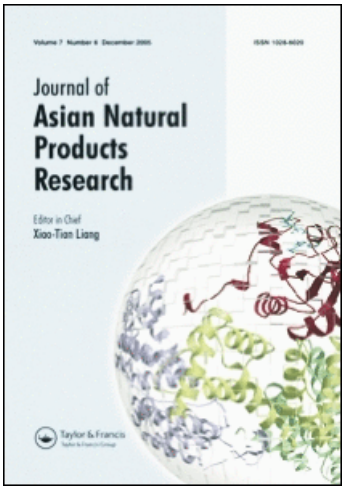


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# Eudesmane-type sesquiterpenoids from the liverwort *Lepidozia fauriana*

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# Eudesmane-type sesquiterpenoids from the liverwort *Lepidozia fauriana*

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Three new (**8**, **12**, **13**) and two revised (**7**, **9**) eudesmane alcohols have been identified from the Taiwanese liverwort *Lepidozia fauriana*, together with a known macrocyclic bisbibenzyl, isoplagiochin D, and other known sesquiterpenoids. Their structures were determined by NMR and X-ray analyses. Three chemotypes of *Lepidozia fauriana* may be classified according to the skeleton of the major sesquiterpenoids identified in the species.

*Keywords*: *Lepidozia fauriana*; Jungermanniales; Lepidoziaceae; Liverwort; Eudesmane alcohols

## 1. Introduction

Previously, two reports have been published on the chemical constituents of the Taiwanese liverwort *Lepidozia fauriana* [1,2]. However, major sesquiterpenoids, such as **1**–**6**, identified in each species, varied from place to place. In the present study, we investigated the species of *L. fauriana* collected from two different locations in Taiwan, and five eudesmane alcohols (**7**–**9**, **12**, **13**) were identified. Among them, alcohols **8**, **12** and **13** were new, while **7** and **9** were reassigned from previously reported structures. In addition, the known isoplagiochin D (**11**) [3] was also isolated from *L. fauriana* collected at Ali Shan. No such macrocyclic bisbibenzyl has ever been reported in the genus *Lepidozia*. The structures of compounds investigated are shown in figure 1.

## 2. Results and discussion

The EtOAc extract of *Lepidozia fauriana* collected at Ali Shan was chromatographed on silica gel and Sephadex LH-20, followed by preparative TLC, if necessary, to afford three oxygenated eudesmanes **7**–**9**, along with (+)-4β,10α-dihydroxyaromadendrane (**10**) [4], (+)-lepidozenolide (**1**) [1], and isoplagiochin D (**11**) [3].

