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UNUSUAL CEMBRANE DITERPENOID ISOLATED FROM THE JAPANESE SOFT CORAL GENUS *SINULARIA*

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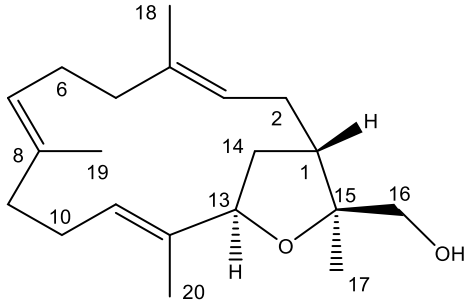
Abstract – A novel cembrane diterpenoid, odosinularol (**1**), featuring an uncommon 13,15-ether bridge, along with nine known casbane diterpenoids (**2**–**10**) were isolated from the Japanese soft coral genus *Sinularia*, which was collected on the main island of Okinawa, Japan. The structure of compound **1** was established through spectroscopic analysis (1D and 2D nuclear magnetic resonance spectroscopy, high resolution electrospray ionization mass spectrometry, and infrared spectroscopy). Furthermore, the bioassay results indicated that the compounds showed moderate cytotoxicity against C2C12 cells (mouse myoblasts).

Marine soft corals contain a wealth of diverse and complex terpenoids,[1–4] which exhibit a wide range of bioactive properties, such as antibacterial, repellent, cytotoxic, and anti-inflammatory activities.[5–8] Among the soft corals, the genus *Sinularia* (order Alcyonacea, family Alcyoniidae) has been thoroughly chemically investigated, resulting in the discovery of several diterpenoids.[9] Cembranes are not only widespread in marine soft corals but also in the liverwort genus *Chandonanthus*.[9] Furthermore, casbanes, characterized by the presence of a dimethyl-cyclopropyl moiety fused to a 14-membered ring, are relatively rare and are mainly found in soft corals of the genus *Sinularia* and *Lobophytum* among all marine organisms.[10,11] Several of the cembranes and casbanes recently discovered in *Sinularia* have been found to exhibit cytotoxic, anti-inflammatory, and antibacterial activities, indicating their potential for use in new drug discovery.[12–14] In

our ongoing search for bioactive diterpenoids from Japanese *Sinularia* soft corals (collected off the coast of Odo Coast, Itoman City, Okinawa), we isolated an unprecedented diterpenoid, odosinularol (**1**) (Chart 1), and nine known casbane diterpenoids **2**–**10** (Figure S1). Herein, we describe the isolation, structural elucidation, and cytotoxicity of these compounds.



1

Chart 1. Chemical structure of new compound **1**

Compound **1** was isolated as a colorless oil with an $[\alpha]_D^{23}$ of −74.4 (*c* 0.1, $CHCl_3$). The molecular formula, $C_{20}H_{32}O_2$, was deduced from the high resolution electrospray ionization mass spectrometry (HR-ESI-MS) data of the compound (*m/z* 305.2475 $[M + H]^+$, calcd. 305.2481), accounting for five degrees of unsaturation. The absorption bands at 3438 and 1049 cm^{-1} in the infrared (IR) spectrum indicated the presence of a hydroxy group and an ether functionality, respectively. The ^1H and ^{13}C nuclear magnetic resonance (NMR) spectra obtained in $CDCl_3$ (Table 1), as well as the distortionless enhancement by polarization transfer (DEPT) and heteronuclear single quantum coherence (HSQC) experiments, indicate the presence of three pairs of double bonds at δ_C 135.8 (C), 134.6 (C), 134.1 (C), 126.6 (CH), 124.7 (CH), and 123.4 (CH) and δ_H 5.41 (1H, t), 5.30 (1H, t), and 4.99 (1H, dd); three vinyl methyls at δ_C 15.2 (CH_3), 14.8 (CH_3), and 14.4 (CH_3) and δ_H 1.63, 1.53, and 1.52 (each 3H, s); an oxygenated methylene signal at δ_C 66.9 (CH_2) and δ_H 3.45 (1H, d) and 3.35 (1H, d); an oxygenated methine signal at δ_C 79.2 (CH) and δ_H 4.28 (1H, d); an oxygenated quaternary carbon signal at δ_C 84.7 (C), one methyl, six methylenes, and one methine. These signals indicate five degrees of unsaturation, suggesting that compound **1** contains a bicyclic ring. A ^1H–^1H correlation spectroscopy (COSY) experiment was conducted, revealing the sequences of the correlations depicted by the bold lines in Chart 2. The chemical shift values for C-16 at δ_C 66.9 and δ_H 3.45 (1H, d) and 3.35 (1H, d) clearly indicate that a hydroxy group is attached to C-16; this conclusion is supported by the HR-MS and IR data. The spectroscopic data of **1** correlates to that previously reported for cembrane 15-hydroxycembra-1,3,7,11-tetraene, however, the data for **1** indicates the absence of the 1,2 double bond and presence of a 13,15-ether bridge.[15] The presence of a methyl-propanol moiety attached to C-1 was confirmed by the heteronuclear multiple bond correlation (HMBC) cross peaks between H_3-17 and C-1, C-

