PERGAMON

Phytochemistry 52 (1999) 105–112

PHYTOCHEMISTRY

Terpenoid constituents of the liverwort *Reboulia hemisphaerica*

Masao Toyota, Mutsumi Konoshima, Asakawa Yoshinori*

Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Yamashiro-cho, Tokushima, 770-8514, Japan

Received 27 January 1999; accepted 17 February 1999

Abstract

Four new sesquiterpenes and nine known compounds were isolated from the ether extract of the liverwort *Reboulia hemisphaerica*. Their structures were established by spectral and chemical evidence. The structures of the new compounds were shown to be gymnomitr-8(12)-en-4-one, *ent*-arist-9-en-8α-ol, 6β,10β-epoxycupar-3-ene and 3(15)-thujopsen-10α-ol.

Keywords: *Reboulia hemisphaerica*; Liverwort; Aytoniaceae; Hepaticae; Sesquiterpenoids; *Ent*-aristolane; Gymnomitrane; Nardosinane

1. Introduction

The liverwort *Reboulia hemisphaerica* which belongs to the subfamily Reboulioideae, family Aytoniaceae grows on rocks or soil and is a rich source of terpenoids and bis-bibenzyls such as aristolane, nardosinane, cadinane, cyclomyltaylane, cuparane and gymnomitrane-type sesquiterpenoids, hopane-type triterpenoids, and riccardine- and marchantin-type bis-bibenzyls (Asakawa, 1995; Morais et al., 1988; Hashimoto et al., 1993; Wei et al., 1995). Further fractionation of the ether extract of this liverwort resulted in the isolation of four new sesquiterpenoids, one of which was biogenetically related to nardosinane-type sesquiterpenoids. Here we report on the isolation and structural determination of these sesquiterpenoids.

2. Results and discussion

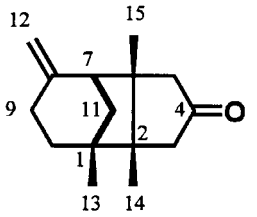
Four collections (Herbarium specimen No. 97001, 97017, 97030 and 97063) grown at different locations were extracted with ether, respectively. The ether extract of *R. hemisphaerica* (No. 97001) showed a major constituent as blue coloration band on TLC. The major constituent was estimated at least at ca 20% constitution of the total extract of this species. The extract was repeatedly chromatographed on silica gel, Sephadex LH-20 and further purified by HPLC on a normal phase column to give a new sesquiterpenoid, gymnomitr-8(12)-en-4-one (**1**), together with the known compounds, 1(10),8-aristoladiene (**2**) (Vidari et al., 1998; Asakawa et al., 1980), rebouliadienol (**3**) (=rulepidanol) (Asakawa, 1995; Hashimoto et al., 1993; Vidari et al., 1998), marchantin O (**4**) (Wei et al., 1995), and zeorin (**5**) (Toyota and Asakawa, 1993; Wenkert et al., 1978). However, the major constituent could not be isolated from this extract. While the isolation procedure of the major constituent was progressing, it was observed that the major constituent was completely converted to 1(10),8-aristoladiene (**2**) on silica gel.

The ^{1}H NMR spectrum of **1** which was first isolated from the present species, showed three tertiary methyl groups (at δ 0.91, 1.07 and 1.19) and two proton signals at δ 4.65 and 4.68 due to an exocyclic methylene. The ^{13}C NMR spectrum exhibited 15 carbons including two sp^2 carbons and carbonyl group at δ 221.4. No further oxygenated carbon signal was observed in its ^{13}C NMR spectrum. The FT-IR spectrum indicated the presence of a five membered ring ketone group at 1732 cm^{-1}. The EI-mass spectrum of **1** showed a molecular ion peak at m/z 218. Accordingly, **1** has 4

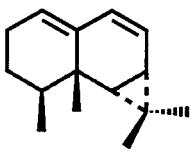
* Corresponding author.

0031-9422/99/$ - see front matter
PII: S0031-9422(99)00137-5

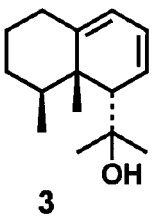
degrees of unsaturation. Resonance of the diagnostic signal at δ 2.35 (*d*, J = 5 Hz) in the ^{1}H NMR of **1** is characteristic to H-7 of gymnomitrane type sesquiterpenoids (Connolly et al., 1974) which have been isolated from many species of liverworts (Asakawa, 1995). The gross structure of **1** was proved by extensive 2-D NMR experiments involving the determination of its COSY, HMQC and HMBC spectra. The stereochemical assignments at the centers C-1, C-2, C-6 and C-7 of **1** were inferred from a 2-D NMR NOESY experiment (Fig. 1).



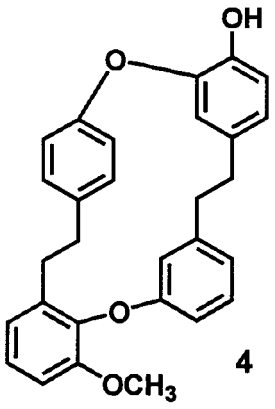
1



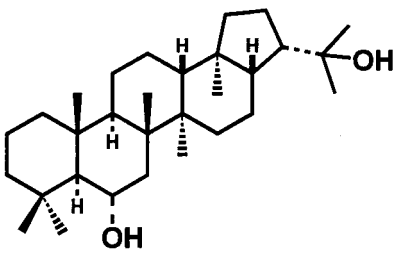
2



3



4



5

It showed cross peaks between (i) H-7 and H-15, (ii) H-15 and H-14, and (iii) H-14 and H-13, indicating that they are on the same side of the molecule. Finally, the absolute configuration of **1** was determined by its CD spectrum. The octant projection of **1** presumed appearance a weak positive Cotton effect in the CD spectrum as shown in Fig. 2. Actually the Cotton

