was washed with $H_2O$, dried over $Na_2SO_4$, and concentrated under reduced pressure. The oily residue was treated with 10% HCl–MeOH and the resulting crystals were recrystallized from ether–EtOH to afford **5** as hydrochlorides. These results are summarized in Table I.

**General Procedure for the Preparation of α-Substituted Cyclic α-Imino Acids (6)**——A mixture of **5** (0.01 mol), 10% NaOH (10 ml) and EtOH (50 ml) was heated under reflux for 30 min with stirring, then EtOH was evaporated off *in vacuo*. The resulting residue was applied to a strong cation exchange resin (Amberlite IR 120) and eluted with 4 N $NH_4OH$. After removal of the eluent by evaporation, the residue was recrystallized from aqueous EtOH to obtain the desired cyclic α-imino acids **6**. Furthermore, treatment with 10% HCl–dioxane, followed by recrystallization from EtOH–ether gave analytically pure **6**·HCl as colorless prisms. These results are summarized in Table II.

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# Sulfonoglycolipid from the Sea Urchin *Anthocidaris crassispina* A. Agassiz

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On the basis of chemical and physicochemical evidence, a sulfonoglycolipid isolated from the shell of the sea urchin *Anthocidaris crassispina* A. Agassiz has been shown to be a 96:4 mixture of 1′-O-palmitoyl-3′-O-(6-sulfo-α-D-quinovopyranosyl)glycerol and its myristoyl counterpart.

**Keywords**——sea urchin; *Anthocidaris crassispina*; sulfonoglycolipid; $^1$H-NMR; $^{13}$C-NMR; FD-MS

Among five classes in the phylum Echinodermata, *Holothuroidea* (sea cucumber) and *Asteroidea* (starfish) are of particular interest in view of their oligoglycosidic metabolites, which exhibit various biological activities. We have already elucidated the chemical structures of some of those: *e.g.* thornasteroside A (from *Acanthaster planci* L.),[2] holotoxins A and B (from *Stichopus japonicus* Selenka),[3] and holothurins A[4] and B[5] (from *Holothuria leucospilota* Brandt). As a continuing study on the metabolites of Echinodermata, we have been examining the chemical constituents of the sea urchin *Anthocidaris crassispina* A. Agassiz (Japanese name, "murasaki-uni") and have isolated a sulfonoglycolipid (designated as Ant-1) from the shell. This paper deals with a structural study of Ant-1 (**1**) showing that it consists of 1′-O-palmitoyl-3′-O-(6-sulfo-α-D-quinovopyranosyl)glycerol and the myristoyl counterpart in a 96:4 ratio.

The aqueous methanolic extract of the shell of *A. crassispina* was subjected to solvent partition, column chromatography, and droplet counter-current chromatography (DCC) (Chart 1). A fraction containing Ant-1 thus obtained was purified by silica gel column chromatog-

