Stoloniolide I and II, New Marine Lactonic Steroids with an Unprecedented 1,10-Secoergostane Skeleton, Isolated from the Okinawan Soft Coral, *Clavularia viridis*

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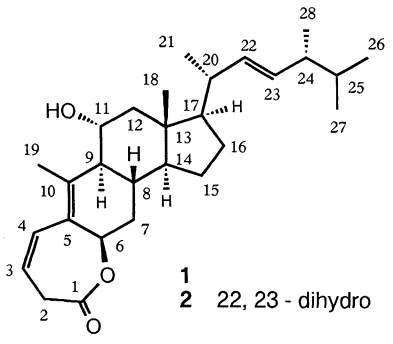
Two novel lactonic steroids, stoloniolide I and II, were isolated from the Okinawan soft coral, *Clavularia viridis* Quoy and Gaimard. Their structures were determined by spectroscopic analysis. They each possessed a unique 1,10-secoergostane skeleton unprecedented in the field of steroids.

The Okinawan soft coral, *Clavularia viridis* Quoy and Gaimard (class Anthozoa, Subclass Octocorallia, Order Stolonifera), is an invertebrate from which potent antitumor marine prostanoids[1,2] such as clavulones can be obtained in abundance. In the soft coral, structurally unique and biologically active steroids (stoloniferones) were also found.[3] During the course of our study on minor chemical substances from *C. viridis*, two novel lactonic steroids, stoloniolide I and II, were isolated. These compounds are the first examples of steroids each having a unique 1,10-secoergostane skeleton. This paper describes their structures as determined by spectroscopic analysis.

Wet specimens of *C. viridis* (3.3 kg), collected on the coral reef of Ishigaki Island, were immersed in methanol at room temperature. The methanol solution was diluted with water and extracted successively with hexane, ethyl acetate and butanol. Repeated silica gel column chromatography (first normal phase and then reversed phase) of the ethyl acetate extract (12.1 g) gave stoloniolide I (**1**)[4] (4 mg) and II (**2**)[5] (3.5 mg).

The IR spectrum (film) of **1** ($C_{28}H_{42}O_3$) showed absorptions due to hydroxyl (3416 cm^{-1}) and ester (1720 cm^{-1}) groups. The presence of a secondary hydroxyl group was indicated by ^{1}H-NMR [δ 3.90 (1H, dt) ppm] and ^{13}C-NMR [δ 69.8 (CH) ppm] spectra. The presence of an ester group of CO-O-CH was confirmed by the NMR data; δ_C 172.7 (C), 73.3 (CH), δ_H 5.10 (1H, br s) ppm. The NMR spectra also showed signals due to three carbon-carbon double bonds (two disubstituted and one tetrasubstituted) as shown in Tables 1 and 2. The presence of a conjugated diene system, CH=CH-C=C-CH_3 was demonstrated by the UV (EtOH) [λ 246 nm (ε 13100)], HMBC and NOESY spectra. Long-range ^{1}H-^{13}C correlations (two or three bonds) between H-4 [δ 6.62 (dd) ppm] and C-10 [δ 142.7 (C) ppm], and H-3 [δ 5.51 (br dd) ppm] and C-5 [δ 125.8 (C) ppm] were evident in the HMBC spectrum as shown in Figure 1. An NOE correlation between H-4 and H-19 [δ 2.09 (s) ppm] was also found. Except for the above groups, ^{1}H and ^{13}C-NMR spectra showed signals due to five methyls, five methylenes, seven methines, and one quaternary carbon in **1**. On consideration of these findings and the degree of unsaturation (8), stoloniolide I was concluded to possibly be a tetracyclic steroid.

A comparison of ^{1}H and ^{13}C-NMR spectra of **1** with those of known steroids, ergosterol[6] and 24-epicyclonervilasterol,[7] indicated the presence of a side chain the same as that of



1
2 22, 23 - dihydro

Table 1. ^{1}H-NMR data[a] for stoloniolide I (**1**) and II (**2**)

