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CLERODANE- AND HALIMANE-TYPE DITERPENOIDS FROM THE LIVERWORT *JUNGERMANNIA HYALINA*

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Key Word Index—*Jungermannia hyalina*; Jungermanniaceae; Hepaticae; *ent*-3β,4β-epoxyclerod-13*E*-en-15-al; *ent*-3β,4β-epoxyclerod-13*Z*-en-15-al; kolavenol; *ent*-3β,4β-epoxyclerod-13*E*-en-15-ol; 3*R**,4*R**-dihydroxyclerod-13*E*-en-15-al; 3*R**,4*R**-dihydroxyclerod-13*Z*-en-15-al; 3ξ-hydroxy-5(10), 13*E*-halimadien-15-al; clerodane; halimane; diterpenoid.

Abstract—Three new clerodane- and one new halimane-type diterpenoids: *ent*-3β,4β-epoxyclerod-13*E*-en-15-ol; 3*R**, 4*R**-dihydroxyclerod-13*E*-en-15-al; 3*R**, 4*R**-dihydroxyclerod-13*Z*-en-15-al; and 3ξ-hydroxy-5(10), 13*E*-halimadien-15-al, were isolated from the German liverwort *Jungermannia hyalina*, together with the previously known clerodane-type diterpenoids *ent*-3β,4β-epoxyclerod-13*E*-en-15-al; *ent*-3β,4β-epoxyclerod-13*Z*-en-15-al and kolavenol. The structures were determined by extensive NMR techniques and chemical degradation.

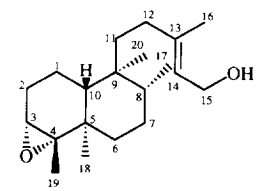
INTRODUCTION

The liverworts of the genus *Jungermannia* are rich sources of diterpenoids [1, 2], and their morphological classification is very difficult because each species is very small. As part of a chemosystematic study of liverworts and a search for biologically active substances [1, 2] we investigated the chemical constituents of *J. hyalina* Leyll collected in Germany. In this paper we report on the isolation and characterization of the structures of three new clerodane-type (**1**–**3**) and a new halimane-type (**4**) diterpenoid along with three previously known clerodane-type diterpenoids (**5**–**7**).

RESULTS AND DISCUSSION

A combination of column chromatography, prep. TLC and prep. HPLC of the ether extract of *J. hyalina* gave three new clerodane-type (**1**–**3**) and a new halimane-type (**4**) diterpenoids as well as the previously known clerodane-type diterpenoids *ent*-3β,4β-epoxyclerod-13*Z*-en-15-al (**5**) [3], *ent*-3β,4β-epoxyclerod-13*E*-en-15-al (**6**) [3] and kolavenol (**7**) [4–6].

The molecular formula of **1** was determined to be $C_{20}H_{34}O_2$ (anal. 306.2563) by HR-mass spectrometry. The IR spectrum showed the presence of a hydroxyl group (3400 cm^{-1}). The ^1H and ^{13}C NMR spectra (Tables 1 and 2) resembled those of **5** and **6**, except for the presence of a hydroxyl group in place of the aldehyde



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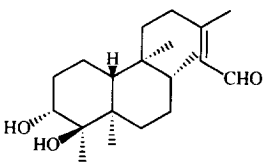

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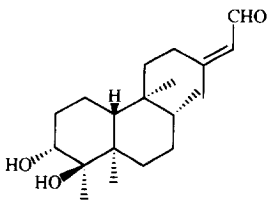

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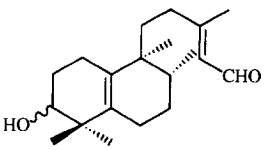

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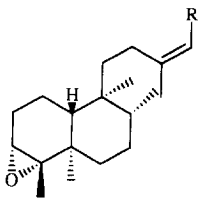

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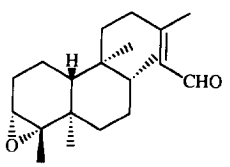
8 R=CH_2OH



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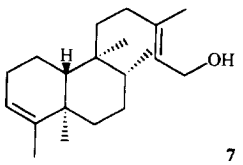

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†Author to whom correspondence should be addressed.

