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ASTEROSAPONIN P_1 FROM THE STARFISH PATIRIA PECTINIFERA

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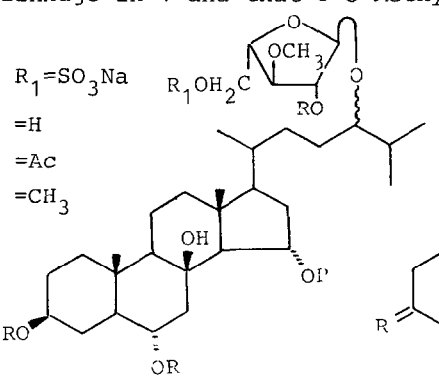
Abstract.-A novel steroidal glycoside has been isolated from the starfish Patiria pectinifera and its structure was determined as 5′-O- sulfate 24-(α-3-O-methyl-L-arabinofuranosyl)-3β,6α, 8β, 15α, 24ξ- pentaoxy-5α- cholestane.

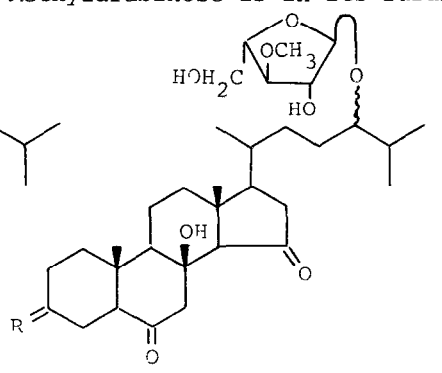
For the long time only two asterosaponin types have been known: either the glycosides with 3β-sulfoxy-6α-hydroxycholest-9(11)-ene aglycone and oligosaccharide part attached at C-6[1] or the glycosides with 3β,6β-dihydroxycholest-7-ene aglycone and the carbohydrate chain cyclized between C-3 and C-6 of the aglycone[2]. We now report the occurence in the Pacific starfish Patiria pectinifera of a steroidal glycoside, asterosaponin P_1, which represents as well as recently described nodososide[3] new type of asterosaponins.

Asterosaponin P_1 (1), $C_{33}H_{57}O_{12}SNa$, mp 191-192°C,$[\alpha]_D$ + 3.0° (C=0.6, MeOH), was obtained in 0,4% yield from the ethanol extract of the starfish pyloric caeca by the column chromatography on Polychrome-1 (USSR), silica gel, florisil and Sephadex LH-20. On acid hydrolysis it liberated a single monosaccharide identified as 3-o-methyl-L-arabinose. In fact, demethylation of the monosaccharide with BF_3 in CH_2Cl_2 gave L-arabinose (glc,pc, $[\alpha]_D$). Mass spectrum of the aldononitrile peracetate obtained from acid hydrolisis product of 1 showed the peaks at m/z 214, 189, 142 and 129, which are characteristic of corresponding 3-O-methylpentoses derivatives[4]

Solvolysis of 1($C_4H_8O_2$-C_5H_5N,90°) produced desulfated derivative (2), mp 213-214°C, $[\alpha]_D$ ± 0°(C=1.4, MeOH) Some structural pecularities of the carbohydrate moiety was determined by comparing ^{13}C NMR spectra of the compounds 1 and 2 (table I). Signals at 109 7, 81 1, 88.8, 84.0 and 63 2 ppm for C-1′-C-5′ in the spectrum of 2 were in close agreement with those of the α-methyl-3-O-methylarabinofuranoside spectrum[5] This allowed us to establish α-configuration for the glycoside linkage in 1 and that 3-O-methylarabinose is in its furanose form.

