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Isolation and structure elucidation of natural products of three soft corals and a sponge from the coast of Madagascar†

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We investigated the three soft corals *Sarcophyton stellatum*, *Capnella fungiformis* and *Lobophytum crassum* and the sponge *Pseudoceratina arabica*, which have been collected at the coast of Madagascar. In addition to previously known marine natural products, *S. stellatum* provided the new (+)-enantiomer of the cembranoid (1*E*,3*E*,11*E*)-7,8-epoxycembra-1,3,11,15-tetraene (**2**). *Capnella fungiformis* afforded three new natural products, ethyl 5-[(1*E*,5*Z*)-2,6-dimethylocta-1,5,7-trienyl]furan-3-carboxylate (**6**), ethyl 5-[(1*E*,5*E*)-2,6-dimethylocta-1,5,7-trienyl]furan-3-carboxylate (**7**) and the diepoxyguaiane sesquiterpene oxyfungiformin (**9a**). The extracts of all three soft corals exhibited moderate activities against the malarial parasite *Plasmodium falciparum*. Extracts of the sponge *Pseudoceratina arabica* proved to be very active against a series of Gram-positive and Gram-negative bacteria.

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Introduction

In continuation of our studies on the chemical constituents of marine organisms from the coast of Madagascar including red algae,¹ brown algae,² and soft corals,³ we herein turned our attention to soft corals of the species *Sarcophyton stellatum*, *Capnella fungiformis*, and *Lobophytum crassum*. A sponge of the species *Pseudoceratina arabica* has also been included in the present investigation. Soft corals and sponges have been frequently investigated and found to be rich sources of biologically active compounds.^{4–14} However, only a few reports are available on soft corals and sponges from the coast of Madagascar.^{3,15–17} This region is of particular interest since it is known that the marine environment of the Southern Africa and Western Indian Ocean region contains a wealth of endemic species, which increases the chance to find new natural products with useful biological activities.^{18–20} In our

previous study on Madagascan soft corals, we have isolated five new compounds including the cadinane-type sesquiterpene vanderlandin, the spatane-type diterpene gravilin and the cembranoid diterpene isodecaryiol.³ These results encouraged us to extend our search for new and potentially bioactive compounds by investigating further soft coral species and a sponge from this region.

Results and discussion

Sarcophyton stellatum

Several species of soft corals of the genus *Sarcophyton* have been investigated for their chemical constituents.^{6,7,11,13,14,21} However, no such reports are available for the species *S. stellatum*. We collected samples of this species at the inner reef of Mahambo, Madagascar, at a depth of 5–6 m. The bodies of *S. stellatum* were sliced and exhaustively extracted with methanol at room temperature. The methanol extract of *S. stellatum* exhibited moderate antimalarial activity with an IC₅₀ value of 35.20 ± 5.02 μg mL⁻¹ for the inhibition of the FCM29 strain of *Plasmodium falciparum*. Further solvent partitioning of the crude extract afforded a diethyl ether and a dichloromethane extract. Three known cembranoid diterpenes and the unknown enantiomer of a fourth representative were isolated from the diethyl ether and dichloromethane extracts of *S. stellatum*. Three of these compounds were isolated from both extracts, namely (+)-(7*S*,8*S*)-epoxy-7,8-dihydrocembrene

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† Electronic supplementary information (ESI) available: Tables S1–S13; Fig. S1–S22 (copies of the ¹H, ¹³C and 2D NMR spectra of compounds **6**, **7** and **9a**); details of the antimalarial assay. CCDC 1526876. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c7ob00191f



C [(+)-1], (+)-(1E,3E,11E)-7,8-epoxycembra-1,3,11,15-tetraene [(+)-2] and (-)-(2R,7R,8R)-sarcophytoxide [(-)-4] (Fig. 1). The fourth compound, (+)-(7R,8R,14S,1Z,3E,11E)-14-acetoxy-7,8-epoxycembra-1,3,11-triene [(+)-3], was found only in the diethyl ether extract. In addition, the diethyl ether extract of *S. stellatum* contained a small amount of (*S*)-3-*O*-octadecylglycerol (batyl alcohol) (5), the scaffold structure for a variety of ether lipids of the 1-*O*-alkyl-2,3-*O*-diacyl-*sn*-glycerol type (Fig. 2).²²

Compound (+)-1 was isolated as a colorless oil with a specific optical rotation of $[\alpha]_{\text{D}}^{20} = +44.6$ (*c* 0.5, MeOH). CD spectroscopy revealed a molar circular dichroism of $\Delta\epsilon = +0.23$ (242 nm, MeOH). The molecular formula of (+)-1 was established as C₂₀H₃₂O by the monoisotopic mass of 288 obtained from the EI mass spectrum in combination with the number and intensities of the ¹H and ¹³C NMR signals. The structure of this compound was deduced from extensive 1D and 2D NMR measurements (COSY, HSQC, HMBC, and NOESY). Our NMR data were generally in agreement with those reported for (+)-(7*S*,8*S*)-epoxy-7,8-dihydrocembrene C [(+)-1] in the literature (Table S1†),²³ except for the presence of an additional signal at $\delta_{\text{H}} = 1.51$ ppm which we assigned to H-9b. In addition, we revised the assignment of the ¹³C NMR signals for C-6 and C-9 and of some proton signals. The isolation of (+)-(7*S*,8*S*)-epoxy-7,8-dihydrocembrene C [(+)-1] has been reported before by Deng and coworkers from the soft coral *S. molle* with a specific optical rotation of $[\alpha]_{\text{D}}^{25} = +99.0$ (*c* 1.9, acetone)²⁴ and by Seifert *et al.* from *S. ehrenbergi* with $[\alpha]_{\text{D}}^{25} = +19$ (*c* 0.5, CHCl₃).²³ (-)-(7*R*,8*R*)-Epoxy-7,8-dihydrocembrene C [(-)-1] has been isolated first from the soft coral *S. crassocaule* by Bowden *et al.*

with an $[\alpha]_{\text{D}}$ value of -22.5 (*c* 0.19, CHCl₃).²⁵ Subsequently, the same compound has been obtained from an unidentified species of a Caribbean gorgonian of the genus *Eunicea*²⁶ and from the soft coral *S. trocheliophorum*.²⁷ The absolute stereochemistry of (-)-1 was assigned as (7*R*,8*R*) via enantioselective total synthesis in 2000 by Li *et al.*²⁸ They reported a specific optical rotation of $[\alpha]_{\text{D}}^{20} = -25.2$ (*c* 0.21, CHCl₃) which is in good agreement with the value of the natural product originally isolated by Bowden. We obtained (+)-(7*S*,8*S*)-epoxy-7,8-dihydrocembrene C [(+)-1] for the first time from the soft coral *S. stellatum*. Seifert *et al.* reported moderate antiproliferative activity of (+)-1 against the cell lines HUVEC and K-562 and a moderate cytotoxicity against the HeLa cell line.²³

The structurally related metabolite (+)-2 was isolated as a colorless oil with a specific optical rotation of $[\alpha]_{\text{D}}^{20} = +12.0$ (*c* 0.5, MeOH). The CD spectrum of (+)-2 in methanol exhibited a molar circular dichroism of $\Delta\epsilon = -0.11$ (251 nm). The $[M + H]^+$ ion at *m/z* = 287 in the ESI mass spectrum in combination with the number and intensities of the ¹H and ¹³C NMR signals suggested a molecular formula of C₂₀H₃₀O. The structure of metabolite (+)-2 was elucidated based on the analysis of the 1D and 2D NMR spectra. The NMR data were in reasonable agreement with those reported in the literature (Table S2†) for the enantiomeric diterpene (-)-(1E,3E,11E)-7,8-epoxycembra-1,3,11,15-tetraene [(-)-2], which has been originally obtained from the soft coral *S. crassocaule*²⁵ with an $[\alpha]_{\text{D}}$ value of -14.4 (*c* 0.1, CHCl₃) and subsequently also from a *Lobophytum* species.²⁹ Thus, we have isolated for the first time the opposite enantiomer, (+)-(1E,3E,11E)-7,8-epoxycembra-1,3,11,15-tetraene [(+)-2]. The absolute configuration of this natural product still remains to be determined.

Compound (+)-3 was isolated as a colorless oil with a specific optical rotation of $[\alpha]_{\text{D}}^{20} = +171.8$ (*c* 0.1, MeOH). The EI mass spectrum with a molecular ion peak at *m/z* = 346 and the ¹H and ¹³C NMR data were in agreement with the molecular formula of C₂₂H₃₄O₃. The IR spectrum of (+)-3 showed an absorption band for an ester group (1724 cm⁻¹). This assignment was confirmed by the ¹H and ¹³C NMR spectra displaying signals for an acetate moiety [$\delta_{\text{H}} = 2.04$ ppm (s), $\delta_{\text{C}} = 170.19$ (C) and 21.37 ppm (CH₃)]. The ¹³C NMR spectrum showed signals for 22 resolved carbon atoms including six methyl groups, five methylene groups, six methine groups, and five quaternary carbon atoms. The analytical data proved to be identical with those of (+)-(7*R*,8*R*,14*S*,1*Z*,3*E*,11*E*)-14-acetoxy-7,8-epoxycembra-1,3,11-triene [(+)-3] (Table S3†), which has been isolated by Bowden *et al.* from the soft coral *S. trocheliophorum*.³⁰ Prior to that report, Kobayashi's group obtained (+)-3 as a semi-synthetic oxidation product of sarcophytol A.³¹ They deduced the (1*S*)-configuration of (+)-3 from sarcophytol A which had been determined by using Horeau's method. Accordingly, the (7*R*)-configuration was assigned by isomerization of (+)-3 to the corresponding allyl alcohol (Δ^8 -7,14-dihydroxy compound) and determination of the absolute configuration by the same method. Bowden *et al.* also determined a (1*S*)-configuration for the corresponding 14-hydroxy compound, which was interconvertible with (+)-3, by using

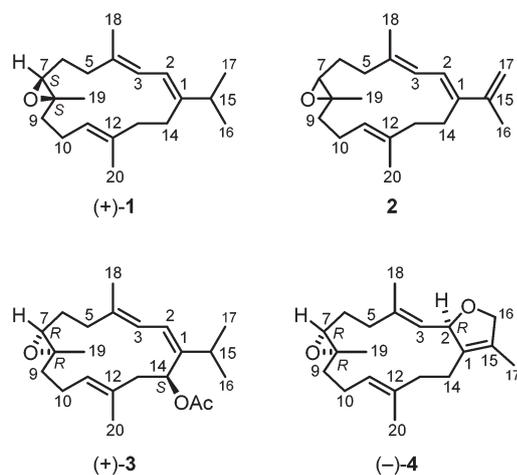


Fig. 1 Structures of the cembranoid diterpenes isolated from *Sarcophyton stellatum*.

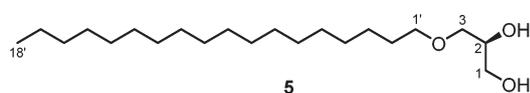


Fig. 2 Structure of (*S*)-3-*O*-octadecylglycerol (batyl alcohol) (5).



Mosher's method.³⁰ They assigned a (7*R*)-configuration for compound (+)-3 in analogy to a related $\Delta^{8-7,14}$ -dihydroxy compound which was isolated along with (+)-3. Knowing the configuration at C-7, the (8*R*)-configuration was derived following Bowden's reasoning.³⁰ The specific optical rotation values of (+)-3 reported by Kobayashi ($[\alpha]_D^{20} = +150$ (*c* 1.02, CHCl₃))³¹ and Bowden ($[\alpha]_D^{20} = +136$ (*c* 1.1, CHCl₃))³⁰ confirm that we have obtained the same enantiomer ($[\alpha]_D^{20} = +171.8$ (*c* 0.1, MeOH)). Thus, we have isolated (+)-(7*R*,8*R*,14*S*,1*Z*,3*E*,11*E*)-14-acetoxy-7,8-epoxycembra-1,3,11-triene [(+)-3] for the first time from the soft coral *S. stellatum*. It should be noted that (+)-3 exhibited a moderate cytotoxicity against P388 (murine leukaemia), A549 (human lung carcinoma), HT29 (human colon carcinoma) and MEL28 (human melanoma) cell lines.³⁰

Compound (–)-4 was isolated as colorless crystals with a melting point of 60–61 °C and a value for the specific optical rotation of $[\alpha]_D^{20} = -129.4$ (*c* 0.1, MeOH). The CD spectrum of (–)-4 in MeOH showed molar circular dichroism values of $\Delta\epsilon = +0.01$ (247 nm) and -0.01 (274 nm). The molecular formula of C₂₀H₃₀O₂ was derived from the EI-MS, ¹H and ¹³C NMR data. Analysis of a set of 1D and 2D NMR spectra led to the identification of (–)-4 as (–)-sarcophytoxide. This was confirmed by comparison of the ¹H and ¹³C NMR data with those of (–)-sarcophytoxide isolated by Seifert *et al.* from *S. ehrenbergi*²³ and Bowden and coworkers^{32,33} (Table S4†).

Recrystallization of (–)-4 from diethyl ether provided single crystals which were suitable for X-ray diffraction. The X-ray crystal structure determination unequivocally confirmed the constitution and relative configuration of (–)-sarcophytoxide [(–)-4] (Fig. 3). In addition, the absolute configuration of (–)-4 has been assigned by the anomalous dispersion as (2*R*,7*R*,8*R*) (Flack parameter: $\chi = 0.1(0)$). (+)-Sarcophytoxide [(+)-4] with a specific optical rotation of $[\alpha]_D^{20} = +25$ (*c* 2.2, MeOH) was isolated first in 1974 by Kashman and coworkers from *S. glaucum*.³⁴ A few years later, Bowden *et al.* obtained the enantiomer (–)-4 with a specific rotation of $[\alpha]_D^{20} = -191$ (*c* 0.4, MeOH) from the species *S. ehrenbergi*³² and *S. birklandi*.³³ The absolute (2*S*,7*S*,8*S*)-configuration of (+)-4 from *Sarcophyton* sp.

