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The Structures of Six Antifungal Oligoglycosides, Stichlorosides A_1, A_2, B_1, B_2, C_1, and C_2, from the Sea Cucumber *Stichopus Chloronotus* (BRANDT)

Chemical structures are reported for six antifungal lanostane-type triterpene-oligoglycosides from the Okinawan sea cucumber *Stichopus chloronotus* (BRANDT). The compounds are: stichlorosides A_1 (**9**), B_1 (**15**), C_1 (**20**) [having stichlorogenol (**3**) as the aglycone] and A_2 (**10**), B_2 (**16**), C_2 (**21**) [dehydrostichlorogenol (**4**) as the aglycone].

Keywords——sea cucumber; *Stichopus chloronotus*; stichloroside A_1; stichloroside A_2; stichloroside B_1; stichloroside B_2; stichloroside C_1; stichloroside C_2; lanost-7-ene type triterpene

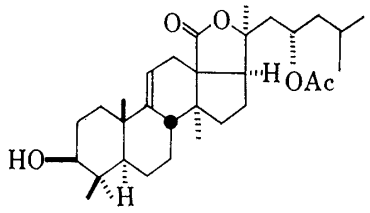
In a recent communication,[1] we reported the isolation of six antifungal triterpene-oligoglycosides, stichlorosides A_1, A_2, B_1, B_2, C_1, and C_2, from the Okinawan sea cucumber *Stichopus chloronotus* (BRANDT) and clarified the structure of their aglycones named stichlorogenol (**3**) and dehydrostichlorogenol (**4**), which are respectively the common genuine aglycones of stichlorosides A_1, B_1, C_1, and A_2, B_2, C_2. This is a report on the structure of the parent oligoglycosides, stichlorosides A_1 (**9**), A_2 (**10**), B_1 (**15**), B_2 (**16**), C_1 (**20**), and C_2 (**21**).

Stichloroside A_1 (**9**)[1] is a hexaglycoside having two moles each of xylose (xyl), glucose (glu), and 3-O-methylglucose (3-Me-glu) in its oligosaccharide moiety.[2] The ^{13}C NMR spectrum of A_1 shows the β-glycosidic nature of these six monosaccharide moieties as judged by the anomeric carbon signals [δ_c 106.4, 105.5, 103.3, and 102.9 (all d)[3]] and also shows the presence of one acetoxyl group [δ_c 170.7(s)]. On acidic hydrolysis (2N aq. H_2SO_4), A_1 liberated two artifact aglycones **1**[1] and **2**,[1] showing that the oilgosaccharide moiety of A_1 attaches to 3β-OH and the acetyl group to 23-OH of stichlorogenol (**3**).

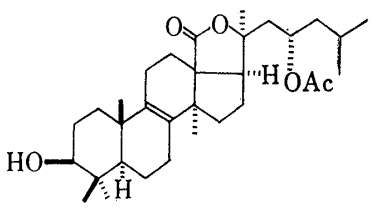
Alkaline treatment (1/6 N NaOMe–MeOH) of A_1 (**9**) gave desacetylstichloroside A_1 (**8**), $C_{66}H_{108}O_{32}\cdot 3H_2O$,[4] mp 211—212°C, $[\alpha]_D^{15}$ −36° (pyr.), which, on enzymic hydrolysis with crude naringinase,[5] yielded stichlorogenol (**3**) and three partial hydrolysates: A-pro-1 (**5**), $C_{35}H_{56}O_8$, mp 270—271°C, $[\alpha]_D^{21}$ −45° (pyr.), (monosaccharide composition[2]: xyl×1), A-pro-2 (**6**), $C_{48}H_{78}O_{18}\cdot 2H_2O$, mp 253—255°C, $[\alpha]_D^{18}$ −38° (pyr.), (xyl×1, glu×1, 3-Me-glu×1), and A-pro-3 (**7**), $C_{53}H_{86}O_{22}\cdot 2H_2O$, mp 256—257°C, $[\alpha]_D^{21}$ −44° (pyr.), (xyl×2, glu×1, 3-Me-glu×1).

