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ENT-KAURANE-TYPE DITERPENOIDS FROM THE LIVERWORT *JUNGERMANNIA ROTUNDATA*

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Key Word Index—*Jungermannia rotundata*; *Jungermannia*; Hepaticae; rotundeic acid A; rotundeic acid B; rotundeic acid C; *ent*-kaurane-type diterpenic acid.

Abstract—Three new *ent*-kaurane-type diterpenoids, named rotundeic acids A–C, have been isolated from the Japanese liverwort *Jungermannia rotundata*. Their structures were determined to be *ent*-15α-hydroxykaur-16-en-20-oic acid, *ent*-15α-acetoxykaur-16-en-20-oic acid and *ent*-9α-hydroxykaur-16-en-20-oic acid by extensive NMR techniques. Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

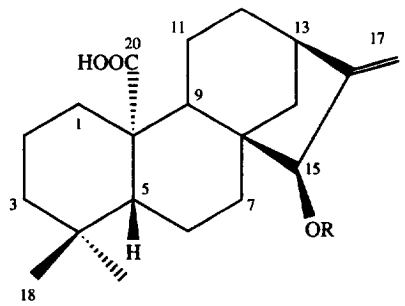
As part of a search for biologically active substances of the Hepaticae, we are continuing to study the chemical constituents of liverworts. Most liverworts contain mono-, sesqui- and di-terpenoids and/or aromatic compounds, such as bisbibenzyl derivatives [1, 2]. Members of the genus *Jungermannia* contain large amounts of diterpenoids of the *ent*-kaurane-, clerodane-, trachylobane-, pimarane- and labdane-type [1, 2]. The chemical constituents of *J. rotundata* have not been reported hitherto. In this paper, we report on the isolation and characterization of three new *ent*-kaurane-type diterpenoids (**1**–**3**) from Japanese *J. rotundata*.

RESULTS AND DISCUSSION

A combination of CC on silica gel, Sephadex LH-20 and prep. HPLC of an ether extract of *J. rotundata* gave three new *ent*-kaurane-type diterpenoids, named rotundeic acid A (**1**), B (**2**), C (**3**).

The IR and ^{13}C NMR spectra of **1**, *m/z* 318.2199 $C_{20}H_{30}O_3$, indicated the presence of a secondary hydroxyl group (3400 cm^{-1}, δ_c 82.2 *d*) and a carboxylic acid (3200–2600, 1700 cm^{-1}, δ_c 179.6 *s*). The ^1H (Table 1) and ^{13}C NMR (Table 2) spectra also showed the presence of two tertiary methyls, an *exo*-methylene (δ_H 4.94 *d*, 5.08 *s*; δ_C 104.5 *t*, 157.9 *s*) and a methine (δ_H 3.76 *t*; δ_C 82.2 *d*) bearing an oxygen atom. The ^{13}C NMR spectrum showed 20 carbons which were assigned to two methyls, nine methylenes, four methines and five quaternary carbons by the DEPT spectrum. The above spectral data indicated that compound **1** was a tetracyclic diterpenoid with a secondary hydroxyl group and carboxylic acid group.

