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Eremophilane-type sesquiterpenes from cultured lichen mycobionts of *Sarcographa tricosa*

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This paper is dedicated to the memory of Prof. Dr. Meinhart H. Zenk.

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ABSTRACT

Spore-derived mycobionts of the crustose lichen *Sarcographa tricosa* were cultivated on a malt-yeast extract medium supplemented with 10% sucrose. Chemical investigation of the cultivated colonies led to isolation of three eremophilane-type sesquiterpenes, 3-*epi*-petasol (**1**), dihydropetasol (**2**) and sarcographol (**3**), together with six known eremophilanes and ergosterol peroxide. These structures were elucidated by spectroscopic and chemical methods. This is the first report of eremophilane-type sesquiterpenes from the cultured mycobionts of lichen.

1. Introduction

Lichens are symbiotic organisms of fungi (mycobionts) and photoautotrophic algal partners, namely, green algae and/or cyanobacteria. About 18,500 different lichen taxa have been described worldwide. Lichens have adapted to extreme ecological conditions, being dominant at high altitudes and in Arctic boreal and tropical habitats. Vietnam has a tropical monsoon climate that is favorable for diverse tropical lichens, but previous studies on the Vietnamese lichens focused mainly on their taxonomy and not on their chemical constituents (Aptroot and Sparrius, 2006). Lichens produce many unique compounds, which are considered to have important biological and ecological functions, such as antimicrobial activity (Ahmadjian, 1993; Huneck, 1999, 2001). Most of these metabolites are produced by the fungal partner, in symbiosis or in the aposymbiotic state. Cultures of isolated lichen mycobionts, however, often exhibit the ability under osmotically stressed conditions to produce substances that have never been detected in the lichenized state (Miyagawa et al., 1994; Tanahashi et al., 1997; Takenaka et al., 2003, 2010). From our interest in Vietnamese lichens and the metabolic ability of isolated lichen mycobionts, the spore-derived mycobionts of the crustose lichen *Sarcographa tricosa* collected in Vietnam were cultured and three new eremophilane-type sesquiterpenes together with seven known compounds were isolated. In this paper, the isolation and the structural determination of the new sesquiterpenes are described. Detailed spectroscopic data and further studies on the absolute stereochemistry of two known sesquiterpenes are also reported.

2. Results and discussion

Specimens of *S. tricosa* (Ach.) Müll. Arg. were collected from tree bark in Dong Nai Province, Vietnam, in 2008. The polyspore-derived mycobionts were cultivated on a malt-yeast extract medium supplemented with 10% sucrose at 18 °C in the dark. After several months, the cultures were harvested and extracted with *n*-hexane and Et_2O. These extracts were separated by chromatographic procedures to afford three new eremophilane-type sesquiterpenes **1**–**3** together with six known eremophilanes **4**–**9** and ergosterol peroxide (**10**) (Fig. 1).

Among the isolated compounds from the culture, petasol (**4**) and dihydrosporogen-AO 1 (**5**) were the major products. Petasol (**4**) was first isolated from *Petasites fragrans* in its free form (Sugama et al., 1983), although its esters such as petasin (**11**) and S-petasin (**12**) have since been isolated. Since alkaline hydrolysis of the esters failed to yield petasol (**4**), but afforded isopetasol (**9**), the absolute stereostructure was investigated on its esters but not on petasol (**4**) itself (Aebi and Djerassi, 1959; Herbst and Djerassi, 1960; Neuenschwander et al., 1979). Dihydrosporogen-AO 1 (**5**) was isolated from the culture broth of fungus *Alternaria citri*. Its absolute configuration was determined previously by the CD spectrum of its dibenzoate derivative (Kono et al., 1989). As the ^{1}H NMR spectroscopic data of **4** and **5** have not been fully

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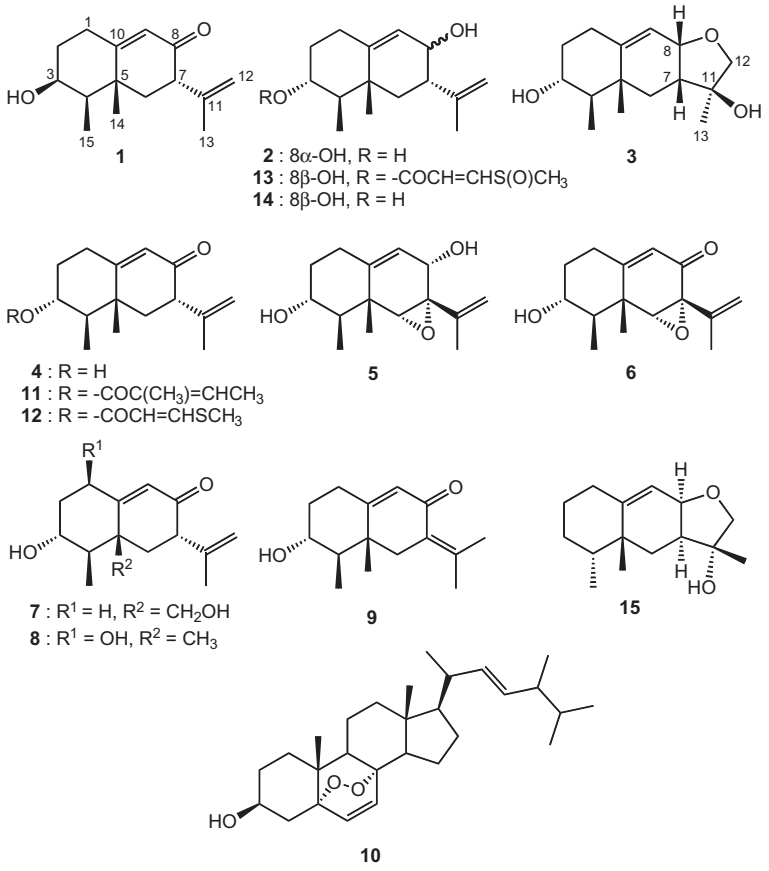
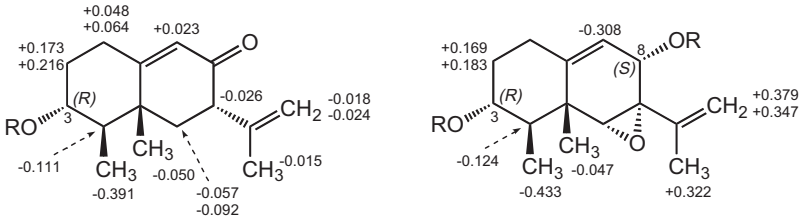


