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Two new bicyclic cembranolides from a new *Sarcophyton* species and determination of the absolute configuration of sarcoglaucol-16-one †

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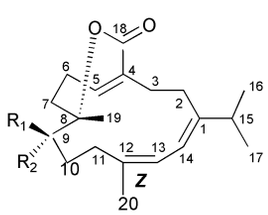
During the chemical investigation of a new species of soft coral belonging to the genus *Sarcophyton*, two novel cembranoid compounds with a 12*Z* double bond (**1** and **2**) were obtained. Determination of the double bond configurations was done using NMR spectroscopic data and the results of molecular modeling studies. Compound **3**, one of several known compounds (**3**–**5**), was found to be cytotoxic toward an MCF7 tumor cell line.

Additionally, we report the absolute stereochemistry of the cembrane sarcoglaucol-16-one (**6**), which was determined using the modified Mosher's method.

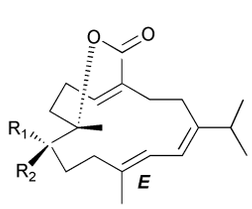
Introduction

The chemistry of soft coral is dominated by terpenes, mainly diterpenes of the cembranoid type.[1] They are produced to defend the animal and its larvae against predators, such as other coral or fish, and against settlement of microorganisms like fungi or bacteria.[2] Cembranoids are also interesting for pharmacological research, since many derivatives show significant biological activity, including antimicrobial, Ca-antagonistic and anti-inflammatory properties. The antitumor effect of cembranes is, however one of the most important activities of this class of natural products.[3,4,5]

In the course of our ongoing studies on marine natural products, we investigated a new *Sarcophyton* species and found two new cembranoid compounds with a 12*Z* double bond (**1** and **2**), along with three known compounds (**3**–**5**). The cembrane derivatives with a 12*Z* double bond (**1** and **2**) represent very rare structures in nature. There are only five examples of such compounds known in the marine literature, *i.e.* (1*Z*,3*Z*,11*E*)- and (1*E*,3*Z*,11*E*)-1,3,11-cembratrien-6-one from the Caribbean sea whip *Eunicea calyculata,*[6] sarcophytols N and J (1*Z*,3*Z*,7*E*,11*E*),[7] and sarcophytol T (1*E*,3*Z*,7*E*,11*E*)[8] from *Sarcophyton glaucum*.

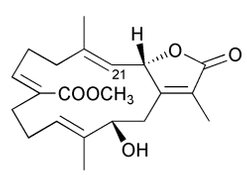


(**1**) R_1=H, R_2=OH
(**2**) R_1, R_2 = O



(**3**) R_1=H, R_2=OH
(**4**) R_1, R_2 = O
(**5**) R_1=H, R_2=OAc

In a previous study, we isolated several cembranoid diterpenes from *S. cherbonnieri*.[5] The deduction of the stereochemistry of these compounds was based on the comparison of their CD spectra with that of sarcophine (**7**). In contrast to many statements in the literature,[9–14] the absolute stereochemistry of sarcophine (**7**) was never established (Y. Kashman, personal communication). Our present work unambiguously establishes the absolute stereochemistry of **6**, and thus also of **7**, by applying Mosher's method.



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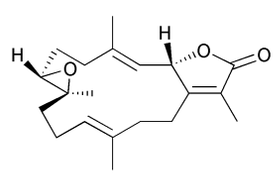

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Results and discussion

HR-MS and ^{13}C NMR analysis of **1** indicated it to have the molecular formula $C_{20}H_{30}O_3$. From ^{1}H, ^{13}C NMR, MS, and IR data, it was evident that the molecule contained one alcohol function (66.7 ppm, 3422 cm^{-1}), three carbon–carbon double bonds (δ 119.9 d, 123.5 d, 133.7 s, 135.8 s, 140.3 d, 145.2 s) and one ester carbonyl group (δ 166.9 s, 1665 cm^{-1}). As the molecular formula of **1** required it to have six elements of unsaturation, **1** had to be bicyclic. More detailed analysis of its spectroscopic data suggested that **1** was a cembranoid-based diterpene.

The ^{1}H–^{1}H COSY spectrum resolved the partial structures **A**–**D** (Fig. 1), which were connected using HMBC correlations. The HMBC spectrum demonstrated coupling between H_3-16 and carbon C-17, and also showed methyl protons, H_3-16 and H_3-17, to be coupled to carbon C-1 and C-15. Thus, fragment **A** forming an isopropyl group was bound to C-1. Substructure **B** was confirmed by long range CH couplings observed between H-5 and C-3, C-6, C-7 and C-18. The methyl protons H_3-19

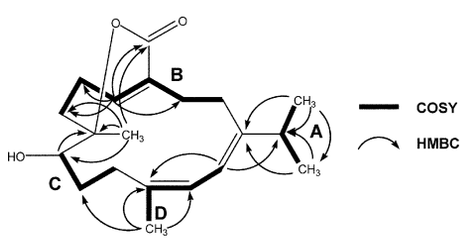

Fig. 1 ^{1}H–^{1}H-COSY and ^{1}H–^{13}C long range correlations of **1**.

