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Marine Natural Products. XIV.[1] Structures of Echinosides A and B, Antifungal Lanostane-Oligosides from the Sea Cucumber *Actinopyga echinites* (JAEGER)

ISAO KITAGAWA,*[,a] MOTOMASA KOBAYASHI,[a] TATSUYA INAMOTO,[a] MASAKO FUCHIDA,[a] and YOSHIMASA KYOGOKU[b]

Faculty of Pharmaceutical Sciences, Osaka University,[a] 1–6, Yamada-oka, Suita, Osaka 565, Japan and Institute for Protein Research, Osaka University,[b] 3–2, Yamada-oka, Suita, Osaka 565, Japan

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Two antifungal lanostane-type triterpene oligosides, named echinosides A (**11**) and B (**9**), were isolated from the sea cucumber *Actinopyga echinites* (JAEGER) collected in Okinawa Prefecture. On the basis of chemical and physicochemical evidence, the structures of echinosides A and B have been elucidated respectively as 3-*O*-[β-D-3-*O*-methylglucopyranosyl(1→3)-β-D-glucopyranosyl-(1→4)-β-D-quinovopyranosyl(1→2)-β-D-(4-*O*-sodiosulfonato)xylopyranosyl]-3β,12α,17α,20(*S*)-tetrahydroxy-lanost-9(11)-en-18,20-olide (**11**) and 3-*O*-[β-D-quinovopyranosyl(1→2)-β-D-(4-*O*-sodiosulfonato)xylopyranosyl]-3β,12α,17α,20(*S*)-tetrahydroxylanost-9(11)-en-18,20-olide (**9**). The antifungal activities of echinosides and allied compounds are discussed.

Keywords——sea cucumber; *Actinopyga echinites*; echinoside A; echinoside B; lanostane-type triterpene; oligoglycoside antifungal activity; oligoglycoside ^{13}C-NMR; oligoglycoside T_1; snail enzyme hydrolysis

As a part of our search for new biologically active substances from marine organisms,[1] we have been investigating various bioactive oligosides contained in starfish[2] and sea cucumber[3] (echinoderms). We have so far reported full details of structural studies on a starfish saponin, thornasteroside A, the major steroidal oligoside of *Acanthaster planci* L. (crown of thorns)[2] and on several sea cucumber saponins such as holotoxins A and B from *Stichopus japonicus* (ma-namako in Japanese),[3a] holothurins A[3b] and *B*[3c] from *Holothuria leucospilota* (nise-kuro-namako), and holothurin A and 24-dehydroholothurin A from the Bahamean sea cucumber *Actinopyga agassizi*.[3d] In this paper, we present a full account of the structure elucidation of two antifungal triterpene-oligosides named echinosides A (**11**) and B (**9**), which were isolated from the sea cucumber *Actinopyga echinites* (JAEGER) (kutsu-namako).[4]

The body-wall of the sea cucumber, which was collected in July at Kudaka-jima in Okinawa Prefecture, was extracted with methanol and the extract was partitioned into a 1-butanol–water mixture. Chromatographic separation of the 1-butanol soluble portion provided two triterpene-oligosides named echinoside A (**11**) and echinoside B (**9**) in 0.7 and 4% yields, respectively, from the methanol extract.

The major oligoside echinoside B (**9**) showed no ultraviolet (UV) absorption maximum above 210 nm. The infrared (IR) spectrum of **9** showed absorption bands at 3400 and 1050 cm^{-1} reminiscent of glycosidic structure and at 1738 cm^{-1} ascribable to a γ-lactone group, the presence of which was further suggested by a negative maximum of $[\theta]_{223}$ −6000 (due to the n→π* transition) observed in the circular dichroism (CD) spectrum. Echinoside B (**9**) showed a positive rhodizonate test[2,5] and the presence of a sulfate group in its molecule was supported by the elemental analysis.

Acidic hydrolysis of echinoside B (**9**) provided two artifact sapogenols (**1**, **2**) and one mole each of xylose and quinovose. The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum of **9** showed two signals (δc 105.1, 105.5) suggestive of the β-anomeric configurations of these monosaccharide moieties. It also showed signals due to a γ-lactone group (δc 174.6) and a carbon–carbon double bond (δc 153.9, 115.6) (Table I).

In order to elucidate the structure of **1**, one of the artifact sapogenols, the following examinations were undertaken. The sapogenol (**1**) gave a UV absorption curve due to the heteroannular diene chromophore. The CD spectrum of **1** showed a positive maximum at 223 nm due to the γ-lactone moiety and a negative maximum at 246 nm ascribable to the heteroannular diene chromophore. Lithium aluminum hydride ($LiAlH_4$) reduction of **1** followed by ordinary acetylation furnished a tetraol-diacetate (**3**). The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **3** showed signals due to the 13β-acetoxymethyl function and the 3β-acetoxyl group. Lead tetraacetate [$Pb(OAc)_4$] oxidation of **3** provided an octanortriterpene diacetate (**4**) and a volatile ketone (**5**) which was derived from the side chain of **3**. The octanortriterpene diacetate (**4**) was found to be identical with the one prepared *via* a similar degradation of 22,25-oxidoholothurinogenin, which was previously obtained by acidic hydrolysis of holothurin B.[3c] Due to its high volatility, the ketone (**5**) was identified as isohexyl methyl ketone after converting it to the crystalline semicarbazone (**6**). Thus, the structure of **1** has been determined except for the C-20 configuration.

The C-20 configuration in **1** was elucidated by examination of the solvent-induced shift[3c,6] in the ^{1}H-NMR spectra of a monoacetate (**1a**) of **1**. As reported in the preliminary communication,[4] signals due to 14α-CH_3 and 20α-CH_3 groups are shifted lower (0.21 ppm) in the spectrum taken in pentadeutero(d_5)-pyridine than in the spectrum taken in deuterochloroform ($CDCl_3$), which indicated the 17α-OH configuration and consequently the 20(*S*) configuration in **1a**.

Another sapogenol **2**, which was obtained above together with **1** by acidic hydrolysis of echinoside B (**9**), showed no UV absorption maximum above 210 nm. On acidic treatment, **2** was readily converted to **1**. The ^{1}H-NMR spectrum of **2** showed signals assignable to adjacent 12α-H (δ 4.51, m, geminal to 12β-OH) and 11-H (δ 5.12, br s, olefinic)[3c] which suggested the presence of a 9(11)-en-12β-ol structure in **2** from the coupling pattern. On the other hand, solvolytic removal of the sulfate group in echinoside B (**9**) with a dioxane–pyridine mixture[2] furnished a diglycoside (**8**). The ^{1}H-NMR spectrum of **8** showed a one-proton doublet (J= 5 Hz) at δ 4.94 which was assigned to 12β-H (geminal to 12α-OH), as was experienced in the case of holothurin B.[3c] Thus, the genuine aglycone of echinoside B (**9**) was shown to possess a 9(11)-en-12α-ol structure, and the sapogenol (**2**) has been clarified to be a secondary product formed during the process of acidic hydrolysis.

Next, in order to clarify the structure of the carbohydrate moiety in echinoside B (**9**), the following examinations were undertaken. Methylation of the desulfated diglycoside (**8**) with methyl iodide and dimsyl carbanion[2,3] afforded a hepta-*O*-methyl derivative (**8a**). The ^{1}H-NMR spectrum of **8a** showed two one-proton doublets (J= 7 Hz each) assignable to two anomeric protons of β-pyranosides. Methanolysis of **8a** liberated methyl 2,3,4-tri-*O*-methylquinovopyranoside and methyl 3,4-di-*O*-methylxylopyranoside. Furthermore, methanolysis of a hexa-*O*-methyl derivative (**9a**), which was prepared from echinoside B (**9**) by methylation with methyl iodide and dimsyl carbanion in the presence of tetrahydrofuran (THF), liberated methyl 2,3,4-tri-*O*-methylquinovopyranoside and methyl 3-*O*-methylxylopyranoside. Consequently, the carbohydrate moiety of echinoside B (**9**) has been elucidated to be a β-D-quinovopyranosyl(1→2)-β-D-xylopyranosyl structure in which the 4-OH in the xylosyl part is sulfated.