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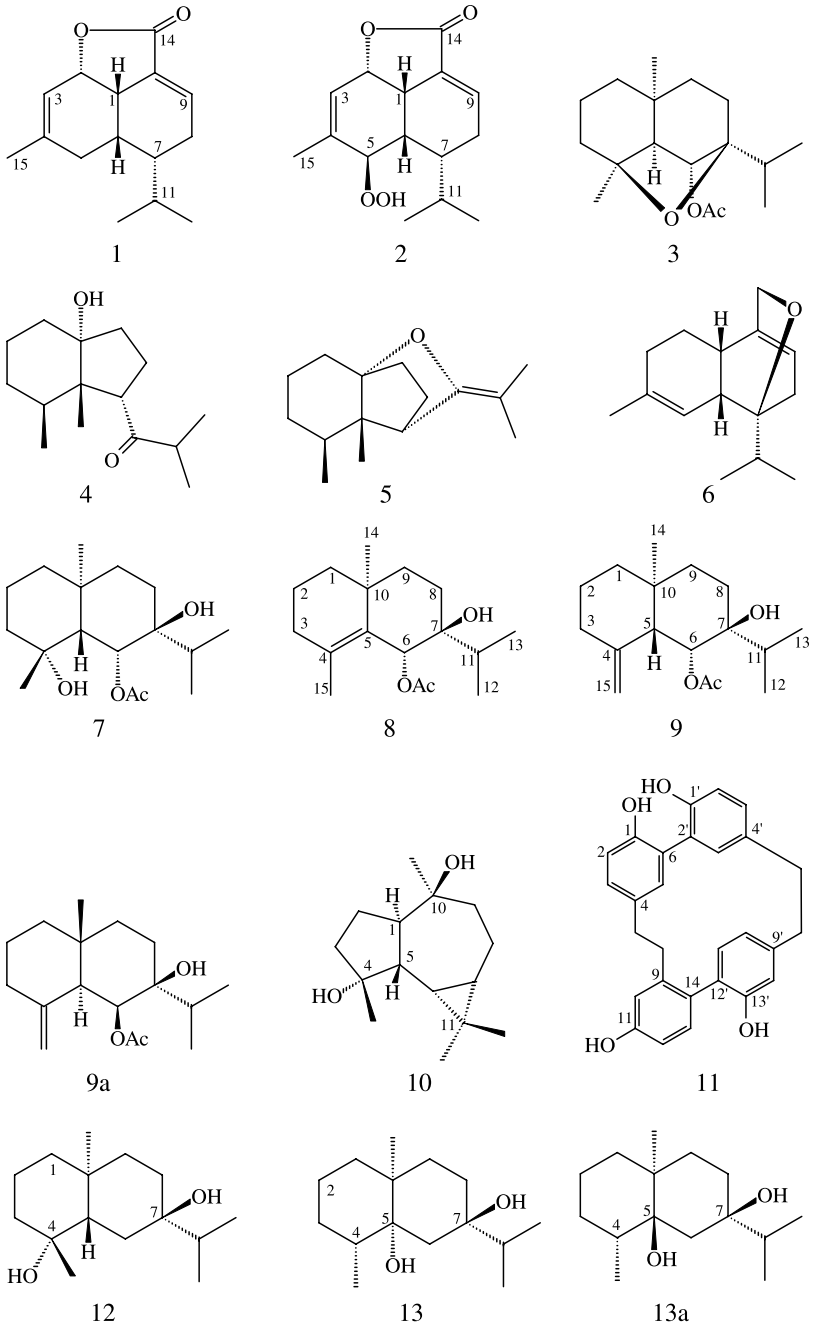



Figure 1. Structures of compounds **1**–**13a**.

Compound **7** was obtained in crystalline form. Its $^{1}$H NMR and $^{13}$C NMR spectra (tables 1 and 2) indicated the identity with those of 6β-acetoxyvitranoxide (**3**) that was formerly isolated from *Lepidozia vitrea* and identified in *L. fauriana* [1] as well. The sample was subjected to X-ray analysis, which was not performed previously since crystals were not obtained. To our surprise, the crystallography (figure 2) revealed a

Table 1. $^{1}$H NMR data of compounds **7**–**9**, **12**, **13** (in $CDCl_3$).

| *Atom* | | ***7*** | ***8*** | ***9*** | ***12***[a] | ***13***[a] |
|---|---|---|---|---|---|---|
| 1 | Eq | 1.23 | 1.47 | 1.39 | 1.40 | 1.19 (br d, 13.3) |
| | Ax | 1.47 (td, 13.4, 3.5) | 1.30 (dt, 3.7, 12.6) | 1.33 (td, 12.5, 5.0) | 1.13 | 1.61 (dt, 3.7, 13.3) |
| 2 | Eq | 1.37 | 1.55 | 1.55 | 1.42 | 1.42 |
| | Ax | 1.81 (qt, 13.4, 3.5) | | | 1.81 (tq, 3.5, 13.7) | 1.58 (tq, 3.7, 13.3) |
| 3 | Eq | 1.57 | 1.99 | 2.30 (d, qnt, 13.3, 2.0) | 1.65 (dm, 13.7) | 1.39 (dm, 13.3) |
| | Ax | 1.47 (td, 13.4, 3.5) | 2.05 | 2.05 | 1.45 | 1.26 (dq, 3.7, 13.3) |
| 4 | Ax | – | – | – | – | 2.49 (m) |
| 5 | Ax | 1.61 | – | 2.54 (br s) | 1.43 | – |
| 6 | Eq | 5.29 (t, 1.8) | 5.64 (d, 1.5) | 5.21 (t, 1.7) | 1.53 (dt, 12.8, 2.3) | 1.81 (dd, 2.0, 14.5) |
| | Ax | | | | 1.47 (t, 12.9) | 1.42 (d, 14.5) |
| 8 | Eq | 1.57 | 1.64 | 1.55 | 1.35 (ddt, 14.1, 4.2, 2.5) | 1.39 (dm, 13.7) |
| | Ax | 1.74 (td, 14.1, 4.1) | 1.83 | 1.66 (td, 12.1, 3.0) | 1.61 (dt, 4.2, 14.1) | 1.66 (dt, 4.5, 13.7) |
| 9 | Eq | 1.37 | 1.45 | 1.37 | 1.12 | 0.97 (ddd, 2.5, 4.5, 13.7) |
| | Ax | 1.13 (td, 14.1, 4.1) | | 1.62 | 1.43 | 2.23 (dt, 4.5, 13.7) |
| 11 | | 1.68 (sept., 6.9) | 1.76 (sept., 6.9) | 1.50 (sept., 6.8) | 1.58 (sept., 6.9) | 1.53 (sept., 6.9) |
| 12 | | 0.87 (d, 6.9) | 0.88 (d, 6.9) | 0.87 (d, 6.8) | 0.93 (d, 6.9) | 0.89 (d, 6.9) |
| 13 | | 0.87 (d, 6.9) | 0.92 (d, 6.9) | 0.94 (d, 6.8) | 0.92 (d, 6.9) | 0.90 (d, 6.9) |
| 14 | | 1.24 (s) | 1.10 (s) | 0.92 (s) | 0.97 (s) | 0.91 (s) |
| 15 | | 1.34 (s) | 1.84 (s) | 4.50 (dd, 1.8, 3.1) | 1.13 (s) | 0.88 (d, 6.7) |
| | | | | 4.70 (dd, 1.8, 3.1) | | |
| OAc | | 2.00 (s) | 1.98 (s) | 2.01 (s) | | |