† Electronic supplementary information (ESI) available: minimum energy conformations of compounds **1** and **2**. See http://www.rsc.org/suppdata/ob/b3/b314332e/

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Table 1 ^{1}H-NMR spectral data for compounds **1**–**5** (δ in ppm, J in Hz)[a,b]

Proton	**1**	**2**	**3**	**4**	**5**
2	2.05[c] (1H, m) 2.48[c] (1H, m)	2.25 (1H, m) 2.50 (1H, m)	2.09[c] (2H, m)	2.05[c] (1H, m) 2.49[c] (1H, m)	
3	2.57[c] (2H, m)	2.11 (1H, m) 2.45 (1H, m)	2.53[c] (2H, m)	2.30[c] (1H, m) 2.60[c] (1H, m)	
5	6.19 (1H, t, J = 4.4)	6.27 (1H, br t, J = 4.4)	6.11 (1H, br t, J = 4.4)	6.03 (1H, t, J = 4.4)	6.11 (1H, dd, J = 3.7, 4.8)
6	2.16 (1H, m) 2.47 (1H, m)	2.33 (2H, m)	2.05 (1H, m) 2.23 (1H, m)	1.94 (1H, m) 2.40 (1H, m)	
7	2.21 (1H, m) 3.15 (1H, m)	2.23 (1H, m) 3.14 (1H, m)	1.85 (1H, m) 3.16 (1H, m)	1.88 (1H, m) 3.10 (1H, m)	3.11 (2H, m)
9	4.11 (1H, d, J = 11.0)	—	4.29 (1H, d, J = 10.3)	—	5.56 (1H, d, J = 7.7)
10	1.45 (1H, m) 2.47 (1H, m)	3.56 (2H, m)	1.54 (1H, m) 2.24 (1H, m)	2.39 (1H, m) 3.52 (1H, m)	
11	1.99 (1H, m) 2.18 (1H, m)	1.96 (1H, m) 2.39 (1H, m)	2.15 (1H, m) 2.40 (1H, m)	2.25 (1H, m) 2.51 (1H, m)	
13	6.02 (1H, d, J = 8.8)	5.79 (1H, d, J = 8.8)	5.57 (1H, d, J = 11.3)	5.47 (1H, d, J = 11.0)	5.59 (1H, d, J = 11.0)
14	5.76 (1H, d, J = 8.8)	5.79 (1H, d, J = 8.8)	6.14 (1H, d, J = 11.3)	6.04 (1H, d, J = 11.0)	6.18 (1H, d, J = 11.0)
15	2.48 (1H, m)	2.47 (1H, m)	2.32 (1H, m)	2.28 (1H, m)	
16	0.99 (3H, d, J = 6.6)	1.10 (3H, d, J = 7.0)	1.08 (3H, d, J = 7.0)	1.06 (3H, d, J = 7.0)	1.08 (3H, d, J = 7.0)
17	0.93 (3H, d, J = 6.6)	0.98 (3H, d, J = 7.0)	1.05 (3H, d, J = 7.0)	1.03 (3H, d, J = 7.0)	1.04 (3H, d, J = 7.0)
19	1.28 (3H, s)	1.46 (3H, s)	1.35 (3H, s)	1.54 (3H, s)	1.46 (3H, s)
20	1.83 (3H, s)	1.87 (3H, s)	1.78 (3H, s)	1.76 (3H, s)	1.71 (3H, s)
21	—	—	—	—	2.05 (3H, s)

[a] All assignments are based on extensive 1D and 2D NMR measurements (HMBC, HSQC, ^{1}H–^{1}H-COSY). [b] $CDCl_3$, 300 MHz. [c] Interchangeable.

exhibited HMBC couplings to carbons C-7, C-8, and C-9, which allowed the connection of partial structure **C** to **B**. H_3-20 showed long range couplings in the HMBC spectrum with C-12 and C-13, identifying C-20 as the methyl group on the Δ^{12} double bond. The connectivity of **C** and **D** *via* carbon C-11 resulted from cross peaks in the HMBC spectrum between H_3-20 and C-10. Coupling between H-14 and C-15 placed partial structure **D** adjacent to **A** *via* carbon C-1. The remaining linkage between **A** and **B** to obtain the first ring closure followed by deduction. The chemical shift of carbon C-8 (δ 82.6 ppm) and a cross peak between H_3-19 and carbon C-18 in the HMBC spectrum indicated the second ring closure to occur *via* an oxygen, forming a lactone function.