The IR and ^1H NMR spectra of **2**, $[M]^+$ *m/z* 360.2301 $C_{22}H_{32}O_4$, showed the presence of a carboxylic acid (3400–2400, 1700 cm^{-1}) and an acetoxyl group (1750, 1250 cm^{-1}; δ_H 2.17 *s*). The ^1H (Table 1) and ^{13}C NMR (Table 2) spectra of **2** were similar to those of **1** except for the presence of the acetoxyl group and the methine proton (δ5.18 *t*) on a carbon bearing an acetoxyl group. From the above evidence, the structure of **2** was presumed to be that of the acetoxyl derivative of **1**. This assumption was confirmed by reduction of **2** to furnish a secondary alcohol whose spectral data were completely identical with those of **1**. Thus, if the structure of **2** could be determined, the structure of **1** followed easily. The ^1H–^1H, ^{13}C–^1H COSYs of **2** supported that **1** and **2** were a kaurane-type diterpenoid with the carboxylic acid group at C-18, 19 or 20. *ent*-15α-Hydroxykaur-16-en-19-oic acid (**5**) and its acetate (**6**) have been isolated from *Xylopia aethiopica* [3, 4]. However, their spectral data were not in agreement with those of **1** and **2**. Therefore, the measurement of their ^1H–^1H, ^{13}C–^1H COSY and HMBC spectra were carried out. The HMBC spectrum of **2** indicated correlations between the methine proton (δ 1.61) and a carboxyl carbon (δ 184.1) and three methylene carbons (δ 39.4, 17.9, 31.4), and between the methine proton (δ 0.98) and a carboxyl carbon, two methyls (δ 22.1, 32.8) and a quaternary carbon (δ 47.9). The detailed analysis of HMBC spectrum (Fig. 1) indicated that **2** was a kaurane-type diterpenoid with a carboxylic acid at C-10 and an acetoxyl group at C-15. Therefore, the structure of **1** was established to be a kaurane-type with a carboxylic acid at C-10 and a hydroxyl group at C-15. The stereochemistry of **2** was revealed by the NOESY spectrum in which NOEs were observed between (i) H-15 and H-14β, (ii) H-15 and H-7, (iii) H-1α and H-11α,



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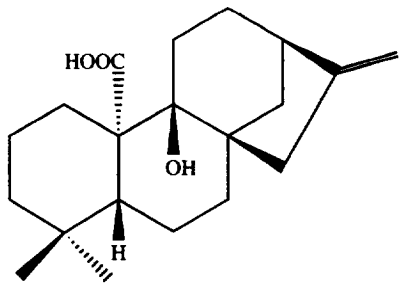
2 R = Ac



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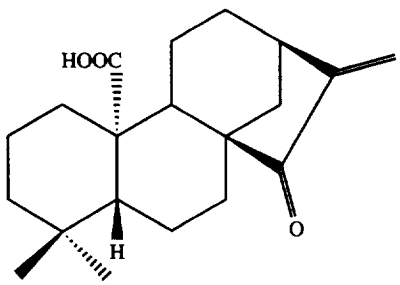

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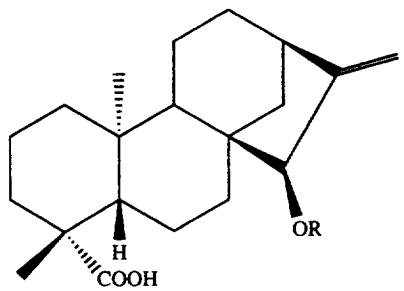

5 R = H
6 R = Ac

(iv) H-9 and H-1β and (v) H-9 and H-5. From the above results, the stereochemistry of the acetoxyl group at C-15 was β, however, the stereochemistry of the carboxylic acid at C-10 could not be clarified. Reduction of **2** to the diol derivative was attempted by several methods. However, only a monoalcohol was obtained from the resulting mixtures. It is known that the reduction of *ent*-kauranoids with a carboxyl group or methoxy carbonyl at C-10α does not proceed even under vigorous conditions [5]. On the basis of the formation of a monoalcohol (**1**) by reduction of **2** and the results of the NOESY spectrum, the stereochemistry of the carboxylic acid at C-10 of **1** and **2** was assigned as α. The CD spectrum of the enone, **4**, derived from **1** by oxidation with pyridinium dichromate (PDC), showed a negative Cotton effect (348 nm), indicating that compound **4** was an *ent*-kauranetype diterpenoid. Thus, the structures of rotundeic acid A and B were established to be *ent*-15α-hydroxykaur-16-en-20-oic acid (**1**) and *ent*-15α-acetoxykaur-16-en-20-oic acid (**2**).