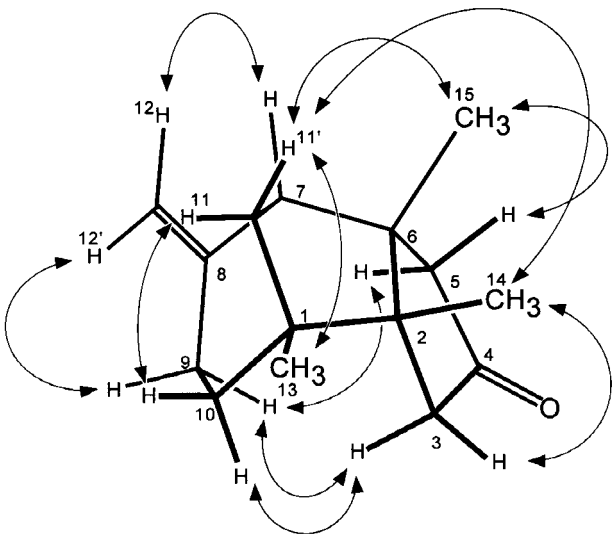
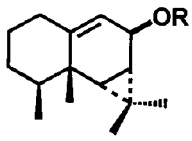
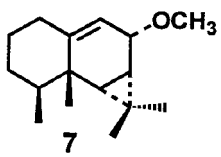


Fig. 1. The arrows display NOE correlations of compound **1**.

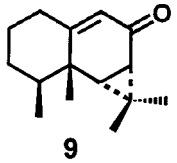
effect at 312 nm ($\Delta\epsilon$ +0.07) was observed in its CD spectrum. The absolute configuration



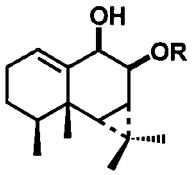
6 R=CH_3
8 R=H



7



9



10 R=H
11 R=Bz-dma

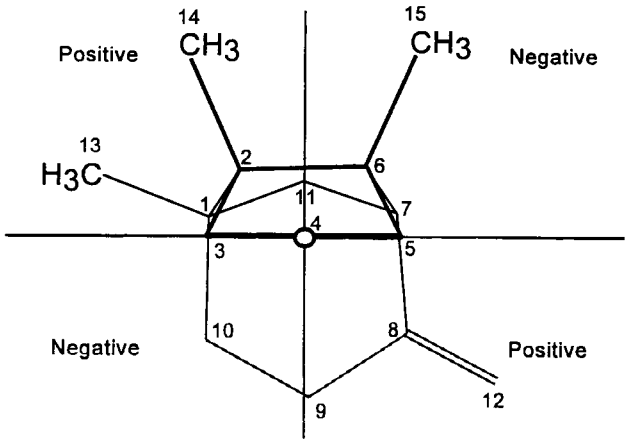


Fig. 2. The octant project of **1**.

of **1** was identical to those of gymnomitrane-type sesquiterpenoids obtained from many other liverworts. Accordingly, the structure of **1** is gymnomitr-8(12)-en-4-one.

In order to isolate the major constituent of this species, another ether extract of *R. hemisphaerica* (No. 97017) was directly chromatographed on Sephadex LH-20 using CH_2Cl_2–CH_3OH (1:1) to yield 8β-methoxyaristol-9-ene (**6**) and 8α-methoxyaristol-9-ene (**7**). Compounds **6** and **7** changed slowly to 1(10),8-aristoladiene (**2**) on silica gel. The structures of **6** and **7** were established by 2D-NMR spectra. Compounds **6** and **7** might be artefacts, since both were absent in the extract before the isolation procedure. This was apparent from the comparative TLC analysis. Again, the major constituent has not been isolated from this extract. Finally, the ether extract of *R. hemisphaerica* (No. 97030) afforded on flash chromatography on silica gel the major compound **8**. The ^{1}H NMR showed signals for three tertiary methyl groups at δ 0.93, 1.02 and 1.28, a secondary methyl group at δ 0.91 (*d*, J = 6.8 Hz), a carbinyl proton at δ 4.52 (*br s*), an olefinic proton at δ 5.30 (*br s*) and cyclopropane ring protons at δ 0.74 (*d*, J = 10 Hz) and 1.13 (*m*). While its structural elucidation was in progress, almost all of the compound decomposed. However, PDC oxidation of the remaining product **8** afforded an α,β-unsaturated ketone **9** whose spectral data were identical to those of aristolone, except for the sign of the optical rotation. It is apparent that the structure of **8** is *ent*-arist-9-en-8-ol. In order to confirm the absolute configuration of **8**, further experiments were performed as follows. Catalytic asymmetric dihydroxylation (Sharpless et al., 1992) of **2**, which was produced during the isolation procedure of the major constituent **8** on silica gel chromatography, gave a diol **10**. Esterification of **10** with *p*-dimethylaminobenzoic acid afforded a benzoate **11** whose CD spectrum showed a positive Cotton effect at 305 nm ($\Delta\epsilon$ +1.08). It was strongly suggested by the homoallyl benzoate rule (Fig. 3) (Humpf et al., 1995; Manabe et al., 1985; Nishino et al., 1984) that the absolute configuration of **8** is opposite to that of aristolone found in higher plants. The axial configuration of C-8 was tentatively assigned, since compound **8** is easily dehydrated.

The ether extract of *R. hemisphaerica* (No. 97063) was chromatographed on silica gel to give two new sesquiterpenoids, 6β,10β-epoxycupar-3-ene (**12**) and 3(15)-thujopsen-10α-ol (**13**), in addition to the known sesquiterpenoids, (−)-cyclopropanecuparenol (**14**) (Asakawa, 1995; Asakawa et al., 1984; Toyota, 1987), rebouliadienol (**3**) (=rulepidanol), gymnomitrol (**15**) (Toyota et al., 1988), (+)-gymnomitr-8(12)-en-9α-ol (**16**) (Morais et al., 1988), marchantin C (**17**) and marchantinquinone (**18**) (Wei et al., 1995).

