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New cytotoxic cembrane based diterpenes from the soft corals *Sarcophyton cherbonnieri* and *Nephthea* sp.†

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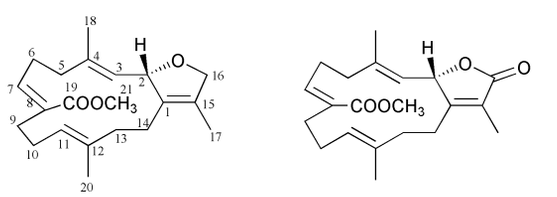
Chemical investigation of the two soft corals *Nephthea* sp. and *Sarcophyton cherbonnieri*, collected from the Fiji Islands and the Great Barrier Reef, respectively, led to the isolation of three new furano-cembranoids (**1**–**3**), two seco-cembranoid acetates (**5**, **6**), along with the known compounds sarcoglaucol (**4**) and decaryiol (**7**). The structures of the new compounds were elucidated by employing spectroscopic techniques (NMR, MS, UV, IR and CD). Seco-cembranoids are extremely rare structures. Compounds **1**, **3**, and **7** were found to be cytotoxic towards several tumor cell lines (GI_{50} values ranged from 0.15 to 8.6 μg mL^{-1}). Compound **7** arrests the cell cycle in the G2/M phase.

Introduction

Soft corals are known sources of terpenes, mainly diterpenes of the cembrane type.[1,2] Cembranoids often found in high concentrations (up to 5% dry weight) in soft corals were shown to have possible chemical defense roles against other reef organisms.[3,4] In biological assay systems some cembranoids display significant ichthyotoxic, cytotoxic,[5] antiinflammatory,[6] and Ca-antagonistic potential.[7] The current report focuses on the isolation, characterization, and *in vitro* cytotoxicity testing of three new and two known cembranoids, and two new seco-cembranoid acetates. Amongst the numerous cembranoids already isolated from coelenterates compounds **1**, **2**, and **3** represent rare examples of cembranoids functionalized at C-19. Seco-cembranoids such as compounds **5** and **6** are very unusual structures.[14–16] The few examples of seco-cembranoids in the literature include seco-sethukarailin from the soft coral *Sinularia dissecta*[15] and mayolide A from the soft coral *Sinularia mayi*.[16]



1 **2** **3** **4** **5** **6** **7** **8**

Results and discussion

The current samples of *Nephthea* sp. and *Sarcophyton cherbonnieri* Tixier-Durivault, 1946, were collected from Ra-Ra Reef, Fiji Islands and Stanley Reef, Australia, respectively. After extraction with CH_2Cl_2 and MeOH the organic extracts of the two soft corals were evaluated for biological activity. Simultaneously with these assays, investigation of the secondary metabolite chemistry of the samples was started. Chromatographic separation of the CH_2Cl_2 and MeOH extracts using Si gel and C_{18} reversed phase vacuum liquid chromatography (VLC), SPE, and HPLC yielded three new compounds (**1**–**3**) from the *Sarcophyton cherbonnieri*, and two new acetates (**5**, **6**) from the *Nephthea* sp., together with the known compounds sarcoglaucol (**4**) from the *Sarcophyton cherbonnieri*, and decaryiol (**7**) from the *Nephthea* sp.

The molecular formula of **1** was found to be $C_{21}H_{30}O_3$ by accurate mass measurement. From its ^1H, ^{13}C NMR, UV, and IR data it was evident that the molecule contained four unconjugated carbon–carbon double bonds (see Tables 1 and 2), and an ester carbonyl (δ 169.6, ν 1716 cm^{-1}), as the only multiple bonds within the molecule. As neither OH nor further CO absorptions were detected in the IR spectrum of **1** the remaining oxygen had to be present as an ether. Since the molecular formula of **1** required it to have seven elements of

† Electronic supplementary information (ESI) available: Mosher results for compound **3**. See http://www.rsc.org/suppdata/ob/b2/b210039h/

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Table 1 ^{1}H NMR data for compounds **1**, **2**, **3**, and **4** (δ in ppm, J in Hz)[a]