Hence, **1** possessed the same basic structure as the known compound **3**,[15,16] and the two molecules must differ in the stereochemistry of either, the chiral centers C-8, C-9 and/or the geometry of the Δ^4, Δ^{12} or $\Delta^{1,14}$ double bonds. When comparing the NMR data of **1** and **3**, remarkable differences of the ^{1}H NMR chemical shifts for H-7, H-10 and the diene protons H-13 and H-14 were seen (Table 1). Also, the ^{13}C NMR chemical shift for C-20 of **1**, was shifted downfield relative to **3** (C-20 for **1**: δ 27.9 *versus* C-20 for **3**: δ 19.0). The latter fact suggested the geometry of the Δ^{12} double bond to be *Z* instead of *E* as in the case of **3**.[17] For the known compound **3** (4*Z*,12*E*,14*E*,8*S*,9*R*) and its C-9 epimer (4*Z*,12*E*,14*E*,8*S*,9*S*) an upfield shift of the ^{1}H NMR resonance of H-9 (δ 4.30 *versus* 4.15) and H_3-19 (δ 1.38 *versus* 1.16) was evident, when comparing NMR data for the 9*R* and 9*S* derivative.[15,16] Taking these characteristic ^{1}H NMR shifts into account, the 8*S*,9*S* configuration is most probable for compound **1** (H-9: δ 4.11 and H_3-19: δ 1.28). The stereochemistry is also reflected in the size of the optical rotation. For the known 8*S*,9*R* derivative (**3**) a considerably larger $[\alpha]_D$ value (Bowden *et al.*[15] +161.0°, Uchio *et al.*[16] +160.0°), is observed than for the C-9 epimer (Bowden *et al.*[15] +61.0°, Uchio *et al.*[16] +70.9°). Thus, the optical rotation of compound **1** ($[\alpha]_D$ = +84.0°) also supports the 8*S*,9*S* configuration.

The geometry of the Δ^4 double bond must be *Z*, otherwise the formation of the seven membered lactone ring is not possible. This is supported by very similar ^{13}C NMR shifts for C-4 and C-5, and ^{1}H NMR shifts for H-5 as well as the $J_{5,6}$ ^{1}H–^{1}H-coupling constant of **1** compared with the known compound **3**.

This left the configuration of the $\Delta^{1,14}$ double bond to be established. Due to the instability of the compound, however it had decomposed before the measurement of NOE experiments was possible. Extensive literature searches for cembranoids, with a conjugated diene structure, revealed that the ^{1}H–^{1}H coupling constant $J_{13,14}$ of *E*,*E*- and *Z*,*Z*-dienes showed values above 11 Hz, while coupling constants for *Z*,*E*-derivatives were found to be smaller.[6–8] Since in **1** $J_{13,14}$ is 8.8 Hz, the 12*Z*,14*E* configuration is suggested for this compound.

The $\Delta^{1,14}$ double bond geometry was confirmed by molecular modeling calculations. Minimum energy conformations of the two possible isomers of **1** (12*Z*,14*E* and 12*Z*,14*Z*) were calculated and the torsion angle between the coupled nuclei H-13 and H-14 was acquired from this model (Figs 2 and 3). This model-derived torsion angle was compared with the one calculated from the size of the vicinal ^{1}H–^{1}H coupling constant (^3J) through the Karplus equation (see Table 3).[18] The torsion angle found in the modelled and minimized structure of **1** and the one calculated from $J_{13,14}$ through the Karplus equation fitted best for the 12*Z*,14*E* configuration. Compound **1** is thus

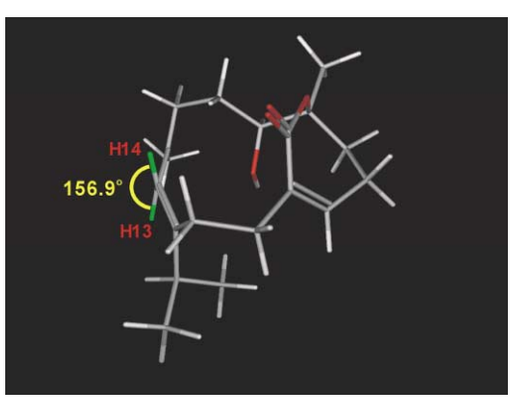

Fig. 2 Minimum energy conformation of the 12*Z*,14*Z* derivative of **1**.

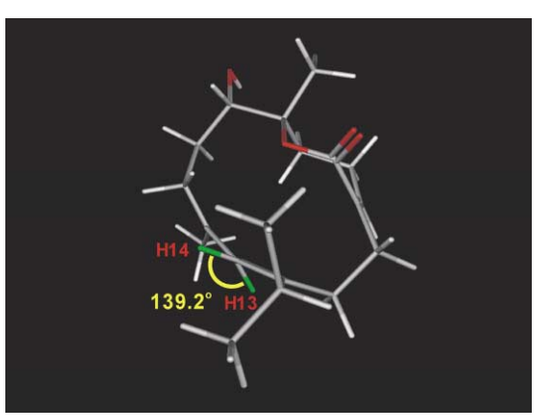

Fig. 3 Minimum energy conformation of the 12*Z*,14*E* derivative of **1**.

proven to be (4*Z*,8*S*,9*S*,12*Z*,14*E*)-9-hydroxy-1-isopropyl-8,12-dimethyl-oxabicyclo[9.3.2]-hexadeca-4,12,14-trien-18-one.

