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STOLONIFERONE-A, -B, -C, AND -D, FOUR NEW CYTOTOXIC STEROIDS FROM THE OKINAWAN SOFT CORAL CLAVULARIA VIRIDIS

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Summary: Four new cytotoxic steroids named stoloniferone-a (7a), -b (7b), -c (7c), and -d (7d) were isolated from the stoloniferan Okinawan soft coral *Clavularia viridis* and their absolute stereostructures were elucidated.

As a continuing study of the bioactive constituents from various marine organisms,[1] we recently isolated six new prostanoids named claviridenone-a (1), -b (2), -c (3), and -d (4), 20-acetoxy-claviridenone-b (5), and -c (6), from the stoloniferan Okinawan soft coral *Clavularia viridis* (Octocorallia) and elucidated their absolute stereostructures.[2] Claviridenones are characteristic not only of their unusual prostanoid structures but also of their cytotoxic and anti-tumor activities. Further examinations of the chemical constituents of the same stolonifer have led us to isolate four new cytotoxic steroids[3] named stoloniferone-a (7a), -b (7b), -c (7c), and -d (7d). The elucidations of their absolute stereostructures are the subjects of this communication.

The acetone extractive of the fresh stolonifer, collected in July at Koha-ma-jima, Okinawa Prefecture, was partitioned into an AcOEt-water mixture. Purification of the AcOEt soluble portion by SiO_2 column chromatography (hexane-AcOEt) and subsequent HPLC (Cosmosil $5C_{18}$, MeOH-H_2O) effected the isolations of stoloniferone-a (7a), -b (7b), -c (7c), and -d (7d) in 0.2, 1.0, 1.1, and 0.4% yields (from the AcOEt soluble portion), respectively.

Stoloniferone-c (7c), mp 148°C (CH_3CN), $C_{28}H_{44}O_3$,[4] $[\alpha]_D$ +40° ($CHCl_3$), was shown to possess a hydroxyl group (ν 3360 cm^{-1}) and an enone moiety (1658 cm^{-1}; λ_{max} 225 nm, ε=7800). The ^1H NMR spectrum (500 MHz, $CDCl_3$, δ) of 7c showed signals due to two olefinic protons [6.10 (1H, dd, J=10.0, 2.5 Hz, 2-H), 6.97 (1H, ddd, J=10.0, 6.0, 2.5, 3-H)], a proton geminal to a hydroxyl group [3.70 (1H, dddd, J=11.0, 11.0, 5.5, 4.5, 11β-H)], an epoxidic proton [3.17 (1H, d, J=3.0, 6α-H)], two *tert.* methyls [1.57 (3H, s, 10-CH_3), 0.71 (3H, s, 13-CH_3)], four *sec.* methyls [0.94 (3H, d, J=6.5, 20-CH_3), 0.76 (3H, d, J=6.5, 24-CH_3), 0.77, 0.84 (both 3H, d, J=6.5, 25-$(CH_3)_2$)]. The ^{13}C NMR data for 7c could be assigned as given in Table I. Comparisons in detail of ^1H NMR[5] and ^{13}C NMR[6]

data for known steroids (*e.g.* dihydrobrassicasterol) with those for stoloniferones showed that stoloniferone-c (7c) was a steroid possessing a 24S-methylcholesterol side chain.

Treatment of stoloniferone-c (7c) with 47% aq. HBr provided two bromohydrins: 8c, $C_{28}H_{45}O_3Br$, λ_{max} 232 nm (ε=7000), δ 4.22 (1H, br s, 6α-H) and 9c, $C_{28}H_{45}O_3Br$, λ_{max} 225 nm (ε=8000), δ 4.51 (1H, dd, J=12.5, 4.5, 6β-H). PCC oxidation followed by a NaOMe-MeOH treatment of 8c furnished a dienedione (10c), $C_{28}H_{42}O_3$, λ_{max} 310 nm (ε=5600), δ 6.31 (1H, dd, J=9.5, 1.0, 2-H), 7.11 (1H, dd, J=9.5, 6.0, 3-H), 6.70 (1H, dd, J=6.0, 1.0, 4-H), 4.23 (1H, m, 11β-H). The ^1H NMR examinations in detail (500 MHz) including the decoupling experiments of 7c, 8c, 9c, and 10c have led us to presume 7c having an 11α-hydroxy-5β,6β-epoxy-2-en-1-one structure for stoloniferone-c.

