Communications to the Editor

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Structures of Echinoside A and B, Two Antifungal Oligoglycosides from the Sea Cucumber *Actinopyga echinites* (JAEGER)

On the basis of chemical and physicochemical evidence, the structures of two antifungal oligoglycosides, echinoside A and B from the sea cucumber *Actinopyga echinites* (JAEGER), have been elucidated as **5** and **3**, respectively.

Keywords——sea cucumber; *Actinopyga echinites* (JAEGER); lanostane-type triterpene; oligoglycoside; ^{1}H-NMR; ^{13}C-NMR; snail enzyme

In a continuing study on biologically active oligoglycosides originated from sea cucumber[1] and starfish,[2] we have elucidated the chemical structures of two antifungal oligoglycosides named echinoside A (**5**) and B (**3**) which were isolated from the sea cucumber *Actinopyga echinites* (JAEGER). This paper communicates evidence supporting the proposed structures.

Chromatographic purifications of the MeOH ext. of the body wall of *A. echinites* (collected in Okinawa Pref. in July) furnished echinoside A and B (0.7% and 4% from the ext.). The major one, echinoside B (**3**), $C_{41}H_{65}O_{16}SNa\cdot H_2O$,[3] mp 203.5—204.5°, $[\alpha]_D$ −2° (pyr.), UV (MeOH): transparent above 210 nm, IR (KBr) cm^{-1}: 3400 (br), 1738 (br, γ-lactone), 1230 (br, sulfate),[4] CD (MeOH): $[\theta]_{233}$ −6000 (neg. max.), exhibits the positive potassium rhodizonate test.[5] On acid hydrolysis, echinoside B liberated one mole each of D-xylose and D-quinovose together with a dienic triterpene-lactone (**6**), $C_{30}H_{46}O_4$, mp 257—260° (dec.), UV (MeOH) max.: 237 nm (ε=15000), 244 (16000), 252 (11000).

$LiAlH_4$ reduction followed by acetylation of **6** yielded a tetraol-diacetate (**7a**), $C_{34}H_{54}O_6$, mp 181—182.5°, which, on $Pb(OAc)_4$ oxidation, was decomposed to give 6-methylheptan-2-one (**9**) and an octanortriterpene diacetate (**8a**) which was identical with an authentic sample prepared from 22,25-epoxy-holosta-7,9(11)-diene-3β,17α-diol (**10**)[1] through the analogous procedure. The 20(*S*) configuration in **6** has been assumed on the basis of a fairly large pyridine-induced downfield shift[1,6] of 20-Me proton signal of its 3-monoacetate (**6a**) (Table I).

Solvolysis[2,7] of echinoside B (**3**) furnished a desulfated derivative (**2**), $C_{41}H_{66}O_{13}\cdot H_2O$, mp 226—228°, which was methylated[8] to give a hepta-O-methyl derivative (**2a**) [two β-anomeric protons in ^{1}H-NMR spectrum: δ 4.17, 4.52 (1H both, d, J=7 Hz)]. On methanolysis, **2a** liberated Me 2,3,4-tri-O-Me-quinovopyranoside and Me 3,4-di-O-Me-xylopyranoside, while a hexa-O-methyl derivative (**3a**) of echinoside B yielded Me 2,3,4-tri-O-Me-quinovopyranoside and Me 3-O-Me-xylopyranoside, thus showing that the sulfate group in echinoside B attaches to 4′-OH of the xyloside moiety.

