

Several New Isoprenoids from the Soft Coral *Sinularia erecta*

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Eight compounds were isolated from the Indo-Pacific soft coral *Sinularia erecta* (Tixier-Durivault, 1945) collected from the lagoon of Mayotte, Comoros Islands, northwest of Madagascar. Four of the compounds, namely the sesquiterpene germacrene D, the diterpenoids nephthenol and decaryiol, and norcembrene, are known. The other four are the new secondary metabolites germacrene E (**1**), *epi*-norcembrene (**3**), and two bis-pukalide diterpenes, mayotolides A and B (**4** and **5**). The structure of each of the compounds was determined by means of 1D and 2D NMR and MS spectroscopy in addition to some chemical transformations.

Marine organisms, especially soft corals, provide many secondary metabolites that exhibit varying degrees of biological activities.¹ Among the most abundant soft coral genera on many coral reefs are *Sinularia* and *Sarcophyton*, which tend to form large monospecific "carpets" of up to several square meters.^{2,3} Both genera contain sesqui- and diterpenes.^{1,3–5} All known *Sinularia* diterpenoid metabolites are based on the cembrane nucleus, mainly 14-membered rings but also 13- and 15-membered ones.³ Among the first diterpenes isolated from the genus *Sinularia*^{3,5} were the sinulariolides from the Indonesian *S. flexibilis*⁷ and pukalide from the Hawaiian *S. abrupta*.⁸ The stereochemistry of pukalide, *vide infra*, was later determined by an X-ray crystallography study⁹ together with the disclosure of other related compounds.¹⁰

Results and Discussion

We now report the investigation of the soft coral *Sinularia erecta* (Tixier-Durivault, 1945, family Alcyoniidae) from the lagoon of Mayotte, Comoros Islands, northwest of Madagascar. The specimen was collected at a depth of 10 m and kept frozen until use. Eight compounds were isolated from the CHCl₃–MeOH (1:2) extract of the soft coral, namely the known diterpenoids nephthenol,¹¹ decaryiol,¹² and norcembrene¹³ and the sesquiterpene germacrene D,¹⁴ as well as four new compounds (**1** and **3–5**). Earlier investigation of the Red Sea *S. erecta* resulted only in high yields (1.5%) of the sesquiterpene $\Delta^{9(15)}$ -africanene⁶ and no significant amounts of other sesqui- or diterpenoids. The nonpolar hexane fraction from a solvent partition of the crude extract of the coral afforded two C₁₅H₂₄ sesquiterpene olefins, the known germacrene D¹⁴ and a new one, designated germacrene E (**1**). The NMR data of **1** pointed clearly to three double bonds, i.e., –CH=C(CH₃)– (δ 5.08 d, 1.55 s (3H), 130.7 d, 131.4 s, and 17.0 q), trans –CH=CH– (δ 5.02 dd, 5.58 dd, 139.3 d, and 126.4 d), and –C(CH₃)=CH₂ (δ 4.64 s, 4.66 s, 1.69 s (3H), 108.1 t, 150.0 s, and 21.4 q), as well as a methine (δ

2.83 m, 54.5 d) and four methylenes (δ 24.0, 33.3, 26.0, and 41.5, all triplets). 2D-NMR experiments, COSY, HMQC, and HMBC (Table 1), established the structure of **1** unequivocally as 4,10-dimethyl-7-(methylethenyl)-1(10),5-cyclodecadiene, an unprecedented marine germacrene. Germacrenes A, C, and D were previously reported independently from *Eunicea mamosa*,¹⁵ *Sinularia polydactyla*, *Lithophyton arboreum*, *Stereonephthea cundabuluensis*,⁴ and *Sinularia mayi*.¹⁴ Finding germacrenes in the Mayotte *S. erecta* as opposed to africanene, the major sesquiterpene in the northern Red Sea coral,⁶ supports the notion that sesquiterpenes in *Sinularia* are produced by symbionts or by a symbiotic relationship with microorganisms. Thus, the use of sesquiterpene character as an aid to taxonomic discrimination, as suggested previously and based on GC derived fingerprints,⁴ seems to be limited to soft corals from a single habitat.

