which in the latter, features quinovose (two units), fucose, galactose, and xylose (one unit each).

The sequence and linkages of the sugars in the oligosaccharide chains in AV1, AV2, and AV3 are currently under investigation.



1, R=(Quinovose)$_3$ (Fucose)$_2$
2, R=(Quinovose)$_3$ (Sugar X)$_1$
3, R=(Quinovose)$_3$ (Fucose)$_1$



4

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Analytical and preparative tlc was performed using precoated silica gel G plates (Kieselgel 60, F-254). A Waters Associates hplc unit equipped with a model 440 Absorbance and R401 refractive index detectors and a Partisil M20, 10/25, ODS-3 column (25 mm ID × 25 cm) was used. A Perkin-Elmer model 990 gas chromatograph equipped with a flame ionization detector and a 3% OV-101, 60-80 mesh column (2 mm ID × 10′) was used at a column temperature of 140° and a helium flow rate of 50 ml/min.

ISOLATION OF SAPONIN FRACTION FROM *A. VULGARIS*.—This was done as described earlier (7).

SEPARATION OF SAPONINS AV1, AV2, AND AV3.—Saponin mixture (1.0 g) was subjected to rapid chromatography on a silica gel G columm (30 cm × 4 cm) packed in $CHCl_3$.[1] The saponin mixture was first dissolved in the minimum quantity of distilled H_2O, adsorbed on a small portion of silica gel, and then the H_2O was removed by evaporation at 40° and the impregnated silica gel loaded onto the column. Elution with $CHCl_3$-MeOH-H_2O (65:35:2) gave fractions that were combined on the basis of tlc to provide five partially purified saponins which, on reverse-phase silica gel tlc, were each found to contain one major component. Further purification was accomplished by hplc using a semi-preparative reverse-phase silica column. The three major saponins were subjected to hplc individually, using the conditions below, and provided purified saponins AV1 (42 mg), AV2 (36 mg), and AV3 (11 mg).

hplc solvent system:	MeOH 60% in H_2O
Flow rate:	9.9 ml/min
Pressure:	2000-3000 psi
Elution time:	10-14 min

ISOLATION OF SUGARS FROM SAPONINS.—The sugars were all obtained in the manner described for the isolation of monosaccharides from AV1 as follows:

Saponin AV1 (2.0 mg) was dissolved in 2 N HCl (10 ml) and refluxed at 90° for 6 h. The mixture was cooled, neutralized with $NaHCO_3$, and extracted with $CHCl_3$. The aqueous extract was evaporated to complete dryness, and the sugars were taken up in absolute EtOH (25 ml). Following evaporation of the EtOH, the sugar fractions were examined by gc analyses as below.

IDENTIFICATION OF SUGARS BY GC.—The sugar fractions were treated with TRI-SIL reagent (1.0 ml) for 5 min, flushed with N_2 to remove solvent, and dissolved in EtOAc (0.5 ml). After concentration to 0.1 ml, the TMS ether derivatives of the anomers were examined by gc, using authentic derivatized samples of quinovose and fucose anomers as controls. The results were as follows:

Sugar Source	Quinovose	Fucose	Unidentified Sugar
AV1	3	2	—
AV2	3	1	—
AV3	3	0	1[2]

[1]We have observed that prolonged exposure (>24 h) of the saponin to a silica gel column sometimes leads to substantial decomposition.

[2]The unidentified sugar, which gave rise to a four- rather than a two- (anomeric) component mixture, was shown to be non-identical with appropriately treated xylose, glucose, galactose, or mannose.

ACKNOWLEDGMENTS

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