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Stichlorogenol and Dehydrostichlorogenol, Genuine Aglycones of Stichlorosides A_1, B_1, C_1 and A_2, B_2, C_2, from the Sea Cucumber *Stichopus Chloronotus* (BRANDT)

Six new antifungal triterpene-oligoglycosides named stichlorosides A_1, A_2, B_1, B_2, C_1, and C_2 have been isolated from the sea cucumber *Stichopus chloronotus* (BRANDT). The structures of their genuine aglycones stichlorogenol (**1**) (for A_1, B_1, and C_1) and dehydrostichlorogenol (**2**) (for A_2, B_2, and C_2) have been elucidated on the bases of chemical and physicochemical evidence together with the X-ray analysis of **1**. They are the first examples of lanost-7-ene type aglycones isolated from sea cucumber.

Keywords——sea cucumber; *Stichopus chloronotus*; lanost-7-ene type triterpene; oligoglycoside; stichlorogenol; dehydrostichlorogenol; stichloroside; CD; ^{13}C NMR; X-ray analysis

As a continuing study in search of bioactive echinoderm metabolites,1) we have been working on the chemical constituents of the sea cucumber *Stichopus chloronotus* (BRANDT) (Japanese name: shikaku-namako) and isolated six new antifungal triterpene-oligoglycosides named stichlorosides A_1, $C_{68}H_{110}O_{33}\cdot H_2O$,2) mp 213—215°, $[\alpha]_D^{15}$ −47° (pyr.), A_2, $C_{68}H_{108}O_{33}\cdot 3H_2O$, mp 205—207°, $[\alpha]_D^{15}$ −33° (pyr.), B_1, $C_{68}H_{110}O_{33}\cdot 2H_2O$, mp 270—271°, $[\alpha]_D^{15}$ −44° (pyr.), B_2, $C_{68}H_{108}O_{33}\cdot H_2O$, mp 265—266°, $[\alpha]_D^{15}$ −37° (pyr.), C_1, $C_{68}H_{110}O_{32}$, mp 251—253°, $[\alpha]_D^{15}$ −50° (pyr.), and C_2, $C_{68}H_{108}O_{32}$, mp 250—251°, $[\alpha]_D^{15}$ −45° (pyr.). During our structural investigation on these oligoglycosides, we have recently become aware of a preliminary report by Elyakov, *et al.*3) which comments on the genuine aglycone of the same sea cucumber. In this communication, we wish to present evidence verifying structures of stichlorogenol (**1**) and dehydrostichlorogenol (**2**) which are respectively genuine aglycones of stichlorosides A_1, B_1, C_1 and A_2, B_2, C_2.

In 1973, Djerassi, *et al.* reported a structural study of an aglycone (**3**, without definition of C-8, -20, and -23 configurations)4) which was obtained by acidic hydrolysis of the oligoglycosidic fraction of the same kind of sea cucumber collected in Australia. We collected the title sea cucumber in Okinawa Prefecture in July. After purification of the oligoglycosidic fraction from the body walls of the sea cucumber through successive column chromatography and high performance liquid chromatography, we isolated above-mentioned six stichlorosides (A_1, A_2, B_1, B_2, C_1, and C_2).