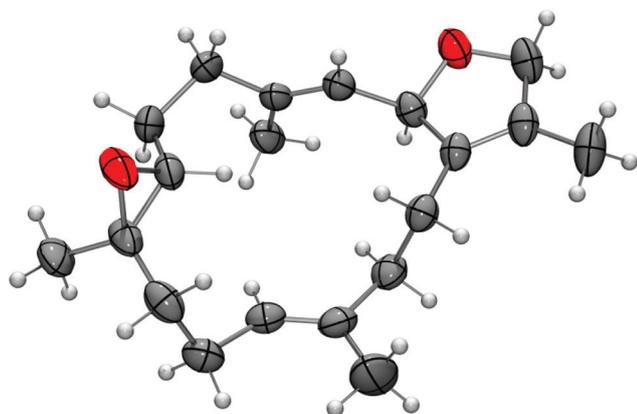


Fig. 3 X-Ray crystal structure of (–)-sarcophytoxide [(–)-4] (thermal ellipsoids at the 50% probability level).

with a specific optical rotation of $[\alpha]_D^{20} = +135$ (*c* 0.93, CHCl₃) was assigned by Faulkner *et al.* by chemical correlation with the structurally known (+)-sarcophine.^{4,35} Similarly, Bowden *et al.* correlated the structure of (–)-4 with the known (2*R*)-sarcophytonin by reductive cleavage of the epoxide with a Zn–Cu couple and secured the absolute configuration at C-2.³³ X-ray structural determination of (+)-sarcophytoxide [(+)-4], obtained from *Sarcophyton* sp. with a specific rotation of $[\alpha]_D^{20} = +157$ (*c* 1.0, MeOH), was reported by Kobayashi and coworkers.³⁶ (+)-Sarcophytoxide [(+)-4] has been isolated in a study of the relationship between soft coral diversity and cembranoid diterpene production of *Sarcophyton* specimens from Okinawa, Japan.³⁷ A more recent isolation of (–)-sarcophytoxide [(–)-4] has been reported by Seifert and coworkers from *S. ehrenbergi* with $[\alpha]_D^{25} = -128$ (*c* 1.0, CHCl₃), which corresponds to our value ($[\alpha]_D^{20} = -129.4$ (*c* 0.1, MeOH)).²³ We have isolated (–)-sarcophytoxide [(–)-4] for the first time from the soft coral *S. stellatum*.

Compound 5 (Fig. 2) was isolated as a yellow solid with a melting point of 69–70 °C and a specific optical rotation of $[\alpha]_D^{20} = +2.6$ (*c* 0.07, MeOH). The analytical studies of compound 5 including 2D NMR experiments suggested that compound 5 is identical with (*S*)-3-*O*-octadecylglycerol (batyl alcohol), one of the most common core compounds of 1-*O*-alkyl-2,3-*O*-diacyl-*sn*-glycerols, which are widespread components of the non-polar lipid fractions of aquatic and terrestrial animals.²² Our analytical data are in agreement with those reported by Haraldsson and coworkers for a synthetic sample.³⁸

Capnella fungiformis

So far, soft corals assigned to the species *Capnella fungiformis* have not been studied for their chemical constituents. We obtained samples of *C. fungiformis* from the inner reef of Mahambo, Madagascar, at a depth of about 5–6 m. The fresh material of *C. fungiformis* was cut and soaked in methanol immediately after collection. After removal of the solvent, the crude methanol extract was tested in an antimalarial assay and found to exhibit moderate activity with an IC₅₀ value of 32.80 ± 3.03 $\mu\text{g mL}^{-1}$ for the inhibition of the FCM29 strain of *Plasmodium falciparum*. The methanol extract of *C. fungiformis* was further extracted with diethyl ether and separated by column chromatography over silica gel into two fractions eluting with pentane–diethyl ether (gradient 10 : 1 to 7 : 3). Fraction F-1 was purified by two additional column chromatographic steps (pentane–diethyl ether, 10 : 1 and 20 : 1) affording an inseparable mixture of ethyl 5-[(1*E*,5*Z*)-2,6-dimethylocta-1,5,7-trienyl]furan-3-carboxylate (6) and the corresponding (1*E*,5*E*)-isomer 7 (subfraction F-1-1-1) along with octadecan-2-one (8) (subfraction F-1-1-2) (Fig. 4).

The mixture of 6 and 7 was obtained as a yellow solid. The ratio of the *Z/E*-isomers was determined to be 3 : 2 (6 : 7) by GC-MS and NMR spectroscopy. Their molecular formula C₁₇H₂₂O₃, corresponding to seven double bond equivalents, was determined on the basis of the molecular ion peak at *m/z* = 274 in the EI mass spectra in combination with the number



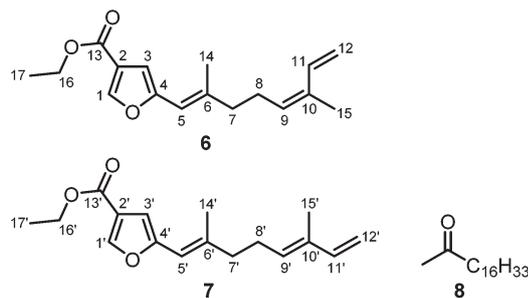


Fig. 4 Structures of the ethyl furancarboxylates **6** and **7** (terpene numbering according to Coll *et al.*³⁹) and octadecan-2-one (**8**).

and intensities of the ^1H and ^{13}C NMR signals. The ^1H and ^{13}C NMR data for the isomers **6** and **7** have been assigned unambiguously from the spectra of the mixture. In combination with the DEPT spectrum, signals for three methyl groups, four methylene groups, and five methine groups were identified for each isomer. The ^{13}C NMR spectrum displayed resonances for 17 carbon atoms, including one carbonyl group at $\delta = 163.38$ ppm (C-13/13') and four quaternary carbon atoms at $\delta = 120.63$ (C-2/2'), 154.61 (C-4/4'), 140.50 (C-6/6'), and 132.84 ppm (C-10/10'). The proton signals at $\delta = 7.88$ (s, H-1/1') and 6.49 ppm (s, H-3/3') and the ^{13}C signals at $\delta = 145.42$ (C-1/1'), 120.63 (C-2/2'), 106.74 (C-3/3'), and 154.61 ppm (C-4/4') were assigned to a 2,4-disubstituted furan ring. Three double bonds were identified in each isomer by signals for olefinic protons at $\delta = 6.05$ (br s, H-5/5'), 5.37 (br t, $J = 7.3$ Hz, H-9), 5.47 (br t, $J = 7.2$ Hz, H-9'), 6.76 (ddd, $J = 17.3, 10.8, 0.9$ Hz, H-11), 6.35 (dd, $J = 17.1, 10.7$ Hz, H-11'), 5.29 (br d, $J = 17.3$ Hz, H-12a), 5.09 (d, $J = 17.3$ Hz, H-12a'), 5.09 (dt, $J = 10.5, 1.5$ Hz, H-12b), and 4.93 ppm (d, $J = 10.9$ Hz, H-12b'), and by ^{13}C NMR signals at 113.65 (C-5/5'), 140.50 (C-6/6'), 129.75 (C-9), 131.75 (C-9'), 132.84 (C-10/10'), 133.46 (C-11), 141.34 (C-11'), 113.77 (C-12), and 110.84 ppm (C-12'). The assignment of the protons to the corresponding carbon atoms was achieved by analysis of the HSQC correlations. The positions of the double bonds and the methyl groups at the side chain were confirmed by the HMBC correlations of the olefinic protons H-5/5' with C-6/6', C-7/7', and C-14/14', of H-9/9' with C-7/7', C-8/8', C-11/11', and C-15/15', of H-11/11' with C-9/9', C-10/10', and C-15/15', of H-12a/12a' with C-10/10' and C-11/11', and of H-12b/12b' with C-10/10' (Fig. 5, Tables S5 and S6[†]). Furthermore, the HMBC correlations of H-1/1' with C-2/2', C-3/3', and C-4/4' and of H-3/3' with C-1/1', C-2/2', C-4/4', and C-13/13' established the furan ring and the location of the ester carbonyl group at C-2/2'. The correlation of H₃-14/14' with C-6/6', C-5/5', and C-4/4' suggested that the methyl group is located at C-6/6' of the Δ^5/Δ^5 -double bond which is attached to C-4/4' of the furan ring. The geometry of the double bonds in **6** and **7** was determined by analysis of the NOE correlations (Fig. 5). The protons H₂-8 and H-11 of **6** exhibited a strong NOE correlation, whereas the protons H₂-8' and H-11' in **7** showed no such interaction. Accordingly, NOESY cross-peaks have been observed for the protons H₂-8' and H₃-15' in **7** but not for H₂-8

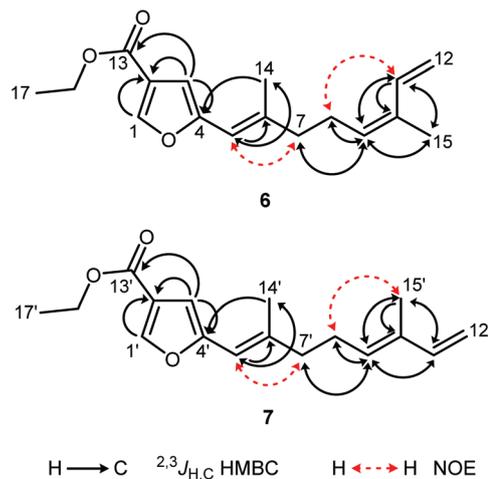


Fig. 5 Characteristic HMBC and NOESY correlations of compounds **6** and **7**.

and H₃-15 in **6**. This fact strongly suggests a *Z*-configuration of the Δ^9 -double bond in **6** and an *E*-configuration for the corresponding double bond in compound **7**. The geometry of the Δ^5/Δ^5 -double bond was determined to be *E* in both isomers due to strong NOE interactions between H₂-7/7' and H-5/5' as compared to weak NOE correlations of the protons H₃-14/14' with the protons H-5/5'.

The ethyl furancarboxylates **6** and **7** are sesquiterpenes which have not been reported as natural products so far. In 1977, Coll *et al.* isolated 5-[(1*E*,5*Z*)-2,6-dimethylocta-1,5,7-trienyl]furan-3-carboxylic acid, corresponding to compound **6**, from the Australian soft coral *Sinularia gonatodes* Kolonko.³⁹ Derivatization with diazomethane afforded the corresponding methyl ester. Subsequently, Bowden and Coll obtained both, the (1*E*,5*Z*) and the (1*E*,5*E*)-furan-3-carboxylic acids, along with their methyl esters from *Sinularia cappilosa*.⁴⁰ 5-[(1*E*,5*Z*)-2,6-Dimethylocta-1,5,7-trienyl]furan-3-carboxylic acid exhibited anti-inflammatory activity as shown by inactivation of bee venom phospholipase A₂.⁴¹ More recent isolations of 5-[(1*E*,5*Z*)-2,6-dimethylocta-1,5,7-trienyl]furan-3-carboxylic acid, the corresponding (1*E*,5*E*)-isomer, and their methyl esters have been reported by Venkateswarlu and coworkers from *Sinularia kavarrattensis*.⁴² A single report for the (1*E*,5*E*)-ethyl ester **7** has been published by Faulkner *et al.* as a synthetic intermediate towards the corresponding furancarboxylic acid.⁴³ The corresponding ethyl 5-[(1*E*,5*Z*)-2,6-dimethylocta-1,5,7-trienyl]furan-3-carboxylate (**6**) has not been reported previously. Since we have not used ethanol during the isolation process, the ethyl furancarboxylates **6** and **7** represent new natural products.

Octadecan-2-one (**8**) was obtained from subfraction F-1-1-2 of the diethyl ether extract of *C. fungiformis* as a colorless solid with a melting point of 57–58 °C. Compound **8** has been isolated previously from various natural sources and the analytical data (IR, ^1H NMR, EI-MS) are in agreement with those reported in the literature.⁴⁴



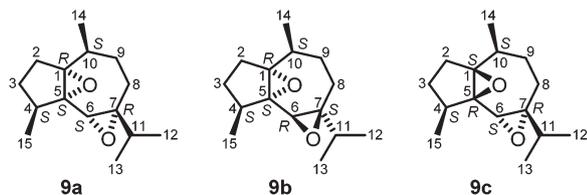


Fig. 6 Structures of compound **9a**, isolated from *Capnella fungiformis*, the hypothetical diastereoisomer **9b**, and compound **9c**, isolated from *Sinularia kavarrattiensis*.^{42b}

Subfraction F-1-2 of the diethyl ether extract of *C. fungiformis* afforded compound **9a** (Fig. 6) as a colorless solid with a specific optical rotation of $[\alpha]_D^{20} = +38.0$ (*c* 0.05, MeOH) and a melting point of 66–66.5 °C. The ESI mass spectrum of **9a** displayed peaks at 237 and 254 mass units for $[M + H]^+$ and $[M + NH_4]^+$ ions, respectively. Detailed analysis of the 2D NMR spectra led to the assignment of all chemical shifts in the 1H and ^{13}C NMR spectra for compound **9a** (Table 1). Signals of four methyl groups, four methylene groups, four methine groups, and three quaternary carbon atoms were identified by 1H NMR, ^{13}C NMR, and DEPT measurements. The COSY experiment revealed the presence of four spin systems (Fig. 7). The first one consists of two coupled methylene groups which further interact with the methine proton H-4 and the methyl protons H₃-15 to give the fragment $-CH_2-CH_2-CH(CH_3)-$. The second spin system extends from H-8 α /H-8 β via H-9 α /H-9 β and H-10 to the methyl protons H₃-14 representing the same structural fragment. A third spin system is formed by the isopropyl group (H-11, H₃-12, H₃-13) and, finally, the isolated proton H-6 completes the number of observed spin systems. A complete proton to carbon assignment was achieved by analysis of the HSQC spectrum. The presence of a

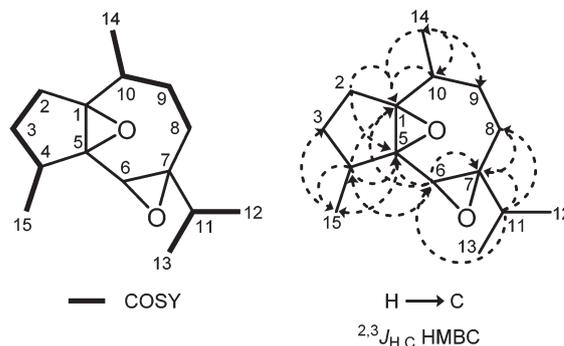


Fig. 7 Characteristic COSY and HMBC correlations of compound **9a**.

guaiane sesquiterpene skeleton was confirmed by the HMBC spectrum. Characteristic HMBC interactions, which led to the elucidation of the fused seven- and five-membered ring system by connecting the proton spin systems, include those of C-1/H-10, C-1/H-6, C-1/H-4, C-1/H-2 α , C-5/H-6, C-5/H-4, C-5/H-2 α , C-4/H-6, C-7/H-6, C-7/H-8 α , and C-7/H-8 β (Fig. 7 and Table 1). The position of the methyl group at C-10 was established based on HMBC cross-peaks between C-10/H₃-14, C-14/H-10, C-14/H-9 α , C-14/H-9 β , C-9/H₃-14, and C-1/H₃-14. Accordingly, the position of the 4-Me group was derived from HMBC interactions between C-15/H-4, C-15/H-3 α , C-15/H-3 β , C-5/H₃-15, and C-3/H₃-15. HMBC cross-peaks between C-7/H-11, C-8/H-11, and C-6/H-11 unambiguously clarified the location of the isopropyl group at C-7. The relative stereochemistry of compound **9a** was tentatively assigned by a NOESY experiment (Fig. 8, 9 and Table 1) in combination with the analysis of coupling constants (Tables 1, 2, S7 and S8[†]) and a comparison of calculated (GIAO) and experimental 1H and ^{13}C NMR shift values (Tables S9 and S10[†]). NOE interactions have been exploited to deter-

Table 1 NMR spectroscopic data of compound **9a** from *Capnella fungiformis* recorded at 600 MHz (1H) and 150 MHz (^{13}C) in CDCl₃