Methylation of these hydrolysates with CH_3I-NaH–tetrahydrofuran[6] afforded their respective fully methylated derivatives: **5a** [anom. H at δ 4.21 (1H, d, J=7 Hz)], **6a** [δ 4.26 (2H, d, J=7), 4.63 (1H, d, J=8)], and **7a** [δ 4.24 (1H, d, J=8), 4.30 (1H, d, J=6), 4.62 (2H, d, J=6)]. Methanolysis of these methylated derivatives liberated the following methyl glycosides: Me 2,3,4-tri-O-Me-xylopyranoside from **5a**, Me 2,3,4,6-tetra-O-Me-glucopyranoside, Me 2,4,6-tri-O-Me-glucopyranoside, and Me 2,3-di-O-Me-xylopyranoside from **6a**, and Me 2,3,4-tri-O-Me-xylopyranoside, Me 2,3,4,6-tetra-O-Me-glucopyranoside, Me 2,4,6-tri-O-Me-glucopyranoside, and Me 3-O-Me-xylopyranoside from **7a**. Based on these findings, the structures of A-pro-1 (**5**), A-pro-2 (**6**), and A-pro-3 (**7**) have been substantiated.

Methylation[6] of desacetylstichloroside A_1 (**8**) gave an octadeca-O-methyl derivative (**8a**),



1



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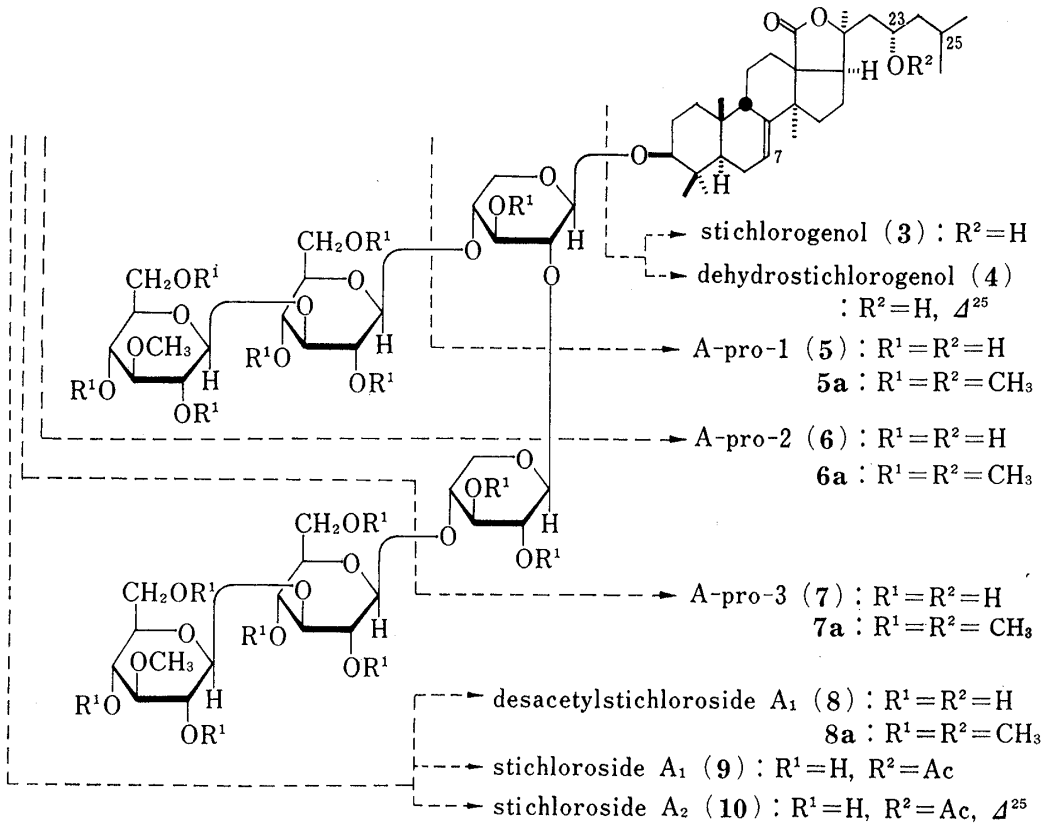

