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Stolonilactone, a Novel Terpenoid-Related Compound, Isolated from the Okinawan Soft Coral *Clavularia koellikeri*

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A novel terpenoid-related compound, stolonilactone (**1**), was isolated from the Okinawan soft coral *Clavularia koellikeri*. The structure of **1** was elucidated on the basis of spectroscopic analysis. A possible biogenesis of **1** through the [4 + 2]-cycloaddition of a trisnorsesquiterpenoid-type diene and a cembranolide-type dienophile is proposed.

Introduction

The Okinawan soft corals of the genus *Clavularia* (class Anthozoa, subclass Octocorallia, order Stolonifera) have been recognized as rich sources for prostanoids, terpenoids, and steroids with unique structural features and significant biological activities. For example, *Clavularia viridis* comprises antitumor prostanoids exemplified by clavulones[1] and cytotoxic steroids such as stolonoferones.[2] *Clavularia koellikeri* contains cytotoxic terpenoids such as kericembrenolides,[3] and other *Clavularia* soft corals, which bear strong morphorogical resemblance to *C. koellikeri*, also gave cytotoxic terpenoids such as stolonidiol.[4] Interestingly, Okinawan *C. viridis* produces prostanoids as major secondary metabolites, while terpenoids were found only as a trace amount from *C. viridis*. On the other hand, terpenoids are the main components in other *Clavularia* species involving *C. koellikeri*, and no prostanoid was detected in these soft corals. Recently, we reported the isolation and structural determination of new terpenoids from *C. koellikeri*.[5−7] Further investigation on secondary metabolites from *C. koellikeri* resulted in the isolation of a novel terpenoid-related compound, stolonilactone (**1**). The unique skeletal carbon framework of **1** is unprecedented in the field of natural products. This paper describes the isolation, structural determination, and possible biogenesis of stolonilactone (**1**).

Results and Discussion

The methanol extract of *C. koellikeri*, collected on a coral reef off Ishigaki Island (Okinawa, Japan), was partitioned between ethyl acetate and water to afford an ethyl acetate soluble portion (71.4 g). A part (39.4 g) of the ethyl acetate soluble portion was chromatographed on a silica gel column by elution with hexane, hexanes−ethyl acetate (2:1), ethyl acetate, and methanol, in turn, to afford four fractions. The second fraction (11.7 g) was further chromatographed on a silica gel column by elution with hexanes−ethyl acetate (10:1) to afford nine fractions (fractions A−I). Further repeated chromatogrphic separation and purification of fraction I (2.59 g) afforded compound **1** (2.7 mg) along with new sesquiterpenoids.[7] Compound **1** was designated as stolonilactone according to the order name (Stolonifera) of *C. koellikeri*.

The molecular formula of stolonilactone (**1**) was found to be $C_{32}H_{44}O_2$ from HREIMS and ^{13}C NMR data. All 32 carbons appeared in the ^{13}C NMR spectrum. The DEPT spectrum indicated five methyls, 10 sp^3 methylenes, four sp^3 methines including one oxymethine, two sp^3 quaternary carbons, five sp^2 methines, and six sp^2 quaternary carbons including one carbonyl carbon. The presence of a γ-lactone moiety was suggested by the IR absorption at 1750 cm^{-1} and the ^{13}C NMR signal at δ_C 181.2 (C) ppm. The UV absorption at 269 nm (ϵ 4200) indicated the presence of a conjugated homoannular diene. The ^1H spectrum[8] of **1** (Table 1) disclosed the signals due to five olefinic protons [δ_H 4.71 (1H, br d, J = 11.0 Hz, H-7), 4.72 (1H, br t, J = 8.0 Hz, H-11), 5.41 (1H, br d, J = 10.6

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(8) A good signal separation in the NMR spectra was obtained in the solution of C_6D_6−$CDCl_3$ (70:30).