Compound **3** showed the same molecular formula, $C_{20}H_{30}O_3$ (HRMS: $[M]^+$ *m/z* 318.2199), as that of rotundeic acid A (**1**). The IR and ^{13}C NMR spectra contained signals for a carboxylic acid (3400–2400, 1710 cm^{-1}; δ_C 182.8 *s*) and a tertiary hydroxyl group (3600 cm^{-1}; δ_C 76.8 *s*). The ^1H (Table 3) and ^{13}C NMR spectra (Table 2) were similar to those of **1**, except for the presence of a tertiary hydroxyl group in place of the secondary hydroxyl group found in **1**, indicating that **3** was an *ent*-kaurane-type diterpenoid with a tertiary alcohol at C-5, C-9 or C-13. The ^1H–^1H and ^{13}C–^1H COSY spectra showed the presence of the three partial structures: (i) CH_2=C—CH_2—, (ii) —CH_2—CH—CH_2— and (iii) —CH_2—CH_2—. The HMBC spectrum of **3** showed connectivity between the quaternary carbon bearing the hydroxyl group (δ 76.8) and H-1, H-11, and H-14. The above results indicated that the tertiary hydroxyl group was located at C-9. The stereochemistry of **3** was clarified by analysis of the NOESY spectrum, although the stereochemistry of the tertiary hydroxyl group at C-9 could not be clarified. Therefore, in the pyridine induced solvent shifts [6], the chemical shifts of H-1β, H-5 and H-7β measured in pyridine-d_5 solution were shifted downfield compared with those measured in $CDCl_3$ solution. The above spectral evidence supported a *β-axial* stereochemistry of the tertiary hydroxyl group at C-9. The stereochemistry of the carboxylic acid at C-10 of **3** was suggested to have the same configuration as in **1** and **2**, by comparison of the spectral data. Thus, the structure of rotundeic acid C was shown to be *ent*-9α-hydroxykaur-16-en-20-oic acid (**3**).

As far as we are aware, this is the first isolation of *ent*-kaurane-type diterpenoids with the carboxylic

Table 1. ^{1}H NMR data of compounds **1** and **2** (600 MHz)

H	**1**		**2**
	$CDCl_3$ + one drop of CD_3OD*	(C_5D_5N)	($CDCl_3$)
1	2.65 *br d*, J = 12.7 Hz, α 0.78–0.92 *m*, β	3.03 *br d*, J = 12.5 Hz, α 0.92 *m*, β	2.69 *br d*, J = 13.2 Hz, α 0.34 *ddd*, J = 13.2, 13.2, 3.7 Hz, β
2	1.36–1.71 2H, *m*	1.53–1.59 *m* 1.90–2.00 *m*	1.40–1.59 2H, *m*
3	1.36–1.71 *m*, α 1.20 *ddd*, J = 13.7, 13.7, 4.4 Hz, β	1.40 *br d*, J = 13.2 Hz, α 1.13–1.26 *m*	1.40–1.59 *m*, α 1.22 *ddd*, J = 13.4, 13.4, 4.4 Hz, β
5	1.02 *dd*, J = 12.7, 2.4 Hz	1.08 *m*	0.98 1H, *dd*, J = 12.7, 2.2 Hz
6	2.34, *dddd*, J = 12.7, 12.7, 12.7, 4.4 Hz, α 1.36–1.71 *m*, β	2.89 *dddd*, J = 13.2, 13.2, 13.2, 2.9 Hz, α 1.73 *br d*, J = 11.7 Hz, β	2.30, *dddd*, J = 12.7, 12.7, 12.7, 4.2 Hz, α 1.40–1.59 *m*
7	1.36–1.71 2H, *m*	1.53–1.59 *m*, α 2.13 *ddd*, J = 13.2, 13.2, 3.7 Hz, β	1.40–1.59 *m*
9	1.36–1.71 *m*	2.21–2.29 *m*	1.61 *m*
11	1.36–1.71 *m* 1.86 *br d*, J = 13.7 Hz	2.21–2.29 2H, *m*	1.90 *br d*, α 1.77 *m* β
12	1.36–1.71 2H, *m*	1.53–1.59 *m* 1.90–2.00 *m*	1.40–1.59 2H, *m*
13	2.55 *br s*	2.66 (1H, *br s*)	2.55 *br s*
14	2.36 *d*, J = 12.2 Hz, α 0.78–0.92 *m*, β	2.97 *d*, J = 11.7 Hz, α 1.13–1.26 *m*, β	2.38 *d*, J = 12.5 Hz, α 1.04 *dd*, J = 2.4 Hz, β
15	3.76 *t*, J = 2.9 Hz	4.20 *br s*	5.18 *t*, J = 2.4 Hz
17	4.94 *d*, J = 2.9 Hz 5.08 *s*	5.12 *s* 5.52 *s*	4.89 *s* 4.94 *d*, J = 2.7 Hz
18	0.90 3H, *s*	1.09 3H, *s*	0.91 3H, *s*
19	0.80 3H, *s*	0.93 3H, *s*	0.82 3H, *s*
OAc			2.17 3H, *s*