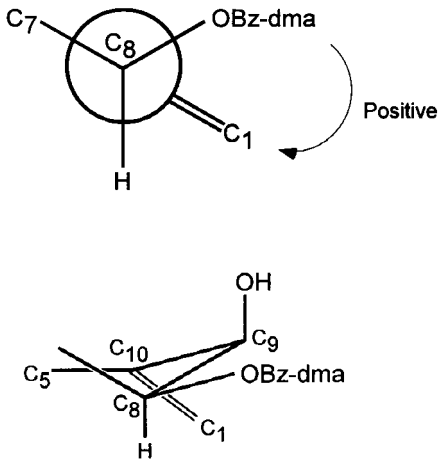
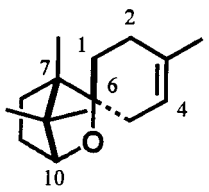
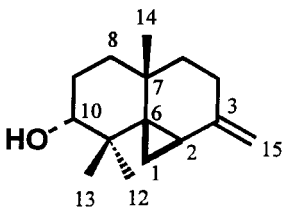


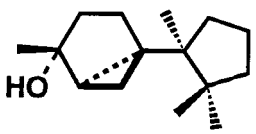
Fig. 3. The Newman's projection formula of **11**.



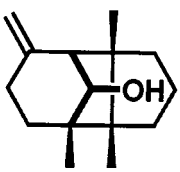
12



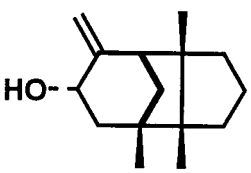
13



14



15



16

The EI-mass spectrum of **12** showed a molecular ion peak at m/z 220. The ^{1}H and ^{13}C NMR spectral data indicated the presence of three tertiary methyl groups [at δ_H 0.86, 0.93 and 1.16], a vinyl methyl group [at δ_H 1.69 and 5.40; δ_C 23.2, 119.5 (*d*) and 134.8 (*s*)] and two oxygenated carbons [at δ_C 80.9 (*s*) and 84.1 (*d*); δ_H 3.60]. Since the IR spectrum of **12** showed no absorption band for a hydroxyl and carbonyl group, it

was inferred that the two oxygenated carbons were assigned to an ether or endoperoxide linkage. Further confirmation of the presence of the ether linkage was provided by a negative colour test for peroxides (Knappe and Peteri, 1962; Abraham et al., 1957). The absence of a fragment peak at m/z 187 (M^+-33) in the EI-mass spectrum of **12** provided further evidence for the absence of the endoperoxide linkage (Mruzek et al., 1987). The DEPT spectra of **12** indicated the presence of 24 protons and 15 carbons. The HREI-mass spectra exhibited a molecular formula $C_{15}H_{24}O$, confirming 4 degrees of unsaturation. The above spectral data showed that **12** was a tricyclic compound. Analysis of the HMQC and HMBC spectra (summarized in Table 1) supported the structural assignment. In particular, the long range ^1H–^{13}C correlation of C-6 with H-1, H-2, H-5, H-8, H-10 and H-14, indicated the positioning of the ether linkage between at C-6 and C-10. Furthermore, the 2D NOESY spectrum provided evidence for the relative stereochemistry of **12** as shown in Fig. 4. Accordingly, the structure of **12** is 6β,10β-epoxycupar-3-ene.

The FT-IR spectrum of **13** showed the absorption band for a hydroxyl group at 3362 cm^{-1}. The presence of a secondary hydroxyl group was apparent from the resonance at δ 3.95 in ^1H NMR spectrum of **13**. The ^{13}C NMR spectrum showed 15 carbons, including the carbinyl carbon at δ 80.4 (d). The ^1H NMR spectrum of **13** gave signals for three tertiary methyl groups (at δ 0.91, 1.00 and 1.02), an exocyclic methylene group (at δ 4.62 and 4.81) and a cyclopropane ring (at δ 0.72, 1.22 and 1.38). The EI-mass spectrum of **13** showed a molecular ion peak at m/z 220. The high-resolution measurement of the molecular ion peak confirmed the formula as $C_{15}H_{24}O$. Therefore compound

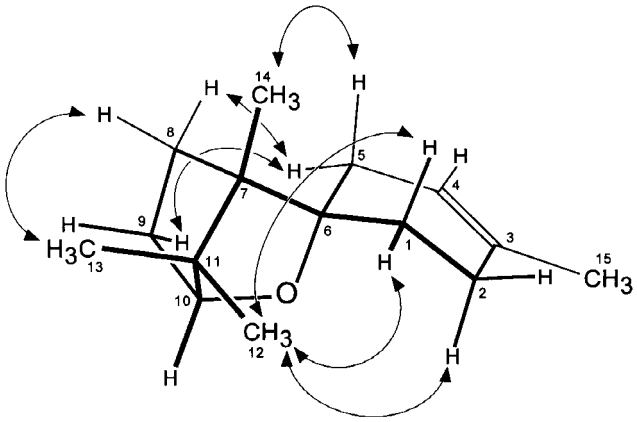


Fig. 4. The arrows display NOE correlations of compound **12**.

13 is a tricarbocyclic compound. Acetylation of **13** gave a monoacetate whose FT-IR spectrum showed the presence of an acetyl group at 1728 and 1246 cm^{-1}, and exhibited no further absorption band for a hydroxyl group. The 2D-NMR experiments involving the determination of ^1H–^1H COSY and HMQC spectra of **13** were effective for complete assignments (Table 2) of all carbons and protons, and the carbon–carbon connectivities of the molecule of **13** were established by HMBC spectrum. The relative configuration was shown to be **13** in figure, since a cross peak between (i) H-12 and H-1α and 2, (ii) H-13 and H-10 and (iii) H-14 and H-10 was observed in its NOESY spectrum.