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TABLE 1. ^{13}C and ^{1}H NMR Data of Compound 1 [C_6D_6−$CDCl_3$ (70:30), δ][a]

no.	^{13}C	^{1}H	no.	^{13}C	^{1}H
1	43.1 (CH)	1.82 (1H, br dd, 3.1, 13.3)	16	40.6 (CH_2)	1.33 (1H, dd, 2.9, 12.0)
2	77.7 (CH)	4.75 (1H, br d, 10.6)			1.95 (1H, d, 12.0)
3	125.0 (CH)	5.41 (1H, br d, 10.6)	17	181.2 (C)	
4	140.1 (C)		18	14.7 (CH_3)	1.46 (3H, br s)
5	39.67 (CH_2)[b]	2.01 (1H, m)	19	14.6 (CH_3)	1.42 (3H, br s)
		2.10 (1H, m)	20	15.0 (CH_3)	1.30 (3H, br s)
6	24.3 (CH_2)	1.93 (1H, m)	1′	149.9 (C)	
		2.20 (1H, m)	2′	38.3 (CH)	2.59 (1H, sextet, 6.4)
7	125.8 (CH)	4.71 (1H, br d, 11.0)	3′	33.4 (CH_2)	1.55 (2H, q, 5.2)
8	133.4 (C)		4′	27.0 (CH_2)	2.00 (1H, m)
9	39.71 (CH_2)[b]	1.76 (1H, dt, 3.9, 12.4)			2.13 (1H, m)
		2.10 (1H, m)	5′	133.7 (CH)	5.76 (1H, td, 5.4, 11.2)
10	24.1 (CH_2)	1.90 (1H, m)	6′	121.6 (CH)	5.80 (1H, d, 11.2)
		2.05 (1H, m)	7′	141.8 (C)	
11	126.1 (CH)	4.72 (1H, br t, 8.0)	8′	51.4 (C)	
12	131.5 (C)		9′	51.8 (CH_2)	1.17 (1H, ddd, 1.7, 2.9, 8.4)
13	37.0 (CH_2)	1.80 (1H, dd, 2.8, 15.9)			2.19 (1H, dd, 1.3, 8.4)
		1.83 (1H, m)	10′	55.5 (CH)	2.84 (1H, br s)
14	28.6 (CH_2)	0.95 (1H, m)	11′	20.7 (CH_3)	0.99 (3H, d, 7.0)
		1.32 (1H, m)	12′	17.9 (CH_3)	1.23 (3H, s)
15	56.6 (C)				

[a] ^{13}C NMR: 125 MHz. ^{1}H NMR: 500 MHz. J in Hz. Assignments of the ^{13}C and ^{1}H signals were made on the basis of HMQC. [b] Values with the superscript may be interchanged.

Hz, H-3), 5.76 (1H, td, J = 5.4, 11.2 Hz, H-5′), 5.80 (1H, d, J = 11.2 Hz, H-6′], three olefinic methyls [δ_H 1.30 (3H, br s, H-20), 1.42 (3H, br s, H-19), 1.46 (3H, br s, H-18)], one secondary methyl [δ_H 0.99 (3H, d, J = 7.0 Hz, H-11′)], one tertiary methyl [δ_H 1.23 (3H, s, H-12′)], and one oxymethine [δ_H 4.75 (1H, br d, J = 10.6 Hz, H-2)]. The ^{13}C NMR spectrum (Table 1) showed the signals of the olefinic carbons [δ_C 121.6 (CH, C-6′), 125.0 (CH, C-3), 125.8 (CH, C-7), 126.1 (CH, C-11), 131.5 (C, C-12), 133.4 (C, C-8), 133.7 (CH, C-5′), 140.1 (C, C-4), 141.8 (C, C-7′), 149.9 (C, C-1′)], and one oxymethine carbon [δ_C 77.7 (CH, C-2)]. These spectral data, coupled with the degrees of unsaturation (11), suggested that stolonilactone (**1**) is a pentacyclic terpenoid-related compound with a γ-lactone group.

The HMQC analysis revealed the assignment of each direct C−H bonding in **1** as summarized in Table 1. The ^{1}H−^{1}H correlations obtained from the ^{1}H−^{1}H COSY exhibited partial structures **a**−**f** as depicted in Figure 1. The ^{1}H signal at δ 1.17 (1H, ddd, J = 1.7, 2.9, 8.4 Hz, H-9′) in partial structure **e** shows the coupling with the ^{1}H signal at δ 1.33 (1H, dd, J = 2.9, 12.0 Hz, H-16) in partial structure **f** (J = 2.9 Hz). It is reasonable to consider that these protons correlate each other by a long-range W-shaped type coupling rather than a vicinal coupling because the structure with the vicinal relationship of these protons could not explain the coupling patterns of the neighboring protons at δ 2.19 (1H, dd) and 1.95 (1H, d).