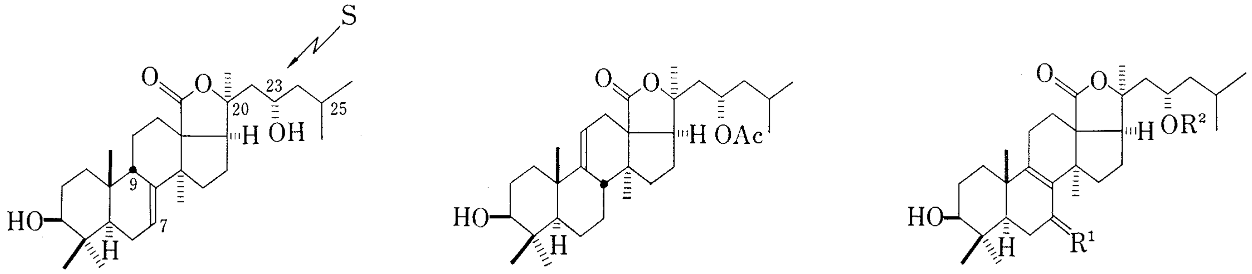
Acidic hydrolysis (2 N aq. H_2SO_4) of the total oligoglycoside yielded **3**4) and two aglycones which were respectively identical with two aglycones (**4** and **5**, without definition of the C-23 configuration)5) which were isolated by Tursch, *et al.* from the sea cucumber *Thelonota ananas* Jaeger. As shown in Table I, stichlorosides A_1, B_1, and C_1 comprise a γ-lactone moiety (exhibiting a positive CD maximum) and a trisubstituted double bond (a strong negative CD maximum) in their triterpenoidal portions whereas stichlorosides A_2, B_2, and C_2 comprise an additional terminal methylene moiety as judged by the ^{13}C NMR data.6) Since the ^{13}C NMR and CD data for **3**, **4**, and **5** were inconsistent with those for triterpenoidal portions of stichlorosides, the three aglycones have been considered artifacts.6)

After acetylation, when total stichloroside was subjected to NBS–$CaCO_3$-fluorescent lamp oxidation7) followed by deacetylation and acidic hydrolysis, a major aglycone (**6**), $C_{30}H_{46}O_5$, mp 247—248°, $[\alpha]_D^{25}$ +33° ($CHCl_3$), was obtained. This new aglycone possesses an 8-en-7-one moiety as shown by UV λ_{max}^{MeOH} nm: 255 (ε 8000) and CD (MeOH): $[\theta]_{324}$ +4700 (pos. max.), $[\theta]_{255}$ −10000 (neg. max.), $[\theta]_{218}$ +30000 (pos. max.).8) The isolation of this aglycone (**6**) and the above-mentioned ^{13}C NMR and CD data for stichlorosides have led us to presume that the genuine aglycone may comprise a 7-ene moiety9,10) instead of a 9(11)-ene moiety

TABLE I. ^{13}C NMR and CD Data

	δ_c (d_5-pyridine)[a]	CD maxima (MeOH)
Stichloroside A_1	120.1(d), 146.7(s)	$[\theta]_{200}$ −40000 (neg.), $[\theta]_{217}$ +8500 (pos.)
Stichloroside A_2	120.1(d), 146.7(s) 114.2(t), 141.7(s)	$[\theta]_{200}$ −33000 (neg.), $[\theta]_{216}$ +6700 (pos.)
Stichloroside B_1	120.0(d), 146.6(s)	$[\theta]_{200}$ −30000 (neg.), $[\theta]_{216}$ +7700 (pos.)
Stichloroside B_2	120.1(d), 146.7(s) 114.3(t), 141.7(s)	$[\theta]_{200}$ −40000 (neg.), $[\theta]_{216}$ +8500 (pos.)
Stichloroside C_1	120.0(d), 146.7(s)	$[\theta]_{200}$ −37000 (neg.), $[\theta]_{217}$ +6200 (pos.)
Stichloroside C_2	120.1(d), 146.7(s) 114.3(t), 141.7(s)	$[\theta]_{200}$ −34000 (neg.), $[\theta]_{216}$ +8000 (pos.)
Stichlorogenol (1)	120.1(d), 146.9(s)	$[\theta]_{200}$ −30000 (neg.), $[\theta]_{216}$ +6000 (pos.)
Dehydrostichlorogenol (2)	120.1(d), 146.9(s) 113.3(t), 143.3(s)	$[\theta]_{200}$ −30000 (neg.), $[\theta]_{218}$ +6400 (pos.)
3	111.2(d), 151.7(s)	$[\theta]_{200}$ +40000 (pos.), $[\theta]_{222}$ −20000 (neg.)
4	[b]	$[\theta]_{202}$ +41000 (pos.), $[\theta]_{222}$ −18000 (neg.)
5	[b]	$[\theta]_{203}$ −19000 (neg.), $[\theta]_{230}$ +17000 (pos.)
Abieslactone[9] (9β-lanost-7-ene deriv.)	[c]	$[\theta]_{205}$ −30000!
9α-lanost-7-ene[10]	[c]	$[\theta]_{209}$ −29000 (neg.)
Holotoxin A[14] (8β-lanost-9(11)-ene deriv.)	112.2(d), 151.6(s) 110.4(t), 145.5(s)	$[\theta]_{200}$ +49000 (pos.), $[\theta]_{234}$ −24000 (neg.), $[\theta]_{305}$ −19000 (neg. max.)

