



EUDESMANE-TYPE SESQUITERPENE LACTONES FROM THE JAPANESE LIVERWORT *FRULLANIA DENSILOBA*

FUMIHIRO NAGASHIMA, HIRONAO TANAKA, SHIGERU TAKAOKA and YOSHINORI ASAKAWA

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

(Received 18 November 1996)

Key Word Index—*Frullania densiloba*; Frullaniaceae; Hepaticae; *ent*- α -cyclodihydrocostunolide; densilobolide-A; densilobolide-B; eudesmane-type; sesquiterpenoid.

Abstract—Three new eudesmane-type sesquiterpene lactones, *ent*- α -cyclodihydrocostunolide, and densilobolide-A and -B, were isolated from the Japanese liverwort *Frullania densiloba*. Their structures were determined by extensive NMR techniques, chemical degradation and X-ray crystallographic analysis. ©1997 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Epiphytic liverworts of the genus *Frullania* are rich sources of sesquiterpene lactones and/or bibenzyl derivatives [1, 2]. Eremophilane- and eudesmane-type sesquiterpene lactones which cause intense allergenic contact dermatitis and show cytotoxic activity have been isolated from several *Frullania* species [1–4]. The isolation of *ent*-eudesmane- and aromadendrane-type sesquiterpenoids has been reported from Japanese *F. densiloba* [1–5]. Because the same liverworts have geographical races like *F. dilatata* [6] and *Jungermannia infusca* [7], we reinvestigated the chemical constituents of *F. densiloba* collected in Tokushima, Japan. This species produced different eudesmane-type sesquiterpene lactones from those found in the same plant in a previous study [5]. In this paper, we report on the isolation and characterization of three new eudesmane-type sesquiterpene lactones.

RESULTS AND DISCUSSION

A combination of column chromatography on silica gel, Sephadex LH-20 and preparative HPLC of the ether extract of *F. densiloba* gave three new eudesmane-type sesquiterpene lactones, *ent*- α -cyclodihydrocostunolide (**1**), densilobolide-A (**2**) and -B (**3**), respectively.

The molecular formula of **1** was determined to be $C_{15}H_{22}O_2$ ($[M]^+$ m/z 234.1613) by high-resolution mass spectroscopy. The ^{13}C NMR and IR spectra showed the presence of a γ -lactone group (1780 cm^{-1} ; δ_C 82.0 *d*, 179.8 *s*). The 1H and ^{13}C NMR spectra (Tables 1 and 2) contained signals assignable to a secondary methyl (δ_H 1.21 *d*, $J = 6.8$ Hz), two tertiary methyls (δ_H 0.91, 1.80 each *s*), a methine with a γ -

lactone group (δ_H 3.88 *dd*, $J = 11.7, 9.8$ Hz, δ_C 82.0 *d*) and a trisubstituted olefin (δ_H 5.37 *br s*, δ_C 122.3 *d*, 133.3 *s*). The ^{13}C NMR (Table 2) also indicated the presence of three methyls, four methylenes, three methines, a quaternary carbon, and a carbonyl carbon on a γ -lactone. Detailed analysis of the H-H, C-H COSYs and heteronuclear multiple bond correlation (HMBC) spectra revealed that the structure of **1** was completely identical with α -cyclodihydrocostunolide (**4**) derived from the known α -santonin (**5**) [8], except for the optical rotation (**1**: $[\alpha]_D -92.5^\circ$, **4** [**8**]: $[\alpha]_D + 71.8^\circ$). In the ^{13}C NMR spectrum, the signals at C-13 and 14 of **4** were assigned at δ 17.3, and 12.4, however, these assignments should be reversed from the analysis of C-H COSY and HMBC spectra. From the above spectral evidence, the structure of **1** was established to be *ent*- α -cyclodihydrocostunolide.

