Clerodane-type Diterpenoids from the Japanese Liverwort *Jungermannia infusca* (MITT.) STEPH.

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Three new clerodane-type diterpenoids have been isolated from the Japanese liverwort *Jungermannia infusca* (MITT.) STEPH., together with previously known compounds, nine clerodane- and four labdane-type diterpenoids, and δ-tocopherol. The structures of the new compounds were confirmed by 2D NMR experiments and X-ray crystallographic analysis.

Key words *Jungermannia infusca*; liverwort; clerodane-type; labdane-type; infuscolide A; diterpenoid

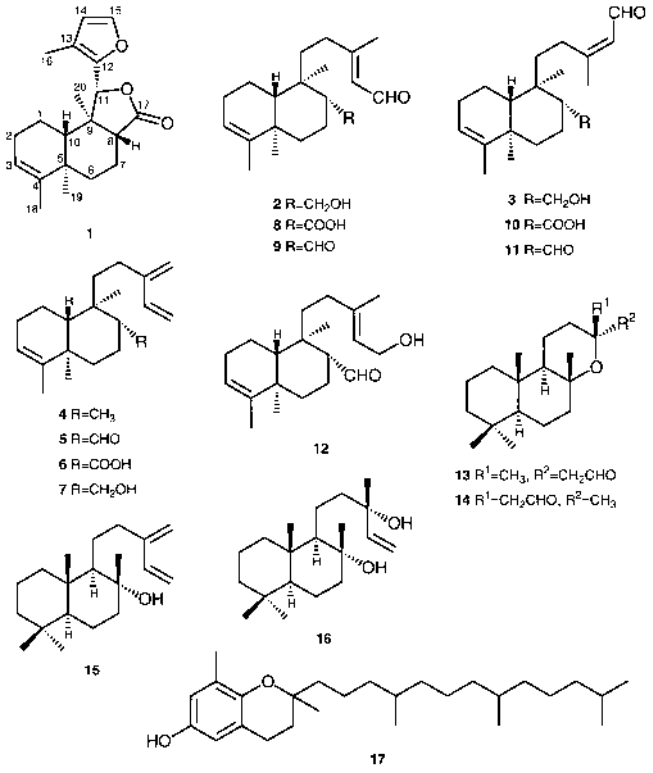
A number of liverworts (Hepaticae) have characteristic fragrant odors and an intense hot and bitter taste. Generally, liverworts are not damaged by insects, snails, slugs and other small animals. Furthermore, some liverworts cause potent allergenic contact dermatitis. Most of the Hepaticae possess cellular oil bodies which are very important markers for the classification of liverworts.[1—3] We are continuing our studies of the chemical constituents of Hepaticae by determining the structures and biological activity of the isolated compounds and investigating the chemosystematics of Hepaticae. It has been shown that most liverworts contain lipophilic mono-, sesqui- and diterpenoids and phenolic compounds which are present in the oil bodies and the biological activity of liverwort is due to these substances.[4]

As a part of the search for novel skeletal terpenoids, aromatic compounds and biologically active compounds in liverworts, we are focussing on the *Jungermannia* L. belonging to the Jungermanniaceae (Jungermanniales) because many of them have a very characteristic fragrance and/or potent bitter taste. There are 41 *Jungermannia* species in Japan. Among them, *J. infusca* is not only a morphologically but also a chemically interesting species because it is polymorphic and its chemical constituents are very dependent on the collection site.[5] In our previous paper,[6] we reported the isolation and structure characterization of several sesqui- and diterpenoids of *J. infusca.* In order to confirm the chemo-type of this species, we collected it in Ehime prefecture and reinvestigated its chemical constituents. We were able to isolate three new clerodane-type diterpenoids (**1**—**3**), along with their related clerodane- (**4**—**12**) and labdane-type diterpenoids (**13**—**16**) and δ-tocopherol. Here we wish to report the structure determination of the new compounds and the chemosystematics of the present species.