Fig. 1. Structures of isolated compounds **1**–**10** and related compounds.

reported, a detailed spectroscopic analysis including 2D-NMR experiments was performed to assign all proton signals of both compounds and to confirm their relative configurations. Furthermore, the absolute stereochemistry of **4** and **5** was re-examined by the ^{1}H NMR analyses on the corresponding methylphenylacetic acid (MPA) esters (Latypov et al., 1996). Preparation of the (*R*)- and (*S*)-MPA esters **4a** and **4b** from petasol (**4**) and **5a** and **5b** from dihydrosporogen-AO 1 (**5**) led us to determine that **4** and **5** possessed 3*R* and 3*R*, 8*S* configurations, respectively (Fig. 2). Consequently, the stereochemistry of petasol (**4**) was confirmed as 3*R*, 4*R*, 5*R*, 7*S*, and the absolute configuration of dihydrosporogen-AO 1 (**5**) was established as 3*R*, 4*R*, 5*R*, 6*R*, 7*S*, 8*S*.

The other known compounds were identified as sporogen-AO 1 (**6**) (Tirilly et al., 1983; Tanaka et al., 1984), JBIR-27 (**7**) (Motohashi et al., 2009), 1β-hydroxypetasol (**8**) (Sugawara et al., 1993), isopetasol (**9**) (Neuenschwander et al., 1979), and ergosterol peroxide (**10**) (Fisch et al., 1973; Takaishi et al., 1991; Sgarbi et al., 1997) by comparison of their physical and spectroscopic ($[\alpha]_D$, UV, IR, NMR and MS) data with reported information. A detailed analysis of the 2D-NMR spectra of **8** has shown that the literature assignment resonances of C-13, C-14 and C-15 (Sugawara et al., 1993) must be interchanged.

Compound **1** was obtained as colorless needles and the molecular formula was established as $C_{15}H_{22}O_2$ by HR-ESIMS. Its UV spectrum showed a maximum at 240 nm, and its IR spectrum exhibited absorption bands at 3479 and 1657 cm^{-1}, indicating the presence of hydroxyl and α,β-unsaturated carbonyl groups. The ^{1}H NMR spectrum of **1** showed signals for three methyls at δ 1.11 (d, J = 7.0 Hz), 1.39 (s), and 1.74 (br s), one olefinic proton at δ 5.80 (d, J = 1.5 Hz), two vinyl protons at δ 4.82 (br s) and 4.98 (quint, J = 1.5 Hz), three methines at δ 1.52 (qd, J = 7.0, 2.5 Hz), 3.17 (dd, J = 14.0, 4.5 Hz) and 3.95 (q, J = 2.5 Hz), and three sets of methylene protons at δ 1.72–2.83 (Table 1). Analysis of the ^{13}C NMR and DEPT spectra of **1** indicated 15 carbon signals due to



4a : R = (*R*)-MPA
4b : R = (*S*)-MPA

5a : R = (*R*)-MPA
5b : R = (*S*)-MPA

Fig. 2. Values of Δ δ_{RS} (δ_R–δ_S) obtained from **4a**/**4b** and **5a**/**5b**.

Table 1
^{1}H NMR spectroscopic data for **1**–**4** and **14** ($CDCl_3$, 500 MHz).

H	**1**		**2**		**3**		**4**		**14**	
1ax	2.83	tdd (14.5, 5.0, 1.5)	2.29	tdt (14.5, 4.5, 1.5)	2.29	tdt (14.5, 4.5, 2.0)	2.45	tdd (15.0, 4.5, 2.0)	2.26	tdt (14.0, 4.5, 1.5)
1eq	2.14	ddd (14.0, 4.0, 2.5)	2.17	ddd (14.5, 5.0, 3.0)	2.23	ddd (14.5, 4.5, 3.0)	2.34	ddd (15.0, 4.5, 2.5)	2.14	ddd (14.0, 5.0, 2.5)
2ax	1.72	tdd (14.5, 4.0, 2.5)	1.36	dddd (14.5, 12.0, 11.0, 5.0)	1.36	m	1.45	dddd (15.0, 12.0, 11.0, 2.5)	1.30	m
2eq	2.03	ddt (14.5, 5.0, 2.5)	2.07	dtd (12.0, 4.5, 3.0)	2.03	dtd (12.0, 4.5, 3.0)	2.16	dtd (12.0, 4.5, 2.5)	2.08	dtd (12.0, 4.5, 2.0)
3	3.95	q (2.5)	3.54	td (11.0, 4.5)	3.53	td (11.0, 4.5)	3.62	td (11.0, 4.5)	3.53	td (10.5, 4.5)
4	1.52	qd (7.0, 2.5)	1.25	m	1.18	dq (11.0, 6.5)	1.34	dq (11.0, 6.5)	1.14	dq (10.5, 6.5)
6ax	1.83	br t (13.5)	1.57	m	0.92	t (13.5)	1.88	t (14.0)	1.32	br t (13.0)
6eq	1.97	dd (13.0, 4.5)	1.60	m	1.76	dd (13.5, 5.0)	2.02	dd (14.0, 4.5)	1.68	dd (13.0, 3.0)
7	3.17	dd (14.0, 4.5)	2.25	br dt (11.5, 3.5)	2.00	dt (13.5, 5.0)	3.11	dd (14.0, 4.5)	2.19	ddd (13.0, 9.5, 3.0)
8			4.09	br	4.52	td (5.0, 2.0)			4.08	br d (9.5)
9	5.80	d (1.5)	5.63	dd (5.5, 1.5)	5.59	dd (5.0, 2.0)	5.78	d (2.0)	5.39	t (1.5)
12	4.82	br s	4.83	br s	3.73	d (10.0)	4.82	br s	4.89	br s
	4.98	quint (1.5)	5.03	br s	3.83	d (10.0)	4.98	quint (1.5)	4.92	quint (1.5)
13	1.74	br s	1.84	br s	1.34	s	1.74	br s	1.74	dd (1.5, 1.0)
14	1.39	s	0.96	s	0.93	s	1.18	s	1.02	d (1.0)
15	1.11	d (7.0)	1.06	d (6.5)	1.06	d (6.5)	1.08	d (6.5)	1.00	d (6.5)