	δ_H^a (J in Hz)	δ_C^b	HMBC ^c	NOESY ^d
1		73.69 C	2 α , 3 α , 4, 6, 9 α , 9 β , 10, 14	
2 α	1.69 dd (13.8, 8.4)	26.75 CH ₂	3 α , 3 β	3 β , 10, 14
2 β	1.79 ddd (13.8, 10.4, 8.3)			3 α , 3 β , 9 β , 14, 15
3 α	1.64 ddddq (12.2, 10.4, 8.4, 7.6, 0.5)	26.72 CH ₂	2 α , 2 β , 4, 15	2 β , 4
3 β	1.12 dd (12.0, 8.3)			2 α , 2 β , 4, 15
4	2.37 dq (7.6, 7.4)	37.60 CH	2 α , 3 α , 3 β , 6, 15	3 α , 3 β , 6, 15
5		69.34 C	2 α , 3 α , 4, 6, 15	
6	3.05 s	58.08 CH	8 α , 8 β , 11	4, 11, 12, 13
7		68.59 C	4, 6, 8 α , 8 β , 9 α , 9 β , 11, 12, 13	
8 α	1.87 dddd (15.6, 6.3, 4.0, 0.9)	22.44 CH ₂	9 α , 9 β , 11	9 α , 9 β , 10, 11, 12, 13, 14
8 β	1.91 ddd (15.6, 10.8, 4.1)			9 β , 12, 13, 14
9 α	1.77 dddd (14.6, 10.8, 4.2, 4.0)	26.27 CH ₂	8 α , 8 β , 9, 14	8 α , 10
9 β	1.23 dddd (14.6, 7.5, 6.3, 4.1)			2 β , 8 α , 8 β , 10, 14
10	2.36 dqdd (7.5, 7.2, 4.2, 0.9)	31.45 CH	8 α , 8 β , 9 α , 9 β , 14	2 α , 8 α , 9 α , 9 β , 14
11	1.49 qq (7.0/6.8)	36.50 CH	6, 8 α , 12, 13	6, 8 α , 12, 13
12	0.92 d (7.0)	17.81 CH ₃	11, 13	6, 8 α , 8 β , 11
13	0.969 d (6.8)	17.86 CH ₃	11, 12	6, 8 α , 8 β , 11
14	1.02 d (7.2)	17.30 CH ₃	10, 9 α , 9 β	2 α , 2 β , 8 α , 8 β , 9 β , 10
15	0.974 dd (7.4, 0.5)	16.01 CH ₃	3 α , 3 β , 4	2 β , 3 β , 4

^a Shift, multiplicity and coupling constants derived from the simulated spectrum (Fig. S11–S17). ^b The number of attached protons as determined by the DEPT experiment. ^c HMBC correlations from carbon atoms to protons. ^d Only the most significant signals are shown.



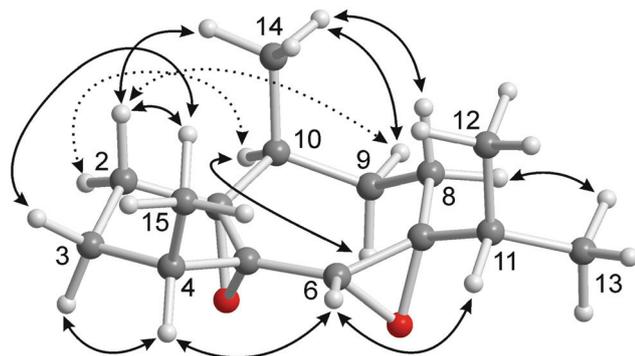


Fig. 8 Characteristic NOE correlations of compound **9a** shown at a computer-generated model of a minimum energy conformation calculated using PBE0-D3(BJ)/def2-TZVP-F.

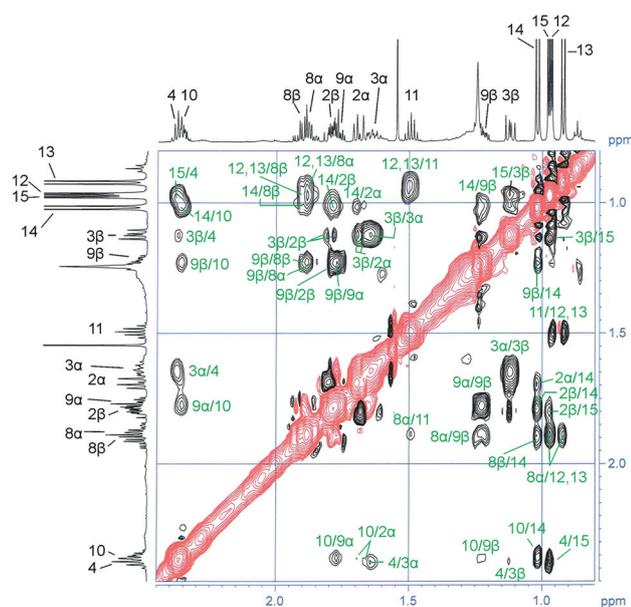


Fig. 9 Expansion of the NOESY spectrum of compound **9a**.

mine the positions of the protons of the seven-membered ring and of the 10-Me group (starting point of the assignment, arbitrarily defined as β) relative to those in the five-membered ring. Thus, the observed NOE cross-peaks for H₃-14/H-2 β and H-10/H-2 α are indicative of a *cis*-arrangement of the corresponding protons (Fig. 8). Moreover, the same signals also suggest an α -position of the 1,5-epoxy group. Support for this assignment is obtained from a weak NOE interaction of H-9 β with H-2 β and the absence of NOE correlations between α -protons in the five-membered ring with those in the seven-membered ring. Due to ambiguous NOE signals for the interactions of H-2 α and H-2 β with the protons at C-3, the direct assignment of H-3 α and H-3 β and the resulting determination of the configuration at C-4 could not be achieved. However, the position of the protons at C-3 relative to H₃-15 and H-4 has

Table 2 Comparison of the estimated and experimental $^3J_{\text{HH}}$ coupling constants of the protons in the five-membered ring of compound **9a**

Torsion angles	θ^a [°]	$^3J_{\text{HH}}$ [Hz] estimated ^b	$^3J_{\text{HH}}$ [Hz] experimental ^c
H2 α -C2-C3-H3 α	-34.3	8.0	8.4
H2 α -C2-C3-H3 β	85.8	2.0	<1
H2 β -C2-C3-H3 α	-155.2	11.1	10.4
H2 β -C2-C3-H3 β	-35.1	7.9	8.3
H3 α -C2-C3-H4	32.5	8.3	7.6
H3 β -C2-C3-H4	-87.3	2.0	<1

^aTorsion angles from a minimum energy conformation obtained by quantum chemical calculations using PBE0-D3(BJ)/def2-TZVP-F (D-PCM, solvent: CDCl₃). ^bCoupling constants estimated from the torsion angles using the Bothner-By equation ($^3J_{\text{HH}} = 7 - \cos \theta + 5 \cos 2\theta$).⁴⁵ ^cCoupling constants obtained from the experimental ¹H NMR signals (CDCl₃) by iterative approximation of simulated signals.

been unambiguously identified by NOE interactions. The *cis*-arrangement of H₃-15/H-3*cis* (*cis* with respect to 4-Me) and of H-4/H-3*trans* is concluded from distinct NOE cross-peaks. No NOE signal is observed for H₃-15/H-3*trans* and only a weak signal for H-4/H-3*cis*.

Coupling constants have been used to correlate the proton assignments of the five-membered ring. For this purpose, the experimental coupling constants in the five-membered ring have been determined from the complex signals in the ¹H NMR spectrum by iterative approximation of simulated coupling patterns (Fig. S11–S17[†]). The minimum energy conformations for all eight possible diastereoisomers of **9** have been obtained by quantum chemical calculations using PBE0-D3 (BJ)/def2-TZVP-F (D-PCM, solvent: CDCl₃). The envelope conformation of the five-membered ring is identical for all eight diastereoisomers and corresponds to a *syn*-arrangement of the epoxy moiety and C-3. This is in full agreement with X-ray crystal structures of related epoxyguaiane sesquiterpenes.⁴⁶ The estimated coupling constants obtained from the torsion angles of the calculated minimum energy conformations (Tables 2 and S7[†]) using the Bothner-By equation⁴⁵ have been compared with the experimental data (Tables 2 and S8[†]). Only the *cis*-diepoxide **9a** and the diastereoisomeric *trans*-diepoxide **9b** (Fig. 6) show good agreement between experimental and estimated data (Table S8[†]). Both compounds have two very small coupling constants (estimated: 2 Hz, found: <1 Hz, Table 2) for H-2 α /H-3*cis* and H-3*cis*/H-4 because of torsion angles of approximately 90° between these pseudoequatorial protons (*cis* and *trans* denote the orientation of the corresponding proton relative to the 4-Me group). Consequently, the 4-Me group adopts the β -position which is also supported by an NOE signal for the interaction of H-2 β with H₃-15. At this point, it becomes possible to assign H-3*cis* as H-3 β and H-3*trans* as H-3 α . A distinction between the structures **9a** and **9b** by NOE interactions proved to be difficult. A strong NOE signal has been observed for H-4/H-6 (Fig. S22[†]); however, this signal could not be used to assign the relative orientation of the corresponding protons. Ambiguous NOE signals between the protons of the isopropyl group and the adjacent protons



H-8 α and H-8 β prevented a direct assignment of the configuration for the isopropyl group relative to the 10-Me group. Both structures, **9a** and **9b**, are in agreement with the observed NOE signals and the coupling constants. In order to differentiate between **9a** and **9b**, we have compared the calculated chemical ^1H and ^{13}C NMR shifts (GIAO) with the corresponding experimental data (Tables S9 and S10 \dagger). As a result from this comparison, our natural product was assigned as the *cis*-diepoxide **9a** because for this structure we observed smaller deviations between calculated and experimental values, in particular for the ^{13}C NMR shifts. Missing NOE interactions between H-9 α and/or H₃-12/H₃-13 as well as between H-10 and/or H₃-12/H₃-13 support our assignment.

Based on the spectroscopic data discussed above, the isolated compound was tentatively assigned as (1*R*/5*S*,4*S*/5*S*/6*S*/7*R*,7*S*/10*S*/R)-1,5,6,7-diepoxyguaiane (**9a**) (Fig. 6) with a *cis*-configuration of the 4-Me and the 10-Me group as known for the common sesquiterpenoid guaial. Compound **9a** represents a novel diepoxyguaiane sesquiterpene which we have named oxyfungiformin. Biogenetically **9a** may derive from a twofold epoxidation of 4 α H,10 α H-guaia-1(5),6-diene (γ -guaiene)⁴⁷ or 4 β H,10 β H-guaia-1(5),6-diene.⁴⁸ Recently, Venkateswarlu and coworkers reported the isolation of the diastereoisomeric compound **9c** (Fig. 6) from *Sinularia kavarattiensis* (India).^{42b}

Subfraction F-2-2 of the diethyl ether extract of *C. fungiformis* contained a mixture of sterols. This fraction was additionally purified by reversed-phase HPLC using acetonitrile–water (9 : 1) as the mobile phase to afford the three known sterols 24-methylenecholesterol (**10**), (24*S*)-24-methylcholesterol (**11**) and gorgosterol (**12**) (Fig. 10). Compound **10** was obtained as a colorless solid and, based on MS, IR, 1D, and 2D NMR data, identified as 24-methylenecholesterol (**10**).^{1,3,49–52} Comparison of the ^1H NMR data of **10** with those reported by Su *et al.*⁵² for a synthetic sample (Table S11 \dagger) and of the ^{13}C NMR shift values with those reported for a sample isolated from *Litophyton viridis*⁵¹ (Table S12 \dagger) confirmed the assignment. 24-Methylenecholesterol (**10**) has been found in various soft corals, for example, *Capnella imbricata*,⁵³ *Litophyton viridis*,⁵¹ *Sinularia flexibilis*,⁵⁴ *Sinularia maxima*,⁵⁵ and *Sinularia vander-*

landi.³ It has not been obtained previously from the soft coral *C. fungiformis*.

(24*S*)-24-Methylcholesterol (**11**) was isolated as a colorless solid and assigned based on comparison of the ^1H and ^{13}C NMR data with those reported in the literature (Tables S11 and S12 \dagger).^{56–58} (24*S*)-24-Methylcholesterol (**11**), the C-24 epimer of campesterol, has been isolated previously from diverse natural sources, in particular from soft corals (*e.g.* from *Sinularia* sp.,⁵⁹ *Sinularia dura*,⁶⁰ *Lobophytum* sp.⁶¹ and others). The first synthesis of compound **11** has been described in 2012 by McCarthy and coworkers.⁵⁶ We have isolated (24*S*)-24-methylcholesterol (**11**) for the first time from the soft coral *C. fungiformis*.

The third sterol was identified as gorgosterol (**12**) (Fig. 10).^{62–64} It features unusual ^1H NMR signals at $\delta_{\text{H}} = -0.14$ (1H, ddd, $J = 5.8, 4.4, 1.3$ Hz), 0.13–0.19 (1H, m), and 0.45 ppm (1H, ddd, $J = 9.1, 4.3, 2.6$ Hz), which are characteristic of the protons of the cyclopropane ring. The structure of the compound was unambiguously confirmed by comparison of the ^{13}C NMR signals with those reported for gorgosterol (**12**) isolated from the soft coral *Alcyonium molle* (Table S12 \dagger).⁶⁵ Soft corals are a rich source of gorgosterol and several species have been shown to contain this sterol, for example *Sarcophyton glaucum*,⁶⁶ *Alcyonium molle*,⁶⁵ *Sinularia kavarattiensis*,⁶⁷ and *Asterospicularia lauriae*.⁶⁸ The soft coral *C. fungiformis* investigated herein represents a new source of this sterol.

Lobophytum crassum

Soft corals of the species *L. crassum* have been frequently studied for their chemical constituents. The first report appeared in 1977 by Bowden *et al.* who isolated a cembranoid diterpene from a specimen collected in Australia.⁶⁹ Cembranoids have been repeatedly described as major components of *L. crassum*.^{70–72} In the present study, we investigated for the first time the chemical constituents of *L. crassum* collected at the coast of Madagascar. A specimen of this soft coral was minced and extracted with methanol at room temperature. After evaporation of the solvent, the crude extract was taken up with diethyl ether. Purification of the resulting diethyl ether extract by column chromatography provided a 3.5 : 1 mixture of (24*S*)-24-methylcholesterol (**11**) and cholesterol. The antimalarial assay of the crude methanol extract of *L. crassum* exhibited an IC₅₀ value of 33.15 \pm 2.90 $\mu\text{g mL}^{-1}$ for the inhibition of the FCM29 strain of *Plasmodium falciparum*, indicating moderate activity against this pathogen.

