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STRUCTURE OF GLYCOSIDE B_2, A STEROIDAL SAPONIN IN THE OVARY OF THE STARFISH, ASTERIAS AMURENSIS

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Summary The starfish *Asterias amurensis* ovary contains a saponin, 20ξ-hydroxyl-6α-O-{β-D-quinovopyranosyl(1→2)-β-D-galactopyranosyl(1→4)-[β-D-quinovopyranosyl(1→2)]-β-D-xylopyranosyl-(1→3)-β-D-quinovopyranosyl}-3β-sulfo-oxy-5α-cholest-9(11)-en-23-one.

Steroidal glycoside sulfates called asterosaponins in the ovary of the starfish *Asterias amurensis* inhibit the production of 1-methyladenine in the follicle cell and this suppression is overcome by the action of a peptide hormone released from the nervous tissue.[1] 1-Methyladenine acts directly on the oocyte to induce meiotic maturational divisions. The saponins are closely related glycosides and their structures have not yet been fully elucidated. We have purified one such glycoside designated glycoside B_2 from the ovary containing immature oocytes and here we describe experimental results leading to the determination of its structure.

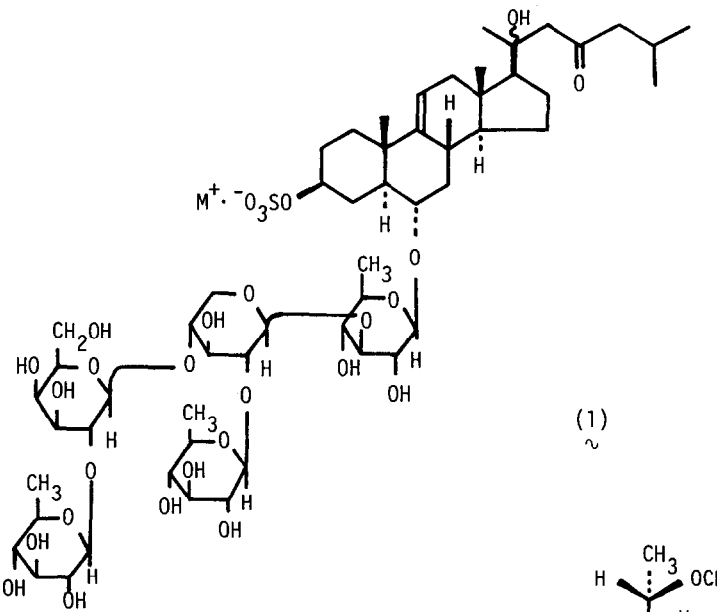
Ovaries were removed from *Asterias amurensis* at the height of the breeding season, washed thoroughly with sea water and freeze-dried. They were extracted with $CHCl_3$-MeOH (2:1, v/v) and then with $CHCl_3$-MeOH-H_2O (20:40:3, v/v). The extracts were combined and partitioned between $CHCl_3$-MeOH-H_2O (10:5:3, v/v). The upper phase was separated and condensed *in vacuo*. The residual aqueous solution was acidified to pH 4.0 with dilute HCl and extracted with *n*-butanol. The *n*-butanol layer was separated, added with water and condensed *in vacuo* to afford an aqueous solution, which was dialyzed against water. The non-dialyzable fraction was applied to a DEAE-Sephadex (acetate form) column. The column was washed with $CHCl_3$-MeOH-H_2O (30:60:8, v/v) and glycoside sulfates were eluted by $CHCl_3$-MeOH-0.8M CH_3COONa (30:60:8, v/v). They were applied to a silica gel column which was developed with $CHCl_3$-MeOH-H_2O (10:5:1, v/v). Fractions which released galactose on acid hydrolysis were collected and designated glycoside B_2 (1). Although free from fucose-containing asterosaponins A and B, this glycoside fraction was always accompanied by another component, glycoside B_1, which was devoid of galactose residue in the molecule. All attempts to remove the component were unsuccessful. Therefore, the