The ^{13}C NMR and IR spectra of **2**, $C_{17}H_{24}O_4$ (HR-MS m/z 292.1657 $[M]^+$), showed the presence of a γ -lactone (1795 cm^{-1} , δ_C 178.7 *s*) and an acetoxy group ($1755, 1250\text{ cm}^{-1}$, δ_C 21.4 *q*, 170.1 *s*). The 1H and ^{13}C NMR spectra (Tables 1 and 2) of **2** showed the presence of two tertiary methyls, a secondary methyl, an acetoxy methyl, a methine proton bearing an acetoxy group (δ_H 5.68 *d*, $J = 11.7$ Hz, δ_C 73.6 *d*), a tetra-substituted olefin (δ_C 129.2, 130.7 each *s*) and a γ -lactone group (δ_H 4.21 *ddd*, $J = 12.2, 11.7, 4.4$ Hz, δ_C 77.0 *d*, 178.7 *s*). Furthermore, the DEPT spectrum indicated four methylenes, two methines and a quaternary carbon, respectively. The above spectral data suggested a eudesmane-type sesquiterpenoid. Analysis of the H-H and C-H COSY spectra suggested the presence of two partial structures as shown in Fig. 1. In the HMBC spectrum (Fig. 2), the tertiary methyl (δ_H 1.19) was correlated with a methylene carbon (δ_C 40.7, C-1) in partial structure. A, a methylene carbon

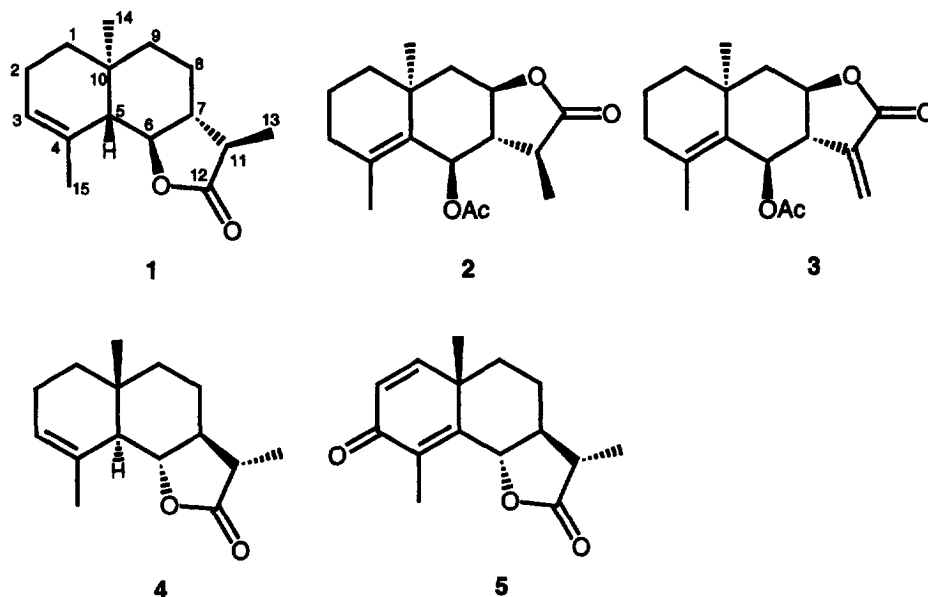
Table 1. ^1H NMR data of compounds 1–3 (TMS, CDCl_3 , 400 MHz)