Chart 1

which shows six anomeric proton signals [δ 4.22 (2H, d, J=7 Hz), 4.33 (1H, d, J=8), 4.61 (2H, d, J=7), and 4.66 (1H, d, J=7)] in its ^{1}H NMR spectrum. Methanolysis of **8a** liberated Me 2,3,4,6-tetra-O-Me-glucopyranoside, Me 2,4,6-tri-O-Me-glucopyranodsie, Me 2,3-di-O-Me-xylopyranoside, and Me 3-O-Me-xylopyranoside, supporting the monosaccharide sequence of desacetylstichlorodise A_1 as shown in **8**, thus the total structure of stichloroside A_1 was formulated as **9**.

Catalytic hydrogenation over 5% Pd-C of stichloroside A_2 (**10**), which is a hexaglycoside of dehydrostichlorogenol (**4**),[1] gave quantitatively stichloroside A_1 (**9**) as identified by TLC (SiO_2-$AgNO_3$), HPLC (μBondapak C_{18}), and $[\alpha]_D$ comparisons and mixed mp determination. Therefore, the structure **10** for stichloroside A_2 has been evidenced.

Stichloroside B_1 (**15**) is a hexaglycoside (xyl×2, glu×2,3-Me-glu×2)[2] of stichlorogenol (**3**).[1] As in the case of stichloroside A_1 (**9**), the ^{13}C NMR examination and the acidic hydrolysis of B_1 have shown that the sugar moiety and the acetyl group in B_1 attach to 3β-OH and 23-OH of the aglycone. Alkaline treatment of B_1 gave desacetylstichloroside B_1 (**14**), $C_{66}H_{108}O_{32}\cdot 2H_2O$, mp 265—266°C, $[\alpha]_D^{15}$ −34° (pyr.), which on acidic hydrolysis (0.5 N aq. HCl–*n*-BuOH),[7] yielded three hydrolysates[2]: B-pro-1 (**11**),[8] $C_{35}H_{56}O_8$, mp 283—285°C, $[\alpha]_D^{15}$ +0.4° (pyr.),

(xyl×1), B-pro-2 (**12**),[8] $C_{48}H_{78}O_{18}\cdot 3H_2O$, mp 265—267°C, $[\alpha]_D^{15}$ −21° (pyr.), (xyl×1, glu×1, 3-Me-glu×1), and B-pro-3 (**13**),[8] $C_{54}H_{88}O_{23}\cdot 2H_2O$, mp 285—287°C, $[\alpha]_D^{17}$ −23° (DMSO), (xyl×1, glu×2, 3-Me-glu×1). Methylation[6] of these hydrolysates furnished their fully methylated derivatives: **11a**, **12a**, **13a**, and **14a**.

The structures of the oligosaccharide moieties are based on the physical properties of the methyl ethers and their methanolysis as carried out for stichloroside A_1 and its hydrolysates. Final evidence for the total structure of desacetylstichloroside B_1 (**14**) and stichloroside B_1 (**15**) comes from the fact that periodate oxidation of desacetylstichloroside B_1 (**14**) followed by $NaBH_4$ reduction and methanolysis furnished erythritol.[9]

The structure of stichloroside B_2 (**16**),[1] which is another hexaglycoside of dehydrostichlorogenol (**4**), has been elucidated on the same basis as that described for A_2 (**10**). Catalytic hydrogenation of B_2 over 5% Pd-C quantitatively gave B_1 (**15**).