[a] Data obtained on 800 MHz NMR. The rest were by 500 MHz NMR.

Table 2. $^{13}C$ NMR data of compounds **7**–**9**, **12**, **13** (in $CDCl_3$, 125 MHz).

| *Atom* | ***7*** | ***8*** | ***9*** | ***12*** | ***13*** |
|---|---|---|---|---|---|
| 1 | 38.6 | 41.7 | 44.3 | 41.2 | 36.8 |
| 2 | 18.0 | 18.7 | 22.7 | 18.1 | 21.3 |
| 3 | 43.6 | 34.1 | 37.6 | 41.6 | 30.8 |
| 4 | 72.6 | 138.1 | 147.4 | 72.1 | 34.1 |
| 5 | 47.9 | 129.7 | 47.2 | 46.0 | 75.1 |
| 6 | 72.0 | 72.1 | 72.7 | 29.5 | 38.3 |
| 7 | 74.5 | 74.1 | 74.6 | 74.4 | 75.6 |
| 8 | 28.0 | 27.3 | 27.1 | 29.3 | 30.2 |
| 9 | 44.3 | 36.8 | 36.0 | 39.2 | 29.8 |
| 10 | 33.8 | 33.4 | 35.2 | 33.6 | 37.8 |
| 11 | 32.7 | 33.1 | 31.7 | 39.2 | 39.7 |
| 12 | 15.9 | 16.0 | 16.3 | 16.8 | 16.66 |
| 13 | 16.2 | 16.3 | 16.3 | 16.9 | 16.74 |
| 14 | 20.7 | 26.5 | 19.8 | 17.7 | 22.5 |
| 15 | 30.0 | 19.8 | 106.8 | 30.1 | 16.1 |
| OAc | 21.8 | 21.4 | 21.5 | | |
| | 170.7 | 169.5 | 170.0 | | |



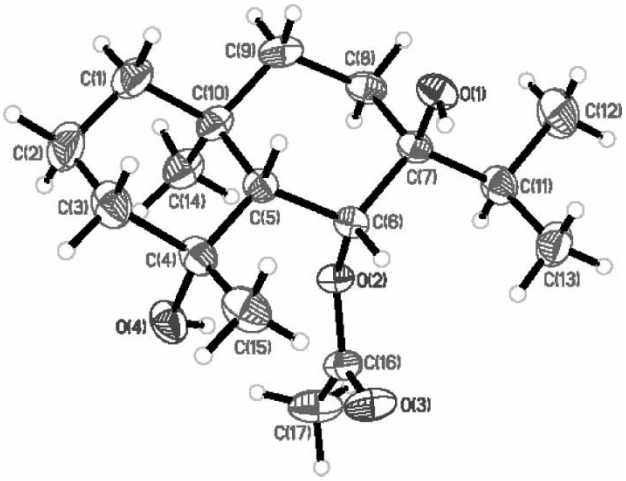



Figure 2. X-ray crystal structure of compound **7**.