Recently, Garlaschelli et al. reported the isolation of **2** and **3** from the mushroom *Russula lepida* (Vidari et al., 1998). On the other hand, our previous work on the chemical constituents of the liverwort *R. hemisphaerica* resulted in the isolation of **2** and **3** (Asakawa, 1995; Hashimoto et al., 1993). The absolute configuration of **2** and nardosinane-type sesquiterpe-

Table 1
^1H–^{13}C long range correlations for compound **12**

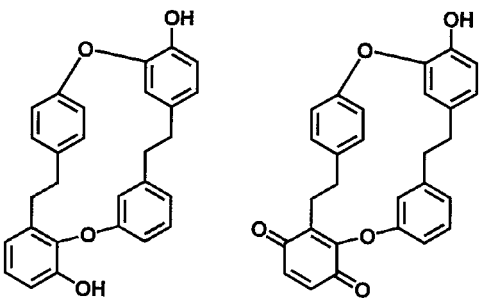
^1H	^{13}C
H-1ax	C-2, 5, 6
H-1eq	C-2, 5, 6
H-2eq	C-1, 4, 6, 15
H-2ax	C-4
H-4	C-2, 5, 6, 15
H-5eq	C-1, 4, 6
H-5ax	C-1, 4
H-8β	C-6, 7, 9, 14
H-8α	C-6, 7, 9, 10, 11
H-9α	C-7, 8, 10, 11
H-9β	C-7, 8, 10
H-10β	C-6, 7, 8, 9
H-12	C-6, 7, 10, 11, 13
H-13	C-7, 10, 11, 12
H-14	C-6, 7, 8, 11
H-15	C-2, 4

Table 2
^1H–^{13}C long range correlations for compound **13**

^1H	^{13}C
H-1β	C-2, 3, 6
H-1α	C-2, 3, 6, 7
H-2	C-3, 4, 6
H-4β	C-3, 5, 7, 15
H-4α	C-2, 3, 5, 7, 15
H-5α	C-3, 4, 7
H-5β	C-4, 7
H-8α	C-6, 7, 9, 10, 14
H-8β	C-6, 7, 9, 10, 14
H-9β	C-8, 10
H-9α	C-8, 10, 11
H-10	C-11, 12, 13
H-15	C-2, 4
H-15′	C-2, 4

noid **3** and its biogenetically related constituent **8** (Fig. 5) found in the liverworts was identical to those found in the mushroom, although both showed opposite configuration to aristolane-type sesquiterpenoids found in higher plants. It is expected to detect *ent*-aristol-9-en-8α-ol (**8**) in the mushroom *Russula lepida* also. The absolute configuration of α-zeorin, $[\alpha]_D$ +70.5 (monoacetate; $CHCl_3$; *c* 2.86) found in the present species was confirmed to be identical to that found in fungi by the ^1H NMR analysis of its (+)- and (−)-MTPA esters (Kusumi et al., 1988).

There are two chemical races in *R. hemisphaerica*. Material grown in Japan produces mainly aristolane-type sesquiterpenoids, while European material elaborates gymnomitrane and cuparane-type sesquiterpenoids and no aristolane-type sesquiterpenoids (Morais et al., 1988). All the present species contained mainly *ent*-aristol-9-en-8α-ol (**8**), whose constitution was estimated at least at ca 20% by TLC analysis of each crude extracts. Some specimens produce the gymnomitrane and nardosinane-type sesquiterpenoids, **1** and **3** (specimen No. 97001), and gymnomitrane, cuparane and thujopsane-type sesquiterpenoids, **15**, **16**, **12**, **14** and **13** (No. 97063) as minor constituents, respectively. Since bis-bibenzyls have been isolated from many species of Marchantiaceae, the occurrence of **4**, **17** and **18** in *R. hemisphaerica* suggests that Aytoniaceae have closer affinities to the Marchantiaceae.



17 **18**

3. Experimental

3.1. General

TLC was carried out on silica gel precoated glass plates with *n*-hexane–EtOAc (1:1 and 4:1). Detection was with Godin reagent (Godin, 1954). For normal phase column chromatography (CC), silica gel 60 (40–63 μm) was used. The mix. of CH_2Cl_2–MeOH (1:1) was used for CC on Sephadex LH-20 as solvent.

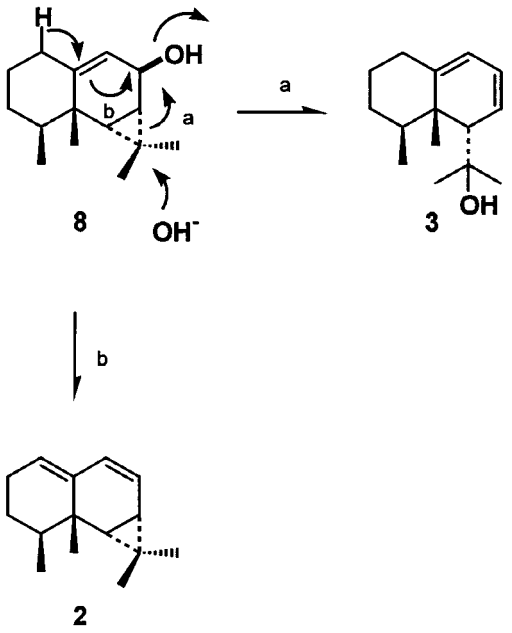


Fig. 5. (a) Possible biogenetic pathway of **3**. (b) The mechanism of the formation of **2** from **8** on silica gel.

3.2. Spectral data

NMR spectra were recorded at 150, 100 or 50 MHz for ^{13}C and 600, 400 or 200 MHz for ^1H. EIMS were measured at 70 eV. The temperature programming of GC-mass analysis performed from 50° isothermal for 3 min, then 50–250° at 5° min^{-1}, and finally isothermal at 250° for 15 min. Injection temp. was 250°. A fused silica column coated with DB-17 (30 m × 0.25 mm i.d., film thickness 0.25 μm) was used.

3.3. Plant material

R. hemisphaerica (L.) Raddi subsp. *orientalis* Schust. (Herbarium specimen No. 97001; dry wt. 554.1 g) was collected in March 1997 at Kaminaka-cho, Tokushima in Japan. *R. hemisphaerica* (No. 97017 and 97030; dry weight 131 and 20.5 g) was collected in March 1997 at Kitagawa-village, Kochi in Japan. *R. hemisphaerica* (No. 97063; dry weight 207.2 g) was collected in May 1997 at Aioi-cho, Tokushima in Japan. Voucher specimens are deposited at the Faculty of Pharmaceutical Sciences, Tokushima Bunri University.