^{1}H–^{1}H coupling constants (Hz) are in parentheses.

three methyls, three sp^3 methylenes, one sp^2 methylene, three sp^3 methines, one sp^2 methine and four quaternary carbons, including two olefinic and one carbonyl carbons (Table 2). These spectroscopic features of **1** closely resembled those of petasol (**4**) (Sugama et al., 1983). The only marked difference in their ^{1}H NMR spectra was that the oxygenated methine proton signal of **1** was observed at δ 3.95 as a quartet (J = 2.5 Hz) instead of a triplet of doublets (δ 3.62, J = 11.0, 4.5 Hz) as seen in petasol (**4**). The ^{1}H–^{1}H COSY and HMBC experiments indicated that compound **1** possessed the same eremophilane backbone as petasol (**4**) and differed from **4** only in configuration at C-3. The detailed inspection of the coupling constants enabled assignment of the signals for methylene protons at δ 1.72, 1.83, 1.97, 2.03, 2.14 and 2.83 to H-2ax, H-6ax, H-6eq, H-2eq, H-1eq and H-1ax, respectively. The NOESY correlations observed between H-1ax/H_3-14, H_3-14/H_3-15, H-4/H-6ax, H-6eq/H_3-14, H-6eq/H_3-15 and H-7/H_3-14 indicated the β-orientation of H-1ax, H-6eq, H-7, H_3-14 and H_3-15 and the α-orientation of H-4, H-6ax, the isopropenyl group as in petasol (**4**) (Fig. 3). The ^{1}H–^{1}H coupling constant (J = 2.5 Hz) of H-3 with H-2ax, H-2eq and H-4 implied an equatorial orientation of H-3 and thus an axial orientation of the hydroxyl group on the upward face of the ring system. Accordingly, compound **1** was formulated as shown and designated as 3-*epi*-petasol.

Compound **2** was isolated as a colorless crystalline solid. The HR-ESIMS established a molecular formula of $C_{15}H_{24}O_2$, that is, two mass units more than that of petasol (**4**). The IR spectrum showed absorption bands at 3341, 1450, 1022 and 884 cm^{-1}, and the UV spectrum exhibited an end absorption, indicating the presence of hydroxyl group(s) but the absence of an α,β-unsaturated carbonyl group. The ^{1}H and ^{13}C NMR spectra of **2** (Tables 1 and 2) together with DEPT and HMBC experiments suggested that the structure of **2** was closely related to **4**. Differences between two isolates were ascribable to the absence of the carbonyl carbon and the presence of an additional oxygenated methine [δ_H 4.09 and δ_C 63.6] in **2**. These findings implied that the carbonyl group in **4** was reduced to a hydroxyl group in **2**. The substitution of the hydroxyl group at C-8 was evidenced from the COSY correlations between H-7 (δ 2.25) and an oxygenated methine at δ 4.09 (H-8), and between H-8 and an olefinic proton at δ 5.63 (H-9), as well as HMBC correlation from H-9 to an oxygenated methine carbon at δ_C 63.6 (C-8) (Fig. 4).

Table 2
^{13}C NMR spectroscopic data for **1**–**5** and **14** ($CDCl_3$, 125 MHz).

C	**1**	**2**	**3**	**4**	**5**	**14**
1	27.3	30.5	30.7	31.0	30.2	30.3
2	33.8	36.2	35.7	35.1	35.7	36.6
3	71.4	71.9	72.0	71.0	71.9	72.0
4	46.4	50.8	50.1	50.2	43.6	50.8
5	39.4	39.3	38.4	39.8	38.8	39.1
6	42.2	35.4	36.2	41.7	67.9	41.3
7	50.5	42.0	44.9	50.3	65.6	47.3
8	199.0	63.6	74.4	198.8	65.3	68.7
9	124.1	121.0	118.6	124.4	119.5	123.1
10	170.2	147.9	148.0	167.9	140.4	146.3
11	143.7	146.3	81.9	143.4	142.1	144.6
12	114.3	111.9	77.9	114.4	113.4	112.6
13	20.0	22.8	20.4	20.0	19.6	19.4
14	18.9	18.1	18.1	17.3	17.6	18.8
15	11.9	10.8	11.1	10.4	11.3	10.4