Proton	**1**[b]	**2**[c]	**3**[c]	**4**[c]
2	5.57 (1H, dd, J = 4.9, 9.8)	5.75 (1H, m)	5.85 (1H, brd, J = 10.3)	5.67 (1H, m)
3	5.16 (1H, d, J = 9.8)	4.98 (1H, d, J = 9.5)	5.00 (1H, brd, J = 10.3)	5.14 (1H, d, J = 10.3)
5	2.30 (1H, m), 2.41 (1H, m)	2.39 (2H, m)	2.45 (2H, m)	2.27 (1H, m), 2.40 (1H, m)
6	3.05 (2H, m)	2.96 (2H, m)	3.17 (2H, m)	2.38 (1H, m), 3.15 (1H, m)
7	5.72 (1H, dd, J = 4.6, 8.5)	5.75 (1H, m)	5.68 (1H, dd, J = 3.3, 9.2)	5.67 (1H, m)
9	2.09 (1H, m), 2.60 (1H, m)	2.51 (2H, m)	1.99 (1H, m), 2.74 (1H, m)	1.95 (1H, m), 2.72 (1H, m)
10	2.13 (1H, m), 2.19 (1H, m)	2.20 (2H, m)	1.99 (1H, m), 2.41 (1H, m)	1.98 (1H, m), 2.37 (1H, m)
11	5.09 (1H, dd, J = 6.7, 7.6)	5.12 (1H, t, J = 6.6)	5.28 (1H, dd, J = 4.4, 10.3)	5.22 (1H, dd, J = 4.4, 10.3)
13	1.99 (2H, m)	2.23 (2H, m)	4.12 (1H, d, J = 10.3)	3.98 (1H, d, J = 10.3)
14	2.46 (2H, m)	2.40 (1H, m), 2.61 (1H, m)	2.27 (1H, m), 2.99 (1H, m)	1.93 (1H, m), 2.67 (1H, m)
16	4.50 (2H, t, J = 4.9)			4.54 (2H, br s)
17	1.71 (3H, t, J = 1.5)	1.83 (3H, t, J = 1.8)	1.88 (3H, t, J = 1.5)	1.74 (3H, br s)
18	1.80 (3H, s)	1.89 (3H, s)	1.94 (3H, s)	1.82 (3H, s)
20	1.63 (3H, s)	1.63 (3H, s)	1.72 (3H, s)	1.68 (3H, s)
21	3.78 (3H, s)	3.78 (3H, s)	3.79 (3H, s)	3.78 (3H, s)

[a] All assignments are based on extensive 1D and 2D NMR measurements (HMBC, HMQC, COSY). [b] CD_3OD, 500 MHz. [c] CD_3OD, 300 MHz.

Table 2 ^{13}C NMR data for compounds **1**, **2**, **3**, and **4** (δ in ppm)

	1[a,c]		**2**[a,c]		**3**[c]		**4**[a]
Carbon	^{13}C	HMBC[e]	^{13}C	HMBC[e]	^{13}C[a]	HMBC[b,e]	^{13}C
1	134.6 (s)[d]	14, 16, 17	166.0 (s)	2, 13, 14, 17	163.8 (s)	2, 13, 14, 17	132.5 (s)
2	85.1 (d)	14	81.1 (d)	3, 14	80.3 (d)	3, 17	84.8 (d)
3	126.8 (d)	5, 18	121.1 (d)	2, 5, 18	122.0 (d)	2, 5, 18	127.5 (d)
4	141.6 (s)	5, 6, 18	146.2 (s)	2, 6, 18	147.0 (s)	2, 5, 6, 18	141.8 (s)
5	39.7 (t)	3, 6, 7, 18	39.6 (t)	3, 6, 18	39.5 (t)	3, 6, 7, 18	39.8 (t)
6	27.1 (t)	5, 7	27.3 (t)	5, 7	27.4 (t)	5, 7	27.4 (t)
7	144.1 (d)	5, 6, 9	143.9 (d)	6, 9, 10	143.7 (d)	5, 6, 9	144.5 (d)
8	131.4 (s)	6, 9, 10	132.0 (s)	6, 9, 10	132.0 (s)	6, 10, 21	131.5 (s)
9	36.4 (t)	7, 11	36.2 (t)	7, 11	36.5 (t)	7, 10, 11	36.6 (t)
10	25.4 (t)	11, 20	25.7 (t)	11	25.0 (t)	9, 11	25.0 (t)
11	124.1 (d)	9, 10, 13, 20	124.7 (d)	9, 10, 13, 20	126.1 (d)	9, 10, 13, 14, 20	125.5 (d)
12	137.2 (s)	10, 20	136.4 (s)	10, 13, 14, 20	139.9 (s)	10, 14, 20	140.4 (s)
13	37.7 (t)	11, 14, 20	37.2 (t)	11, 20	77.7 (d)	11, 14, 20	77.1 (d)
14	26.9 (t)	2, 13	27.7 (t)	2	36.9 (t)	11, 13	35.0 (t)
15	128.3 (s)	14, 16, 17	123.1 (s)	2, 14, 17	125.1 (s)	17	130.9 (s)
16	78.8 (t)	17	177.5 (s)	17	177.2 (s)	17	79.2 (t)
17	9.8 (q)	16	8.8 (q)		8.8 (q)	2	10.2 (q)
18	15.1 (q)	3, 5	16.1 (q)	3, 5	15.7 (q)	3, 5	15.2 (q)
19	169.6 (s)	7, 21	169.7 (s)	7, 10, 21	169.6 (s)	7, 10, 21	169.6 (s)
20	15.5 (q)	10, 11, 13	16.0 (q)	11, 13	9.9 (q)	11, 13	9.9 (q)
21	51.3 (q)		51.7 (q)		51.7 (q)		51.7 (q)

[a] CD_3OD, 75.5 MHz. [b] CD_3COCD_3, 75.5 MHz. [c] Assignments are based on extensive 2D NMR measurements (HMBC, HMQC, COSY). [d] Implied multiplicities determined by DEPT (C = s; CH = d; CH_2 = t; CH_3 = q). [e] Numbers refer to proton resonances.