15, and C-16. The 14-membered cembrane structure of **1** was established based on key HMBC cross peaks between H_3-18 and C-3, C-4, and C-5; H_3-19 and C-7, C-8, and C-9; H_3-20 and C-11, C-12, and C-13; and H-13 and C-11, C-12, and C-15. Therefore, the overall structure of **1** was determined and is shown in Chart 2.

Table 1. ^{1}H and ^{13}C NMR data (400 MHz and 100 MHz, $CDCl_3$ ([a]) and C_6D_6 ([b])) for **1** (δ in ppm, *J* in Hz)

Position	δ_C[a]	δ_H (mult., *J* in Hz)[a]	δ_C[b]	δ_H (mult., *J* in Hz)[b]
1	44.0 (d)	1.95-2.00 m	44.1 (d)	1.96-2.02 m
2	26.5 (t)	2.01-2.06 m	26.5 (t)	1.84-1.95 m
3	124.7 (d)	5.41 t (7.3)	125.1 (d)	5.33 t (7.3)
4	135.8 (s)		135.1 (s)	
5	39.2 (t)	2.10-2.15 m	39.2 (t)	1.96-2.02 m
		2.20-2.25 m		2.05-2.10 m
6	25.7 (t)	2.01-2.06 m	25.8 (t)	1.84-1.95 m
		2.40-2.45 m		2.28-3.30 m
7	126.6 (d)	4.99 dd (4.1, 9.6)	126.5 (d)	4.92 dd (4.1, 10.1)
8	134.1 (s)		133.8 (s)	
9	39.5 (t)	1.95-2.00 m	39.6 (t)	1.84-1.95 m
		2.15-2.20 m		1.96-2.02 m
10	22.8 (t)	2.01-2.05 m	22.7 (t)	1.84-1.95 m
		2.05-2.10 m		1.84-1.95 m
11	123.4 (d)	5.30 t (5.5)	122.8 (d)	5.27 t (5.0)
12	134.6 (s)		135.0 (s)	
13	79.2 (d)	4.28 d (7.3)	78.7 (d)	4.20 d (7.3)
14	33.3 (t)	1.78 ddd (5.0, 7.3, 12.4)	33.2 (t)	1.49 ddd (5.5, 7.3, 12.8)
		2.34 dd (5.0, 11.9)		2.18 dd (5.0, 12.4)
15	84.7 (s)		84.4 (s)	
16	66.9 (t)	3.35 d (11.5)	66.9 (t)	3.30 d (11.5)
		3.45 d (11.0)		3.45 d (11.0)
17	18.8 (q)	0.98 s	18.7 (q)	0.84 s
18	14.4 (q)	1.53 s	14.1 (q)	1.45 s
19	14.8 (q)	1.52 s	14.4 (q)	1.41 s
20	15.2 (q)	1.63 s	14.9 (q)	1.58 s