Three new clerodane-type diterpenoids, infuscolide A (**1**), 17-hydroxy-3,13*E*-clerodadien-15-al (**2**) and 17-hydroxy-3,13*Z*-clerodadien-15-al (**3**), were isolated from the ether extract of *J. infusca*, together with known clerodane-types: cleroda-3,13(16),14-triene (**4**),[7] cleroda-3,13(16),14-trien-17-al (**5**),[8] infuscaic acid (**6**) which possesses superoxide anion radical release inhibitory activity,[9] cleroda-3,13(16),14-trien-17-ol (**7**),[9] 15-oxo-3,13*E*-clerodadien-17-oic acid (**8**),[8,10] cleroda-3,13*E*-diene-15,17-dial (**9**),[8] 15-oxo-3,13*Z*-clerodadien-17-oic acid (**10**),[8,10] cleroda-3,13*Z*-diene-15,17-dial (**11**)[8] and 15-hydroxy-3,13*E*-clerodadien-17-al (**12**),[9] labdane-types: gomeraldehyde (**13**),[9] *epi*-gomeraldehyde (**14**),[9] isoabienol (**15**)[11] and 13-*epi*-sclareol (**16**),[12] and δ-tocopherol (**17**).[13]

The IR spectrum of **1** showed the presence of a γ-lactone (1780 cm^{-1}). The molecular formula was found to be $C_{20}H_{36}O_3$ (obs. *m/z* 314.1870 $[M]^+$) by high-resolution electron impact mass spectrometry (HR-EIMS), indicating eight degrees of unsaturation. The ^{1}H- and ^{13}C-NMR (Tables 1 and 2) spectra of **1** showed the presence of a trisubstituted double bond, two methines and carbonyl carbon of the lactone ring, and two methines and two quaternary carbons of an α,β-disubstituted furan ring, together with two tertiary methyls, two olefinic methyls, four methylenes, two methines and two quaternary carbons. The gross structure of **1** was suggested to be a *trans*-clerodane-type diterpenoid by comparison of the above spectral data with those of compounds **4**—**12**.

The ^{1}H–^{1}H correlated spectroscopy (^{1}H–^{1}H COSY) and the heteronuclear multiple quantum coherence (HMQC) of **1** showed two partial structures, (i) –C=CH–CH_2–CH_2–CH–



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and (ii) $-CH_2-CH_2-CH-$. Moreover, long-range $^1H-^{13}C$ correlations were observed in the heteronuclear multiple bond correlation (HMBC) experiment as shown in Fig. 1. Subsequently, the phase sensitive nuclear Overhauser enhancement and exchange spectroscopy (NOESY) spectrum of **1** showed NOEs between (i) H-11 and H-8, H-10, (ii) H-10 and H-8, H-6β, (iii) H-19 and H-1α, H-6α, H-7α, H-20, and (iv) H-20 and H-1α, H-7α, H-19, respectively. Conclusive evidence of the structure of **1** was obtained by X-ray crystallographic analysis as shown in Fig. 2. Thus, the relative stereochemistry of infuscolide A was assigned as 12,15-epoxy-3,12,14-clerodatrien-17,11-olide (**1**). The circular dichroism (CD) spectrum of **1** indicated first positive (296 nm), second negative (240 nm) and third positive (226 nm) Cotton effects, however, the absolute configuration of **1** was not obtained by this analysis.

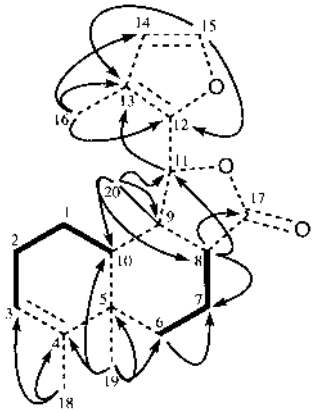

Fig. 1. $^1H-^1H$ Correlations (Bold Lines) and Long-Range $^1H-^{13}C$ Correlations (Arrows) of **1**