unsaturation, it had to be bicyclic. After extensive 2D NMR measurements had been completed, it was possible to deduce major fragments of **1** from the resultant spectra. Thus, H_3-18 showed long-range couplings in the HMBC spectrum with C-3, C-4, and C-5. This identified the allylic methyl group connected to C-4 as CH_3-18 and showed $\Delta^{3,4}$ to be attached to C-5. CH_2-6 was shown to bond to both C-5 and C-7 based on ^{1}H–^{1}H couplings observed between H_2-6 and both H_2-5 and H-7. H-7 exhibited HMBC couplings to C-9 and the carbonyl C-19. As C-8 showed long-range couplings to H_2-6 and H_2-9, C-7, C-9, and C-19 all had to be attached to C-8. The linkage between the methoxyl group CH_3-21 and C-19 was evident from the long-range CH coupling seen between C-19 and H_3-21. The ^{1}H–^{1}H COSY spectrum of **1** further showed H_2-10 to couple to H_2-9 and H-11, thus giving evidence for the C-9, C-10, and C-11 sequence. HMBC long range correlations between the resonance of H_3-20 and those of C-11, C-12, and C-13 revealed CH_3-20 to bond to C-12, and $\Delta^{11,12}$ to be adjacent to C-13. The H_2-13 resonance showed in the HMBC spectrum a cross peak to the resonance of C-14, placing CH_2-14 next to C-13. Further HMBCs, this time between the resonance of H_2-14 and those of C-1, C-2, and C-15, and between that of H_3-17 and those of C-1, C-15, and C-16 confirmed the C-1, C-2, C-14 part of **1**. The observed ^{1}H–^{1}H coupling between H-2 and H-3 in the COSY spectrum of **1** led to completion of the planar structure of **1**. The low field chemical shifts of CH_2-16 (δ 78.8) and CH-2 (δ 85.1) showed the ether linkage to be between C-2 and C-16, a deduction that was supported by long range ^{1}H–^{13}C couplings between H_2-16 and C-2, and COSY couplings between H-2 and H_2-16. With the planar structure of **1** determined, the geometry of three carbon–carbon double bonds and the configuration at C-2 required resolution. Comparison of the ^{13}C NMR chemical shifts for CH_3-18 and CH_3-20 (< 20 ppm; see Table 2), and C-7 and C-8, with literature values,[8,9] clearly showed the carbon–carbon double bonds $\Delta^{3,4}$ and $\Delta^{11,12}$ to have *E*, and $\Delta^{7,8}$ to have *Z* geometries.

Bowden *et al.* demonstrated for some very similar cembranoid structures that the absolute configuration at C-2 can be related to the sign of the specific optical rotation.[10] For compounds with the *S* configuration a large positive and for those with *R* configuration a large negative optical rotation was found. Thus, the absolute configuration at C-2 of **1** was tentatively assigned as *S* based on the optical rotation ($[a]_D$ +100.8°). For **1** the trivial name 13-dehydroxysarcoglaucol is proposed.

Compound **2** analysed for $C_{21}H_{28}O_4$ by accurate mass measurement. Comparison of the ^{1}H, ^{13}C, and HMBC NMR

data of **2** with those of **1** indicated the two molecules to be closely related. The obvious spectroscopic differences between the two compounds resulted from the presence of a carbonyl group (δ 177.5) at C-16 in **2** instead of the CH_2 group found in **1**. The geometries of the double bonds of **2** were deduced to be the same as in **1** on the basis of ^{13}C NMR chemical shifts. The stereochemistry at C-2 was defined by CD measurements. The CD spectrum of **2** (Fig. 1) showed ε values of -7.57 at 249.8 nm and $+88.24$ at 222.8 nm, compared with -6.5 (246 nm) and $+22.6$ (223 nm) for sarcophine (**8**).[22–25] These data demonstrated that the stereochemistry at C-2 of **2** and sarcophine (**8**) is identical and *S*. For **2**, 13-dehydroxysarcoglaucol-16-one is proposed as the trivial name.

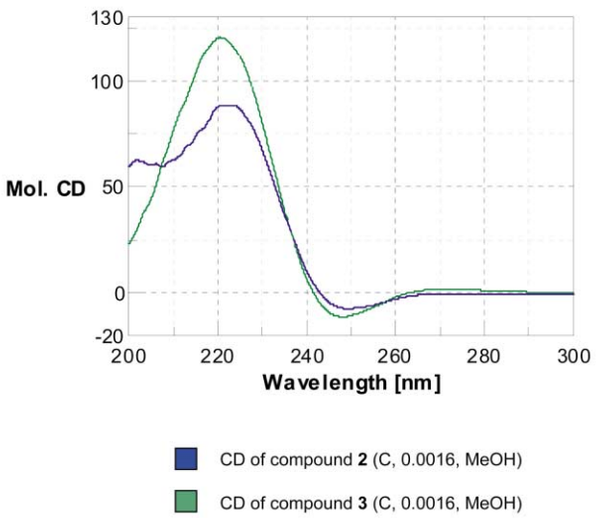

Fig. 1 CD spectra of compounds **2** and **3**.