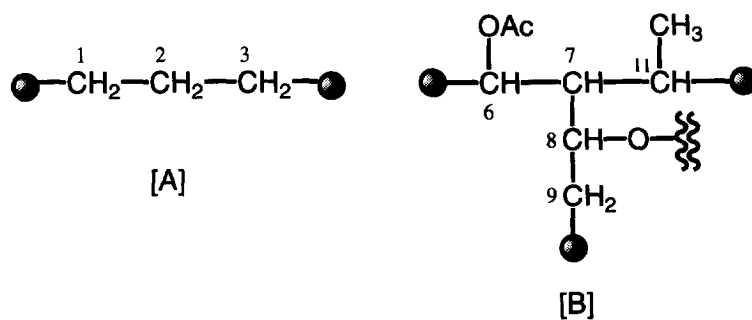
H	1	2	3
1	1.28–1.51 <i>m</i> 1.54–1.62 <i>m</i>	1.43–1.50 <i>m</i> 1.53–1.64 <i>m</i>	1.44–1.67 2H, <i>m</i>
2	1.54–1.62 <i>m</i> 2.04–2.11 <i>m</i>	1.53–1.64 2H, <i>m</i>	1.44–1.67 2H, <i>m</i>
3	5.37 <i>br s</i>	1.92–2.08 2H, <i>m</i>	2.01 2H, <i>m</i>
5	2.21 <i>br d</i>		
6	3.88 <i>dd</i> , $J = 11.7, 9.8$ Hz	5.68 <i>d</i> , $J = 11.7$ Hz	5.73 <i>dd</i> , $J = 11.2, 1.5$ Hz
7	1.54–1.62 <i>m</i>	1.89 <i>q</i> , $J = 11.7$ Hz	2.79 <i>tt</i> , $J = 11.2, 11.2, 2.9$ Hz
8	1.82 <i>m</i> 2.04–2.11 <i>m</i>	4.21 <i>ddd</i> , $J = 12.2, 11.7, 4.4$ Hz	4.19 <i>ddd</i> , $J = 12.2, 11.2, 3.9$ Hz
9	1.28–1.51 2H, <i>m</i>	1.43–1.50 <i>m</i> 2.07 <i>dd</i> , $J = 12.2, 4.4$ Hz	1.44–1.67 <i>m</i> 2.11 <i>dd</i> , $J = 11.7, 3.9$ Hz
11	2.28 <i>quit. like</i>	2.55 <i>six.</i> , $J = 6.8$ Hz	
13	1.21 3H, <i>d</i> , $J = 6.8$ Hz	1.24 3H, <i>d</i> , $J = 6.8$ Hz	5.53 <i>d</i> , $J = 2.9$ Hz 6.13 <i>d</i> , $J = 2.9$ Hz
14	0.91 3H, <i>s</i>	1.19 3H, <i>s</i>	1.20 3H, <i>s</i>
15	1.80 3H, <i>s</i>	1.76 3H, <i>s</i>	1.74 3H, <i>s</i>
OAc		2.13 3H, <i>s</i>	2.17 3H, <i>s</i>

(δ_{C} 44.7, C-9) in partial structure B, a tetrasubstituted olefinic carbon (δ_{C} 130.7, C-5) and a quaternary carbon (δ_{C} 37.2, C-10). The olefinic methyl (δ_{H} 1.76) was correlated with two tetrasubstituted olefinic carbons (δ_{C} 129.2, 130.7, C-4, C-5), a methylene carbon (δ_{C} 36.1, C-3) in partial structure A and a methylene carbon (δ_{C} 72.5, C-6) in partial structure B. The secondary methyl (δ_{H} 1.24) was correlated with a carbonyl carbon (δ_{C} 178.7, C-12) and two methine carbons. The methine proton (δ_{H} 5.68) bearing acetoxy group was correlated with two tetrasubstituted olefinic carbons (C-4 and C-5). Thus, the structure of **2** is that of a eudesman-8,12-olide. The stereochemistry of **2** was demonstrated by the difference NOE spectrum. NOEs were observed between (i) H-

14 and H-6 α ; (ii) H-14 and H-8 α ; and (iii) H-8 α and H-11 α , respectively. Furthermore, X-ray crystallographic analysis of **2** was carried out and the ORTEP drawing is shown in Fig. 3. Thus, the stereostructure of densilobolide-A is depicted as **2**.