Pseudoceratina arabica

Samples of the sponge *P. arabica* from the Red Sea have already been investigated for their chemical constituents, resulting in the isolation of brominated compounds derived from tyrosine.^{73–75} Specimens of *P. arabica* were obtained from the waters around Nosy Be, Madagascar, at a depth of about 18–25 m. The samples were homogenized and then extracted with methanol at room temperature. The combined organic layers were filtered and concentrated *in vacuo* to obtain the crude methanol extract, which was extracted with hexane and

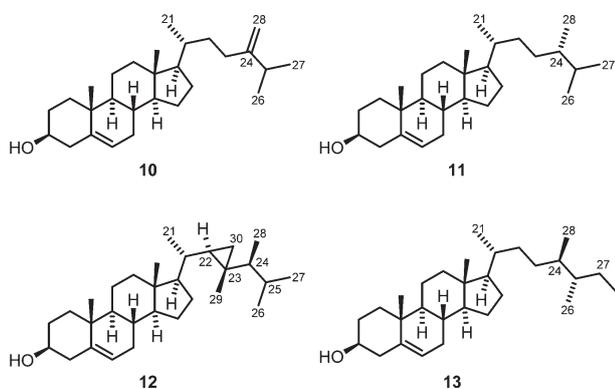


Fig. 10 Chemical structures of 24-methylenecholesterol (**10**), (24*S*)-24-methylcholesterol (**11**), gorgosterol (**12**) and aplysterol (**13**).



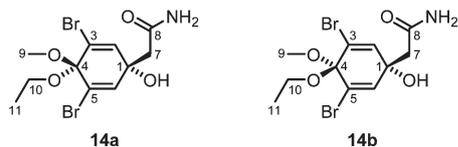


Fig. 11 Structures of compounds **14a** and **14b**.

then dichloromethane. The major compound of the hexane extract (F-1) was identified as aplysterol (**13**) (Fig. 10). Compound **13** was obtained as a colorless solid with a melting point of 125–126 °C and a specific optical rotation of $[\alpha]_D^{20} = -27.0$ (c 0.05, MeOH). The NMR data of **13** were in agreement with those reported in the literature (Tables S11 and S12†).^{76,77} Aplysterol (**13**) was previously isolated from other marine sponges, e.g. *Aplysina aerophoba*⁷⁸ and *Aplysina fistularis*,^{77,79} and also from terrestrial plants, such as *Mussaenda macrophylla*,⁸⁰ but not from *P. arabica*. Further fractionation of the dichloromethane extract (F-2) of *P. arabica* followed by column chromatography over silica gel afforded additional aplysterol (**13**) and a mixture of the dibromotyrosine metabolites **14a** and **14b** (Fig. 11). Compounds **14a** and **14b** were identified by IR spectroscopy, EI mass spectrometry, ¹H and ¹³C NMR spectroscopy, and a set of 2D NMR methods (COSY, HSQC, HMBC, NOESY). The structure elucidation was confirmed by comparison with the ¹H and ¹³C NMR data of the compounds isolated from *Aplysina* sp. (Table S13†).⁸¹ The GC-MS chromatogram showed only a single peak. However, a double set of signals for H₃-9, H₂-10 and H₃-11 in the ¹H NMR spectrum indicated the presence of two diastereoisomers in a ratio of about 3.5 : 1 in favor of the *cis*-isomer **14a** (*cis* and *trans* denote the position of the ethoxy group relative to the hydroxy group), the same ratio as reported by Santalova *et al.*⁸² The predominant *cis*-isomer **14a** was identified by a strong NOE correlation between H₃-9

and H₂-7. The *trans*-isomer **14b** showed NOE cross-peaks between H₂-10 and H₂-7.

The diastereoisomeric compounds **14a** and **14b** have been isolated first from a sponge of the genus *Verongia*⁸³ and subsequently from *Aplysina fistularis*,⁸⁴ *Aplysina* sp.,^{81,82,85,86} *Pseudoceratina* sp.,⁸⁷ *Suberea mollis*,⁸⁸ *Suberea* sp.,⁸⁹ and *Ircinia variabilis*.⁹⁰ Occasionally, they have been described as possible artifacts.^{83,85} However, since we have not used ethanol during the isolation process, we consider **14a** and **14b** to be original natural products of *P. arabica*. Even if not explicitly mentioned, the diastereoisomeric compounds **14a** and **14b** most likely have always been obtained as a mixture.^{83,87,89} Separation of the two diastereoisomers by HPLC was achieved by Lin and co-workers⁹¹ and Santalova *et al.*,⁸¹ who also provided ¹H NMR data for the separated isomers. Even though the sponge *P. arabica* has already been investigated for its chemical constituents,^{73–75} compounds **14a** and **14b** have not been isolated from this species so far.

The dichloromethane fraction (F-2) of *P. arabica* and the ethyl acetate (F-2-1), *n*-butanol (F-2-2), and aqueous (F-2-3) fractions derived therefrom (see the Experimental, Extraction and isolation section) have been investigated for their activity against a series of microbes (Table 3). It appeared that the parent dichloromethane (F-2) and the ethyl acetate (F-2-1) extracts of *P. arabica* are very active against the Gram-negative bacteria *Enterobacter cloacae*, *Klebsiella oxytoca*, *Shigella boydii*, *Escherichia coli*, and *Salmonella enteridis* and against the Gram-positive bacteria *Bacillus cereus*, *Staphylococcus aureus*, and *Streptococcus pneumoniae*. Exceptionally high activity was observed for the dichloromethane extract (F-2) of *P. arabica* against *S. boydii*. The butanol extract (F-2-2) was very active against *S. boydii* and significantly active against *S. aureus* and *S. pneumoniae*. The aqueous extract (F-2-3) was very active against *E. coli* and *S. enteridis*. It should be noted that compounds **14a** and **14b**, which we isolated from the dichloro-

Table 3 Antimicrobial activities of four different fractions from the sponge *Pseudoceratina arabica* obtained by the agar diffusion test^a

Microbes	Zone of inhibition ^b (Ø in mm)			
	CH ₂ Cl ₂ (F-2)	EtOAc (F-2-1)	<i>n</i> -BuOH (F-2-2)	H ₂ O (F-2-3)
Gram-negative bacteria				
<i>Enterobacter cloacae</i> ATCC 700323	12	17	6.5	6.5
<i>Klebsiella oxytoca</i> ATCC 8724	14	15.5	6	6
<i>Shigella boydii</i> ATCC 9207	22	14	10	6
<i>Escherichia coli</i>	11	15	6	10
<i>Salmonella enteridis</i>	12.5	13	6	12
Gram-positive bacteria				
<i>Bacillus cereus</i> ATCC 13061	15	11	6	6
<i>Staphylococcus aureus</i> ATCC 11632	14	13.5	8.5	6
<i>Streptococcus pneumoniae</i> ATCC 6301	14	15	8	6
Yeast				
<i>Candida albicans</i>	6	6	6	6

^a Each test was run in triplicate and the mean values are given; the solvent (methanol) was used as the negative control. ^b Concentration of the extracts: 1 mg mL⁻¹, 10 µL solution per 6 mm disc; Ø < 7 mm: inactive, 7 mm ≤ Ø < 8 mm: slightly active, 8 mm ≤ Ø < 9 mm: significantly active, Ø ≥ 9 mm: very active.



methane extract, were reported to show no antimicrobial activity.^{84,86,88,89} In contrast, the parent ketone and related derivatives are described to be active against a variety of pathogens.^{73,88,89,92} Some derivatives show antimigratory activity against the highly metastatic MDA-MB-231 human breast cancer cell line,^{74,75} induce apoptosis in human breast tumor cells,⁹³ or inhibit Na⁺-K⁺-ATPase activity.⁹⁴ It can be assumed that some as yet unidentified components, possibly also dibromotyrosine derivatives, are responsible for the antimicrobial activity of the extracts. None of the four extracts of *P. arabica* investigated in our antimicrobial assay exhibited activity against the yeast *Candida albicans*. The crude methanol extract of *P. arabica* was also inactive against the FCM29 strains of *Plasmodium falciparum*.

Conclusions

We have investigated the chemical constituents of the Madagascan soft corals *Sarcophyton stellatum*, *Capnella fungiformis*, and *Lobophytum crassum* and the sponge *Pseudoceratina arabica*. The chemical constituents of *S. stellatum* and *C. fungiformis* have been studied for the first time. The crude extracts of the three soft corals showed moderate antimalarial activities (*Plasmodium falciparum*). The extracts of the sponge *P. arabica* exhibited strong activity against a series of Gram-positive and Gram-negative bacteria. From *S. stellatum* we have isolated the previously unknown (+)-enantiomer of the cembranoid diterpene (1*E*,3*E*,11*E*)-7,8-epoxycembra-1,3,11,15-tetraene [(+)-2]. *C. fungiformis* provided a mixture of the two novel sesquiterpenes ethyl 5-[(1*E*,5*Z*)-2,6-dimethylocta-1,5,7-trienyl]furan-3-carboxylate (6) and ethyl 5-[(1*E*,5*E*)-2,6-dimethylocta-1,5,7-trienyl]furan-3-carboxylate (7). Moreover, we isolated from *C. fungiformis* the novel diepoxyguaiane sesquiterpene oxyfungiformin (9a).

Experimental

General methods

Thin layer chromatography was performed on aluminum plates from Merck (60 F₂₅₄) coated with silica gel. For visualization, the plates were analyzed under UV light or treated with a solution of 0.5 g vanillin dissolved in 100 mL of 80/20 (v/v) sulfuric acid/dry ethanol and subsequently heated. Preparative TLC was carried out with glass plates (20 × 20 cm, Merck) coated with a 0.25 mm layer of silica gel (60 F₂₅₄). Analytical HPLC was carried out using an Agilent 1100 instrument equipped with a G1315B UV-DAD (detection at 215, 260 and 560 nm), a G1321A fluorescence and an evaporative light scattering detector (ELS 1000, Polymer Laboratories) using a Vydac 208TP104 column (reversed-phase C8, 4.6 × 250 mm) under the following conditions: flow rate: 1.0 mL min⁻¹; eluent A: H₂O + 0.1% TFA; eluent B: MeCN + 0.1% TFA; gradient from 20 to 90% B in 35 min. Preparative HPLC was performed using a Varian PrepStar system with a Varian ProStar Model 320 UV and an evaporative light scattering detector (ELS 1000,

Polymer Laboratories) connected *via* a Sunchrom Quick-Split splitter. Column chromatography was performed using silica gel from Acros Organics (0.035–0.070 mm). Melting points were measured using a Gallenkamp MPD 350 melting point apparatus. Optical rotations were determined with a PerkinElmer 341 polarimeter at a wavelength of 589 nm (sodium D line) using a 1.0 decimeter cell with a total volume of 1.0 mL. CD spectra were recorded on a JASCO J-815 CD spectrometer. UV spectra were measured with a PerkinElmer Lambda 25 UV-Vis spectrometer. Fluorescence spectra were measured on a Varian Cary Eclipse fluorescence spectrometer. IR spectra were recorded on a Thermo Nicolet Avatar 360 FT-IR spectrometer using the ATR technique (attenuated total reflectance). NMR measurements were performed with a Bruker AVANCE III 600 spectrometer. The chemical shifts δ are reported in ppm using the solvent as the internal standard (¹H: δ_{H} 7.25 ppm CHCl₃; ¹³C: δ_{C} 77.00 ppm CDCl₃). The following abbreviations have been used: s: singlet, d: doublet, t: triplet, q: quartet, sp: septet, m: multiplet and br: broad. Assignment of the ¹H NMR and ¹³C NMR signals was achieved using the 2D NMR methods COSY, HSQC, HMBC and NOESY. Mass spectra were measured by GC-MS coupling with an Agilent 6890 N GC System equipped with a 5973 Mass Selective Detector or with HRMS on a Finnigan MAT 95 mass spectrometer (electron impact, 70 eV). ESI-MS spectra were recorded on a Bruker Esquire mass spectrometer with an ion trap mass analyzer; positive and negative ions were detected. Elemental analyses were performed using a EuroVector EuroEA3000 elemental analyzer. X-ray single crystal structure analysis: X-ray diffraction data were collected on a Bruker AXS Kappa APEX Duo diffractometer with a microfocus tube using Cu K α radiation ($\lambda = 1.54178 \text{ \AA}$). The programs used were: for data collection: APEX2,⁹⁵ for data reduction: SAINT,⁹⁶ for absorption correction: SADABS version 2.10,⁹⁷ for structure solution: SIR2002,⁹⁸ and for structure refinement by full-matrix least-squares against F^2 : SHELXL.⁹⁹ Hydrogen atoms were placed in calculated positions and refined as riding atoms at carbon atoms. The figure was generated using the programs ORTEP-III¹⁰⁰ and POV-Ray 3.7.

Natural sources

Samples of the soft corals *C. fungiformis*, *S. stellatum* and *L. crassum* were collected by hand using scuba in March 2006 in the inner reef of Mahambo (17°29'15.0" S; 49°28'32.1" E), located in the Tamatave province, at the east coast of Madagascar, at a depth of 5–6 m. The sponge *P. arabica* was collected using scuba in April 2005 from the waters around Nosy Be, an island off Madagascar located in the Mozambique Channel, near the northwest coast of Madagascar at a depth of 18–25 m. The collection site was determined at Banc des Gorgones 13°25'03.5" S and 48°12'25.5" E. The species were assigned by Dr Shirley Parker-Nance (Nelson Mandela Metropolitan University, Port Elizabeth, and South African Institute for Aquatic Biodiversity (SAIAB), Grahamstown, South Africa). Voucher specimens of the three soft corals and the sponge investigated in this study have been deposited at the



SAIAB (collection numbers: 201734 for *S. stellatum*, 201730 for *C. fungiformis*, 201729 for *L. crassum* and 201727 for *P. arabica*).

Extraction and isolation

General. Each sample was labeled, immediately frozen, and kept at $-20\text{ }^{\circ}\text{C}$ until being processed unless otherwise described.

A specimen of *S. stellatum* (1 kg, wet weight) was minced and then extracted with methanol at room temperature. The mixture was filtered and the organic phase was concentrated under reduced pressure to yield the crude extract (29 g), which was partitioned between water/diethyl ether and water/dichloromethane, successively. The diethyl ether extract (11 g) was subjected to column chromatography over silica gel using pentane with increasing proportions of diethyl ether and then diethyl ether–ethyl acetate (10 : 1) as eluents to afford the three fractions F-1, F-2 and F-3, respectively. Fraction F-1 (2.0 g) eluted with pentane–diethyl ether (10 : 1) was then separated by column chromatography over silica gel using increasing concentrations of diethyl ether in pentane to give two fractions (F-1-1 and F-1-2) according to TLC detection. Fraction F-1-1 (1.53 g) was further purified by column chromatography over silica gel with pentane–diethyl ether (12 : 1) to afford (+)-(7*S*,8*S*)-epoxy-7,8-dihydrocembrene C [(+)-1] (814 mg) and (+)-(1*E*,3*E*,11*E*)-7,8-epoxycembra-1,3,11,15-tetraene [(+)-2] (316 mg). Fraction F-1-2 (459 mg) was separated by column chromatography over silica gel with pentane–diethyl ether (10 : 1) as the eluent to obtain (+)-(7*R*,8*R*,14*S*,1*Z*,3*E*,11*E*)-14-acetoxy-7,8-epoxycembra-1,3,11-triene [(+)-3] (207 mg). Fraction F-2 (3.71 g) was subjected to column chromatography over silica gel using pentane–diethyl ether (4 : 1) as the mobile phase to afford (–)-(2*R*,7*R*,8*R*)-sarcophytoxide [(–)-4] (3.37 g), which crystallized from a small amount of diethyl ether. Fraction F-3 (300 mg) was further purified by preparative HPLC (column: Vydac 208TP1030, reversed phase C8, 30 × 250 mm; flow rate: 20 mL min⁻¹; eluent A: H₂O + 0.1% TFA, eluent B: THF + 0.1% TFA; gradient from 50 to 80% B in 20 min) to yield (*S*)-3-*O*-octadecylglycerol (batyl alcohol) (5) (11 mg). The dichloromethane extract of *S. stellatum* (270 mg) was subjected to chromatography over silica gel using pentane with increasing proportions of diethyl ether as the eluent to afford (+)-1 (3 mg), (+)-3 (3 mg) and (–)-4 (10 mg).