^1H, ^{13}C, HMBC NMR, IR, and MS analyses ($C_{21}H_{28}O_5$) of **3** showed it to be very similar to **2**. The major differences between the two data sets concerned C-13. The aforementioned differences can be explained by the presence of a hydroxyl group at C-13 in **3**, making it the 16-keto derivative of sarcoglaucol (**4**), and the 13-hydroxy derivative of **2**. The stereochemistry at C-2 was deduced to be the same as in **2** on the basis of a CD spectrum with ε values (-11.52 at 248.2 nm and $+120.4$ at 220.4 nm) comparable to those of sarcophine (**8**) (-6.5 at 246 nm and $+22.6$ at 223 nm).[22–25] Thus, the configuration at C-2 of **3** was assigned as *S*. The ^1H NMR spectrum shows for H-13 the same large coupling constant $J_{13/14\alpha}$ (10.3 Hz) as seen in **4**. According to the Karplus equation the dihedral angle between H-13 and one of the H_2-14 protons (H-14_α) must therefore approach 180°. Since no ^1H–^1H coupling can be observed between H-13 and H-14_β, the dihedral angle between these two protons must be about 90°. Molecular modeling calculations were used to find the minimum energy conformations of the 2*S*, 13*R* (Fig. 2) and 2*S*, 13*S* (Fig. 3) epimers, showing that the torsion angles are $\sphericalangle$(H-13-H-14_α) = 164.0° and $\sphericalangle$(H-13-H-14_β) = 48.4° for the 13*R* configuration, and $\sphericalangle$(H-13-H-14_α) = 166.6° and $\sphericalangle$(H-13-H-14_β) = 76.9° for the 13*S* configuration (Figs. 2 and 3). The calculation clearly shows that the dihedral angles, formed by H-13-H-14_α and H-13-H-14_β, predicted from the ^1H–^1H couplings and the Karplus equation are most probable when C-13 has the *S* configuration. Taking the coordinates of the X-ray data for compound **4** from the Cambridge Crystallographic Database (relative configuration) made it possible to construct the 3D structure for **4** and subsequently to overlay it with the minimum energy conformation of the 2*S*, 13*S* epimer of **3**. The reliability of our calculations is obvious from the alignment (Fig. 4) of our calculated model with the X-ray structure of the known sarcoglaucol (**4**), showing an excellent fit for the C-5 to C-11 part of both molecules. Some differences are seen concerning the

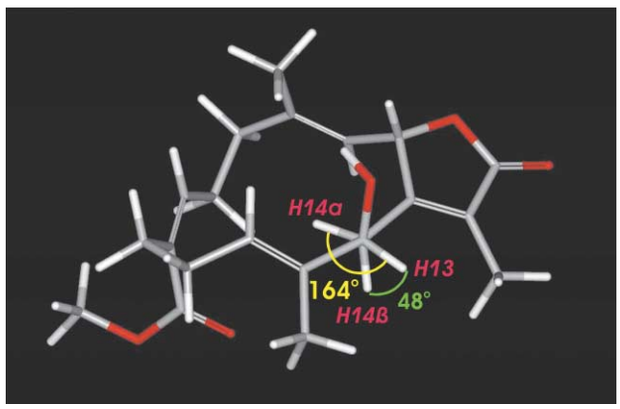

Fig. 2 Minimum energy conformation of the 2*S*, 13*R* epimer of **3**.

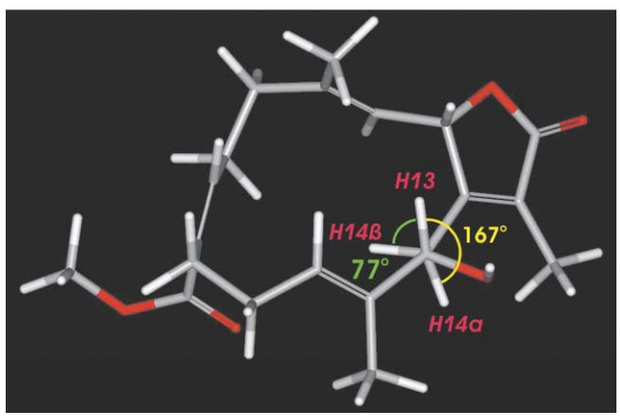

Fig. 3 Minimum energy conformation of the 2*S*, 13*S* epimer of **3**.

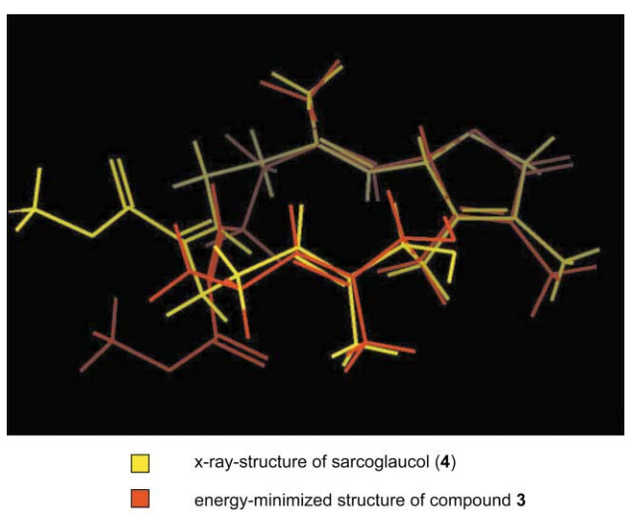

Fig. 4 Alignment of the minimum energy conformation of the 2*S*, 13*S* epimer of **3** with the X-ray structure of **4**.