For the structural elucidation of stichloroside C_1 (**20**), which is a hexaglycoside [xyl×2, quinovose (qui)×1, glu×1, 3-Me-glu×2][2] of stichlorogenol (**3**),[1] and stichloroside C_2 (**21**), a sequence of investigations were carried out similar to those described for B_1 (**15**) and B_2 (**16**) with the preparation of the following derivatives: desacetylstichloroside C_1 (**19**), $C_{66}H_{108}O_{31}\cdot 2H_2O$, mp 250.5—251.5°C, $[\alpha]_D^{15}$ −41° (pyr.); and three acidic hydrolysates of **19**[7]: C-pro-1 (**17**),[8] $C_{41}H_{66}O_{12}\cdot 2H_2O$, mp 276—278°C, $[\alpha]_D^{15}$ −20° (pyr.), (xyl×1, qui×1), C-pro-2(=B-pro-2) (**12**),[8] C-pro-3 (**18**),[8] $C_{54}H_{88}O_{22}\cdot 3H_2O$, mp 271.5—273.5°C, $[\alpha]_D^{17}$ −34° (DMSO), (xyl×1, qui×1, glu×1, 3-Me-glu×1), and their fully methylated[6] derivatives: **17a**, **18a**, and **19a**. Finally, catalytic hydrogenation of stichloroside C_2 giving C_1 (**20**) has corroborated the structure **21**