The material of *C. fungiformis* (770 g, wet weight) was cut and then extracted with methanol. The methanol extract was concentrated under reduced pressure and the residue (17 g) was dissolved in dry diethyl ether. The diethyl ether extract (8.0 g) was subjected to column chromatography over silica gel using pentane with increasing proportions of diethyl ether as the eluent to afford two main fractions. The first fraction (F-1) (2.2 g) was separated by column chromatography over silica gel eluting with a gradient of pentane–diethyl ether to obtain three subfractions (F-1-1, F-1-2 and F-1-3) according to TLC detection. Fraction F-1-1 (1.8 g) was purified by column chromatography over silica gel with pentane–diethyl ether (20 : 1) as mobile phase, affording a mixture of furan sesquiterpenes 6

and 7 (F-1-1-1, 15 mg) in a ratio of about 3 : 2 (6 : 7) (GC-MS, ¹H NMR) and octadecan-2-one (8) (F-1-1-2, 13 mg). Subfraction F-1-2 (224 mg) was further purified by repeated column chromatography over silica gel with pentane–diethyl ether (10 : 1) to afford 23 mg of the diepoxyguaiane sesquiterpene 9a. Subfraction F-1-3 (17 mg) contained a mixture of monoalkyl-diacylglycerols, which was not investigated in detail. The second fraction of the diethyl ether extract (F-2, 574 mg) was repeatedly subjected to column chromatography over silica gel eluting with pentane–diethyl ether (9 : 1) to give two fractions (F-2-1 and F-2-2). Fraction F-2-1 (51 mg) contained a mixture of monoalkylmonoacylglycerols. Fraction F-2-2 (156 mg) was further separated by reverse phase HPLC (acetonitrile–water, 9 : 1) to afford 24-methylenecholesterol (10) (26 mg), (24*S*)-24-methylcholesterol (11) (77 mg), and gorgosterol (12) (43 mg).

A sample of the fresh soft coral *L. crassum* (0.5 kg, wet weight) was homogenized and exhaustively extracted with methanol. The combined extracts were concentrated to yield a crude mass of 15 g which was extracted with diethyl ether. The dried diethyl ether extract was concentrated under reduced pressure and the residue (2 g) was subjected to column chromatography over silica gel with pentane–diethyl ether (4 : 1) to collect a portion (480 mg) showing a main spot on TLC. This portion was subsequently further purified by two column chromatographic steps over silica gel using pentane–ethyl acetate (9 : 1) and pentane–diethyl ether (7 : 3) as mobile phases to yield a mixture of (24*S*)-24-methylcholesterol (11) and cholesterol in a ratio of 3.5 : 1 according to the GC-MS chromatogram.

The specimens of the sponge *P. arabica* (957 g, wet weight) were homogenized and extracted with methanol at room temperature. The combined extracts were concentrated to obtain 34 g of a crude extract, which was extracted successively with hexane and dichloromethane to afford 9.6 g of the hexane extract (F-1) and 4.3 g of the dichloromethane extract (F-2). A portion of 782 mg of the hexane extract (F-1) was subjected to column chromatography over silica gel with a stepped solvent gradient from pentane to diethyl ether as the mobile phase. Fractions eluted with pentane–diethyl ether (4 : 1) were combined to provide 201 mg of aplysterol (13). The dichloromethane extract (F-2) (4.3 g) was partitioned between water and ethyl acetate. After separation of the ethyl acetate layer, the aqueous layer was extracted with *n*-butanol. Evaporation of the ethyl acetate, *n*-butanol and the aqueous layers provided residues of 1.3 g (F-2-1), 1.9 g (F-2-2) and 1.0 g (F-2-3), respectively. A 1.0 g portion of the ethyl acetate extract (F-2-1) was extracted with dichloromethane to give 356 mg of a dichloromethane extract (F-2-1-1), which was subjected to column chromatography over silica gel using pentane with increasing proportions of diethyl ether as the eluent. The fractions eluted with pentane–diethyl ether (4 : 1) were combined to give a fraction of 240 mg, which was subjected to column chromatography over silica gel to provide another 97 mg of aplysterol (13). A 657 mg portion of the *n*-butanol extract (F-2-2) was further extracted with dichloromethane to afford a residue of 234 mg (F-2-2-1). The dichloromethane extract (F-2-2-1) was



subjected to column chromatography over silica gel eluting with: (1) pentane–diethyl ether (4 : 1) and (2) diethyl ether–dichloromethane (3 : 2) to afford two main fractions F-2-2-1-1 and F-2-2-1-2. Fraction F-2-2-1-1 (77 mg) was purified by column chromatography over silica gel with pentane–diethyl ether (4 : 1) providing 40 mg of aplysterol (**13**). Fraction F-2-2-1-2 (157 mg) was subjected to column chromatography over silica gel using diethyl ether with increasing proportions of dichloromethane as the eluent to collect 100 mg of a fraction showing one main spot on TLC. This fraction was subsequently purified by preparative TLC over silica gel with diethyl ether–dichloromethane (3 : 2) to afford a 3.5 : 1 mixture of the acetamides **14a** and **14b** (26 mg). The aqueous extract (F-2-3, 1.0 g) was extracted with methanol. Column chromatography of the methanol extract (201 mg) over silica gel with ethyl acetate afforded a fraction (116 mg) which showed one main spot on TLC. Another column chromatographic purification over silica gel using diethyl ether–ethyl acetate (3 : 2) as the mobile phase afforded 49 mg of a mixture of the diastereoisomeric acetamides **14a** and **14b**.

Biological activity

Antimalarial assay. The antiplasmodial activity against the FCM29 strains of *Plasmodium falciparum* was determined by using the microfluorimetric assay previously reported (see the ESI†).^{101–103} Results are given as IC₅₀ values in µg mL⁻¹. Quinine (IC₅₀ = 3.5 µg mL⁻¹) was used as the positive control and the solvent (methanol) as the negative control.

Antimicrobial assay. The antimicrobial activities were determined using the agar diffusion technique in Petri dishes. The different extracts from the sponge *P. arabica* were tested for their antimicrobial activity against five Gram-negative bacteria: *Enterobacter cloacae* (ATCC 700323), *Klebsiella oxytoca* (ATCC 8724), *Shigella boydii* (ATCC 9207), *Escherichia coli*, *Salmonella enteridis*; three Gram-positive bacteria: *Bacillus cereus* (ATCC 13061), *Staphylococcus aureus* (ATCC 11632), *Streptococcus pneumoniae* (ATCC 6301); and against one yeast: *Candida albicans*. The pathogens were supplied by the Laboratoire de Microbiologie de l'Environnement (LME), Centre National de Recherche sur l'Environnement (CNRE), Antananarivo, Madagascar. The fractions were dissolved in methanol at a concentration of 1 mg mL⁻¹. A sample (10 µL) of each solution was added *via* a pipette onto a sterile filter disc of 6 mm diameter and oven dried at 40–50 °C. The discs were placed on Müller–Hinton agar plates that had been inoculated with the microorganisms mentioned above. The plates were incubated for 24 h at 37 °C for the bacteria and for 48 h at 25 °C for the yeast. The diameters of the inhibition zones generated around the discs were measured (Ø in mm). The tests were performed in triplicate and the mean values were determined. Methanol, used to dissolve the extracts, was checked for the absence of antimicrobial activity. The diameters of the halos of inhibition can be rationalized on a qualitative basis as follows: Ø < 7 mm: inactive, 7 mm ≤ Ø < 8 mm: slightly active, 8 mm ≤ Ø < 9 mm: significantly active, Ø ≥ 9 mm: very active.

Molecular modeling

Conformational energy minimization for compound **9a** (Fig. 8) and for the seven alternative diastereoisomers **9b–h** was achieved by quantum chemical calculations using PBE0-D3 (BJ)/def2-TZVP-F (D-PCM, solvent: CDCl₃).

(+)-(7S,8S)-Epoxy-7,8-dihydrocembrene C [(+)-1]. Colorless oil. $[\alpha]_{\text{D}}^{20} = +44.6$ (c 0.5, MeOH) (lit.: $[\alpha]_{\text{D}}^{25} = +99.0$ (c 1.90, acetone),²⁴ $[\alpha]_{\text{D}}^{25} = +19$ (c 0.5, CHCl₃),²³ lit. (–)-1: $[\alpha]_{\text{D}} = -22.5$ (c 0.19, CHCl₃)²⁵, $[\alpha]_{\text{D}}^{20} = -25.2$ (c 0.21, CHCl₃)²⁸). CD (MeOH): $\Delta\epsilon$ (λ) +0.23 (242 nm). UV (MeOH): λ = 249 nm. Fluorescence (MeOH): λ_{ex} = 249 nm, λ_{em} = 311 nm. IR (ATR): ν = 2958, 2927, 2871, 1713, 1667, 1446, 1381, 1162, 1062, 987, 860 cm⁻¹. ¹H NMR (600 MHz, CDCl₃): δ = 1.04 (3H, d, J = 7.1 Hz, H₃-16 or H₃-17), 1.06 (3H, d, J = 6.8 Hz, H₃-16 or H₃-17), 1.26 (3H, s, H₃-19), 1.51 (1H, ddd, J = 13.9, 10.5, 3.0 Hz, H-9b), 1.58 (3H, d, J = 0.8 Hz, H₃-20), 1.66–1.72 (1H, m, H-6b), 1.75 (3H, s, H₃-18), 1.77–1.83 (1H, m, H-6a), 1.92 (1H, ddd, J = 14.3, 7.5, 2.3 Hz, H-9a) 1.98–2.06 (3H, m, H-13b, H₂-10), 2.17–2.31 (5H, m, H₂-5, H-13a, H₂-14), 2.33 (1H, sp, J = 6.7 Hz, H-15), 2.84 (1H, t, J = 5.6 Hz, H-7), 5.06 (1H, t, J = 7.1 Hz, H-11), 5.96 (1H, dq, J = 11.3, 1.1 Hz, H-3), 6.03 (1H, d, J = 10.9 Hz, H-2). ¹³C NMR and DEPT (150 MHz, CDCl₃): δ = 16.95 (C-20), 17.10 (C-18), 17.84 (C-19), 22.14, 22.32 (C-16, C-17), 22.49 (C-10), 25.72 (C-6), 28.23 (C-14), 34.89 (C-15), 35.75 (C-5), 37.48 (C-9), 39.44 (C-13), 60.09 (C-8), 61.61 (C-7), 118.40 (C-2), 121.40 (C-3), 125.54 (C-11), 134.21 (C-4), 135.63 (C-12), 148.16 (C-1). MS (EI, 70 eV): m/z (%) = 288 [M⁺] (6), 245 (7), 227 (5), 205 (8), 203 (7), 163 (14), 136 (53), 121 (100), 107 (55), 93 (86), 79 (37), 69 (40), 55 (34), 43 (58). ESI-MS (+25 V): m/z = 271 [M + H – H₂O]⁺, 289 [M + H]⁺.

(+)-(1E,3E,11E)-7,8-Epoxyembrene-1,3,11,15-tetraene [(+)-2]. Colorless oil. $[\alpha]_{\text{D}}^{20} = +12.0$ (c 0.5, MeOH) (lit. (–)-2: $[\alpha]_{\text{D}} = -14.4$ (c 0.1, CHCl₃)²⁵). CD (MeOH): $\Delta\epsilon$ (λ) –0.11 (251 nm). UV (MeOH): λ = 282 nm. IR (ATR): ν = 2923, 1723, 1662, 1473, 1442, 1380, 1253, 1163, 1063, 877 cm⁻¹. ¹H NMR (600 MHz, CDCl₃): δ = 1.27 (3H, s, H₃-19), 1.55 (3H, d, J = 1.1 Hz, H₃-20), 1.60 (1H, ddd, J = 14.3, 9.4, 3.0 Hz, H-9b), 1.68–1.74 (1H, m, H-6b), 1.79–1.86 (1H, m, H-6a), 1.81 (3H, s, H₃-18), 1.89 (1H, ddd, J = 14.4, 7.7, 2.6 Hz, H-9a), 1.95–1.99 (2H, m, H₂-10), 1.96 (3H, s, H₃-16), 2.05 (1H, dt, J = 13.6, 6.5 Hz, H-13b), 2.20–2.35 (3H, m, H-13a, H-5a, H-5b), 2.54 (2H, t, J = 6.4 Hz, H₂-14), 2.83 (1H, t, J = 5.6 Hz, H-7), 4.98 (1H, s, H-17b), 5.02–5.06 (1H, m, H-11), 5.08 (1H, s, H-17a), 6.10 (1H, dq, J = 11.1, 1.3 Hz, H-3), 6.39 (1H, d, J = 10.9 Hz, H-2). ¹³C NMR and DEPT (150 MHz, CDCl₃): δ = 17.23 (C-18), 17.31 (C-20), 18.21 (C-19), 21.22 (C-16), 22.28 (C-10), 25.62 (C-6), 22.81 (C-14), 36.10 (C-5), 37.04 (C-9), 39.12 (C-13), 60.16 (C-8), 61.03 (C-7), 112.15 (C-17), 122.24 (C-3), 122.58 (C-2), 126.00 (C-11), 135.84 (C-12), 137.70 (C-4), 139.81 (C-1), 143.28 (C-15). MS (EI, 70 eV): m/z (%) = 286 [M⁺] (5), 271 (3), 252 (3), 215 (2), 201 (6), 187 (7), 173 (8), 159 (12), 145 (15), 133 (67), 119 (100), 105 (48), 91 (57), 77 (28), 69 (24), 55 (27), 41 (44). ESI-MS (+10 V): m/z = 287 [M + H]⁺.

