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Cyclolobatriene, a novel prenylated germacrene diterpene, from the soft coral *Lobophytum pauciflorum*

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ABSTRACT

A new 10-membered-ring diterpene, cyclolobatriene (**1**), along with three other known diterpenes, lobatriene (**2**), eunicol (**3**), and fuscol (**4**), were isolated from the Okinawan soft coral *Lobophytum pauciflorum*. Their structures were established by extensive NMR spectroscopic analyses. Cyclolobatriene (**1**) is an additional example of rare prenylated germacrenes. Although **1**, due to a 10-membered-ring structure, exists as an equilibrium mixture of three conformers, the NMR measurement in $CDCl_3$ at 7 °C enabled us to assign the NMR signals of the three, which is the first example of the complete NMR assignment of all the existing conformers of germacrene-type compounds. Cyclolobatriene (**1**) was thermally unstable and converted into **2** through Cope rearrangement upon heating at 70 °C. Eunicol (**3**) also possesses the same prenylated germacrene structure as **1**, showing similar physico-chemical properties to **1**. All four compounds **1**–**4** showed cytotoxic effect with IC_{50}'s of 0.64, 0.41, 0.35 and 0.52 μM, respectively, against human epidermoid carcinoma A431 cells.

1. Introduction

Soft corals of the subclass Alcyonaria (Octocorallia) are known as rich sources of lipids, sesquiterpenes, diterpenes and steroids with wide structural diversity and pronounced bioactivities.[1] As a study on marine metabolites with biological activities, we have tested the cytotoxicity of an extracts library established from hundreds of marine organisms including a number of soft corals. As a result, we found a relatively high activity for an extract from the Okinawan soft coral *Lobophytum pauciflorum* Ehrenberg (Family Alcyoniidae), from which we have isolated a novel diterpene, cyclolobatriene (**1**), and three related metabolites [lobatriene (**2**), eunicol (**3**) and fuscol (**4**)] (Fig. 1). These metabolites are structurally interesting, because they possess a 'prenylated' germacrene (**1** and **3**) or a 'prenylated' elemene (**2** and **4**) skeleton which are not common as natural products. Several prenylated elemenes (or lobanes[2]) have been isolated from a gorgonian *Eunicea fusca*[3,4] and some soft corals belong to the genus *Lobophytum*,[2,5] *Sarcophyton*,[6] and *Sinularia*.[7–9] But very few prenylated germacrenes like **1** have been isolated so far; one from brown algae *Dilophus ligulatus*,[10] and three from a soft coral *Lobophytum* sp.[11] and the gorgonian *Eunicea fusca*.[12] Therefore, cyclolobatriene (**1**) is an additional example of the rare prenylated germacrenes in nature. In the present paper, we describe isolation, cytotoxicity, and structure elucidation of **1**–**4** (Fig. 1), with complete NMR assignment of three conformers of **1** and **3**.

2. Results and discussion

The soft coral *Lobophytum pauciflorum* was collected off the Okinawan coast of Japan and extracted with methanol. The ethyl acetate-soluble fraction of the methanol extract was subjected to silica gel flash column chromatography followed by recycling HPLC gave cyclolobatriene (**1**) and the known related compounds lobatriene (**2**), eunicol (**3**), and fuscol (**4**). All four compounds **1**–**4** showed cytotoxic effect with IC_{50}'s of 0.64, 0.41, 0.35 and 0.52 μM, respectively, against human epidermoid carcinoma A431 cells.

Cyclolobatriene (**1**), $[\alpha]_D^{26}$ +57 (*c* 0.139, $CHCl_3$), appears as a colorless oil. It exhibited IR absorption at 3472 cm^{-1} due to hydroxyl group. It showed a molecular ion peak at *m/z* 327.2292 $[M+Na]^+$ in the high-resolution ESI-TOF-MS that is compatible with the molecular composition of $C_{20}H_{32}O_2$. Broadened ^1H NMR ($CDCl_3$, 27 °C) signals in the olefin region with <0.5 integral values indicated the existence of more than one conformer (Fig. 2 and 27 °C). This phenomenon made the structure elucidation challenging.

Attempts to measure the average NMR spectrum of the conformers at 70 °C were discouraged by thermal instability of **1**

