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ENT-KAURENE DITERPENE FROM THE LIVERWORT *PLAGIOCHILA PULCHERRIMA*

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Key Word Index—*Plagiochila pulcherrima*, Jungermanniales, Hepaticeae; *ent*-kaur-16-en-7α,15β-diol, *ent*-kaurene diterpene, plagiochilines A and B

Abstract—From the pungent liverwort *Plagiochila pulcherrima*, a new *ent*-dihydroxykaurene diterpene has been isolated together with *ent*-2,3-secoaromadendrane-type sesquiterpenoids plagiochilines A and B. Its structure has been determined to be *ent*-kaur-16-en-7α,15β-diol on the basis of spectral data and chemical evidence.

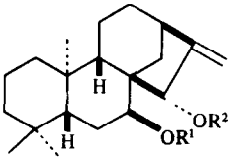
INTRODUCTION

The liverworts of the *Plagiochila* species are widespread in the world and more than 1500 species have been identified. These are divided into two groups which comprise pungent and non-pungent species. The species of the former group are well known to elaborate a series of the unique *ent*-2,3-secoaromadendrane-type sesquiterpene hemiacetals [1], which exhibit a variety of significant activities, e.g. insect antifeedant [2], cytotoxicity [3] and some enzyme inhibition. The latter group of liverworts elaborate bibenzyl derivatives [4], *ent*-aromadendrane-type sesquiterpenoids [1] or highly oxygenated fusicoccane-type diterpenoids [5] In the course of our continuing search for biologically active substances of the pungent species we have examined *Plagiochila pulcherrima*. We have isolated a new *ent*-kaurene diterpene, together with the previously known sesquiterpenoids, plagiochilines A (**3**) and B (**4**), and spathulenol (**5**) and bicyclogermacrene (**6**) and a phytosterol, stigmasterol (**7**) [1, 6]. This paper describes the structure of the new diterpenoid.

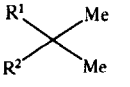
RESULTS AND DISCUSSION

An ether extract of *P. pulcherrima* was chromatographed on silica gel and then on alumina followed by Sephadex LH-20 to give compounds **1**–**7**.

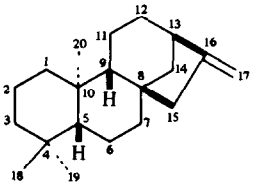
Compound **1**, mp 206–208°, obtained as colourless needles revealed an intense molecular ion peak on FDMS at *m/z* 304 and a dehydrated fragment peak at *m/z* 286 and its molecular formula was estimated as $C_{20}H_{32}O_2$ which was in accord with the ^{13}C NMR data (Table 1). The IR spectrum of **1** showed the presence of hydroxy groups (3600 and 3550 cm^{-1}). Its ^1H NMR spectrum contained signals for three tertiary methyl groups, an exomethylene group (δ5.10 and 5.22), as well as two secondary hydroxy groups (δ2.30, *d*, $J = 3.9$ Hz and 2.60, *br s*, disappeared with D_2O exchange), the



1 $R^1 = R^2 = H$
1a $R^1 = R^2 = Ac$
1b $R^1 = H$, $R^2 = Ac$
1c $R^1 = R^2 = Bz$



1d



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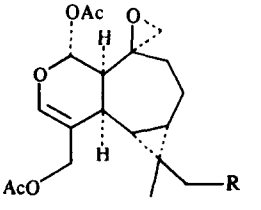

3 R = H
4 R = OAc

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Table 1 ^{13}C NMR spectral data of compound **1** and *ent*-kaurene (**2**)

C	1*	2†
1	40 12	40 5
2	18 59	18 9
3	41 94	42 2
4	32 71	33 3
5	49 36	56 2
6	26 75	20 4
7	73 07	41 3
8	51 47	44 3
9	46 28	56 1
10	39 25	39 5
11	17 53	18 3
12	33 35	33 5
13	42 87	44 3
14	35 14	40 0
15	81 22	49 5
16	158 64	155 8
17	108 50	103 4
18	33 35	33 7
19	21 55	21 2
20	17 24	17 7