[a] Measured at 60°. Abbreviations given in the parentheses denote the signal patterns observed in the off-resonance experiments.
[b] Not measured.
[c] Unavailable.



stichlorogenol (1)
dehydrostichlorogenol (2) : Δ25

3
4: Δ25

5: $R^1=H_2$, $R^2=Ac$
6: $R^1=O$, $R^2=H$

Chart 1

which is common in hitherto known sea cucumber aglycones.[1]

On enzymic hydrolysis using crude naringinase,[11] stichlorosides A_1 and A_2 respectively liberated stichlorogenol (**1**), $C_{30}H_{48}O_4 \cdot CH_3OH$, mp 225—226°, $[\alpha]_D^{20}$ −42° ($CHCl_3$), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1756 (γ-lactone), ^1H NMR ($CDCl_3$, δ): 0.90, 1.02, 1.03, 1.13 (all 3H, s, *tert.* $CH_3 \times 4$), 0.93 (6H, d, $J=7$ Hz, *sec.* CH_3), 1.53 (3H, s, 20-CH_3), 3.20 (1H, t-like, 3-H),[12] 3.45 (s, OCH_3),[13] 3.90 (1H, m, 23-H), 5.53 (1H, m, 7-H), and dehydrostichlorogenol (**2**), $C_{30}H_{46}O_4$, mp 239—240°, $[\alpha]_D^{17}$ −35° ($CHCl_3$), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1757 (γ-lactone), 892 (terminal methylene), ^1H NMR: 0.88 (3H), 1.03 (6H), 1.13 (3H) (all s, *tert.* $CH_3 \times 4$), 1.55 (3H, s, 20-CH_3), 1.75 (3H, s, 25-CH_3), 3.22 (1H, t-like, 3-H), 3.91 (1H, m, 23-H), 4.78, 4.87 (1H each, br.s, 26-H_2), 5.57 (1H, m, 7-H). Catalytic hydrogenation of **2** over Pd–C furnished **1**, thus **2** being substantiated to be the 25-dehydro derivative of **1**.

Based on the above evidence and by comparison of the ^{13}C NMR and CD data for stichlorogenol and dehydrostichlorogenol with those for stichlorosides (Table I), it has become evident that i) stichlorogenol and dehydrostichlorogenol are respectively genuine aglycones of stichlorosides A_1, B_1, C_1 and A_2, B_2, C_2, and ii) stichlorogenol and dehydrostichlorogenol correspond to 7-en-23-ol analogs of **3** and **4**, respectively.

In order to clarify the whole structure of stichlorogenol (**1**) including the C-9, C-20, and C-23 configurations, the X-ray analysis was carried out.

Crystal data: $C_{30}H_{48}O_4 \cdot CH_3OH$, F.W.=504.75, monoclinic, $P2_1$, $a=7.596(7)$, $b=18.200(15)$, $c=10.773(6)$ Å, $\beta=92.89(6)°$, $Z=2$, $Dm=1.11$ g/cm^3, $Dx=1.12$ g/cm^3, $\mu=$(Mo Kα)$=0.8$ cm^{-1}. The cell dimensions and intensities were measured on a Syntex R3 four-circle diffractometer with a graphite-monochromated Mo Kα radiation with ω-scan mode within 2θ less than 55°. A total of 3525 independent reflections were collected, among which 2131 reflections ($I>1.96\sigma(I)$) were stored as observed. The structure was solved by the direct method using MULTAN in Syntex XTL program. All hydrogen atoms except four were found on a difference Fourier map. Block-diagonal least-squares method was applied to the refinement, the final R-value being 0.069. A perspective view of the molecule of stichlorogenol(**1**) with 23S configuration is presented in Fig. 1.