The relative configuration of **2** was deduced from the coupling constants and NOESY correlations. The NOESY cross-peaks observed between H-1ax/H_3-14, H-3/H_3-14, and H_3-14/H-7 indicated that H-1ax, H-3, H_3-14 and H-7 were axially oriented on the same face on the ring system. The coupling constants (J = 11.0 or 11.5 Hz) of H-3/H-2ax, H-3/H-4 and H-6ax/H-7 implied a *trans*-diaxial relationship between each pair of protons and indicated that compound **2** had the same configurations at C-3, C-4, C-5 and C-7 as petasol (**4**). The coupling constant (J = 3.5 Hz) of H-7/H-8 was different from that of petasinol (**13**) (J = 9.6 Hz) (Lin et al., 1998), indicating a *cis* relationship of H-7 and H-8 and furthermore a quasi-equatorial orientation of H-8 (Fig. 4). Finally, the absolute stereochemistry of **2** was determined by its chemical correlation with petasol (**4**). Reduction of petasol (**4**) with $NaBH_4$-$CeCl_3$ (Luche, 1978) yielded **2** as a minor product along with its 8-epimer **14** (Fig. 5), whose structure was confirmed by spectroscopic means and comparison with the reported data for petasinol (**13**) (Lin et al., 1998). Thus, the structure of **2** was established as shown and designated dihydropetasol.

Compound **3**, named sarcographol, was obtained as a crystalline solid, which was shown to have a molecular formula of $C_{15}H_{24}O_3$ by HR-ESIMS. Its IR spectrum exhibited absorption bands at 3441, 3375 and 1659 cm^{-1}, characteristic of a hydroxyl group(s) and a double bond. The ^{1}H and ^{13}C NMR spectra of **3** (Tables 1 and 2) closely resembled those of **2** except that the double bond C-11/C-12 was replaced by an oxygenated quaternary carbon at δ_C 81.9 (C-11) and an oxygenated methylene at δ_H 3.73 and 3.83 (each d, J = 10.0 Hz, H_2-12), and δ_C 77.9 (C-12). The HMBC correlations from H-7 (δ_H 2.00) to C-11, from H_2-12 to C-7 (δ_C 44.9), C-8 (δ_C 74.4) and C-11, and from H_3-13 (δ_H 1.34) to C-7, C-11 and C-12 suggested that an ether linkage between C-8 and C-12 forms a tetrahydrofuran ring with methyl and hydroxyl groups at C-11 as in cyclodebneyol (**15**) (Burden et al., 1986). Furthermore, the

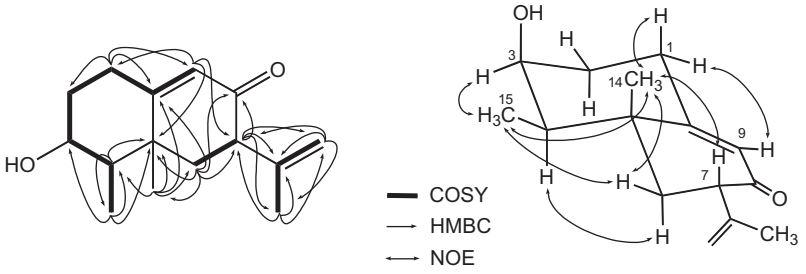


Fig. 3. Key COSY, HMBC and NOESY correlations of **1**.

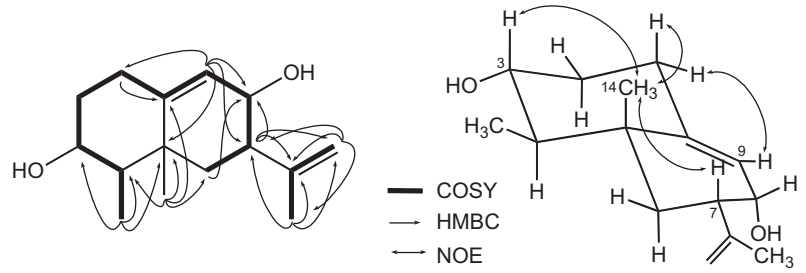


Fig. 4. Key COSY, HMBC and NOESY correlations of **2**.

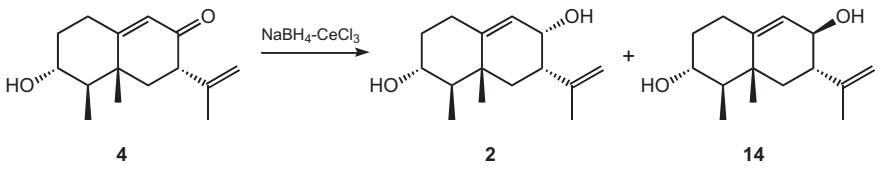


Fig. 5. Reduction of **4** to **2** and **14**.

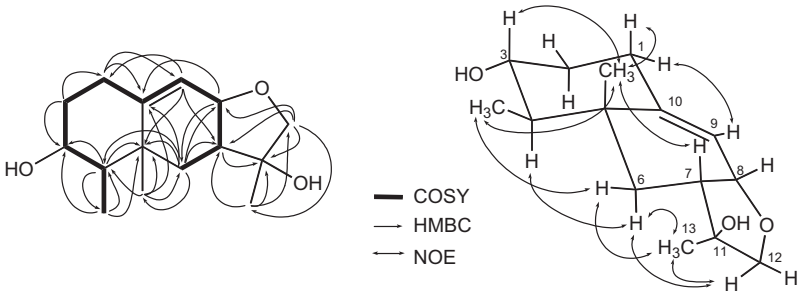


Fig. 6. Key COSY, HMBC and NOESY correlations of **3**.

molecular formula of **3** and chemical shifts of ^{13}C NMR spectrum indicated the location of a hydroxyl group at C-3.