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1) Location: *133-1, Yamada-kami, Suita, Osaka 565, Japan.*
2) *a*) I. Kitagawa, M. Kobayashi, and T. Sugawara, *Chem. Pharm. Bull.* (Tokyo), **26**, 1852 (1978); *b*) I. Kitagawa and M. Kobayashi, *ibid.*, **26**, 1864 (1978).
3) I. Kitagawa, H. Yamanaka, M. Kobayashi, T. Nishino, I. Yosioka, and T. Sugawara, *Chem. Pharm. Bull.* (Tokyo), **26**, 3722 (1978).
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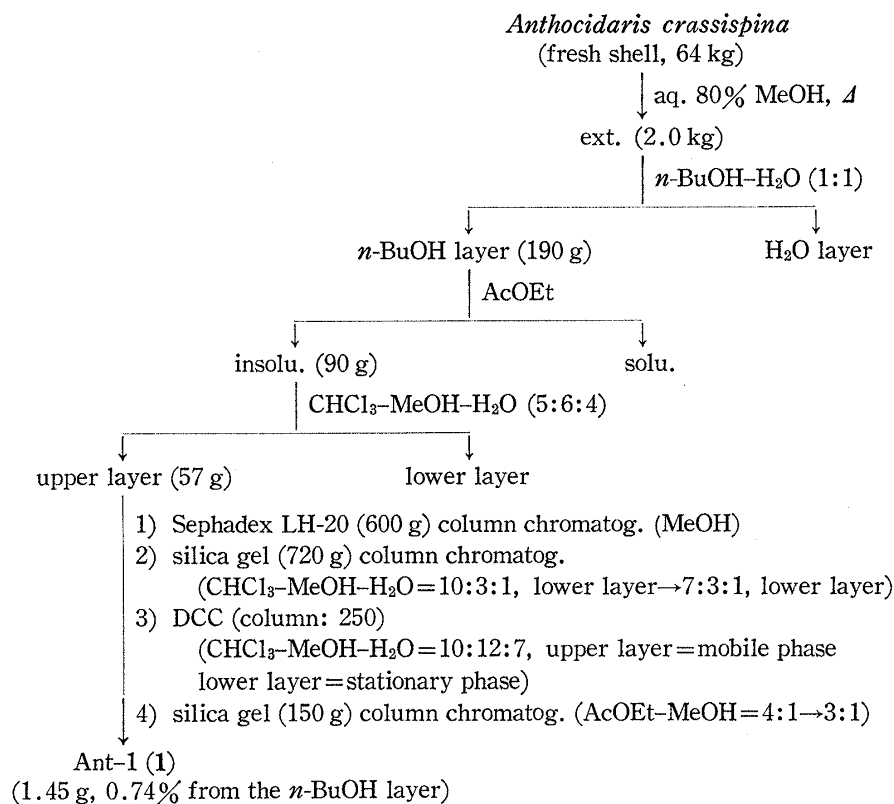
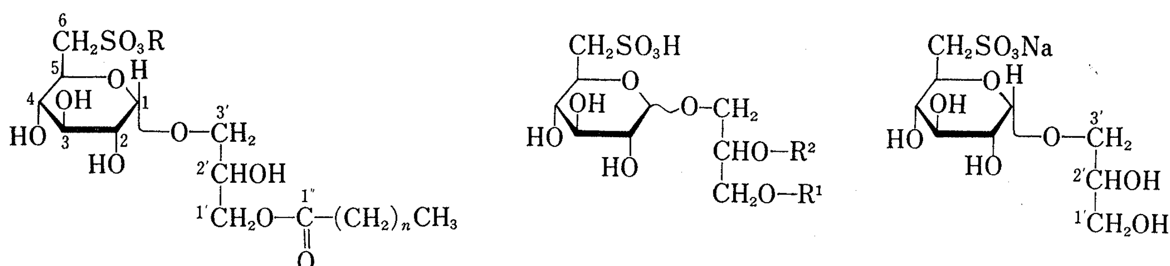



Chart 1




**1** (Ant-1) : R=Na $n$=14 (96%), $n$=12 (4%)
**2** : R=K, $n$=14

**3** : $R^1$, $R^2$=palmitoyl myristoyl

**4**

Chart 2

raphy to yield Ant-1 as a white amorphous substance in 0.74% yield from the $n$-butanol layer.

The infrared (IR) spectrum of Ant-1 (**1**) shows absorption bands at 3400 (br, hydroxyl), 1745 (ester), 1170 and 1035 (sulfonate) $cm^{-1}$, while the proton magnetic resonance ($^1H$-NMR) spectrum shows a characteristic signal pattern due to a glyceroglycolipid[6]: *e.g.* a triplet (3H) at $\delta$ 0.87 (terminal methyl), a broad signal (*ca.* 26H) centered at $\delta$ 1.25 (methylene in the fatty acid chain), and a broad singlet (1H) at $\delta$ 4.87 (anomeric proton). The field-desorption mass spectrum (FD-MS) of Ant-1 gives a base peak at $m/e$ 601 which corresponds to $(M+Na)^+$ (Fig. 1). Ant-1 exhibits an absorption maximum at 587 nm in the anthrone method,[7] suggesting that it contains a sulfoquinovose moiety[8] in its molecule.

On treatment with sodium methoxide in methanol, Ant-1 furnished approximately equal amounts of a glycerol-glycoside and a mixture of fatty acid methyl esters. The composition of the fatty acid methyl esters was shown to be methyl palmitate and methyl myristate

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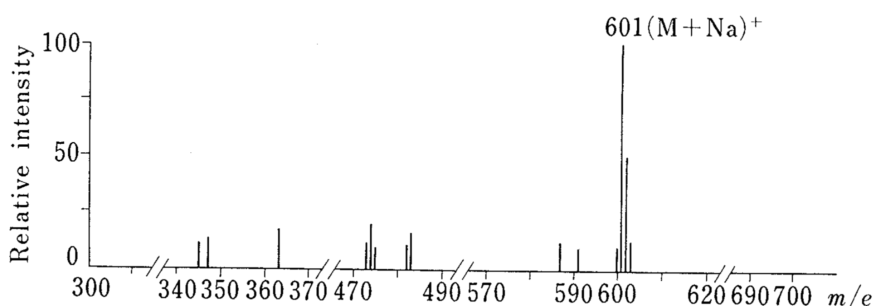