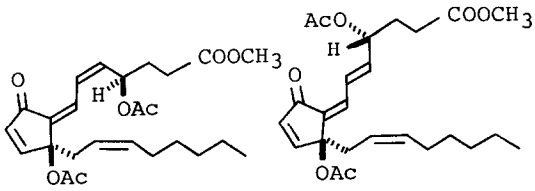
The ^{13}C NMR data for stoloniferone-a (7a), mp 148°C (CH_3CN), $C_{28}H_{42}O_3$, λ_{max} 225 nm (ε=8200), $[\alpha]_D$ +38.5° ($CHCl_3$), stoloniferone-b (7b), mp 150°C(CH_3CN), $C_{28}H_{42}O_3$, λ_{max} 225 nm (ε=8000), $[\alpha]_D$ +33° ($CHCl_3$), and stoloniferone-d (7d), mp 142°C (CH_3CN), $C_{29}H_{44}O_3$, λ_{max} 225 nm (ε=8000), $[\alpha]_D$ +31° ($CHCl_3$), have been assigned as shown in Table I. It has been shown that four stoloniferones (7a, 7b, 7c, 7d) possess a common 11α-hydroxy-5β,6β-epoxy-2-en-1-one structure in their steroidal skeletons but differ in their side chain structures.

Catalytic hydrogenations (10% Pd-C) of 7a, 7b, and 7c furnished an identical compound 11c, $C_{28}H_{46}O_3$, ν 1695 cm^{-1}. In the ^1H NMR spectra (500 MHz, $CDCl_3$) of 7a, 7b, and 7d, signals due to protons in their side chains have been assigned as follows: δ 0.97 (3H, d, J=6.5, 20-CH_3), 1.02, 1.03 (both 3H, d, J=6.5, 25-$(CH_3)_2$), 4.71 (1H, s, 28-H), 4.65 (1H, d, J=1.0, 28-H) in 7a; δ 1.02 (3H, d, J=6.5, 20-CH_3), 0.80, 0.82 (both 3H, d, J=6.5, 25-$(CH_3)_2$), 0.89 (3H, d, J=6.5, 24-CH_3), 5.12, 5.19 (2H, dABq, J=8.0, 15.0, 22,23-H) in 7b; δ 0.95 (3H, d, J=6.5, 20-CH_3), 0.89 (3H, d, J=7.0, 25-CH_3), 0.90 (3H, d, J=6.5, 25-CH_3), 0.86 (3H, d, J=6.5, 24-CH_3), 0.28 (1H, m, 22-H), 0.52 (1H, m, 23-H) in 7d. Comparisons in detail of these data with those reported for known steroids[5] have led us to formulate the structures of stoloniferone-a (7a), -b (7b), and -d (7d) with a 24-methylene moiety, a 24R-methyl-22-ene moiety, and a demethylgorgosterol-type moiety in their side chains, respectively.

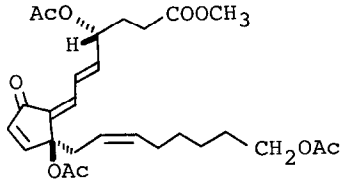
In order to substantiate above presumption, the X-ray crystallographic analysis of stoloniferone-d (7d)[7] was carried out. As shown in Fig. 1, the relative stereostructure of stoloniferone-d (7d) has been established. Furthermore, the CD data for 11c: $[\theta]_{285}$ -7200 (neg. max.) and 8c: $[\theta]_{333}$ -7300 (neg. max.), substantiated the absolute stereostructures of 7a, 7b, 7c, and 7d.