The other six isolated compounds were diterpenoids or diterpenoid derivatives. Besides nephthenol¹¹ and decaryiol,¹² a mixture of two closely related compounds, **2** and **3**, which analyzed for C₁₉H₂₄O₅ by combined spectral methods, have been isolated. Attempts to separate the mixture of **2** and **3** failed. However, the ratio of **2** to **3** changed upon various chromatographies from about 3:1 in the natural extract¹⁶ to about 2:1, confirming the existence of two isomers rather than two conformers.¹⁷ Prolonged mild basic treatment (0.1% K₂CO₃ in MeOH for several weeks) led to a 2:3 mixture of compounds **2** and **3**, respectively. The natural 3:1 mixture of the two isomers enabled the identification of compound **2** by means of MS and 1D and 2D NMR spectra in C₆D₆ as used for norcembrene reported previously from *Sinularia numerosa*.¹³ Comparison of the NMR data of compound **3** (Experimental Section) with those of **2** and especially the slow transformation of compound **2** to **3**, indicated that **3** is a stereoisomer of **2** (Scheme 1). From the structure of **2** and **3** it is clear that epimerization can occur on both sides of the THF ethereal bond: at C-5, which is α to C(6)=O and also β to C(3)=O (enabling a retro-Michael addition), and C-8, which is β to C(6)=O and can also undergo a retro-Michael addition. Indeed, the major observed proton chemical shift changes in **3** are in the THF

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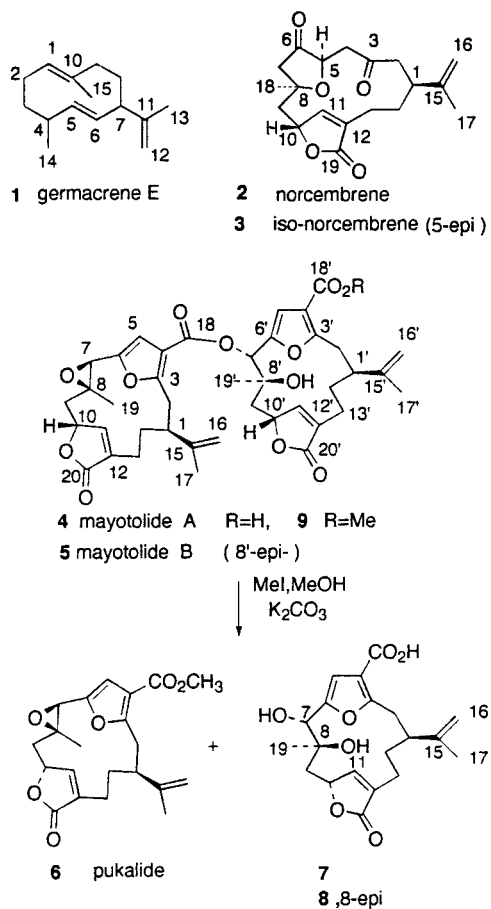
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Table 1. ^1H (500 MHz) and ^{13}C NMR (125 MHz) Data of Germacrene E (**1**)^a

no.	^{13}C	^1H	HMBC C to H	COSY
1	130.7 d	5.08 d (11)	2b, 15	2a, 2b, 15
2a	24.0 t	1.90 brd (14)		1, 2b, 3a, 3b
b		2.42 m		1, 2a, 3a, 3b
3a	33.3 t	1.68 m	5, 6, 14	2a, 2b, 3b, 4
b		1.52 m		2a, 2b, 3a, 4
4	33.9 d	2.42 m	14	3a, 3b, 5, 14
5	139.3 d	5.58 dd (15.8, 3.5)	3b, 14	4, 6
6	126.4 d	5.02 dd (15.8, 1.9)		
7	54.5 d	2.83 brt (10)	6, 9b, 12a, 12b, 13a	6, 8a, 8b, 12a, 12b
8a	26.0 t	1.47 m	6	7, 9a, 9b
b		1.47 m		
9a	41.5 t	2.29 m	1, 15	8a, 8b
b		2.29 m		
10	131.4 s		8a, 8b, 9a, 9b, 15	
11	150.0 s		13a	
12a	108.1 t	4.66 s	13a	7, 13b
b		4.64 s		7, 13a
13	21.4 q	1.69 s	12a, 12b	7, 12a, 12b
14	15.0 q	1.10 d (6.9)	5	4
15	17.0 q	1.56 s	1, 9a, 9b	1

