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AGLYCONES FROM THE SAPONIN OF THE STARFISH *ASTERIAS VULGARIS*

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ABSTRACT.—The partial hydrolysis products of the saponin from *Asterias vulgaris* have been examined, and 13 compounds have been identified, including the glucosides 3β-hydroxy-5α-cholesta-9(11),20(22)-dien-23-one-6α-yl-β-D-6′-deoxyglucoside and 3β-hydroxy-5α-chol-9(11)-en-23-one-6α-yl-β-D-6′-deoxyglucoside.

There has been widespread interest in echinoderm saponins because of their toxicity, broad range of pharmacological activities, and ability to induce unusual behavior in other marine organisms (1). In relatively few instances has the complete structure of pure saponins been reported, and most bioassays have been performed on the complex mixtures because of the difficulty of separating these highly polar compounds. Little is known about the saponin of the starfish *Asterias vulgaris*, which, together with the closely related *Asterias forbesi*, is very abundant in the Bay of Fundy. With a view, ultimately, to determining the structures of the major saponins of these species, we report here on the hydrolysis and partial hydrolysis products of the saponin of *A. vulgaris*.

Crude saponin, obtained by the method of Gilgan (2), was treated with aqueous HCl (2 N) at 90° in a two-phase system with $CHCl_3$. The $CHCl_3$ layer was removed and replaced with fresh aliquots several times, and the total $CHCl_3$ extract was evaporated to yield an aglycone mixture that was acetylated and subjected to chromatographic separation on a silica gel column. Fractions were further purified by hplc or ptlc to provide 13 compounds as indicated in table 1. Thus, all compounds were characterized initially as their acetates, and, in some cases, these derivatives were hydrolyzed to provide free aglycones.

TABLE 1. Chromatography of aglycone acetates from *Asterias vulgaris*

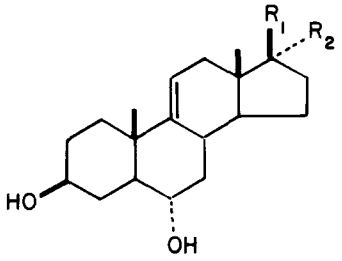
Compounds in order of elution	Column solvent (EtAc-C_6H_{14}-MeOH)	% Yield (of total aglycone)	ptlc solvent (EtAc-C_6H_{14}-MeOH)	References
11	1:9:0	1.0	1:4:0	
8[a]	1:9:0	3.2	1:4:0	
9[a]	1:9:0	2.6	1:4:0	3
10[a]	1:9:0	0.6	1:4:0	
4	1:3:0	4.6	1:3:0	4,5,6
5[b]	1:3:0	7.0		6
6[b]	1:3:0	0.6		6
7[b]	1:3:0	2.9		6
1	2:3:0	10.1	15:5:1	4,6,7,8
2	2:3:0	1.4	15:5:1	9
3	2:3:0	2.7	12:7:5	10
12	10:10:1	0.1	13:7:5	11
13	10:10:1	0.1	13:7:5	

[a]Three component mixture.
[b]Three component mixture.

The major aglycone in our partial hydrolysis was shown to be asterone (**1**) in admixture with isoasterone, the 17α isomer (**2**). While this isomer mixture has been previously obtained from several other starfish saponins, there are no reports of separating **1** and **2** for characterization as homogeneous compounds. We have succeeded in separat-

ing these isomers as their diacetates using a hplc system incorporating a silica gel column and a hexane-ethyl acetate-methanol (95:5:2.5) system.

The pure diacetates of **1** and **2** show significant spectral differences. Thus, in contrast to asterone diacetate *m/e* 416, isoasterone does not show a molecular ion, and the lower regions of their spectra are very similar but not identical. The notable difference in the ^{1}H-nmr spectra of these isomers is in the signal for the C-13 methyl found at δ 0.55 in asterone **1** and at δ 0.80 in isoasterone. This difference is due to the different shielding of the side-chain carbonyl.