(+)-(7R,8R,14S,1Z,3E,11E)-14-Acetoxy-7,8-epoxyembrene-1,3,11-triene [(+)-3]. Colorless oil. $[\alpha]_{\text{D}}^{20} = +171.8$ (c 0.1, MeOH) (lit.: $[\alpha]_{\text{D}} = +136$ (c 1.1, CHCl₃),³⁰ $[\alpha]_{\text{D}} = +150$ (c 1.02, CHCl₃)³¹). UV



(MeOH): $\lambda = 250$ nm. Fluorescence (MeOH): $\lambda_{\text{ex}} = 250$ nm, $\lambda_{\text{em}} = 299$ nm. IR (ATR): $\nu = 2958, 2922, 2863, 1738, 1438, 1370, 1238, 1017, 961, 840$ cm^{-1} . ^1H NMR (600 MHz, CDCl_3): $\delta = 1.05$ (3H, d, $J = 6.8$ Hz, H_3 -16), 1.15 (3H, d, $J = 7.2$ Hz, H_3 -17), 1.29 (3H, s, H_3 -19), 1.50 (3H, s, H_3 -20), 1.72–1.77 (2H, m, H_2 -6), 1.78 (3H, s, H_3 -18), 1.80–1.85 (2H, m, H_2 -9), 1.91 (2H, q, $J = 5.5$ Hz, H_2 -10), 2.04 (3H, s, COCH_3), 2.19–2.27 (3H, m, H_2 -5, H -13b), 2.43 (1H, dd, $J = 13.6, 3.8$ Hz, H -13a), 2.53 (1H, sp, $J = 6.8$ Hz, H -15), 2.78 (1H, t, $J = 5.8$ Hz, H -7), 5.18 (1H, t, $J = 6.4$ Hz, H -11), 5.90 (1H, dd, $J = 9.4, 3.8$ Hz, H -14), 6.07 (1H, d, $J = 11.7$ Hz, H -3), 6.23 (1H, d, $J = 11.3$ Hz, H -2). ^{13}C NMR and DEPT (150 MHz, CDCl_3): 16.90 (C-18), 17.96 (C-20), 18.62 (C-19), 21.37 (COCH_3), 21.44 (C-10), 23.92 (C-17), 24.72 (C-16), 25.12 (C-6), 28.22 (C-15), 35.70 (C-5), 35.92 (C-9), 43.63 (C-13), 59.61 (C-2, C-7, C-8), 73.93 (C-14), 120.19 (C-3), 121.02 (C-2), 129.75 (C-11), 131.28 (C-12), 136.22 (C-4), 143.83 (C-1), 170.19 (C=O). MS (EI, 70 eV): m/z (%) = 346 [M^+] (1), 331 (1), 286 (4), 263 (2), 243 (5), 221 (7), 194 (5), 175 (8), 161 (10), 152 (28), 137 (100), 109 (65), 43 (74).

(-)-(2R,7R,8R)-Sarcophytoxide [(−)-4]. Colorless crystals; m.p. 60–61 °C (lit.: m.p. 79–81 °C,³² m.p. 78–79 °C²⁵). $[\alpha]_{\text{D}}^{20} = -129.4$ (c 0.1, MeOH) (lit.: $[\alpha]_{\text{D}} = -191$ (c 0.4, CHCl_3),³² $[\alpha]_{\text{D}} = -183$ (c 0.1),²⁵ $[\alpha]_{\text{D}}^{25} = -128$ (c 1.0, CHCl_3)²³). CD (MeOH): $\Delta\epsilon$ (λ) +0.01 (247 nm), −0.01 (274 nm). Fluorescence (MeOH): $\lambda_{\text{ex}} = 203$ nm, $\lambda_{\text{em}} = 300$ nm. IR (ATR): $\nu = 2929, 2853, 1755, 1662, 1446, 1384, 1245, 1040, 940, 838$ cm^{-1} . ^1H NMR (600 MHz, CDCl_3): $\delta = 1.00$ (1H, td, $J = 13.2, 3.0$ Hz, H -9b), 1.26 (3H, s, H_3 -19), 1.59 (3H, s, H_3 -20), 1.60–1.65 (2H, m, H -6b, H -14b), 1.64 (3H, s, H_3 -17), 1.81 (3H, s, H_3 -18), 1.86–1.94 (4H, m, H -6a, H -10b, H_2 -13), 2.10 (1H, ddd, $J = 13.1, 5.2, 2.8$ Hz, H -9a), 2.25 (1H, dddd, $J = 14.3, 10.2, 4.9, 3.4$ Hz, H -10a), 2.30–2.38 (2H, m, H_2 -5), 2.51–2.59 (1H, m, H -14a), 2.71 (1H, t, $J = 4.1$ Hz, H -7), 4.45–4.53 (2H, m, H_2 -16), 5.09 (1H, dd, $J = 9.8, 5.3$ Hz, H -11), 5.22 (1H, d, $J = 9.8$ Hz, H -3), 5.50–5.56 (1H, m, H -2). ^{13}C NMR and DEPT (150 MHz, CDCl_3): $\delta = 10.20$ (C-17), 15.06 (C-20), 15.58 (C-18), 16.91 (C-19), 23.52 (C-10), 25.28 (C-6), 26.12 (C-14), 36.66 (C-13), 37.65 (C-5), 39.83 (C-9), 59.84 (C-8), 61.90 (C-7), 78.40 (C-16), 83.63 (C-2), 123.59 (C-11), 126.27 (C-3), 127.86 (C-15), 133.19 (C-1), 136.84 (C-12), 139.30 (C-4). MS (EI, 70 eV): m/z (%) = 302 [M^+] (9), 287 (11), 259 (4), 231 (7), 217 (6), 203 (10), 189 (13), 175 (29), 163 (100), 149 (78), 135 (99), 121 (53), 109 (59), 95 (77), 91 (56), 79 (49), 67 (63), 55 (53), 43 (75). ESI-MS (+10 V): $m/z = 285$ [$\text{M} + \text{H} - \text{H}_2\text{O}$]⁺, 303 [$\text{M} + \text{H}$]⁺, 320 [$\text{M} + \text{NH}_4$]⁺. Anal. calcd for $\text{C}_{20}\text{H}_{30}\text{O}_2$: C 79.42, H 10.00 found C 79.15, H 10.34.

Crystal data for (−)-4. $\text{C}_{20}\text{H}_{30}\text{O}_2$, $M = 302.44$ g mol^{−1}, crystal size: 0.23 × 0.30 × 0.45 mm³, monoclinic, space group $P2_1$, $a = 5.6825(3)$ Å, $b = 14.2013(7)$ Å, $c = 11.4613(6)$ Å, $\beta = 94.933(2)^\circ$, $V = 921.49(8)$ Å³, $Z = 2$, $\rho_{\text{calcd}} = 1.090$ g cm^{−3}, $\mu = 0.526$ mm^{−1}, $T = 150$ (2) K, $\lambda = 1.54178$ Å, θ range = 3.87–68.72°, reflections collected 31 136, independent reflections 3258 ($R_{\text{int}} = 0.0316$), 204 parameters, $R_1 = 0.0264$, $wR_2 = 0.0747$ [$I > 2\sigma(I)$]; maximal residual electron density: 0.123 e Å^{−3}. Flack parameter: $\chi = 0.1$ (0). CCDC 1526876 contains the supplementary crystallographic data for this structure.

(S)-3-O-Octadecylglycerol (batyl alcohol) (5). Yellow solid; m.p. 69–70 °C (lit.: m.p. 70.6–71.1 °C³⁸). $[\alpha]_{\text{D}}^{20} = +2.6$ (c 0.07,

MeOH) (lit.: $[\alpha]_{\text{D}}^{20} = +2.0$ (c 4.40, CHCl_3)³⁸). IR (ATR): $\nu = 3355$ (br), 2957, 2916, 2849, 1469, 1330, 1258, 1117, 1061, 1021, 936, 865, 798, 717, 677 cm^{-1} . ^1H NMR (600 MHz, CDCl_3): $\delta = 0.87$ (3H, t, $J = 7.0$ Hz, H_3 -18'), 1.24 (28H, s br, H_2 -4'– H_2 -17'), 1.25–1.32 (2H, m, H_2 -3'), 1.57 (2H, tt, $J = 7.6, 6.3$ Hz, H_2 -2'), 3.42–3.50 (2H, m, H_2 -1'), 3.50 (1H, dd, $J = 9.4, 6.0$ Hz, H -3b), 3.54 (1H, dd, $J = 9.8, 3.8$ Hz, H -3a), 3.67 (1H, dd, $J = 11.7, 5.3$ Hz, H -1b), 3.73 (1H, dd, $J = 11.7, 3.8$ Hz, H -1a), 3.88 (1H, tt, $J = 5.3, 4.1$ Hz, H -2). ^{13}C NMR and DEPT (150 MHz, CDCl_3): $\delta = 14.11$ (C-18'), 22.68 (C-17'), 26.07 (C-3'), 29.35, 29.44, 29.57, 29.60, 29.65, 29.68 (C-2', C-4'–C-15'), 31.91 (C-16'), 64.33 (C-1), 70.34 (C-2), 71.88 (C-1'), 72.53 (C-3). MS (EI, 70 eV): m/z (%) = 313 [$[\text{M} - \text{CH}_2\text{OH}]^+$] (2), 283 (8), 253 (6), 125 (7), 111 (13), 97 (26), 85 (45), 71 (60), 57 (100), 43 (65). ESI-MS (+25 V): $m/z = 345$ [$\text{M} + \text{H}$]⁺, 362 [$\text{M} + \text{NH}_4$]⁺.

Mixture of ethyl 5-[(1E,5Z)-2,6-dimethylocta-1,5,7-trienyl]furan-3-carboxylate (6) and ethyl 5-[(1E,5E)-2,6-dimethylocta-1,5,7-trienyl]furan-3-carboxylate (7) (3 : 2). Yellow solid. ESI-MS (+10 V): $m/z = 275$ [$\text{M} + \text{H}$]⁺.

6: ^1H NMR (600 MHz, CDCl_3): $\delta = 1.34$ (3H, t, $J = 7.2$ Hz, H_3 -17), 1.80 (3H, q, $J = 1.1$ Hz, H_3 -15), 1.96 (3H, d, $J = 1.1$ Hz, H_3 -14), 2.22 (2H, t, $J = 7.9$ Hz, H_2 -7), 2.36 (2H, q, $J = 7.5$ Hz, H_2 -8), 4.29 (2H, q, $J = 7.1$ Hz, H_2 -16), 5.09 (1H, dt, $J = 10.5, 1.5$ Hz, H -12b), 5.29 (1H, br d, $J = 17.3$ Hz, H -12a), 5.37 (1H, br t, $J = 7.3$ Hz, H -9), 6.05 (1H, br s, H -5), 6.49 (1H, s, H -3), 6.76 (1H, ddd, $J = 17.3, 10.8, 0.9$ Hz, H -11), 7.88 (1H, s, H -1). ^{13}C NMR and DEPT (150 MHz, CDCl_3): $\delta = 14.11$ (C-17), 18.71 (C-14), 19.74 (C-15), 25.80 (C-8), 40.66 (C-7), 60.38 (C-16), 106.74 (C-3), 113.65 (C-5), 113.77 (C-12), 120.63 (C-2), 129.75 (C-9), 132.84 (C-10), 133.46 (C-11), 140.50 (C-6), 145.42 (C-1), 154.61 (C-4), 163.38 (C-13). MS (EI, 70 eV): m/z (%) = 274 [M^+] (5), 229 (6), 193 (44), 147 (100), 119 (8), 91 (20), 81 (10), 79 (10), 65 (7), 53 (8), 41 (8).

7: ^1H NMR (600 MHz, CDCl_3): $\delta = 1.34$ (3H, t, $J = 7.2$ Hz, H_3 -17'), 1.74 (3H, s, H_3 -15'), 1.97 (3H, d, $J = 1.1$ Hz, H_3 -14'), 2.24 (2H, t, $J = 7.9$ Hz, H_2 -7'), 2.33 (2H, q, $J = 7.9$ Hz, H_2 -8'), 4.29 (2H, q, $J = 7.1$ Hz, H_2 -16'), 4.93 (1H, d, $J = 10.9$ Hz, H -12b'), 5.09 (1H, d, $J = 17.3$ Hz, H -12a'), 5.47 (1H, br t, $J = 7.2$ Hz, H -9'), 6.05 (1H, br s, H -5'), 6.35 (1H, dd, $J = 17.1, 10.7$ Hz, H -11'), 6.49 (1H, s, H -3'), 7.88 (1H, s, H -1'); ^{13}C NMR and DEPT (150 MHz, CDCl_3): $\delta = 11.69$ (C-15'), 14.32 (C-17'), 18.71 (C-14'), 26.70 (C-8'), 40.27 (C-7'), 60.38 (C-16'), 106.74 (C-3'), 110.84 (C-12'), 113.65 (C-5'), 120.63 (C-2'), 131.75 (C-9'), 132.84 (C-10'), 140.50 (C-6'), 141.34 (C-11'), 145.42 (C-1'), 154.61 (C-4'), 163.38 (C-13'). MS (EI, 70 eV): m/z (%) = 274 [M^+] (5), 229 (4), 193 (43), 147 (100), 119 (8), 91 (20), 81 (10), 79 (9), 65 (6), 53 (8), 41 (7).

Octadecan-2-one (8). Colorless solid; m.p. 57–58 °C (lit.: m.p. 45–50 °C,^{44a} m.p. = 52 °C¹⁰⁴). Fluorescence (MeOH): $\lambda_{\text{ex}} = 202$ nm, $\lambda_{\text{em}} = 303$ nm. IR (ATR): $\nu = 2954, 2916, 2848, 1709, 1462, 1372, 1165, 720$ cm^{-1} . ^1H NMR (600 MHz, CDCl_3): $\delta = 0.87$ (3H, t, $J = 7.0$ Hz, H_3 -18), 1.22–1.29 (26H, m, H -5– H -17), 1.52–1.58 (2H, m, H_2 -4), 2.12 (3H, s, H_3 -1), 2.40 (2H, t, $J = 7.5$ Hz, H_2 -3). ^{13}C NMR and DEPT (150 MHz, CDCl_3): $\delta = 14.12$ (C-18), 22.69 (C-17), 23.88 (C-4), 29.35, 29.47, 29.60, 29.65, 29.68 (11 CH_2 , C-5–C-15), 29.85 (C-1), 31.92 (C-16), 43.84 (C-3), 209.43 (C-2). MS (EI, 70 eV): m/z (%) = 268 [M^+] (8), 96 (10),



85 (13), 71 (42), 58 (90), 43 (100). ESI-MS (+10 V): $m/z = 286 [M + NH_4]^+$, 559 $[2M + Na]^+$.

Oxyfungifomin (9a). Colorless solid; m.p. 66–66.5 °C. $[\alpha]_D^{20} = +38.0$ (c 0.05, MeOH). IR (ATR): $\nu = 2960, 2927, 2871, 2855, 1738, 1458, 1377, 1167, 979, 920, 891, 848$ cm^{-1} . 1H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR and DEPT (150 MHz, $CDCl_3$): see Table 1. EI-MS (EI, 70 eV): m/z (%) = 236 $[M^+]$ (1), 221 (3), 203 (5), 193 (17), 175 (26), 165 (30), 151 (42), 147 (79), 138 (38), 135 (51), 123 (63), 121 (57), 109 (100), 107 (67), 105 (64), 95 (43), 81 (56), 67 (27), 55 (38). ESI-MS (+25 V): $m/z = 219 [M + H - H_2O]^+$, 237 $[M + H]^+$, 254 $[M + NH_4]^+$, 490 $[2M + NH_4]^+$, 495 $[2M + Na]^+$. HRMS: m/z calcd for $C_{15}H_{24}O_2^+ [M^+]$: 236.1771, found: 236.1762; calcd for $C_{14}H_{21}O_2^+ [(M - CH_3)^+]$: 221.1536, found: 221.1549; calcd for $C_{14}H_{19}O^+ [(M - CH_3 - H_2O)^+]$: 203.1430, found: 203.1423.