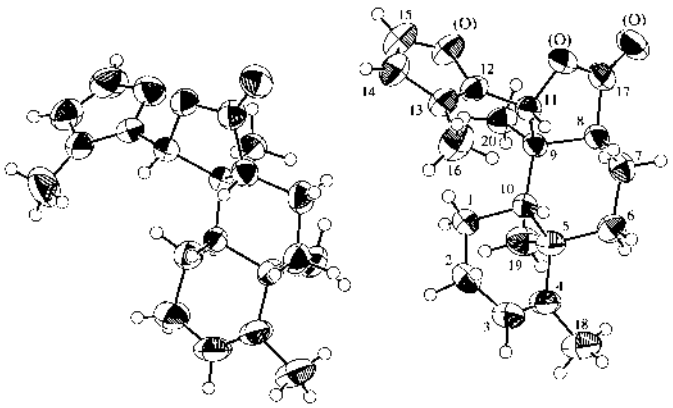

Fig. 2. The ORTEP Drawing of **1**

The EIMS of **2** showed *m/z* 304 $[M]^+$, and its molecular formula was found to be $C_{20}H_{32}O_2$ by HR-EIMS. The IR spectrum confirmed the presence of a hydroxyl (3420 cm^{-1}) and a carbonyl group (1670 cm^{-1}). Its ^1H-NMR (Table 1) showed the signals of a formyl proton, a methylene proton bearing a hydroxyl group, two olefinic protons and two tertiary methyls and two olefinic methyls. The ^{13}C-NMR (Table 2) and distortionless enhancement by polarization transfer (DEPT) spectra suggested the presence of two trisubstituted double bonds, a formyl carbon, a methylene bearing a hydroxyl group, together with four methyls, six methylenes, two methines and two quaternary carbons, respectively. These NMR spectra resembled those of *trans*-clerodanes **4**—**12**. The $^1H-^1H$ and long-range $^1H-^{13}C$ correlations of **2** were confirmed by the $^1H-^1H$ COSY, HMQC and HMBC spectra as shown in Fig. 3. On the basis of the above spectral evidence, the structure of **2** was suggested to be a *trans*-clerodane-type diterpenoid with a hydroxyl and a formyl group. In addition, the NOESY spectrum showed NOEs between (i) H-15 and H-16, (ii) H-12 and H-14, and (iii) H-20 and H-19, H-17. From these results, the geometry of the 13,14-double bond is *E*. Thus, the structure of **2** was established as 17-hydroxy-3,13*E*-clerodadien-15-al.

The IR spectrum of **3** showed the presence of a hydroxyl

Table 1. ^1H-NMR Data of **1**—**3** (600 MHz, C_6D_6)

H	1*	2	3
1	1.48 m, α	1.25—1.56 2H, m	1.25—1.43 2H, m
	1.25 m, β		
2	1.96—2.02 2H, m	1.88 m	1.91 m
		1.97 br d (16.2)	1.97 br d (16.2)
3	5.16 br s	5.20 br s	5.20 br s
6	1.94 dt (13.2, 3.3)† α	1.07 ddd (14.3, 14.3, 4.9)	1.08 ddd (12.4, 12.4, 3.3)
	1.29 ddd (13.2, 13.2, 4.1) β	1.64—1.68 m	1.62—1.70 m
7	1.69 dddd (12.9, 12.9, 12.9, 3.3) α	1.20—1.23 m	1.20 m
	1.96—2.02 m, β	1.64—1.68 m	1.62—1.70 m
8	2.30 dd (12.9, 3.0)	1.25—1.56 m	1.25—1.43 m
10	1.75 dd (12.9, 2.2)	1.20—1.23 m	1.23 br d (11.8)
11	4.96 s	1.25—1.56 2H, m	1.25—1.43 2H, m
12		1.63 m	1.89 ddd (13.2, 13.2, 5.2)
		1.80 ddd (12.4, 12.4, 5.2)	2.26 ddd (12.4, 12.4, 4.7)
14	6.24 d (1.9)	5.91 dd (8.0, 1.1)	5.80 dd (7.4, 1.4)
15	7.36 d (1.9)	9.91 d (8.0)	9.96 d (7.4)
16	2.07 s	1.59 s	1.48 d (1.4)
17		3.00 t (10.2)	3.01 dd (10.4, 6.9)
		3.41 dd (10.2, 4.4)	3.42 dd (10.4, 5.2)
18	1.62 dd (3.6, 2.2)	1.59 d (1.1)	1.59 sext. like
19	1.03 s	0.96 s	0.95 s
20	1.14 s	0.60 s	0.58 s

* Measured in $CDCl_3$. † Coupling constants (*J* in Hz) are given in parentheses.