^a Spectra taken in CDCl_3 .**Scheme 1**

surroundings, i.e., H-5 and CH_3 -18. However, because of changes in the macrocycle conformation and, hence, of the transannular interactions, chemical shift changes of H-11 and even H-16a,16b could also clearly be observed. The suggested difference between compounds **2** and **3** is based on d-NOE measurements. In case of norcembrene (**2**), there is a strong (6%) NOE between CH_3 -18 and H-5, which are both on the same (α) side of the THF ring. A weaker effect, observable between CH_3 -18 and H-16a (1%), points clearly to a puckered conformation of the macrocycle, which brings H-16a into

proximity with CH_3 -18. In **3**, on the other hand, there is no NOE between CH_3 -18 and H-5; therefore, they have to be on opposite sides of the THF ring. A weak NOE between H-5 and H-11 (0.5%) (not existing in **2**) is in better agreement with epimerization at C-5. Thus, tentatively, compound **3** is suggested to be 5-*epi*-norcembrene.

The last pair of compounds isolated from the coral were compounds **4** and **5**, both of which analyzed for $\text{C}_{40}\text{H}_{44}\text{O}_{12}\text{Na}$ by combined FAB-MS and NMR data. The NMR data of compound **4**, mayotolide A (Table 2), suggested it to be assembled from two closely related 20 carbon halves. The presence in each half, of a methylethenyl (C-15–C-17), a quaternary methyl (C-19), and two conjugated lactone/ester functionalities (C-18 and -20), originating, most likely, biogenetically from methyl groups (δ_{C} 145.5 s, 113.1 t, 18.1 q; 18.7 q; and 162.3 s and 174.7 s for the “left” part of **4**, respectively, Table 2) suggested a diterpene structure for each half. The carbon atom resonances of the left half of **4** further suggested an epoxide (C-7,8) and that one of the above-mentioned carbonyl groups is conjugated to a furan (C-3–C-6 and C-18) and the other is part of an unsaturated γ -lactone (C-10–C-12, and C-20) (Table 2).

For the “right” part, we see the same functionalities except for replacement of the 7,8 epoxide by an alcohol (on C-8') and an ester group (on C-7'). Comparison of the NMR data of mayotolide A (**4**) with known marine cembrane-derived diterpenoids showed a high similarity between the left part and pukalide.⁸ Indeed, mild basic methylation–methanolysis of **4** (K_2CO_3 , MeI, MeOH)¹⁸ afforded two diterpenoids, **6** and **7** (Scheme 1), which were separated by silica gel chromatography. Compound **6** was found to be identical to pukalide (NMR, MS, IR)⁸ and **7** to be a pukalide derivative, namely, 7 α ,8 β -dihydroxy-18*O*-demethyldeopypukalide. The connection between the two halves of **4** became evident from a CH–connectivity (HMBC experiment) between CO_2 (18) and H-7', that is, an ester linkage between the original left CO_2H group and the right C-7'-hydroxyl. The latter ester bond makes clear the biogenesis of this bispukalide **4**, namely, acid-catalyzed or autocatalyzed opening of the epoxide of 18*O*-demethylpukalide (the acid of **6** before methylation) by the carboxylic group of a second 18*O*-demethyl pukalide molecule to form the dimeric bispukalide structure. Micro- CH_2N_2 methylation of **4** (1 mg) afforded the expected 18'-methyl ester (**9**) (δ_{H} 3.82 s, 3H), confirming the 18'-carboxylic group of **4**. Peculiar in the NMR spectrum of **4** (and **5**) was the absence of the H-11' signal, which could, however, be deduced from the HMQC experiment, in contrast to the sharp singlet of H-11 in compound **7**. This pointed clearly to the strong influence of the left pukalide molecule on the conformation of the macrocycle of the right part of mayotolide A (and B). Slow tumbling macrocycles resulting in strong broadening to total disappearance of proton signals are not uncommon within the 14-membered terpenoids.