The relative configuration of **3** could be deduced from analysis of ^{1}H–^{1}H coupling constants and NOESY correlations (Fig. 6). The coupling constants $J_{2ax,3}$ (11.0 Hz), $J_{3,4}$ (11.0 Hz) and $J_{6ax,7}$ (13.5 Hz) as well as NOESY correlations of H-1ax/H_3-14, H-3/H_3-14, H_3-14/H_3-15 and H-7/H_3-14 indicated that the relative configurations at C-3, C-4, C-5 and C-7 of **3** were consistent with those of **2**. Moreover, from the coupling constants $J_{7,8}$ (5.0 Hz) and $J_{8,9}$ (5.0 Hz), and the magnitudes of allylic coupling $J_{9,1ax}$ (2.0 Hz) and homoallylic coupling $J_{8,1ax}$ (2.0 Hz), the dihedral angles between H-1ax and the C1-C10-C9 plane (Garbisch, 1964), and between H-8 and the plane formed by C8-C9-C10 (Barfield and Sternhell, 1972), were estimated as ca. 90° and ca. 140°, respectively. These findings established a quasi-equatorial orientation of H-8 and a *cis*-fused tetrahydrofuran ring. Furthermore, the NOESY spectrum showed significant cross peaks of H-6eq/H_3-13 and H-6ax/H_3-13, demonstrating that H_3-13 was in close proximity to H_2-6, hence the configuration of C-11 was assigned as *S**. Accordingly, the structure of sarcographol was thereby elucidated as **3**.

The absolute stereochemistry of **1** and **3** could not be chemically determined owing to the minute amount of the isolated compounds. Compounds **1**–**9** isolated in this study could be expected to have a close biosynthetic relationship. Compounds **1**–**3** were different from **4** only in the oxidation pattern. Since the absolute configuration of **4** was determined, it is reasonable to assign the same absolute configurations for the chiral centers C-4, C-5 and C-7 within molecules **1**–**4** on the basis of biogenetic considerations.

3. Concluding remarks

Diverse eremophilane-type sesquiterpenes have been isolated from plants (Lin et al., 1998; Liu et al., 2008; Sugama et al., 1983) and fungi (Tanaka et al., 1984; Tirilly et al., 1983; Kono et al., 1989; Sugawara et al., 1993; Motohashi et al., 2009), and exhibited a variety of biological activities, such as anti-HIV (Singh et al., 1999), antibacterial (Mohamed and Ahmed, 2005), and cytotoxic activities (Beattie et al., 2011; Liu et al., 2008; Motohashi et al., 2009). This type of sesquiterpenes has never been isolated from lichen thalli or lichen mycobionts. This investigation of cultured lichen mycobionts of *S. tricosa* yielded three new eremophilane-type sesquiterpenes **1**–**3** along with six known eremophilanes **4**–**9** and ergosterol peroxide (**10**). This is the first instance of isolation of eremophilane-type sesquiterpenes from lichen mycobionts in culture. It is also noteworthy that the metabolic ability was expressed only in the isolated mycobionts.

4. Experimental

4.1. General

Melting points were measured on a Yanaco micro melting point apparatus and are uncorrected. UV spectra were recorded on a Shimadzu UV-240 spectrophotometer and IR spectra were obtained with a Shimadzu FTIR-8200 infrared spectrophotometer. Optical rotations were measured on a Jasco DIP-370 digital polarimeter. HR-EIMS data were obtained with a Hitachi M-4100 mass spectrometer. The NMR experiments were performed with Varian VXR-500, Varian UNITY INOVA and Gemini-300 spectrometers, with TMS as internal standard. HPLC was performed using a Waters system (600E Multisolvent Delivery System, 2487 Dual λ Absorbance Detector). Silica gel 60 (Merck) was used for column chromatography (CC). Thin-layer chromatography (TLC) was performed on precoated Kieselgel 60F_{254} plates (Merck) and spots were visualized under UV light or sprayed with phosphomolybdic acid solution (H_2O 500 ml, H_3PO_4 85% 7.5 ml, concentrated H_2SO_4 25 ml, $H_3(PMo_{12}O_{40})\cdot nH_2O$ 12.1 g), then heated.

4.2. Plant material

Specimens of *S. tricosa* (Ach.) Müll. Arg. were collected from tree bark in Ma Da forest, Dong Nai Province, Vietnam (94 m alt.), in September 2008 by D.H.L. The voucher specimens were identified by Prof. H. Miyawaki, Saga University, Japan, and was deposited in Saga University, Japan (registration No. LHD068). Mycobionts were obtained from the spores discharged from apothecia of a thallus, and were cultivated in test tubes containing modified MY10 medium (malt extract 10 g, yeast extract 4 g, sucrose 100 g, agar 15 g, H_2O 1 l, pH 7) at 18 °C in the dark. After cultivation for 4–8 months, the colonies were harvested.

4.3. Extraction and isolation

The harvested colonies (285 test tubes, dry weight 185.5 g) were extracted with *n*-hexane (2 × 800 ml) and Et_2O (3 × 400 ml) at room temperature, and the combined extracts were concentrated under reduced pressure to give *n*-hexane (0.23 g) and Et_2O (0.92 g) extracts, respectively. The *n*-hexane extract was separated by preparative TLC (Et_2O-EtOAc, 10:1; toluene-EtOAc, 6:4, 1:1 or 1:2) and by preparative HPLC (Sunfire, *n*-hexane-2-propanol, 9:1), giving **1** (1.2 mg), **4** (49.3 mg), **5** (12.6 mg), **6** (9.3 mg, colorless oil, $[\alpha]_D^{22}$ + 221 (*c* 0.80, MeOH)), **10** (30.3 mg, colorless crystalline solid, mp. 174–175 °C ($CHCl_3$); $[\alpha]_D^{22}$ − 34.0 (*c* 1.07, $CHCl_3$)) and fatty compounds (58.2 mg).