diol **7** instead of an oxide **3**. In the earlier study [1], the isolated sample deteriorated and no IR spectrum was available. In addition, the results of its FAB-MS always showed a quasi-molecular ion at *m/z* 281 ($[M + H]^+$). The molecular ion of *m/z* 298 was undetectable. Obviously, the diol was very unstable, and easily dehydrated to lose one molecule of water even under mild FAB conditions. The NOESY correlations observed in a previous experiment [1], such as H-6/H-12, H-13, H-15 and H-14/H-acetoxyl as shown in figure 3, still applied to the *trans*-fused relative structure **7**, which was now given the name 6α-acetoxy-4α,7β-dihydroxyeudesmane.

Compounds **8** and **9** were isolated from $AgNO_3$-impregnated PTLC plates. They both revealed very similar $^1H$ NMR and $^{13}C$ NMR spectra except for the difference in the olefinic region. It was easily realised that compound **8** was the tetra-substituted double bond isomer, and compound **9** was the di-substituted, exocyclic double bond isomer. Since all spectra of

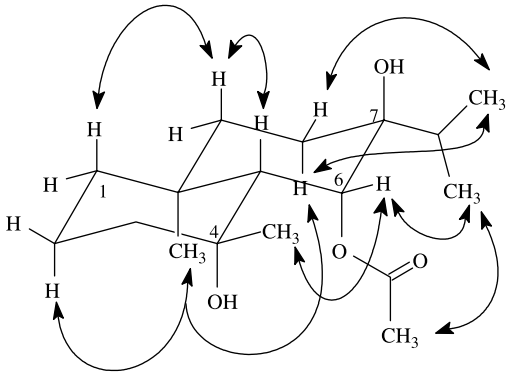
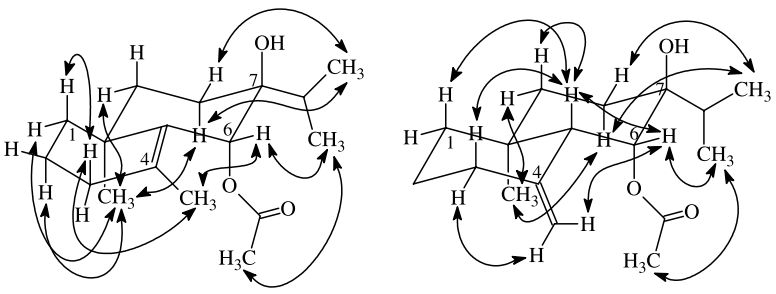


Figure 3. Key NOE correlations of compound **7**.



both compounds displayed close similarity to those of compound **7**, their structures were accordingly assigned. Both HMQC and HMBC spectra confirmed the assignments. The NOESY data also supported the relative configuration of compound **8** as depicted in figure 4. Compound **8** has not been reported in the literature, whereas the $^{1}$H NMR and $^{13}$C NMR spectra (tables 1 and 2) of compound **9** appeared fairly comparable with those of a known sesquiterpene alcohol (**9a**) isolated from another liverwort *Bazzania tridens* [5]. Upon close examination of the NOESY correlations observed (figure 4), obviously the relative stereostructure **9a** of the eudesmane alcohol from *B. tridens* was erroneously assigned and should be revised as **9**. The absolute configurations depicted here were assumed since all eudesmane alcohols so far reported from the *Lepidozia* species [1,2] have α-configuration of the C-10 methyl group.

Two other eudesmane diols (**12** and **13**) were again isolated as the major components of its EtOAc extract from the same *L. fauriana* species, yet was collected at a northern location of Taiwan, Fu Shan. Neither molecular ion were detected from their GC-MS spectra. Nevertheless, both $^{1}$H NMR and $^{13}$C NMR spectra (tables 1 and 2) of these two compounds indicated that they both possessed two tertiary hydroxyl groups with a saturated eudesmane skeleton. The positions of the two hydroxyl groups could be easily deduced as shown in structures **12** and **13** on the basis of their respective HMQC and HMBC spectra. As to the relative stereochemistry of compound **12**, a *trans*-fused ring junction of the eudesmane



8 9

Figure 4. Key NOE correlations of compounds **8** and **9**.