1 R=H R_1=SO_3Na
2. R=R_1 =H
3. R=R_1 =Ac
4 R=R_1 =CH_3





5.R=O
6.R=OH / H

Table I. ^{13}C NMR (py-d_5) shifts[a] of 1-6 in δ (TMS = 0)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1[b]	39.1	32.3	71.2	32.9	53.4	66.5	50.4	75.4	56.5	37.1	19.0	42.0	44.6	66.5
2	39.2	32.0	71.3	33.0	53.7	66.5	50.8	75.5	56.8	37 2	19.2	42.2	44.8	66.9
3[c]	38.0[e]	26.7	73.2	27.9[g]	49.5	70.0	45.7	74.9	55.6[f]	37.0	18.3	41.2	44.0	62.3
4[d]	38.8	27.9	80.3[e]	28.5	51.3	73.5	45.5	75.1	56.5	37.4	19.1	42.0	44.4	64.5
5	39.4	37.4	207.8[e]	37.4	57.3[f]	210.1[e]	53.3	77.5	55.6[f]	42.3	19.0	41.1	43.9	69.0
6	38.7	30.9	70.2	31.4	57.5[e]	209.4	53.7	77.5	56.3[e]	42.3	19.1	41.2	43.9	69.3

	15	16	17	18	19	20	21	22	23	24	25	26	27
1[b]	69.1	41.3	55.3	15.4	14.2	35.3	18.6	31.8	28.5	81.3	31.3	18.1	17.9
2	69.0	41.6	55.2	15.5	14.4	35.3	18.8	32.0	28.3	83.2	30.9	18.1	18.1
3[c]	72.1	37.8[e]	55.0[f]	14.8	13.5	35.0	18.3	31.4	27.6[g]	83.4	30.2	17.9[h]	17.8[h]
4[d]	78.8	36.4	55.4	15.6	14.2	35.5	18.8	32.2	28.5	81.2[ef]	30.8	18.3[g]	18.0[g]
5	212.7	42.3	52.4	14.9	13.4	35.3	19.0	31.8	27.9	82.4	30.5	18.3[g]	17.9[g]
6	212.9	42.3	52.6	14.9	14.4	35.3	19.1	31.9	27.9	82.4	30.6	18.3[f]	17.9[f]

^{13}C NMR shifts of sugar carbons

	1′	2′	3′	4′	5′	OMe
1[b]	109.4	80.8	89.0	83.6	68.4	57.6
2	109.7	81.1	88.8	84.0	63.2	57.6
3[c]	106.1	81.5	86.3	80.2	64.0	58.2
4[d]	106.2	91.3	86.8	83.4[f]	73.5	

[a] ^{13}C signals of C-3, C-4, C-6, C-7, C-14, C-15, C-18, C-19, C-21, C-24, C-26, C-27 and C-1′-C-5′ for 2 and C-1′-C-5′ for 3 were assigned using ^{1}H single-frequency off-resonance decoupling. [b] Measured at 333°K. [C] Measured in $CDCl_3$. The resonances of the acethoxy-carbon atoms are δ 20.8(x2), 21.1, 21.2, 21.4. [d] The resonances of the methoxy-carbon atoms are δ 56.1(x3), 57.5, 57.8, 59.7.
[efgh] Assignments may be reversed.

The downfield position for C-5′ signal in the spectrum of 1 (68.4 ppm), ca. 5 ppm shifted relative to 2, indicated the location of the sulfate group at C-5 in the monosaccharide.

A set of artefact sapogenols produced by acid hydrolysis of 1 prevented establishing of the genuine aglycone structure. Its structure was determined using high-temperature catalytic reduction[6], others chemical transformations and analysing ^{1}H and ^{13}C NMR spectra of 1 and its derivatives. It was concluded that aglycone has a cholestane skeleton after identification of 5α-cholestane(glc,glc-ms) as a product of the glycoside treatment over $Pd/CaCO_3$ catalyst at 330°C in hydrogen atmosphere

A comparison of ^{1}H and ^{13}C NMR spectra for 1,2, pentaacetate (3), hexa-O-met-

Table II. 250 MHz ^{1}H NMR (py-d_5) data of 2,3,5 and 6 in δ (TMS=0)