The Et_2O extract was subjected to silica gel CC and eluted by the solvent system *n*-hexane-Et_2O with increasing Et_2O ratios to obtain four fractions, fr-I (0–10% Et_2O, 170 mg), fr-II (20–30% Et_2O, 135 mg), fr-III (50–70% Et_2O, 284 mg) and fr-IV (70–100% Et_2O, 245 mg). Fr-I was a mixture of fatty compounds (168.9 mg). Fr-II was purified by preparative TLC (toluene–EtOAc, 3:2, 1:1), giving **1** (2.1 mg) and **10** (10.5 mg). Fr-III was separated by preparative TLC (toluene–EtOAc, 1:1, 1:2) to yield **4** (83.7 mg), **5** (119.8 mg) and **9** (8.0 mg, colorless crystalline solid, mp. 120–121 °C (*n*-hexane-$CHCl_3$); $[\alpha]_D^{20}$ + 106 (*c* 0.25, $CHCl_3$)). Purification of fr-IV by preparative TLC (*n*-hexane-EtOAc, 1:4; toluene-EtOAc-AcOH, 5:5:0.2) afforded **2** (6.9 mg), **3** (2.6 mg), **7** (2.3 mg, colorless solid, $[\alpha]_D^{22}$ + 166 (*c* 0.13, MeOH)) and **8** (4.9 mg).

4.3.1. 3-Epi-petasol (**1**)

Colorless needles, mp. 130–131 °C (*n*-hexane-$CHCl_3$); $[\alpha]_D^{23}$ + 114 (*c* 0.17, $CHCl_3$); UV λ_{max}^{EtOH} nm (log ε): 240 (4.02); IR ν_{max}^{KBr} cm^{-1}: 3479, 2937, 1657, 1444, 1250, 1208, 949, 900; for ^1H NMR and ^{13}C NMR spectroscopic data, see Tables 1 and 2; HR-ESIMS *m/z*: 235.1691 (calcd for $C_{15}H_{23}O_2$: 235.1699 $[M+H]^+$).

4.3.2. Dihydropetasol (**2**)

Colorless crystalline solid, mp. 138–140 °C (*n*-hexane-$CHCl_3$); $[\alpha]_D^{21}$ + 208 (*c* 0.38, $CHCl_3$); IR ν_{max}^{KBr} cm^{-1}: 3341, 2934, 1450, 1022, 884; for ^1H NMR and ^{13}C NMR spectroscopic data, see Tables 1 and 2; HR-ESIMS *m/z*: 259.1669 (calcd for $C_{15}H_{24}O_2Na$: 259.1675 $[M+Na]^+$).

4.3.3. Sarcographol (**3**)

Colorless crystalline solid, mp. 224–225 °C (*n*-hexane-$CHCl_3$); $[\alpha]_D^{23}$ + 102 (*c* 0.11, $CHCl_3$); IR ν_{max}^{KBr} cm^{-1}: 3441, 3375, 2953, 2878, 1659, 1443, 1378, 1262, 1120, 1036, 1012, 918, 881; for ^1H NMR and ^{13}C NMR spectroscopic data, see Tables 1 and 2; HR-ESIMS *m/z*: 253.1800 (calcd for $C_{15}H_{25}O_3$: 253.1805 $[M+H]^+$).

4.3.4. Petasol (**4**)

Colorless oil; $[\alpha]_D^{20}$ + 130 (*c* 0.82, $CHCl_3$); UV λ_{max}^{EtOH} nm (log ε): 236.5 (4.12); IR ν_{max}^{KBr} cm^{-1}: 3433, 2971, 2942, 1669, 1625, 1444, 1374, 1330, 1252, 1216, 1195, 1070, 1038, 898; for ^1H NMR and ^{13}C NMR spectroscopic data, see Tables 1 and 2; NOESY: H-1ax/H-3, H-1ax/H_3-14, H-1eq/H-9, H-3/H_3-14, H-4/H-6ax, H-6eq/H_3-15, H-7/H_3-14, H_3-14/H_3-15; HMBC: H_2-1 → C-2, 5, 9; H_2-2 → C-3; H-4 → C-3, 14, 15; H_2-6 → C-4, 5, 7, 8, 10, 11, 14; H-7 → C-6, 8, 11, 12, 13; H-9 → C-1, 5, 7; H_2-12 → C-7, 11, 13; H_3-13 → C-7, 11, 12; H_3-14 → C-4, 5, 6, 10; H_3-15 → C-3, 4, 5; HR-ESIMS *m/z*: 235.1695 (calcd for $C_{15}H_{23}O_2$: 235.1699 $[M+H]^+$).

4.3.5. Dihydrosporogen-AO 1 (**5**)

Colorless needles, mp. 124–125 °C (*n*-hexane-CH_2Cl_2); $[\alpha]_D^{24}$ + 104 (*c* 0.84, MeOH); IR ν_{max}^{KBr} cm^{-1}: 3348, 2968, 2935, 2872, 1675, 1648, 1455, 1380, 1264, 1138, 1037, 958, 917, 882, 841; ^1H NMR ($CDCl_3$, 500 MHz): δ 1.01 (3H, s, H_3-14), 1.16 (3H, d, *J* = 6.5 Hz, H_3-15), 1.33 (1H, dddd, *J* = 14.0, 11.5, 10.5, 5.0 Hz, H-2ax), 1.62 (1H, dq, *J* = 10.5, 6.5 Hz, H-4), 1.87 (3H, br s, H_3-13), 2.04 (1H, dtd, *J* = 11.5, 5.0, 2.5 Hz, H-2eq), 2.17 (1H, ddd, *J* = 14.0, 5.0, 2.5 Hz, H-1eq), 2.29 (1H, tddd, *J* = 14.0, 5.0, 3.0, 2.0 Hz, H-1ax), 3.08 (1H, s, H-6), 3.52 (1H, td, *J* = 10.5, 5.0 Hz, H-3), 4.52 (1H, t, *J* = 3.0 Hz, H-8), 5.06 (1H, quint, *J* = 1.5 Hz, H-12), 5.17 (1H, br s, H-12), 5.25 (1H, br t, *J* = 2.0 Hz, H-9); ^{13}C NMR: Table 2; NOESY: H-1ax/H_3-14, H-1eq/H-9, H-3/H_3-14, H-3/H_3-15, H-6/H_3-14, H-6/H_3-15, H-8/H_3-13, H_3-14/H_3-15; HMBC: H_2-1 → C-2; H_2-2 → C-3; H-4 → C-3, 5, 14, 15; H-6 → C-5, 7, 10, 11, 14; H_2-12 → C-7, 11, 13; H_3-13 → C-7, 11, 12; H_3-14 → C-4, 5, 6, 10; H_3-15 → C-3, 4, 5; HR-ESIMS *m/z*: 273.1463 (calcd for $C_{15}H_{22}O_3Na$: 273.1468 $[M+Na]^+$).