1) *a*) I. Kitagawa, T. Nishino, T. Matsuno, H. Akutsu, and Y. Kyogoku, *Tetrahedron Lett.*, **1978**, 985; *b*) I. Kitagawa, T. Nishino, and Y. Kyogoku, *ibid.*, **1979**, 1419; *c*) The 22 (S) configuration in **10** has been determined recently: I. Kitagawa and T. Nishino, presented at the 99th Annual Meeting of Pharmaceutical Society of Japan (Sapporo, Aug. 28—30, 1979), Abstract Papers, p. 168.
2) *a*) I. Kitagawa, M. Kobayashi, and T. Sugawara, *Chem. Pharm. Bull.*, **26**, 1852 (1978); *b*) I. Kitagawa and M. Kobayashi, *ibid.*, **26**, 1864 (1978).
3) Compounds given with the chemical formulae gave the satisfactory analytical values.
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5) *a*) D.P. Burma, *Anal. Chim. Acta*, **9**, 513 (1953); *b*) J.J. Schneider and M.L. Lewbart, *J. Biol. Chem.*, **222**, 787 (1956).
6) *a*) P.V. Demarco, E. Farkas, D. Doddrell, N.L. Mylari, and E. Wenkert, *J. Am. Chem. Soc.*, **90**, 5480 (1968); *b*) I. Kitagawa, M. Yoshikawa, and I. Yosioka, *Tetrahedron Lett.*, **1974**, 469.
7) J. McKenna and J.K. Norymberski, *J. Chem. Soc.*, **1957**, 3889.
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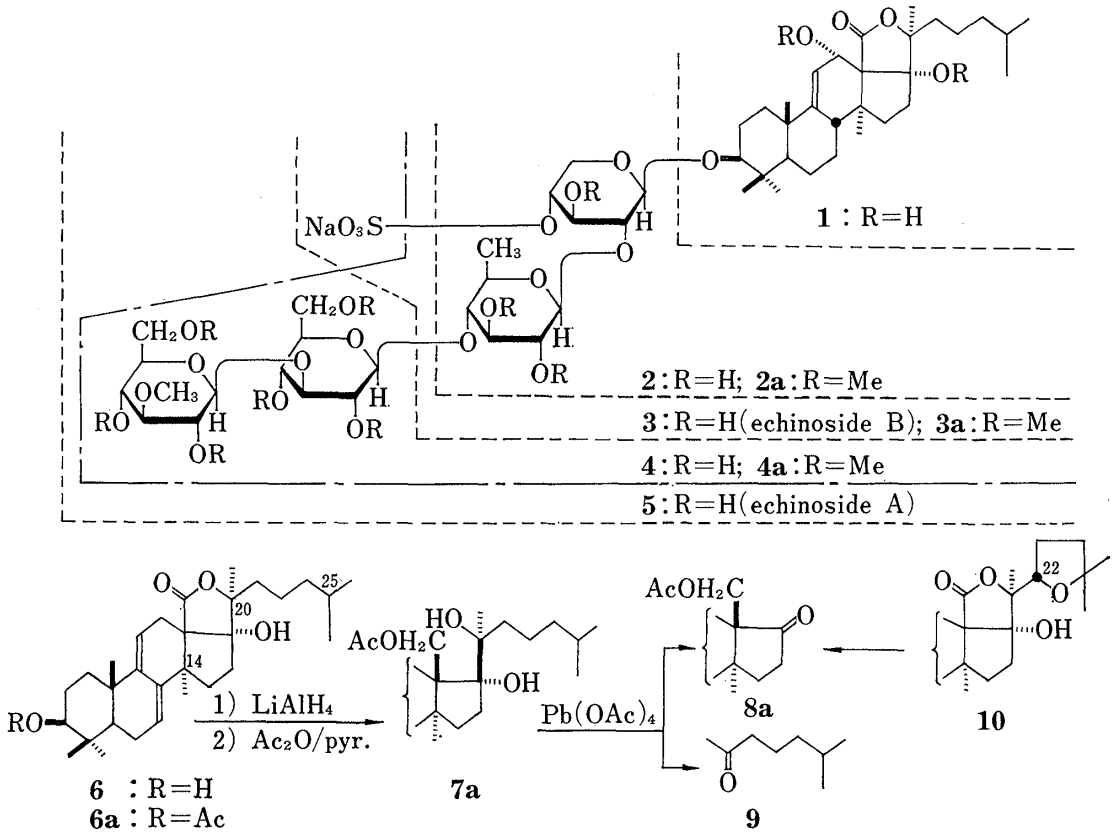

Chart 1

Table I. ^{1}H-NMR Data for **6a** (90 MHz)

Solvent	4-Me$_2$	10-Me	14-Me	20-Me	25-Me$_2$	7-H	11-H
$CDCl_3$	0.89, 0.97	1.12[a]	1.14[a]	1.40	0.90	5.48(m)	5.26(m)
d_5-pyr.	0.91, 1.01	1.34	1.48	1.61	0.81	5.61(m)	5.35(m)

a) The assignments are interexchangeable.

As for the structure of genuine aglycone (**1**) of echinoside B (**3**), the presence of 9(11)-en-12α-ol structure, as proved in holothurin A[1b] and B,[1a] has been presumed on the basis of ^{1}H-NMR analysis (d_5-pyr.) [δ 5.58 (d, J=4 Hz, 11-H), 4.94 (d, J=4 Hz, 12β-H)][1] and ^{13}C-NMR analysis (d_5-pyr.) of **3** [δ 153.9 (s, 9-C), 115.6 (d, 11-C), 71.4 (d, 12-C)].[1] Furthermore, the glycosidation shift[1,9] observed for 3-C [δ 88.7 (d)] of **3** shows that the disaccharide moiety attaches at 3β-OH of the aglycone (**1**), thus the chemical structure of echinoside B being elucidated as **3**.