While the stereochemistry of the left part of **4** was determined by its transformation to pukalide, a 7 α ,8 β -dihydroxy stereochemistry (the methylethenyl defined as β) was suggested for the right part on the basis of measured NOE's of compound **7**. Most significant were the observed NOE's of CH_3 -19, of **7**, with H-5, -7, and

Table 2. ^1H and ^{13}C NMR Data of Mayotolides A and B (**4** and **5**)^a

no.	4			5	
	^{13}C	^1H	HMBC (C to H)	^{13}C	^1H
1	40.4 d	3.45 t (10.7)	2a, 2b, 13a, 13b, 17	40.5 d	3.40 t (10.8)
2a	32.4 t	2.98 dd (18.3, 12.6)	1	32.7 t	2.98 dd (18.2, 12.5)
b		2.82 d (18.3)			2.75 d (18.2)
3	148.3 s		1, 5	148.3 s	
4	113.1 s		2a, 2b, 5	113.1 s	
5	106.1 d	6.25 s	7	106.1 d	6.25 s
6	148.4 s		5, 7	148.3 s	
7	54.8 d	3.96 s	9a, 19	54.8 d	3.95 s
8	57.2 s		7, 9a, 9b, 10, 19	57.2 s	
9a	39.5 t	2.50 m	19	39.7 t	2.45 m
b		2.08 m			2.05 m
10	78.2 d	5.07 bs	11	78.0 d	5.02 s
11	148.9 d	7.07 s	14a, 14b	148.5 d	7.04 s
12	137.1 s		11, 13a, 13b, 14b	137.1 s	
13a	22.5 t	2.33 m		22.7 t	2.32 m
b		2.33 m			2.32 m
14a	32.5 t	1.80 m		32.8 t	1.75 m
b		1.05 m			1.00 m
15	145.5 s		17	145.6 s	
16a	113.0 t	5.03 s	17	113.2 t	4.98 s
b		4.75 s			4.70 s
17	18.1 q	1.68 s		18.3 q	1.55 s
18	162.3 s		2a, 2b, 5, 7'	162.3 s	
19	18.7 q	0.96 s		18.8 q	0.90 s
20	174.7 s		11, 13a, 13b	174.7 s	
1'	43.8 d	2.10 m	2'a, 2'b, 13'a, 13'b, 17'	43.9 d	2.05 m
2'a	31.5 t	3.41 dd (17.5, 14.5)	1'	31.7 t	3.55 dd (17.6, 14.4)
b		2.61 d (17.5)			2.55 (17.6)
3'	149.6 s		1', 5'	150.0 s	
4'	118.0 s		2'b, 5'	118.1 s	
5'	110.6 d	6.49 s	7'	110.5 d	6.46 s
6'	148.4 s		5', 17'	148.4 s	
7'	75.5 d	5.56 s	9'a, 9'b, 19'	75.1 d	5.52 s
8'	71.9 s		7', 9'a, 9'b, 19'	72.3 s	
9'a	43.6 t	2.46 m	19'	43.4 t	2.45 m
b		1.92 m			1.85 m
10'	78.9 d	4.86 d (10.5)	9'a, 9'b	78.7 d	4.82 d (10.0)
11'	148.9 d	5.85 ^b	7'	148.8 d	5.84 ^b
12'	132.8 s		13'a, 13'b, 14'b	132.8 s	
13'a	21.4 t	2.25 m	1'	21.6 t	2.18 m
b		2.05 m			2.02 m
14'a	28.1 t	1.75 m		28.1 t	1.65 m
b		1.53 m			1.43 m
15'	146.5 s		17'	148.5 s	
16'a	111.7 t	4.65 s	1', 17'	112.1 t	4.60 s
b		4.60 s			4.55 s
17'	18.1 q	1.67 s		18.3 q	1.56 s
18'	158.0 s		2'a, 2'b, 5'	158.0 s	
19'	19.5 q	1.43 s		19.8 q	1.35 s
20'	174.1 s		11, 13a, 13b	174.1 s	