It is interestingly pointed out that marine steroids, isolated here from a stoloniferan soft coral, comprise an epoxy-enone moiety, which has been known as an important partial structure of cytotoxic Solanaceous plant products.[8]

Acknowledgement The authors are grateful to the Ministry of Education, Science, and Culture of Japan for a Grant-in-Aid for Scientific Research (Grant No. 59470121) and to The Naito Foundation Research Grant for 1983 for financial support.



claviridenone-a(1) claviridenone-b(2) claviridenone-c(3) claviridenone-d(4)



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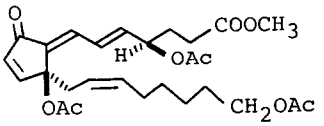

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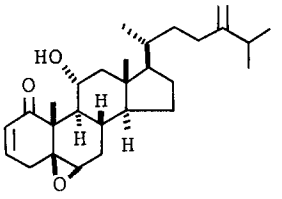
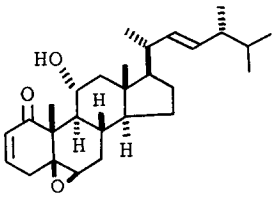


stoloniferone-a (7a)



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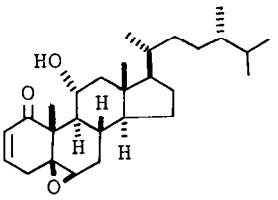

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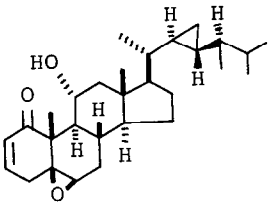

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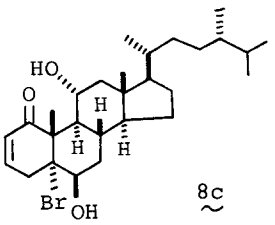
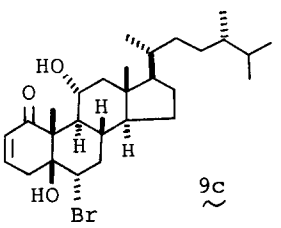
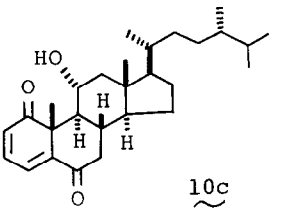


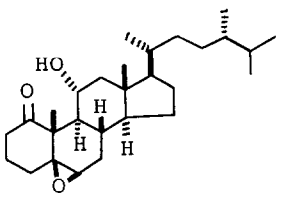
8c



9c



10c



11c

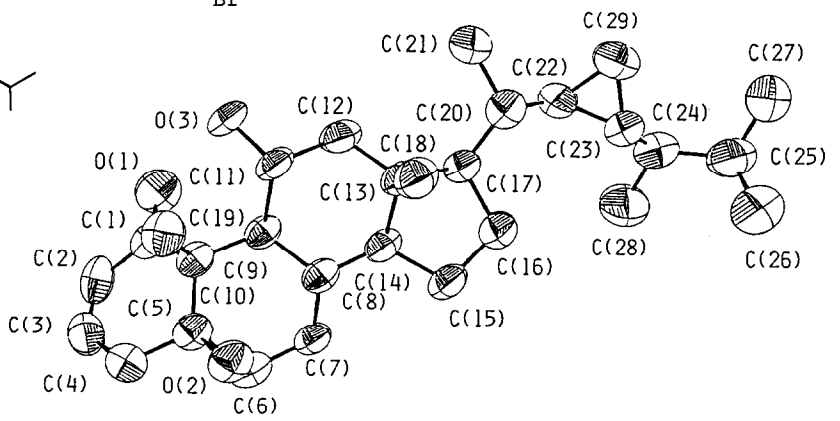


Fig 1. Since the conformations of two crystallographically independent molecules (A and B) are almost identical except their terminal isopropyl residues (C_{25} - C_{27}), the ORTEP drawing of the molecule B is shown here.

Table I ^{13}C NMR Data[a)]