number	1	2
2	3.19 (1H, dd, *J* 8.6, 17.7, β-H)	3.19 (1H, dd, *J* 8.6, 17.5, β-H)
	3.82 (1H, br d, *J* 17.7, α-H)	3.82 (1H, br d, *J* 17.5, α-H)
3	5.51 (1H, br dd, *J* 8.6, 11.6)	5.51 (1H, ddd, *J* 2.2, 8.6, 11.5)
4	6.62 (1H, dd, *J* 3.1, 11.6)	6.62 (1H, dd, *J* 3.4, 11.5)
6	5.10 (1H, br s)	5.10 (1H, br d, *J* 2.5)
7	2.12 (1H, br d, *J* 14.1)	2.12 (1H, br d, *J* 14.1)
8	1.69 (1H, m)	1.72 (1H, m)
9	1.69 (1H, m)	1.68 (1H, m)
11	3.90 (1H, dt, *J* 5.5, 10.4)	3.90 (1H, m)
12	2.37 (1H, dd, *J* 5.5, 12.2)	2.39 (1H, dd, *J* 5.1, 12.2)
18	0.73 (3H, s)	0.72 (3H, s)
19	2.09 (3H, s)	2.09 (3H, s)
20	2.03 (1H, m)	
21	1.01 (3H, d, *J* 6.7)	0.94 (3H, d, *J* 6.6)
22	5.14 (1H, dd, *J* 8.6, 15.3)	
23	5.22 (1H, dd, *J* 7.3, 15.3)	
24	1.85 (1H, m)	
25	1.48 (1H, m)	
26,27	0.82 (3H, d, *J* 7.3)	0.778 (3H, d, *J* 6.9)[b]
	0.84 (3H, d, *J* 6.7)	0.86 (3H, d, *J* 6.7)
28	0.91 (3H, d, *J* 6.7)	0.784 (3H, d, *J* 6.7)[b]

[a] δppm, 500 MHz, $CDCl_3$, *J* in Hz.
[b] Assignments may be interchangeable.

Table 2. ^{13}C-NMR data[a] for stoloniolide I (**1**) and II (**2**)

number	1	2	number	1	2
1	172.7 (C)	172.7 (C)	15	23.4 (CH_2)	23.5 (CH_2)
2	35.6 (CH_2)	35.6 (CH_2)	16	28.7 (CH_2)	28.3 (CH_2)
3	117.6 (CH)	117.6 (CH)	17	56.0 (CH)	56.0 (CH)
4	129.6 (CH)	129.6 (CH)	18	13.1 (CH_3)	12.9 (CH_3)
5	125.8 (C)	125.8 (C)	19	17.4 (CH_3)	17.4 (CH_3)
6	73.3 (CH)	73.3 (CH)	20	40.1 (CH)	36.1 (CH)
7	34.2 (CH_2)	34.3 (CH_2)	21	20.8 (CH_3)	18.8 (CH_3)
8	31.3 (CH)	31.3 (CH)	22	135.3 (CH)	33.5 (CH_2)
9	53.7 (CH)	53.7 (CH)	23	132.2 (CH)	30.5 (CH_2)
10	142.7 (C)	142.8 (C)	24	42.8 (CH)	39.0 (CH)
11	69.8 (CH)	69.8 (CH)	25	33.1 (CH)	31.5 (CH)
12	51.6 (CH_2)	51.8 (CH_2)	26,27	19.6 (CH_3)	17.6 (CH_3)
13	43.5 (C)	43.6 (C)		20.0 (CH_3)	20.5 (CH_3)
14	54.0 (CH)	53.9 (CH)	28	17.6 (CH_3)	15.4 (CH_3)

[a] δ_{ppm}, 125 MHz, $CDCl_3$.

ergosterol and 24-epicyclonervilasterol. The methylene protons at C-2 [δ 3.19 (dd), 3.82 (br d) ppm] unusually shifted to a low field, suggesting the methylene to be situated between an ester carbonyl group and carbon-carbon double bond. Based on this finding and ^{1}H-^{13}C long-range correrations (Figure 1), a seven-membered β,γ-unsaturated lactonic moiety was considered. The structure of the B ring and connectivity of C-9, -11, -12, -13, -14, -17 and -18 positions were also established by ^{1}H-^{13}C long-range correlations as shown in Figure 1. The remaining two methylenes may logically be placed at C-15 and -16 positions. All these findings considered together, the plane structure of stoloniolide I (**1**) was concluded.

The *E* configuration of the carbon-carbon double bond at C-22 in the side chain was based on the coupling constant (J = 15.3 Hz) between H-22 and -23. The stereochemistry at the C-20 and -24 positions was demonstrated by the similarity of the ^{13}C chemical shifts of the side-chain carbons with those of ergosterol and 24-epicyclonervilasterol. The stereochemistry of the chiral centers at the C-8, -11, -13 and -17 positions was determined from NOE measurement: the NOESY spectrum of **1** showed NOEs between H-8 and -18, H-11 and -18, and H-18 and -21. The stereochemistry at C-9 was shown by the coupling constant (J = 10.4 Hz) between H-9 and -11, indicating a *trans*-diaxial configuration of these protons. The β-configuration of the lactonic bond at C-6 was indicated by analysis of NOE correlation and coupling pattern discussed in the following. The NOESY spectrum showed an NOE between H-6 [δ 5.10 (1H, br s) ppm] and H-2α [δ 3.82 (1H, br d, J = 17.7 Hz) ppm] as shown in Figure 2. The small coupling constant between H-2α and H-3 indicates the dihedral angle between these protons to be about 90°. The broad singlet signal of H-6 demonstrates that H-6 bisects the methylene protons at C-7. These findings can be reasonably explained in the case of the β-configuration of the lactonic bond and the conformation as shown in Figure 2, but not be explained in the case of the α-configuration of the lactonic bond. Although evidence was not obtained yet, H-14 should have the α-configuration in a consideration of the biogenesis discussed below.