^a Spectra taken in CDCl_3 . ^b Signal deduced from the HMQC experiment.

10 simultaneously (4.5, 2.5, and 6%, respectively), and the NOE's between CH_3 -17 and H-7 (1%), together proving all the latter protons to be on the same β side of the molecule. The $7\alpha,8\beta$ -dihydroxy configuration of **7** also defines, of course, the stereochemistry of these centers in (numbers with a prime) **4**. Similar NOE's were also observed for **4**, namely, CH_3 -19' to H-5', -7', -10' (2.5% for all) and, in addition, an NOE between CH_3 -19' of the "right" part to H-5 of the left part (4%).

The NMR data of compound **5**, mayotolide B (Table 2), were very similar to those of mayotolide A (**4**) and, under the same basic methylation–methanolysis conditions, *vide supra*, **5** also gave pukalide (**6**) and an isomer of **7**, compound **8** (Scheme 1). The major difference between the NMR data of **7** and **8** were differences in the d-NOE experiment. Irradiation of CH_3 -19, of **8**, exhibited NOE's with H-5, -7, and -10 (6, 2.5, and 6.5%, respectively), as in **7**, but also to H-11 (4%) best

rationalized by an 8-epimeric structure. The still-existing NOE between CH_3 -17 and H-7 confirmed that the configuration of C-1 and C-7 remained unchanged. Therefore, compound **5** is the $8'\alpha$ -hydroxy epimer of **4**.

While mayotolide-A (**4**), as explained above, seems to be obtained directly from 18*O*-demethylpukalide, compound **5**, mayotolide B, is assumed to be derived from **4** by acid-catalyzed epimerization of the tertiary 8-hydroxy group.

Experimental Section

General Experimental Procedures. IR spectra were recorded on a Nicolet 205 FT-IR spectrophotometer. LRMS and HRMS were recorded on a Fisons, Autospec Q instrument. ^1H and ^{13}C NMR spectra were recorded on a Bruker ARX-500 spectrometer. All chemical shifts are reported with respect to residual CHCl_3 (δ 7.25 for ^1H and 77 ppm for ^{13}C). Optical rotations

were measured on a Perkin-Elmer Model 141 polarimeter using a 1 cm microcell.

Biological Material. The soft coral *S. erecta* was collected at the lagoon of Mayotte, Comoros Islands, northwest of Madagascar, by scuba at a depth of 10 m in May 1995. A voucher specimen is deposited at Tel-Aviv University (AMS-22).

Extraction and Isolation. The soft coral (300 g) was homogenized and extracted with $\text{CHCl}_3/\text{MeOH}$ (1:2) to give a brown gum (1 g) after evaporation. The gum (800 mg) was partitioned into aqueous MeOH and hexane, CCl_4 , and CHCl_3 . The hexane fraction (450 mg) was subjected to silica gel chromatography to give germacrene D (12 mg) and germacrene E (1, 9 mg). The CCl_4 fraction (160 mg) gave, upon silica gel chromatography, a 3:1 mixture of compounds **2** and **3** (25 mg). The CHCl_3 fraction (150 mg) afforded, upon repeated Sephadex LH-20 chromatography eluted with $\text{MeOH}/\text{CHCl}_3$ (1:1), mayotolide A (**4**, 20 mg) and mayotolide B (**5**, 8 mg).