carbon	7a	7b	7c	7d
1	207.8	207.9	207.8	207.9
2	128.7	128.7	128.7	128.7
3	147.3	147.3	147.3	147.3
4	34.0[b]	34.0[b]	34.0[b]	34.0[b]
5	62.3	62.3	62.3	62.3
6	63.6	63.6	63.6	63.7
7	30.5[b]	30.5[b]	30.5[b]	30.5[b]
8	28.7	28.7	28.7	28.7
9	50.5	50.6	50.5	50.6
10	50.1	50.1	50.0	50.1
11	67.0	67.0	67.1	67.1
12	50.8	50.7	50.8	50.8
13	43.1	42.9	43.0	43.4
14	55.6	55.8	55.6	55.4
15	24.0	24.0	24.0	24.3
16	28.4	28.7	28.4	28.7
17	56.0	56.0	56.0	57.5
18	13.0	13.2	13.0	12.9
19	14.9	14.9	14.9	14.9
20	35.8	40.2	36.2	40.0[c]
21	18.5	19.7[c]	18.8	19.0
22	34.6	132.1	33.7	24.0[d]
23	31.1	135.6	30.7	25.1[d]
24	156.8	42.9	39.2	45.0[c]
25	33.9	33.2	31.6	32.9
26	22.1	17.7	17.7	18.6
27	21.9	20.8	20.5	20.7
28	106.1	20.0[c]	15.6	15.9
29				10.5

a) Measured at 22.5 MHz in $CDCl_3$. b~d) The assignments for these signals within the same vertical column may be interchanged.

References and Notes

1) A preceding paper: M. Kobayashi, B. W. Son, T. Fujiwara, Y. Kyogoku, and I. Kitagawa, Tetrahedron Lett., submitted.

2) a) M. Kobayashi, T. Yasuzawa, M. Yoshihara, H. Akutsu, Y. Kyogoku, and I. Kitagawa, Tetrahedron Lett., 23, 5331 (1982); b) M. Kobayashi, T. Yasuzawa, M. Yoshihara, B. W. Son, Y. Kyogoku, and I. Kitagawa, Chem. Pharm. Bull., 31, 1440 (1983); c) I. Kitagawa, M. Kobayashi, T. Yasuzawa, B. W. Son, M. Yoshihara, and Y. Kyogoku, Tetrahedron, in the press.

3) Growth inhibition against P-388 leukemia cells: 69% at 1 μg/ml.

4) The molecular compositions of compounds with the chemical formulae were determined by high resolution mass spectrometry.

5) a) I. Rubinstein, L. J. Goad, A. D. H. Clague, and L. J. Mulheirn, Phytochem., 15, 195 (1976); b) P. A. Blanc and C. Djerassi, J. Am. Chem. Soc., 102, 7113 (1980).

6) N. Koizumi, Y. Fujimoto, T. Takeshita, and N. Ikekawa, Chem. Pharm. Bull., 27, 38 (1979).

7) A colorless crystal having approximate dimensions of 0.40x0.35x0.15 mm was mounted on a glass fiber in a random orientation. Preliminary examination and data collection were performed on an Enraf-Nonius CAD4 diffractometer with graphite-monochromated Cu Kα radiation (λ=1.5418 Å). Cell constants were obtained from least squares refinement, using the setting angles of 25 reflections in the range $18°<\theta<25°$. Crystal data are: $C_{29}H_{44}O_3$, M.W. =440.7, orthorhombic, space group $P2_12_12_1$, a=10.193(1), b=27.125(2), c= 10.193(1) Å, V=5194.5 Å^3, Z=8, Dx=1.13 gcm^{-3}, μ(Cu Kα)=5.2 cm^{-1}.

Reflections within $2\theta<130°$ were collected by $\omega/2\theta$ scans of variable rate. A total of 4918 unique data was collected, of which 2864 had $I>3\sigma(I)$, and were used in the refinement. The structure was solved by direct methods and refined by full-matrix least squares, treating non-hydrogen atoms anisotropically. Positions of most of the hydrogen atoms were obtained from difference maps, but included as fixed contributions in calculated positions where possible, with B=5.0 Å^2. Convergence was achieved with R=0.056 for 577 variables, and maximum residual electron density 0.23 eÅ^{-3}.

All calculations were performed on a PDP 11/34 computer using Enraf-Nonius SDP-PLUS programs. Tables of atomic coordinates, temperature factors, bond distances and angles have been deposited with the Cambridge Crystallographic Data Centre.

8) *e.g.* a) D. Lavie, I. Kirson, E. Glotter, D. Rabinovich, and Z. Shakked, J. Chem. Soc. Chem. Commun., 1972, 877; b) E. Glotter, I. Kirson, A. Abraham, P. D. Sethi, and S. S. Subramanian, J. Chem. Soc. Perkin I, 1975, 1370.

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