*Recorded at 100 16 MHz, $CDCl_3$, TMS as internal standard
†Quoted from the literature [7]

protons adjacent to which were largely shifted downfield (δ3.91–>4 96, 4 12–>5.40) upon acetylation of **1** On the other hand, the ^{13}C NMR data (Table 1) for **1** indicated the presence of twenty carbons comprised of three Me, seven CH_2, three CH, three C, and two O-bearing CH as well as two olefinic carbons composing an exomethylene moiety These spectral features disclosed a diterpenoid nature of the kaurene type bearing two hydroxy substituents The 2D-COSY spectrum of **1** revealed cross peaks between the two broad singlet signals due to an exomethylene group and the carbinyl proton signal at δ4.12 indicating the location of the one hydroxy group at C-15, whereas the other hydroxy group should be placed at C-1, C-3 or C-7 since the carbinyl proton at δ3 91 was coupled only with two geminal protons which were poorly resolved. The position of the remaining hydroxy group, however, was verified to be at C-7 by ready formation of the acetonide (**1d**) on treatment of **1** with 2,2-dimethoxypropane in the presence of *p*-toluenesulphonic acid In addition, the ^{13}C NMR data suggested the kaurene skeleton dihydroxylated at C-7 and C-15 was plausible for **1** in comparison with the spectrum of *ent*-kaur-16-ene (**2**) [7] Furthermore, the axial configuration of the C-7 hydroxyl group was evident from the following data. The acetylation rate of the C-7 hydroxy group was unusually slow using the conventional method, and small J values (3.9 and 2 4 Hz) of the carbinyl proton at C-7 in the diacetate (**1a**) were observed. On the other hand, the relative stereochemistry for the C-15 hydroxy group could not be unambiguously assigned although an α-orientation was assumed due to no detection of the NOE between H-7 and H-15. Fujita *et al* [8] reported convergent syntheses of both *ent*-kaur-16-en-7α,15β-diol and the 7α,15α-diol starting from epicandicandiol The ^{1}H NMR data of compound **1** is in

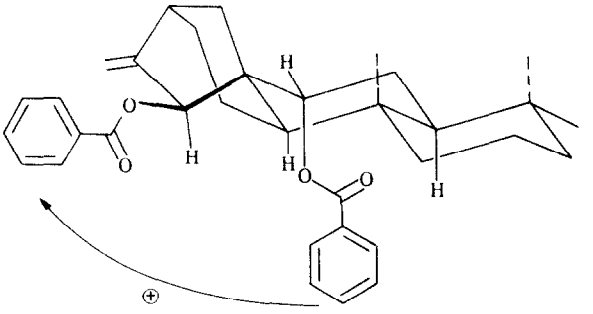

Fig 1. A positive exciton chirality between two benzoate chromophores

good accordance with those of the 7β,15α-diol reported in the literature [8] Accordingly, the hydroxy group at C-15 was assigned to the α-orientation The absolute configuration of **1** was examined by the CD spectrum of the dibenzoate derivative (**1c**) of **1** [9]. The CD spectrum of **1c** had a positive first Cotton effect at 238 nm and negative second Cotton effect at 222 nm The positive sign of the first Cotton effect leads to the conclusion that **1** was *ent*-kaur-16-en-7α,15β-diol as depicted in Fig. 1.