The molecular formula of **2**, $C_{20}H_{28}O_3$ was deduced from accurate mass measurement. It required seven double bond equivalents, five of them being accounted for by the presence of two carbonyl functionalities (ketone: δ 211.4, ester: δ 166.8) and three carbon–carbon double bonds, indicating the bicyclic nature of **2**. Comparison of the ^{1}H, ^{13}C, and HMBC NMR data of **2** with those of **1** suggested, that the two molecules were closely related. Further examination of their spectroscopic data revealed the differences of the two compounds to result from the presence of a carbonyl group (δ 211.4) at C-9 in **2** instead of the alcohol function (δ 66.7) found in **1**. On the basis of the ^{13}C NMR chemical shift for C-20 (δ 26.7),[17] the geometry of the Δ^{12} double bond was defined as *Z*. Molecular modeling calculations to prove the 12*Z*,14*E* geometry of the diene led to similar results as in the case of compound **1** (see Table 3). Hence, **2** is the 12*Z*-derivative of the known compound sarcophytolide (**4**).[15,16] This was also supported by comparison of the ^{13}C-NMR shift values of **2** with those of **4**. Only significant differences for C-11, C-12, C-13 and C-20 were observed due to the difference in the Δ^{12} configuration. For **2** the trivial name 4*Z*,12*Z*,14*E* sarcophytolide is proposed.

The identity of compounds **3**–**5** was established by direct comparison of the $[\alpha]_D^{20}$, IR, HR-EIMS, ^{1}H NMR, and ^{13}C NMR data with literature data.[15,16] The ^{1}H and ^{13}C NMR chemical shifts of **3**–**5**, which were not thoroughly assigned in the previous literature,[15,16] are reported in Tables 1 and 2.

From a further *Sarcophyton* species, *S. cherbonnieri*, compound **6**, sarcoglaucol-16-one, was isolated in a previous study, and its relative configuration determined.[5] The configuration of **6** and related compounds was based on that of sarcophine (**7**).[19,20] Several reports take the absolute configuration of sarcophine (**7**) as established.[9–14] To our knowledge, however, only the relative configuration was deduced by X-ray and CD studies.[19,20] Hence, we applied Mosher′s method to determine the absolute stereochemistry, for sarcoglaucol-16-one (**6**) and thus, since CD spectra for **6** and **7** are nearly congruent, also for sarcophine (**7**). At first methoxy-trifluoromethylphenylacetic acid chloride (MTPA-Cl) was applied, as used successfully by Kakisawa′s group to determine the absolute stereochemistry of the structurally closely related cembrane **8**.[21] (*R*)- and (*S*)-esters were obtained, but regrettably the ^{1}H NMR $\Delta\delta$ values of the esters were irregularly distributed on both sides of the MTPA plane and stereochemical deductions were therefore ambiguous. According to Riguera *et al.*,[22] methoxyphenylacetic acid (MPA) can be used instead of MTPA in such a case. The ^{1}H–^{13}C HSQC NMR spectra of the obtained (*R*)- and (*S*)-MPA esters[23] of **6** were assigned, and the calculated values [δ of protons in the (*R*)-MPA-ester − δ of the corresponding protons in the (*S*)-MPA ester] are shown in Fig. 4. The interpretation of positive and negative $\Delta\delta$ values led to the assignment of the *S* absolute stereochemistry for C-13 of **6** (Fig. 5). From the results of selective gradient NOE-experiments, previously described,[5] the relative stereochemistry at the C-13 and C-2 stereogenic center was determined. Thus, compound **6** was assigned the 2*S*,13*S* absolute stereochemistry. The absolute configuration at C-2 of sarcophine (**7**) was shown to be the same as in **6** from CD measurements.[5] Sarcophine (**7**) thus has the 2*S*,7*S*,8*S* configuration.

Table 2 ^{13}C NMR spectral data for compounds **1**–**5** (δ in ppm)[a,b,c]

Carbon	1	2	3	4	5
1	145.2 (s)	146.0 (s)	146.1 (s)	145.4 (s)	145.1 (s)
2	27.7 (t)	25.7 (t)	27.0[d] (t)	27.2[d] (t)	26.6[d] (t)
3	27.8 (t)	27.5 (t)	27.4[d] (t)	27.2[d] (t)	27.1[d] (t)
4	133.7 (s)	132.1 (s)	133.4 (s)	134.0 (s)	133.0 (s)
5	140.3 (d)	143.6 (d)	140.2 (d)	143.2 (d)	140.7 (d)
6	32.0 (t)	34.0 (t)	34.5 (t)	33.7 (t)	34.5 (t)
7	34.3 (t)	32.8 (t)	37.4 (t)	36.8 (t)	37.7 (t)
8	82.6 (s)	87.3 (s)	83.4 (s)	86.7 (s)	82.3 (s)
9	66.7 (d)	211.4 (s)	66.2 (d)	209.5 (s)	69.1 (d)
10	27.3 (t)	31.9 (t)	26.7 (t)	31.9 (t)	24.8 (t)
11	34.8 (t)	34.0 (t)	31.4 (t)	29.7 (t)	31.9 (t)
12	135.8 (s)	137.8 (s)	132.2 (s)	131.0 (s)	132.9 (s)
13	123.5 (d)	122.5 (d)	121.0 (d)	119.5 (d)	120.7 (d)
14	119.9 (d)	119.6 (d)	118.8 (d)	118.9 (d)	119.4 (d)
15	30.2 (d)	31.3 (d)	35.7 (d)	35.3 (d)	35.8 (d)
16	19.9 (q)	20.5 (q)	22.1 (q)	22.0 (q)	22.3 (q)
17	23.3 (q)	23.0 (q)	22.6 (q)	22.9 (q)	22.8 (q)
18	166.9 (s)	166.8 (s)	166.9 (s)	166.5 (s)	166.7 (s)
19	22.3 (q)	29.4 (q)	21.8 (q)	29.5 (q)	21.1 (q)
20	27.9 (q)	26.7 (q)	19.0 (q)	18.9 (q)	18.6 (q)
21	—	—	—	—	170.1 (s)
22	—	—	—	—	23.7 (q)