* Measured by 400 MHz.

Table 2. ^{13}C NMR data of **1–3** (100 MHz) and **5**

C	**1***(†)	**2***	**3**‡(†)	**5*** [3]
1	39.2 (39.9)	39.4	32.7 (33.7)	40.6
2	20.4 (21.2)	20.5	20.5 (21.5)	19.2
3	42.3 (42.9)	42.3	42.3 (43.1)	38.0
4	33.7 (34.3)	34.1	34.3 (34.5)	43.7
5	56.0 (56.6)	56.1	49.3 (49.3)	56.4
6	19.8 (20.7)	19.8	20.3 (21.3)	21.6
7	38.2 (39.1)	38.2	35.8 (36.7)	38.9
8	45.7 (46.6)	46.0	50.1 (51.0)	45.7
9	45.2 (45.8)	46.7	76.8 (76.3)	45.5
10	47.4 (48.0)	47.9	54.1 (54.7)	39.3
11	17.8 (18.6)	17.9	29.7 (30.2)	18.3
12	31.1 (32.3)	31.4	33.2 (33.8)	33.1
13	39.7 (40.6)	40.3	41.9 (42.9)	40.0
14	34.4 (35.3)	37.4	39.9 (40.4)	36.3
15	82.2 (82.6)	81.8	44.1 (44.8)	82.5
16	157.9 (159.9)	153.4	155.1 (156.9)	158.3
17	104.5 (104.4)	106.5	103.4 (103.0)	104.8
18	32.7 (33.1)	32.8	33.6 (34.1)	29.0
19	21.8 (22.7)	22.1	22.6 (23.3)	183.1
20	179.6 (179.4)	184.1	182.8 (179.6)	15.7
OAc		21.3 171.3		

* In $CDCl_3$; † in C_5D_5N; ‡ $CDCl_3$ + one drop of CD_3OD.

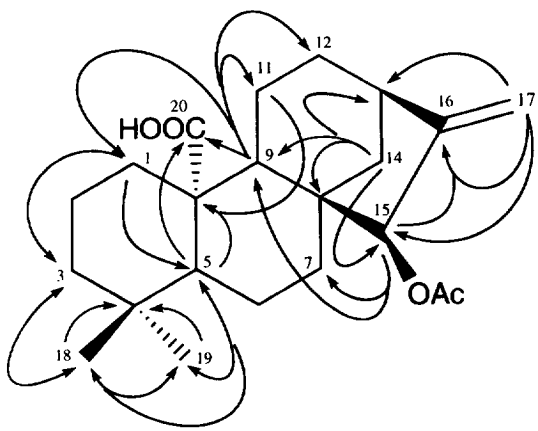

Fig. 1.

acid at C-10 from byrophytes or higher plants [7] although *ent*-kaurane-10-carboxylic acid is a known oxidation product of *ent*-kaurane-20-ol [5].