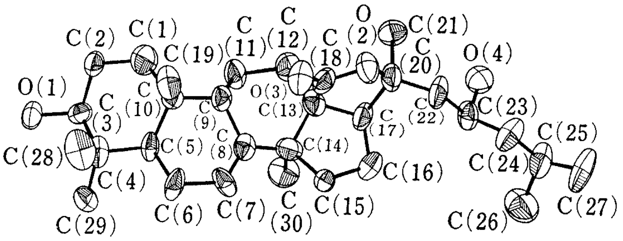


Fig. 1

As is apparent from this view, the C-ring of stichlorogenol (**1**) with 9β-H configuration is in a strained boat form, which may be a principal reason for ready conversion from genuine 7-ene moieties (**1**, **2**) to artifact 8-ene and 9(11)-ene moieties (*e.g.* **3**, **4**, **5**)[9] during acidic hydrolysis of parent stichlorosides. It should also be pointed out that the isomerization from the 7-ene structure (**1**, **2**) to the 9(11)-ene structure (**3**, **4**) causes inversion of the sign of CD maximum due to the γ-lactone moiety from positive to negative (*cf.* holotoxins[14]). Furthermore, structures of **3**, **4**, and **5** have now been fully characterized. Stichlorogenol (**1**) and dehydrostichlorogenol (**2**) are the first examples of lanost-7-ene type aglycones among hitherto known sea cucumber aglycones.

Glycosidic structures of stichlorosides will be reported in our forthcoming paper.

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References and Notes

1) I. Kitagawa, M. Kobayashi, M. Hori, and Y. Kyogoku, *Chem. Pharm. Bull.*, **29**, 282 (1981), and preceding papers cited therein.
2) Elemental compositions of all compounds given with the chemical formulae were confirmed by elemental analyses or high resolution mass spectrometry.
3) A.I. Kalinovsky, V.F. Sharypov, V.A. Stonik, A.K. Dzizenko, and G.B. Elyakov, *Bioorg. Khim.*, **6**, 86 (1980).
4) I. Rothberg, B.M. Tursch, and C. Djerassi, *J. Org. Chem.*, **38**, 209 (1973).
5) A. Kelecom, D. Daloze, and B. Tursch, *Tetrahedron*, **32**, 2353 (1976).
6) The detail will be reported in our full paper.
7) B.W. Finucane and J.B. Thomson, *Chem. Commun.*, **1969**, 1220.
8) These data have been assigned on the basis of the helicity rule: C. Djerassi, R. Records, E. Bunnenberg, K. Mislow, and A. Moscowitz, *J. Am. Chem. Soc.*, **84**, 870 (1962).
9) H. Irie, S. Uyeo, and K. Kuriyama, *Tetrahedron Lett.*, **1971**, 3467.
10) J. Hudec and D.N. Kirk, *Tetrahedron*, **32**, 2475 (1976).
11) I. Kitagawa and M. Kobayashi, *Chem. Pharm. Bull.*, **26**, 1864 (1978).
12) This signal is overlapped by the signal of 9β-H which is shifted lower presumably due to an anisotropic effect of the γ-lactone carbonyl. In d_6-benzene–$CDCl_3$ (2: 1), signals due to 3-H and 9-H are observed at δ 3.06 (t-like) and 3.32 (m).
13) The signal intensity was smaller than 3H (*ca.* 1.5—2 H), the reason is yet obscure.
14) I. Kitagawa, H. Yamanaka, M. Kobayashi, T. Nishino, I. Yosioka, and T. Sugawara, *Chem. Pharm. Bull.*, **26**, 3722 (1978), and preceding papers cited therein.

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