Acetylation of desulfated echinoside B (**8**) with acetic anhydride and pyridine furnished a hexa-*O*-acetyl derivative (**8b**), in which the 17α-OH group was not acetylated, as shown by its

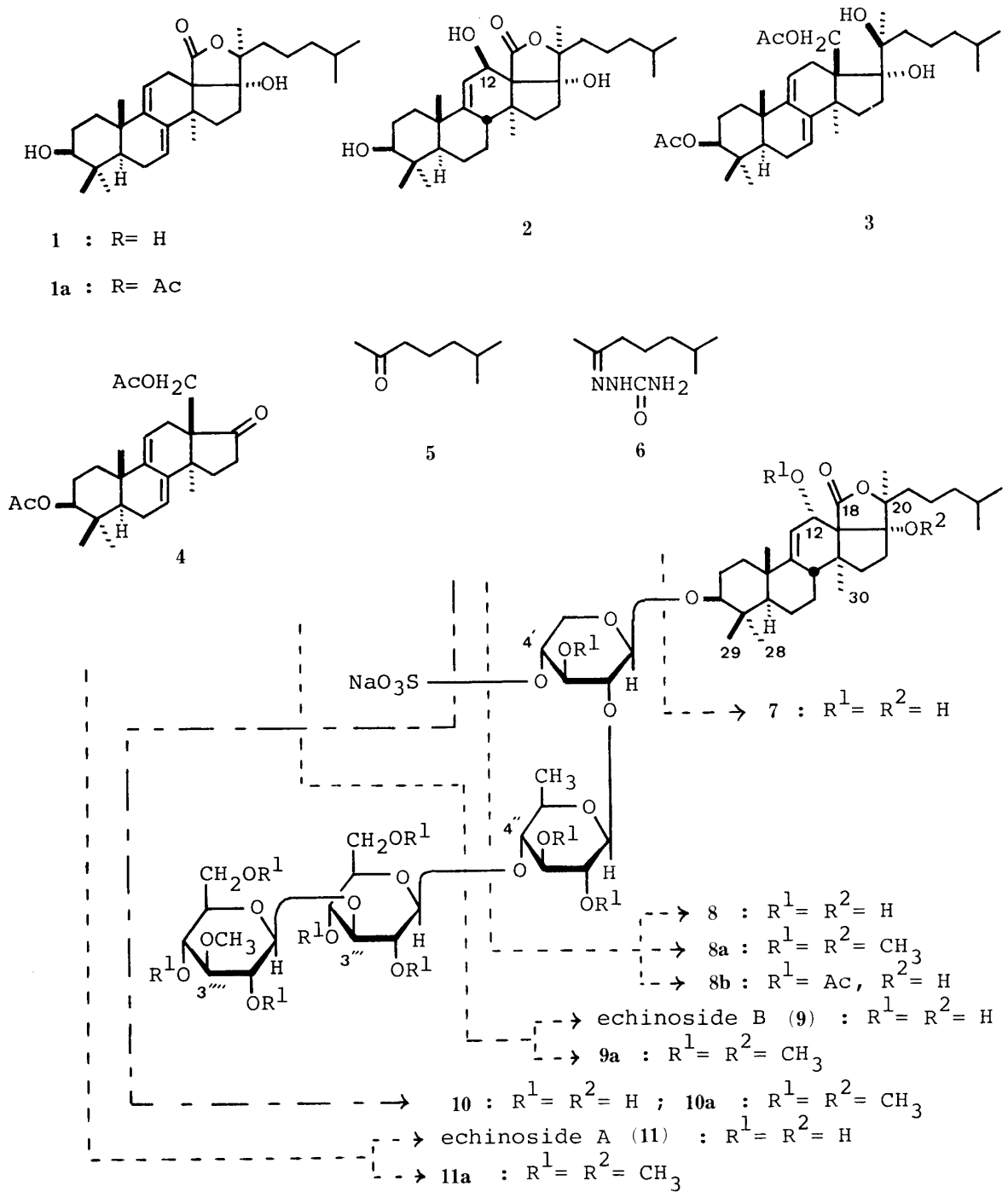

Chart 1

IR spectrum. Oxidation of the allylic hydroxyl function (12α-OH) in echinoside B (**9**) with chromium trioxide in a 1-butanol–sulfuric acid mixture[7] provided a 9(11)-en-12-one derivative (**12**). The CD spectrum of **12** clearly indicated the formation of an enone moiety in **12**: $[\theta]_{326} -8500$ (neg. max.), $[\theta]_{255} +21000$ (pos. max.). Thus, the carbohydrate moiety in echinoside B (**9**) was shown to be attached to the 3-OH function of the aglycone (**7**), and the total structure of echinoside B (**9**) has been established as shown in Chart 1.

Echinoside A (**11**) showed no UV absorption maximum above 210 nm. The IR spectrum of **11** showed absorption bands characteristic of glycosidic nature (3380, 1070 cm^{-1}) and the γ-lactone moiety (1745 cm^{-1}). Based on the positive potassium rhodizonate test[2,5] and the

TABLE I. ^{13}C-NMR Data for Echinoside B (**9**), Echinoside A (**11**), and Their Desulfated Derivatives (**8**, **10**) (25 or 50 MHz, in d_5-Pyridine, δc)[a]

Carbon	**8**	**9**	**10**	**11**	Carbon	**8**	**9**	**10**	**11**
1	36.5[b]	36.7[b]	36.4[b]	36.4[b]	1′	105.7	105.1	104.6	104.4
2	27.1[c]	27.0[c]	27.1[c]	27.0[c]	2′	84.0	83.3	84.0	83.1
3	88.8	88.7	88.6	88.5	3′	78.1	76.6	77.9	76.1
4	40.1[d]	40.0[d]	40.0[d]	39.9[d]	4′	70.8	75.1	70.6	74.7
5	52.8	52.7	52.7	52.7	5′	66.7	63.9	66.5	63.9
6	21.2[e]	21.3[e]	21.2[e]	21.2[e]	1″	106.2	105.6	105.3	104.9
7	28.0[c]	28.0[c]	28.3[c]	28.2[c]	2″	76.7	76.0	76.2	74.7
8	40.9	40.9	40.8	40.8	3″	77.8	77.4	75.8	75.6
9	154.0	153.9	154.2	154.0	4″	77.1	76.6	87.2	86.2
10	39.7[d]	39.7[d]	39.6[d]	39.6[d]	5″	73.4	73.3	71.5	71.8
11	115.7	115.6	115.6	115.6	6″	18.7	18.5	18.2	17.9
12	71.3	71.4	71.2	71.3	1‴			105.3	105.2
13	58.5	58.6	58.5	58.5	2‴			73.5	73.9
14	46.3	46.3	46.2	46.3	3‴			87.9	87.6
15	27.1[c]	27.0[c]	27.1[c]	27.0[c]	4‴			69.7	69.2
16	35.8[b]	35.9[b]	35.8[b]	35.8[b]	5‴			77.7	77.2
17	89.2	89.3	89.1	89.1	6‴			62.1	61.4
18	174.5	174.6	174.5	174.7	1⁗			105.3	105.6
19	20.0	20.1	20.0	20.0	2⁗			74.8	74.7
20	87.0	87.1	86.8	86.9	3⁗			87.7	87.6
21	22.6	22.7	22.9[f]	23.0[f]	4⁗			70.4	70.4
22	36.5[b]	36.7[b]	36.6[b]	36.5[b]	5⁗			78.0	77.9
23	22.3[e]	22.3[e]	22.1[e]	22.2[e]	6⁗			62.1	62.0
24	38.9	38.9	38.8	38.8	3⁗-OMe			60.5	60.5
25	28.2	28.1	28.3	28.2					
26	22.6	22.7	22.6[f]	22.6[f]					
27	22.6	22.7	22.7[f]	22.6[f]					
28	28.2	28.1	27.9	28.0					
29	16.8	16.8	16.7	16.7					
30	22.6	22.7	22.6[f]	22.7[f]					

a) Measured at 22 °C for **8**, **9** and at 35 °C for **10**, **11**. All assignments were supported by the off-resonance experiments. *b*—*f*) Assignments may be interchangeable within the same column.