EXPERIMENTAL

Mps uncorr. The solvents used for spectral measurement were TMS–$CDCl_3$ [^{1}H- and ^{13}C NMR]; $CHCl_3$ ([α]$_D$); EtOH (UV). MeOH–CH_2Cl_2 (1:1) was used for Sephadex LH-20 CC. TLC: silica gel and the

Table 3. ^{1}H NMR data of compound **3** (600 MHz)

H	3	
	$CDCl_3$	C_5D_5N
1	1.44–1.61 *m*	1.87 *ddd*, *J* = 13.7, 13.7, 6.6 Hz, α
	2.27–2.36 *m*	2.78 *dd*, *J* = 13.9, 4.9 Hz, β
2	1.44–1.61 2H, *m*	1.65–1.69 *m*
		1.93 *m*
3	1.22 *m*	1.34 *m*
	1.37 *m*	1.43–1.46 *m*
5	1.44–1.61 *m*	2.15 *dd*, *J* = 13.2, 2.7 Hz
6	1.44–1.61 *m*	2.92 *dddd*, *J* = 13.2, 13.2, 3.4 Hz, α
	2.27–2.36 *m*	1.76 1H, *br d*, β
7	1.32 *m*, α	1.43–1.46 *m*, α
	1.88 *ddd*, *J* = 13.4, 13.4, 3.9 Hz, β	2.43 *ddd*, *J* = 13.4, 13.4, 4.2 Hz, β
11	1.44–1.61 *m*	2.14 *m*
	2.27–2.36 *m*	2.83 *br d*, *J* = 12.7 Hz
12	1.33 *m*	1.65–1.69 *m*
	1.44–1.61 *m*	2.05 *dddd*, *J* = 13.4, 13.4, 5.4, 2.4 Hz
13	2.51 *br s*	2.60 1H *br s*
14	2.27–2.36 *m*, α	3.06 *dd*, *J* = 12.2, 2.7 Hz, α
	1.18 *m*, β	1.33 *m*, β
15	1.81, *dt*, *J* = 17.3, 2.9 Hz	1.95 1H *like dd*
	2.78 *d*, *J* = 17.3 Hz	3.39 *br d*, *J* = 16.8 Hz
17	4.78 *s*	4.90 2H, *s*
	4.80 *s*	
18	0.94 3H, *s*	1.01 3H, *s*
19	0.84 3H, *s*	1.18 3H, *s*

detection of spots was with 30% H_2SO_4 or Godin reagent [8].

Plant material. Jungermannia rotundata (Amak.) was collected in Tokushima, Japan, 1994 and identified by Dr M. Mizutani. The voucher specimens were deposited at the Faculty of Pharmaceutical Sciences, Tokushima Bunri University.

Extraction and isolation. The ground material of *J. rotundata* (28.6 g) was extracted with Et_2O for one month. The crude extract (530 mg) was divided into five fractions by CC on Sephadex LH-20 using CH_2Cl_2–MeOH (1:1) as solvent system. Fr. 4 was rechromatographed on silica gel (*n*-hex.–EtOAc 9:1) to afford rotundeic acid A (**1**) (4.0 mg) and C (**3**) (9.6 mg). Fr. 5 was rechromatographed on silica gel (*n* hex.–EtOAc gradient) and finally purified by prep. HPLC (Nucleosil 50-5; *n*-hex.–Et_2O 4:1) to give rotundeic acid B (**2**) (52 mg).

Rotundeic acid A (**1**). mp 215–217°; $[\alpha]_D$ −15.2° (*c* 0.62); HREIMS: Found $[M]^+$ 318.2199 $C_{20}H_{30}O_3$ requires 318.2195; FTIR ν_{max} cm^{-1}: 3400 (OH), 3200–2600 (*broad*), 1700 (COOH); ^{1}H and ^{13}C NMR: Tables 1 and 2; EIMS *m/z* (rel. int.): 318 $[M]^+$ (25), 300 (22), 290 (14), 272 (100), 255 (27), 245 (41), 215 (29), 203 (12), 189 (18), 163 (23), 149 (21), 135 (25), 119 (22), 105 (20), 91 (30), 79 (21), 69 (23), 55 (21), 41 (26).

