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SESQUI- AND DITERPENOIDS FROM THE LIVERWORTS *PORELLA DENSIFOLIA* SUBSP. *APPENDICULATA* AND *PORELLA DENSIFOLIA* VAR. *FALLAX**

YOSHINORI ASAKAWA, KEIKO TAKIKAWA, MASAO TOYOTA, AKIKO UEDA, MOTOO TORI and SUDARSHAN S. KUMAR†

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

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Key Word Index—*Porella densifolia* subsp. *appendiculata*; *Porella densifolia* var. *fallax*; Jungermanniales; Hepaticae; pinguisane-type sesquiterpenoids; *ent*-kaurane-type diterpenoids; chemosystematics.

Abstract—A new diterpenic acid, *ent*-kauren-15-one-18-oic acid, was isolated from the Indian liverwort *Porella densifolia* subsp. *appendiculata* together with the previously known *ent*-18-hydroxykauren-15-one and norpinguisone methyl ester. Further investigation of the chemical constituents of the Japanese *P. densifolia* var. *fallax* resulted in the isolation of three known *ent*-kaurane-type diterpenoids. *P. densifolia* subsp. *appendiculata* is chemically very close to *P. densifolia* var. *fallax*.

INTRODUCTION

The liverwort *Porella* species are a rich source of sesqui- and diterpenoids [1–14]. They are divided into two types: those containing the pungent sesquiterpene dialdehyde polygodial and those containing no polygodial [12]. The Indian *P. densifolia* subsp. *appendiculata* and the Japanese *P. densifolia* var. *fallax* belong to the latter type. As part of a chemosystematic investigation of bryophytes, we examined the chemical constituents of *P. densifolia* subsp. *appendiculata* and isolated a new *ent*-kaurane-type diterpenic acid, together with related kaurane-type diterpenoids and pinguisane-type sesquiterpenoids. Chemical re-examination of the Japanese *P. densifolia* var. *fallax* led to the isolation of three previously known *ent*-kaurane-type diterpenoids. In this paper, we report the chemical structure of a new *ent*-kaurenic acid and discuss the chemosystematics of the two *Porella* species.

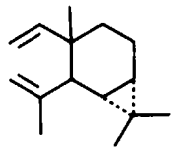
RESULTS AND DISCUSSION

The methanol extract of *P. densifolia* subsp. *appendiculata* was analysed by TLC, GC and GC/MS. α-Pinene, β-pinene, camphene, bicycloelemene (**1**), β-chamigrene (**2**), β-caryophyllene (**3**), bicyclogermacrene (**4**) [12], striatene (**5**), striatol (**6**) [15], spathulenol (**7**), deoxopinguisone (**9**), norpinguisone (**11**), norpinguisone methyl ester (**12**), kaurene (**13**), phytol, 15-hydroxykaurene (**14**), kauren-15-one (**15**), 18-hydroxykauren-15-one (**16**), campesterol, stigmasterol and sitosterol [12] were detected. The compounds **4**, **5**, **12** and **16** were the major components detected on GC of the crude extract. The remaining extract was chromatographed on silica gel and Sephadex LH-20 to give norpinguisone methyl ester (**12**) [12], *ent*-18-hydroxykauren-15-one (**16**) [12, 14] and a new *ent*-kaurenic acid (**21**). The spectral data of **21** indicated the presence of a carboxylic group (3600–2400 cm^{-1}; 1720 cm^{-1}; δ_C 184.3 ppm), an exocyclic methylene group [δ_C 114.7 (*t*), δ_H 5.25 (*s*), 5.95 (*s*)] conjugated with a carbonyl group [232 nm; 1690 cm^{-1}; δ_C 210.3 (*s*)] and two tertiary methyl groups [δ_H 1.13 (*s*), 1.18 (*s*)]. These data resembled closely those of the co-occuring *ent*-18-hydroxykauren-15-one (**16**), except for the absence of the AB doublet signals of a hydroxymethyl group, indicating that **21** might be 15-oxo-kauren-18-oic acid. To confirm this assumption, **16** was oxidized by the Jones reagent to afford *ent*-kauren-15-one-18-oic acid (**21**) whose physical and spectral data were in good agreement with those of the natural *ent*-kaurenic acid.