C-10 to C-6 part, most probably due to the fact that the conformation of a crystalline compound, *i.e.* **4**, is compared with a conformation, *i.e.* **3**, modelled in vacuum. The final evidence of the absolute stereochemistry of **3** came from CD measurements in combination with the results of selective gradient NOESY experiments. With the knowledge about the configuration at C-2, the stereochemistry at C-13 was defined on the basis of selective gradient NOESY experiments as shown in Fig. 5. Thus, low power irradiation at δ 5.85 (H-2, $CDCl_3$) led to the enhancement of the resonances for H-13 and H_3-18. Enhancement of the signals for H-2 and H-11 was observed following low-power irradiation at δ 4.12 (H-13, $CDCl_3$). Inspection of the energy minimized models of **3** (Figs. 2, 3) indicated that these NOE interactions were only possible for the 2*S*, 13*S* configuration.

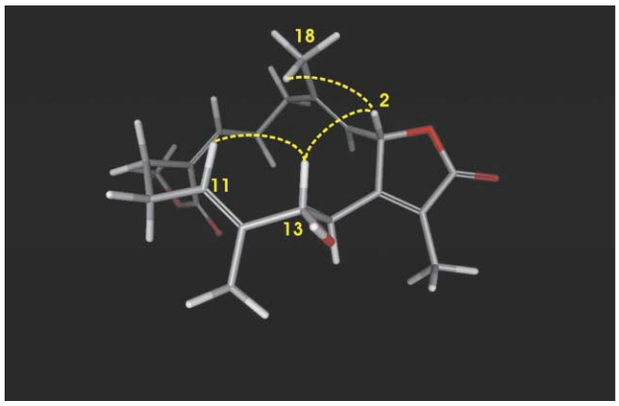

Fig. 5 NOESY correlations of **3**.

Determination of the absolute configuration of compounds **1**–**3** is based on that of sarcophine (**8**). Several reports take the absolute configuration of sarcophine (**8**) as established.[10,25,28,29] There is some doubt, however, since X-ray and CD studies of Kashman[23] only established the relative configuration. Thus additional attempts were made to solve the absolute stereochemistry of **3** applying Mosher's method.[26,27] (*R*)- and (*S*)-MTPA-esters were obtained, but regrettably ^{1}H NMR chemical shift differences are not unambiguous (see supplementary data †). For **3** the trivial name of sarcoglaucol-16-one is proposed.

Compounds **5** and **6** were obtained as an inseparable mixture. ^{1}H, ^{13}C NMR, and MS analyses indicated **5** to have the molecular formula $C_{22}H_{36}O_4$ and showed it to have five elements of unsaturation. The IR spectrum had a characteristic band at 3448 cm^{-1}, consistent with the presence of a hydroxyl functionality. Its ^{13}C NMR data contained a total of 22 resonances for 6 × CH_3, 6 × CH_2, 5 × CH groups, and five quaternary carbons. These data also revealed the presence of five double bonds (3 × C=C; 2 × C=O, an ester and an aldehyde) as the only multiple bonds within the molecule; **5** was thus acyclic. The planar structure of **5** was determined by analysis of its 1D and 2D NMR spectral data. After assignment of all protons to their directly bonded carbon atom *via* a one bond ^{1}H–^{13}C 2D NMR shift correlated measurement (HMQC), major fragments of the molecule were deduced from the results of a ^{1}H–^{1}H COSY measurement. Thus, analysis of the COSY spectrum gave evidence for connectivities from C-3 to C-13, *via* C-1. A second fragment, from C-5 to C-7, was established on the basis of couplings observed between H_2-5 and H_2-6, which in turn coupled to H-7. In addition, the aldehyde proton H-10 coupled to H-11. The planar structure of **5** was further elaborated by interpretation of a long-range ^{1}H–^{13}C 2D NMR shift correlated spectrum recorded for **5** (HMBC; see Table 4). Thus, the HMBC data showed both CH_3-16 and CH_3-17 to bond to C-15, CH_3-18 to bond to C-4, CH_3-19 to bond to C-8, and CH_3-20 to bond to C-12. The *exo*-methylene group, CH_2-9, had to be connected to C-8, because of its couplings with H-7 and H_3-19. Further long-range CH correlations observed between the resonance of C-8 and that of H-7, C-4 and H_2-5, C-3 and H_3-18, C-12 and H_2-13, and C-11 and H_3-20, clearly delineated all of the so far unaccounted for C–C bonds in the C-9 to C-10 continuous chain. Finally, HMBC coupling between C-15 and H-1 indicated C-15 to bond to C-1, leaving CH_3-22 to be connected to C-21 of the acetyl group, a deduction supported by the HMBC coupling between C-21 and H_3-22. Selective absolute NOESY experiments showed NOEs between H_3-22 and both H_3-16 and H_3-17 and revealed the acetyl group to reside at C-15 and by deduction the hydroxyl group at C-7. The geometry of both the double bonds $\Delta^{3,4}$ and $\Delta^{11,12}$ was assigned as *E* on the basis of the ^{13}C NMR chemical shifts of CH_3-18 (δ 16.3) and CH_3-20 (δ 17.6). The relative configuration of the molecule was not established due to its conformational instability.

Compound **6** was found to be identical to **5** in all respects except for the geometry of the $\Delta^{11,12}$ double bond. The chemical shift of C-20 in **6** (δ 22.6) clearly showed $\Delta^{11,12}$ to have the *Z* geometry. Compounds **5** and **6** presumably derive from a cembranoid precursor through cleavage of the C-9–C-10 bond. Seco-cembranoids of this type are unprecedented.

