Short Communication

Separation of Ovarian Asterosaponins in the Starfish *Asterias amurensis*

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In 1965~1967, Yasumoto and Hashimoto reported that the starfish *Asterias amurensis* contained asterosaponin A, mp 185~190°C, $[\alpha]_D +0.03^\circ$, which consists of one steroidal aglycone, one sulfuric acid, 2 mol each of D-quinovose and D-fucose,¹⁾ and asterosaponin B, mp 189~191°C, $[\alpha]_D -2.66^\circ$, which consists of one steroidal aglycone, one sulfuric acid, 2 mol of D-quinovose and one mol each of D-fucose, D-galactose and D-xylose.²⁾ In 1972, Ikegami *et al.*³⁾ obtained asterosaponin A as a spawning inhibitor and reported the structure as 6 α -O-{6-deoxy- α -D-galactopyranosyl(1 $\rightarrow$ 4)-6-deoxy- α -D-galactopyranosyl(1 $\rightarrow$ 4)-6-deoxy- α -D-glucopyranosyl(1 $\rightarrow$ 4)-6-deoxy-D-glucopyranosyl}-3 β -sulfo-oxy-5 α -pregn-9(11)-en-20-one.⁴⁾ In 1978, however, Kitagawa *et al.*⁵⁾ suggested that 6 α -hydroxy-3 β -sulfo-oxy-5 α -pregn-9(11)-en-20-one (**I**) is an artifact and the genuine one is 6 α ,20 ξ -di-hydroxy-3 β -sulfo-oxy-5 α -cholest-9(11)-en-23-one (**II**) in the studies of the saponin, thornasteroside A, in the starfish *Acanthaster planci*. We had some doubt if the structure of asterosaponin A were incorrect and examined ¹³C-NMR of asterosaponin A that had been obtained in 1972. The spectrum (C₅D₅N-H₂O, 5:1, v/v) depicted the presence of C-23 ketone of **II** rather than C-20 ketone of **I** at 212.7 ppm, five anomeric carbons at 106.2, 105.5, 104.4, 103.5 and 102.2 ppm, and a hydrated ketone at 92.8 ppm with signals due to some impurities. This result shows that the proposed structure of asterosaponin A is incorrect. Therefore, we re-

examined purification and structures of saponins obtained from ovaries of *Asterias amurensis*. As a result, we isolated five saponins, the physico-chemical properties being different from those of asterosaponin A and B, and designated ovarian asterosaponins (OA)-1, 2, 3, 4 and 5.

The isolation procedure for ovarian asterosaponins is as follows. Ovaries were removed from *Asterias amurensis* at the height of its breeding season and freeze-dried. The dry powder (100 g) was extracted with 1 liter of CHCl₃-MeOH (2:1, v/v) and then 1 liter of CHCl₃-MeOH-H₂O (20:40:3, v/v). The extracts were combined and partitioned with CHCl₃-MeOH-H₂O (10:5:3, v/v). The upper phase was separated and concentrated *in vacuo*. The residual aqueous solution was partitioned between *n*-BuOH and H₂O (pH 4.0). The upper phase was separated, neutralized and condensed. The residue was dissolved in a small amount of H₂O and dialyzed twice against 5 liters of H₂O. The non-dialyzable fraction was dissolved in 60 ml of CHCl₃-MeOH-H₂O (30:60:8, v/v) and applied to a DEAE-Sephadex A-25 (acetate form) column (2.2 $\times$ 12.7 cm). The column was washed with 300 ml of CHCl₃-MeOH-H₂O (30:60:8, v/v) and eluted with 750 ml of CHCl₃-MeOH-0.8 M NaOAc (30:60:8, v/v). The eluate was concentrated and dialyzed against H₂O to afford crude saponins. Then, they were applied to partition column chromatography on silanized silica gel (4.2 $\times$ 37 cm, 35% aqueous

FIG. 1. The Isolation Procedure for Ovarian Asterosaponins.

TABLE I. PHYSICAL AND CHEMICAL PROPERTIES OF OVARIAN ASTEROSAPONINS

	Saponins				
	OA-1	OA-2	OA-3	OA-4	OA-5
<i>R_f</i> values in TLC (1)*	0.20 (0.24**)	0.16	0.21 (0.25**)	0.16	0.16
<i>R_f</i> values in TLC (2)*	0.63	0.64	0.45	0.47	0.40
mp (°C)	203~204	204~205.5	205~208	207~209	216~218
$[\alpha]_D^{22}$ (H ₂ O)	-2°	-11.8°	+11.7°	-1.5°	-5°
CD (H ₂ O)	$[\theta]_{285}$ -4100	$[\theta]_{287}$ -4200	—	—	—
UV $\lambda_{\max}^{H_2O}$ (ε)	—	—	243 (6900)	—	—
Sugars***	Qui 2 Fuc 2 (DXHU 1)	Qui 3 Xyl 1 Gal 1	Qui 2 Fuc 2 (DXHU 1)	Qui 3 Xyl 1 Glc 1	Qui 3 Xyl 1 Glc 1

* TLC (1) was carried out on silica gel 60 plates with CHCl₃-MeOH-H₂O (10:5:1, v/v), following by spraying with 20% phosphotungstic acid in EtOH; for TLC (2), silanized silica gel 60 plates were used and they were developed with 50% aqueous MeOH.

** In two dimensional TLC (1), the saponin shows 4 spots, indicating that these spots are convertible with each other. Number in parenthesis indicates *R_f* value of the minor spot.

*** The sugar compositions were analyzed by GLC (2% Silicone OV-1, 175°C) as TMS ethers of methanolizates and hydrolyzates. But, DXHU was undetectable by the above analysis and its presence was shown by the procedures as described in the following paper.⁷⁾ Abbreviations: Qui, quinovose (6-deoxy glucose); Fuc, fucose (6-deoxy galactose); DXHU, 6-deoxy-xylo-hexos-4-ulose; Xyl, xylose; Gal, galactose; Glc, glucose.

MeOH) and three fractions-a, -b and -c were eluted out in this order. Fraction-a was further separated by medium pressure column chromatography on Kiesel gel P [1.4×50 cm, CHCl₃-MeOH-H₂O (10:5:1, v/v)] to afford OA-1 and OA-2 fractions. Each of them was further purified by chromatography on a Lobar column RP-8 (Size B, 50% aqueous MeOH) to afford pure OA-1 and OA-2. Fractions-b and -c were purified by use of the same column as fraction-a in the order as shown in Fig. 1.

Physical and chemical properties of the five saponins are shown in Table I. We formerly obtained glucose- and galactose-containing fractions from the starfish ovary and designated tentatively glycoside B₁ and B₂, respectively, although they were not separated with each other in the preceding study.⁶⁾ The

structure of OA-2 is the same as that proposed for glycoside B₂ and OA-4 corresponds to glycoside B₁. ¹³C-NMR spectrum and other data obtained in earlier studies of asterosaponin A showed that asterosaponin A is mainly composed of OA-1 with contaminating OA-2 and OA-3. Since acid hydrolysis of OA-1 resulted in severe degradation of both aglycone and sugar moieties, structural elucidation derived solely from the analyses of acid hydrolyzates led to be an error. It is necessary for structural elucidation of so complicated molecules as saponins to examine hydrolyzates obtained with glycosidases and to analyze spectrometrically intact molecules with ¹³C-NMR or other instruments.

The structure of OA-1 will be reported in the following paper.⁷⁾

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