THE ABSOLUTE STRUCTURES OF NEW 1β-HYDROXY-SACCULATANE-TYPE DITERPENOIDS WITH PISCICIDAL ACTIVITY FROM THE LIVERWORT *PELLIA ENDIVIIFOLIA*

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Eight new sacculatane-type diterpenoids, named 12-deoxy-1β, 11α-dihydroxy-sacculatanolide (**1**), 11α-hydroxysacculatanolide (**2**), pellianolactones A, B (**3, 4**), 1β, 11α-dihydroxysacculatanolide (**5**), 1β-hydroxysacculatal (**6**), 1β-hydroxy-isosacculatal (**7**), and 1β-hydroxysacculatanolide (**8**), have been isolated from the liverwort *Pellia endiviifolia.*

KEY WORDS liverwort; *Pellia endiviifolia*; sacculatane-type diterpenoid; piscicidal activity; X-ray crystallographic analysis

Previously, we reported the isolation and structure elucidation of a pungent diterpene dialdehyde, sacculatal (**9**),[1] together with a non-pungent isosacculatal (**10**)[1] from the liverwort *Pellia endiviifolia* (male thallus). In pursuit of pharmacologically interesting substances found in liverworts, we have further investigated the chemical constituents of Et_2O extract of *P. endiviifolia* , and isolated eight new sacculatane-type diterpenoids (**1**~**8**) as well as three known diterpenes, **9**, **10**, and sacculatanolide (**11**).[1] Here we wish to report on the structure elucidation of **1**~**8**.

The molecular formula of 12-deoxy-1β, 11α-dihydroxysacculatanolide (**1**)[2] was determined to be $C_{20}H_{32}O_3$ by HRMS. Acetylation of **1** afforded diacetate (**12**) [^{1}H NMR ($CDCl_3$): δ 1.98, 2.04 (each *s*, 3H)], and the Jones oxidation of **1** gave a γ-lactone (**13**) (IR: 1775 and 1710 cm^{-1}) indicating the presence of secondary and hemiacetal hydroxyl groups [IR: 3385 cm^{-1} ; ^{13}C NMR: δ_C 79.2 (*d*) and 98.8 (*d*)]. The relative structure of **1** was deduced from careful analysis of the 2D NMR spectra including DQF-COSY, HMQC, HMBC and NOESY, and finally established by X-ray crystallographic analysis[3] as shown in Fig. 1. The CD spectra of *p*-bromodibenzoate (**14**) showed the negative first Cotton effect at 253 nm (Δε –47.3) and the positive second Cotton effect at 235 nm (Δε +20.4), indicating that the absolute configurations at C-1 and C-11 of **1** were represented as *R*, respectively.

The IR, and ^{1}H and ^{13}C NMR spectra of 11α-hydroxysacculatanolide (**2**)[4] ($C_{20}H_{30}O_3$) indicated the presence of a lactol group [3385 and 1738 cm^{-1}; δ_H 5.68 (*d*, J=5.9 Hz); δ_C 99.4 (*d*) and 168.6 (*s*)]. Acetylation of **2** afforded monoacetate (**15**). The relative structure of **2** was established by X-ray crystallographic analysis.[5] The absolute structure of **2** was determined from the CD specta of **2** [λ_{max} 243 nm (Δε +2.75)] and **15** [λ_{max} 239 nm (Δε +1.53)], and the experimental result indicated that Jones oxidation (CrO_3-H_2SO_4/ acetone/ 0-5°) of sacculatal (**9**) afforded **2**.

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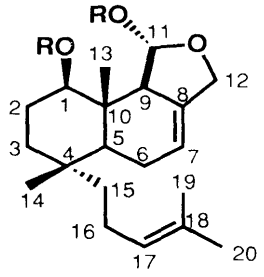
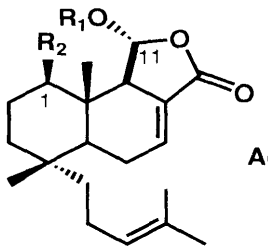
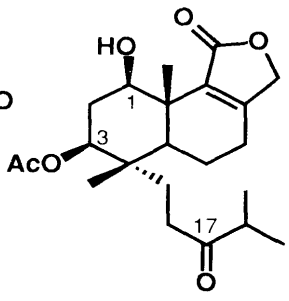
The IR, and ^1H and ^{13}C NMR spectra of pellianolactone A (**3**)[6] ($C_{22}H_{32}O_6$) contained the absorption bands and the signals due to a secondary hydroxyl group [3375 cm^{-1}; δ_H 3.76 (*dd*, J= 10.3, 4.4 Hz)], a ketone group [1715 cm^{-1}; δ_C 214.2 (*s*)] and an acetoxyl group [1735 cm^{-1}; δ_H 2.06 (*s*)]. The relative structure of **3** was deduced from careful analysis of the 2D NMR spectra, and finally established by X-ray crystallographic analysis[7] as shown in Fig. 2. The absolute structure of **3** was determined by the negative single Cotton effect [λ_{max} 235 nm ($\Delta\varepsilon$ -5.03)] in its CD spectrum.