The IR, ^1H and ^{13}C NMR spectra (Tables 1 and 2) of **3**, $\text{C}_{17}\text{H}_{22}\text{O}_4$ (HR-MS m/z 290.1507 $[\text{M}]^+$), were similar to those of **2** indicating that **3** is an eudesman-8,12-olide. In the ^1H NMR spectrum (Table 1) of **3**, the secondary methyl H₃-13 found in **2** disappeared and was replaced by *exo*-methylenic protons (δ_{H} 5.53, 6.13). Thus, compound **3** was eudesman-8,12-olide with an *exo*-methylene group at C-11. Furthermore, reduction of **3** by sodium borohydride gave a dihydroderivative, the spectral data of which were com-



Fig. 1. The partial structures of **2**.

pletely identical with those of **2**. From the above data, the stereostructure of densilobolide-B is as depicted in **3**. Considering the co-occurrence of *ent*- α -cyclodihydrocostunolide (**1**) in this species, the absolute configuration of **2** and **3** are suggested to be the same as **1**.

In a previous paper, we reported that there are six chemo-types in *Frullania* species [9]. The present species contained eudesmane-type, sesquiterpene lactones as major components, thus, it is classified as a sesquiterpene lactone-type (Type-II). Generally, *Frullania* species produce various sesquiterpene lactones [1, 2]. This is the first report of the isolation of eudesman-8,12-olides from the Frullaniaceae.

EXPERIMENTAL

Mp: uncorr.; ^1H and ^{13}C NMR: 400 MHz (^1H NMR) and 100.16 or 50.31 MHz (^{13}C NMR). Chemical shift values were expressed in δ (ppm) downfield from TMS as int. standard (^1H NMR) and δ 77.03 (ppm) from CHCl_3 as a standard (^{13}C NMR). TLC: visualized under UV (254 nm) light and by spraying

with 10% H_2SO_4 or Godin reagent [10] followed by heating.

Plant material. *Frullania densiloba* Evans was collected in Tokushima, Japan, in May, 1995, and identified by Dr M. Mizutani, Hattori Botanical Laboratory, Miyazaki, Japan. A voucher specimen was deposited at the Institute of Pharmacognosy, Tokushima Bunri University.

Extraction and isolation. Dried *F. densiloba* (77.8 g) was mechanically ground and extracted with Et_2O for 4 weeks. The crude extract was filtered and evaporated *in vacuo* to give a residue (966 mg) which was divided into 5 frs by CC on Sephadex LH-20 (CH_2Cl_2 -MeOH 1:1). Fr. 3 was chromatographed on silica gel (*n*-hexane-EtOAc gradient) to give frs A-E. Fr. C was rechromatographed on MPLC (Lobar[®] column, *n*-hexane-EtOAc 19:1) and recrystallized from pentane to give *ent*- α -cyclodihydrocostunolide (**1**) (10 mg). Fr. D was rechromatographed on MPLC (Lobar[®] column, *n*-hexane-EtOAc 9:1) and prep. HPLC (NUCLEOSIL 50-5, *n*-hexane-EtOAc 9:1) to afford densilobolide-B (**3**) (45 mg) and densilobolide-A (**2**) (18 mg).

Ent- α -cyclodihydrocostunolide (1). Mp 133–135°; $[\alpha]_{\text{D}} -92.5^\circ$ ($c = 3.6$; CHCl_3). FTIR ν_{max} cm^{-1} : 1780 (γ -lactone); EI-MS m/z (rel. int.): 234 [M^+] (36), 219 (100), 205 (8), 191 (12), 179 (7), 161 (24), 145 (23), 137 (9), 119 (13), 107 (24), 93 (17), 81 (12), 67 (8), 55 (13), 41 (11); HR-MS m/z 234.1613. Calcd for $\text{C}_{15}\text{H}_{22}\text{O}_2$: 234.1619; ^1H and ^{13}C NMR: Tables 1 and 2; CD: $\Delta\epsilon$ (nm) -3.69 (219) ($c = 5.3 \times 10^{-4}$; MeOH).