1 $R_1 = COCH_3$, $R_2 = H$

2 $R_1 = H$, $R_2 = COCH_3$

3 $R_1 = CHOHCH_3$, $R_2 = H$

4 $R_1 = (E)C(CH_3)=CHCOCH_2CH(CH_3)_2$, $R_2 = H$

Other aglycones shown to be present, isolated as their acetates and identified by comparing their spectra with literature data (see table 1), are: 20(R),5α-pregn-(11)-ene-3β,6α,20-triol (**3**) (asterogenol), 20(E),3β,6α-dihydroxy-5α-cholesta-9(11),20(22)-dien-23-one, (**4**), 17(Z),3β,6αdihydroxy-5α-cholesta-9(11),17(20)-dien-23-one, (**5**), 17(E),3β,6α-dihydroxy-5α-cholesta-9(11),17(20)-dien-23-one, (**7**), 17(E),3β,6α-dihydroxy-5α-cholesta-9(11),17(20)-24-triene, (**8**). The presence of a weak ion of *m/e* 512 and the ms of **5** suggested the presence of traces of the 24-methyl homolog (**6**), while an ion *m/e* 496 in the ms of the triene (**8**) is indicative of the presence of the 24-methyl homolog (**10**). This latter assignment is supported by the observation that the signal for the C-24 olefinic proton in the 400 MHz ^{1}H-nmr spectrum of **8** integrates for less than one hydrogen.

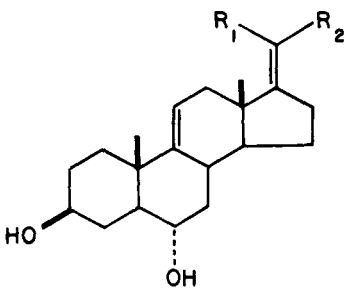
The first fraction eluted by silica gel chromatography of the acetates proved to be a complex mixture that was then purified by hplc to provide a compound tentatively assigned structure **11**. The 400 MHz ^{1}H-nmr shows the usual features of this ring system with secondary 3β- and 6α-acetate groups and the 9(11) double bond. The location of a singlet, δ 0.76, is consistent with the proximity of the C-13 methyl and the 17(20) double bond, while another singlet, δ 1.56, is typical for the vinylic methyl at C-20. The unique feature of this spectrum is the presence of a triplet (J=6 Hz) at δ 4.05, assigned to the ethereal proton at C-21. The downfield region (δ 5.72-6.34) shows weak signals indicating the presence (≃10%) of a minor component featuring additional olefinic protons. Evidence for the existence of conjugated double bonds in this minor component comes from the uv spectrum (λ max 232).

Glycoside **12** features an isomarthasterone aglycone attached via C-6 to a quinovose moiety with a β-anomeric linkage and was previously obtained by hydrolysis of the saponin from *Acanthaster planci*. The tetraacetate of 3β-hydroxy-5α-cholesta-9(11)-en-23-one-6α-yl-β-D-6′-deoxyglucoside (**12**) shows no molecular ion in its ei ms, and the highest substantial (>1%) ion is found at *m/e* 439 (m-289), corresponding to cleavage of the C-6 bond to oxygen. The next notable high mass ion is *m/e* 378, corresponding to loss of acetic acid from ion *m/e* 439.

The ms of the tetraacetate of **13** is similar in that it displays prominent ions at *m/e* 397 (M-289) and 337 (M-289-60) and has no molecular ion. Another prominent feature of both spectra is an ion at *m/e* 273 ($C_{12}H_{17}O_7$), derived from the quinovose acetate moiety. The ir spectrum of **13** features absorptions at 1735 (acetate) and 1710 (ketone) cm^{-1}. The 360 MHz ^{1}H-nmr spectrum of this compound depicts singlets at δ 0.54,