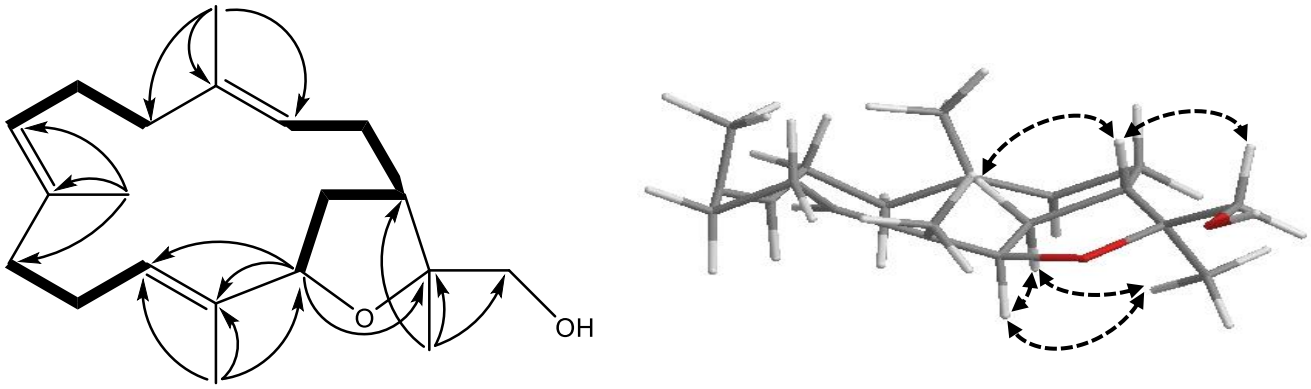


Chart 2. The ^{1}H–^{1}H COSY, key HMBC, and NOESY correlations of **1**

The relative stereochemistry of compound **1** was deduced using a nuclear Overhauser effect spectroscopy (NOESY) experiment and ^{13}C NMR chemical shifts. The ^{13}C NMR chemical shifts of the vinyl methyl groups at C-18, C-19, and C-20 at δ_C 14.4, 14.8, and 15.2, respectively, suggest that all the trisubstituted double bonds have *E* configurations.[16,17] These *E* configurations were further supported by observation of NOESY correlations between H-3/H_2-5, H-7/H_2-9, and H-11/H-13. The relative stereochemistry of tetrahydrofuran ring at C-1, C-13, and C-15 was deduced based on the strong NOE correlations between H_3-17, H-13, and H_2-14α, suggesting they positioned at the same orientation. While, opposite orientation of this tetrahydrofuran ring was determined based on NOE correlations between H_2-16 and H-1, as well as H-1 and H_2-14β, as shown in Chart 2. Therefore, compound **1** can be described as (3*E*,7*E*,11*E*)-16-hydroxycembra-13,15-ether-3,7,11-triene, named odosinularol. To the best of our knowledge, the tetrahydrofuran ring that connects C-13 to C-15 is limited to three norcembranoid diterpenes,[18–20] chloroscabrolides A–B found in Indonesian soft coral *Sinularia* sp.,[21] and sinularectin found in Kenyan soft coral *S. erecta*.[22] In contrast, odosinularol (**1**) is the first example of cembranoid diterpene that contains a tetrahydrofuran ring which connect C-13 to C-15. In response to these results, we named this unusual skeleton as odosinulane.

Compounds **2**–**10** were identified as depressin (**2**), 1-*epi*-depressin (**3**), 10-oxo-3,4,11,12-tetrahydrodepressin (**4**), 10-oxo-11,12-dihydrodepressin (**5**), 1-*epi*-10-oxodepressin (**6**), sinularcasbane F (**7**), sinularcasbane E (**8**), 10-hydroxydepressin (**9**), and 1-*epi*-10-hydroxydepressin (**10**) by comparing their spectroscopic data with those reported in literature.[23–25] The isolation of compound **3** from Chinese *S. erecta* has very recently been reported.[23] There are very few reports on the assessment of the bioactivity of these known compounds; therefore, we tested their cytotoxicity using mouse myoblast C2C12 cells. Compounds **1**, **5**–**7**, and **9** showed cytotoxicity at 50 μg/mL, while compound **8** showed efficient cytotoxicity at even only 10 μg/mL. Compounds **2**–**4** and **10** showed lower levels of cytotoxicity at 50 μg/mL. Based on the results obtained by conducting a series of experiments, the *Sinularia* species treated in this study protect themselves from predators using casbane-type diterpenes accumulated at high concentrations. In conclusion, the chemical structures of the isolated compounds are useful for elucidating the evolution and speciation of *Sinularia* soft corals and for analyzing their taxonomic correlations.