Echinoside A (**5**), $C_{54}H_{87}O_{26}SNa\cdot 2H_2O$, mp 228—230°, $[\alpha]_D$ −6.0° (pyr.), UV (MeOH): transparent above 210 nm, IR (KBr) cm^{-1}: 3380 (br), 1745 (br), 1260 (br), CD (MeOH): $[\theta]_{223}$ −4000 (neg. max.), also shows the positive potassium rhodizonate test. On acid hydrolysis, echinoside A gave the artifact aglycone (**6**) along with one mole each of D-xylose, D-quinovose, D-glucose, and 3-O-Me-D-glucose, while it furnished echinoside B (**3**) and **2** on enzymatic hydrolysis with the glycosidase fraction prepared from snail.[10] Solvolysis of

9) *a*) R. Kasai, M. Suzuo, J. Asakawa, and O. Tanaka, *Tetrahedron Lett.*, **1977**, 175; *b*) K. Tori, S. Seo, Y. Yoshimura, H. Arita, and Y. Tomita, *ibid.*, **1977**, 179.

10) Prepared from snail by employing the method reported by A. Okano, K. Hoji, T. Miki, and M. Miyatake, *Chem. Pharm. Bull.*, **5**, 167 (1957).

echinoside A gave a desulfated derivative (**4**), $C_{54}H_{88}O_{23}\cdot 2H_2O$, mp 237—239°. On methanolysis, the trideca-O-methyl derivative (**4a**) [four β-anomeric protons: all doublets at δ 4.33 (J=7 Hz), 4.37 (J=8 Hz), 4.66 (J=7 Hz), 4.68 (J=7 Hz)] liberated one part each of Me 3,4-di-O-Me-xylopyranoside, Me 2,3-di-O-Me-quinovopyranoside, Me 2,4,6-tri-O-Me-glucopyranoside, and Me 2,3,4,6-tetra-O-Me-glucopyranoside. Consequently, the chemical structure of echinoside A has been elucidated as **5**.

The structures **3** and **5** proposed for echinoside B and A are further supported by the ^{13}C-NMR data for echinoside B, desulfated echinoside B, and echinoside A which will be reported in detail later together with the antifungal activities of echinoside A (**5**), B (**3**), and their derivatives.

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Faculty of Pharmaceutical Sciences,
Osaka University,
133-1 Yamada-kami, Suita,
Osaka 565 Japan

ISAO KITAGAWA
TATSUYA INAMOTO
MASAKO FUCHIDA
SHINJI OKADA
MOTOMASA KOBAYASHI
TAKAO NISHINO

Institute for Protein Research,
Osaka University,
5311, Yamada-kami, Suita,
Osaka 565, Japan

YOSHIMASA KYOGOKU

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Formation of *m*-Tyrosine and *o*-Tyrosine from L-Phenylalanine by Rat Brain Homogenate

Incubation of L-phenylalanine with rat brain homogenate in the presence of a pteridine co-factor and 2-mercaptoethanol gave rise to three hydroxylated products which were identified with high-performance liquid chromatography as *p*-tyrosine, *m*-tyrosine and *o*-tyrosine.

Keywords——enzymatic hydroxylation of phenylalanine; *m*-tyrosine; *o*-tyrosine; rat brain; fluorescence high-performance liquid chromatography

It is well known that phenylalanine is in large part metabolized by conversion to *p*-tyrosine (tyrosine) in mammal.[1] In addition, Tong *et al.*[2] reported the formation of *m*-tyrosine (*m*-hydroxyphenylalanine) from phenylalanine by beef adrenal medulla preparation *in vitro*. However, the formation of *o*-tyrosine (*o*-hydroxyphenylalanine) by mammalian tissues has not been found. The present communication describes that when phenylalanine is incubated with rat brain homogenate in the presence of a pteridine co-factor, besides *p*- and *m*-tyrosines, *o*-tyrosine is also formed in the reaction mixture.

1) A. Meister (ed.), "Biochemistry of the Amino Acids," Vol. 2, Academic Press, New York, 1965, p. 909.
2) J.H. Tong, A.D' Iorio, and N.L. Benoiton, *Biochem. Biophys. Res. Commun.*, **44**, 229 (1971).