3.4. Extraction and isolation

R. hemisphaerica (No. 97001) was extracted with Et_2O for two weeks. The ether extract (11.8 g) was chromatographed on silica gel and divided into 7 frs (fr. I–VII). Fr. IV was rechromatographed on Sephadex LH-20 and purified by prep.-HPLC on silica gel column using *n*-hexane to give gymnomitr-8(12)-en-

4-one (**1**) (34.3 mg; 0.3% for the total extract), together with known compounds, 1(10),8-aristoladiene (**2**) (90.7 mg; 0.8%), rebouliadienol (**3**) (=rulepidanol) (40.0 mg; 0.3%), marchantin O (**4**) (32.7 mg; 0.3%) and marchantin C (**17**) (78 mg; 0.7%). Fr. V was rechromatographed on Sephadex LH-20 and recrystallization from EtOAc to give zeorin (**5**) (117.1 mg; 1.0%). The ether extract of *R. hemisphaerica* (No. 97017; 3.7 g) was directly chromatographed on Sephadex LH-20, then purified by prep.-TLC to give 8α-methoxyaristol-9-ene (**6**) (4.2 mg; 0.1%), 8β-methoxyaristol-9-ene (**7**) (6.8 mg; 0.2%), 1(10),8-aristoladiene (**2**) (120.3 mg; 3.3%) and rebouliadienol (**3**) (=rulepidanol) (18 mg; 0.5%). The ether extract of *R. hemisphaerica* (No. 97030; 0.44 g) was subjected to flash chromatography on silica gel using *n*-hexane–EtOAc (9:1 v/v) to afford *ent*-aristol-9-en-8α-ol (**8**) (8 mg; 1.8%) and marchantin C (**17**) (30.1 mg; 6.8%). The ether extract of *R. hemisphaerica* (No. 97063; 7.9 g) was repeatedly chromatographed on silica gel, Sephadex LH-20 and prep.-HPLC to give rebouliadienol (**3**) (=rulepidanol) (30.3 mg; 0.4%), 6β,10β-epoxycupar-3-ene (**12**) (15.5 mg; 0.2%), 3(15)-thujopsen-10α-ol (**13**) (154.2 mg; 2.0%), (−)-cyclopropanecuparenol (**14**) (4.1 mg; 0.05%), gymnomitrol (**15**) (11.3 mg; 0.1%), (+)-gymnomitr-8(12)-en-9α-ol (**16**) (9.2 mg; 0.1%), marchantin C (**17**) (201.5 mg; 2.6%) and marchantinquinone (**18**) (61.8 mg; 0.8%).

Gymnomitr-8(12)-*en*-4-*one* (**1**): Oil; $[\alpha]_D$ −26.7 ($CHCl_3$; *c* 1.71), FT-IR ν_{max} (neat) cm^{-1}: 3071, 1732, 1464, 1404, 1176, 887, 582. EIMS *m/z* (rel. int.): 218 $[M]^+$ (40), 200 (13), 185 (12), 161 (8), 122 (16), 110 (100), 93 (57), 79 (27), 67 (11), 55 (6), 41 (10); CD: $\Delta\epsilon_{302}$ +0.071 ($CHCl_3$; *c* 0.05). ^1H NMR (600 MHz, $CDCl_3$): δ 0.91 (H-13), 1.07 (H-14) and 1.19 (H-15) (each 3H, *s*), 1.34 (1H, *d, J* = 11.7 Hz, H-11), 1.46 (1H, *ddd, J* = 14, 12.7, 7.9 Hz, H-10), 1.68 (1H, *ddd, J* = 14, 7.9, 3 Hz, H-10′), 1.78 (1H, *ddddd, J* = 17.3, 12.7, 7.7, 2.5, 2.5 Hz, H-9), 1.92 (1H, *dd, J* = 20.3, 1.7 Hz, H-3), 1.98 (1H, *ddd, J* = 11.7, 4.9, 3 Hz, H-11′), 2.02 (1H, *dd, J* = 20.3, 1.7 Hz, H-5), 2.22 (1H, *dd, J* = 17.3, 7.7 Hz, H-9′), 2.35 (1H, *d, J* = 4.9 Hz, H-7), 2.62 (1H, *dd, J* = 20.3, 1.7 Hz, H-5′), 2.66 (1H, *dd, J* = 20.3, 1.7 Hz, H-3′), 4.65 (1H, *dd, J* = 2.5, 2.5 Hz, H-12), 4.68 (1H, *ddd, J* = 2.5, 2.5, 0.6 Hz, H-12′). ^{13}C NMR (150 MHz, $CDCl_3$); δ 24.0 (C-13), 24.8 (C-14), 27.9 (C-15), 28.2 (C-9), 37.9 (C-10), 43.6 (C-11), 44.9 (C-1), 48.9 (C-8), 49.8 (C-2 or 6), 50.1 (C-5), 50.7 (C-6 or 2), 57.1 (C-7), 109.9 (C-15), 149.4 (C-3), 221.4 (C-4).

ent-8α-*Methoxyaristol*-9-*ene* (**6**): Oil; ^1H NMR (600 MHz; C_6D_6): δ 0.65 (1H, *d, J* = 9 Hz, H-6), 0.90 (3H, *d, J* = 6.8 Hz, H-15), 1.02 (H-14), 1.13 (H-13) and 1.15 (H-12) (each 3H, *s*), 1.09 (1H, *dd, J* = 9.1, 1.9 Hz, H-7), 1.23 (1H, *m*, H-3ax), 1.35 (1H, *m*, H-3eq), 1.54 (1H, *m*, H-2ax), 1.56 (1H, *m*, H-2eq), 1.62 (1H, *m*, H-4), 1.96 (1H, *m*, H-1eq), 2.20 (1H, *m*, H-1ax), 3.29 (3H, *s*, OMe), 3.94 (1H, *brd, J* = 4.0 Hz, H-8), 5.37 (1H, *ddd, J* = 4, 1.9, 1.9 Hz, H-9).