Table 1. ^{1}H NMR data of compounds **1–4**

H	1*	2†	3†	4†
1	1.08–1.18 *m*‡	1.11–1.17 *m*‡	1.13–1.45 *m*‡	1.60 *m*
		1.49–1.56 *m*§	1.47–1.63 *m*§	1.87–1.92 *m*‡
2	1.38–1.47 *m*§	1.43 *m*	1.98 *dddd*, *J* = 13.7, 13.7, 4.6, 3.7 H z	1.45–1.54 *m*§
	2.00 *br d*	1.97 like *tt*	1.13–1.45 *m*‡	
3	2.71 *s*	3.21 *br s*	3.19 *br s*	3.24 *dd*, *J* = 10.7, 4.2 Hz
6	1.08–1.18 *m*‡	1.22–1.33 *m*‖	1.13–1.45 *m* ‡	1.87–1.92 *m*‡
	1.38–1.47 *m*§	1.49–1.56 *m*§	1.47–1.63 *m*§	1.82 *m*
7	1.24–1.36 *m*	1.22–1.33 *m*‖	1.13–1.45 *m*‡	1.26–1.37 *m*‖
		1.38 *m*		
8	1.24–1.36 *m*‖	1.22–1.33 *m*‖	1.13–1.45 *m*‡	1.26–1.37 *m*‖
10	0.82 *d*, *J* = 11.0 Hz	1.74–1.79 *m*¶	1.79 *dd*, *J* = 12.5, 2.2 Hz	
11	1.24–1.36 *m*‖	1.11–1.17 *m*‡	1.13–1.45 *m*‡	1.23 2H, *m*
		1.22–1.33 *m*‡		
12	1.63 *ddd*, *J* = 12.5, 12.5, 5.1 Hz	1.74–1.79 *m*¶	2.03 *ddd*, *J* = 12.5, 12.5, 4.4 Hz	1.72 *m*
	1.80 *ddd*, *J* = 13.2, 13.2, 5.1 Hz		2.18 *ddd*, *J* = 12.5, 12.5, 5.1 Hz	1.45–1.54 *m*§
14	5.42 *br t*	5.89 *d*, *J* = 7.8 Hz	5.79 *dd*, *J* = 8.1, 1.2 Hz	5.91 *dd*, *J* = 7.8, 1.2 Hz
15	4.00 2H, *d*, *J* = 6.6 Hz	9.88 *d*, *J* = 7.8 Hz	10.03 *d*, *J* = 7.6 Hz	9.91 *d*, *J* = 7.8 Hz
16	1.50 3H, *s*	1.54 3H, *s*	1.43 3H, *d*, *J* = 1.0 Hz	1.57 3H, *d*, *J* = 1.2 Hz
17	0.74 3H, *d*, *J* = 5.9 Hz	0.68 3H, *d*, *J* = 6.6 Hz	0.68 3H, *d*, *J* = 6.6 Hz	0.72 3H, *d*, *J* = 6.8 Hz
18	1.15 3H, *s*	1.15 3H, *s*	1.14 3H, *d*, *J* = 1.0 Hz	1.04 3H, *s*[a]
19	1.10 3H, *s*	1.01 3H, *s*	1.02 3H, *s*	1.00 3H, *s*[a]
20	0.60 3H, *s*	0.67 3H, *s*	0.64 3H, *s*	0.71 3H, *s*

*In $CDCl_3$ (400 MHz).
†In C_6D_6 (600 MHz).
‡,§,‖,¶Overlapped signals in each column.
[a]May be interchanged.

group, indicating that **1** might be a clerodane-type diterpenoid with a hydroxyl group at C-15. Compounds **5** and **6** were reduced with $LiAlH_4$ to afford monohydroxy alcohols **8** and **1**. The physical and spectral data of **8** and **1** were completely identical with those of the natural product **1**. From the above spectral and chemical evidence, the absolute structure of **1** was established as *ent*-3β,4β-epoxyclerod-13*E*-en-15-ol.