elemental analysis, echinoside A (**11**) was shown to possess a sulfate residue. Acidic hydrolysis of echinoside A (**11**) yielded one mole each of xylose, quinovose, glucose, and 3-*O*-methylglucose, together with the above-mentioned two artifact sapogenols (**1** and **2**). The ^{13}C-NMR spectrum of **11** showed signals (δc 104.4, 104.9, 105.2, 105.6) due to four β-anomeric carbons in pyranosyl structures. In addition, almost all of the carbon signals due to the aglycone of **11** [including the γ-lactone and 9(11)-en-12α-ol moieties] were superimposable on those of the aglycone of echinoside B (**9**). Thus, the genuine aglycone of echinoside A (**11**) was suggested to be **7**, the same as in echinoside B (**9**) (Table I).

Enzymatic hydrolysis of echinoside A (**11**) with a glycosidase preparation, which was prepared from the snail *Euhadra sandai communis* PILSBRY (Japanese name: nami-maimai) according to Okano's procedure,[8] or with crude naringinase furnished echinoside B (**9**) quantitatively. Consequently, echinoside A (**11**) was shown to contain the structure of echinoside B (**9**) in its molecule. Solvolytic degradation of echinoside A (**11**) with dioxane and pyridine as mentioned above afforded a desulfated derivative (**10**), which, by complete methylation, was converted to a trideca-*O*-methyl derivative (**10a**). The ^{1}H-NMR spectrum of **10a** showed four β-anomeric carbon signals and methanolysis of **10a** liberated methyl 2,3,4,6-tetra-*O*-methylglucopyranoside, methyl 2,4,6-tri-*O*-methylglucopyranoside, methyl 2,3-di-*O*-

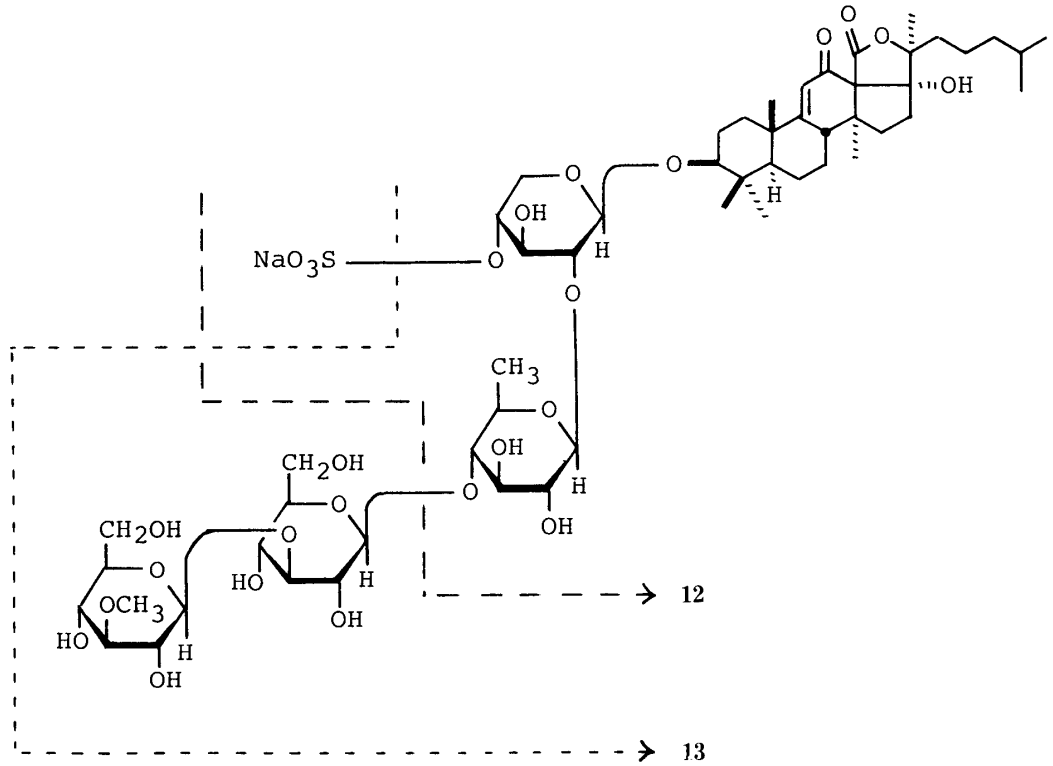

Chart 2

TABLE II. Minimum Growth Inhibitory Concentrations of **8**—**13** for Various Microorganisms (μg/ml)

	8	Echinoside B (**9**)	**12**	**10**	Echinoside A (**11**)	**13**
Aspergillus niger	25	3.12	>100	1.56	3.12	6.25
Aspergillus oryzae	25	3.12	6.25	1.56	3.12	6.25
Penicillium citrinum	>100	3.12	>100	3.12	3.12	25
Penicillium chrysogenum	50	3.12	>100	1.56	3.12	6.25
Mucor spinescens	>100	>100	>100	100	50.0	>100
Cladosporium herbarum	>100	>100	>100	100	25.0	>100
Rhodotorula rubra	100	12.5	>100	3.12	6.25	>100
Trichophyton mentagrophytes	>100	12.5	>100	6.25	6.25	>100
Trichophyton rubrum	100	6.25	100	3.12	6.25	12.5
Candida albicans	>100	>100	>100	50	12.5	>100
Candida utilis	>100	12.5	>100	3.12	6.25	12.5

methylquinovopyranoside, and methyl 3,4-di-*O*-methylxylopyranoside. Based on these findings, the structure of echinoside A has been determined to be 3-*O*-methyl-β-D-glucopyranosyl(1''''→3''')-β-D-glucopyranosyl(1'''→4'')echinoside B (**11**).

The structures of echinoside B (**9**) and echinoside A (**11**) were further confirmed by detailed ^{13}C-NMR analyses of **9**, **11**, and their desulfated derivatives (**8**, **10**), and all carbon signals were assigned as indicated in Table I.

Since hitherto isolated sea cucumber oligosides (*e.g.* holotoxins A and B[3a] and holothurins A[3b] and B[3c]) are known to exhibit antifungal activities, the present compounds, echinosides A (**11**) and B (**9**), were examined from the same point of view. A 9(11)-en-12-one derivative of echinoside A (**13**) was also prepared from echinoside A (**11**) by a similar oxidation procedure[7] to that described above for the synthesis of **12** from echinoside B (**9**).