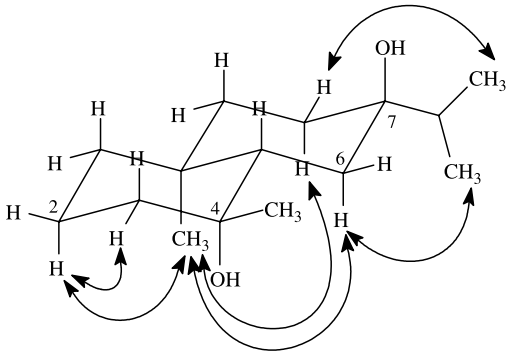


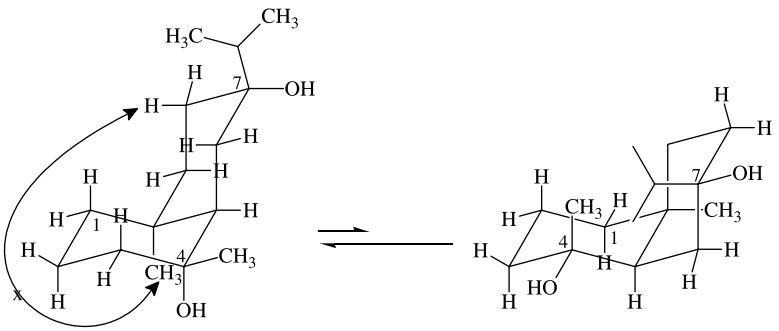
Figure 5. Key NOE correlations of compound **12**.



skeleton with identical relative configurations at C-4 and C-7 as those of compound **7** was supported from the NOESY correlations, as shown in figure 5. The most convincing evidence for a *trans*-fused ring was NOE correlations observed between $CH_3$-14 and H-6a, H-8a and H-2a. Such three correlations would be impossible to see in a *cis*-fused more comfortable conformation, as demonstrated in figure 6. In other words, structure **12** has exactly the same relative configuration as that of structure **7** except for a missing acetoxy substitution at C-7.

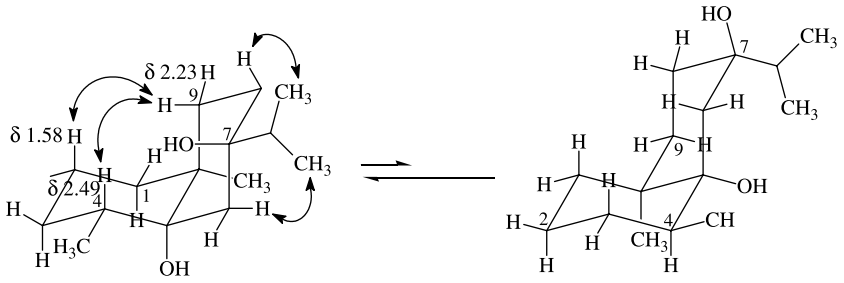
The relative configuration of compound **13** was interesting. The NOESY correlations of H-9a ($\delta$ 2.23) with H-2a ($\delta$ 1.58) and H-4a ($\delta$ 2.49) definitely could not be explained by a *trans*-fused ring arrangement (**13a** in figure 7), while a *cis*-fused ring (figure 8) justified the observations very well. In addition, the rather downfield-shifted absorptions of H-9a ($\delta$ 2.23) and H-4a ($\delta$ 2.49) were good evidence of an axial hydroxyl group at C-7 in proximity to a *cis*-fused eudesmane configuration. The chemical shift of H-4a would not be accountable in a *trans*-fused stereostructure, as shown in **13a**. The absolute configuration depicted was also assumed as stated above.

Three other known minor compounds were also isolated from the EtOAc extracts of *L. fauriana* collected at Ali Shan. Lepidozenolide (**1**) was identified by GC-MS, $^1$H NMR and $^{13}$C NMR spectra. However, upon standing overnight this compound was oxidised to compound **2**, 5β-hydroperoxylepidozenolide, as indicated by GC-MS, $^1$H NMR and $^{13}$C



12a

Figure 6. Impossible NOE correlations in a *cis*-fused **12a**.