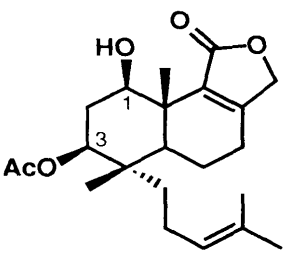
1 : R= H
12 : R= Ac
14 : R= *p*-Br-Bz



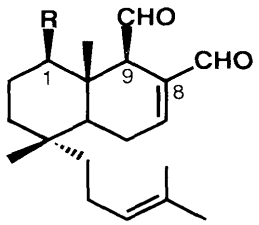
2 : R_1=R_2=H
5 : R_1=H, R_2=OH
15 : R_1=Ac, R_2=H



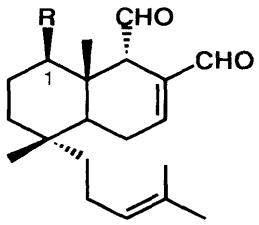
3



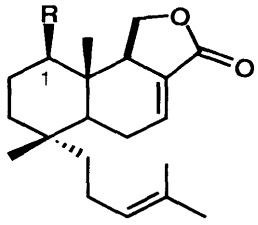
4



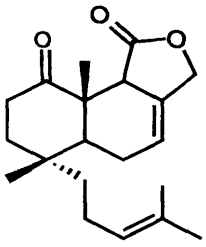
6 : R= OH
9 : R= H



7 : R= OH
10 : R= H



8 : R= OH
11 : R= H



13

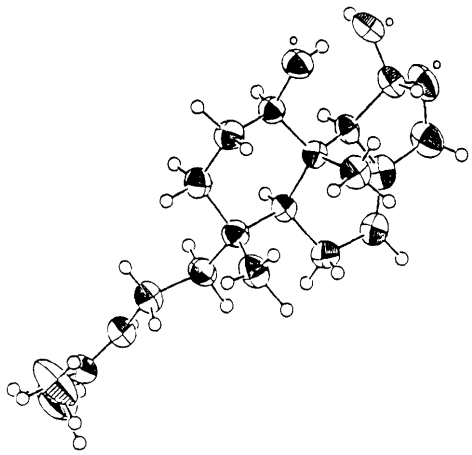


Fig. 1. ORTEP Drawing of **1**

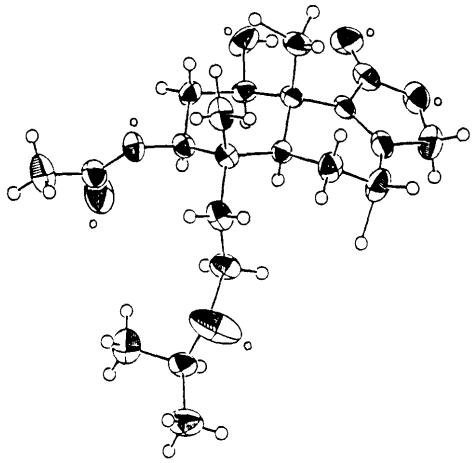


Fig. 2. ORTEP Drawing of **3**

In the ^{13}C NMR spectra of **5**~**8**, the signals for C-1, C-2 and C-10 appeared 10~40 ppm downfield compared with the corresponding resonances in **2** and **9**~**11**, and NOEs between H-1 and H-5, and H-1 and H-9 of **5**~**8** were observed. The relative and absolute structures of diterpenes **4**[8], **5**[9], **6**[10], **7**[11] and **8**[12] were determined as 1β–hydroxylated compounds from the careful analysis of 2D NMR spectra, and CD spectra.

Compounds **1** and **3**~**8** were the first naturally occurring sacculatane-type diterpenoids bearing 1β-hydroxyl group. Piscicidal activity of compounds **1**~**11** was tested. Pungent-tasting 1β-

hydroxysacculatal (**6**) and sacculatal (**9**) possess potent piscicidal activity against killie-fish, which died within 20 min at a concentration of 1 ppm, indicating that both 8- and 9β-diformyl groups were essential to the activity.[1)]

REFERENCES AND NOTES

1) Asakawa Y., in *Studies in Natural Products Chemistry* Vol. 2 (Atta-ur-Rahman, ed.), p. 277, Elsevier (1988).