*4.3.6. 1β-Hydroxypetasol (**8**)*

Colorless oil. $[\alpha]_D^{23}$ + 47.7 (*c* 0.42, MeOH). UV λ_{max}^{EtOH} nm (log ε): 233.0 (3.98); IR ν_{max}^{KBr} cm^{-1}: 3401, 2946, 1665, 1028. ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 1.11 (3H, d, *J* = 7.0 Hz, H_3-15), 1.35 (1H, m, H-4), 1.38 (3H, s, H_3-14), 1.65 (1H, ddd, *J* = 14.5, 11.0, 3.0 Hz, H-2ax), 1.73 (3H, dd, *J* = 1.0, 0.5 Hz, H_3-13), 1.92 (1H, t, *J* = 14.0 Hz, H-6ax), 2.03 (1H, dd, *J* = 13.0, 5.0 Hz, H-6eq), 2.35 (1H, ddd, *J* = 14.5, 4.5, 3.0 Hz, H-2eq), 3.22 (1H, dd, *J* = 14.0, 5.0 Hz, H-7), 4.03 (1H, td, *J* = 11.0, 4.5 Hz, H-3), 4.48 (1H, t, *J* = 3.0 Hz, H-1), 4.84 (1H, br t, *J* = 1.0 Hz, H-12), 5.00 (1H, quint, *J* = 1.0 Hz, H-12), 5.87 (1H, s, H-9). ^{13}C-NMR ($CDCl_3$, 125 MHz): δ 10.4 (C-15), 19.5 (C-14), 20.0 (C-13), 39.2 (C-5), 41.6 (C-2), 43.1 (C-6), 50.0 (C-4), 50.8 (C-7), 67.2 (C-3), 73.7 (C-1), 114.6 (C-12), 126.8 (C-9), 143.1 (C-11), 165.4 (C-10), 199.5 (C-8). HR-ESIMS *m/z*: 251.1643 (calcd for $C_{15}H_{23}O_3$: 251.1648 $[M+H]^+$).

*4.4. Preparation of (R)- and (S)-MPA esters of **4***

To a solution of **4** (6.9 mg) in dry CH_2Cl_2 (1 ml) were added (*R*)-MPA (24.5 mg), *N*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide (39.6 mg) and a catalytic amount of 4-pyrrolidinopyridine, and the mixture was stirred at room temperature for 15 h. The reaction mixture was poured into 1 N HCl (10 ml) and extracted with $CHCl_3$ (3 × 10 ml). The $CHCl_3$ layer was dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by preparative TLC ($CHCl_3$–EtOAc, 9:1) to yield **4a** (6.4 mg). Compound **4** (8.4 mg) was treated with (*S*)-MPA (29.8 mg) as described above to yield **4b** (8.6 mg). **4a**: ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.504 (3H, d, *J* = 6.9 Hz, H_3-15), 1.149 (3H, s, H_3-14), 1.478 (1H, m, H-2ax), 1.490 (1H, m, H-4), 1.712 (3H, br s, H_3-13), 1.809 (1H, t, *J* = 13.5 Hz, H-6ax), 1.930 (1H, dd, *J* = 13.5, 4.8 Hz, H-6eq), 2.194 (1H, dtd, *J* = 12.3, 4.5, 2.4 Hz, H-2eq), 2.335 (1H, ddd, *J* = 15.3, 4.2, 2.4 Hz, H-1eq), 2.484 (1H, tdd, *J* = 15.3, 4.5, 1.5 Hz, H-1ax), 3.068 (1H, dd, *J* = 13.5, 4.8 Hz, H-7), 3.410 (3H, s, MPA-OC$\underline{H_3}$), 4.744 (1H, s, MPA-C$\underline{H}$), 4.787 (1H, br s, H-12), 4.859 (1H, td, *J* = 10.8, 4.5 Hz, H-3), 4.966 (1H, quint, *J* = 1.5 Hz, H-12), 5.764 (1H, d, *J* = 1.5 Hz, H-9), 7.329–7.454 (5H, m, MPA-$\underline{Ph}$); HR-ESIMS *m/z*: 383.2220 (calcd for $C_{24}H_{31}O_4$: 383.2224 $[M+H]^+$). **4b**: ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.895 (3H, d, *J* = 6.9 Hz, H_3-15), 1.199 (3H, s, H_3-14), 1.306 (1H, m, H-2ax), 1.601 (1H, dq, *J* = 10.8, 4.5 Hz, H-4), 1.727 (3H, br s, H_3-13), 1.866 (1H, t, *J* = 14.1 Hz, H-6ax), 1.978 (1H, m, H-2eq), 2.022 (1H, dd, *J* = 14.1, 4.8 Hz, H-6eq), 2.271 (1H, ddd, *J* = 15.0, 4.5, 2.7 Hz, H-1eq), 2.436 (1H, tdd, *J* = 15.0, 4.8, 1.5 Hz, H-1ax), 3.093 (1H, dd, *J* = 14.1, 4.8 Hz, H-7), 3.425 (3H, s, MPA-OC$\underline{H_3}$), 4.773 (1H, s, MPA-C$\underline{H}$), 4.811 (1H, br s, H-12), 4.914 (1H, td, *J* = 10.8, 4.5 Hz, H-3), 4.984 (1H, quint, *J* = 1.0 Hz, H-12), 5.741 (1H, d, *J* = 1.5 Hz, H-9), 7.198–7.448 (5H, m, MPA-$\underline{Ph}$); HR-ESIMS *m/z*: 383.2219 (calcd for $C_{24}H_{31}O_4$: 383.2224 $[M+H]^+$).