0.98, and 2.15, assigned to methyls at C-13, C-10, and C-24, respectively, while four overlapping acetate methyl singlets are centered at δ 2.05 and a doublet for the C-21 methyl at δ 0.93 (J=6 Hz). A doublet at δ 1.24 (J=6 Hz) is attributed to the C-5′ methyl. The β-configuration of the anomeric carbon is inferred from the large coupling constant for the signal at δ 4.52 (J=8 Hz) due to C-1′H. Two broad multiplets, one at δ 3.45 for the 6 H, another at δ 3.56 for the 5′H are observed. Furthermore, the downfield region shows a broad multiplet at δ 4.63 for the acetate methine at C-3, a triplet at δ 4.82 (J=9.5 Hz) for the 2′H, a triplet at δ 4.49 (J=8.5 Hz) for the 4′H, a triplet at δ5.14 (J=9.5 Hz) for the 3′H, and a broad multiplet at δ 5.25 for the olefinic proton at C-11.

Isolation of these glycosides indicates that in the parent saponins of *A. vulgaris*, the oligosaccharide chain is attached to the C-6 position of a steroidal aglycone via a β-anomeric linkage and that the first sugar in some components is 6-deoxyglucose (quinovose). It is well known (1) that many aglycones obtained by acid hydrolysis of asterosaponins are artifacts whose side-chain structural features have been altered during hydrolysis. Thus, it is possible that most or all of compounds, **4-12** arise by dehydration of a C-20 hydroxyl aglycone precursor and that **1** and **2** result from β-hydroxyketone cleavage of a 3β,6α,20-trihydroxy-5α-cholesta-9(11)-en-23-one aglycone moiety. This conclusion has been confirmed by the isolation and characterization of several pure saponins with these features, which will be discussed in a forthcoming publication.



5 $R_1 = CH_2COCH_2CH(CH_3)_2$, $R_2 = CH_3$

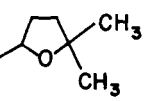
6 $R_1 = CH_2COCHCH_3CH(CH_3)_2$, $R_2 = CH_3$

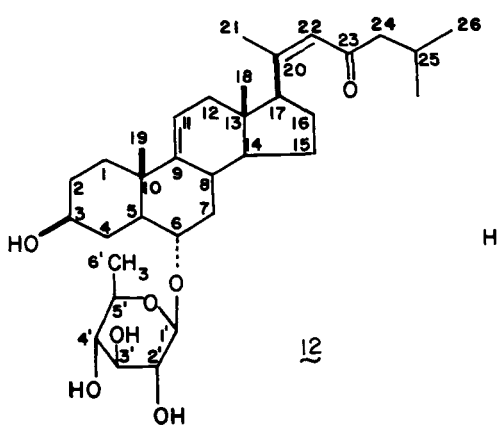
7 $R_1 = CH_3$, $R_2 = CH_2COCH_2CH(CH_3)_2$

8 $R_1 = CH_3$, $R_2 = CH_2CH_2CH{=}C(CH_3)_2$

9 $R_1 = CH_2CH_2CH{=}C(CH_3)_2$, $R_2 = CH_3$

10 $R_1 = CH_3$, $R_2 = CH_2CH_2C(CH_3){=}C(CH_3)_2$

11 $R_1 = CH_3$, $R_2 =$ 



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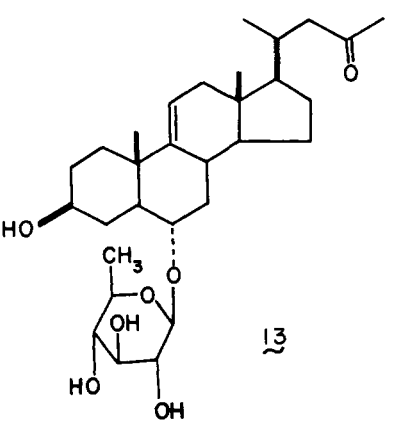