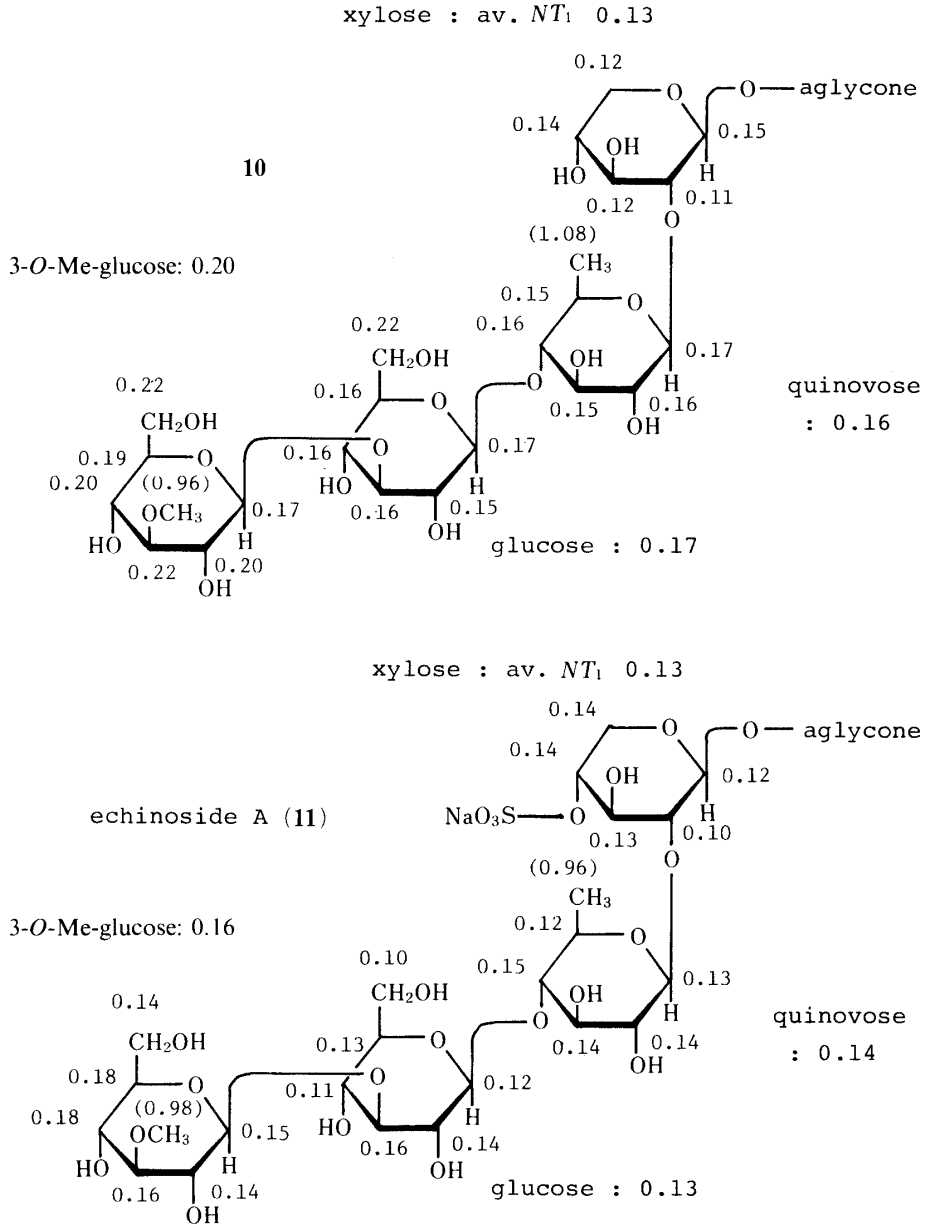

Fig. 1. NT_1 Values (s)[9] for **10** and **11**

The antifungal activities of echinoside B (**9**), echinoside A (**11**), their desulfated derivatives (**8**, **10**), and the enone derivatives (**12**, **13**) are summarized in Table II. As can be seen from those minimum growth inhibitory concentrations, echinosides B (**9**) and A (**11**) exhibited stronger antifungal activities than their desulfated and enone counterparts (**8**, **12**, **13**) except **10**. It is interesting that **10** was found to exhibit somewhat stronger growth inhibitory activities than echinoside A (**11**) against some microorganisms. It can be concluded that the presence of a tetrasaccharide sugar chain in echinoside A (**11**) and the presence of a sulfate group in echinoside B (**9**) are important in relation to the antifungal activities.

Since the carbohydrate moiety and the sulfate group have been elucidated to have some connection with the antifungal activities and in order to evaluate the physicochemical properties of the carbohydrate moiety with or without the sulfate function in echinoside A (**11**), the spin-lattice relaxation time (T_1) of all carbons in the carbohydrate moieties of echinoside A (**11**) and the desulfated derivative (**10**) were measured in the ^{13}C-NMR spectra.[3b]

The T_1 values of all carbons in the carbohydrate moieties of **10** and **11** were as shown in Fig. 1. The average NT_1 values[9,10] for the four monosaccharide constituents in **10** were 0.13 s (for xylose), 0.16 s (quinovose), 0.17 s (glucose), and 0.20 s (3-*O*-methylglucose), which are in increasing order from the reducing terminal to the non-reducing terminal. On the other hand, in the case of echinoside A (**11**) which possesses a sulfate group at the reducing terminal xylose, the NT_1 values for xylose (0.13 s), quinovose (0.14 s), and glucose (0.13 s) were almost identical except for the NT_1 value for non-reducing terminal 3-*O*-methylglucose (0.16 s). These findings may be of interest in connection with the above-mentioned relation between the carbohydrate structures and antifungal activities of **10** and **11**, which will be a subject of future investigation at our laboratory.

Finally, it is interesting to note that echinoside A (**11**) had a significant inhibitory effect against Na^+, K^+-adenosine triphosphatase (Na^+, K^+-ATPase, IC_{50}: 2×10^{-5} M), whereas the desulfated derivative (**10**) showed a decreased inhibitory effect (IC_{50}: 2×10^{-4} M), and echinoside B (**9**) and the desulfated derivative (**8**) did not show such an effect.[11] It should be added here that echinoside A (**11**) was shown to produce contraction of smooth muscle in the rat.[11]

Experimental

The instruments used to obtain physical data and the experimental conditions for chromatography were the same as described in our previous paper.[1] T_1 values were obtained by the inversion recovery method (35 °C, 0.1 mol/l).

Isolation of Echinoside A (11) and Echinoside B (9)——Body walls of the fresh sea cucumber *Actinopyga echinites* (6.5 kg, collected in July in Okinawa Prefecture) were cut and extracted with MeOH three times (20 l each, with heating under reflux for 4 h). Removal of the solvent under reduced pressure from the combined extracts provided the MeOH extract (560 g). The extract was partitioned into a 1-butanol–water mixture and removal of the solvent under reduced pressure from the 1-butanol phase furnished the 1-butanol soluble portion (125 g). A part of this 1-butanol extract (60 g) was subjected repeatedly to column chromatography (Kieselgel 60, 1 kg, $CHCl_3$–MeOH–H_2O = 7 : 3 : 1, lower phase) to furnish echinoside A (4 g, 0.7% from the MeOH extract) and echinoside B (20 g, 4% from the MeOH extract). Echinoside A (**11**), colorless needles, mp 228—230 °C (aq. EtOH), $[\alpha]_D^{12}$ −6.0° (c = 1.36, pyridine). *Anal.* Calcd for $C_{54}H_{87}NaO_{26}S \cdot 2H_2O$: C, 52.16; H, 7.38; S, 2.58. Found: C, 52.41; H, 7.37; S, 2.42. IR ν_{max}^{KBr} cm^{-1}: 3380, 1745, 1260, 1070. UV λ_{max}^{MeOH}: transparent above 210 nm. CD ($c = 1.96 \times 10^{-2}$, MeOH): $[\theta]_{248}$ 0, $[\theta]_{223}$ −4000 (neg. max.), $[\theta]_{216}$ 0, $[\theta]_{212}$ +4500! Potassium rhodizonate test[2]: yellow. ^{13}C-NMR (50 MHz, d_5-pyridine, δc): Table I. Echinoside B (**9**), colorless needles, mp 203.5—204.5 °C (aq. MeOH), $[\alpha]_D^{12}$ −2.2° (c = 0.88, pyridine). *Anal.* Calcd for $C_{41}H_{65}NaO_{16}S \cdot H_2O$: C, 55.51; H, 7.61; S, 3.61. Found: C, 55.35; H, 7.67; S, 3.34. IR ν_{max}^{KBr} cm^{-1}: 3400, 1738, 1230, 1050. UV λ_{max}^{MeOH}: transparent above 210 nm. CD ($c = 1.65 \times 10^{-1}$, MeOH): $[\theta]_{258}$ 0, $[\theta]_{223}$ −6000 (neg. max.), $[\theta]_{216}$ 0, $[\theta]_{214}$ +3000! Potassium rhodizonate test: yellow. ^{13}C-NMR (50 MHz, $CDCl_3$, δc): Table I.