Densilobolide-A (2). Mp 105–107°. $[\alpha]_{\text{D}} +71.7^\circ$ ($c = 3.56$; CHCl_3). FTIR ν_{max} cm^{-1} : 1795 (γ -lactone),

Table 2. ^{13}C NMR data of compounds **1–3** (CDCl_3 , 100 MHz)

C	1	2	3
1	39.6	40.7	40.7
2	23.2	18.3	18.2
3	122.3	36.1	36.1
4	133.3	129.2	129.5
5	50.7	130.7	130.7
6	82.0	73.6	72.5
7	54.1	56.8	52.6
8	22.9	77.0	77.6
9	37.9	44.7	44.9
10	35.9	37.2	37.2
11	40.8	40.9	137.1
12	179.8	178.7	169.9*
13	12.5	14.2	119.6
14	17.4	26.5	26.1
15	23.6	20.5	20.0
OAc		21.4	21.2
		170.1	170.1*

* May be interchanged.

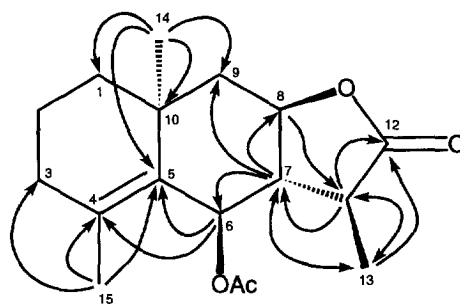
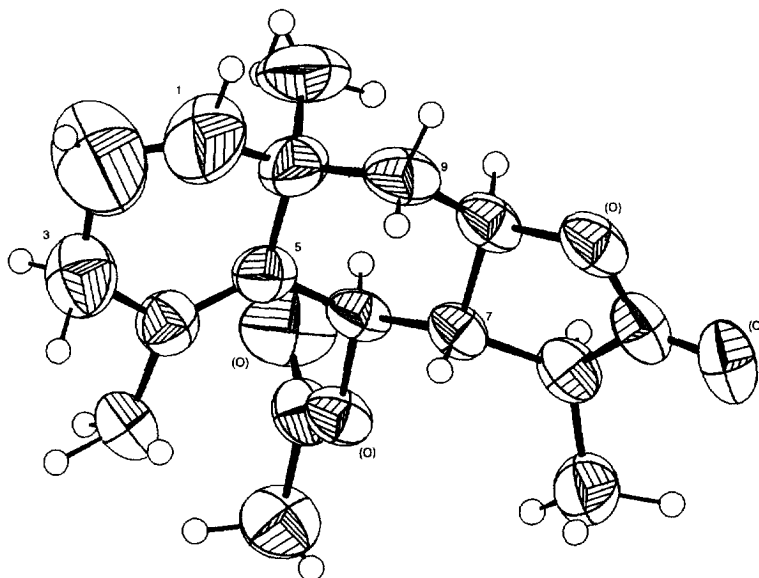


Fig. 2. Long-range C-H correlations in the HMBC spectrum of **2**.

Fig. 3. ORTEP drawing of **2**.

1750, 1250 (OAc); EI-MS m/z (rel. int.): 292 $[M]^+$ (7), 250 (19), 232 (100), 217 (37), 204 (27), 188 (77), 176 (66), 161 (78), 143 (55), 119 (47), 105 (57), 91 (54), 79 (30), 67 (18), 55 (25), 43 (54). HR-MS m/z 292.1657. Calcd for $C_{17}H_{24}O_4$: 292.1675; 1H and ^{13}C NMR: Tables 1 and 2. CD: $\Delta\epsilon_{213\text{ nm}} -1.17$ ($c = 3.5 \times 10^{-4}$, MeOH).