Cembrane based diterpenes have been reported to be cytotoxic towards several cell lines.[5,17] Prominent examples are sinularin (ED_{50} 0.3 μg mL^{-1} towards KB and P388 cell lines) and sarcophytol A, which was further examined in several animal experiments.[18–21] Consequently, the cytotoxic effects of compounds **1**–**4**, and **7** against HM02 (gastric adenocarcinoma), HepG2 (hepatocellular carcinoma), and MCF7 (breast adenocarcinoma) cell lines were investigated. Compounds **1**, **3**, and **7** inhibited the growth of these tumor cells (Table 5), compounds **2** and **4** were found to be inactive at the 10 μg mL^{-1} level. The activities found for **1** and **3** must be judged as moderate when compared with those reported for other cembranoids,[5] and for **7**.

Compound **7** showed remarkable concentration-dependent inhibition of cell growth; thus, further cell cycle analyses were carried out in HM02 cells. HM02 cells showed a significant increase in the number of cells in the G2/M phase of the cell cycle after exposure to 10 μg mL^{-1} decaryiol for 24 hours (see Table 6). This increase was accompanied by a marked reduction of cells in the G1 and S phase. The percentage of cells in the sub G1 phase was significantly increased, indicating apoptosis. Compound **7** is thus a cell cycle specific inhibitor of cell growth.

Experimental

HPLC was carried out using a Merck-Hitachi system consisting of an L-6200 A pump, an L-4500 A photodiode array detector and a D-6000 A interface, together with a Knauer K-2300 differential refractometer as detector. ^{1}H (1D, 2D COSY, selective NOESY, 2D NOESY, 2D ROESY) and ^{13}C (1D, DEPT 135, 2D HMQC, 2D HMBC) NMR spectra were recorded on Bruker Avance 300 DPX and Bruker Avance 500 DRX spectrometers in CD_3OD, CD_3OH, and $CDCl_3$. Spectra were referenced to residual solvent signals with resonances at $\delta_{H/C}$ 3.35/49.0 (CD_3OD) and $\delta_{H/C}$ 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 with a Jasco DIP 140 polarimeter. CD spectra were taken on a Jasco J-810 spectropolarimeter. HREIMS were recorded using a Kratos MS 50 spectrometer. All other experimental details were as previously reported.[11]

Animal material

The soft coral *Sarcophyton cherbonnieri* Tixier-Durivault, 1946, was collected in May 1983 from Stanley Reef, Great Barrier Reef, Australia, from a depth of 7 m, freeze dried and stored at −20 °C until workup. The *Nephthea* sp. was collected in March 1999 from Ra-Ra reef, Raki Raki, Fiji Islands, at a depth of 5 m, and stored in EtOH at −20 °C until workup. Voucher specimens have been deposited at the Museum and Art Galleries of the Northern Territory, Darwin, Australia, voucher number NTM C13645 (*Sarcophyton cherbonnieri* sample) and at the Institute for Pharmaceutical Biology, University of Bonn, voucher number CT199 4A (*Nephthea* sp. sample).

Extraction and isolation

The freeze dried soft coral *Sarcophyton cherbonnieri* (35.7 g dry wt.) was extracted with CH_2Cl_2 (5 × 1 L), followed by MeOH (3 × 1 L). The CH_2Cl_2 extract was fractionated by vacuum liquid chromatography (VLC) over Si 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

Table 3 ^{1}H NMR data for compounds **5** and **6** (δ in ppm, J in Hz)[a]

Proton	**5**[b]	**6**[b]
1	1.99 (1H, m)	2.01 (1H, m)
2	1.98 (1H, m), 2.18 (1H, m)	1.98 (1H, m), 2.18 (1H, m)
3	5.19 (1H, m)	5.19 (1H, m)
5	2.01 (1H, m), 2.09 (1H, m)	2.01 (1H, m), 2.09 (1H, m)
6	1.64 (2H, m)	1.64 (2H, m)
7	4.03 (1H, dd, J = 6.6, 6.6)	4.03 (1H, dd, J = 6.6, 6.6)
9	4.84 (1H, s), 4.94 (1H, s)	4.84 (1H, s), 4.94 (1H, s)
10	9.97 (1H, d, J = 8.2)	9.88 (1H, d, J = 8.2)
11	5.85 (1H, ddd, J = 1.2, 1.2, 8.2)	5.85 (1H, ddd, J = 1.2, 1.2, 8.2)
13	2.19 (1H, m), 2.31 (1H, m)	2.19 (1H, m), 2.31 (1H, m)
14	1.35 (1H, m), 1.67 (1H, m)	1.35 (1H, m), 1.67 (1H, m)
16	1.43 (3H, s)	1.43 (3H, s)
17	1.48 (3H, s)	1.48 (3H, s)
18	1.64 (3H, s)	1.64 (3H, s)
19	1.72 (3H, s)	1.72 (3H, s)
20	2.15 (3H, d, J = 1.2)	1.96 (3H, d, J = 1.2)
22	1.97 (3H, s)	1.97 (3H, s)

[a] All assignments are based on extensive 1D and 2D NMR measurements (HMBC, HMQC, COSY). [b] $CDCl_3$, 300 MHz.