Germacrene E (1): oil; $[\alpha]_D +2.1^\circ$ (c 0.23, MeOH); ^1H and ^{13}C NMR, see Table 1; HREIMS m/z 204.1892 $[\text{M}^+]$ (calcd for $\text{C}_{15}\text{H}_{24}$, 204.1897).

Norcembrene (2) and epi-norcembrene (3): oil (3:1 mixture). The ^{13}C NMR (CDCl_3) and ^1H NMR (C_6D_6) of the major compound (**2**) were identical with the reported data for norcembrene.⁵

Compound 3: oil; ^1H NMR (C_6D_6) δ 0.92 (3H, s, Me-18), 1.03 (1H, dd, $J = 15.3, 3.0$ Hz, H-9b), 1.47 (3H, s, Me-17), 1.52 (1H, m, H-9a), 2.00 (1H, m, H-13b), 2.11 (1H, dd, $J = 14.2, 11.2$ Hz, H-4b), 2.29 (1H, d, $J = 14.2$ Hz, H-4a), 2.38 (1H, m, H-13a), 4.08 (1H, d, $J = 11.2$ Hz, H-5), 4.28 (1H, m, H-10), 4.78 (1H, s, H-16b), 4.81 (1H, s, H-16a), 6.41 (1H, s, H-11); ^{13}C NMR δ 212.0 (s, C-6), 207.9 (s, C-3), 151.6 (d, C-11), 145.2 (s, C-15), 130.8 (s, C-12), 113.0 (t, C-16), 78.6 (s, C-8), 78.4 (d, C-10), 74.7 (d, C-5), 51.0 (t, C-7), 50.1 (t, C-9), 43.9, 41.6 (t, C-2,4), 38.7 (d, C-1), 29.1, 17.9 (t, C-13,14), 27.8 (q, CH_3 -18), 18.3 (q, CH_3 -17).

Compound 4: oil; $[\alpha]_D +43.0^\circ$ (c 0.33, MeOH); IR (KBr) ν_{max} 3423, 2950, 1742, 1620, 1223, 1066, 1037 cm^{-1} ; ^1H and ^{13}C NMR, see Table 2; correlations observed in a COSY experiment, H-1/2a,2b,14a,14b; H-5/7; H-10/9a,9b,11; H-13a/13b, 14a,14b; H-1'/2'a,2'b, 14'a,14'b; H-5'/7'; H-10'/9'a,9'b,11'; H-13'a/13'b, 14'a, 14'b; FABMS m/z 739 $[\text{C}_{40}\text{H}_{44}\text{O}_{12}\text{Na}, \text{M}^+ + \text{Na}]$.

Compound 5: oil; $[\alpha]_D +48.5^\circ$ (c 0.23, MeOH); IR (KBr) ν_{max} 3420, 2950, 1742, 1621, 1066, 1040 cm^{-1} ; ^1H and ^{13}C NMR, see Table 2; FABMS m/z 739

$[\text{M}^+ + \text{Na}]$.

Basic Hydrolysis of Compounds 4 and 5. Mayotolide A (**4**, 20 mg) in a mixture of MeOH (1 mL), acetone (10 mL), and MeI (0.5 mL) in the presence of anhydrous K_2CO_3 (10 mg) was left at room temperature for 20 h. The carbonate was then filtered away, the solvent evaporated under vacuum, and the residue subjected to a silica gel column to afford pukalide (**6**, 3 mg) and compound **7** (4 mg). Hydrolysis of mayotolide B (**5**, 8 mg), under the same conditions as described for **4**, afforded pukalide (**6**, 2 mg) and compounds **8** (3 mg) and **9** (2 mg).