[a] $CDCl_3$, 75.5 MHz. [b] Assignments are based on extensive 2D NMR measurements (HMBC, HSQC, ^{1}H–^{1}H-COSY). [c] Implied multiplicities determined by DEPT (C = s; CH = d; CH_2 = t; CH_3 = q). [d] Interchangeable.

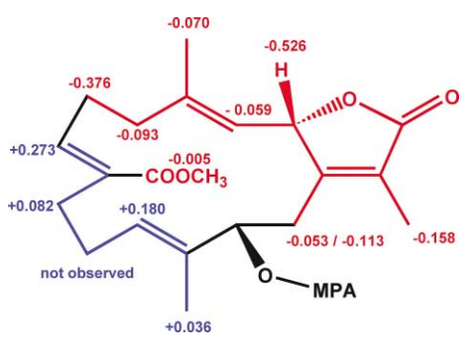

Fig. 4 δ^{RS}-values of sarcoglaucol-16-one (**6**) –MPA ester.

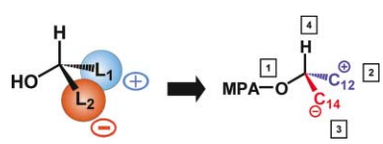

Fig. 5 Deduction of the absolute stereochemistry of **6**. Digits in boxes label the priority according to CIP rules.

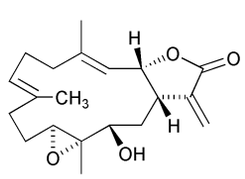

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Table 3 Torsion angles ∢(H-13-H-14) of compounds **1** and **2**

		Calculated torsion angle from model	
Compound	Karplus equation[a]	12*Z*,14*E*	12*Z*,14*Z*
1	8.8 Hz ⟶ 141°	139.2° ≡ 8.4 Hz[b]	156.9° ≡ 11.3 Hz[b]
2	8.8 Hz ⟶ 141°	140.2° ≡ 8.6 Hz	144.4° ≡ 9.3 Hz

[a] ∢(H-13-H-14) was calculated according to $J = 9.50\cos^2(\theta) - 1.60\cos(\theta) + 1.80$, using the Sweet-J program. [b] See Fig. 2 and Fig. 3.

Table 4 Cytotoxic activity (μg mL^{-1}) of compound **3** compared with that of sarcoglaucol-16-one[5] (**6**) and clinically relevant anticancer agents

	HM02	HepG2	MCF7
3	GI_{50} [a] > 10 TGI [b] > 10	GI_{50} > 10 TGI > 10	GI_{50} 6.5 TGI 9.8
6	GI_{50} 7.1 TGI > 10	GI_{50} 8.6 TGI > 10	GI_{50} 6.1 TGI > 10
Cisplatin	GI_{50} 0.8 TGI 1.5	GI_{50} 0.65 TGI 0.95	GI_{50} 0.095 TGI 0.19
5-Fluorouracil	GI_{50} 4.2 TGI 9.9	GI_{50} 15.0 TGI > 50	GI_{50} 45.0 TGI > 50
Doxorubicin	GI_{50} 0.006 TGI 0.012	GI_{50} 0.15 TGI 0.58	GI_{50} 0.087 TGI 0.13

[a] Drug concentration causing 50% growth inhibition. [b] Drug concentration causing 100% growth inhibition.

Cembrane derivatives containing a 12*Z* double bond, like compounds **1** and **2**, are very rare.[6–8] Additionally, some of them are merely designated as *Z* due to the formal use of CIP rules and the further substitution of neighbouring carbon atoms. These latter cembranes with a 12*Z* double bond are not relevant when considering biosynthetic implications.[24]

Assuming that the terpene metabolism of marine organisms uses the same biosynthetic pathways and mechanisms as described for plants and microorganisms, diterpenes, such as **1** and **2**, are derived from the universal acyclic precursor (all-*E*)-geranylgeranyl pyrophosphate (GGPP).[25,26] Subsequent cyclization through diterpene synthases would lead to the macrocyclic cembranes with all-*E*-configuration. Our knowledge about diterpene cyclases, especially in marine organisms, is currently very scarce. From some mono- and sesquiterpene cyclases it is known, that they are capable of catalysing a multistep process, whereby the enzyme may carry out an isomerization reaction, as well as the cyclization reaction itself.[27,28] Such a combined isomerization–cyclization step was, however never described for diterpene synthases. In the case of cembranes **1** and **2** the concerned diterpene synthase would have to convert geranylgeranyl-PP [GGPP] into geranylneryl-PP [GNPP], which would then cyclize to the cembranoid ring containing a 12*Z* double bond (see Fig. 6). Hence, a multifunctional diterpene cyclase present in the studied animal would be a possible explanation for the occurrence of cembranes with a 12*Z* double bond. Due to the presence of both, 4*E*,12*E*,14*E* and 4*E*,12*Z*,14*E*-isomers, the existence of two diterpene cyclases in this new species of soft coral may be postulated.