To our knowledge, this is the first isolation of an *ent*-kaurene oxidized only at C-7 and C-15 and it is considered to be one of the dioxygenated derivatives formed at an early stage in the biosynthetic route from *ent*-kaurene-16-ene (**2**) [10] The occurrence of a kaurene-type diterpene is very rare in *Plagiochila* species Only kaurene (**2**) has been detected in *P dura* De Not [11]

EXPERIMENTAL

Mps uncorr, ^{1}H NMR (400 and 90 MHz) and ^{13}C NMR (100 16 MHz) $CDCl_3$, TMS as int standard, CC silica gel (Merck, 70–230 mesh and Wakogel C-300); TLC and GC-MS were carried out as previously reported [12]

Plant material The liverworts, *Plagiochila pulcherrima* Hovik were collected in Yakushima Island, Japan on Apr. 26, 1986 and identified by Y A and M Mizutani A voucher specimen has been deposited at the Herbarium of Tokushima Bunri University

Extraction and isolation Air-dried powdered whole plants (3 kg) were extracted twice with Et_2O (20 l) at room temp for 2 weeks The combined Et_2O extract was evaporated *in vacuo* to give a crude extract (29 g) A small amount of the extract was checked by TLC and GC-MS and α-barbatene, δ-elemene, bicyclogermacrene, kaurene, spathulenol, stigmasterol, plagiochilines A (**3**) an B (**4**) were detected [1, 6] The remaining material was divided into eight fractions by CC on silica gel frs 1, 2 (CH_2Cl_2) (2 3 g), frs 3, 4 (EtOAc–CH_2Cl_2, 1:9) (5 7 g), fr 5 (EtOAc–CH_2Cl_2, 1 9) (3 8 g), frs 6, 7 (EtOAc–CH_2Cl_2, 3 7) (3 3 g), fr 8 (EtOAc–CH_2Cl_2, 2 3) (2 5 g) From fr 1 a combination of chromatography of Sephadex LH-20 (*n*-hexane–CH_2Cl_2, 1 4) and silica gel ($CHCl_3$–*n*-hexane, 1 9) gave α-barbatene (400 mg), bicyclogermacrene (50 mg) [1] Fr 4 was rechromatographed on silica gel (*n*-hexane–EtOAc, 9 1) to afford spathulenol (610 mg) [1] Repeated chromatography on silica gel ($CHCl_3$–EtOAc, 9 1), Sephadex LH-20 (MeOH–$CHCl_3$, 1 1) and silica gel (*n*-hexane–EtOAc, 7 3) of fr 5 afforded stigmasterol (250 mg) and plagiochiline A (114 mg) [1]. Fr 6 was purified by Sephadex LH-20 (MeOH, 100%) to give plagiochiline A (600 mg) Fr 7 (1 3 g) was passed through a

short alumina column eluting with CH_2Cl_2–EtOAc (7:3) to give a colourless oil (1 g), which was purified by Sephadex LH-20 chromatography (MeOH–CH_2Cl_2, 7:3) to yield *ent*-kaur-16-en-7α,15β-diol (**1**) (14 mg) as colourless needles (from MeOH), mp 206–208° [lit. [8] 202–206°]; $[\alpha]_D^{24}$ −35.8° ($CHCl_3$, *c* 0.56); IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3600 (OH), 3550 (OH), FDMS *m/z* (rel. int.): 304 $[M]^+$ (10), 286 $[M-18]^+$ (100); EIMS *m/z* (rel. int.): 286 $[M-18]^+$ (100), 271 (85), 253 (30); ^{1}H NMR (400 MHz). δ 0.82, 0.89 and 1.02 (each 3 H, *s*, 3 × Me), 2.30 (1 H, *d*, *J* = 3.9 Hz, OH, disappeared with D_2O exchange), 2.60 (1H, *br s*, OH, disappeared with D_2O exchange). 2.79 (1H, *m*, H-13), 3.91 (1H, *m*, H-7), 4.12 (1H, *m*, H-15), 5.10 and 5.22 (each 1H, *br s*, H-17), ^{13}CNMR Table 1. Fr. 8 was further chromatographed on alumina (CH_2Cl_2–EtOAc, 1·1), Sephadex LH-20 (MeOH–CH_2Cl_2, 7:3) and finally silica gel (CH_2Cl_2–MeOH, 24:1) to give plagiochiline B (25 mg).