The IR and NMR spectra (Tables 1 and 2) of **2**, $C_{20}H_{34}O_3$ (anal. 322.2486), indicated the presence of a secondary hydroxyl group (3500 cm^{-1}; δ_H3.21 *br s*; δ_C76.4 *d*), a conjugated aldehyde (1670 cm^{-1}; δ_H9.88 *d*; δ_C189.5 *d*) and a tertiary alcohol (δ75.9 *s*) which was confirmed by the formation of a mono acetate **9** ($C_{22}H_{36}O_4$, anal. 364.2601; 1750, 1260 cm^{-1}; δ1.70, 3H, *s*). The spectral data of **2** were very similar to those of **5**, suggesting that **2** was a clerodane-type diol with the same functional groups as those of **5** except for the absence of an epoxide group. This assumption was confirmed as follows. Analysis of ^{1}H–^{1}H COSY and heteronuclear single-quantum coherence (HSQC) spectra indicated the presence of three partial segments; (A) CH_3(17)–CH(8)–CH_2(7)–, (B) –(HO)CH(3)–CH_2(2)–CH_2(1)–CH(10)– and (C) –CH_2(11)–CH_2(12)–. Furthermore, the HMBC spectrum showed crosspeaks between (i) Me-16 and C-12, C-13 and C-14, (ii) Me-17 and C-7, C-8 and C-9, (iii) Me-18 and C-4, C-5, C-6 and C-10, (iv) Me-19 and C-3, C-4 and C-5, (v) Me-20 and C-8, C-9, C-10 and C-11, (vi) H-3 and C-1, C-4 and C-5 and (vii) H-15 and C-14, respectively. This spectral evidence confirmed that **2** was a clerodane-type diterpenoid with two hydroxyl groups at C-3 and C-4 and an aldehyde at C-15. The stereochemistry of **2** was clarified by the NOESY spectrum of the mono acetate **9**. As a NOE was observed between H-15 and Me-16, the $\Delta^{13,14}$ double bond was assigned the *E* configuration. Moreover, NOEs were observed between (i) H-3 and H-2α, (ii) H-3 and H-2β, (iii) H-3 and H-10, (iv) H-10 and H-2β, (v) H-10 and H-8, (vi) Me-18 and Me-19 and (vii) Me-18 and Me-20. Thus the structure of **2** was established to be 3*R**,4*R**-dihydroxyclerod-13*E*-en-15-al.

The ^{13}C NMR spectral data (Table 2) of **3**, $C_{20}H_{34}O_3$ (anal. 322.2486), were almost identical to those of **2**, indicating that **3** might be the geometrical isomer at C-13/C-14 of **2**. This assumption and the stereochemistry of **3** were confirmed by the NOESY spectrum, in which NOEs were observed between (i) Me-16 and H-14, (ii) H-15 and H-12, (iii) H-3 and H-2, (iv) Me-19 and H-3, (v) Me-18 and Me-20 and (vi) H-10 and H-12, respectively. Thus the structure of **3** was characterized as 3*R**,4*R**-dihydroxyclerod-13*Z*-en-15-al. The absolute configuration of **2** and **3** might be the same as in the *ent*-series, in view of the co-occurrence of compound **1** and the previously known *ent*-clerodanes **5–7**.