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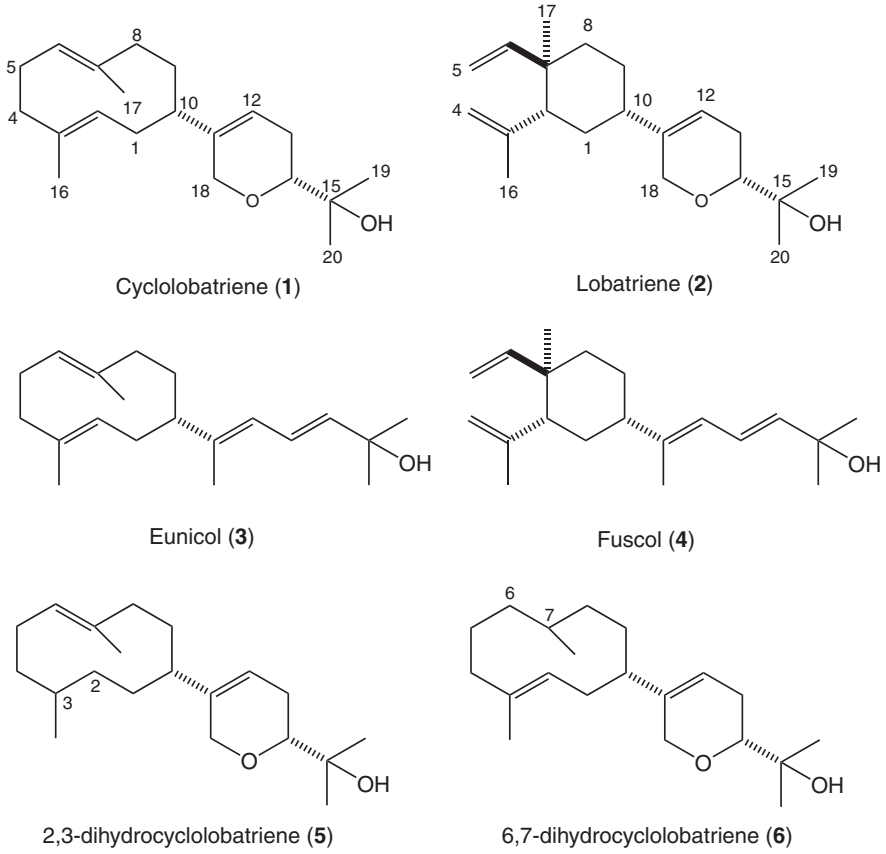

Figure 1. The molecular structures of compounds **1**–**6**.

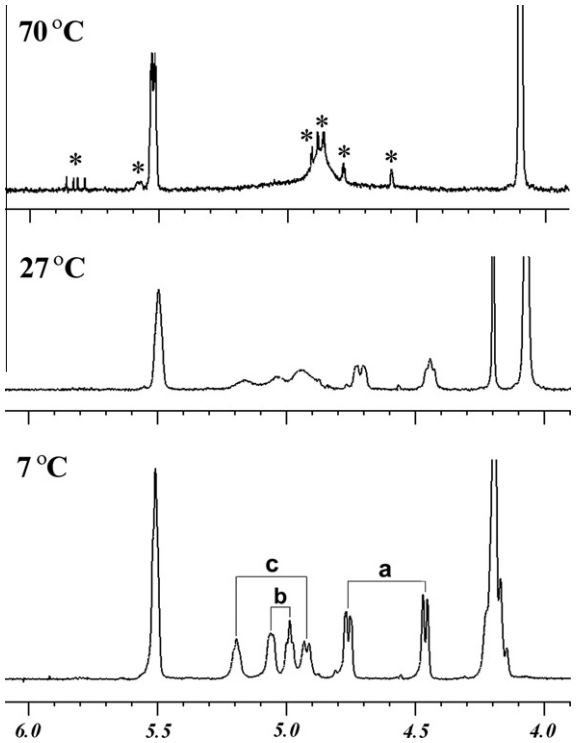

Figure 2. Olefinic ^{1}H NMR signals of cyclolobatriene (**1**) at different temperatures. The asterisks (∗) indicate signals due to commencement of isomerization to lobatriene (**2**). The signals of three conformers are indicated by the letters **a**, **b**, and **c**.

towards partial isomerisation to **2** (Fig. 2 and 70 °C). Such a rearrangement was reported in the conversion of germacrene and its derivatives toward their 10-membered ring elemene congeners.[13–17] However, the broadened NMR signals of **1** decoalesced and became distinct when the temperature decreased to 7 °C in $CDCl_3$, therefore the NMR analyses were performed under these conditions (Fig. 2 and 7 °C).

The decoalesced NMR signals enabled us to assign the NMR data separately to each conformer (**a**, **b** and **c**). The presence of six distinct signals [δ_H 4.46 (br d, H2, **a**), 4.76 (br d, H6, **a**), 4.92 (br d, H6, **c**), 4.99 (br t, H6, **b**), 5.06 (m, H2, **b**), 5.19 (br t, H2, **c**)] in the olefinic region due to the two olefinic protons indicated the existence of three conformational isomers (Table 1). The integral values of these peaks revealed that the populations of the three conformers (**a**, **b** and **c**) were in 44:32:24 ratios at 7 °C in $CDCl_3$. The proton-carbon direct connectivities were established by the HMQC spectrum. There were signals for three oxygen-bearing carbons [C14 (δ_C 80.3), C15 (δ_C 71.7) and C18 (δ_C 67.5) for conformer **a**] in ^{13}C NMR spectrum (Table 2). The HMBC correlation of H18 methylene protons (δ_H 4.19) with two olefinic carbons [C11 (δ_C 144.7), C12 (δ_C 115.4)] and an oxygen-bearing carbon atom [C14 (δ_C 80.3)] and the DQF-COSY correlation of H12–H13–H14 (Fig. 3) suggested the presence of an oxene as a partial structure of **1**. The occurrence of a $(CH_3)_2C(OH)$– group was inferred by HMBC correlations (Fig. 3) between the ^{1}H methyl signals at δ 1.16 (3H, s, H19), δ 1.21 (3H, s, H20) and the quaternary ^{13}C signal at δ 71.7 (C15). The NMR signals of the 10-membered-ring part were distinctive for each of the three conformers, which was extremely helpful to assign the chemical shifts of three conformers (**a**, **b** and **c**). The DQF-COSY correlations of the two olefinic protons (H2 and H6) with their adjacent methylene groups (H1 and H5) and the HMBC