Table 2. ^{13}C-NMR Data of **1**—**3** (150 MHz, C_6D_6)

C	1*	2	3†
1	19.2	18.0	18.16
2	26.0	27.0	27.1
3	120.6	120.9	120.9
4	143.0	143.9	144.0
5	38.0	38.17‡	38.2
6	37.1	36.6	36.7
7	16.6	22.3	22.6
8	54.4	44.3	44.4
9	49.1	38.23‡	38.7
10	51.0	46.7	46.6
11	83.3	36.3	38.0
12	143.9	34.2	26.8
13	120.9	163.1	163.8
14	113.1	127.5	127.7
15	142.4	189.9	189.9
16	10.2	17.2	24.8
17	175.1	64.0	64.3
18	18.3	18.2	18.24
19	21.5	20.0	20.0
20	11.4	19.3	19.1

∗ Measured in $CDCl_3$. † Measured by 100 MHz. ‡ May be interchanged.

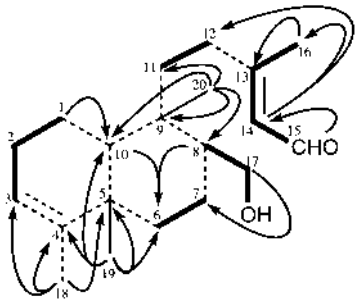

Fig. 3. ^{1}H–^{1}H Correlations (Bold Lines) and Long-Range ^{1}H–^{13}C Correlations (Arrow) of **2**

(3325 cm^{-1}) and an unsaturated carbonyl group (1670 cm^{-1}). The molecular formula of **3**, $C_{20}H_{32}O_2$ (*m/z* 304.2380 $[M]^+$), was confirmed by HR-EIMS. The ^{1}H- and ^{13}C-NMR spectra (Tables 1 and 2) were almost similar to those of **2**, indicating that compound **3** was a *trans*-clerodane-type diterpenoid. Furthermore, detailed analysis of ^{1}H–^{1}H COSY, HMQC and HMBC spectra showed that compound **3** was the geometrical isomer of **2**. The NOESY spectrum of **3** showed NOEs between (i) H-16 and H-14, (ii) H-15 and H-12, (iii) H-20 and H-19, H-17, respectively. Thus, the structure of **3** was confirmed as 17-hydroxy-3,13*Z*-clerodadien-15-al.

The absolute configuration of **1**—**3** could be the same as that of **8** which was established by X-ray crystallographic analysis of the carbamate derived from **8**,[7] coexisting in the same species.

At present, four chemo-types of *J. infusca*; [I] *ent*-kaurane-type, [II] clerodane- and labdane-type, [III] bis(bibenzyl)-type and [IV] cuparane- and diterpene-type, are known.[3,6] The present *J. infusca*, which contains clerodane- and labdane-type diterpenoids as its main constituents, is classified as type [II].