To connect the partial structures for constructing the gross structure, HMBC analysis was undertaken; the key correlations observed in the HMBC spectrum are shown by broken arrows in Figure 2. The HMBC correlations from H-5 [δ 2.01 (m)] to C-4 and C-3 and from H-3 to C-5 indicated the connectivity between C-5 of partial structure **b** and C-4 of **a**. The connection between C-8 of partial structure **b** and C-9 of **c** was indicated by the correlations from H-9 [δ 1.76 (dt)] to C-7, C-8, and C-19. The correlations from H-11 to C-13 and from H-20 to C-13 indicated the connectivity between C-12 of partial structure **c** and C-13 of **a**. These findings disclosed the presence of a 14-membered carbocyclic ring in **1**. Among the five carbon−carbon double bonds in **1**, the three trisubstituted double bonds constitute the 14-membered carbocyclic ring. Therefore, the remaining two double bonds (disubstituted and tetrasubstituted) should form the above-mentioned conjugated homoannular diene. The presence of this conjugated diene in the seven-membered carbocyclic ring involving partial structure **d** was indicated by the HMBC correlations from H-6′ to C-7′, from H-5′ to C-7′, from H-2′ to C-1′, and from H-11′ to C-1′. The location of the γ-lactone ring on the C-1 and C-2 positions of the 14-membered carbocyclic ring was determined by the correlations from the oxymethine proton (H-2) to the lactonic carbonyl carbon (C-17) and the quaternary carbon (C-15), and from H-1 to C-15 and C-17. Finally, the HMBC analysis of the remaining C_5H_8 unit involving the partial structures (**e** and **f** in Figure 1) and the tertiary methyl group exhibited the presence of a bicyclo[2.2.1]heptene moiety in **1**. The HMBC correlation from H-16 [δ 1.95 (1H, d, J = 12.0 Hz)] to the quaternary carbon (C-15) on the γ-lactone moiety indicated the connectivity between C-15 and C-16 (partial structure **f**). Further HMBC correlations from H-16 [δ 1.95 (d)] to C-8′ and C-12′ [δ 17.9 (CH_3)], from H-12′ to C-8′ and C-16, from H-9′ [δ 2.19 (1H, dd, J = 1.3, 8.4 Hz)] to C-8′, from H-9′ [δ 1.17 (1H, ddd, J = 1.7, 2.9, 8.4 Hz)] to C-15, and from H-10′ to C-8′ and C-16 demonstrated the presence of a methylcyclopentane unit, which is connected with the γ-lactone moiety by holding the quaternary carbon at C-15 in a spiro manner. The final two connections between C-8′ and C-7′, and between C-10′ and C-1′ constituting a bicyclo[2.2.1]heptene moiety were indicated by the HMBC correlations from H-9′ [δ 2.19 (dd)] to C-7′ and C-1′, and from H-10′ to C-2′. The presence of this bicyclo[2.2.1]heptene moiety was supported by the following ^{1}H NMR data. The W-shaped long-range coupling (J = 2.9 Hz), which is characteristic of the norbornane-type bicyclic system, was observed between H-9′ (Ha) and H-16 (Ha) shown by bold lines in Figure 3.