Table 1
^{1}H NMR data of the three conformers of cyclolobatriene (**1**) at 7 °C (600 MHz, $CDCl_3$)

Position	Conformer **a**	Conformer **b**	Conformer **c**
	δ_H (mult., *J* in Hz)	δ_H (mult., *J* in Hz)	δ_H (mult., *J* in Hz)
1	2.01 (m), 2.16 (m)	1.71 (m), 2.19 (m)	1.71 (m), 2.13 (m)
2	4.46 (br d, 10.2)	5.06 (m)	5.19 (br t, 7.6)
4	1.86 (m), 2.17 (m)	2.13 (m)	2.08 (m), 2.17 (m)
5	2.08 (m), 2.23 (m)	2.08 (m), 2.35 (m)	2.08 (m), 2.37 (m)
6	4.76 (br d, 11.2)	4.99 (br t, 7.6)	4.92 (br d, 11.2)
8	2.02 (m), 2.37 (m)	1.78 (m), 2.10 (m)	2.30 (m), 1.55 (m)
9	1.58 (m), 1.73 (m)	1.44 (m), 1.60 (m)	1.58 (m), 1.73 (m)
10	1.82 (m)	1.71 (m)	1.79 (m)
12	5.51 (br s)	5.51 (br s)	5.51 (br s)
13	1.94 (m), 2.12 (m)	1.94 (m), 2.13 (m)	1.94 (m), 2.12 (m)
14	3.26 (m)	3.26 (m)	3.26 (m)
16	1.51 (s)	1.46 (s)	1.47 (s)
17	1.37 (s)	1.53 (s)	1.66 (s)
18	4.19 (m)	4.19 (m)	4.19 (m)
19	1.16 (s)	1.16 (s)	1.16 (s)
20	1.21 (s)	1.21 (s)	1.21 (s)
OH	2.51 (s)	2.51 (s)	2.51 (s)

Table 2
^{13}C NMR chemical shifts of the three conformers of cyclolobatriene (**1**) at 7° C (150 MHz, $CDCl_3$)

Position	Conformer **a**	Conformer **b**	Conformer **c**
1	34.8	32.2	30.1
2	131.4	124.8	126.4
3	129.2	136.2	137.0
4	39.3	36.9	38.6
5	26.7	24.1	24.1
6	126.4	121.7	128.8
7	138.1	138.1	134.5
8	41.6	41.3	34.4
9	33.9	31.8	33.9
10	47.3	44.8	42.5
11	144.7	143.3	142.0
12	115.4	115.5	116.0
13	25.2[a]	25.1[a]	25.1[a]
14	80.3[b]	80.2[b]	80.2[b]
15	71.7	71.7	71.7
16	16.7	15.4	15.4
17	16.2	16.7	22.1
18	67.5	67.5	67.5
19	23.5	23.5	23.5
20	26.1	26.1	26.1

[a,b] Interchangeable within the same sign.

relationship of methyl groups (H16 and H17) with C2, C3, C4, and C6, C7, C8, respectively, enabled us to establish the two partial structures of –CH=C(CH_3)–CH_2– (Fig. 3). These 2D NMR correlations were similar in all three conformers. The structure **1** for cyclolobatriene was finally established by connecting these partial structures to each other by HMBC and HOHAHA spectra.

Attempts to identify the double bond geometries at C2 and C6 using NOESY correlations at 7 °C were unsuccessful due to the overlapping of the correlating signals of the three conformers. However, the *E* geometry of the two trisubstituted olefins at C2 and C6 were determined by partial hydrogenation followed by the NOESY analysis of the two dihydro derivatives **5** and **6** (Fig. 1). The structures of **5** and **6** were confirmed by comparisons of the ^{1}H NMR data with that of **1** (7 °C) and also by analysing the 2D NMR spectra. The broad olefinic signals of H2 and H6 of **1** were replaced by a signal at 5.45 (1H, m, H6) in **5**, and 5.36 (1H, br s, H2) in **6**. Correspondingly, a vinylogous methyl signal was shifted to relatively high field of 0.89 (d, *J* = 6.9 Hz, H16) in **5** and 0.81 (d, *J* = 6.2 Hz, H17) in **6** when compared to **1**. These facts suggest that **5** and **6** are 2,3-dihydrocyclolobatriene and 6,7-dihydrocyclolobatriene, respectively. In compound **5**, the DQF-COSY correlations