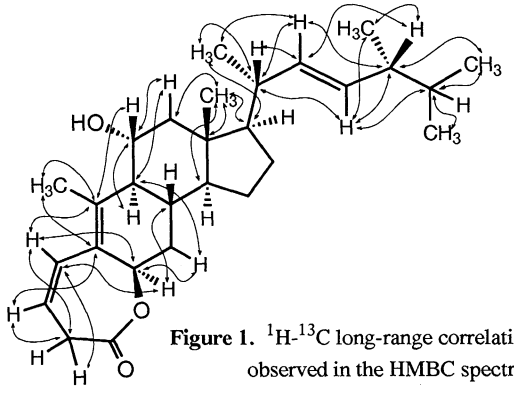


Figure 1. ^{1}H-^{13}C long-range correlations observed in the HMBC spectrum of **1**.

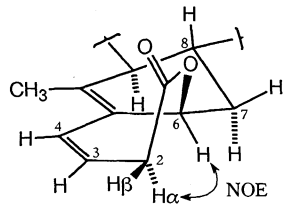


Figure 2.

Stoloniolide II (**2**) was shown to have the molecular formula, $C_{28}H_{44}O_3$, with two hydrogens more than that of **1**. ^{1}H- and ^{13}C-NMR spectra due to the nuclear part of **2** were virtually the same as those of **1**, showing the nuclear part of **2** to be the same as that of **1** including stereochemistry. Difference was observed in the side chain part: NMR signals due to the carbon-carbon double bond at C-22 and -23 of **1** were absent in **2**, but two methylene signals were observed in **2** instead. These findings indicate that stoloniolide II is 22,23-dihydrostoloniolide I. The stereochemistry at C-20 and -24 in the side chain was deduced by comparison of ^{13}C-NMR data with those of 24-epidihydrocyclonervilasterol.[7]

Stoloniolides may possibly be biosynthesized from stoloniferones which coexisted with stoloniolides in *C. viridis*. As shown in Figure 3, nucleophilic attack of a nucleophile (X^-) to the carbonyl group at C-1 of **a**, possibly formed by 1,4-addition of R'OH to the conjugated enone system of stoloniferones, gives an intermediate **b**. Fragmentation of **b** to **c** followed by lactonization and elimination of R'OH gives stoloniolides. The biological activity of **1** and **2** is currently under investigation.

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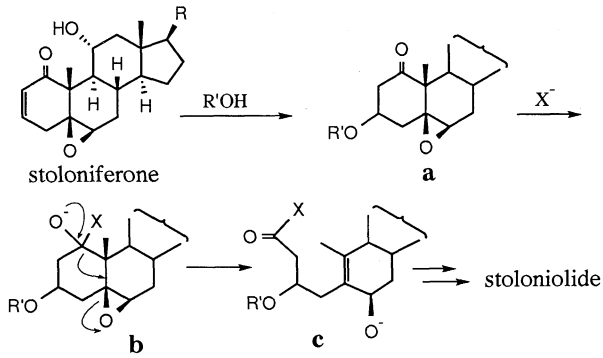

Figure 3. Possible biogenesis for stoloniolides.

References and Notes

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3 M. Kobayashi, N. K. Lee, B. W. Son, K. Yanagi, Y. Kyogoku, and I. Kitagawa, *Tetrahedron Lett.*, **25**, 5925(1984).
4 **1**: colorless viscous oil; $[\alpha]_D$ -4.8° (*c* 0.17, $CHCl_3$). HRMS *m/z* 408.2998 (M^+-H_2O, $C_{28}H_{40}O_2$ requires 408.3028).
5 **2**: colorless viscous oil; $[\alpha]_D$ -12.7° (*c* 0.055, $CHCl_3$). HRMS *m/z* 428.3312 (M^+, $C_{28}H_{44}O_3$ requires 428.3290).
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7 T. Kikuchi, S. Kadota, S. Matsuda, and H. Suehara, *Tetrahedron Lett.*, **25**, 2565(1984).