Experimental

Melting points were measured on a Yanagimoto micromelting points apparatus without correction. Optical rotations were measured on a Jasco DIP-1000 polarimeter with $CHCl_3$. IR spectra were recorded on a Jasco FT/IR-5300 infrared spectrophotometer. The ^{1}H- and ^{13}C-NMR spectra were measured on Varian Unity-600 (^{1}H; 600 MHz, ^{13}C; 150 MHz) and Jeol Eclipse-400 (^{1}H; 400 MHz, ^{13}C; 100 MHz) instruments. Chemical shift values expressed in δ (ppm) downfield from tetramethylsilane as an internal standard (^{1}H-NMR), and δ 77.03 (ppm) from $CDCl_3$ and δ 128.0 (ppm) from C_6D_6 as a standard (^{13}C-NMR). Mass spectra were obtained on a Jeol JMS AX-500 instrument. X-ray crystallographic analysis was carried out on a Mac Science DIP-2020 instrument. TLC was carried out using Silica gel 60F_{254} plates (Merck). Column chromatography was performed on Silica gel 60 (Merck, 230—400 and 35—70 mesh), reverse phase silica gel (Cosmosil 140C_{18}, Nacalai), Sephadex™ LH-20 (Amersham Pharmacia Biotech, sol. CH_2Cl_2–MeOH 1 : 1) and Lobar® (LiChroprep, Merck) columns. TLC plates were examined under UV (254 nm) light and by spraying with 10% H_2SO_4 or Godin reagent,[14] followed by heating.

Plant Material *Jungermannia infusca* (Mitt.) Steph. (9509501) was collected in Higashikawadani, Ehime pref. Japan. Dr. M. Mizutani (The Hattori Botanical Laboratory, Miyazaki, Japan) identified this species and a voucher specimen was deposited at the Institute of Pharmacognosy, Tokushima Bunri University.

Extraction and Isolation The ether extract (4.5 g) of *J. infusca* was divided into nine fractions by column chromatography (CC) on silica gel (35—70 mesh) using an *n*-hexane–EtOAc gradient solvent system. Cleroda-3,13(16),14-triene (**4**, 30 mg) was isolated from fr. 1. fr. 2 was chromatographed on silica gel (230—400mesh, *n*-hexane–EtOAc 19 : 1) and Sephadex™ LH-20 to give clerod-3,13(16),14-trien-17-al (**5**, 30 mg). Fraction 3 was divided into five fractions (3-1—3-5) by CC on Sephadex™ LH-20 and silica gel (230—400 mesh, *n*-hexane–EtOAc 9 : 1). Gomeraldehyde (**13**, 163 mg) and isoabienol (**15**, 391 mg) were isolated from fr. 3-2 and fr. 3-4, respectively. *epi*-Gomeraldehyde (**14**, 37 mg) was purified by preparative HPLC (NUCLEOSIL 50-5, *n*-hexane–EtOAc 9 : 1) of fr. 3-3. Fraction 3-5 was also purified by preparative HPLC (NUCLEOSIL 50-5, *n*-hexane–EtOAc 9 : 1) to give δ-tocopherol (**17**, 16 mg) ($[\alpha]_D^{19}$ +1.0° *c*=1.53; lit.[15] $[\alpha]_D^{25}$ +3.4° *c*=15.48, EtOH. $[\alpha]_D^{25}$ +1.1° *c*=10.86, benzene).

Fraction 4 was divided into three fractions (4-1—4-3) by CC on Sephadex™ LH-20 and reverse phase silica gel (Cosmosil 140C_{18}, CH_3CN). Fraction 4-1 was subjected to preparative HPLC (NUCLEOSIL 50-5, *n*-hexane–EtOAc 4 : 1) to give infuscolide A (**1**, 3 mg) and cleroda-3,13*Z*-diene-15,17-dial (**11**, 4 mg). Fraction 4-2 was methylated, and rechromatographed on silica gel (230—400 mesh, *n*-hexane–EtOAc 4 : 1) to give the methyl ester (33 mg) of infuscaic acid (**6**) and cleroda-3,13(16),14-trien-17-ol (**7**, 34 mg). Fraction 5 was rechromatographed on Sephadex™ LH-20 and silica gel (230—400 mesh, CH_2Cl_2–Et_2O 97 : 3) to give infuscaic acid (**6**, 306 mg) and cleroda-3,13*E*-diene-15,17-dial (**9**, 25 mg).