The ^{1}H NMR and IR spectra of **4**, $C_{20}H_{32}O_2$ (anal. 304.2400), showed the presence of a secondary hydroxyl (δ3.24 *dd*; 3450 cm^{-1}) and an aldehyde (δ9.91 *d*; 1680 cm^{-1}) group. The ^{1}H NMR spectrum of **4** (Table 1)

Table 2. ^{13}C NMR data compounds of **1–4**

C	1*	2†	3†	4†
1	15.7	16.8	16.9	24.6
2	28.6	30.9	30.9	27.9
3	61.8	76.4	76.5	75.9
4	65.7	75.9	75.9	40.2
5	37.5	41.6	41.6	137.8
6	37.4	32.6	32.6	25.9
7	28.5	26.9	26.7	27.4
8	36.3	36.4	36.3	33.7
9	39.2	38.9	39.3	40.8
10	48.2	40.8	40.7	131.2
11	37.2	36.6	38.1	33.9
12	33.5	34.5	26.9	35.5
13	138.9	163.1	163.8	162.7
14	124.6	127.6	127.6	127.4
15	59.4	189.8	189.2	189.8
16	16.4	17.1	24.7	17.2
17	16.1	16.1	16.1	16.1
18	17.2	17.4	17.4	25.3
19	19.9	21.7	21.7	20.2
20	18.9	18.4	18.3	21.1

*In $CDCl_3$ (100 MHz).
†In C_6D_6 (120 MHz).

Table 3. Long-range correlation between ^1H and ^{13}C for compound **4**

H	C
11	9, 10, 12
12	11, 13, 14, 16
14	12, 16
15	14
16	13, 14
17	7, 8
18	3, 4, 5, 19
19	3, 4, 5, 18
20	8, 9, 10

indicated the presence of three tertiary methyls, a secondary methyl, a methine proton bearing a hydroxyl group (δ3.24 *dd*, J = 10.7, 4.2 Hz), a trisubstituted olefinic proton (δ5.91 *d*, J = 7.8 Hz) and an aldehyde proton (δ9.91 *d*, J = 7.8 Hz). The ^{13}C NMR spectrum (Table 2) also displayed 20 carbons; five methyls, six methylenes, a methine and two quaternary carbons, as well as a methine (δ75.9) bearing a hydroxyl group, an aldehyde methine (δ189.8), two trisubstituted olefinic carbons (δ127.4 *d*, 162.7 *s*) and two tetrasubstituted olefinic carbons (δ131.2, 137.8 each *s*). Analysis of the ^1H–^1H COSY and HSQC spectra supported the presence of four partial segments: (A) –CH_2(6)–CH_2(7)–CH(8)(CH_3)–, (B) –CH_2(11)–CH_2(12)–, (C) –(CH_3)C(13) = CH(14)–CHO and (D) –CH_2(1)–CH_2(2)–CH(3)(OH)–. The connections of the partial segments were deduced from the HMBC spectrum, as shown in Table 3. This spectral evidence indicated a halimane-type diterpenoid structure, with an aldehyde at C-15 and a hydroxyl group at C-3, for compound **4**. The geometry of the C-13/C-14 double bond was determined to be *E* on the basis a NOE between Me-16 and H-15. The stereochemistry of the hydroxyl group at C-3 was presumed to be equatorial from the coupling constant (*dd*, J = 10.7, 4.2 Hz) of H-3. In order to clarify the stereochemistry of **4**, the NOESY spectrum was measured in detail; however, no useful information was obtained. The α-configuration of Me-17 and Me-20 was suggested on the basis of the co-occurrence of the clerodane-type diterpenoids in the same plant. Thus, the gross structure of **4** was assigned as 3ξ-hydroxy-5(10),13*E*-halimadien-15-al.

The occurrence of halimane-type diterpenoids in nature is very rare [7]. This is the third report of a halimane-type diterpenoid from liverworts [8, 9]. Compounds **5–7** from the present species have already been isolated from English *Jungermannia paroica* [3], which is morphologically similar to *J. hyalina* (Dr Mizutani, private communication). The present and other *Jungermannia* species mainly biosynthesize diterpenoids of the kaurane, clerodane and labdane types [1, 2]. These diterpenoids might be important chemical markers of the genus *Jungermannia*.