Acidic Hydrolysis of Echinoside B (9)——A mixture of echinoside B (**9**) (1.01 g) in aq. 3 N HCl (66 ml) was heated under reflux for 4 h, then cooled. The reaction mixture was extracted with AcOEt. Work-up of the AcOEt extract in the usual manner and removal of the solvent under reduced pressure from the AcOEt extract gave a residue (455 mg). Crystallization of the residue (450 mg) from MeOH afforded a sapogenol mixture (256 mg), which was purified by preparative thin-layer chromatography (TLC, benzene–acetone = 5 : 1) to furnish **1** (66 mg) and **2** (35 mg). **1**, colorless needles, mp 257—260 °C (dec.) (MeOH), $[\alpha]_D^{25}$ −16° (c = 0.6, $CHCl_3$). *Anal.* Calcd for $C_{30}H_{46}O_4 \cdot 1/2H_2O$: C, 75.11; H, 9.88. Found: C, 75.27; H, 9.67. IR ν_{max}^{KBr} cm^{-1}: 3600, 1760. UV λ_{max}^{MeOH} nm (ε): 237 (15000), 244 (16000), 252 (11000). CD ($c = 2.58 \times 10^{-2}$, MeOH): $[\theta]_{266}$ 0, $[\theta]_{246}$ −35000 (neg. max.), $[\theta]_{235}$ 0, $[\theta]_{223}$ +25000 (pos. max.). ^{1}H-NMR (90 MHz, d_5-pyridine, δ): 0.82 (6H, d, J = 6 Hz), 1.12, 1.20, 1.40, 1.46, 1.60 (each 3H, s), 3.53 (1H, t-like), 5.46 (1H, m), 5.71 (1H, m). MS m/z (%): 470 (M^+, 92), 437 ($M^+ - CH_3 - H_2O$, 50), 383 (M^+ − ring A, 82). **2**, colorless needles, mp 270—275 °C (MeOH), $[\alpha]_D^{24}$ −14° (c = 0.3, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400, 3250, 1735. UV λ_{max}^{MeOH}: transparent above 210 nm. ^{1}H-NMR (90 MHz, $CDCl_3$, δ): 0.85 (3H, s), 0.88 (6H, d, J = 6 Hz), 1.00, 1.02, 1.18, 1.47 (each 3H, s), 3.22 (1H, m, 3α-H), 4.51 (1H, m, 12α-H), 5.12 (1H, br s, $W_{1/2}$ = 6 Hz, 11-H). ^{13}C-NMR (25 MHz, d_5-pyridine, δc): 174.5 (s, 18-C), 151.2 (s, 9-C), 119.0 (d, 11-C), 87.1 (s, 17-C), 78.0 (d, 3-C), 66.9 (d, 12-C). MS m/z (%): 488 (M^+, 6), 470 ($M^+ - H_2O$, 34). High resolution MS: Found 488.349. Calcd for $C_{30}H_{48}O_5$: 488.349.

Carbohydrate Compositions of Echinoside A (11) and Echinoside B (9)——A solution of echinoside A (**11**) or echinoside B (**9**) (10 mg each) in 2.5 N AcCl–MeOH (1.0 ml) was heated under reflux for 2 h, then cooled. The reaction mixture was neutralized with Ag_2CO_3 and filtered. Removal of the solvent from the filtrate under reduced pressure gave a residue, which was dissolved in pyridine (0.1 ml) and treated with *N*,*O*-bis(trimethylsilyl)trifluoroacet-

amide (BSTFA, 0.2 ml) at room temperature for 5 min. The reaction mixture was subjected to gas-liquid chromatographic (GLC) analysis (column of 5% SE-52 on Chromosorb WAW DMCS 80—100 mesh, 3 mm × 2 m, temp. 170 °C, N_2 flow rate 30 ml/min). From echinoside A (**11**): xylose (t_R 6′25″, 7′00″), quinovose (7′55″), 3-*O*-Me-glucose (9′05″), and glucose (17′30″, 19′00″). Echinoside B (**9**): xylose and quinovose.

$LiAlH_4$ Reduction of 1 Followed by Acetylation——A solution of **1** (296 mg) in THF (15 ml) was added dropwise to a suspension of $LiAlH_4$ (70 mg) in THF (45 ml), and the mixture was heated under reflux for 5 h, then cooled. The reaction was quenched with aq. ether and the whole mixture was washed successively with aq. dil. HCl, aq. sat. $NaHCO_3$, and aq. sat. NaCl, then dried over Na_2SO_4. Removal of the solvent from the filtrate under reduced pressure afforded a product (342 mg), which was treated with Ac_2O (2 ml) and pyridine (4 ml) at 42 °C for 12 h. Work-up of the reaction mixture in the usual manner gave a product (319 mg) which was crystallized from *n*-hexane to furnish **3** (186 mg). **3**: Colorless needles, mp 181—182.5 °C, $[\alpha]_D^{25}$ +43° (c=0.4, $CHCl_3$). *Anal.* Calcd for $C_{34}H_{54}O_6$: C, 73.08; H, 9.74. Found: C, 72.85; H, 9.61. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3550 (OH), 1740 (OAc). UV λ_{max}^{MeOH} nm (ε): 237 (8400), 244 (9600), 253 (6400). ^{1}H-NMR (90 MHz, $CDCl_3$, δ): 0.88 (3H, s), 0.88 (6H, d, J=6 Hz), 0.94, 0.96, 1.27, 1.29 (each 3H, s), 2.00, 2.04 (both 3H, s), 3.90, 4.04 (2H, ABq, J=12 Hz), 4.50 (1H, t, J=7 Hz), 5.20—5.60 (2H, m). MS m/z (%): 558 (M^+, 1), 429 (M^+ − ring A, 3), 370 (25), 352 (370 − H_2O, 16).

$Pb(OAc)_4$ Degradation of 3——A solution of **3** (163 mg) in dry benzene (5 ml) was treated with $Pb(OAc)_4$ (329 mg) and the mixture was stirred in the dark for 4 h. The benzene was partly removed from the solution to leave a volume of 2 ml, and the resulting mixture was treated with *n*-hexane (2 ml). The whole mixture was applied to a column of silica gel (4 g) and the column was eluted with *n*-hexane–AcOEt (10 : 1) to furnish fractions containing **5** and **4**. **4** (60 mg) was obtained by recrystallization of the latter fraction from *n*-hexane. The earlier eluate containing **5** was concentrated to a volume of 2 ml and purified by preparative GLC (column of 15% polyethylene glycol succinate (PEGS) on Chromosorb WAW, 10 mm × 1 m, temp. 34 °C) to give **5**, which was treated with an aqueous solution (0.2 ml) of semicarbazide hydrochloride (37 mg) and $AcONa \cdot 3H_2O$ (60 mg). The resulting white precipitate was collected by filtration and crystallized from 95% EtOH to furnish a semicarbazone (**6**) (22 mg). **4**: Colorless needles, mp 159.5—160.5 °C (mixed mp with an authentic sample[3c] 158—159 °C), $[\alpha]_D^{28}$ +90° (c=0.5, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1745 (OAc), 1727 (sh). UV λ_{max}^{MeOH} nm (ε): 236 (6700), 242 (7300), 251 (4900). CD (c=8 × 10^{-3}, MeOH): $[\theta]_{326}$ 0, $[\theta]_{295}$ +15000 (pos. max.), $[\theta]_{262}$ +2500 (pos. min.), $[\theta]_{242}$ +21000 (pos. max.), $[\theta]_{218}$ 0. ^{1}H-NMR (90 MHz, $CDCl_3$, δ): 0.90 (6H, s), 0.96, 1.04 (both 3H, s), 1.99, 2.05 (both 3H, s), 3.69, 4.27 (2H, ABq, J=12 Hz), 4.51 (1H, t, J=6 Hz), 5.43, 5.55 (both 1H, m). MS m/z (%): 428 (M^+, 2), 355 (80). **6**: Colorless plates, mp 154—156 °C (mixed mp with an authentic sample synthesized (*vide infra*) from 6-methyl-5-hepten-2-one, 154—156 °C). IR ν_{max}^{KBr} cm^{-1}: 3180, 3150, 1690, 1655, 1582. MS m/z (%): 185 (M^+, 36), 149 (20), 141 (20).