Fenical observed the simultaneous appearance of 4*E*,12*E*,-14*E* and 4*E*,12*Z*,14*E* and 4*E*,12*Z*,14*Z* cembrane isomers in a gorgonian. In this case the photochemical interconversion of 4*E*,12*E*,14*E*-cembratrienes into 4*E*,12*Z*,14*E* and 4*E*,12*Z*,14*Z* isomers was demonstrated.[6,29] The photochemical formation of **1** and **2** from their *E*-configured precursors is out of the question because in our study no 12*Z*,14*Z*-derivative (that should also be generated in an comparable amount to the 12*Z*,14*E* isomer) and no cubitanes were isolated.

The cytotoxic effects of compounds **1**–**4** against HM02, HepG2 and MCF7 cell lines were investigated, and compound **3** showed significant growth inhibitory activity (GI) towards MCF7 tumor cells (see Table 4). This activity is in the same range as that of other cembranes, *e.g.* **6** and partially better than that of 5-fluorouracil. Overall, the cytotoxicity of **3** must however be judged as moderate compared to anticancer agents in clinical use (Table 4). Compounds **1**, **2** and **4** were found to be inactive, which may be explained by the chemical instability of these compounds.

Experimental

HPLC was carried out using a Merck-Hitachi system consisting of a L-6200A pump, a L-4500 A photodiode array detector and a D-6000 A interface. ^{1}H (1D, 2D COSY) and ^{13}C (1D, DEPT 135, 2D HMQC, 2D HSQC, 2D HMBC) NMR spectra were recorded on a Bruker Avance 300 DPX and Bruker Avance 500 DRX spectrometers in $CDCl_3$. Spectra were referenced to residual solvent signals with resonances at $\delta_{H/C}$ 7.26/77.0 ($CDCl_3$). UV and IR spectra were obtained employing Perkin-Elmer Lambda 40 and Perkin-Elmer Spectrum BX instruments, respectively. Optical rotations were measured on a Jasco DIP 140 polarimeter. HREIMS were recorded on a Kratos MS 50 spectrometer.

Animal material

Sarcophyton n. sp. was collected in May 1983 from Stanley Reef, Great Barrier Reef, Australia, from a depth of 9–12 m, freeze dried and stored at −20 °C until workup. A voucher specimen has been deposited at the Museum and Art Galleries of the Northern Territory, Darwin, Australia, voucher number NTM C13646.

Extraction and isolation

The freeze dried soft coral *Sarcophyton* n. sp. (306.5 g dry wt) was extracted with CH_2Cl_2 (3 × 0.5 L), followed by MeOH (3 × 0.5 L). The CH_2Cl_2 extract (22.7 g) was fractionated by vacuum liquid chromatography (VLC) over Silica gel (Merck, 5–40 μm) using gradient elution from petroleum ether (100%) to MeOH (100%), to yield four fractions. ^{1}H NMR investigations of these indicated VLC-fractions 2 and 3 to be of interest. VLC-fraction 2 was further fractionated using RP-HPLC (column: Knauer C_{18} Eurospher-100, 250 × 8 mm, 5 μm; MeOH–H_2O (75 : 25), 1.7 mL min^{-1}) to yield four more fractions. Fraction 2 was rechromatographed by RP-HPLC (column: Aluspher 100 RP-Select B 250 × 4.0 mm, 5 μm; MeOH–H_2O (45 : 55), 0.8 mL min^{-1}) to yield 6.4 mg of compound **1** and 12.5 mg of **3**. Purification of fraction 4 by RP-HPLC (column: Phenomenex Max C_{12}, 250 × 4.6 mm, 5 μm; MeOH–H_2O (80 : 20), 1.0 mL min^{-1}) afforded 5.5 mg of sarcophytolide (**4**) and a mixture of compounds **2** and **5**, which were separated by RP-HPLC (column: XTerra C_{18} 250 × 4.6 mm, 5 μm; MeOH–H_2O (66 : 34), 0.8 mL min^{-1}) to give 1.3 mg of compound **2** and 1.1 mg of compound **5**. VLC-fraction 3 was further separated in 2 fractions using RP-HPLC (column: Knauer C_{18} Eurospher-100, 250 × 8 mm, 5 μm; MeOH–H_2O (70 : 30), 1.5 mL min^{-1}). Fraction 2 was rechromatographed by RP-HPLC (column: Aluspher 100 RP-Select B 250 × 4.0 mm, 5 μm; MeOH–H_2O (45 : 55), 0.8 mL min^{-1}) to yield further amounts of compound **1** (8.6 mg) and **3** (5.3 mg). Extraction and isolation details of the *Sarcophyton cherbonnieri* sample were as previously reported.[5]

(4*Z*,8*S*,9*S*,12*Z*,14*E*)-9-Hydroxy-1-isopropyl-8,12-dimethyl-oxabicyclo[9.3.2]-hexadeca-4,12,14-trien-18-one (1). Yellow oil (15.0 mg, 0.005%); $[\alpha]_D^{23}$ +84.0° (*c* 0.2, CH_2Cl_2); UV (EtOH) λ_{max} 234 nm (ε 8767), 253sh nm (ε 6261), 290sh nm (ε 2040); IR (ATR) ν_{max} 3422, 2928, 1665 cm^{-1}; ^{1}H and ^{13}C NMR data (see Tables 1 and 2); EIMS *m*/*z* (rel int) 318 (36), 133 (61), 121 (83), 107 (80), 93 (100); HREIMS *m*/*z* 318.2194 (calcd for $C_{20}H_{30}O_3$, 318.2195).