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EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Ir spectra were recorded on a Perkin-Elmer 727B infrared spectrometer with polystyrene calibration at 1601 cm^{-1}; uv spectra were recorded using a Beckman

model 25 spectrophotometer. The 360 MHz ^{1}H-nmr spectra were recorded on a Nicolet NIC-360 spectrometer at Toronto Biomedical NMR Centre, and 400 MHz ^{1}H-nmr spectra were recorded on Brüker WH-400 facility at Laboratoire Regional de RMN à Haut Champ, Département de Chimie, Université de Montréal. The 60 MHz ^{1}H-nmr spectra were recorded on a Varian T-60 spectrometer. Tetramethylsilane was used as an internal standard for all ^{1}H-nmr spectra. Low resolution mass spectra were recorded on an Hitachi Perkin-Elmer RMU-6D spectrometer and high resolution mass spectra were recorded on an AE-I-MS-50 spectrometer at the Mass Spectrometry Lab, University of Alberta. A Waters Associates (Melford, MA, USA) hplc instrument was used, with Model 6000A pump, U6K Universal injector, model 440 absorbance detector, and μ-Porasil (10 microns), 3.9 mm (id)×30 cm column.

ISOLATION OF SAPONINS FROM *ASTERIAS VULGARIS*.—Frozen animals (30 kg) were defrosted in methanol-water (3:1) and chopped into small pieces. The solvent was decanted after 24 h, and fresh solvent (methanol-water, 1:1) was added. This procedure was repeated three times, and the total aqueous methanol extract was concentrated *in vacuo* (<40°) to remove all methanol. The aqueous extract thus obtained was passed through an XAD-2 amberlite resin column, and the saponins were isolated by the method of Gilgan. The crude brown saponin mixture (4.8 g) thus obtained was dissolved in the minimum quantity of water, precipitated with acetone, filtered, and dried completely. The resulting light brown solid was stirred with anhydrous benzene for 6 h at room temperature and filtered, yielding 4.61 g of saponin.

HYDROLYSIS OF SAPONINS.—Saponin mixture (3.0 g) was dissolved in HCl (2 N, 250 ml), and chloroform (250 ml) was added. The reaction mixture was refluxed for 10 h at 90°, and chloroform was replaced three times by the fresh aliquots of chloroform (250 ml). The total chloroform extract was evaporated to dryness yielding a mixture of aglycones (0.91 g).

ACETYLATION OF AGLYCONE MIXTURE.—The aglycone mixture (0.91 g) was dissolved in pyridine (10 ml) and acetic anhydride (10 ml) was added. The reaction mixture was warmed for 0.25 h at 50° and then left overnight at 36°. After evaporating the mixture *in vacuo*, crushed ice was added and a brown solid separated out, which was extracted into chloroform. The chloroform layer was dried over anhydrous $CaCl_2$ and evaporated, affording a mixture of aglycone acetates (0.95 g).

SEPARATION OF AGLYCONE ACETATES.—Initial separation was achieved by the column chromatography on a 6′×24 cm column packed with silica gel G in hexane. Initial elution was carried out with 5% ethyl acetate in hexane. Polarity of the solvent was increased slowly up to 50% within 72 h.

Fractions were further purified by ptlc and finally by hplc using μ-Porasil (10 μ), 3.9 mm (id)×30 cm column with uv detector under conditions shown in table 2.

TABLE 2. Hplc purification of aglycone acetates

Acetate of Compound No.	Solvent System	Flow rate (ml/min)	Elution time (min)
11	hexane-ethyl acetate (95:5)	3	26.0
8,9,10	hexane-ethyl acetate (95:5)	3	32.3
7	hexane-ethyl acetate (95:5)	3	45.4
5,6	hexane-ethyl acetate (95:5)	3	41.6
2	hexane-ethyl acetate-methanol (95:5:2.5)	1.5	16.8
1	hexane-ethyl acetate-methanol (95:5:2.5)	1.5	19.0
12	hexane-ethyl acetate-methanol (95:5:2.5)	2	14.8