24-Methylenecholesterol (10). Colorless solid. 1H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$): see ref. 3 and Tables S11 and S12.† MS (EI, 70 eV): m/z (%) = 398 $[M^+]$ (8), 383 (13), 365 (10), 314 (100), 299 (34), 281 (34), 271 (55), 229 (27), 213 (26), 159 (27), 145 (36), 105 (48), 91 (47), 69 (80), 55 (100) 41 (64). ESI-MS (+10 V): $m/z = 416.4 [M + NH_4]^+$, 819.9 $[2M + Na]^+$. For additional data, see ref. 3.

(24S)-24-Methylcholesterol (11). Colorless solid. 1H NMR (600 MHz, $CDCl_3$): $\delta = 0.66$ (3H, s, H_3-18), 0.76 (3H, d, $J = 6.8$ Hz, H_3-28), 0.77 (3H, d, $J = 6.8$ Hz, H_3-26), 0.84 (3H, d, $J = 6.8$ Hz, H_3-27), 0.91 (3H, d, $J = 6.8$ Hz, H_3-21), 1.00 (3H, s, H_3-19). ^{13}C NMR and DEPT (150 MHz, $CDCl_3$): $\delta = 11.85$ (C-18), 15.44 (C-28), 17.58 (C-26), 18.88 (C-21), 19.40 (C-19), 20.52 (C-27), 21.08 (C-11), 24.29 (C-15), 28.19 (C-16), 30.56 (C-23), 31.45 (C-25), 31.67 (C-2), 31.90 (2C, C-7, C-8), 33.71 (C-22), 36.18 (C-20), 36.50 (C-10), 37.25 (C-1), 39.06 (C-24), 39.76 (C-12), 42.31 (2C, C-4, C-13), 50.12 (C-9), 55.98 (C-17), 56.75 (C-14), 71.81 (C-3), 121.73 (C-6), 140.76 (C-5). MS (EI, 70 eV): m/z (%) = 400 $[M^+]$ (42), 382 (25), 340 (6), 315 (27), 289 (26), 273 (13), 255 (13), 231 (10), 213 (22), 197 (7), 178 (6), 161 (14), 145 (21), 121 (14), 105 (31), 79 (22), 43 (100). ESI-MS (+10 V): $m/z = 418 [M + NH_4]^+$, 823 $[2M + Na]^+$.

Gorgosterol (12). Colorless solid. 1H NMR (600 MHz, $CDCl_3$): $\delta = -0.14$ (1H, td, $J = 5.8, 4.4, 1.3$ Hz, H-30b), 0.13–0.19 (1H, m, H-22), 0.24 (1H, dqd, $J = 8.8, 7.0, 1.8$ Hz, H-24), 0.45 (1H, ddd, $J = 9.1, 4.3, 2.6$ Hz, H-30a), 0.65 (3H, s, H_3-18), 0.85 (3H, d, $J = 6.4$ Hz, H_3-26), 0.89 (3H, s, H_3-29), 0.93 (3H, d, $J = 7.5$ Hz, H_3-27), 0.94 (3H, d, $J = 6.9$ Hz, H_3-28), 0.98–1.01 (8H, m, H-14, H_3-19 , H-20, H_3-21). ^{13}C NMR and DEPT (150 MHz, $CDCl_3$): $\delta = 11.90$ (C-18), 14.27 (C-29), 15.45 (C-28), 19.40 (C-19), 21.07 (C-11), 21.17 (C-21), 21.29 (C-30), 21.53 (C-26), 22.18 (C-27), 24.51 (C-15), 25.80 (C-23), 28.21 (C-16), 31.61 (C-2), 31.86 (C-7), 31.96, 32.03, 32.14 (C-8, C-22, C-25), 35.28 (C-20), 36.51 (C-10), 37.23 (C-1), 39.85 (C-12), 42.23 (C-4), 42.76 (C-13), 50.14 (C-9), 50.80 (C-24), 56.49 (C-14), 57.90 (C-17), 71.92 (C-3), 121.80 (C-6), 140.68 (C-5).

Apysterol (13). Colorless solid; m.p. 125–126 °C (lit.: m.p. 132–134 °C,¹⁰⁵ m.p. 135–136 °C⁷⁸). $[\alpha]_D^{20} = -27.0$ (c 0.05, MeOH) (lit.: $[\alpha]_D = -25$ ($CHCl_3$)⁷⁸). CD (MeOH): $\Delta\epsilon$ (λ) +0.19 (201 nm). Fluorescence (MeOH): $\lambda_{ex} = 203$ nm, $\lambda_{em} = 299$ nm. IR (ATR): $\nu = 3339$ (br), 2955, 2933, 2866, 1456, 1377, 1056,

1023, 957, 925, 886, 841, 799, 738 cm^{-1} . 1H NMR (600 MHz, $CDCl_3$): $\delta = 0.67$ (3H, s, H_3-18), 0.79 (3H, d, $J = 6.8$ Hz, H_3-28), 0.80 (3H, d, $J = 7.2$ Hz, H_3-26), 0.85 (3H, t, $J = 7.3$ Hz, H_3-27 -Me), 0.90 (3H, d, $J = 6.8$ Hz, H_3-21), 0.90–0.92 (1H, m, H-9), 0.96–0.99 (1H, m, H-14), 1.00 (3H, s, H_3-19), 1.03–1.11 (6H, m, H-1b, H-15b, H-17, H-22b, H-23b, H-27b), 1.14 (1H, td, $J = 12.8, 4.9$ Hz, H-12b), 1.17–1.20^a (1H, m, H-23a), 1.23–1.27 (2H, m, H-16b, H-25), 1.27–1.32 (2H, m, H-22a, H-24), 1.32–1.37 (2H, m, H-20, H-27a), 1.40–1.47 (1H, m, H-8), 1.47–1.57 (4H, m, H-2b, H-7b, H-11a, H-11b), 1.55–1.60 (1H, m, H-15a), 1.80–1.86 (3H, m, H-1a, H-2a, H-16a), 1.96 (1H, dtd, $J = 17.3, 4.9, 2.6$ Hz, H-7a), 2.00 (1H, dt, $J = 12.4, 3.4$ Hz, H-12a), 2.19–2.26 (1H, m, H-4b), 2.29 (1H, ddd, $J = 13.2, 5.3, 2.3$ Hz, H-4a), 3.51 (1H, tt, $J = 11.2, 4.7$ Hz, H-3), 5.34 (1H, dt, $J = 5.0, 2.6$ Hz, H-6);

^aChemical shift derived from the HSQC spectrum. ^{13}C NMR and DEPT (150 MHz, $CDCl_3$): $\delta = 11.87$ (C-18), 12.22 (C-27-Me), 15.90 (C-26), 16.54 (C-28), 18.71 (C-21), 19.40 (C-19), 21.10 (C-11), 24.30 (C-15), 25.78 (C-27), 28.23 (C-16), 29.04 (C-23), 31.70 (C-2), 31.93 (C-7, C-8), 33.90 (C-22), 35.88 (C-20), 36.52 (C-10), 37.27 (C-1), 37.53 (C-24), 39.80 (C-12), 39.86 (C-25), 42.34 (C-4, C-13), 50.17 (C-9), 56.15 (C-17), 56.79 (C-14), 71.83 (C-3), 121.72 (C-6), 140.78 (C-5). MS (EI, 70 eV): m/z (%) = 414 $[M^+]$ (95), 399 (36), 396 (49), 381 (34), 354 (9), 329 (52), 303 (49), 273 (27), 255 (32), 231 (20), 213 (39), 145 (51), 105 (57), 57 (100). ESI-MS (+10 V): $m/z = 397 [M + H - H_2O]^+$, 432 $[M + NH_4]^+$.

Mixture of 2-[(1*s*,4*s*)-3,5-dibromo-4-ethoxy-1-hydroxy-4-methoxycyclohexa-2,5-dien-1-yl]acetamide (14a) and 2-[(1*r*,4*r*)-3,5-dibromo-4-ethoxy-1-hydroxy-4-methoxycyclohexa-2,5-dien-1-yl]acetamide (14b) (3.5 : 1). Amorphous solid. Fluorescence (MeOH): $\lambda_{ex} = 205$ nm, $\lambda_{em} = 300$ nm. IR (ATR): $\nu = 3417, 3350, 3196, 2979, 2930, 2845, 1662, 1395, 1321, 1214, 1089, 1059, 966, 870, 815, 698$ cm^{-1} . MS (EI, 70 eV): m/z (%) = 297 (3), 295 (3), 283 (15), 281 (33), 279 (16), 269 (17), 267 (33), 265 (17), 247 (8), 245 (9), 219 (56), 217 (61), 187 (100), 185 (97), 159 (21), 157 (22), 131 (12), 129 (9), 53 (78). ESI-MS (+10 V): $m/z = 401 [M + NH_4]^+$, 403, 405, 789 $[2M + Na]^+$, 791, 793, 795, 797.

14a: 1H NMR (600 MHz, $CDCl_3$): $\delta = 1.26$ (3H, t, $J = 7.0$ Hz, H_3-11), 2.50 (2H, s, H_2-7), 3.14 (3H, s, H_3-9), 3.34 (2H, q, $J = 7.2$ Hz, H_2-10), 5.20 (1H, s, OH), 5.59 (1H, br s, N- H_b), 5.73 (1H, br s, N- H_a), 6.72 (2H, s, H-2/H-6). ^{13}C NMR and DEPT (150 MHz, $CDCl_3$): $\delta = 14.94$ (C-11), 43.67 (C-7), 50.88 (C-9), 59.74 (C-10), 70.92 (C-1), 96.12 (C-4), 124.41 (C-3/C-5), 139.44 (C-2/C-6), 172.31 (C-8).

14b: 1H NMR (600 MHz, $CDCl_3$): $\delta = 1.27$ (3H, t, $J = 6.8$ Hz, H_3-11), 2.50 (2H, s, H_2-7), 3.20 (3H, s, H_3-9), 3.24 (2H, q, $J = 7.2$ Hz, H_2-10), 5.16 (1H, s, OH), 5.59 (1H, br s, N- H_b), 5.73 (1H, br s, N- H_a), 6.72 (2H, s, H-2/H-6). ^{13}C NMR and DEPT (150 MHz, $CDCl_3$): $\delta = 15.07$ (C-11), 43.71 (C-7), 51.16 (C-9), 59.34 (C-10), 70.90 (C-1), 96.13 (C-4), 124.42 (C-3/C-5), 139.42 (C-2/C-6), 172.31 (C-8).

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Notes and references

- M. P. Rahelivao, M. Gruner, H. Andriamanantoanina, B. Andriamihaja, I. Bauer and H.-J. Knölker, *Mar. Drugs*, 2015, **13**, 4197–4216.
- M. P. Rahelivao, M. Gruner, H. Andriamanantoanina, I. Bauer and H.-J. Knölker, *Nat. Prod. Bioprospect.*, 2015, **5**, 223–235.
- M. P. Rahelivao, M. Gruner, T. Lübken, D. Islamov, O. Kataeva, H. Andriamanantoanina, I. Bauer and H.-J. Knölker, *Org. Biomol. Chem.*, 2016, **14**, 989–1001.
- D. J. Faulkner, *Nat. Prod. Rep.*, 1984, **1**, 551–598.
- W. Fenical, *J. Nat. Prod.*, 1987, **50**, 1001–1008.
- A. S. R. Anjaneyulu and G. V. Rao, *J. Indian Chem. Soc.*, 1997, **74**, 272–278.
- Y. Li, L. Peng and T. Zhang, in *Medicinal Chemistry of Bioactive Natural Products*, ed. X.-T. Liang and W.-S. Fang, Wiley, Hoboken, NJ, USA, 2006, pp. 257–300.
- P. Radhika, *Biochem. Syst. Ecol.*, 2006, **34**, 781–789.
- D. Kelman, Y. Kashman, R. T. Hill, E. Rosenberg and Y. Loya, *Pure Appl. Chem.*, 2009, **81**, 1113–1121.
- J. Hu, B. Yang, X. Lin, X. Zhou, X. Yang, L. Long and Y. Liu, *Chem. Biodiversity*, 2011, **8**, 1011–1032.
- L.-F. Liang and Y.-W. Guo, *Chem. Biodiversity*, 2013, **10**, 2161–2196.
- R. Y. P. Burhan, E. M. M. Putri, Y. Zetra, R. Herdhiansyah and M. Y. Putra, *J. Chem. Pharm. Res.*, 2014, **6**, 585–595.
- Q. Yu, P. Sun, B.-s. Liu, H. Tang and W. Zhang, *Dier Junyi Daxue Xuebao*, 2014, **35**, 206–215.
- T. A. Hussien and T. A. Mohamed, *Res. J. Pharm., Biol. Chem. Sci.*, 2015, **6**, 1301–1319.
- A. Longeon, M.-L. Bourguet-Kondracki and M. Guyot, *Tetrahedron Lett.*, 2002, **43**, 5937–5939.
- J. J. Poza, R. Fernández, F. Reyes, J. Rodríguez and C. Jiménez, *J. Org. Chem.*, 2008, **73**, 7978–7984.
- Y. Hou and L. Harinantenaina, *Curr. Med. Chem.*, 2010, **17**, 1191–1219.
- C. L. Griffiths, *Indian J. Mar. Sci.*, 2005, **34**, 35–41.
- Drug Discovery in Africa*, ed. K. Chibale, M. Davies-Coleman and C. Masimirembwa, Springer, Berlin, Germany, 2012.
- Vlaams Institute voor de Zee, Assessing potential World Heritage marine sites in the Western Indian Ocean. <http://www.vliz.be/projects/marineworldheritage/index.php> (accessed 11 January 2017).
- M. S. Zubair, K. O. Al-Footy, S.-E. N. Ayyad, S. S. Al-Lihaibi and W. M. Alarif, *Nat. Prod. Res.*, 2016, **30**, 869–879.
- Ether Lipids, Biochemical and Biomedical Aspects*, ed. H. K. Mangold and F. Paltauf, Academic Press, New York, USA, 1983.
- K. H. Shaker, M. Müller, M. A. Ghani, H.-M. Dahse and K. Seifert, *Chem. Biodiversity*, 2010, **7**, 2007–2015.
- Z.-G. Rao, S.-Z. Deng, F.-Y. Li, H.-M. Wu and Z.-S. Shi, *Chin. J. Org. Chem.*, 1997, **17**, 252–255.
- B. F. Bowden, J. C. Coll and S. J. Mitchell, *Aust. J. Chem.*, 1980, **33**, 879–884.
- J. Shin, W. Fenical, T. J. Stout and J. Clardy, *Tetrahedron*, 1993, **49**, 515–524.
- H. Dong, Y.-L. Gou, R. M. Kini, H.-X. Xu, S.-X. Chen, S. L. M. Teo and P. P.-H. But, *Chem. Pharm. Bull.*, 2000, **48**, 1087–1089.
- Z. Liu, W. Z. Li, L. Peng, Y. Li and Y. Li, *J. Chem. Soc., Perkin Trans. 1*, 2000, 4250–4257.
- J. C. Coll, B. F. Bowden, G. M. König, R. Braslau and I. R. Price, *Bull. Soc. Chim. Belg.*, 1986, **95**, 815–834.
- G. J. Greenland and B. F. Bowden, *Aust. J. Chem.*, 1994, **47**, 2013–2021.
- M. Kobayashi, K. Kondo, K. Osabe and H. Mitsushashi, *Chem. Pharm. Bull.*, 1988, **36**, 2331–2341.
- B. F. Bowden, J. C. Coll, W. Hicks, R. Kazlauskas and S. J. Mitchell, *Aust. J. Chem.*, 1978, **31**, 2707–2712.
- B. F. Bowden, J. C. Coll, A. Heaton, G. König, M. A. Bruck, R. E. Cramer, D. M. Klein and P. J. Scheuer, *J. Nat. Prod.*, 1987, **50**, 650–659.
- Y. Kashman, E. Zadock and I. Néeman, *Tetrahedron*, 1974, **30**, 3615–3620.
- J. M. Frincke, D. E. McIntyre and D. J. Faulkner, *Tetrahedron Lett.*, 1980, **21**, 735–738.
- J. Kobayashi, Y. Ohizumi, H. Nakamura, T. Yamakado, T. Matsuzaki and Y. Hirata, *Experientia*, 1983, **39**, 67–69.
- S. Aratake, T. Tomura, S. Saitoh, R. Yokokura, Y. Kawanishi, R. Shinjo, J. D. Reimer, J. Tanaka and H. Maekawa, *PLoS One*, 2012, **7**, e30410.
- C. D. Magnusson, A. V. Gudmundsdottir and G. G. Haraldsson, *Tetrahedron*, 2011, **67**, 1821–1836.
- J. C. Coll, S. J. Mitchell and G. J. Stokie, *Tetrahedron Lett.*, 1977, **18**, 1539–1542.
- B. F. Bowden, J. C. Coll, E. D. de Silva and M. S. L. de Costa, *Aust. J. Chem.*, 1983, **36**, 371–376.
- K. J. S. Grace, D. Zavortink and R. S. Jacobs, *Biochem. Pharmacol.*, 1994, **47**, 1427–1434.
- (a) S. Rajaram, U. Ramulu, D. Ramesh, D. Srikanth, P. Bhattacharya, P. Prabhakar, S. V. Kalivendi, K. S. Babu, Y. Venkateswarlu and S. Navath, *Bioorg. Med. Chem. Lett.*, 2013, **23**, 6234–6238; (b) S. Rajaram, D. Ramesh, U. Ramulu, M. Anjum, P. Kumar, U. S. N. Murthy,