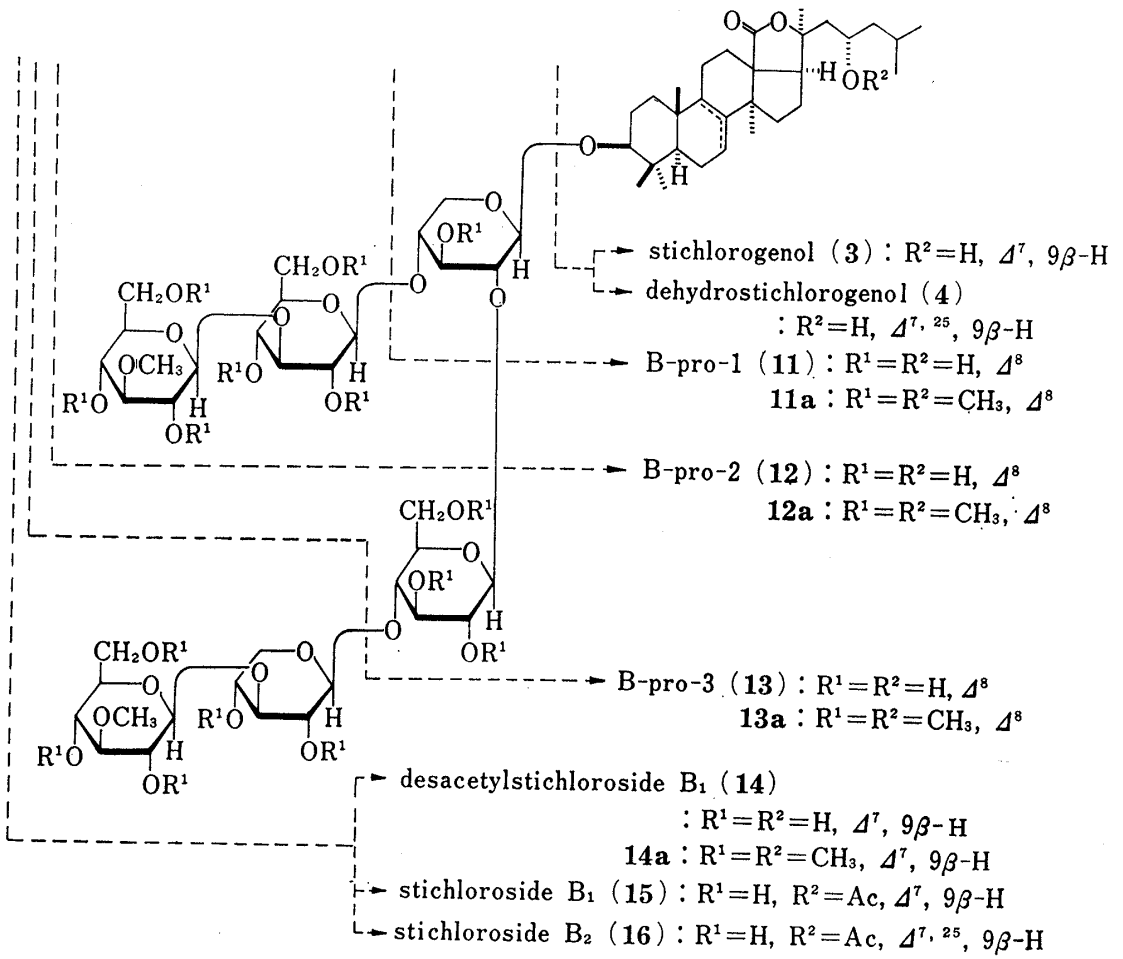

Chart 2

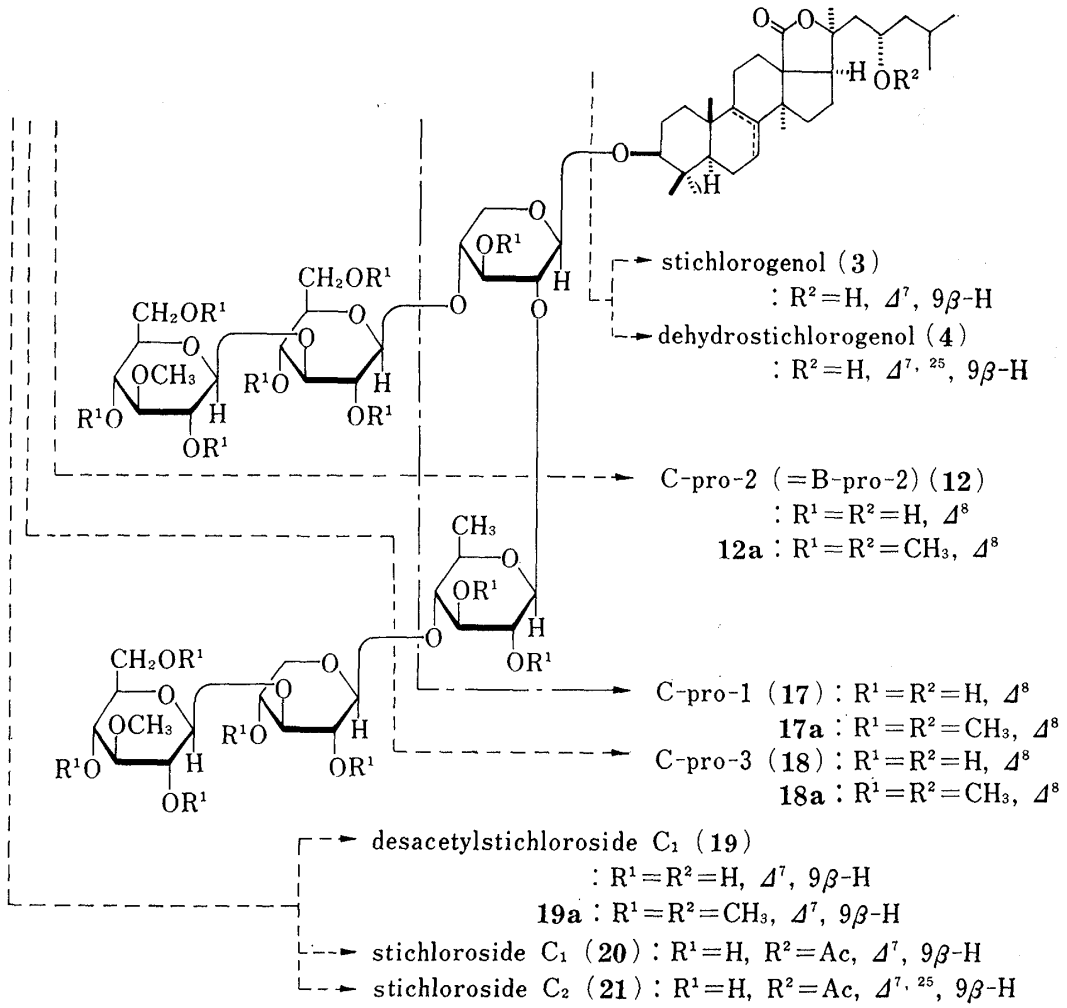

Chart 3

for C_2.