Fig. 1. FD–MS Diagram (*m/e* 300—700) of Ant-1 (**1**)

(96 : 4 mixture) by gas–liquid chromatography–mass spectrometry (GC–MS). The glycerol-glycoside was shown to be 6-sulfo-O-α-quinovopyranosyl-(1→1')-glycerol (**4**) on the basis of acid hydrolysis, which liberated 6-sulfoquinovose and glycerol and from its carbon magnetic resonance ($^{13}$C–NMR) spectrum.[9] The anomeric carbon signal observed at $\delta$ 99.3 indicates the presence of an α-glycosidic linkage in **4**. Comparison of the $^{13}$C–NMR data of the glycerol moieties in Ant-1 and **4** suggests that the fatty acid moiety in Ant-1 is attached at C-1' of the glycerol moiety rather than C-2' (Table I).

Table I. $^{13}$C–NMR Data for Ant-1(**1**) and **4**

| Carbon | Ant-1 (**1**) (major) | **4** |
|---|---|---|
| 1 | 99.6(d)[a] | 99.3(d) |
| 2 | 72.5(d) | 72.4(d) |
| 3 | 73.9(d) | 74.0(d) |
| 4 | 73.5(d) | 73.5(d) |
| 5 | 69.2(d) | 69.1(d) |
| 6 | 53.3(t) | 53.1(t) |
| 1' | 66.4(t) | 63.7(t)[b] |
| 2' | 69.2(d) | 71.8(d) |
| 3' | 70.0(t) | 69.9(t) |
| 1'' | 176.1(s) | |
| 2'' | 34.9 | |
| 3'' | 25.7 | |
| 4''—6'' | 30.5 | |
| 7''—12'' | 31.0 | |
| 13'' | 30.3 | |
| 14'' | 32.9 | |
| 15'' | 23.6 | |
| 16'' | 14.8(q) | |

*a*) Abbreviations given in parentheses denote the signal patterns observed in off-resonance experiments: d=doublet, q=quartet, s=singlet, t=triplet.
*b*) The carbon numbering in the glycerol moiety of **4** follows that in **1** for convenience.

On the basis of the above evidence, Ant-1(**1**) has been elucidated to be a 96 : 4 mixture of the sodium salts of 1'-O-palmitoyl- and 1'-O-myristoyl-3'-O-(6-sulfo-α-D-quinovopyranosyl)-glycerol. The signals (assigned to the major component) observed in the $^{13}$C–NMR spectrum of Ant-1 (**1**) (Table I) are consistent with this.

In 1968, Nagai, *et al.*[10] reported that 6-sulfoquinovosyl-diglyceride (**3**), a substance closely related to Ant-1 (**1**), had a respiration-activating effect on the sperm of the sea urchin *Pseudo-*

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11) N. Fusetani and Y. Hashimoto, *Agric. Biol. Chem.*, **39**, 2021 (1975).

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*centrotus depressus* A. AGASSIZ (Japanese name, "aka-uni"); this was the first sulfonolipid to be isolated from a member of the animal kingdom. Recently, Fusetani and Hashimoto reported[11] the isolation of the potassium salt of the major constituent of Ant-1 (2) from the green alga *Ulva pertusa* KJELLMAN (Japanese name, "ana-aosa") as one of two water-soluble hemolysins. Direct comparison of Ant-1 (**1**) with **2**[11] confirms their structural similarity; both compounds run with the same *Rf* value on a thin-layer chromatogram (TLC) and show almost identical IR spectra. It is interesting from a food-chain point of view that the green alga (*U. pertusa*) and the sea urchin (*A. crassispina*) both inhabit the same sea areas. However, the true metabolic origin of Ant-1 (**1**) in the sea urchin is unclear as yet.