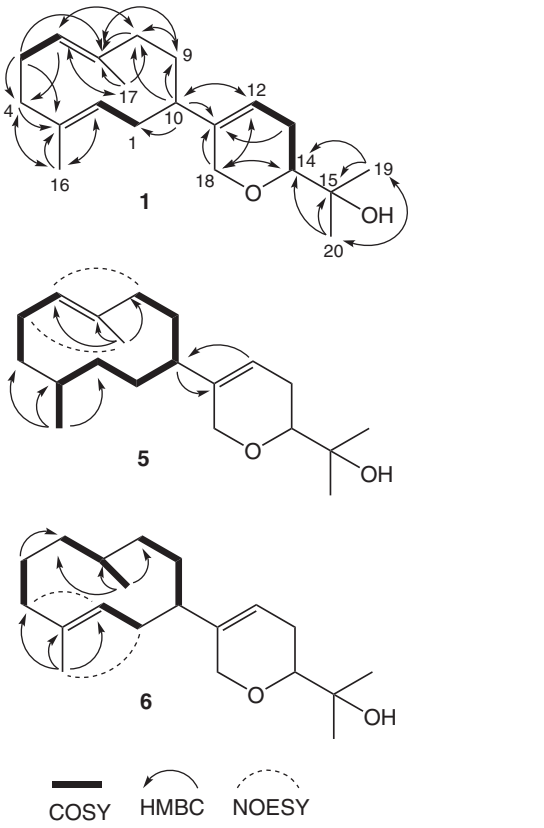

Figure 3. 2D NMR correlations in cyclolobatriene (**1**), 2,3-dihydrocyclolobatriene (**5**) and 6,7-dihydrocyclolobatriene (**6**).

of H16–H3—H2—H1–H10–H9–H8 and H4–H5–H6, and the HMBC correlations of H16—C2, C3, C4; H17–C6, C7, C8; H12–C10; and H10–C11 confirmed the 10-membered-ring structure with a double bond between C6–C7 (Fig. 3). In **6**, the DQF-COSY correlations of H1–H2, H4–H5, H6–H7–H–17, H8–H9–H10 and the HMBC correlations of H16–C2, C3, C4; H17–C6, C7, C8; H5–C6 confirmed the double bond between C2–C3 (Fig. 3). Finally, the NOE correlations shown in Fig. 3 confirmed the *E* geometry of C6–C7 in **5** and C2–C3 in **6**, confirming the structure of **1**. The three dimensional structures of the conformers **a**–**c** are discussed in the last part.

The heat-induced Cope rearrangement of **1** into **2** that occurred during NMR measurement at 70 °C was beneficial in determining its absolute configuration. Since the NMR data and optical rotation ($[\alpha]_D^{26}$ +85) of the isomerised product **2** were in good agreement with the reported data of lobatriene ($[\alpha]_D^{26}$ +86.7)[9], we concluded that the absolute configurations at C-10 and C-14 of **1** are same as that of **2**.

Eunicol (**3**) is another 10-membered-ring compound isolated in the present study. The isolation has been reported previously.[11] In this compound as well, the broadened NMR signals of the three conformers (**a**:**b**:**c** = 44:32:24) decoalesced when temperature decreased to 7 °C as in the case of **1**. This facilitated the complete assignment of the NMR data for the three conformers (see Section 3.2.3), whereas the previous authors reported the NMR data of one major conformer only.[12]

Eunicol (**3**), like **1**, may underwent thermal isomerization to an elemene congener, fuscol (**4**), which was also isolated in this study and identified by comparing the ^{1}H NMR data with published data (see Section 3.2.4). [3,5] The thermal instability of these 10-membered-ring diterpenes (**1** and **3**) towards their corresponding elemene congeners (**2** and **4**) via the Cope rearrangement implies that most of the elemenes or elemene-type diterpenes are probably

artifacts of their precursor germacrenes or germacrene-type 10-membered-ring diterpenes. To best of our knowledge, the present study provided the first complete NMR assignment of all the existing conformers of germacrene-type compounds.

The 10-membered-ring sesquiterpenes have been well studied for their conformational isomers as well as their geometry in transannular reactions through variable-temperature NMR studies, molecular mechanics, NOE spectrums and X-ray analysis.[18–23] Germacrene A and related germacrene-type sesquiterpenes, for example, germacrene B, costunolide, hedycaryol, and dehydrogermacrene A, are known to have the four possible conformers, namely UU, UD, DU and DD, in reference to the up (U) and down (D) orientations of C16 and C17 methyl groups to the plane of the 10-membered-ring (Fig. 4). A high-filed ^{1}H NMR study of germacrene A at variable temperatures was reported and confirmed the presence of three conformational isomers **a**, **b** and **c** in a ratio of 52:29:19 at −20 °C.[24] The major conformer **a** exhibited relatively high-field signals at 4.51 (H2), 4.78 (H6), 1.37 (H17), and 1.52 ppm (H16) (Table 3), as suggested for the UU conformer of hedycaryol and dihydropregeijerene.[23,24] Similarly, the major conformer **a** of cyclolobatriene (**1**) exhibited relatively high-field signals at 4.46 (H2), 4.76 (H6), 1.37 (H17), and 1.51 (H16) (Table 3), suggesting that the major conformer **a** of **1** possesses the UU conformation (Fig. 4). The other two downfield sets of ^{1}H NMR signals of **1** correspond to the less populated conformers **b** (32%) and **c** (24%), which were predicted to have UD or DU conformations. In germacrene A, the second most populated conformer **b** and the third populated **c** exhibit as having UD and DU conformations, respectively.[23] In the present study, the relatively downfield olefinic signals [4.99 (H6), 5.06 (H2)] and methyl singlets [1.46 (H16), 1.53 (H17)] of the conformer **b** of cyclolobtriene (**1**) are in agreement with the UD conformer of germacrene A (Table 3).[23] The NMR data of the third conformer **c** of **1** at 4.92 (H6), 5.19 (H2), 1.47 (H16), and 1.66 (H17) are in parallel to those of the DU conformer of germacrene A (Table 3).[23] These conformational assignments were supported by the NOESY correlations as shown in Figure 4. Likewise, in eunicol (**3**), the comparable NMR chemical shifts (Table 3) and NOESY correlations (Fig. 4) to those of **1** indicated that the three conformers **a**, **b**, and **c** of **3** possess UU, UD, and DU conformations, respectively. Despite the existence of the three conformational isomers for **1** and **3**, they exclusively isomerise to **2** and **4**, respectively, because the Cope rearrangement of germacrene-type compounds is known to proceed through the most stable chair–chair transition state, to which the UU conformer **a** is structurally closest.[19,25]