(4*Z*,8*S*,12*Z*,14*E*)-1-Isopropyl-8,12-dimethyl-oxabicyclo-[9.3.2]hexadeca-4,12,14-trien-9,18-dione, 4*Z*,12*Z*,14*E*-sarcophytolide (2). Colorless oil (1.3 mg, 0.0004%); $[\alpha]_D^{23}$ +147.9° (*c* 0.1, CH_2Cl_2); UV (EtOH) λ_{max} 224 nm (ε 12849), 251sh nm (ε 6847), 288sh nm (ε 1313); IR (ATR) ν_{max} 2960, 1716, 1682

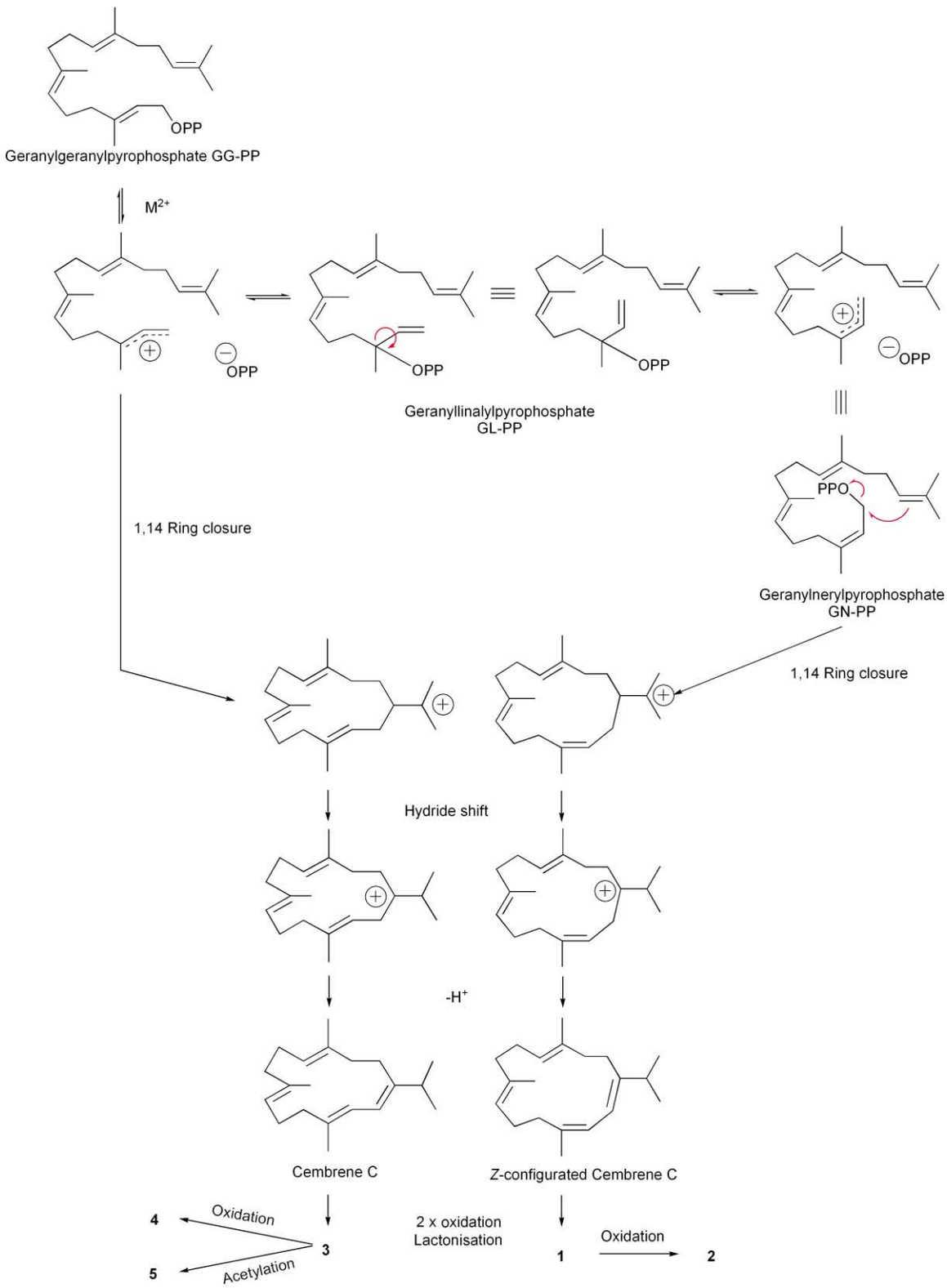

Fig. 6 Proposed biosynthetic pathway for compounds **1**–**5**.

cm^{-1}; ^{1}H and ^{13}C NMR data (see Tables 1 and 2); EIMS *m*/*z* (rel int) 316 (39), 133 (70), 121 (83), 107 (76), 93 (100); HREIMS *m*/*z* 316.2044 (calcd for $C_{20}H_{28}O_3$, 316.2038).

