Short Paper

Separation of Saturated Glyceryl Ethers from Lipid Unsaponifiable Matter by a Combination of Hydrogenation and Recrystallization

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The pyloric cecal and/or ovarian lipids of starfish *Asterias rubens*,¹⁾ *A. lincki*,²⁾ *Ctenodiscus crispatus*,²⁾ *Pteraster militaris*,²⁾ *Solaster paxillatus*,³⁾ and *A. amurensis*³⁾ contain significant amounts of glyceryl ethers present in the form of diacyl glyceryl ethers. These starfish species are a good source of glyceryl ethers. Glyceryl ethers of marine origin have proven useful as natural surface-active or softening agents in cosmetics and ointments. In the previous report, we demonstrated the separation of highly-purified glyceryl ethers rich in saturates from unsaponifiable matter of the gonatid squid liver lipids by the use of a simple and low-cost recrystallization method.⁴⁾ Also, it was found that glyceryl ethers from the tissue lipids of *S. paxillatus* and *A. amurensis* had saturates with 18:0 and 16:0 alcohols and monoenes with 20:1 and 18:1 ones.³⁾ The present investigation was undertaken to find a convenient method for separation of saturated glyceryl ethers in a reasonable yield from unsaponifiable matter of the starfish tissue lipids.

An aliquot of each of the pyloric cecal and ovarian lipids obtained from *S. paxillatus* and *A. amurensis*,³⁾ was subjected to hydrolysis in alcoholic KOH by boiling under reflux for 1 h. The unsaponifiable matter was extracted from the saponified mixture with diethyl ether. The unsaponifiable matter was then hydrogenated in a methanol solution with 5% palladium catalyst (Nakarai Chemicals), and hydrogen was bubbled at atmospheric pressure and at room temperature for 1 h. Upon recrystallization at 2°C from a *n*-hexane solution of the hydrogenated unsaponifiable matter, the solid fraction was separated as white crystal grains. The methods used for qualitative and quantitative determination of constituent lipids by thin-layer chromatography and an Iatroscan TH-10 flame ionization detector (Iatron Lab. Inc.), preparation of isopropylidene derivatives of glyceryl ethers, and determination of the above compounds by gas-liquid chromatography were performed as described in a previous report.⁵⁾

The pyloric cecal and ovarian lipids of the starfish studied contained 19.5% and 83.1% of diacyl glyceryl ethers for *S. paxillatus* and 19.5% and 13.6% for *A. amurensis*, respectively. The level and class composition of unsaponifiable matter in the tissue lipids from these starfish species are listed in Table 1. Also, Table 1 shows the yield and composition of the solid fraction separated by the first recrystallization from the hydrogenated unsaponifiable matter.

The pyloric cecal and ovarian lipids of the examined starfish contained considerable amounts of unsaponifiable matter, accounting for 13.4% and 32.2% for *S. paxillatus* and 11.9% and 11.7% for *A. amurensis*, respectively. On the other hand, the hydrogenated unsaponifiable matter of the above tissue lipids consisted of 61.0% and 85.7% of glyceryl ethers and 29.1% and 10.2% of sterols for *S. paxillatus* and of 74.4% and 36.6% of the former compounds and 13.5% and 27.8% of the latter ones for *A. amurensis*, respectively. Of both the starfish species, the tissue lipid un-

Table 1. Lipid component and glyceryl ether composition of hydrogenated unsaponifiable matter of starfish tissue lipids and their solid fractions separated by recrystallization