In previous reports [1, 3, 6], we described the distribution of mono- and sesquiterpenoids in the Japanese *P. densifolia* var. *fallax*, and the isolation and structure determination of pinguisane-type sesquiterpenoids. Reinvestigation of the methanol extract of this species resulted in the isolation of the previously known *ent*-spathulenol (**7**), *ent*-11α-hydroxykauren-15-one (**14**) [12, 16], (16*R*)-*ent*-11α-hydroxykauran-15-one (**18**) [12, 16] and *ent*-kauren-18-oic acid (**20**) [12, 14], together with pinguisenol (**8**) [3, 12]. The presence of the monoterpenoids, α- and β-pinenes, camphene, limonene, α-terpinene, and *p*-cymene [12], sesquiterpenoids (**1**–**7**) and diterpene (**16**) was confirmed by comparison of their MS spectra obtained by GC/MS with those of authentic samples. Table 1 shows the distribution of terpenoids in *P. densifolia* subsp. *appendiculata* and *P. densifolia* var. *fallax*. Although four kaurane-type diterpenoids (**17**–**20**) found in the latter species have not been detected in the former species, the present two *Porella* species are chemically very similar.

* Part 21 in the series "Chemosystematics of Bryophytes". For Part 20, see Asakawa, Y., Masuya, T., Tori, M. and Campbell, E. O. (1987) *Phytochemistry* **26**, 735.

† Present address: Botany Department, Panjab University, Chandigarh 160014, India.



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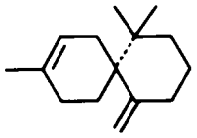

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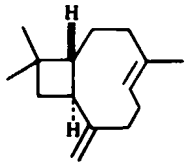

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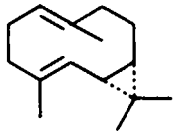

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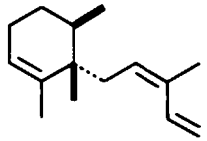

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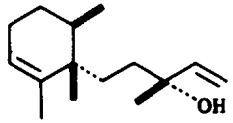

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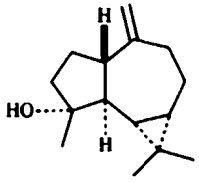

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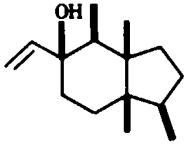

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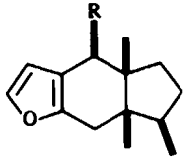

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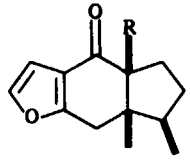
10 R = CO_2Me



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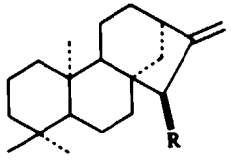
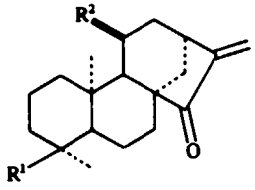
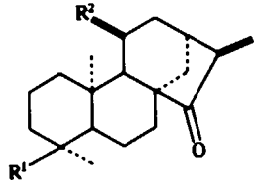
12 R = CO_2Me



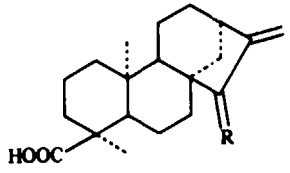
13 R = H
14 R = OH



15 R^1 = Me, R^2 = H
16 R^1 = CH_2OH, R^2 = H
19 R^1 = Me, R^2 = OH



17 R^1 = CH_2OH, R^2 = H
18 R^1 = Me, R^2 = OH



20 R = H_2
21 R = O

Table 1. Distribution of terpenoids in *P. densifolia* subsp. *appendiculata* and *P. densifolia* var. *fallax*

Compounds	*P. densifolia* subsp. *appendiculata*	*P. densifolia* var. *fallax*
Pinguisenol (**8**)		+ [3, 12]
Deoxopinguisone (**9**)	+	+ [1, 12]
Pinguisone methyl ester (**10**)		+ [1, 12]
Norpinguisone (**11**)	+	+ [1, 12]
Norpinguisone methyl ester (**12**)	+	+ [1, 12]
ent-Kaurene (**13**)*	+	+
ent-15-Hydroxykaurene (**14**)*	+ [12]	
ent-Kauren-15-one (**15**)*	+ [12]	
ent-18-Hydroxykauren-15-one (**16**)	+	+ [14]
(16*R*)-*ent*-18-Hydroxykauran-15-one (**17**)		+ [14]
(16*R*)-*ent*-11α-Hydroxykauran-15-one (**18**)		+ [16]
ent-11α-Hydroxykauren-15-one (**19**)		+ [16]
ent-Kauren-18-oic acid (**20**)		+ [14]
ent-Kauren-15-one-18-oic acid (**21**)	+	

*The stereochemistry of compounds **13–15** was tentatively assigned on the basis of the co-occurring *ent*-kaurenes (**16**, **21**).

EXPERIMENTAL

TLC, GC and GC/MS were carried out as previously reported [17]. The solvents used for spectral determination were: TMS–$CDCl_3$ [^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz)]; EtOH (UV); $CHCl_3$ (IR and $[\alpha]_D$).