Synthesis of Semicarbazone (6) from 6-Methyl-5-hepten-2-one——A solution of 6-methyl-5-hepten-2-one (10 g) in EtOH (80 ml) was hydrogenated over 3% Pd–C (3.4 g) for 3 h. Removal of the solvent from the filtrate under reduced pressure gave a product, which was purified by distillation (5 mmHg, bath temp. 95—100 °C, bp 87 °C) to yield **5** (6.5 g). **5**: IR ν_{max}^{film} cm^{-1}: 2950, 1725. ^{1}H-NMR (90 MHz, $CDCl_3$, δ): 0.87 (6H, d, J=6 Hz), 1.0—1.7 (5H), 2.05 (3H, s), 2.35 (2H, t, J=8 Hz). MS m/z (%): 128 (M^+), 43 (100). A solution of **5** (1.00 g) in EtOH (6 ml) was treated at 20 °C dropwise with a solution of $AcONa \cdot 3H_2O$ (1.55 g) and semicarbazide hydrochloride (0.95 g) in water (6 ml). A resulting white precipitate (1.33 g) was collected by filtration and crystallized from 95% EtOH to afford **6** (1.09 g). **6**: Colorless plates, mp 156—157.5 °C. IR ν_{max}^{KBr} cm^{-1}: 3450, 3180, 2950, 1690, 1660, 1582. ^{1}H-NMR (90 MHz, $CDCl_3$, δ): 0.88 (6H, d, J=7 Hz), 1.81 (3H, s), 2.19 (2H, t, J=8 Hz), 5.60 (2H, m, exchangeable with D_2O), 8.15 (1H, br s, exchangeable with D_2O). MS m/z (%): 185 (41), 41 (100). *Anal.* Calcd for $C_9H_{19}N_3O$: C, 58.34; H, 10.34; N, 22.68. Found: C, 58.40; H, 10.53; N, 22.45.

Acetylation of 1 Giving the Monoacetate (1a)——A solution of **1** (55 mg) in pyridine (5 ml) was treated with Ac_2O (5 ml) at room temp. (20 °C) for 15 h. The reaction mixture was poured into ice-water and the whole was extracted with AcOEt. Work-up of the AcOEt extract in the usual manner gave a product (65 mg) which was purified by crystallization from MeOH to furnish **1a** (30 mg). **1a**: Colorless needles, mp 245—246 °C, $[\alpha]_D^{25}$ +17° (c=0.3, $CHCl_3$). *Anal.* Calcd for $C_{32}H_{48}O_5$: C, 74.96; H, 9.44. Found: C, 74.81; H, 9.37. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600, 3450, 1760, 1720. UV λ_{max}^{MeOH} nm (ε): 238 (13000), 245 (14000), 253 (10000). ^{1}H-NMR (90 MHz, $CDCl_3$, δ): 0.89 (3H, s), 0.90 (6H, d, J=6 Hz), 0.97, 1.12, 1.14, 1.40 (each 3H, s), 2.05 (3H, s), 4.50 (1H, m), 5.26, 5.48 (both 1H, m).

Acidic Treatment of 2 Giving 1——A mixture of **2** (20 mg) in 2.5 N AcCl–MeOH (3 ml) was heated under reflux for 2 h. The reaction mixture was poured into ice-water and the whole was extracted with AcOEt. The AcOEt extract was washed with aq. $NaHCO_3$ and aq. sat. NaCl, then dried over $MgSO_4$. Removal of the solvent from the filtrate under reduced pressure gave a product (17 mg), which was purified by column chromatography (SiO_2 10 g, benzene–acetone = 5 : 1) to furnish **1** (10 mg). This product was shown to be identical with an authentic sample by TLC (benzene–acetone = 4 : 1) and ^{1}H-NMR comparisons.

Solvolysis of Echinoside B (9) Giving 8——A mixture of **9** (330 mg) in dioxane (5 ml)–pyridine (5 ml) was heated under reflux for 1 h, then cooled. The reaction mixture was diluted with MeOH (*ca.* 20 ml) and filtered. Removal of the solvent from the filtrate under reduced pressure gave a product (325 mg) which was purified by crystallization from MeOH to furnish **8** (226 mg). **8**: Colorless needles, mp 226—228 °C, $[\alpha]_D^{22}$ +0.7° (c=1.0, pyridine). *Anal.* Calcd for $C_{41}H_{66}O_{13} \cdot H_2O$: C, 62.73; H, 8.73. Found: C, 62.58; H, 8.72. IR ν_{max}^{KBr} cm^{-1}: 3360, 1731. CD (c=6.2 × 10^{-4},

MeOH): $[\theta]_{260}$ 0, $[\theta]_{225}$ −5800 (neg. max.), $[\theta]_{217}$ 0, $[\theta]_{203}$ +35000 (pos. max.). ^{1}H-NMR (90 MHz, d_5-pyridine, δ): 0.85 (6H, d, J=6 Hz), 1.18, 1.32, 1.39, 1.65 (each 3H, s), 1.65 (3H, d, J=7 Hz), 1.75 (3H, s), 4.76 (1H, d, J=7 Hz), 4.94 (1H, d, J=5 Hz), 5.15 (1H, d, J=7 Hz), 5.58 (1H, d, J=5 Hz). ^{13}C-NMR (25 MHz, d_5-pyridine, δc): Table I.

Methylation of 8 Followed by Methanolysis——A solution of **8** (145 mg) in dimethyl sulfoxide (DMSO, 7 ml) was treated with a dimsyl carbanion solution (12 ml) [prepared from NaH (2 g), which was washed with dry *n*-hexane before use, and DMSO (35 ml) by stirring at 70 °C under a nitrogen atmosphere for 1 h], and the mixture was stirred at room temperature (25 °C) under a nitrogen atmosphere for 1 h, then treated with CH_3I (5.2 ml) under ice-cooling. The reaction mixture was stirred at room temperature for a further 1 h, then poured into ice-water and the whole was extracted with AcOEt. The AcOEt extract was washed with water and dried over $MgSO_4$. Removal of the solvent under reduced pressure gave a product (202 mg), which was purified by column chromatography (SiO_2 30 g, *n*-hexane–AcOEt = 10 : 1) to furnish the hepta-*O*-methyl derivative (**8a**, 158 mg). **8a**: Colorless glass, $[\alpha]_D^{22}$ −1.1° (*c* = 0.4, MeOH). *Anal.* Calcd for $C_{48}H_{80}O_{13}$: C, 66.60; H, 9.41. Found: C, 66.83; H, 9.50. IR $\nu_{max}^{CCl_4}$ cm^{-1}: no OH, 1756. CD ($c = 1.0 \times 10^{-3}$, MeOH): $[\theta]_{250}$ 0, $[\theta]_{224}$ −5000 (neg. max.), $[\theta]_{216}$ 0, $[\theta]_{203}$ +24000 (pos. max.). ^{1}H-NMR (90 MHz, CCl_4, δ): 4.17 (1H, d, J=7 Hz), 4.52 (1H, d, J=7 Hz). A solution of **8a** (5 mg) in 2.5 N AcCl–MeOH (1 ml) was heated under reflux for 1 h, then cooled. The reaction mixture was neutralized with Ag_2CO_3 and filtered. The filtrate was subjected to GLC analysis [i) column of 15% neopentyl glycol succinate (NPGS) on Chromosorb WAW (80—100 mesh), 3 mm × 2 m, temp. 170 °C, N_2 flow rate 30 ml/min; ii) column of 15% PEGS on Chromosorb WAW (80—100 mesh), 3 mm × 1 m, temp. 150 °C, N_2 flow rate 30 ml/min] and TLC analysis (*n*-hexane–acetone = 2 : 1) to identify methyl 2,3,4-tri-*O*-methylquinovopyranoside [i) t_R 3′50″, 5′05″; ii) t_R 1′44″, 2′30″; Rf=0.75, 0.65] and methyl 3,4-di-*O*-methylxylopyranoside [i) 12′07″, 14′08″, ii) 9′10″, 11′38″; Rf=0.25, 0.20].