Rotundeic acid B (**2**). mp 195–196°; $[\alpha]_D$ −39.7° (*c* 5.20); HREIMS: Found $[M]^+$ 360.2301 $C_{23}H_{32}O_4$ requires 360.2300; FTIR ν_{max} cm^{-1}: 3400–2400 (*broad*), 1710 (COOH); 1750, 1250 (OAc): ^{1}H and ^{13}C NMR: Tables 1 and 2; EIMS *m/z* (rel. int.): 360 $[M]^+$ (33), 318 (51), 300 (32), 285 (1), 272 (77), 255 (100), 239 (15), 227 (6), 215 (18), 199 (19), 159 (18), 131 (22), 105 (21), 91 (27), 55 (20), 43 (45).

Rotundeic acid C (**3**). mp 255–257°; $[\alpha]_D$ −16.1° (*c* 0.97); HREIMS: Found $[M]^+$ 318.2201 $C_{20}H_{30}O_3$ requires 318.2195; FTIR ν_{max} cm^{-1}: 3600 (OH) 3400–2400 (*broad*), 1710 (COOH); ^{13}C and ^{1}H NMR: Tables 2 and 3; EIMS *m/z* (rel. int.): 318 $[M]^+$ (1), 300 (43), 282 (13), 272 (20), 254 (13), 239 (11), 231 (5), 211 (14), 199 (1), 187 (8), 177 (20), 155 (12), 135 (15), 121 (9), 107 (39), 91 (100), 79 (18), 65 (13), 55 (14), 44 (18).

Reduction of **2**. To a suspension of $LiAlH_4$ (12 mg) in dry Et_2O (2 ml) was added **2** (9 mg) in Et_2O and the mixture stirred at room temp. for 20 min. Work-up gave a monoalcohol (7 mg) whose spectral data were identical with those of **1**.

Oxidation of **1**. To compound **1** (7 mg) in CH_2Cl_2 (3 ml) was added pyridinium dichromate (PDC) (15 mg) and the soln was stirred at room temp. for 40 min. Usual work-up gave an α,β-unsaturated ketone **4** (3 mg): $[\alpha]_D$ −39.7° (*c* 1.04); HREIMS: Found $[M]^+$ 316.2043 $C_{20}H_{28}O_3$ requires 316.2039; FTIR ν_{max} cm^{-1}: 3200–2800 (*broad*), 1700 (COOH), 1740 (C=O); CD: $\Delta\varepsilon_{348}$ −0.57 (*c* 3.16×10^{-4}); UV: λ_{max} nm (log ε) 234 (4.00) (*c* 1.58×10^{-4}); ^{1}H-NMR (400 MHz): δ0.84 (3H, *s*), 0.94 (3H, *s*), 1.16 (1H, *dd*, *J* = 12.7, 2.5 Hz), 1.96 (1H, *ddd*, *J* = 18.1, 13.7, 4.4 Hz), 2.04 (1H, *m*), 2.29 (1H, *ddd*, *J* = 16.6, 13.7, 3.4 Hz), 2.62 (1H, *d*, *J* = 17.6 Hz), 2.66 (1H, *d*, *J* = 12.2 Hz), 2.94 (1H, *br s*), 5.28 (1H, *s*), 5.97 (1H, *s*); EIMS *m/z* (rel. int.): 316 $[M]^+$ (36), 298 (19), 288 (61), 270 (88), 253 (12),

243 (96), 227 (1), 213 (13), 173 (39), 162 (100), 147 (31), 119 (27), 105 (35), 91 (42), 79 (31), 69 (38), 55 (30), 41 (39).

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