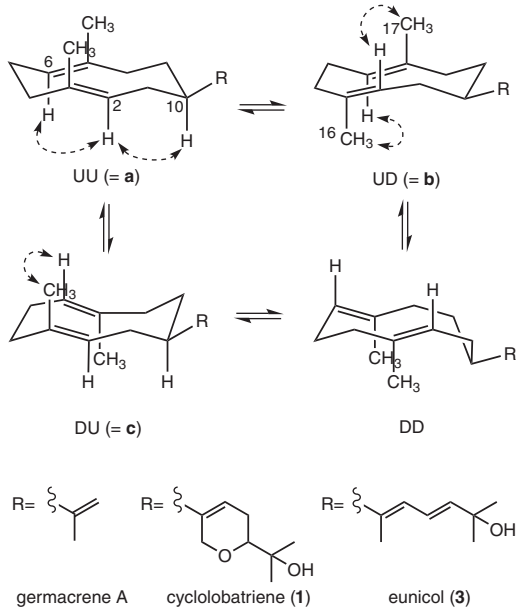

Figure 4. Possible conformations of cyclolobatriene (**1**) and eunicol (**3**) in comparison with those of germacrene A. The conformers are denoted as UU, UD, DU, and DD in reference to the U (up) and D (down) orientations of C16 and C17 methyl groups on the 10-membered ring.[23] The NOESY correlations observed for **1** and **3** are shown with dotted curved arrows.

3. Experimental section

3.1. General experimental procedures

Thin-layer chromatography (TLC) was performed by using precoated silica gel 60 F_{254} plates (Art. 5715, Merck) or ODS RP-18 F_{254} plates (Art. 15389, Merck). Flash column chromatography was performed with an intelligent HPLC pump 880-PU (JASCO Corp.) and a ternary gradient 880-02 unit (JASCO Corp). Silica gel (40 μm) Hi-Flash column (Yamazen Science, Inc) of size L (26 mm i.d. × 100 mm) was used in flash column chromatography. HPLC was performed on a high-pressure gradient system equipped with 980-PU pumps and UV-970 detector or an MD-915 photodiode array UV detector (JASCO Corp.). Recycling system was generated by using a LC-8A pump (Shimadzu Corp.). FT–IR spectrum was recorded on a FT/IR 400 spectrophotometer (JASCO). Mass spectra (MS) were recorded on a Mariner Biospectrometry Workstation (Applied Biosystems, Inc.) in the positive ESI mode. NMR spectra were recorded on an AMX2 600 (600 MHz for ^{1}H) spectrometer (Bruker). The NMR chemical shifts (ppm) were referenced to the solvent peaks of δ_H 7.26 (residual $CHCl_3$) and δ_C 77.0 for $CDCl_3$ solutions. The cytotoxicity assays were carried out on human epidermoid carcinoma A431 cells by a model Zf Coulter counter (Coulter Electronics).[26]

3.2. Isolation of 1–4

The specimens of the soft coral *L. pauciflorum* (wet weight 210 g) were collected by scuba off Taketomijima Island, Okinawa, Japan, and were deposited as KTG-070618-006K in OP Bio Factory. The species was identified by Professor Yehuda Benayahu, Department of Zoology, Tel-Aviv University, Israel. The fresh samples were extracted with MeOH. The MeOH extract was concentrated, and the residue was partitioned between EtOAc and water. The organic layer was concentrated under reduced pressure to yield crude extract (2.259 g). Flash column chromatography of a part of the crude extract (1.023 g) on Silica gel Hi-Flash column (L-size, silica gel 30 g), with linear gradient from 10 to 70% EtOAc (30 min) in hexane gave a total of eight fractions. The fourth fraction (330.1 mg) showed a cytotoxic activity. A portion (20.0 mg) of the fourth fraction was separated by recycling HPLC [ODS YMC-PAK (20 mm i.d. × 250 mm) column, 85% aq MeOH, detected at 205 nm, 2 recycles] to afford **1** (7.8 mg), **2** (1.2 mg), and a mixture of **3** and **4** (1.1 mg). To obtain enough of **3** and **4**, another portion of the fourth fraction (53.6 mg) was purified by the same recycling HPLC (3 recycles) to afford pure **3** (0.5 mg) and **4** (1.8 mg).