The microbial activities of stichlorosides will be reported elsewhere.

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References and Notes

1) I. Kitagawa, M. Kobayashi, T. Inamoto, T. Yasuzawa, Y. Kyogoku, and M. Kido, *Chem. Pharm. Bull.*, **29**, 1189 (1981).
2) The monosaccharide compositions were examined by methanolysis and subsequent GLC analysis (as TMS derivatives) and were determined on the basis of these analyses carried out for the parent oligoglycosides and their partial hydrolysates.
3) Due to overlap, only four signals were observed.
4) All compounds given with the chemical formulae gave satisfactory analytical values.
5) I. Kitagawa and M. Kobayashi, *Chem. Pharm. Bull.*, **26**, 1684 (1978).
6) H. Okabe, Y. Miyahara, T. Yamauchi, K. Miyahara, and T. Kawasaki, *Chem. Pharm. Bull.*, **28**, 2753 (1980).
7) Desacetylstichlorosides B_1 and C_1 were unaffected under enzymic hydrolysis using various kinds of glycosidase.
8) Since no olefinic proton signal was observed in the ^1H NMR spectra of the fully methylated derivatives,

the C-7 (8) double bond in the parent oligoglycoside (**14** or **19**) is presumed to be shifted to C-8 (9) in the hydrolysates during the acidic hydrolysis.[1)]

9) Based on this finding, another possible sugar sequence (3-Me-glu^{3}—xyl^{4}—3-Me-glu^{3}—glu^{4}—xyl^{3}(—glu^{2})—aglycone)

for B_1 has been ruled out. If this alternate sequence is correct, glycerol should be obtained instead of erythritol.

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Anti-Platelet Aggregation Principles from the Bark of *Fraxinus japonica* Blume[1)]

The methanol extract of the bark of *Fraxinus japonica* Blume was fractionated with the guidance of inhibitory activity on rabbit platelet aggregation induced by arachidonic acid. The following active principles were identified: 3-methoxy-4-hydroxyphenylethanol (I) (which also exists in the animal body as a dopamine metabolite), *p*-hydroxyphenylethanol (II), 2,6-dimethoxy-*p*-benzoquinone (III), compounds (IV), (V), and esculetin.

Keywords——the bark of *Fraxinus japonica* Blume; anti-aggregatory activity on platelet; 3-methoxy-4-hydroxyphenylethanol; *p*-hydroxyphenylethanol; 2,6-dimethoxy-*p*-benzoquinone; esculetin; 3,4-dihydroxyphenylethanol; dopamine metabolites

The bark of *Fraxinus japonica* Blume (the Oriental medicine "Shinpi"), which is widely distributed in Japan, has been used as a home remedy diuretic, antifebrile analgesic and so on.

The methanol extract of the bark is reported to have an anti-inflammatory action and to promote the excretion of uric acid.[2,3)] Esculin, a coumarin glycoside which is a main constituent of the extract, is an active principle having these physiological activities.[4)] Until now no other biologically active compound has been found in the bark.

The methanol extract of the bark was bioassayed for inhibitory activity on rabbit platelet aggregation induced by arachidonic acid (AA) and found to be active. We report here the isolation of the active principles guided by this bioassay[5)] and their potencies.

The methanol extract of the bark was partitioned between ethyl acetate and water. The acidic portion, which was fractionated from the ethyl acetate layer in the usual manner and had a significant inhibitory activity, was separated into eight fractions by Sephadex-LH 20 column chromatography. Two of the fractions, which showed a potent inhibitory activity, were further purified by the combination of high performance liquid chromatography (μBondapak C_{18}) and preparative thin-layer chromatography (silica gel). Finally, the compounds I, II, III, IV, V, and esculetin were isolated as the active principles. All except esculetin gave very low yields.