	<i>S. paxillatus</i>		<i>A. amurensis</i>	
	Pyloric cecum	Ovary	Pyloric cecum	Ovary
Unsaponifiable matter%* ¹	13.4	32.2	11.9	11.7
<i>Component %</i> * ²				
Glyceryl ethers	61.0	85.7	74.4	36.6
Sterols	29.1	10.2	13.5	27.8
Free fatty acids	4.5	2.4	4.2	3.3
Polar compounds	5.4	1.7	7.9	32.3
Solid fraction %* ³	53.0	83.5	53.3	36.0
<i>Component %</i> * ⁴				
Glyceryl ethers	96.2	98.6	95.2	90.1
Polar compounds	3.8	1.4	4.8	9.9
<i>Glyceryl ether component %</i>				
15:0* ⁵	0.6	0.4	1.1	1.1
16:0	7.4	20.5	10.2	10.4
17:0	4.1	3.8	6.0	6.0
18:0	60.5	37.8	67.9	64.8
19:0	4.5	3.1	2.8	2.9
20:0	18.4	33.0	5.3	6.8
Br16:0	0.3	tr	1.7	2.2
Br17:0	0.8	0.3	1.8	2.5
Br19:0	2.5	0.6	1.5	1.5
Others* ⁶	0.9	0.5	1.7	1.8

*¹ % to total lipids.*² % to hydrogenated unsaponifiable matter.*³ Solid fraction was separated from hydrogenated unsaponifiable matter by recrystallization.*⁴ % to solid fraction.*⁵ Indicated by chain length and double bond of alkyl moiety.*⁶ Consisted of 14:0, Br15:0, and Br18:0 alcohols.

Br: Branched, tr: Trace (less than 0.05%).

saponifiable matter before hydrogenation contained minute amounts of alkenyl glyceryl ethers (1.9–5.9%), which were converted to saturated glyceryl ethers after hydrogenation. Small amounts of free fatty acids contaminated were also detected in the unsaponifiable matter before and after hydrogenation.

As shown in Table 1, the solid fractions for the pyloric ceca and ovaries of *S. paxillatus* yielded 53.0% and 83.5% of the hydrogenated unsaponifiable matter and consisted of 96.2% and 98.6% of glyceryl ethers, respectively. Also, for the pyloric ceca and ovaries of *A. amurensis*, the solid fractions yielded 53.3% and 36.0% of the hydrogenated unsaponifiable matter and consisted of 95.2% and 90.1% of glyceryl ethers, respectively. These solid fractions of crystalline grains were free from saturated sterols and/or saturated free fatty acids. Thus, the yields of glyceryl ethers in the solid fractions to the amounts of total glyceryl ethers in the hydrogenated unsaponifiable matter were 83.6–97.4% for *S. paxillatus* and 68.2–88.6% for *A. amurensis*.

The glyceryl ethers in the separated solid fractions contained only saturates with 18:0 (batyl alcohol: 37.8–60.5% for *S. paxillatus* and 64.8–67.9% for *A. amurensis*), 20:0 (18.4–33.0%; 5.3–6.8%), and 16:0 (chimyl alcohol: 7.4–20.5%; 10.2–10.4%) alcohols as major constituents. Between the examined tissues of *A. amurensis*, the glyceryl ethers separated from the hydrogenated unsaponifiable

matter were similar in composition; the most predominant component was 18:0 alcohol followed by 16:0 alcohol. On the other hand, between the examined tissues of *S. paxillatus* the separated glyceryl ethers were somewhat different in composition. The predominant components were 18:0 and 20:0 alcohols for pyloric ceca and 18:0, 20:0, and 16:0 ones for ovaries, respectively. The percentages of 20:0 alcohol in *S. paxillatus* were higher than those in *A. amurensis*. These solid fractions also consisted of considerable amounts of odd numbered glyceryl ethers (7.3–10.0%) and branched chain compounds (1.1–7.7%). (7.3–10.0%).

Finally, 0.6–2.1 g of saturated glyceryl ethers (purity: 96–99%) were separated from 100 g of the wet tissues of *S. paxillatus* and 0.1–0.6 g of saturated ones (90–95%) were separated from those of *A. amurensis*. The combination of hydrogenation and recrystallization is applicable to large-scale separation of highly-purified saturated glyceryl ethers from marine resources.

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