fraction was desulfated and treated with alkali according to the procedure described by Kitagawa *et al.*[2] for an *Acanthaster planci* saponin: the glycoside fraction was heated in dioxane-pyridine (1:4, v/v) under reflux for 4 hr, followed by $NaOCH_3$-CH_3OH treatment for 4 hr. Silica gel column chromatography separated clearly alkali-transformed desulfated glycoside B_2 {2, $C_{50}H_{80}O_{24}$, mp 275-276.5°C, $[\alpha]_D^{20}$ +28.5° ($CHCl_3$-MeOH, 1:1, v/v), CD ($CHCl_3$-MeOH, 1:1, v/v): $[\Theta]_{288}$ +6,400 (pos. max.)} from alkali-stable desulfated glycoside B_1. On acid hydrolysis, 2 afforded 5α-pregn-9(11)-en-3β,6α-diol-20-one (3),[3] D-quinovose (6-deoxy-D-glucose), D-xylose and D-galactose in a 1:3:1:1 ratio. Methylation of 2 with CH_3I-NaH-DMSO[4] gave a trideca-O-methyl derivative (4), whose IR spectrum (film) showed the absence of hydroxyl groups in the molecule. The PMR specrum ($CDCl_3$) of 4 exhibited five anomeric protons at δ 4.25 (1H, d, J = 8Hz), 4.34 (1H, d, J = 8Hz), 4.58 (2H, d, J = 7.5Hz) and 4.77 (1H, d, J = 6Hz). Therefore, all anomeric configurations in 4 are β ($^4C_1$ conformation). Formolysis of 4, followed by hydrolysis, $NaBH_4$ reduction and acetylation with Ac_2O-pyridine[5] afforded 2,3,4-tri-O-methyl-1,5-di-O-acetyl-quinovitol, 2,4-di-O-methyl-1,3,5-tri-O-acetyl-quinovitol, 3,4,6-tri-O-methyl-1,2,5-tri-O-acetyl-galactitol and 3-O-methyl-1,2,4,5-tetra-O-acetyl-xylitol in the ratio of 2:1:1:1. The identification and quantification of these alditol acetates were carried out by GLC (Silicone OV-1, 150°C) and GC-MS. On partial acid hydrolysis, 2 gave a triglycoside (5) and a monoglycoside (6). Methylation of 5 with CH_3I-NaH-DMSO gave an octa-O-methyl derivative (7). Alditol acetates obtained from 7 were 2,3,4-tri-O-methyl-1,5-di-O-acetyl-quinovitol, 2,4-di-O-methyl-1,3,5-tri-O-acetyl-quinovitol and 3,4-di-O-methyl-1,2,5-tri-O-acetyl-xylitol in a 1:1:1 ratio. On acid hydrolysis, 6 afforded 3 and quinovose. MS of 6 tetraacetate showed M^+ at m/e 646. The PMR spectrum ($CDCl_3$) of 6 tetraacetate showed the presence of a proton at a methine group forming an O-glycoside linkage at δ 3.51 (1H, m). Therefore, the sugar moiety of 2 is β-D-quinovopyranosyl-(1→2)-β-D-galactopyranosyl(1→4)-[β-D-quinovopyranosyl(1→2)]-β-D-xylopyranosyl(1→3)-β-D-quinovopyranosyl.

Reduction of 2 with $NaBH_4$ gave an epimeric mixture of dihydro derivatives whose IR spectrum (Nujol) showed no carbonyl band and CD spectrum (CH_3OH) showed no maxima in the range of 240-350 nm. Methylation of the mixture with CH_3I-NaH-DMSO followed by methanolysis and oxidation with pyridinium chlorochromate[6] gave a ketone (8), $C_{23}H_{36}O_3$ (M^+ 360.2670, calcd. 360.2662), CD (CH_3OH): $[\Theta]_{295}$ -2,280 (neg. max.). The PMR spectrum ($CDCl_3$) of 8 showed that signals due to 20- and 13-methyl groups were at δ 1.09 and 0.66, respectively, suggesting that the configuration at C-20 is β.[7] The signal due to the 11-proton was at δ 5.54, showing that an oxo-

group is at C-6.[3] The structure of 8 was thus determined to be 3β,20β-dimethoxy-5α-pregn-9(11)-en-6-one. It follows therefore that 6α-hydroxyl group of the aglycone 3 forms an O-acetal linkage with the sugar moiety in the structure of 2.

The compound 1 was treated with a glycosidase mixture of the mollusc *Charonia lampas* to afford thornasterol A sulfate {9 as triethylammonium salt; $C_{33}H_{58}NO_7S$, SO_4^{2-}, obsd. 16.0%, calcd. 15.7%; CD (MeOH): $[\Theta]_{293}$ -1,360 (neg. max.)}. The PMR spectrum ($CDCl_3$) of 9 (triethylammonium salt) showed a signal due to one proton, C<u>H</u>-OSO_3^-, at δ 4.30. Solvolysis of 9 followed by acetylation gave thornasterol A diacetate, whose PMR and MS spectra were in good agreement with those reported by Kitagawa *et al.*[2] Furthermore, 9 was converted to the known sulfate, 5α-pregn-9(11)-en-3β,6α-diol-20-one-3-sulfate (10)[2,3] on $NaOCH_3$ treatment, suggesting that conversion of 1 to 2 is due to the cleavage of C(20)-C(22) bond by retro aldol



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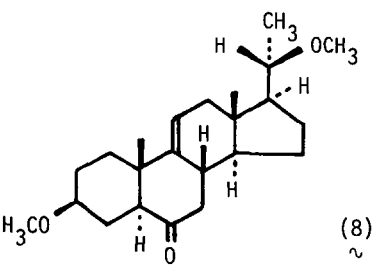

(8)

reaction. Based on these findings, the structure of glycoside B_2 (1) is formulated as 20ξ-hydroxyl-6α-O-{β-D-quinovopyranosyl(1→2)-β-D-galactopyranosyl(1→4)-[β-D-quinovopyranosyl(1→2)]-β-D-xylopyranosyl(1→3)-β-D-quinovopyranosyl}-3β-sulfo-oxy-5α-cholest-9(11)-en-23-one. The action of 1 on the follicle cell to suppress the production of 1-methyladenine needs to be examined.

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(7) The chemical shifts of 20- and 13-methyl groups of 3β,6α,20α-tri-O-methyl-5α-pregn-9(11)-ene were depicted at δ 1.15 and 0.58, respectively. On the other hand, chemical shifts of 20- and 13-methyl groups of 3β,6α,20β-tri-O-methyl-5α-pregn-9(11)-ene were found at δ 1.09 and 0.63, respectively. Therefore, the configuration at C-20 of 8 was assigned to be β.

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