Table 4 ^{13}C NMR data for compounds **5** and **6** (δ in ppm)

	5[a,b]		**6**[a,b]	
Carbon	^{13}C	HMBC[d]	^{13}C	HMBC[d]
1	46.6 (d)[c]	2, 3, 16, 17	47.2 (d)	2, 3, 16, 17
2	28.9 (t)	3	29.0 (t)	3
3	123.9 (d)	18	123.6 (d)	18
4	135.3 (s)	5, 18	135.8 (s)	5, 18
5	35.7 (t)	3, 7, 18	35.7 (t)	3, 7, 18
6	33.2 (t)	7	33.2 (t)	7
7	75.5 (d)	5, 9, 19	75.5 (d)	5, 9, 19
8	147.4 (s)	7, 9, 19	147.4 (s)	7, 9, 19
9	111.1 (t)	7, 19	111.0 (t)	7, 19
10	191.3 (d)	—	190.8 (d)	—
11	127.3 (d)	10, 20	128.1 (d)	10, 20
12	164.1 (s)	13, 20	164.8 (s)	13, 20
13	39.9 (t)	11, 20	39.9 (t)	11, 20
14	28.1 (t)	—	28.1 (t)	—
15	85.5 (s)	1, 16, 17	85.4 (s)	1, 16, 17
16	23.4 (q)	17	23.3 (q)	16
17	24.0 (q)	16	24.0 (q)	16
18	16.3 (q)	3	16.3 (q)	3
19	17.6 (q)	7, 9	17.6 (q)	7, 9
20	17.6 (q)	11	22.6 (q)	—
21	170.3 (s)	22	170.3 (s)	22
22	22.6 (q)	—	22.6 (q)	—

[a] $CDCl_3$, 75.5 MHz. [b] Assignments are based on extensive 2D NMR measurements (HMBC, HMQC, COSY). [c] Implied multiplicities determined by DEPT (C = s; CH = d; CH_2 = t; CH_3 = q). [d] Numbers refer to proton resonances.

Table 5 Activities (μg mL^{-1}) of compounds **1**, **3**, and **7** against selected tumor cell lines

	HM02	HepG2	MCF7
1	GI_{50}[a] 5.4	GI_{50} 6.6	GI_{50} 1.7
	TGI[b] 9.0	TGI > 10	TGI > 10
3	GI_{50} 7.1	GI_{50} 8.6	GI_{50} 6.1
	TGI > 10	TGI > 10	TGI > 10
7	GI_{50} 0.19	GI_{50} 2.0	GI_{50} 0.15
	TGI 7.1	TGI 9.8	TGI 9.1

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

fractions indicated VLC fractions 1, 2, and 3 be of further interest. On the basis of comparable ^{1}H NMR spectra fractions 1 and 2 were combined and then fractionated using RP-HPLC (column: Knauer C_{18} Eurospher-100, 250 × 8 mm, 5 μm; MeOH–H_2O (7 : 3), 1.5 mL min^{-1}) to yield 1.2 mg of **1**. RP-HPLC separation of fraction 3 (column: Knauer C_{18} Eurospher-100, 250 × 8 mm, 5 μm; MeOH–H_2O (7 : 3), 1.5 mL min^{-1}) yielded **2** (8.1 mg), **3** (9.2 mg), and **4** (25.3 mg).

The *Nephthea* sample (18.5 g wet wt.) was extracted with MeOH (3 × 0.2 L). The MeOH extract and the EtOH used for preservation were combined and evaporated to dryness to yield 0.31 g of yellow gum. This material was partitioned between MeOH and H_2O (1 : 1) and CH_2Cl_2. The CH_2Cl_2 phase was fractionated employing solid phase extraction (SPE Baker-bond; Si) using gradient elution from petroleum ether (100%) to MeOH (100%) to yield five fractions. Fraction 1 was rechromatographed by RP-HPLC (column: Phenomenex Max C_{12}, 250 × 4.6 mm, 5 μm; MeOH–H_2O (8 : 2), 1.0 mL min^{-1}) to yield 12.3 mg of **7**,[12] and 3.0 mg of an inseparable 2 : 1 mixture of **5** and **6**.

13-Dehydroxysarcoglaucol (2,5,6,9,10,13,14,16-octahydro-4,12,15-trimethylcyclotetradeca[*b*]furan-8-carboxylic acid methyl ester) (1)

Green-yellow solid (1.2 mg, 0.003%); $[\alpha]_D^{23}$ +100.8° (*c* 0.08 in MeOH); UV λ_{max}(MeOH)/nm 205 (ε/dm^3 mol^{-1} cm^{-1} 10500); IR (ATR) ν_{max}/cm^{-1} 2928 br, 1716 (CO); ^{1}H and ^{13}C NMR data (see Tables 1 and 2); MS(EI) *m*/*z* 330 (M^+, 36), 203 (46), 135 (100); HREIMS *m*/*z* 330.2186 (calcd for $C_{21}H_{30}O_3$, 330.2195).