Plant materials. Porella densifolia (Steph.) Hatt. subsp. *appendiculata* (Steph.) Hatt. and *P. densifolia* (Steph.) Hatt. var. *fallax* (Mass.) Hatt. identified by Dr. S. Hattori were deposited in the Herbarium of Institute of Pharmacognosy, Tokushima Bunri University.

Extraction and isolation. P. densifolia subsp. *appendiculata* collected in Panjab, India, May 1983 was extracted with MeOH. The crude extract (650 mg), after removal of the solvent, was checked by TLC, GC and GC/MS. The components obtained by GC/MS were identified by direct comparison of the MS spectra with those of authentic samples. The remaining extract was chromatographed on silica gel using an *n*-hexane–EtOAc gradient to give 6 fractions. From fraction 2 (8% EtOAc), norpinguisone methyl ester (**12**) (141 mg) was isolated. Fraction 4 (20% EtOAc) (144 mg) was rechromatographed on Sephadex LH-20 using $CHCl_3$–MeOH (1:1) to give two crude kaurene-type diterpenoids which were purified by prep. TLC (C_6H_6–EtOAc, 4:1) to afford *ent*-18-hydroxykauren-15-one (**16**) (30 mg) [12, 14] and *ent*-kauren-15-one-18-oic acid (**21**) (16 mg): mp 227–228°; $[\alpha]_D$ −110° (*c*, 0.12); UV λ_{max} nm (log ε): 232 (3.69); IR ν_{max} cm^{-1}: 3600–2400; 1720 (COOH), 1690 (C=C–C=O), 1640 (C=C), 945; ^{1}H NMR: δ 1.13, 1.18 (each 3H, *s*), 2.40 (1H, *d*, *J* = 12.0 Hz), 3.04 (1H, *br d*, *J* = 3.4 Hz), 5.25, 5.95 (each, 1H, *s*); ^{13}C NMR: δ 16.1 (Me, *q*), 17.6 (CH_2, *t*), 17.8 (Me, *q*), 18.0, 21.5, 32.3, 33.0, 36.6, 36.7, 38.8 (each CH_2, *t*), 38.1, 39.3, 52.4 (each CH, *d*), 39.4, 47.4, 52.6 (each C, *s*), 114.7 (CH_2=, *t*), 149.3 (C=, *s*), 184.3 (COO, *s*), 210.3 (C=O); MS *m/z* (rel. int.): 316 $[M]^+$ (91), 283 (56), 270 (91) 255 (65), 149 (87), 148 (66), 123 (82), 121 (78), 109 (100), 107 (78), 105 (85), 93 (64), 91 (99), 81 (51), 79 (61).

P. densifolia var. *fallax* collected in Kito, Tokushima, Nov. 1983, was extracted with MeOH and the crude extract partitioned between Et_2O and H_2O. On removal of the Et_2O a viscous oil (25.07 g) was obtained. A small amount of the extract was analysed by TLC, GC and GC/MS and known compounds detected by direct comparison of the MS spectra with those of authentic samples. The crude extract (10.0 g) was chromatographed on silica gel using an *n*-hexane–EtOAc gradient to give 7 fractions. Fraction 2 (10% EtOAc) (915 mg) was rechromatographed on Sephadex LH-20 using $CHCl_3$–MeOH (1:1) to furnish pinguisenol (**8**) (337 mg) [3, 12]. Fraction 3 (15% EtOAc) (1.205 g) was rechromatographed on silica gel using *n*-hexane–EtOAc gradient to give *ent*-spathulenol (**7**) (162 mg) [12]. Fraction 5 (50–100% EtOAc) (834 mg) was treated in the same manner as described above to afford two kaurene-type diterpenoids (**18**) (18 mg) and (**19**) (97 mg) the physical and spectral data of which were identical to those of (16*R*)-*ent*-11α-hydroxykauran-15-one and *ent*-11α-hydroxykauren-15-one, respectively [12, 16]. The crude extract (15.07 g) was chromatographed on Sephadex LH-20 using $CHCl_3$–MeOH (1:1) to give 2 fractions. Fraction 2 (10.73 g) was further chromatographed on silica gel using an *n*-hexane–EtOAc gradient to give 7 fractions. Fraction 3 (10% EtOAc) (1.481 g) was rechromatographed on Sephadex LH-20 using the same solvent system described above to give a kaurenic acid (**20**) (184 mg) identical with *ent*-kauren-18-oic acid [12, 14].

Oxidation of **16**. Compound **16** (6 mg) in Me_2CO (1 ml) was oxidized by Jones' reagent (2.2 mol) at 0° for 2 hr. Work up as usual gave *ent*-kauren-15-one-18-oic acid (**21**) (5.8 mg) the physical and spectral data of which were consistent with those of the natural *ent*-kauren-15-one-18-oic acid.

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