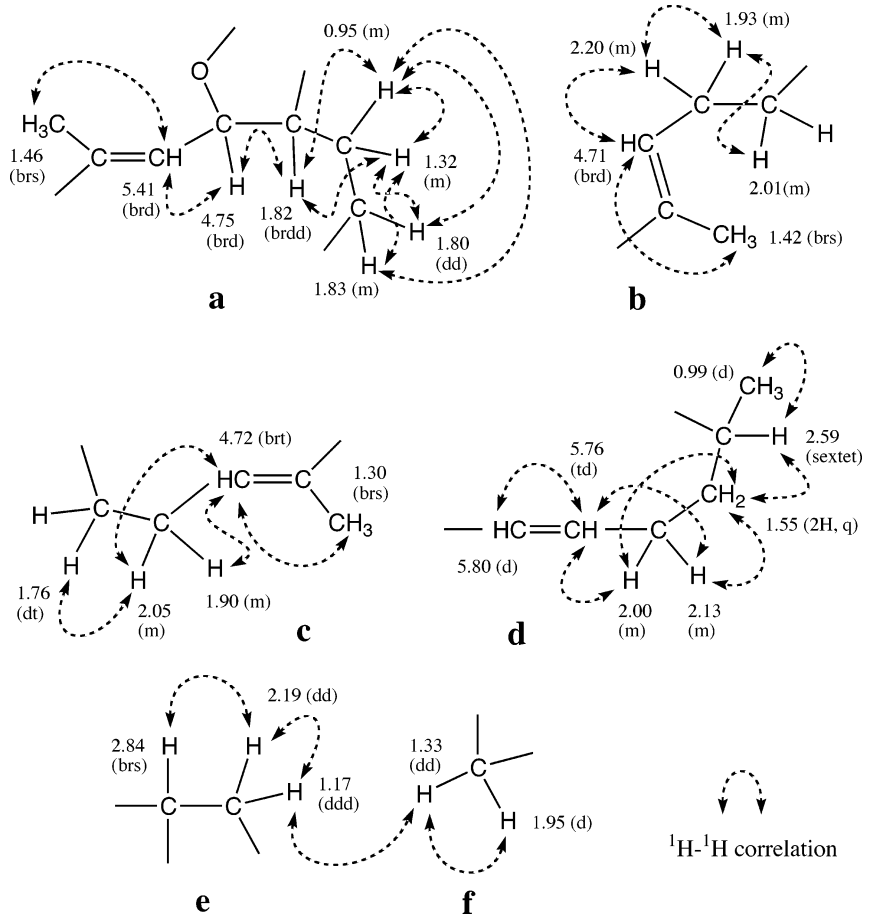


FIGURE 1. Partial structures, ^{1}H NMR data, and ^{1}H−^{1}H correlations for **1**.

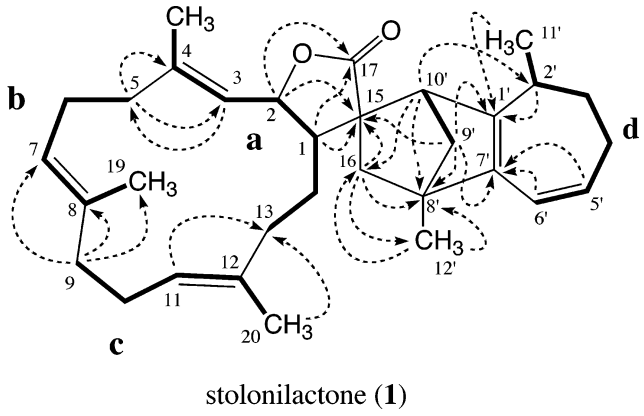


FIGURE 2. Gross structure, ^{1}H−^{1}H correlations (bold lines), and key HMBC correlations (broken lines) for **1**.

Furthermore, H-9′ [Ha, δ 1.17 (ddd)] exhibited a highly shielded chemical shift due to the anisotropic effect of the tetrasubstituted double bond at C-1′ and C-7′, while Hb [δ 2.19 (dd)] at C-9′ showed a highly deshielded value due to the anisotropic effect of the lactonic carbonyl group.

The stereochemistry of the three trisubstituted double bonds in **1** was determined by NOESY analysis. As shown in Figure 3, the NOE correlation between H-2 and H-18 indicated the 3*E* configuration. The 7*E* and 11*E* configurations were also indicated by the NOE correlations between H-6 and H-19 and between H-10 and H-20, respectively.

The relative configurations of six chiral centers at C-1, C-2, C-15, C-8′, C-10′, and C-2′ were determined by NOESY analysis (Figure 3). The trans configuration between H-1 and H-2 was indicated by the NOE correlations between H-1 and H-3, and between H-2 and H-14b [δ 0.95 (1H, m)]. The above-mentioned low-field shift of the bridge-methylene proton Hb at C-9′ demonstrated that the lactonic carbonyl group and the bridge-methylene group oriented to the same direction. This indicated the relative configurations of the C-15, C-8′, and C-10′ positions. The NOE correlation between H-3 and H-10′ disclosed the relative configuration between the C-2 and C-10′ positions. Finally, the relative stereochemistry at C-2′ to C-10′ was indicated by the NOE correlation between H-11′ and H-10′. The absolute stereochemistry of **1** was deduced on the basis of the following biogenesis.