3.2.1. Cyclolobatriene (1)

colorless viscous oil; $[\alpha]_D^{26}$ +57° (*c* 0.139, $CHCl_3$); IR (film) 3472, 1098, 1077, 844, 756 cm^{-1}; MS (ESI^+) *m/z* 287.2 $[M-OH]^+$, 327.1 $[M+Na]^+$; HRMS (ESI) calcd. for $C_{20}H_{32}O_2Na$ $[M+Na]^+$ 327.2295, found 327.2292.

3.2.2. Lobatriene (2)

colorless viscous oil; $[\alpha]_D^{26}$ +85.5 (*c* 0.098, $CHCl_3$) (reported data: $[\alpha]_D^{25}$ +86.7 (*c* 0.19, $CHCl_3$))[9]; ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.81 (1H,

Table 3
Selected ^{1}H NMR data (in $CDCl_3$) of conformers **a**, **b** and **c** of germacrene A, cyclolobatriene (**1**) and eunicol (**3**)

Compound	Conformer	H-2	H-6	H-16	H-17
Germacrene A (−20 °C)[23]	**a** (UU)	4.51 (d, 10.0)	4.78 (dd, 11.2, 4.0)	1.52 (s)	1.37 (s)
	b (UD)	5.07 (dd, 9.5, 6.6)	5.01 (t, 8.1)	1.46 (s)	1.54 (s)
	c (DU)	5.21 (t, 7.9)	4.94 (d, 12.1)	1.48 (s)	1.68 (s)
Cyclolobatriene (**1**) (7 °C)	**a** (UU)	4.46 (br d, 10.2)	4.76 (br d, 11.2)	1.51 (s)	1.37 (s)
	b (UD)	5.06 (m)	4.99 (br t, 7.6)	1.46 (s)	1.53 (s)
	c (DU)	5.19 (br t, 7.6)	4.92 (br d, 11.2)	1.47 (s)	1.66 (s)
Eunicol (**3**) (7 °C)	**a** (UU)	4.52 (br d, 7.4)	4.78 (br d, 10.8)	1.52 (s)	1.39 (s)
	b (UD)	5.07 (br d, 7.4)	5.02 (br t, 6.3)	1.47 (s)	1.54 (s)
	c (DU)	5.19 (br s)	4.93 (br d, 11.5)	1.49 (s)	1.62 (s)

dd, *J* = 18.0, 10.2 Hz, H-6), 5.57 (1H, br d, *J* = 4.8 Hz, H-12), 4.90 (1H, d, *J* = 18.0 Hz, H-5b), 4.89 (1H, d, *J* = 10.2 Hz, H-5a), 4.82 (1H, s, H-4b), 4.58 (1H, s, H-4a), 4.20 (2H, m, H-18), 3.26 (1H, dd, *J* = 10.8, 3.0 Hz, H-14), 2.43 (1H, s, OH), 2.15 (1H, m, H-13b), 1.99 (1H, m, H-2), 1.96 (1H, m, H-13a), 1.84 (1H, m, H-10), 1.70 (3H, s, H-16), 1.57 (1H, m, H-9b), 1.55 (2H, m, H-1), 1.50–1.40 (2H, m, H-8), 1.45 (1H, m, H-9a), 1.21 (3H, s, H-19), 1.17 (3H, s, H-20), 0.99 (3H, s, H-17); ^{13}C NMR (150 MHz; $CDCl_3$) δ 150.1 (C-6), 147.5 (C-3), 141.2 (C-11), 116.4 (C-12), 112.2 (C-4), 110.0 (C-5), 80.3 (C-14), 71.7 (C-15), 68.1 (C-18), 52.7 (C-2), 41.7 (C-10), 39.8 (C-8), 39.7 (C-7), 32.8 (C-1), 27.0 (C-9), 26.1 (C-19), 25.3 (C-13), 24.7 (C-16), 23.7 (C-20), 16.6 (C-17); MS (ESI) *m/z* 287.2 $[M-OH]^+$, 327.1 $[M+Na]^+$.

