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Structures of Echinoid 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, echinoid 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; ^1H -NMR; ^{13}C -NMR; snail enzyme

In a continuing study on biologically active oligoglycosides originated from sea cucumber¹⁾ and starfish,²⁾ we have elucidated the chemical structures of two antifungal oligoglycosides named echinoid 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 echinoid A and B (0.7% and 4% from the ext.). The major one, echinoid B (**3**), $\text{C}_{41}\text{H}_{65}\text{O}_{16}\text{SNa}\cdot\text{H}_2\text{O}$,³⁾ mp 203.5—204.5°, $[\alpha]_{\text{D}} -2^\circ$ (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, echinoid B liberated one mole each of D-xylose and D-quinovose together with a dienic triterpene-lactone (**6**), $\text{C}_{30}\text{H}_{46}\text{O}_4$, mp 257—260° (dec.), UV (MeOH) max.: 237 nm ($\epsilon=15000$), 244 (16000), 252 (11000).

LiAlH_4 reduction followed by acetylation of **6** yielded a tetraol-diacetate (**7a**), $\text{C}_{34}\text{H}_{54}\text{O}_6$, mp 181—182.5°, which, on $\text{Pb}(\text{OAc})_4$ 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-3 β ,17 α -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 echinoid B (**3**) furnished a desulfated derivative (**2**), $\text{C}_{41}\text{H}_{66}\text{O}_{13}\cdot\text{H}_2\text{O}$, mp 226—228°, which was methylated⁸⁾ to give a hepta-O-methyl derivative (**2a**) [two β -anomeric protons in ^1H -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 echinoid B yielded Me 2,3,4-tri-O-Me-quinovopyranoside and Me 3-O-Me-xylopyranoside, thus showing that the sulfate group in echinoid B attaches to 4'-OH of the xyloside moiety.

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- 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).
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- 8) S. Hakomori, *J. Biochem. (Tokyo)*, **55**, 205 (1964).

Chart 1

TABLE I. ¹H-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(w)

a) The assignments are interexchangeable.

As for the structure of genuine aglycone (1) of echinoside B (3), the presence of 9(11)-en-12 α -ol structure, as proved in holothurin A^{1b)} and B,^{1a)} has been presumed on the basis of ¹H-NMR analysis (d₅-pyr.) [δ 5.58 (d, J =4 Hz, 11-H), 4.94 (d, J =4 Hz, 12 β -H)]¹⁾ and ¹³C-NMR analysis (d₅-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 3 β -OH of the aglycone (1), thus the chemical structure of echinoside B being elucidated as 3.

Echinoside A (5), C₅₄H₈₇O₂₆SNa·2H₂O, mp 228–230°, [α]_D –6.0° (pyr.), UV (MeOH): transparent above 210 nm, IR (KBr) cm^{–1}: 3380 (br), 1745 (br), 1260 (br), CD (MeOH): [θ]₂₂₃ –4000 (neg. max.), also shows the positive potassium rhodizionate 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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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).