(4*Z*,8*S*,9*R*,12*E*,14*E*)-9-Hydroxy-1-isopropyl-8,12-dimethyl-oxabicyclo[9.3.2]-hexadeca-4,12,14-trien-18-one (3). Colorless oil (17.8 mg, 0.006%); $[a]_D^{23}$ +112.8° (*c* 0.24, CH_2Cl_2) {lit.[6] +161° (*c* 0.1, $CHCl_3$) and lit.[7] +160.0° (*c* 1.10, $CHCl_3$)}; UV (EtOH) λ_{max} 234 nm (ε 5424), 251sh nm (ε 4855), 292sh nm (ε 1301); IR (ATR) ν_{max} 3420, 2959, 1663 cm^{-1}; ^{1}H and ^{13}C NMR data (see Tables 1 and 2); EIMS *m*/*z* (rel int) 318 (100), 135 (82), 121 (99), 107 (64), 93 (83); HREIMS *m*/*z* 318.2196 (calcd for $C_{20}H_{28}O_3$, 318.2195).

Sarcophytolide (4). Colorless oil (5.5 mg, 0.002%); $[a]_D^{23}$ +144.5° (*c* 0.45, CH_2Cl_2) {lit.[6] +177° (*c* 0.1, $CHCl_3$) and lit.[7] +201.4° (*c* 1.43, $CHCl_3$)}; UV (EtOH) λ_{max} 232sh nm (ε 15576), 240 nm (ε 16177), 256sh nm (ε 11881), 287sh nm (ε 934); IR (ATR) ν_{max} 2957, 1716, 1687 cm^{-1}; ^{1}H and ^{13}C NMR data (see Tables 1 and 2); EIMS *m*/*z* (rel int) 316 (100), 134 (42), 121 (44), 107 (24), 93 (30); HREIMS *m*/*z* 316.2037 (calcd for $C_{20}H_{28}O_3$, 316.2038).

(4*Z*,8*S*,9*R*,12*E*,14*E*)-1-Isopropyl-8,12-dimethyl-18-oxo-oxabicyclo[9.3.2]-hexadeca-4,12,14-trien-2-yl acetate (5). Colorless oil (1.05 mg, 0.0003%); ^{1}H and ^{13}C NMR data (see Tables 1 and 2); further data not available due to the small amount and the instability of the compound.

Biological assays

In vitro growth inhibition effect towards the human cancer cell lines HM02 (gastric adenocarcinoma), HepG2 (hepatocellular carcinoma), and MCF7 (breast adenocarcinoma) was determined according to the NCI guidelines (see Table 4).[30] Inhibition of acetylcholinesterase inhibition was tested for **1**–**4** as previously described.[31] In this test system compounds **1**–**4** were all found to be inactive at the 25 μM level.

Mosher′s method

Preparation of the acid chlorides of (*R*)- and (*S*)-MPA. Oxalyl chloride (103.7 μL, 1.2 mmol) was added to a mixture of the corresponding MPA (20 mg, 0.12 mmol) and DMF (0.94 μL, 0.012 mmol) in hexane at room temperature. After two days, the solvent was evaporated to dryness at reduced pressure to afford 0.12 mmol of MPA-Cl (22.2 mg, 0.12 mmol, 100%).[22,32]

Preparation of the (*R*)- and (*S*)-MPA esters. The corresponding MPA-Cl (8.97 mg, 48.6 μmol) was dissolved in 5 mL of CH_2Cl_2 and added to a solution of compound **3** (3.5 mg, 9.72 μmol), Et_3N (16.5 μL, 116.7 μmol) and DMAP (1.19 mg, 9.72 μmol) in 10 mL of CH_2Cl_2. After 20 min the residue obtained after evaporation of the solvent was applied to preparative Silica-TLC hexane–CH_2Cl_2 (40 : 60) to give pure corresponding MPA ester of **3** (0.74 mg = 15.1% of *R*-MPA ester and 1.66 mg = 33.9% of the *S*-MPA ester, respectively).

Molecular modeling, Karplus calculations

E,Z and *Z,Z*-derivatives of **1** and **2** were calculated by conformation search (grid scan) using an MMFF force field as implemented in the Cerius^2 4.0 (MSI) molecular modeling software package. The models were further refined with 1500 iterations of minimisation. Calculations were performed using a Silicon Graphics O2 workstation (Irix 6.5.6).

The dihedral angles of the *E,Z* and *Z,Z*-derivatives of **1** and **2** were calculated with the Desktop Calculator Sweet-J,[33] operating with trigonal carbon atoms and the alpha-beta-set of the Equation Manager: $J = 9.50\cos^2(\theta) - 1.60\cos(\theta) + 1.80$. Calculations were performed using an Apple iMac G3 (MacOS 9.2).

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