EXPERIMENTAL

The solvents used for spectral measurements were TMS–C_6D_6 [^1H (600 and 400 MHz) and ^{13}C (120 and 50 MHz) NMR]; $CHCl_3$ ([α]$_D$); UV(MeOH). TLC was carried out as previously reported [10].

Plant material. Jungermannia hyalina Leyll was collected by S.H. in Germany, 1992. The voucher specimen was deposited at the Institute of Pharmacognosy, Tokushima Bunri University.

Extraction and isolation. Air-dried *J. hyalina* (46 g) was extracted with Et_2O and the crude extract (890 mg) was divided into two frs by Sephadex LH-20 CC. Fr. 2 was rechromatographed on silica gel (*n*-hexane–EtOAc gradient) to give fractions A–E. Fr. B was further chromatographed on prep. HPLC (Nucleosil 50–5, *n*-hexane–EtOAc 7:3) to give *ent*-3β,4β-epoxyclerod-13*Z*-en-15-al (**5**) (12.4 mg) [3], *ent*-3β,4β-epoxyclerod-13*E*-en-15-al (**6**) (6.4 mg) [3] and kolavenol (**7**) (6.4 mg) [4–6]. Fr. E was rechromatographed on silica gel and Sephadex LH-20, and then purified by prep. TLC to give *ent*-3β,4β-epoxyclerod-13*E*-en-15-ol (**1**) (3.7 mg), (3*R**,4*R**)-dihydroxyclerod-13*E*-en-15-al (**2**) (3.8 mg), (3*R**,4*R**)-dihydroxyclerod-13*Z*-en-15-al (**3**) (1.4 mg) and 3ξ-hydroxy-5(10), 13*E*-halimadien-15-al (**4**) (2.5 mg), respectively.

ent-3β,4β-Epoxyclerod-13E-en-15-ol (**1**). [α]$_D$–31.4° (*c* 0.64); HREIMS: found [M]$^+$ 306.2563; $C_{20}H_{34}O_2$ requires 306.2559; FTIR ν_{max} cm^{-1}: 3400 (OH); ^1H and ^{13}C NMR: Tables 1 and 2; EIMS *m/z* (re.int): 306 [M]$^+$ (21), 291(19), 273(4), 245(9), 221(5), 207(69), 189(65), 177(12), 163(34), 151(91), 138(100), 125(53), 109(56), 95(71), 81(53), 69(43), 55(51), 43(84).

3R,4R*,-Dihydroxyclerod-13E-en-15-al* (**2**). $[\alpha]_D$ −38.4° (*c* 0.38); HREIMS: found $[M]^+$ 322.2486; $C_{20}H_{34}O_3$ requires 322.2508; FTIR ν_{max} cm^{-1}: 3500 (OH), 1670 (C=O); ^1H and ^{13}C NMR: Tables 1 and 2; EIMS *m/z* (rel. int): 322 $[M]^+$ (9), 249(11), 223(71), 207(48), 189(42), 177(19), 163(31), 149(27), 137(44), 123(37), 109(46), 95(62), 84(100), 69(32), 55(29), 43(38).

3R,4R*,-Dihydroxyclerod-13Z-en-15-al* (**3**). $[\alpha]_D$ −45.0° (*c* 0.11); HREIMS: found $[M]^+$ 322.2513; $C_{20}H_{34}O_3$ requires 322.2508; FTIR ν_{max} cm^{-1}: 3500 (OH), 1670 (C=O); ^1H and ^{13}C NMR: Tables 1 and 2; EIMS *m/z* (rel. int): 322 $[M]^+$ (17), 249(6), 239(14), 223(44), 207(39), 189(35), 177(17), 163(24), 147(14), 137(37), 123(54), 109(46), 95(67), 84(100), 69(36), 55(35), 43(51).