ent-8β-*Methoxyaristol*-9-*ene* (**7**): Oil; ^1H NMR (600 MHz; C_6D_6) δ 0.79 (1H, *d, J* = 9.3 Hz, H-6), 0.96 (3H, *d, J* = 6.9 Hz, H-15), 1.00 (H-14), 1.09 (H-13) and 1.42 (H-12) (each 3H, *s*), 1.12 (1H, *ddd, J* = 9.3, 6.9, 1.3 Hz, H-7), 1.22 (1H, *m*, H-3ax), 1.35 (1H, *m*, H-3eq), 1.54 (1H, *m*, H-2ax), 1.58 (1H, *m*, H-2eq), 1.68 (1H, *m*, H-4), 2.02 (1H, *m*, H-1eq), 2.13 (1H, *m*, H-1ax), 3.29 (3H, *s*, OMe), 4.13 (1H, *ddd, J* = 6.9, 3.8, 2.7 Hz, H-8), 5.49 (1H, *dd, J* = 3.8, 3.8 Hz, H-9).

ent-*Aristol*-9-*en*-8α-*ol* (**8**): Oil; ^1H NMR (400 MHz; C_6D_6): δ 0.74 (1H, *d, J* = 10 Hz), 0.91 (3H, *d, J* = 6.8 Hz), 0.93, 1.02 and 1.28 (each 3H, *s*), 1.13 (*m*), 4.52 (1H, *br s*), 5.30 (1H, *br s*).

6β,10β-*Epoxycupar*-3-*ene* (**12**): Oil; $[\alpha]_D$ +69.3 ($CHCl_3$, *c* 1.07); FT-IR ν_{max} (neat) cm^{-1}: 2959, 1444, 1016, 837, 563. EIMS *m/z* (rel. int.): 220 $[M]^+$ (43), 202 (6), 152 (68), 127 (58), 109 (100), 95 (63), 71 (12), 55 (10), 43 (19). HRMS: 220.1814 $[M]^+$. Calcd for $C_{15}H_{24}O$, 220.1828. ^1H NMR (600 MHz; $CDCl_3$): δ 0.86 (3H, *s*, H-14), 0.93 (3H, *s*, H-13), 1.16 (3H, *s*, H-12), 1.23 (1H, *ddd, J* = 13.7, 13.7, 4.9 Hz, H-1ax), 1.44 (1H, *ddd, J* = 12.9, 11.5, 5.5 Hz, H-8β), 1.57 (1H, *ddd, J* = 11.5, 9.0, 5.5 Hz H-9α), 1.69 (3H, *br s*, H-15), 1.69 (1H, *dddd, J* = 11.5, 11.5, 3.0, 3.0 Hz, H-9β), 1.73 (1H, *m*, H-8α), 1.75 (1H, *m*, H-2eq), 1.97 (1H, *dd, J* = 16.5, 2.5 Hz, H-5eq), 2.09 (1H, *dddd, J* = 13.7, 4.9, 2.5, 2.5 Hz, H-1eq), 2.18 (1H, *dddd, J* = 16.5, 6.0, 6.0, 2.5 Hz, H-5ax), 2.32 (1H, *dd, J* = 13.7, 13.7 Hz, H-2ax), 3.70 (1H, *d, J* = 3.0 Hz, H-10), 5.34 (1H, *m*, H-4). ^1H NMR (600 MHz; C_6D_6): δ 0.70 (3H, *s*, H-14), 0.73 (3H, *s*, H-13), 1.13 (3H, *s*, H-12), 1.14 (1H, *ddd, J* = 13.2, 13.2, 4.9 Hz, H-1ax), 1.23 (1H, *ddd, J* = 12.9, 11.3, 5.7 Hz, H-8), 1.47 (1H, *dddd, J* = 11.0, 11.0, 2.7, 2.7 Hz, H-9β), 1.52 (1H, *ddd, J* = 11.0, 9.1, 5.7 Hz, H-9α), 1.60 (*ddd, J* = 12.9, 9.1, 3.5 Hz, H-8α), 1.71 (3H, *s*, H-15), 1.72 (1H, *m*, H-2eq), 1.95 (1H, *brd, J* = 16.8 Hz, H-5eq), 2.02 (1H, *dddd, J* = 13.2, 4.9, 2.5, 2.5 Hz, H-1eq), 2.07 (1H, *dddd, J* = 16.8, 4.4, 4.4, 2.5 Hz, H-5ax), 2.56 (1H, *dd, J* = 15.0, 15.0 Hz, H-2ax), 3.60 (1H, *d, J* = 2.7 Hz, H-10), 5.40 (1H, *m*, H-4). ^{13}C NMR (150 Hz; $CDCl_3$): δ 11.0 (C-14), 20.3 (C-13), 21.6 (C-12), 23.2 (C-15), 26.3 (C-2), 28.1 (C-9), 29.4 (C-8), 30.2 (C-1), 36.0 (C-5), 49.2 (C-11), 51.0 (C-7), 80.9 (C-6), 84.1 (C-10), 119.5 (C-4), 134.8 (C-3).