EXPERIMENTAL

General Experimental Procedures

The optical rotations of the compounds were measured using a Jasco P-1010 polarimeter. NMR spectra were recorded on a JEOL ECA 400 FT-NMR spectrometer using $CDCl_3$ and C_6D_6 as solvent and TMS as internal standard. Infrared spectra were recorded on a Jasco FT/IR-6100 spectrometer. High-resolution

mass spectra were acquired using a Waters SYNAPT HDMS instrument. Semi-preparative high-performance liquid chromatography (HPLC) was performed on a Shimadzu HPLC system (CBM-20A system controller, LC-20AT binary pump, and SPD-20A ultraviolet–visible (UV/VIS) detector) with a Luna 5 μm C18(2) 100 Å column (250 × 10 mm internal diameter). Preparative thin layer chromatography (TLC) was performed using silica gel glass plates (Merck, Kieselgel 60 F_{254}) and column chromatography (CC) with silica gel (Merck, Kieselgel 60, 70–230 mesh). The spots were visualized under UV light and/or by spraying with a 5% phosphomolybdic acid-ethanol solution, followed by heating at 90 °C.

Animal Materials

A specimen of *Lobophytum* sp. was collected from Odo Coast, Itoman City, Okinawa, on February 28, 2021. The voucher specimen was deposited at the Faculty of Agriculture, University of the Ryukyus.

Extraction and Isolation

Fresh soft coral (2.0 kg wet weight) was homogenized in MeOH and an extraction was performed at room temperature over 2 days. The resulting MeOH extract was concentrated *in vacuo* and partitioned into EtOAc/H_2O. The EtOAc fraction (1.27 g) was subjected to CC using an increasing polarity gradient of *n*-hexane and EtOAc. Fraction 2 (182.7 mg) was subjected to repeated preparative TLC using *n*-hexane:EtOAc (95:5) and toluene to yield compounds **2** (7.3 mg), **3** (7.3 mg), and **4** (6.1 mg). Fraction 3 (137.0 mg) was subjected to repeated preparative TLC using *n*-hexane:EtOAc (8:2) to yield compounds **5** (6.2 mg), **6** (6.2 mg), and **9** (11.5 mg), and the residue was further purified in $CHCl_3$:MeOH (99:1) to obtain compounds **1** (3.4 mg), **7** (3.5 mg), and **8** (3.8 mg). Fraction 4 (129.0 mg) was subjected to preparative TLC using *n*-hexane:EtOAc (2:1) to yield compound **10** (2.4 mg). Compound **1** was purified using a C18 column and detected at an UV wavelength of 210 nm using a gradient elution of 50–100% MeCN (0–40 min) and 100% MeCN (40–60 min).

Cell Cultures

C2C12 mouse myoblast cells were purchased from the American Type Culture Collection (Manassas, VA, USA). The cells were maintained in Dulbecco's modified Eagle's medium with high glucose (D-MEM (044-29765), Wako, Osaka, Japan) containing 10% fetal bovine serum (F7524, Lot# BCBT3928, Sigma-Aldrich, St. Louis, MO, USA) and 1% penicillin-streptomycin solution (antibiotics) (168-23191, Wako), and grown at 37 °C in an atmosphere of 5% CO_2.

Determination of Cell Viability

All the compounds were dissolved in EtOH. The C2C12 cells were seeded at 5 x 10^3 cells/well in a 96 well plate, cultured overnight, and treated with 10 and 50 μg/mL of compound over 24 h. To determine cell viability, a WST-8 assay was performed using a Cell Counting Kit-8 (Dojindo, Kumamoto, Japan) according to the manufacturer's protocol. The results were calculated as a percentage of treated no addition or vehicle control cells.

Odosinularol (1): colorless oil; $[\alpha]_D^{23}$: −74.4 (*c* 0.1, $CHCl_3$); IR ν_{max} (cm^{-1}): 3438, 1711, 1449, 1380, and 1049; ^1H and ^{13}C NMR spectral data: see Table 1; HR-ESI-MS *m/z* 305.2475 $[M + H]^+$ (calculated for $C_{20}H_{33}O_2$, 305.2481).