Acetylation of **1**. A mixture of **1** (10 mg), Ac_2O (0.1 ml) and pyridine (0.4 ml) was stood overnight at room temp. The usual work-up afforded an oil, which was purified by CC on silica gel to give *ent*-kaur-16-en-7α, 15β-diacetate (**1a**) (2.5 mg) and *ent*-kaur-16-en-7α-ol-15β-acetate (**1b**) (6.2 mg). Compound **1a**: colourless needles, mp 127–129°, MS *m/z* (rel. int.): 388 $[M]^+$ (3), 346 (5), 328 (58), 286 (71), 268 (92), 253 (75), 43 (100); IR ν_{max}^{KBr} cm^{-1} 1735 (ester C=O), 1250, 900, ^{1}H NMR (400 MHz): δ 0.76, 0.79 and 1.05 (each 3H, *s*, 3 × Me); 1.98 and 2.00 (each 3H, *s*, 2 × Ac), 2.83 (1H, *m*, H-13), 4.96 (1H, *dd*, *J* = 3.9, 2.4 Hz, H-7), 5.05 (1H, *br s*, H-17), 5.20 (1H, *br* s, H-17), 5.40 (1H, *m*, H-15). Compound **1b**: colourless needles, mp 134–135° MS *m/z* (rel. int.): 346 $[M]^+$ (4), 328 $[M-18]^+$ (40), 304 (25), 286 (90), 268 (73), 253 (43), 43 (100), IR ν_{max}^{KBr} cm^{-1}: 3520 (OH), 1730 (ester C=O), 1290; ^{1}H NMR (400 MHz) δ 0.82, 0.85 and 1.07 (each 3H, *s*, 3 × Me), 2.07 (3H, *s*, Ac), 2.83 (1H, *m*, H-13), 3.87 (1H, *m*, H-7), 5.06 (1H, *br s*, H-17), 5.19 (1H, *br s*, H-17), 5.44 (1H, *m*, H-15).

Dibenzoylation of **1**. To a soln of **1** (9 mg) in pyridine (0.5 ml) containing DMAP (2 pieces) was added 5 drops of benzoyl chloride and the reaction mixture was stood at room temp. for 3 hr. The usual work-up afforded an oil, which was purified by prep. TLC (1 mm) (*n*-hexane–EtOAc, 4·1) to give the dibenzoate (**1c**) (10 mg) as colourless prisms after recrystallization from MeOH, mp 198–201° UV λ_{max}^{EtOH} nm (ε) 202 (14 400), 232 (25 000); CD (EtOH): Δ ε_{238} + 22.8, Δ ε_{222} − 15.8, MS *m/z* (rel. int.). 512 $[M]^+$ (2), 390 (5), ^{1}H NMR (90 MHz): δ 0.65, 0.81 and 1.13 (each 3H, *s*, 3 × Me), 2.91 (1H, *m*, H-13), 5.09 (1H, *m*, H-7), 5.31 (2H, *br s*, H-17), 5.76 (1H, *br s*, H-15), 7.35 (5H, *m*), 7.62 (5H, *m*).

Acetonide of **1**. A mixture of **1** (1.5 mg), 2,2-dimethoxypropane (0.3 ml) and *p*-toluenesulphonic acid (2 pieces) was stirred at room temp. overnight. Dry K_2CO_3 was added and stirring continued for 30 min. After filtering, the obtained filtrate was passed through a short silica gel column eluting with $CHCl_3$ to yield the acetonide (**1d**) (1.6 mg), ^{1}H NMR (90 MHz) δ 0.79, 0.87 and 0.96 (each 3H, *s*), 1.36 and 1.41 (each 3H, *s*), 2.75 (1H, *m*, H-13), 3.52 (1H, *dd*, *J* = 3.2, 2.8 Hz, H-7), 3.85 (1H, *m*, H-15), 5.16 (2H, *br s*, H-17).

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