3.2.3. Eunicol (3)

colorless viscous oil; $[\alpha]_D^{29}$ −15 (*c* 0.15, $CHCl_3$) (reported data: $[\alpha]_D^{20}$ −22.8 (*c* 0.00395, CH_2Cl_2))[12]; ^{1}H NMR (600 MHz, $CDCl_3$, 7 °C) conformer **a**: δ 6.44 (1H, dd, *J* = 15.3, 10.9 Hz, H-13), 5.83 (1H, d, *J* = 10.9 Hz, H-12), 5.75 or 5.77 (1H, d, *J* = 15.3 Hz, H-14), 4.78 (1H, br d, *J* = 10.8 Hz, H-6), 4.52 (1H, br d, *J* = 7.4 Hz, H-2), 2.36 (1H, m, H-8b), 2.24 (1H, m, H-5b), 2.18 (1H, m, H-4b), 2.12 (2H, m, H-1), 2.08 (1H, m, H-5a), 2.04 (1H, m, H-8a), 1.97 (1H, m, H-10), 1.87 (1H, m, H-4a), 1.75 or 1.77 (3H, s, H-18), 1.67 (1H, m, H-9b), 1.59 (1H, m, H-9a), 1.52 (3H, s, H-16), 1.39 (3H, s, H-17), 1.35 (6H, s, H-19, 20), conformer **b**: δ 6.44 (1H, dd, *J* = 15.3, 10.9 Hz, H-13), 5.83 (1H, d, *J* = 10.9 Hz, H-12), 5.77 or 5.75 (1H, d, *J* = 15.3 Hz, H-14), 5.07 (1H, br d, *J* = 7.4 Hz, H-2), 5.02 (1H, br t, *J* = 6.3 Hz, H-6), 2.34 (1H, m, H-5b), 2.27 (1H, m, H-8b), 2.12 (1H, m, H-1b), 2.12 (1H, m, H-4b), 2.09 (1H, m, H-5a), 2.06 (1H, m, H-4a), 1.84 (1H, m, H-1a), 1.77 or 1.75 (3H, s, H-18), 1.70 (1H, m, H-10), 1.69 (1H, m, H-8a), 1.59 (1H, m, H-9b), 1.54 (3H, s, H-17), 1.47 (3H, s, H-16), 1.43 (1H, m, H-9a), 1.35 (6H, s, H-19, 20), conformer **c**: δ 6.44 (1H, dd, *J* = 15.3, 10.9 Hz, H-13), 5.83 (1H, d, *J* = 10.9 Hz, H-12), 5.77 or 5.75 (1H, d, *J* = 15.3, H-14), 5.19 (1H, br t, H-2), 4.93 (1H, br d, *J* = 11.5 Hz, H-6), 2.38 (1H, m, H-5b), 2.34 (1H, m, H-8b), 2.17 (1H, m, H-5a), 2.11 (1H, m, H-4b), 2.09 (1H, m, H-8a), 2.06 (1H, m, H-1b), 2.06 (2H, m, H-9), 1.92 (1H, m, H-4a), 1.85 (1H, m, H-1a), 1.77 or 1.75 (3H, s, H-18), 1.62 (3H, s, H-17), 1.49 (3H, s, H-16), 1.35 (6H, s, H-19, 20); ^{13}C NMR (150 MHz, $CDCl_3$, 7 °C) conformer **a**: δ 147.0 (C-11), 139.1 or 138.9 (C-14), 138.9 (C-7), 131.4 (C-2), 129.2 (C-3), 126.3 (C-6), 122.9 (C-13), 121.2 (C-12), 71.0 (C-15), 53.2 (C-10), 41.6 (C-8), 39.4 (C-4), 34.5 (C-1), 33.3 (C-9), 29.82 (C-19 and 20), 26.6 (C-5), 16.7 (C-16), 16.3 (C-17), 14.5 (C-18), conformer **b**: δ 145.5 (C-11), 138.9 or 139.1 (C-14), 138.9 (C-7), 136.2 (C-3), 124.9 (C-2), 122.9 (C-13), 121.6 (C-12), 121.6 (C-6), 71.0 (C-15), 50.1 (C-10), 41.2 (C-8), 37.0 (C-4), 32.4 (C-1), 31.1 (C-9), 29.8 (C-19 and 20), 24.2 (C-5), 16.7 (C-17), 15.45 (C-16), 14.1 (C-18), conformer **c**: δ 144.4 (C-11), 138.9 or 139.1 (C-14), 138.1 (C-3), 135.4 (C-7), 128.8 (C-6), 122.9 (C-13), 126.3 (C-2), 121.8 (C-12), 71.0 (C-15), 48.1 (C-10), 38.8 (C-4), 34.2 (C-8), 30.2 (C-1), 29.8 (C-19 and 20) 26.6 (C-9), 24.2 (C-5), 22.1 (C-17), 15.5 (C-16), 14.1 (C-18); MS (ESI) *m/z* 271.2 $[M-OH]^+$.

3.2.4. Fuscol (4)

colorless oil; $[\alpha]_D^{29}$ +14 (*c* 0.099, $CHCl_3$) (reported data: $[\alpha]_D$ +16.3)[3]; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.49 (1H, dd, *J* = 15.2, 11.0 Hz, H-13), 5.87 (1H, d, *J* = 11.0 Hz, H-12), 5.82 (1H, dd, *J* = 16.8, 11.6 Hz, H-6), 5.76 (1H, d, *J* = 15.2 Hz, H-14), 4.91 (1H, d, *J* = 16.8 Hz, H-5b), 4.90 (1H, d, *J* = 11.6 Hz, H-5a), 4.81 (1H, s, H-4b), 4.58 (1H, s, H-4a), 2.00 (2H, m, H-2, 10), 1.79 (3H, s, H-18), 1.70 (3H, s, H-16), 1.35 (6H, s, H-19, 20), 1.6–1.2 (6H, m, H-1, 8, 9), 1.00 (3H, s, H-17); MS (ESI) *m/z* 271.2 $[M-OH]^+$.