X-ray crystallographic analysis data of 2. X-Ray crystallographic analysis were carried out on a Mac Science MXC 18 diffractometer with $CuK\alpha$ radiation. The structure was solved by direct methods using *Monte-Carlo Multane* and refined by full-matrix least-squares. **2** was recrystallized from *n*-hexane– Et_2O . Crystal data $M_f = C_{17}H_{24}O_4$, $M_r = 292.00$, monoclinic, $P2_1$ (#4), $a = 10.633(3)$, $b = 7.166(2)$, $c = 10.594(3)$ Å, $\beta = 92.57(2)^\circ$, $V = 806.4(4)$ Å³, $Z = 2$, $D_x = 1.30$ g cm⁻³, $\mu = 6.05$ cm⁻¹ ($CuK\alpha$), λ ($CuK\alpha$) = 1.54178, $F(000) = 316$, Maximum $\sin(\theta)/\lambda = 0.579$, total reflections measured 1512, unique reflections 1279, $R_{int} = 0.02$, reflections used 1261, no. of variables 253, $R = 0.068$, $R_w = 0.092$, $S = 1.82$.

Densilobolide-B (3). Mp 133–134°. $[\alpha]_D -75.9^\circ$ ($c = 4.50$). FTIR ν_{max} cm⁻¹: 1785 (γ -lactone), 1750, 1250 (OAc); EI-MS m/z (rel. int.): 290 $[M]^+$ (3), 248 (3), 230 (100), 215 (54), 201 (17), 187 (26), 173 (12), 159 (20), 145 (18), 128 (17), 121 (99), 105 (22), 93 (31), 79 (19), 65 (9), 55 (9), 43 (35). HR-MS m/z 290.1507. Calcd for $C_{17}H_{22}O_4$: 290.1518; 1H and ^{13}C NMR: Tables 1 and 2. UV λ_{max} nm (log ϵ): 212 (3.75) ($c = 3.5 \times 10^{-4}$, MeOH); CD: $\Delta\epsilon_{245\text{ nm}} +0.19$, $\Delta\epsilon_{216\text{ nm}} -0.88$ ($c = 3.5 \times 10^{-4}$, MeOH).

Reduction of 3. To a suspension of **3** (22 mg) in $EtOAc$ (2.5 ml) was added $NaBH_4$ (20 mg) and the mixture stirred for 40 min at room temp. Work-up as usual gave a residue which was chromatographed on silica gel to give a dihydroderivative (21 mg) whose spectral data were completely identical with those of **2**.

Acknowledgements—We thank Dr T. Hashimoto and Miss Y. Okumura (T.B.U.) for collection of the liverwort and Dr M. Mizutani (The Hattori Botanical Laboratory, Miyazaki, Japan) for identification of the species. Thanks are also due to Miss Y. Okamoto (TBU) for measurements of mass spectra.

REFERENCES

- Asakawa, Y., in *Progress in the Chemistry of Organic Natural Products*, Vol. 42, ed. W. Herz, H. Grisebach and G. W. Kirby. Springer, Vienna, 1982, p. 1.
- Asakawa, Y., in *Progress in the Chemistry of Organic Natural Products*, Vol. 65, ed. W. Herz, G. W. Kirby, R. E. Moore, W. Steglich and Ch. Tamm. Springer, Vienna, 1995, p. 1.
- Asakawa, Y., in *Bryophytes Development: Physiology and Biochemistry*, ed. R. N. Chopra and S. C. Bhatla. CRC Press Boca Raton, FL, 1990, p. 259.
- Asakawa, Y., in *Bryophytes: Their Chemistry and Chemical Taxonomy*, ed. H. D. Zinsmeister and R. Mues. Oxford University Press, Oxford, 1990, p. 369.
- Asakawa, Y., Matsuda, R., Toyota, M., Hattori, S. and Mizutani, M., *Phytochemistry*, 1981, **20**, 2187.
- Nagashima, F., Takaoka, S., Asakawa, Y. and Huneck, S., *Phytochemistry*, 1994, **37**, 1317.
- Nagashima, F., Toyota, M. and Asakawa, Y., *Phytochemistry*, 1990, **29**, 2169.
- Cardona, L., García, B., Giménez, J. E. and Pedro, J. R., *Tetrahedron*, 1992, **48**, 851.
- Toyota, M., Nagashima, F. and Asakawa, Y., *Phytochemistry*, 1988, **27**, 1789.
- Godin, P., *Nature (London)*, 1954, **174**, 134.