3-(15)-*Thujopsen*-10α-*ol* (**13**): Mp 47.0–48.5°; FT-IR ν_{max} (neat) cm^{-1}: 3362 (OH), 3075, 1064, 862, 642. EIMS *m/z* (rel. int.): 220 $[M]^+$ (19), 202 (22), 187 (18), 152 (25), 133 (64), 109 (100), 94 (63), 83 (50), 69 (20), 57 (46), 43 (28), 32 (8). HRMS: 220.1834 $[M]^+$. Calcd for $C_{15}H_{24}O$, 220.1827. ^1H NMR (600 MHz; $CDCl_3$): δ 0.72 (1H, *dd, J* = 5.5, 3.8 Hz, H-1β), 0.87 (1H, *ddd, J* = 12.6, 10.2, 5.2 Hz, H-8α), 0.91 (3H, *s*, H-12), 1.00

(3H, *br s*, H-14), 1.02 (3H, *s*, H-13), 1.22 (1H, *ddd, J* = 5.5, 3.8, 1.9 Hz, H-1α), 1.40 (1H, *ddd, J* = 8.2, 5.2, 0.8 Hz, H-9β), 1.43 (1H, *dd, J* = 8.2, 3.8 Hz, H-2), 1.49 (1H, *ddd, J* = 12.6, 12.6, 5.2 Hz, H-8β), 1.74 (1H, *dd, J* = 12.9, 3.2 Hz, H-5α), 2.04 (1H, *m*, H-5β), 2.05 (1H, *m*, H-4β), 2.06 (1H, *m*, H-9α), 2.16 (1H, *ddd, J* = 12.9, 5.5, 0.8 Hz, H-4α), 3.96 (1H, *t, J* = 8.2 Hz, H-10), 4.62 (1H, *br s*, H-15), 4.81 (1H, *br s*, H-15′). ^{13}C NMR (150 Hz; $CDCl_3$): δ 17.3 (C-1), 17.4 (C-12), 22.3 (C-13), 22.6 (C-14), 27.6 (C-2), 28.8 (C-5), 29.3 (C-9), 29.6 (C-4), 30.0 (C-8), 38.0 (C-7), 46.2 (C-6), 47.1 (C-11), 80.4 (C-10), 101.7 (C-15), 154.0 (C-13).

3.5. Pyridinium dichromate (PDC) oxidation of ent-aristol-9-en-8α-ol (8)

Compound **8** (10 mg) in CH_2Cl_2 (1 ml) was added to a soln of PDC (100 mg) in dry CH_2Cl_2 (2 ml), then the mix was stirred for 30 min. Purification of the reaction mixture by prep.-HPLC afforded a ketone, whose spectral data were identical to those of aristolone, except for the sign of the optical rotation.

3.6. Asymmetric dihydroxylation of 1(10),8-aristoladiene (2)

To a mix of *t*-BuOH and H_2O, AD-mix-α (420 mg), $CH_3SO_2NH_2$ (28.5 mg) and **2** (30 mg) were added and the reaction mix was stirred for 24 hr at 0°C. A diol **10** (27.5 mg) was obtained after purifying the mix.

1(10)-*Aristolene*-8β,9β-*diol* (**10**): Oil; EIMS *m/z* (rel. int.); 218 $[M-H_2O]^+$ (100), 200 (66), 185 (47), 175 (48), 136 (65), 119 (56), 105 (95), 91 (82), 83 (54), 41 (54). ^{1}H NMR (600 MHz; $CDCl_3$): δ 0.66 (1H, *d, J* = 9.6 Hz, H-6), 0.72 (1H, *dd, J* = 9.6, 2.0 Hz, H-7), 0.94 (3H, *d, J* = 6.7 Hz, H-15), 0.88 (H-12), 1.00 (H-13) and 1.39 (H-14) (each 3H, *s*), 1.29 (1H, *ddddd, J* = 13.2, 6.7, 2.7, 1.3, 1.3 Hz, H-3eq), 1.48 (1H, *m*, H-3ax), 1.61 (1H, *dddd, J* = 13.7, 6.7, 6.7, 2.7 Hz, H-4), 1.81 (1H, *dddd, J* = 18.2, 13.7, 6.7, 2.7 Hz, H-2ax), 1.88 (1H, *dddd, J* = 18.2, 6.3, 4.5, 1.3 Hz, H-2eq), 3.50 (1H, *brdd, J* = 4.0, 2.0 Hz, H-8), 3.84 (1H, *d, J* = 4.0 Hz, H-9), 5.35 (1H, *brdd, J* = 4.5, 2.7 Hz, H-1). ^{13}C NMR (150 MHz; $CDCl_3$): δ 16.0 (C-15), 17.1 (C-12), 18.0 (C-11), 25.3 (C-14), 26.0 (C-2), 26.2 (C-7), 27.2 (C-3), 29.6 (C-13), 33.4 (C-6), 35.9 (C-5), 36.4 (C-4), 68.1 (C-8), 76.7 (C-9), 127.4 (C-1), 143.8 (C-10).

3.7. Esterification of 1(10)-aristolene-8β,9β-diol (10) by p-dimethylaminobenzoic acid

To a soln of **10** (8.5 mg) in CH_2Cl_2 (1 ml), *p*-dimethylaminobenzoic acid (15 mg), DCC (95 mg) and DMAP (20 mg) were added and the reaction mix was stirred for three days at room temp. After purification of the mix, mono-*p*-dimethylaminobenzoate **11** (6.8 mg) was obtained.

p-*Dimethylaminobenzoate* **11**: Oil; EIMS *m/z* (rel. int.): 383 $[M]^+$ (3), 249 (3), 219 (43), 201 (33), 185 (12), 166 (100), 148 (25), 105 (10), 41 (8). UV λ_{max} nm (log ε): 310 (4.52), 227 (3.98), 203 (4.46). CD: $\Delta\epsilon_{305}$ +1.08 ($CHCl_3$; *c* 4.22×10^{-5}). ^{1}H NMR (600 MHz; $CDCl_3$): δ 0.81 (1H, *d, J* = 9.3 Hz, H-6), 0.89 (1H, *dd, J* = 9.3, 3.0 Hz, H-7), 0.99 (3H, *d, J* = 6.7 Hz, H-15), 1.10 (H-12), 1.12 (H-13) and 1.33 (H-14) (each 3H, *s*), 1.45 (1H, *ddddd, J* = 13.2, 6.7, 2.7, 1.3, 1.3 Hz, H-3eq), 1.57 (1H, *m*, H-3ax), 1.75 (1H, *dddd, J* = 13.7, 6.7, 6.7, 2.7 Hz, H-4), 2.03 (1H, *dddd, J* = 18.2, 13.7, 6.7, 2.7 Hz, H-2ax), 2.08 (1H, *dddd, J* = 18.2, 6.3, 4.4, 1.3 Hz, H-2eq), 3.06 (6H, *s*, N, N′-dimethyls), 4.30 (1H, *d, J* = 3.0 Hz, H-9), 4.90 (1H, *dd, J* = 3.0, 3.0 Hz, H-8), 5.71 (1H, *brd, J* = 4.4, 2.7 Hz, H-1), 6.66 (2H, *d, J* = 9.1 Hz, H-2′, 6′), 7.96 (2H, *d, J* = 9.1 Hz, H-3′, 5′).