13

Figure 7. Key NOE correlations of compound **13**.



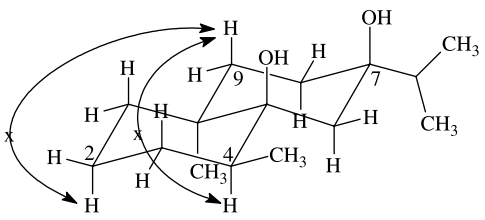
NMR spectral data. Both peaks of compounds **1** and **2** were observable in the GC-MS trace of EtOAc extract and isolated from previous collections at different localities [1]. Another sesquiterpene diol isolated from a rather polar fraction (45–50% EtOAc/n-hexane) was (+)-4α,10β-dihydroxyaromadendrane (**10**), which had been previously found in two other liverworts of *Plagiochila* species [6,7]. All spectral data were consistent with the published data including the optical rotation. From the 50% EtOAc eluate, a bisbibenzyl isoplagiochin D (**11**) [3] was isolated. No such macrocyclic aromatics has ever been reported from the genus *Lepidozia*, although the same compound was identified in the liverwort species *Plagiochila fruticosa* [8] and *Herbertus sakuraii* [9].

So far, six species of *L. fauriana* collected at different sites of Taiwan have been studied chemically. According to the sesquiterpene skeletons, i.e., amorphane, chiloscyphane and eudesmane, of the major constituents of each species investigated, three chemo-types could be classified, as shown in table 3. It is interesting to note that the two eudesmane diols **12** and **13** isolated from the same plant were ring-fused in different configurations.

## 3. Experimental

### *3.1 General experimental procedures*

NMR spectra were measured on Bruker AM-300, DMX-500 and AV-800 in $CDCl_3$. All GC-MS (EI) spectra were taken at 70 eV. A DBWAX, 30 m × 0.25 mm (i.d.), fused silica capillary column was used for GC-MS. The column temperature was programmed from 50 to




13a

Figure 8. Impossible NOE correlations in compound **13a**.

Table 3. Chemo-types of *Lepidozia fauriana*.

| *Locality* | *Major $C_{15}$ skeleton* | *Chemo-type* | *Ref.* |
|---|---|---|---|
| SL | Amorphane | I | [1] |
| YY-1 | Amorphane | I | [1] |
| YY-2 | Amorphane | I | [2] |
| YY-3 | Chiloscyphane | II | [2] |
| AL | Eudesmane | III | [a] |
| FS | Eudesmane | III | [a] |

SL, Shanlin Chi, Nantou Hsien, 1700 m; YY-1, -2, -3, different sites of Yuenyang Lake, Hsinchu Hsien, 1700 m; AL, Ali Shan, Chiayi Hsien, 2400 m; FS, Fu Shan, Ilan Hsien, 600 m.
[a] Present study.

220°C at 5°/min. IR spectra were measured in $CHCl_3$ on a KBr disc after the solvent had evaporated. Optical data were also taken in $CHCl_3$.



### *3.2 Plant material*

*Lepidozia fauriana* was collected at Fu-Shan, Ilan Hsien, 600–800 m, in 1991 and at Ali-Shan, Chiayi Hsien, 2400 m, in 1999. The voucher specimens were identified by Dr. Kohsaku Yamada (Ise-shi, Japan) and deposited at the Department of Chemistry, Tamkang University, Taiwan.

### *3.3 Extraction and isolation*

Plants of *L. fauriana* (27 g) collected at Ali-Shan were powdered and extracted with EtOAc (3 × 100 ml). The crude oil (0.8 g) was chromatographed on silica gel (70–230 mesh) and eluted with n-hexane and EtOAc in gradient. Fractions of 9% and 12% were further chromatographed on $AgNO_3$-impregnated (10%) PTLC to afford compounds **8** (3.8 mg) and 9 (3.4 mg). Compound **1** was obtained from the 15% n-hexane/EtOAc eluate. Compound **7** (41.8 mg) was yielded from the 18% and 21% fractions, followed by further purification on Sephadex LH-20 ($CH_2Cl_2$/MeOH = 1:1) and recrystallised from pure n-hexane. The 45% EtOAc fraction was again chromatographed on PTLC to furnish compounds **10** (1.9 mg) and **11** (2.3 mg).