Methylation of Echinoside B (9) Followed by Methanolysis——A solution of **9** (500 mg) in DMSO (25 ml) and dry THF (5 ml) was treated with a dimsyl carbanion solution (29 ml), and the mixture was stirred at room temp. (18 °C) for 1.5 h, then treated with CH_3I (12.8 ml) under ice-cooling. The reaction mixture was stirred at room temp. in the dark for 2 h, the poured into ice-water and the whole was extracted with 1-BuOH. The 1-BuOH extract was washed successively with aq. 10% $Na_2S_2O_3$, aq. sat. NaCl, and water. Removal of the solvent from the organic phase under reduced pressure yielded a product (1.19 g) which was purified by column chromatography (SiO_2 60 g, $CHCl_3$–MeOH = 13 : 1) to furnish the hexa-*O*-methyl derivative (**9a**) (208 mg). **9a**: Colorless glass, $[\alpha]_D^{22}$ 0° (*c* = 0.2, MeOH). *Anal.* Calcd for $C_{47}H_{77}NaO_{16}S \cdot 2H_2O$[12]: C, 57.06; H, 8.25; S, 3.24. Found: C, 57.17; H, 8.12; S, 2.96. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1753. CD ($c = 6 \times 10^{-3}$, MeOH): $[\theta]_{255}$ 0, $[\theta]_{225}$ −5200 (neg. max.), $[\theta]_{219}$ 0, $[\theta]_{204}$ +33000 (pos. max.). ^{1}H-NMR (90 MHz, d_5-pyridine–D_2O = 2 : 1, δ): 4.74 (1H, d, J=8 Hz), 4.93 (1H, d, J=7 Hz). A solution of **9a** (7 mg) in 2.5 N AcCl–MeOH (1.5 ml) was heated under reflux for 1 h, then cooled. The reaction mixture was neutralized with Ag_2CO_3 and filtered. The filtrate was subjected to GLC analysis [i) NPGS (*vide supra*), column temp. 180 °C; ii) PEGS (*vide supra*), column temp. 170 °C, N_2 flow rate 45 min/min] and TLC analysis (*n*-hexane–acetone = 2 : 1), to identify methyl 2,3,4-tri-*O*-methylquinovopyranoside [i) t_R 3′04″, 3′58″, ii) 1′30″, 2′14″; Rf=0.75, 0.65] and methyl 3-*O*-methylxylopyranoside [i) 20′15″, 29′24″, ii) 9′03″, 14′11″; Rf=0.10].

Acetylation of 8——A solution of **8** (300 mg) in pyridine (16 ml) was treated with Ac_2O (16 ml) and the mixture was left to stand at room temp. (27 °C) for 15 h, then poured into ice-water. The whole was extracted with AcOEt. Work-up of the AcOEt extract in the usual manner gave a product, which was purified by column chromatography (SiO_2 60 g, *n*-hexane–AcOEt = 3 : 1) to furnish the hexaacetate (**8b**) (152 mg). **8b**: Colorless glass, $[\alpha]_D^{22}$ +0.1° (*c* = 1.1, MeOH). *Anal.* Calcd for $C_{53}H_{78}O_{19}$: C, 62.46; H, 7.71. Found: C, 62.17; H, 7.75. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3560, 1747.

Oxidation of Echinoside B (9) Giving 12——1) The CrO_3 reagent:[7] A solution of CrO_3 (500 mg) in pyridine (30 ml) was poured into 1-BuOH (250 ml) saturated with water and the mixture was left to stand for 1 d. Removal of the solvent below 45 °C under reduced pressure yielded a residue which was treated with water (100 ml). The precipitated reagent was collected by filtration, washed with water and $CHCl_3$ repeatedly, then dried in a desiccator under reduced pressure for use. 2) A solution of **9** (505 mg) in dioxane (50 ml)–water (50 ml) was treated with aq. 2 N H_2SO_4 (100 ml) and the CrO_3 reagent (1.4 g) and the mixture was stirred at room temp. (27 °C) for 15 h. The reaction mixture was diluted with water and extracted with 1-BuOH. The 1-BuOH extract was neutralized with aq. dil. NaOH and washed with aq. sat. NaCl and water. Removal of the solvent from the organic phase under reduced pressure yielded a product (1.58 g), which was purified by column chromatography (SiO_2 70 g, $CHCl_3$–MeOH–H_2O = 10 : 3 : 1, lower phase) to furnish **12** (259 mg) and **9** (120 mg, recovered). **12**: Colorless needles, mp 166—167 °C (90% EtOH), $[\alpha]_D^{25}$ −2.7° (*c* = 1.1, pyridine). *Anal.* Calcd for $C_{41}H_{63}NaO_{16}S \cdot H_2O$: C, 55.64; H, 7.40; S, 3.62. Found: C, 55.94; H, 7.46; S, 3.69. IR ν_{max}^{KBr} cm^{-1}: 3425, 1763, 1659. UV λ_{max}^{MeOH} nm (ε): 258 (8500). CD ($c = 2.2 \times 10^{-1}$, MeOH): $[\theta]_{366}$ 0, $[\theta]_{326}$ −8500 (neg. max.), $[\theta]_{286}$ 0, $[\theta]_{255}$ +21000 (pos. max.), $[\theta]_{220}$ 0. ^{13}C-NMR (25 MHz, d_5-pyridine, δc); 105.1 (d, C-1″), 105.7 (d, C-1′), 118.8 (d, C-11), 169.4 (s, C-9), 177.5 (s, C-18), 196.6 (s, C-12).

Acidic Hydrolysis of Echinoside A (11)——A mixture of **11** (50 mg) in aq. 3 N HCl (5 ml) was heated in a boiling water-bath for 4 h. The reaction mixture was extracted with AcOEt and the AcOEt extract was washed with aq. $NaHCO_3$ and aq. sat. NaCl, then dried over $MgSO_4$. The product obtained by usual work-up was purified by column chromatography (SiO_2 20 g, benzene–acetone = 15 : 1) to afford **1** (8 mg) and **2** (3 mg), which were shown to be identical with respective authentic samples by TLC and ^{1}H-NMR comparisons.

Enzymatic Hydrolysis of Echinoside A (11)——1) A solution of **11** (500 mg) in AcONa–AcOH buffer (pH 5.2,

200 ml) was treated with a glycosidase preparation (500 mg), which was prepared from the snail *Euhadra sandai communis* PILSBRY (nami-maimai),[3c,8] and the whole was stirred at 37 °C for 4 d. The reaction mixture was then treated with 1-BuOH (10 ml), heated at 70 °C for 20 min, and then filtered. The filtrate was extracted with 1-BuOH and the 1-BuOH extract was washed with water. Removal of the solvent from the 1-BuOH extract under reduced pressure gave a residue (540 mg), which was purified by column chromatography (SiO_2 50 g, $CHCl_3$–MeOH–H_2O = 10 : 3 : 1, lower phase) to furnish echinoside B (**9**) (50 mg), which was shown to be identical with an authentic sample by mixed mp determination (202—204 °C), $[\alpha]_D$, IR, CD, TLC, and ^{13}C-NMR comparisons. 2) A solution of **11** (100 mg) in AcONa–AcOH buffer (pH 5.2, 20 ml) was treated with crude naringinase (200 mg) (kindly provided by Tanabe Pharm. Co., Lot No. 705140) and the mixture was stirred at 32 °C for 7 d. Work-up of the reaction mixture as described above furnished **9** (40 mg) and **11** (24 mg, recovered).