DIACETATE OF **11**.—This compound was purified on pltc using 20% ethyl acetate in hexane and was further purified by hplc, affording an oil (11 mg) estimated to be >90% pure by 400 MHz ^{1}H-nmr ($CDCl_3$) δ_{TMS} 0.76 (s, 3H, H18), 1.02 (s, 3H, H19), 1.20-1.30 (bs, 6H, H26, H27), 1.56 (s, 3H, H21), 2.02-2.03 (2s, 6h, $2CH_3COO$), 4.05 (t, 1H, J=6 Hz, H22), 4.65 (m, 1H, H3), 4.84 (m, 1H, H6), 5.35 (bs, 1H, H11); ir ($CHCl_3$) ν max 1735 (acetate); ms *m/e*: 498 (7, M^+), 438 (3, M-60), 378 (10, M-2x60), 369 (35), 311 (100), 251 (31), 211 (8), 85 (10), 57 (11).

TETRAACETATE OF 3β-HYDROXY-5α-CHOLESTA-9(11),20(22)-DIEN-23-ONE-6α-YL-β-D-6′-DEOXY-GLUCOSIDE (**12**).—From the silica gel column chromatogram of the acetylated aglycone mixture, a prod-

uct (1.6 mg) was eluted with hexane-ethyl acetate-methanol (10:10:1) and was further purified by ptlc on silica gel using hexane-ethyl acetate-methanol (65:35:5). Final purification by hplc gave the tetraacetate of **12** (0.9 mg): ir ($CHCl_3$) ν max 1730 (ketone), 1755 (acetates); ms *m/e*: 671 (0.4, M-57), 439 (2, M-289), 379 (11, 439-60), 378 (8), 311 (12), 273 (45), 251 (16), 213 (18), 171 (19), 153 (38), 111 (34), 85 (59), 83 (51), 57 (100); 360 MHz ^{1}H-nmr ($CDCl_3$) δ_{TMS}: 0.52 (s, 3H, H18), 0.93 (d, 6H, J=7 Hz, H26, H27), 0.97 (s, 3H, H19), 1.24 (d, 3H, J=6 Hz, H6′), 1.99-2.04 (3s, 12H, 4CH_3COO), 2.14 (s, 3H, H21), 3.45 (m, 1H, H6), 3.56 (m, 1H, H5′), 4.63 (m, 1H, H3), 4.82 (t, 1H, J=9.5 Hz, H2′), 4.94 (t, 1H, J=8.5 Hz, H4′), 5.14 (t, 1H, J=9.5 Hz, H3′), 5.34 (bm, 1H, H11), 6.06 (s, 1H, H22).

TETRAACETATE OF 3β-HYDROXY-5α-CHOL-9(11)-EN-6-YL-β-D-6′-DEOXYGLUCOSE (**13**).—After eluting tetraacetate deoxyglucoside (**12**), another compound (1.2 mg) was recovered from the silica gel column and was further purified by ptlc as before and gave the tetraacetate of **13** (1.0 mg): ir ($CHCl_3$) ν max 1710, 1735 cm^{-1}; ms *m/e* 397 (12, M-289), 337 (9, M-289-60), 273 (81, $C_{12}H_{17}O_7$), 213 (26, 273-60), 171 (27), 153 (39, 213-60), 111 (47); 360 MHz ^{1}H-nmr ($CDCl_3$) δ_{TMS}: 0.54 (s, 3H, H18), 0.93 (d, 3H, J=6 Hz, H21), 0.98 (s, 3H, H19), 1.24 (d, 3H, J=6 Hz, H6′), 2.01-2.05 (4s, 12H, 4×CH_3COO), 2.15 (s, 3H, H24), 3.45 (m, 1H, H6), 3.56 (m, 1H, H5′), 4.52 (d, 1H, J=8 Hz, H1′), 4.63 (m, 1H, H3), 4.82 (t, 1H, J=9.5 Hz, H2′), 4.94 (t, 1H, J=8.5 Hz, H4′), 5.14 (t, 1H, J=9.5 Hz, H3′), 5.34 (bm, 1H, H11).

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