*4.5. Preparation of (R)- and (S)-MPA esters of **5***

Compound **5** (5.5 mg) was treated with (*R*)-MPA (36.5 mg) as described above and the crude product was purified by preparative TLC (*n*-hexane–Et_2O–AcOH, 3:7:0.5) to yield **5a** (10.7 mg). Treatment of **5** (5.8 mg) with (*S*)-MPA (38.5 mg) followed by preparative TLC gave **5b** (11.7 mg). **5a**: ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.530 (3H, d, *J* = 6.6 Hz, H_3-15), 0.966 (3H, s, H_3-14), 1.360 (1H, m, H-2ax), 1.782 (3H, br s, H_3-13), 1.816 (1H, dq, *J* = 11.1, 6.6 Hz, H-4), 2.005 (1H, m, H-2eq), 2.086 (1H, br dt, *J* = 14.4, 4.2 Hz, H-1eq), 2.247 (1H, br tt, *J* = 14.4, 4.2 Hz, H-1ax), 2.862 (1H, s, H-6), 3.392, 3.398 (each 3H, s, 3-MPA-OC$\underline{H_3}$ and 8-MPA-OC$\underline{H_3}$), 4.721, 4.801 (each 1H, s, 3-MPA-C$\underline{H}$ and 8-MPA-C$\underline{H}$), 4.757 (1H, td, *J* = 11.1, 4.5 Hz, H-3), 4.848 (1H, t, *J* = 2.1 Hz, H-9), 4.899 (1H, quint, *J* = 1.5 Hz, H-12), 5.056 (1H, br s, H-12), 5.953 (1H, t, *J* = 2.1 Hz, H-8), 7.280–7.481 (10H, m, 3-MPA-$\underline{Ph}$ and 8-MPA-$\underline{Ph}$); HR-ESIMS *m/z*: 569.2510 (calcd for $C_{33}H_{38}O_7Na$: 569.2517 $[M+Na]^+$). **5b**: ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.962 (3H, d, *J* = 6.6 Hz, H_3-15), 1.013 (3H, s, H_3-14), 1.192 (1H, m, H-2ax), 1.460 (3H, br s, H_3-13), 1.822 (1H, m, H-2eq), 1.940 (1H, dq, *J* = 10.5, 6.6 Hz, H-4), 2.109 (1H, br d, *J* = 14.4 Hz, H-1eq), 2.255 (1H, br t, *J* = 14.4 Hz, H-1ax), 2.894 (1H, s, H-6), 3.401, 3.415 (each 3H, s, 3-MPA-OC$\underline{H_3}$ and 8-MPA-OC$\underline{H_3}$) 4.552, 4.677 (each 1H, br s, H_2-12), 4.751, 4.816 (each 1H, s, 3-MPA-C$\underline{H}$ and 8-MPA-C$\underline{H}$), 4.805 (1H, td, *J* = 10.5, 4.5 Hz, H-3), 5.156 (1H, br s, H-9), 5.830 (1H, br t, *J* = 2.4 Hz, H-8), 7.285–7.440 (10H, m, 3-MPA-$\underline{Ph}$ and 8-MPA-$\underline{Ph}$); HR-ESIMS *m/z*: 569.2512 (calcd for $C_{33}H_{38}O_7Na$: 569.2517 $[M+Na]^+$).

*4.6. Reduction of **4***

To a solution of **4** (7.5 mg) in MeOH (0.4 ml) was added $CeCl_3{\cdot}7H_2O$ (12.0 mg) and then $NaBH_4$ (1.2 mg), and the mixture was stirred at room temperature for 1 h. The reaction mixture was diluted with H_2O and extracted with $CHCl_3$ (3 × 10 ml). The $CHCl_3$ layer was dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was subjected to preparative TLC (*n*-hexane–Et_2O–EtOAc, 1:1:0.3) to give **2** (0.2 mg), **14** (2.8 mg) and **4** (1.3 mg). Compound **2** was identified with the isolated compound from the culture (^{1}H NMR, $[\alpha]_D$). **14** (8-*epi*-dihydropetasol): Colorless crystalline solid, mp. 126–127 °C ($CHCl_3$); $[\alpha]_D^{26}$ − 36 (*c* 0.26, $CHCl_3$); for ^{1}H NMR and ^{13}C NMR spectroscopic data, see Tables 1 and 2; NOESY: H-1ax/H_3-14, H-1eq/H-9, H-3/H_3-14, H-4/H-6ax, H-6eq/H_3-15, H-7/H_3-14; HMBC: H_2-1 → C-2, 5, 9, 10; H_2-2 → C-3; H-4 → C-3, 5, 15; H_2-6 → C-4, 5, 7, 8, 11, 14; H-7 → C-8; H-9 → C-1, 5, 7; H_2-12 → C-7, 11, 13; H_3-13 → C-7, 11, 12; H_3-14 → C-4, 5, 6, 10; H_3-15 → C-3, 4, 5; HR-ESIMS *m/z*: 259.1663 (calcd for $C_{15}H_{24}O_2Na$: 259.1675 $[M+Na]^+$).

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