Air-dried and powdered whole material (6.7 g) of the same plant *L. fauriana* collected at Fu-Shan was also extracted with EtOAc (3 × 20 ml). The crude extract (0.2 g) was chromatographed on silica gel (70–230 mesh) using an n-hexane/EtOAc gradient. Compounds **13** (2.9 mg) and 12 (6.0 mg) were eluted in 9% and 15% fractions, respectively.

**3.3.1 6α-Acetoxy-4α,7β-dihydroxyeudesmane (7)**. Colourless plates (n-hexane); $C_{17}H_{30}O_4$; mp 153–154°C; $[\alpha]_D^{25}$ + 31.6 (*c* 1.08, $CHCl_3$); IR (KBr) $\nu_{max}$ ($cm^{-1}$): 3459, 1720; GC $R_t$ = 40.3 min; GC–MS (EI) *m/z* (rel. int.): 298 ($[M]^+$, 0), 195 (96), 177 (15), 137 (26), 135 (23), 121 (20), 81 (18), 71 (26), 43 (100); $^1$H NMR data: see table 1; $^{13}$C NMR data: see table 2. Compound **7** afforded orthorhombic crystals from pure n-hexane, cell parameters: $a$ = 18.207 (1), $b$ = 8.971 (2), $c$ = 10.698 (3) Å, space group $P2_12_12$ $Z$ = 4. The diffraction intensities were collected on a Kappa CCD diffractometer using monochromated Mo-$K_\alpha$ radiation. The structure was solved by direct methods and the final $R$ value was 0.073 for 4017 reflections.

**3.3.2 6α-Acetoxy-7β-hydroxyeudesm-4-ene (8)**. Yellowish oil, $C_{17}H_{28}O_3$; $[\alpha]_D^{25}$ +45.9 (*c* 0.19, $CHCl_3$); IR (KBr) $\nu_{max}$ ($cm^{-1}$): 3450, 1717; GC $R_t$ = 31.3 min; GC-MS (EI) *m/z* (rel. int.): 280 ($[M]^+$, 1), 177 (15), 139 (23), 122 (42), 121 (100), 100 (27), 93 (25), 79 (15), 43 (42); $^1$H NMR data: see table 1; $^{13}$C NMR data: see table 2.

**3.3.3 6α-Acetoxy-7β-hydroxyeudesm-4(15)-ene (9)**. Colourless oil, $C_{17}H_{28}O_3$; $[\alpha]_D^{25}$ +65.4 (*c* 0.17, $CHCl_3$); IR (KBr) $\nu_{max}$ ($cm^{-1}$): 3522, 1719; GC $R_t$ = 36.1 min; GC-MS (EI) *m/z* (rel. int.): 280 ($[M]^+$, 0), 219 (12), 178 (15), 177 (100), 122 (12), 121 (20), 107 (12), 93 (17), 43 (44); $^1$H NMR data: see table 1; $^{13}$C NMR data: see table 2.

**3.3.4 4α,7β-Dihydroxyeudesmane (12)**. Yellowish oil, $C_{15}H_{28}O_2$; $[\alpha]_D^{25}$ +4.5 (*c* 0.3, $CHCl_3$); IR (KBr) $\nu_{max}$ ($cm^{-1}$): 3469; GC $R_t$ = 33.6 min; GC-MS (EI) *m/z* (rel. int.): 240 ($[M]^+$, 0), 179 (100), 161 (35), 109 (50), 97 (59), 69 (21), 55 (22), 43 (69), 41 (23); $^1$H NMR data: see table 1; $^{13}$C NMR data: see table 2.

**3.3.5 5α,7β-Dihydroxyeudesmane (13)**. Yellowish oil, $C_{15}H_{28}O_2$; $[\alpha]_D^{25}$ +25.7 (*c* 0.14, $CHCl_3$); IR (KBr) $\nu_{max}$ ($cm^{-1}$): 3484; GC $R_t$ = 33.2 min; GC-MS (EI) *m/z* (rel. int.): 240 ($[M]^+$, 0), 197 (35), 179 (86), 126 (65), 109 (57), 71 (69), 55 (41), 43 (100), 41 (43); $^1$H NMR data: see table 1; $^{13}$C NMR data: see table 2.



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