## Experimental[12]

**Isolation of Ant-1** (**1**)——The fresh shell of *A. crassispina* (collected in Hokkaido in July) (64 kg) was crushed and extracted twice with hot aq. 80% MeOH (120 l) under reflux. The methanolic extract was then partitioned into *n*-BuOH–water (1:1) mixture (5.8 l) as usual to give the *n*-BuOH ext. (190 g). The *n*-BuOH ext. was then treated with warm AcOEt (1.2 l) with vigorous stirring, and the supernatant was removed by decantation. The insoluble portion was treated again with AcOEt (0.45 l) in a similar manner to give 90 g of insoluble residue, which was partitioned into $CHCl_3$–MeOH–$H_2O$ (5:6:4, 6.6 l). The lower layer was separated and extracted again with an equal amount of fresh solvent mixture. The combined upper layer was evaporated down under reduced pressure to give a residue (57 g) which was chromatographed repeatedly as shown in Chart 1 to furnish Ant-1 (**1**, 1.45 g, 0.74% from the *n*-BuOH ext.).

Ant-1 (**1**), a white amorphous material, IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3400 (br), 1745, 1170, 1035. $^1H$-NMR ($CDCl_3$–$CD_3OD$) $\delta$: as given previously in the text. $^{13}C$-NMR ($D_2O$): $\delta_C(Me_4Si) = \delta(tsp) - 1.6$ ppm, as given in Table I. FD-MS: as given in Fig. 1.

**Treatment of 1 with MeONa–MeOH**——A solution of **1** (107 mg) in 0.17 N MeONa–MeOH (15 ml) was stirred at room temperature (15°) for 1 hr and neutralized with Dowex 50W×8. After filtration, the filtrate was evaporated down under reduced pressure and the residue was treated with MeOH–*n*-hexane (2:3) (100 ml). The MeOH layer was taken and extracted again with *n*-hexane (40 ml). Concentration of the MeOH layer under reduced pressure gave a glycerol-glycoside (**4**) (52 mg) and the combined *n*-hexane layer gave a mixture of fatty acid methyl esters (49 mg). The mixture of fatty acid methyl esters was subjected to GC–MS for identification; it consisted of methyl palmitate [MS (*m/e*, %): 270, 40, $M^+$; 239, 12; 74, 100] and methyl myristate [MS (*m/e*, %): 242, 39, $M^+$; 211, 13; 74, 100] in a 96:4 ratio. Glycerol-glycoside (**4**), $^1H$-NMR ($CDCl_3$–$CD_3OD$–$D_2O$) $\delta$: 4.90 (1H, d, $J$=4 Hz, anomeric proton), $^{13}C$-NMR ($D_2O$): as given in Table I.

**Acid Hydrolysis of 4**——**4** (72 mg) was heated in 1 N HCl (5 ml) under reflux for 2 hr. The hydrolysis products were subjected to paper partition chromatography (PPC) (Toyo filter paper No. 50, developing with iso-PrOH–pyridine–$H_2O$–AcOH=8:8:4:1 and *n*-BuOH–pyridine–$H_2O$=6:4:3, detection with $AgNO_3$ reagent and periodate reagent) and TLC (Merck Art. 5715 DC-Fertigplatten Kieselgel 60 $F_{254}$, developing with $CHCl_3$–MeOH–$H_2O$=9:13:3, detection with 1% $Ce(SO_4)_2$–10% $H_2SO_4$ with heating) to identify 6-sulfoquinovose.[13] Glycerol was also identified by PPC and GLC (after trimethylsilylation; column, 3% SE-52 on chromosorb WAW (80—100 mesh), 3 mm×2 m; column temp., 120°; carrier gas, $N_2$ at a flow rate of 12 ml/min; $t_R$ (min), 3'22").

**Comparison of Ant-1** (**1**) **with 2**——Both compounds showed *Rf* 0.28 on TLC with $CHCl_3$–MeOH–$H_2O$=65:35:10 (lower layer, developing twice) and *Rf* 0.31 on TLC with AcOEt–MeOH=3:2, and gave almost identical IR spectra (KBr).

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12) The instruments used for obtaining physical data were those reported in our previous paper[2] unless otherwise specified. FD-MS was taken with a JMS-D 300 mass spectrometer (cathode voltage, −6 kV; accelerating voltage, 3 kV; emitter heating current, 19—23 mA), $^{13}C$-NMR with a JEOL FX-100 NMR spectrometer (pulse width, 6 μsec; spectral width, 5000 Hz; data points, 8192 (for **1**) and 16000 (for **4**); number of pulses, 6000 (for **1**) and 2400 (for **4**); temp., 28°) with sodium 3-trimethylsilylpropionate[2,2,3,3-$D_4$] (tsp) as an internal standard, and GC-MS with a Varian 920 GLC-Hitachi RMU-6 mass spectrometer combination.

13) The authentic sample was synthesized by the method of Miyano and Benson.[8]

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