3ξ-Hydroxy-5(10),13E-halimadien-15-al (**4**). $[\alpha]_D$ +104.8° (*c* 0.25); HREIMS: found $[M]^+$ 304.2400; $C_{20}H_{32}O_2$ requires 304.2403; FTIR ν_{max} cm^{-1}: 3450 (OH), 1680 (C=O); ^1H and ^{13}C NMR: Tables 1 and 2; EIMS *m/z* (rel.int): 304 $[M]^+$(1), 286(10), 243(6), 207(100), 173(7), 163(18), 135(31), 119(24), 107(19), 95(19), 81(12), 69(13), 55(15), 41(17).

Acetylation of **2**. Compound **2** (3.8 mg) in Ac_2O (1 ml) and pyridine (1 ml) was kept overnight at room temp. Work-up as usual gave a mono acetate (**9**) (1 mg): HREIMS: found $[M]^+$ 364.2601; $C_{22}H_{36}O_4$ requires 364.2614; FTIR ν_{max} cm^{-1}: 3550 (OH), 1750, 1260 (OAc), 1680 (C=O); ^1H NMR (C_6D_6): δ0.66 (3H, *s*, Me-20), 0.68 (3H, *d*, *J* = 6.6 Hz, Me-17), 0.88 (3H, *s*, Me-19), 1.06 (3H, *s*, Me-18), 1.10–1.18 (*m*), 1.20–1.44 (*m*), 1.53 (3H, *d*, *J* = 1.2 Hz, Me-16), 1.61 (1H, *br t*), 1.70 (3H, *s*, OAc), 1.69–1.74 (3H, *m*), 1.80 (1H, *d*, *J* = 10.7 Hz, H-10), 1.99 (1H, *br t*), 4.82 (1H, *s*, H-3), 5.87 (1H, *d*, *J* = 7.8 Hz, H-14), 9.89 (1H, *d*, *J* = 7.8 Hz, H-15); EIMS *m/z* (rel. int): 364 $[M]^+$ (0.1), 304(4), 286(4), 265(38), 249(7), 207(57), 189(91), 173(7), 163(17), 149(19), 137(29), 123(31), 109(32), 95(49), 84(53), 69(33), 55(38), 43(100).

Reduction of **5**. To a suspension of $LiAlH_4$ (2 mg) in dry Et_2O (2 ml) **5** (14 mg) was added in dry Et_2O (1 ml). The reaction mixture was stirred for 10 min at room temp. and then chromatographed on prep. TLC to give **8** (2 mg): $[\alpha]_D$ −64.3° (*c* 0.14); HREIMS: found $[M]^+$ 306.2560; $C_{20}H_{34}O_2$ requires 306.2559; FTIR ν_{max} cm^{-1}: 3400 (OH); ^1H NMR (400 MHz, C_6D_6): δ0.56 (3H, *s*, Me-20), 0.73 (3H, *d*, *J* = 6.4 Hz, Me-17), 0.81 (1H, *d*, *J* = 10.7 Hz), 1.10 (3H, *s*, Me-19), 1.14 (3H, *s*, Me-18), 1.20 (1H, *m*), 1.62 (3H, *d*, *J* = 1.0 Hz, Me-16), 1.64 (1H, *ddd*, *J* = 12.7, 12.7, 5.4 Hz), 1.78 (1H, *ddd*, *J* = 12.2, 12.2, 4.8 Hz), 2.03 (1H, *br d*), 2.72 (1H, *br s*, H-3), 3.98 (2H, *d*, *J* = 6.4 Hz, H-15), 5.34 (1H, *t*, *J* = 6.3 Hz, H-14), EIMS *m/z* (rel. int): 306 $[M]^+$ (14), 291(13), 273(8), 255(6), 231(1), 207(100), 189(95), 175(13), 163(35), 151(72), 138(75), 119(61), 107(73), 95(86), 81(67), 69(53), 55(58), 43(63).

Reduction of **6**. To a suspension of $LiAlH_4$ (2 mg) in dry Et_2O (2 ml) **6** (7 mg) was added in dry Et_2O (1 ml). The reaction mixture was stirred for 10 min at room temp. and then chromatographed on prep. TLC to give a mono alcohol (6 mg) whose physical and spectral data were identical to those of **1**.

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