- M. A. Hussain, G. N. Sastry and Y. Venkateswarlu, *Indian J. Chem.*, 2014, **53B**, 1086–1090.
- 43 D. H. Williams and D. J. Faulkner, *Tetrahedron*, 1996, **52**, 4245–4256.
- 44 (a) D. H. R. Barton, H. Togo and S. Z. Zard, *Tetrahedron*, 1985, **41**, 5507–5516; (b) A. Ulubelen and T. Baytop, *Phytochemistry*, 1973, **12**, 1824; (c) J. O. Grimalt, L. Angulo, A. López-Galindo, M. C. Comas and J. Albaigés, *Chem. Geol.*, 1990, **82**, 341–363; (d) M. A. Krüge, A. Permanyer, J. Serra and D. Yu, *J. Anal. Appl. Pyrolysis*, 2010, **89**, 204–217.
- 45 (a) A. A. Bothner-By, *Adv. Magn. Opt. Reson.*, 1965, **1**, 195–316; (b) M. J. Minch, *Concepts Magn. Res.*, 1994, **6**, 41–56.
- 46 (a) X.-Q. Ma, C.-Z. Liao, J.-Y. Su and L.-M. Zeng, *Acta Crystallogr., Sect. E: Struct. Rep. Online*, 2003, **59**, o889–o890; (b) A.-C. Huang, C. J. Sumbly, E. R. T. Tiekink and D. K. Taylor, *J. Nat. Prod.*, 2014, **77**, 2522–2536.
- 47 (a) R. B. Bates and R. C. Slagel, *J. Am. Chem. Soc.*, 1962, **84**, 1307–1308; (b) M. V. Kadival, M. S. R. Nair and S. C. Bhattacharyya, *Tetrahedron*, 1967, **23**, 1241–1249.
- 48 (a) C. Ehret and G. Ourisson, *Tetrahedron*, 1969, **25**, 1785–1799; (b) H. D. Friedel and R. Matusch, *Helv. Chim. Acta*, 1987, **70**, 1616–1622; (c) H. D. Friedel and R. Matusch, *J. Chromatogr., A*, 1987, **407**, 343–348.
- 49 G. W. Patterson, *Comp. Biochem. Physiol.*, 1968, **24**, 501–505.
- 50 G. W. Patterson, *Lipids*, 1971, **6**, 120–127.
- 51 K. Iguchi, S. Saitoh and Y. Yamada, *Chem. Pharm. Bull.*, 1989, **37**, 2553–2554.
- 52 W. Lu, C. Zhang, L. Zeng and J. Su, *Steroids*, 2004, **69**, 803–808.
- 53 W. C. M. C. Kokke, S. Epstein, S. A. Look, G. H. Rau, W. Fenical and C. Djerassi, *J. Biol. Chem.*, 1984, **259**, 8168–8173.
- 54 S. Yu, Z. Deng, L. van Ofwegen, P. Proksch and W. Lin, *Steroids*, 2006, **71**, 955–959.
- 55 Y. Venkateswarlu, K. V. Sridevi and M. R. Rao, *J. Nat. Prod.*, 1999, **62**, 756–758.
- 56 N. M. O'Connell, Y. C. O'Callaghan, N. M. O'Brien, A. R. Maguire and F. O. McCarthy, *Tetrahedron*, 2012, **68**, 4995–5004.
- 57 D. Sica, V. Piccialli and A. Masullo, *Phytochemistry*, 1984, **23**, 2609–2611.
- 58 J. Zielinski, H. T. Li and C. Djerassi, *J. Org. Chem.*, 1982, **47**, 620–625.
- 59 M. Y. Putra, G. Bavestrello, C. Cerrano, B. Renga, C. D'Amore, S. Fiorucci, E. Fattorusso and O. Tagliatalata-Scafati, *Steroids*, 2012, **77**, 433–440.
- 60 M. M. Radwan, S. P. Manly, K. A. El Sayed, V. B. Wali, P. W. Sylvester, B. Awate, G. Shah and S. A. Ross, *J. Nat. Prod.*, 2008, **71**, 1468–1471.
- 61 C. Subrahmanyam, C. Venkateswara Rao, V. Anjaneyulu, P. Satyanarayana, P. V. Subba Rao, R. S. Ward and A. Pelter, *Tetrahedron*, 1992, **48**, 3111–3120.
- 62 W. Bergmann, M. J. McLean and D. Lester, *J. Org. Chem.*, 1943, **8**, 271–282.
- 63 N. C. Ling, R. L. Hale and C. Djerassi, *J. Am. Chem. Soc.*, 1970, **92**, 5281–5282.
- 64 R. L. Hale, J. Leclercq, B. Tursch, C. Djerassi, R. A. Gross Jr., A. J. Weinheimer, K. Gupta and P. J. Scheuer, *J. Am. Chem. Soc.*, 1970, **92**, 2179–2180.
- 65 B. F. Bowden, J. C. Coll and M. C. Dai, *Aust. J. Chem.*, 1989, **42**, 665–673.
- 66 M. Kobayashi, A. Tomioka and H. Mitsushashi, *Steroids*, 1979, **34**, 273–284.
- 67 V. Sepe, F. S. Di Leva, C. D'Amore, C. Festa, S. De Marino, B. Renga, M. V. D'Auria, E. Novellino, V. Limongelli, L. D'Souza, M. Majik, A. Zampella and S. Fiorucci, *Mar. Drugs*, 2014, **12**, 3091–3115.
- 68 B. F. Bowden, B. J. Cusack and A. Dangel, *Mar. Drugs*, 2003, **1**, 18–26.
- 69 B. F. Bowden, J. A. Brittle, J. C. Coll, N. Liyanage, S. J. Mitchell and G. J. Stokie, *Tetrahedron Lett.*, 1977, **18**, 3661–3662.
- 70 N. X. Cuong, N. P. Thao, B. T. T. Luyen, N. T. T. Ngan, D. T. T. Thuy, S. B. Song, N. H. Nam, P. V. Kiem, Y. H. Kim and C. V. Minh, *Chem. Pharm. Bull.*, 2014, **62**, 203–208.
- 71 S.-Y. Cheng, S.-K. Wang and C.-Y. Duh, *Mar. Drugs*, 2014, **12**, 6028–6037.
- 72 C.-Y. Kao, J.-H. Su, M.-C. Lu, T.-L. Hwang, W.-H. Wang, J.-J. Chen, J.-H. Sheu, Y.-H. Kuo, C.-F. Weng, L.-S. Fang, Z.-H. Wen and P.-J. Sung, *Mar. Drugs*, 2011, **9**, 1319–1331.
- 73 J. M. Badr, L. A. Shaala, M. I. Abou-Shoer, M. K. Tawfik and A.-A. M. Habib, *J. Nat. Prod.*, 2008, **71**, 1472–1474.
- 74 L. A. Shaala, D. T. A. Youssef, M. Sulaiman, F. A. Behery, A. I. Foudah and K. A. El Sayed, *Mar. Drugs*, 2012, **10**, 2492–2508.
- 75 L. A. Shaala, D. T. A. Youssef, J. M. Badr, M. Sulaiman, A. Khedr and K. A. El Sayed, *Tetrahedron*, 2015, **71**, 7837–7841.
- 76 A. Kelecom, G. J. Kannengiesser and P. M. Baker, *An. Acad. Bras. Cienc.*, 1979, **51**, 643–645.
- 77 C. A. N. Catalan, J. E. Thompson, W. C. M. C. Kokke and C. Djerassi, *Tetrahedron*, 1985, **41**, 1073–1084.
- 78 P. De Luca, M. De Rosa, L. Minale and G. Sodano, *J. Chem. Soc., Perkin Trans. 1*, 1972, 2132–2135.
- 79 N. S. Lira, R. L. Monte-Neto, J. G. B. Marchi, A. C. S. Lins, J. F. Tavares, M. S. Silva, C. S. Dias, J. M. Barbosa-Filho, C. F. Santos, E. V. L. Cunha, U. S. Pinheiro and R. Braz-Filho, *Quim. Nova*, 2012, **35**, 2189–2193.
- 80 B. Dinda, S. Majumder, S. Arima, N. Sato and Y. Harigaya, *J. Nat. Med.*, 2008, **62**, 447–451.
- 81 E. A. Santalova, V. A. Denisenko, V. P. Glazunov, A. I. Kalinovskii, S. D. Anastyuk and V. A. Stonik, *Russ. Chem. Bull.*, 2011, **60**, 570–580.
- 82 E. A. Santalova, V. A. Denisenko and P. S. Dmitrenok, *Chem. Nat. Compd.*, 2013, **49**, 75–78.
- 83 R. J. Andersen and D. J. Faulkner, *Tetrahedron Lett.*, 1973, **14**, 1175–1178.
- 84 Y. M. Goo, *Arch. Pharmacol. Res.*, 1985, **8**, 21–30.
- 85 R. Xynas and R. J. Capon, *Aust. J. Chem.*, 1989, **42**, 1427–1433.



- 86 M. H. Kossuga, S. P. Lira, A. M. Nascimento, M. T. P. Gambardella, R. G. S. Berlinck, Y. R. Torres, G. G. F. Nascimento, E. F. Pimenta, M. Silva, O. H. Thiemann, G. Oliva, A. G. Tempone, M. S. C. Melhem, A. O. Souza, F. C. S. Galetti, C. L. Silva, B. Cavalcanti, C. O. Pessoa, M. O. Moraes, E. Hajdu, S. Peixinho and R. M. Rocha, *Quim. Nova*, 2007, **30**, 1194–1202.
- 87 X.-P. Huang, Z.-W. Deng, R. W. M. van Soest and W.-H. Lin, *J. Asian Nat. Prod. Res.*, 2008, **10**, 253–258.
- 88 L. A. Shaala, S. I. Khalifa, M. K. Mesbah, R. W. M. van Soest and D. T. A. Youssef, *Nat. Prod. Commun.*, 2008, **3**, 219–222.
- 89 K. H. Shaker, H. Zinecker, M. A. Ghani, J. F. Imhoff and B. Schneider, *Chem. Biodiversity*, 2010, **7**, 2880–2887.
- 90 J.-j. Zhao and H.-g. Liu, *Cent. South Pharm.*, 2013, **11**, 185–186.
- 91 X.-P. Huang, Z.-W. Deng, R. W. M. van Soest and W.-H. Lin, *J. Asian Nat. Prod. Res.*, 2008, **10**, 239–242.
- 92 A. A. Salim, Z. G. Khalil and R. J. Capon, *Tetrahedron*, 2012, **68**, 9802–9807.
- 93 F. D. Mora, D. K. Jones, P. V. Desai, A. Patny, M. A. Avery, D. R. Feller, T. Smillie, Y.-D. Zhou and D. G. Nagle, *J. Nat. Prod.*, 2006, **69**, 547–552.
- 94 B. A. Gorshkov, I. A. Gorshkova, T. N. Makarieva and V. A. Stonik, *Toxicol.*, 1982, **20**, 1092–1094.
- 95 Bruker, *APEX2 Software Suite for Crystallographic Programs*, Bruker AXS Inc., Madison, WI, USA, 2009.
- 96 Bruker, *SMART and SAINT. Area Detector Control and Integration Software. Version 5.1*, Bruker Analytical X-ray Instruments Inc., Madison, WI, USA, 1996.
- 97 G. M. Sheldrick, *SADABS. Program for Absorption Correction. Version 2.10*, University of Göttingen, Germany, 1996.
- 98 M. C. Burla, M. Camalli, B. Carrozzini, G. L. Cascarano, C. Giacovazzo, G. Polidori and R. Spagna, *J. Appl. Crystallogr.*, 2003, **36**, 1103–1103.
- 99 G. Sheldrick, *Acta Crystallogr., Sect. A: Fundam. Crystallogr.*, 2008, **64**, 112–122.
- 100 L. J. Farrugia, *J. Appl. Crystallogr.*, 1997, **30**, 565–565.
- 101 Y. Corbett, L. Herrera, J. Gonzalez, L. Cubilla, T. L. Capson, P. D. Coley, T. A. Kursar, L. I. Romero and E. Ortega-Barria, *Am. J. Trop. Med. Hyg.*, 2004, **70**, 119–124.
- 102 W. Trager and J. Jensen, *Science*, 1976, **193**, 673–675.
- 103 W. Trager and J. B. Jensen, *J. Parasitol.*, 2005, **91**, 484–486.
- 104 M. C. Pangborn and R. J. Anderson, *J. Am. Chem. Soc.*, 1936, **58**, 10–14.
- 105 R. Jain, S. Nagpal, S. Jain and S. C. Jain, *J. Med. Aromat. Plant Sci.*, 2004, **26**, 48–50.