	2	3[a]	5	6		2	3
H-3	4.01m	4.67m		3.90m	H-1′	5.54d	5.02s
H-6	4.37td	4.95m			H-2′	4.76dd	5.04d
H-15	4.80td	5.00m			H-3′	4.24dd	3.68dd
H-24	3.57m	3.31m	3.60m	3.60m	H-4′	4.71ddd	4.20m
H-7a	2.20dd	1.43dd	2.88d	2.77d	H-5′	4.29Add	4.20m[b]
H-7e	3.41dd	1.95dd	4.04d	4.02d	H-5′′	4.19Bdd	4.30m[b]
H-14	1.72d	1.56d	2.28s	2.27s	OMe	3.52s	3.40s
CH_3-18	1.301s	0.975s	1.270s	1.260s	OAc		1.965s
CH_3-19	1.436s	1.073s	1.385s	1.314s			2.015s
CH_3-21	1.030d	0.900d	1.050d	1.060d			2.035s
CH_3-26	0.920d	0.870d	0.920d	0.930d			2.095s
CH_3-27	0.920d	0.880d	0.920d	0.930d			2.100s

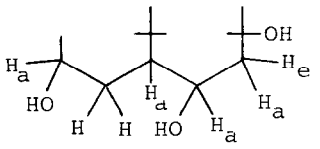
[a]Measured in $CDCl_3$. [b]Assignments may be reversed.

Table III. Chemical shifts (ppm) and the coupling constants (Hz) for some protons of 2

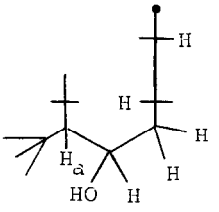
		protons	J	protons	J	protons	J
H-4a	1.86td	3,4a	10.6	6,7a	10.7	16,16′	13.5
H-4e	3.13dm	3,4e	4.6	6,7e	3.8	16,17	8.6
H-5a	1.55td	4a,4e	12.5	7a,7e	13.7	16,17	9.3
H-16	2.24Addd	4a,5	12.6	14,15	9.8	24,25	4.8
H-16′	2.14Bdt	4e,5	2.7	15,16	13.6	26,25	6.6
H-25	1.90m	5,6	10.5	15,16′	9.3		
H-17	1.57m						

hylderivative(4)showed the presence three secondary and one tertiary free hydroxyl groups in the algycone moiety and also one secondary hydroxyl group connected with the monosaccharide residue (table I,II). A comparison of ^{1}H NMR spectra for 2, triketone(5) (oxidation 0f 2 with CrO_3/C_5H_5N) and diketone(6) (oxidation of 2 with CrO_3/CH_3COOH) and assignment of monosaccharide signals(table II) allowed us to find a single-proton multiplet at 3.57 ppm,associated with hydroxymethine group(24-C<u>H</u>-OH) which is the site of glycosidation. The proton difference spin decoupling experiments displayed a single-proton multiplet H-25(1,90 ppm),producing a doublet at irradiation of protons CH_3-26 and CH_3-27 and a septet at irradiation of H-24.

Difference spin decoupling and double resonance techniques established details of two fragments(A and B)in the structure 2. Corresponding chemical shifts and coupling constantsare presented in the tables II and III.



A



B

From these data we determined 3ß, 6α, 8ß and 15α-tetrahydroxy oxidation pattern in 1.

The proposed structure of 1 was confirmed by the following additional data.

1. Signals of CH_3-19 and CH_3-18 protons were downfield shifted in ^1H NMR spectra of 3[7], while C-6, C-11, C-15 in the ^{13}C NMR spectrum of 2 were upfield shifted relative to the spectra of 3ß, 6α-dioxy and 15α-oxycholestanes[8], thus locating the tertiary hydroxyl function at C-8.

2. We have analysed acceptable conformations of the ring D with the substituent at C-15 and have calculated vicinal coupling constants for them using data[9] to find corresponding dihedral angles. A close agreement with the experimentally obtained constants was found for $^{13}T_{14}$ and ^{13}V conformations with 15α-OH Our coupling constants corresponded well to recently obtained[10] corrected for electronegativity of the substituent at C-15

3. 15α - and 6α- configurations for hydroxyl groups were confirmed also by difference NOE experiments with 2. At irradiation of CH_3-18 signal (1,301 ppm) and CH_3-19 signal (1,436 ppm) we revealed only downfield signals for 15-C<u>H</u>-OH and 6-C<u>H</u>-OH, respectively.

On the basis of the above accumulated evidence, the total structure of the asterosaponin P_1 was elucidated as 1.

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