The structure of stolonilactone (**1**) having a 14-membered carbocyclic ring, a spirolactone system, and a bicyclo[2.2.1]heptene moiety fused with the cycloheptene ring in its molecule is extremely unique, and therefore, consideration of the biosynthesis of **1** is very attractive. On considering the fact that the structural unit from C-1 to C-20 corresponds to that of cembrane-type diterpenoid and that the bicyclo[2.2.1]heptene system may be formed by a [4 + 2]-cycloaddition reaction, the molecule **1** could be constructed by the [4 + 2]-cycloaddition of the dienophile **A** with the diene **B** in the manner as depicted in

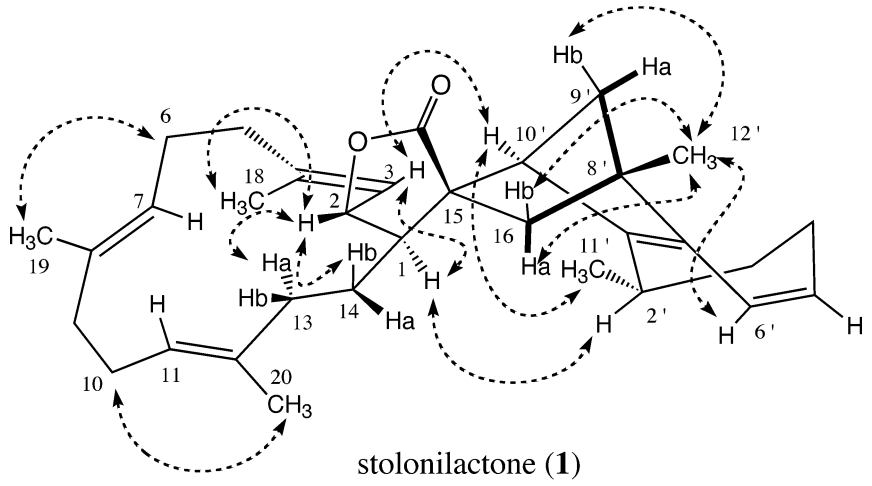
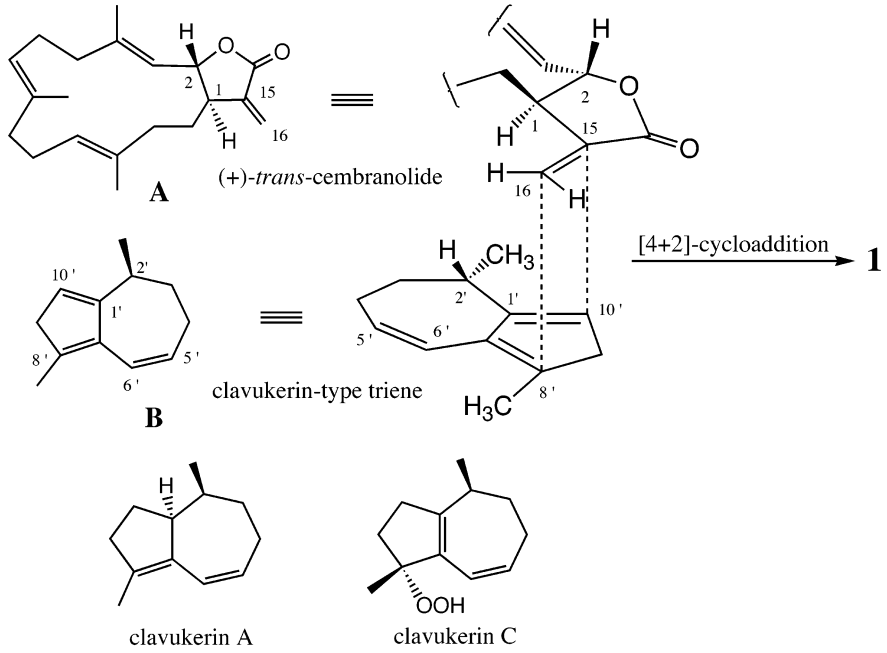


FIGURE 3. Relative stereochemistry and key NOE correlations for **1**.

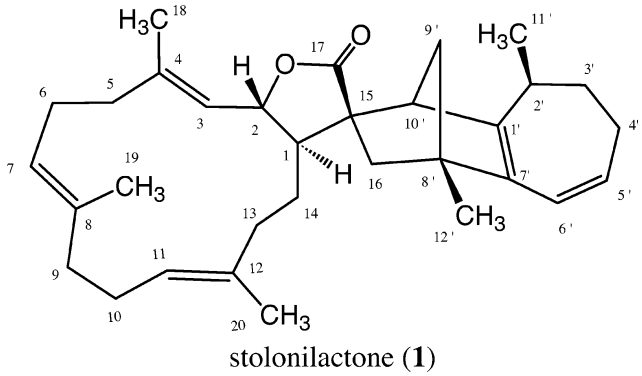
SCHEME 1. Proposed Biogenesis for Stolonilactone (1) and Structures of Clavukerins A and C



Scheme 1. The diterpenoid, (+)-*trans*-cembranolide, which is just the dienophile **A**, was previously isolated from *C. koellikeri* by our group[6] and its absolute configuration was established. Although the diene **B** was not found as a natural product in *C. koellikeri*, clavukerins can possibly be regarded as related natural products. Clavukerins[9] such as clavukerins A and C were trisnorsesquiterpenoids isolated from *C. koellikeri* by Kitagawa et al., and their absolute structures were already determined. Therefore, the absolute stereochemistry of **1** is suggested as shown in Chart 1.