3.3. Hydrogenation of cyclolobatriene (1)

Compound **1** (4.6 mg) was hydrogenated with PtO_2 (2 mg) in EtOH (2 mL) for 2 h. The reaction mixture was filtered through a cotton plug, and the filtrate was concentrated. The crude material was subjected to HPLC [Develosil ODS-UG-5 (10 mm i.d. × 250 mm), 90% aq MeOH, 4 mL/min, detected at 205 nm] to give two dihydro derivatives **5** (0.7 mg, t_R = 15.1 min) and **6** (0.5 mg, t_R = 15.9 min).

3.3.1. 2,3-Dihydrocyclolobatriene (5)

Colorless oil; IR (film) 3469, 1164, 1124, 1098, 1073 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.49 (1H, d, *J* = 4.8 Hz, H-12), 5.45 (1H, m, H-6), 4.18 (1H, d, *J* = 15.0 Hz, H-18b), 4.13 (1H, d, *J* = 15.0 Hz, H-18a) 3.25 (1H, dd, *J* = 10.8, 3.6 Hz, H-14), 2.42 (1H, br s, OH), 2.20 (1H, m, H-5b), 2.19 (1H, m, H-8b), 2.11 (1H, m, H-13b), 1.97 (1H, m, H-8a), 1.95 (1H, m, H-5a), 1.92 (1H, m, H-13a), 1.86 (1H, br s, H-10), 1.67 (3H, s, H-17), 1.56 (1H, m, H-3), 1.51 (1H, m, H-9b), 1.44 (1H, m, H-9a),1.37 (1H, m, H-4b), 1.31 (1H, br s, H-4a), 1.21 (3H, s, H-20), 1.22 (2H, m, H-1), 1.24 (1H, m, H-2b), 1.16 (3H, s, H-19), 1.02 (1H, br s, H-2a), 0.89 (3H, d, *J* = 6.9 Hz, H-16); ^{13}C NMR (150 MHz; $CDCl_3$) δ 141.5 (C-11), 133.8 (C-7), 126.4 (C-6), 116.9 (C-12), 80.4 (C-14), 71.7 (C-15), 67.9 (C-18), 41.2 (br, C-10), 39.8 (C-8), 34.9(C-2), 34.0 (C-4), 31.2 (C-9), 28.7 (C-1 and 3), 26.2 (C-20), 25.6 (C-5), 25.4 (C-13), 23.8 (C-16), 23.7 (C-19), 16.9 (C-17); HRMS (ESI) calcd for $C_{20}H_{34}O_2Na$ $[M+Na]^+$ 329.2451, found 329.2480.

3.3.2. 6,7-Dihydrocyclolobatriene (6)

Colorless oil; IR (film) 3464, 1163, 1129, 1098, 1076 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.52 (1H, d, *J* = 4.2 Hz, H-12), 5.36 (1H, br s, H-2), 4.22 (1H, d, *J* = 15.6 Hz, H-18b), 4.17 (1H, d, *J* = 15.6 Hz, H-18a) 3.27 (1H, dd, *J* = 11.1, 3.0 Hz, H-14), 2.42 (1H, s, OH), 2.17 (1H, m, H-1b), 2.14 (1H, m, H-13b), 2.13 (1H, m, H-4b), 2.00 (1H, m, H-4a), 1.94 (1H, m, H-13a), 1.90 (2H, m, H-1a and H-10), 1.64 (3H, s, H-16), 1.61 (1H, m, H-9b), 1.60 (1H, m, H-5b), 1.38 (1H, m, H-9a), 1.36 (1H, s, H-5a), 1.34 (1H, s, H-6b), 1.26 (1H, m, H-7), 1.21 (3H, s, H-20), 1.19 (1H, m, H-8b), 1.17 (3H, s, H-19), 1.09

(1H, m, H-8a), 0.91 (1H, m, H-6a), 0.81 (3H, d, J = 6.2 Hz, H-17); ^{13}C NMR (150 MHz; $CDCl_3$) δ 142.8 (C-11), 135.0 (C-3), 125.0 (C-2), 116.0 (C-12), 80.4 (C-14), 71.7 (C-15), 67.9 (C-18), 42.0 (C-10), 40.0 (C-4), 36.6 (C-8), 32.1 (C-6), 31.5 (C-1), 29.5 (C-7), 28.5 (C-9), 26.1 (C-20), 25.3 (C-13), 23.7 (C-19), 22.6 (C-5), 22.0 (C-17), 16.5 (C-16); HRMS (ESI) calcd for $C_{20}H_{34}O_2Na$ $[M+Na]^+$ 329.2451, found 329.2453.

3.4. Thermal isomerisation of cyclolobatriene (1) into lobatriene (2)

Compound **1** (4.3 mg) was partially isomerised to **2** while measuring NMR at 70 °C overnight (13 h) in deuterated DMSO (0.7 mL). The resultant mixture was purified by recycling HPLC [ODS YMC-PAK (20 mm i.d. × 250 mm) column, 85% aq MeOH, detected at 205 nm, 2 recycles] to afford **1** (0.6 mg) and **2** (1.8 mg). Lobatriene (**2**): $[\alpha]_D^{26}$ +85 (*c* 0.099, $CHCl_3$) (ref. data:[9] $[\alpha]_D^{26}$ +86.7 (*c* 0.19, $CHCl_3$).

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