Compound 7: oil; ^1H NMR (CDCl_3) δ 1.42 (3H, s, Me-19), 1.50 (1H, m, H-14b), 1.72 (3H, s, Me-17), 1.80 (1H, m, H-14a), 1.85 (1H, dd, $J = 13.6, 12.0$ Hz, H-9b),

2.08 (1H, bd, $J = 13.9$ Hz, H-13b), 2.15 (1H, t, $J = 11.5$ Hz, H-1), 2.37 (1H, t, $J = 13.9$ Hz, H-13a), 2.58 (1H, dd, $J = 11.5, 4.3$ Hz, H-9a), 2.65 (1H, dd, $J = 14.5, 2.1$ Hz, H-2b), 3.40 (1H, dd, $J = 8.9, 7.0$ Hz, H-2a), 3.82 (3H, s, OCH_3), 4.49 (1H, s, H-7), 4.73 (1H, s, H-16b), 4.76 (1H, s, H-16a), 6.96 (1H, d, $J = 11.4$ Hz, H-10), 5.59 (1H, s, H-11), 6.70 (1H, s, H-5); ^{13}C NMR δ 44.1 (d, C-1), 31.7 (t, C-2), 160.5 (s, C-3), 116.8 (s, C-4), 108.8 (d, C-5), 152.5 (s, C-6), 75.6 (d, C-7), 73.6 (s, C-8), 42.7 (t, C-9), 78.6 (d, C-10), 148.0 (d, C-11), 133.6 (s, C-12), 21.6 (t, C-13), 27.7 (t, C-14), 146.1 (s, C-15), 112.6 (t, C-16), 18.9 (q, C-17), 165.0 (s, C-18), 19.3 (q, C-19), 173.3 (s, C-20), 51.6 (q, OCH_3); EIMS m/z 372 $[\text{C}_{21}\text{H}_{24}\text{O}_6, \text{M}^+, 5]$, 340 (10), 223 (100), 168 (80).

Compound 8: oil; ^1H NMR (CDCl_3) δ 1.42 (3H,s), 1.51 (1H, m), 1.71 (3H, s), 1.80 (1H, m), 1.89 (1H, dd, $J = 14.8, 12.0$ Hz), 2.08 (1H, bd, $J = 14.5$ Hz), 2.15 (1H, t, $J = 11.0$ Hz), 2.30 (1H, t, $J = 14.5, 6.0$ Hz), 2.58 (1H, dd, $J = 14.9, 4.1$ Hz), 2.64 (1H, dd, $J = 14.5, 2.0$ Hz), 3.46 (1H, dd, $J = 8.9, 7.0$ Hz), 3.85 (3H, s, OCH_3), 4.56 (1H, s), 4.80 (1H, s), 4.83 (1H, s), 4.94 (1H, bd, $J = 11.7$ Hz), 5.66 (1H, s), 6.74 (1H, s); EIMS m/z 372 $[\text{C}_{21}\text{H}_{24}\text{O}_6, \text{M}^+, 10]$.

Compound 9: oil; $[\alpha]_D +24.0^\circ$ (c 0.25, MeOH); ^1H NMR (CDCl_3) δ 1.04 (3H, s), 1.50 (3H, s), 1.75 (3H, s), 1.76 (3H, s), 3.82 (3H, s), 4.09 (1H, s), 4.80 (1H, s), 4.83 (1H, s), 4.92 (1H, s), 4.94 (1H, d, $J = 4.9$ Hz), 5.18 (1H, s), 5.21 (1H, s), 5.70 (1H, s), 6.34 (1H, s), 6.60 (1H, s), 7.10 (1H, s); EIMS m/z 730 $[\text{C}_{41}\text{H}_{46}\text{O}_{12}, \text{M}^+, 15]$, 712 (20), 372 ($\text{C}_{21}\text{H}_{24}\text{O}_6$, 65), 340 (55), 208 (60), 153 (100).

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- The 3:1 ratio between **2** and **3** is seen already in the NMR spectrum of the crude extract. Moreover, as TFA does not equilibrate the isomers and the basic equilibration is very slow, both epimers are natural products.
- Surprisingly, the mixture of compounds **2** and **3** was not stable on a RP-18 column.
- Traces of the methyl ester of **4** were also obtained during the methylation–methanolysis reaction.
- It is important to note that freeze-drying of soft corals should not be used if the content of sesquiterpenes is of interest, as the volatile compounds will be lost with the water.