Previously, some tetraterpenoids (biscembranolides)[10] exemplified by methyl isosartortuate[10a] were found from soft corals. Although these compounds had the structures formed by [4 + 2]-cycloaddition (Diels−Alder reaction)

CHART 1



between two cembrane-type diterpenoids, the authors of the literature did not mention that these compounds formed enzymatically or not. Recentl,y the reaction mechanism of the enzyme system involving Diels−Alder reaction was reported for the formation of macrophomate,

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a benzoic acid-type secondary metabolite isolated from the fungus *Macrophoma commelinae*.[11] The problem whether stolonilactone (**1**) forms enzymatically or nonenzymatically is interesting and will be solved by further chemical and biochemical investigations.

Experimental Section

General Methods. IR spectra were recorded with an FT-IR spectrophotometer. ^{1}H and ^{13}C NMR spectral data and ^{1}H−^{1}H COSY, HMQC, HMBC, and NOESY experiments were measured with a 500 MHz FT-NMR spectrometer in the mixed solvent of C_6D_6 and $CDCl_3$ (70:30). Chemical shifts are given on a δ (ppm) scale with C_6H_6 (^{1}H; 7.16 ppm) or C_6D_6 (^{13}C; 128.2 ppm) as an internal standard (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad).

Animal and Material. The soft coral *C. koellikeri* (order Stolonifera, family Clavlaridae) was collected from a coral reef off Ishigaki Island, Okinawa Prefecture, Japan, at a depth of 1−2 m in June 1997. A voucher specimen (no. SC-97-1) has been deposited at Tokyo University of Pharmacy and Life Science, Tokyo, Japan.

Wet specimens (5.4 kg) were extracted with MeOH. The MeOH extract (237 g) was partitioned between EtOAc and H_2O to obtain an EtOAc-soluble portion (71.4 g). An aliquot of the EtOAc-soluble portion (39.4 g) was chromatographed on a silica gel column. Stepwise elution with hexane (2 L), hexanes−EtOAc (2:1, 2 L), EtOAc (2 L), and MeOH (2 L) afforded four fractions. The second fraction [22.3 g, eluted with hexanes−EtOAc (2:1)] was further chromatographed on a silica gel column by stepwise elution with hexane, hexanes−EtOAc (10:1 and 4:1), and EtOAc to afford four fractions (fractions I−IV). Silica gel column chromatography of fraction II [11.7 g, eluted with hexanes−EtOAc (10:1)] afforded nine fractions (fractions A−I) by stepwise elution with hexanes−EtOAc (25:1 and 15:1). Further separation and purification of fraction I (2.59 g) using silica gel column chromatography (normal-phase) and medium-pressure liquid chromatography (MPLC, normal-phase) afforded stolonilactone (**1**) (2.7 mg) along with two new sesquiterpenoids[7] and two known diterpenoids, (+)-*trans*-cembranolide[6] (75 mg) and neodolabellenol[12] (144 mg).

Stolonilactone (1): colorless viscous oil; $[\alpha]^{25}_D$ +100 (*c* 0.21, $CHCl_3$); UV (EtOH) λ_{max} 269 nm (ϵ 4200); IR (dry film) ν_{max} 3019, 2950, 2922, 1750, 1667, 1444, 1372, 1329, 1198 cm^{-1}; ^{1}H and ^{13}C NMR (Table 1); HREIMS *m*/*z* 460.3336 [calcd for $C_{32}H_{44}O_2$ (M)$^+$, 460.3341].

Acknowledgment. We thank Dr. Yasuo Shida, Tokyo University of Pharmacy and Life Science, for measurement of mass spectra.

Supporting Information Available: ^{1}H NMR and ^{13}C NMR, ^{1}H−^{1}H COSY, HMQC, HMBC, and NOESY spectra of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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