3.8. Esterification of α-zeorin (5) by R(+) and S(−)-α-methoxy-α-trifluoromethylphenylacetic acid (MTPA)

To a soln of **5** (10 mg) in dry CH_2Cl_2, *R*(+)-MTPA (30 mg), DCC (30 mg) and DMAP (10 mg) were added and the reaction mixture was stirred for 48 hr. To the mix, H_2O was added and the soln was washed with 1N HCl, saturated $NaHCO_3$ and NaCl. After evaporation, the CH_2Cl_2 layer was purified by prep.-HPLC to give a mono *R*(+)-MTPA ester (11.7 mg). *S*(−)-MTPA ester (6.4 mg) of **5** was obtained by *S*(−)-MTPA within the same manner as above.

R(+)-*MTPA ester of* α-*zeorin* (**5**): ^{1}H NMR (600 MHz; $CDCl_3$): δ 0.46 (H-23), 0.49 (H-24), 0.76 (H-28), 0.90 (H-25), 0.96 (H-27), 1.15 (H-26), 1.18 (H-29) and 1.22 (H-30) (each 3H, *s*), 1.11 (1H, *d, J* = 10.9 Hz, H-5ax), 1.68 (1H, *dd, J* = 11.8, 3.8 Hz, H-7eq), 5.31 (1H, *ddd, J* = 10.9, 10.9, 3.8 Hz, H-6ax).

S(−)-*MTPA ester of* α-*zeorin* (**5**): ^{1}H NMR (600 MHz; $CDCl_3$): δ 0.75 (H-28), 0.80 (H-24), 0.81 (H-23), 0.91 (H-27), 0.93 (H-25), 1.14 (H-26), 1.18 (H-29) and 1.22 (H-30) (each 3H, *s*), 1.12 (1H, *d, J* = 10.7 Hz, H-5ax), 1.60 (1H, *dd, J* = 12.1, 3.8 Hz, H-7eq), 5.37 (1H, *ddd, J* = 10.7, 10.7, 3.8 Hz, H-6ax).

Acknowledgements

We thank Dr M. Mizutani (Hattori Botanical Laboratory, Nichinan, Miyazaki, Japan) for his confirmation of our identification of the species. This work was supported by a Grant-in-Aid for Scientific Research (B) (No. 08459026) from the Ministry of Education, Science, Sports and Culture.

References

Abraham, M. H., Davies, A. G., Llewellyn, D. R., & Thain, E. M. (1957). *Analytica Chimica Acta*, *17*, 499.

Asakawa, Y. (1995). In W. Herz, G. W. Kirby, R. E. Moore, W. Steglich, & Ch Tamm, *Progress in the Chemistry of Organic Natural Products,* , *vol. 65* (pp. 1–562). Vienna: Springer.

Asakawa, Y., Yamamura, A., Waki, T., & Takemoto, T. (1980). *Phytochemistry*, *19*, 603.

Asakawa, Y., Toyota, M., Bischler, H., Campbell, E. O., & Hattori, S. (1984). *Journal of Hattori Botanical Laboratory*, *57*, 383.

Connolly J.D., Harding A.E., Thornton M.S. (1974). *Journal of Chemical Society, Perkin Transactions I* 2487.

Godin, P. (1954). *Nature*, *174*, 134.

Hashimoto, T., Asakawa, Y., Nakashima, K., & Tori, M. (1993). *Journal of Hattori Botanical Laboratory*, *74*, 121.

Humpf, H-U., Berova, N., & Nakanishi, K. (1995). *Journal of Organic Chemistry*, *60*, 3539.

Knappe, E., & Peteri, D. (1962). *Zeitschrift für Analytische Chemie*, *109*, 386.

Kusumi, T., Ohtani, I., Inouye, Y., & Kakisawa, H. (1988). *Tetrahedron Letters*, *29*, 4731.

Manabe, S., Enoki, N., & Nishino, C. (1985). *Tetrahedron Letters*, *26*, 2213.

Morais R.M.S.C., Harrison L.J., Becker H. (1988). *Chemical Research (Synopses)* 380.

Mruzek, M. N., Bloodworth, A. J., & Eggelte, H. J. (1987). *Organic Mass Spectrometry*, *22*, 765.

Nishino, C., Manabe, S., Kazui, M., & Matsuzaki, T. (1984). *Tetrahedron Letters*, *25*, 2809.

Sharpless, K. B., Amberg, W., Bennani, Y. L., Crispino, G. A., Hartung, J., Jeong, K. S., Kwong, H. L., Morikawa, K., Wang, Z. M., Xu, D., & Zhang, X. L. (1992). *Journal of Organic Chemistry*, *57*, 2768.

Toyota M. (1987) Ph.D. Thesis, Tokushima Bunrin University.

Toyota, M., & Asakawa, Y. (1993). *Phytochemistry*, *32*, 1235.

Toyota, M., Nagashima, F., & Asakawa, Y. (1988). *Phytochemistry*, *27*, 2161.

Vidari G., Che Z., Garlaschelli L. (1998). *Tetrahedron Letters* 6073.

Wei, H-C., Ma, S-J., & Wu, C-L. (1995). *Phytochemistry*, *39*, 91.

Wenkert, E., Baddeley, G. V., Burfitt, I. R., & Moreno, L. N. (1978). *Organic Magnetic Resonance*, *11*, 337.