Solvolysis of Echinoside A (11)——A solution of **11** (250 mg) in dioxane (4 ml)–pyridine (8 ml) was heated under reflux for 1 h. Work-up of the reaction mixture as described above for the solvolysis of **9** gave a product which was purified by column chromatography (SiO_2 50 g, $CHCl_3$–MeOH–H_2O = 10 : 3 : 1, lower phase) to furnish **10** (125 mg). **10**: Colorless needles, mp 237—239 °C (MeOH), $[\alpha]_D^{25}$ −1.5° (*c* = 0.7, MeOH). *Anal.* Calcd for $C_{54}H_{88}O_{23}\cdot 2H_2O$: C, 56.82; H, 8.12. Found: C, 56.71; H, 7.99. IR ν_{max}^{KBr} cm^{-1}: 3370, 1743, 1050. UV λ_{max}^{MeOH} nm: transparent above 210. CD (*c* = 1.6 × 10^{-1}, MeOH): $[\theta]_{250}$ 0, $[\theta]_{224}$ −5500 (neg. max.), $[\theta]_{216}$0, $[\theta]_{212}$ +6600!. ^{13}C-NMR (50 MHz, d_5-pyridine, δc): Table I.

Methylation of 10 Followed by Methanolysis——A solution of 10 (500 mg) in DMSO (25 mg) was treated with a dimsyl carbanion solution (47 ml) and the mixture was stirred at room temp. (25 °C) under an N_2 atmosphere for 1 h, then treated with CH_3I (20 ml) under ice-cooling. The whole was stirred in the dark at room temp. for a further 1 h. Work-up of the reaction mixture as described above for the methylation of **8** gave a product, which was purified by column chromatography (SiO_2 60 g, *n*-hexane–acetone = 10 : 1) to furnish the trideca-*O*-methyl derivative (**10a**) (437 mg). **10a**: Colorless glass, $[\alpha]_D^{25}$ −3° (*c* = 1.2, MeOH). *Anal.* Calcd for $C_{66}H_{112}O_{23}$: C, 61.14; H, 8.86. Found: C, 61.44; H, 8.90. IR $\nu_{max}^{CCl_4}$ cm^{-1}: no OH, 1760. CD (*c* = 2.5 × 10^{-2}, MeOH): $[\theta]_{250}$ 0, $[\theta]_{223}$ −6400 (neg. max.), $[\theta]_{216}$ 0, $[\theta]_{210}$ +21000!. ^{1}H-NMR (90 MHz, $CDCl_3$, δ): 4.33 (1H, d, *J* = 7 Hz), 4.37 (1H, d, *J* = 8 Hz), 4.66 (1H, d, *J* = 7 Hz), 4.68 (1H, d, *J* = 7 Hz). A solution of **10a** (10 mg) in 2.5 N AcCl–MeOH (1 ml) was heated under reflux for 30 min, then neutralized with Ag_2CO_3 and filtered. The filtrate was subjected to GLC analysis [i) column of NPGS (*vide supra*), temp. 180 °C, N_2 flow rate 32 ml/min; iii) column of 5% butane-1,4-diol succinate (BDS) on Uniport B (80—100 mesh), 3 mm × 2 m, N_2 flow rate 35 ml/min] and TLC analysis (benzene–acetone = 2 : 1) to identify methyl 2,3,4,6-tetra-*O*-methylglucopyranoside [i) t_R 7′56″, 10′44″; iii) column temp. 135 °C, t_R 14′34″, 22′22″; *Rf* = 0.75] methyl 2,4,6-tri-*O*-methylglucopyranoside [i) 20′50″, 38′01″; iii) column temp. 175 °C, 9′50″, 13′51″; *Rf* = 0.20, 0.35], methyl 2,3-di-*O*-methylquinovopyranoside [i) 7′20″, 9′14″; iii) column temp. 135 °C, 13′46″, 19′22″; *Rf* = 0.45, 0.65], and methyl 3,4-di-*O*-methylxylopyranoside [i) 9′14″, 10′44″; iii) column temp. 135 °C, 18′10″, 22′22″; *Rf* = 0.40].

Methylation of Echinoside A (11) Followed by Methanolysis——An ice-cooled solution of **11** (200 mg) in DMSO (10 ml) and dry THF (6 ml) was treated with a dimsyl carbanion solution (15 ml), and the mixture was stirred at room temp. (22 °C) under an N_2 atmosphere for 1 h, then treated with CH_3I (7 ml) under ice-cooling. The whole was stirred in the dark at room temp. for a further 2 h. Work-up of the reaction mixture as described above for methylation of **9** yielded a product, which was purified by column chromatography (SiO_2 40 g, $CHCl_3$–MeOH = 10 : 1) to furnish **11a** (95 mg). **11a**: Colorless glass, $[\alpha]_D^{21}$ −7° (*c* = 0.5, $CHCl_3$). *Anal.* Calcd for $C_{65}H_{109}NaO_{26}S\cdot 2H_2O$[12]: C, 55.84; H, 8.15; S, 2.29. Found: C, 55.54; H, 7.98; S, 2.29. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3430 (w), 1750. A solution of **11a** (7 mg) in 2.5 N AcCl–MeOH (1 ml) was heated under reflux for 1 h, then cooled. The reaction mixture was neutralized with Ag_2CO_3 and filtered. The filtrate was subjected to GLC analysis [i) column of NPGS (*vide supra*), temp. 180 °C, N_2 flow rate 32 ml/min; and iii) column of BDS (*vide supra*), temp. 185 °C, N_2 flow rate 35 ml/min] and TLC analysis (benzene–acetone = 2 : 1) to identify methyl 2,3,4,6-tetra-*O*-methylglucopyranoside [i) 5′46″, 7′48″; *Rf* = 0.75], methyl 2,4,6-tri-*O*-methylglucopyranoside [iii) 5′52″, 8′06″; *Rf* = 0.20, 0.35], methyl 2,3-di-*O*-methylquinovopyranoside [i) 5′22″, 6′45″; *Rf* = 0.65], and methyl 3-*O*-methylxylopyranoside [iii) 6′28″, 9′33″; *Rf* = 0.10, 0.15].

Oxidation of 10 Giving 13——A solution of **10** (400 mg) in dioxane (50 ml)–water (50 ml) was treated with aq. 2 N H_2SO_4 (100 ml) and the CrO_3 reagent (1.2 g) (*vide supra*),[7] and the mixture was stirred at room temp. (26 °C) for 19 h. Work-up of the reaction mixture as described above for the oxidation of **9** gave a product (493 mg), which was purified by column chromatography (SiO_2 50 g, $CHCl_3$–MeOH–H_2O = 12 : 3 : 1, lower phase) to furnish **13** (175 mg) and **10** (80 mg, recovered). **13**: Colorless needles, mp 206—207.5 °C (MeOH), $[\alpha]_D^{13}$ +1.5° (*c* = 1.0, MeOH). *Anal.* Calcd for $C_{54}H_{86}O_{23}\cdot 2H_2O$: C, 56.93; H, 7.96. Found: C, 56.92; H, 7.83. IR ν_{max}^{KBr} cm^{-1}: 3492, 1755, 1655. UV λ_{max}^{MeOH} nm (ε): 258 (9600). CD (*c* = 1.5 × 10^{-1}, MeOH): $[\theta]_{385}$ 0, $[\theta]_{327}$ −18500 (neg. max.), $[\theta]_{287}$ 0, $[\theta]_{256}$ +54000 (pos. max.), $[\theta]_{220}$ 0, $[\theta]_{212}$ −20000 (neg. max.), $[\theta]_{206}$ 0. ^{13}C-NMR (25 MHz, d_5-pyridine, δc): 104.6 (d), 105.5 (d), 118.8 (d, C-11), 169.1 (s, C-9), 177.5 (s, C-18), 196.7 (s, C-12).

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References and Notes

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