Fraction 7 was chromatographed on Sephadex™ LH-20 and reverse phase silica gel (Cosmosil 140C_{18}, CH_3CN) to yield 15-oxo-3,13*Z*-clerodadien-17-oic acid (**10**, 86 mg) and a mixture of diterpenes. The diterpene fraction was rechromatographed on silica gel (230—400 mesh, CH_2Cl_2–EtOAc 19 : 1) to give 17-hydroxy-3,13*E*-clerodadien-15-al (**2**, 5 mg), 17-hydroxy-3,13*Z*-clerodadien-15-al (**3**, 3 mg) and 15-hydroxy-3,13*E*-clerodadien-17-al (**12**, 3 mg). Fraction 8 was purified by CC on Sephadex™ LH-20 and Lobar® (RP-8 40—63 μm, CH_3CN) to give 13-*epi*-sclareol (**16**, 147 mg) and 15-oxo-3,13*E*-clerodadien-17-oic acid (**8**, 223 mg).

Infuscolide A (**1**): mp 130—132 °C. $[\alpha]_D^{23}$ −90.9° (*c*=1.22). FT-IR cm^{-1}: 1780 (lactone). UV (MeOH) λ_{max} (log ε): 222 nm (3.73) (*c*=3.4×10^{-4}). CD (MeOH): $\Delta\varepsilon_{296\,nm}$ +0.82, $\Delta\varepsilon_{240\,nm}$ −0.17, $\Delta\varepsilon_{226\,nm}$ +0.25 (*c*=3.4×10^{-4}). ^{1}H- and ^{13}C-NMR: Tables 1 and 2. HR-EIMS: obs. 314.1870 $C_{20}H_{26}O_3$ requires 314.1882. EIMS *m/z* (int.): 314 $[M]^+$ (26), 176 (73), 161 (100), 147 (12), 133 (32), 121 (28), 111 (53), 95 (19), 81 (15), 69 (10), 55 (12), 44 (27).

X-ray crystallographic analysis of **1**: All diagrams were prepared and calculations were performed using maXus (MacScience, Japan). Data collection: DIP Image plate. Data reduction: maXus. Program used to solve the structure: maXus *SIR92*. Program used to refine the structure: maXus. Crystal data: $C_{20}H_{26}O_3$, M.W.=314, monoclinic, $P2_1$, *a*=0.377(0) Å, *b*=10.021(0) Å, *c*=17.158001(0) Å, *V*=1774.199951(0) Å^3, *Z*=4, MoKα (λ=0.71073 Å), D_x=1.530 Mg/cm^3, θ=1—20°, μ=0.77 mm^{-1}, 2823 reflections, 426 parameters, *R*=0.056, R_w=0.141, *S*=2.325, only coordinates of H atoms refined, Extinction correction: no atomic scattering factors.[16]

17-Hydroxy-3,13*E*-clerodadien-15-al (**2**): $[\alpha]_D^{20}$ −94.5° (*c*=0.44). FT-IR cm^{-1}: 3418 (OH), 1670 (C=O). ^{1}H- and ^{13}C-NMR: Tables 1 and 2. HR-EIMS: obs. 304.2430 $C_{20}H_{32}O_2$ requires 304.2402. EIMS *m/z* (int.): 304 $[M]^+$ (11), 302 (16), 286 (24), 252 (19), 205 (33), 187 (41), 145 (33), 119 (60), 107 (55), 95 (100), 81 (52), 69 (31), 55 (35), 45 (6).

17-Hydroxy-3,13*Z*-clerodadien-15-al (**3**): $[\alpha]_D^{14}$ −152.9° (*c*=0.48). FT-IR cm^{-1}: 3325 (OH), 1670 (C=O). ^{1}H- and ^{13}C-NMR: Tables 1 and 2. HR-

EIMS obs. *m/z* 304.2380 $C_{20}H_{32}O_2$ requires 304.2402. EIMS *m/z* (int.): 304 $[M]^+$ (24), 271 (6), 253 (9), 241 (5), 205 (52), 187 (20), 159 (19), 145 (28), 119 (41), 107 (51), 95 (100), 81 (36), 67 (18), 55 (22), 41 (21).

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