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REFERENCES

1. J. Liu, M.-J. Wu, H. Li, H. Wang, W. Tang, Y.-C. Gu, X.-W. Li, and Y.-W. Guo, *Bioorg. Chem.*, 2021, **114**, 105028.
2. C.-S. Phan, C. S. Yee, C. S. Vairappan, T. Ishii, and T. Kamada, *Chem. Nat. Compd.*, 2019, **55**, 285.
3. G. Li, H. Li, Y.-W. Guo, and X.-W. Li, *Org. Lett.*, 2019, **21**, 5660.
4. T. Kamada, I. I. Zanil, C.-S. Phan, and C. S. Vairappan, *Nat. Prod. Commun.*, 2018, **13**, 123.
5. C.-S. Phan, S.-Y. Ng, T. Kamada, and C. S. Vairappan, *Nat. Prod. Commun.*, 2016, **11**, 899.
6. T. Ishii, T. Kamada, C.-S. Phan, and C. S. Vairappan, *Sains Malays.*, 2018, **47**, 319.
7. C.-S. Phan, T. Kamada, T. Ishii, T. Hamada, and C. S. Vairappan, *Nat. Prod. Commun.*, 2018, **13**, 15.
8. K.-H. Lai, W.-J. You, M. EI-Shazly, Z.-J. Liao, and J.-H. Su, *Mar. Drugs*, 2017, **15**, 327.
9. I. Komal, T. Ito, F. Nagashima, Y. Yagi, M. Kawahata, K. Yamaguchi, and Y. Asakawa, *Phytochemistry*, 2010, **71**, 1387.
10. X. Yan, J. Liu, X. Leng, and H. Ouyang, *Mar. Drugs*, 2021, **19**, 335.
11. P. K. Roy, R. Ashimine, H. Miyazato, J. Taira, and K. Ueda, *Molecules*, 2016, **21**, 679.
12. H. Sun, F. Liu, M.-R. Feng, Q. Peng, X.-J. Liao, T.-T. Liu, J. Zhang, and S.-H. Xu, *Nat. Prod. Res.*, 2016, **30**, 2819.
13. Z.-R. Zeng, W.-S. Li, B. Nay, P. Hu, H.-Y. Zhang, H. Wang, X.-W. Li, and Y.-W. Guo, *Org. Lett.*, 2021, **21**, 7575.
14. X. Yan, H. Ouyang, W. Wang, J. Liu, T. Li, B. Wu, X. Yan, and S. He, *Mar. Drugs*, 2021, **19**, 294.
15. T. Kamada, C.-S. Phan, H.-S. Tin, C. S. Vairappan, and S. T. M. Tengku, *Nat. Prod. Commun.*, 2016, **11**, 1077.
16. A. J. Blackman, B. F. Bowden, J. C. Coll, B. Frick, M. Mahendran, and S. J. Mitchell, *Aust. J. Chem.*, 1982, **35**, 1873.

17. C.-Y. Duh, S.-K. Wang, S.-G. Chung, G.-C. Chou, and C.-F. Dai, *J. Nat. Prod.*, 2000, **63**, 1634.
18. W.-T. Chen, Y. Li, and Y.-W. Guo, *Acta Pharm. Sinica B*, 2012, **2**, 227.
19. I. G. Rodrigues, M. G. Miguel, and W. Mnif, *Molecules*, 2019, **24**, 781.
20. M. Y. Nurrachma, D. Sakaraga, A. Y. Nugraha, S. I. Rahmawati, A. Bayu, L. Sukmarini, A. Atikana, A. Prasetyoputri, F. Izzati, M. F. Warsito, and M. Y. Putra, *Nat. Prod. Bioprospect.*, 2021, **11**, 243.
21. E. Fattorusso, P. Luciano, M. Y. Putra, O. Taglialatela-Scafati, A. Ianaro, E. Panza, G. Bavestrello, and C. Cerrano, *Tetrahedron*, 2011, **67**, 7983.
22. A. Rudi, G. Shmul, Y. Benayahu, and Y. Kashman, *Tetrahedron Lett.*, 2006, **47**, 2937.
23. Y. Li, M. Carbone, R. M. Vitale, P. Amodeo, F. Castelluccio, G. Sicilia, E. Mollo, M. Nappo, G. Cimino, Y.-W. Guo, and M. Gavagnin, *J. Nat. Prod.*, 2010, **73**, 133.
24. J. Yin, M. Zhao, M. Ma, Y. Xu, Z. Xiang, Y. Cai, J. Dong, X. Lei, K. Huang, and P. Yan, *Mar. Drugs*, 2013, **11**, 455.
25. J. Liu, H. Li, M.-J. Wu, W. Tang, J.-R. Wang, Y.-C. Gu, H. Wang, X.-W. Li, and Y.-W. Guo, *J. Org. Chem.*, 2021, **86**, 10975.