13-Dehydroxysarcoglaucol-16-one (2,5,6,9,10,13,14,16-octahydro-4,12,15-trimethyl-16-oxocyclotetradeca[*b*]furan-8-carboxylic acid methyl ester) (2)

Green-yellow solid (8.1 mg, 0.023%); $[\alpha]_D^{23}$ +79.0° (*c* 0.1 in MeOH); UV λ_{max}(MeOH)/nm 211 (ε/dm^3 mol^{-1} cm^{-1} 8040), 290 (3600); IR (ATR) ν_{max}/cm^{-1} 2924, 1750 (CO), 1715 (CO); ^{1}H and ^{13}C NMR data (see Tables 1 and 2); MS(EI) *m*/*z* 344 (M^+, 80), 312 (100); HREIMS *m*/*z* 344.1998 (calcd for $C_{21}H_{28}O_4$, 344.1988).

Sarcoglaucol-16-one (2,5,6,9,10,13,14,16-octahydro-13-hydroxy-4,12,15-trimethyl-16-oxocyclotetradeca[*b*]furan-8-carboxylic acid methyl ester) (3)

Green-yellow solid (16.6 mg, 0.046%); $[\alpha]_D^{23}$ +119.8° (*c* 0.26 in MeOH); UV λ_{max}(MeOH)/nm 217 (ε/dm^3 mol^{-1} cm^{-1} 15260), 285 (2720); IR (ATR) ν_{max}/cm^{-1} 3444 (OH), 2948, 1716 (CO); ^{1}H and ^{13}C NMR data (see Tables 1 and 2); MS(EI) *m*/*z* 360 (M^+, 54), 165 (100); HREIMS *m*/*z* 360.1947 (calcd for $C_{21}H_{28}O_5$, 360.1937).

Sarcoglaucol (4)

Green-yellow solid (25.3 mg, 0.071%); $[\alpha]_D^{23}$ +127.7° (*c* 0.31 in MeOH) {lit.,[9] +177° (*c* 0.31 in MeOH)}; UV λ_{max}(MeOH)/nm

Table 6 Cell cycle analysis of HM02 cells exposed to 10 μg mL^{-1} of compound **7**[a]

	SubG1-phase	G1-phase	S-phase	G2/M-phase
Control	9.1 ± 1.6	58 ± 0.7	18.2 ± 1.7	14.7 ± 0.4
Compound **7**	18.5 ± 1*	42 ± 1*	13.2 ± 1.8*	25.7 ± 0.4*

[a] Data represent percentage of cells in each stage of the cell cycle. Values are mean ± SE of four experiments. *$p < 0.05$ *versus* control (t-test).

216 (ε/dm^3 mol^{-1} cm^{-1} 9420); IR (ATR) ν_{max}/cm^{-1} 3423 (OH), 2944, 1706 (CO); ^1H and ^{13}C NMR data (see Tables 1 and 2); MS(EI) *m*/*z* 315 (M^+, 35), 175 (100); HREIMS *m*/*z* 346.2132 (calcd for $C_{21}H_{30}O_4$, 346.2144).

Mixture (2 : 1) of (3*E*)-7-hydroxy-4,8,15,15-tetramethyl-1-[(*E*)-12-methyl-10-oxo-12-pentenyl]-3,8-decadienyl acetate (5) and (3*E*)-7-hydroxy-4,8,15,15-tetramethyl-1-[(*Z*)-12-methyl-10-oxo-12-pentenyl]-3,8-decadienyl acetate (6)‡

Colorless oil (3.0 mg, 0.017%); $[\alpha]_D^{23}$ 0° (*c* 0.23 in $CHCl_3$); UV λ_{max}($CHCl_3$)/nm 246 (ε/dm^3 mol^{-1} cm^{-1} 5100); IR (ATR) ν_{max}/cm^{-1} 3448 (OH), 2926, 1727 (CO), 1670 (CO), 1456, 1370, 1255; ^1H and ^{13}C NMR data (see Tables 3 and 4); MS(EI) *m*/*z* 304 (10, $M^+ - C_2H_4O_2$), 286 (6), 125 (100).

Decaryiol (7)

Pale yellow solid (12.3 mg, 0.068%); $[\alpha]_D^{23}$ +67.4° (*c* 1.03 in $CHCl_3$) {lit.,[12] +69° (*c* 1.3 in $CHCl_3$)}; ^1H, ^{13}C NMR, UV, and IR data (see ref. 12); MS(EI) *m*/*z* 306 (M^+, 65), 288 (100).

Biological assays

In vitro growth inhibition effect was determined according to the NCI guidelines in the human cancer cell lines HM02 (gastric adenocarcinoma), HepG2 (hepatocellular carcinoma), and MCF 7 (breast adenocarcinoma).[13]

Cell cycle distribution was determined by staining DNA with propidium iodide. Cells were treated for 24 h with 10 μg mL^{-1} sample, harvested by trypsination, washed with RPMI 1640 containing 1% fetal bovine serum, and resuspended in 125 μL of a solution containing 150 μg mL^{-1} propidium iodide, 1% Triton X-100, 1% bovine serum albumin, and 4 mM sodium citrate-buffer, pH 7.4. After 15 min incubation at room temperature under light exclusion, the same volume of RNase A (10 mg mL^{-1} in 10 mM Tris and 15 mM NaCl, pH 7.4) was added and cells were incubated for an additional 30 min at room temperature. At the end of the incubation period cells were analyzed using a Becton Dickinson FACSscan and Lysis II software.

Molecular modeling

Epimers of **3** 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).

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‡ The numbering of compounds **5** and **6** corresponds to that of the furano-cembranoids.

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