2) **1** : mp 111-112°; $[\alpha]_D^{24}$+13.8° (*c* 0.80, $CHCl_3$); HR-MS: *m/z* 320.2299, $C_{20}H_{32}O_3$ requires 320.2351; EI-MS: *m/z* 320 (M^+), 302, 287, 69 (100%); FT-IR (KBr) cm^{-1}: 3385 (OH), 2926, 1638 (C=C); ^1H NMR ($CDCl_3$): δ 3.46 (1H, *dd*, J=2.2, 6.8 Hz, H-1), 5.26 (1H, *d*, J=6.8 Hz, H-11).

3) The crystal data for **1** : orthorhombic; space group $P2_12_12_1$ with a=7.839 (2), b=35.506 (8), c=6.648 (2) Å, V=1850.1 (8) $Å^3$, Z=4, and μ (Cu K-α)=5.17 cm^{-1}. Final R value was 0.052 for 1551 reflections.

4) **2** : mp 155-156°; $[\alpha]_D^{25}$-49.3° (*c* 0.51, $CHCl_3$); HR-MS: *m/z* 318.2202, $C_{20}H_{30}O_3$ requires 318.2215; EI-MS: *m/z* 318 (M^+), 300, 275, 257, 69 (100%); FT-IR (KBr) cm^{-1}: 3385 (OH), 1738 (CO), 1201; CD (EtOH) λ_{max} nm (Δε): 245 (+1.61), 198 (-4.33); UV (EtOH) λ_{max}nm (log ε): 205 (4.04), 220 (3.85); ^1H NMR ($CDCl_3$): δ 5.68 (1H, *d*, J=5.9 Hz, H-11), ^{13}C NMR ($CDCl_3$): δ 99.4 (*d*, C-11).

5) The crystal data for **2** : monoclinic; space group $P2_1$ with a=12.062 (3), b=9.234 (2), c=8.506 (3) Å, V= 942.2 (4) $Å^3$, Z=2, and μ(Cu K-α)=5.479 cm^{-1}. Final R value was 0.083 for 1439 reflections.

6) **3** : mp 145-146°; $[\alpha]_D^{21}$-1.7° (*c* 0.64, $CHCl_3$); HR-MS: *m/z* 392.2217, $C_{22}H_{32}O_6$ requires 392.2199; EI-MS: *m/z* 392 (M^+), 332, 307, 289, 234, 151, 121, 71 (100%); FT-IR (KBr) cm^{-1}: 3375 (OH), 1715 (CO), 1655, 1240; CD (EtOH) λ_{max} nm (Δε): 235 (-5.03); UV (EtOH) λ_{max} nm (log ε): 218 (3.94); ^1H NMR ($CDCl_3$): δ 2.06 (3H, *s*, -OAc), 3.76 (1H, *dd*, J=4.6, 12.4 Hz, H-1), 4.82 (1H, *dd*, J=4.6, 12.2 Hz, H-3), ^{13}C NMR ($CDCl_3$): δ 214.2 (*s*, C-17).

7) The crystal data for **3** : orthorhombic; space group $P2_12_12_1$ with a=10.539 (9), b=24.71 (2), c=8.155 (9) Å, V=942.2 (4) $Å^3$, Z=2 and μ(Cu K-α)=6.37 cm^{-1}. Final R value was 0.063 for 1973 reflections.

8) **4** : $[\alpha]_D^{20}$ +14.1° (*c* 0.39, $CHCl_3$); HR-MS: *m/z* 376.2251, $C_{22}H_{32}O_5$ requires 376.2250; EI-MS: *m/z* 376 (M^+), 316, 235 , 217, 151; 109 (100%); FT-IR (KBr) cm^{-1}: 3364 (OH), 1723 (CO), 1655, 1242, 1028; CD (EtOH): λ_{max}nm (Δε): 236 (-5.97). UV (EtOH) λ_{max} nm (log ε): 219 (3.85); ^1H NMR ($CDCl_3$): δ 2.06 (3H, *s*, -OAc), 5.01 (1H,*br. t*, J=5.9 Hz, H-17), ^{13}C NMR ($CDCl_3$): δ 123.7 (*d*, C-17).

9) **5** : $[\alpha]_D^{21}$ -12.5° (*c* 0.91, $CHCl_3$); HR-MS: *m/z* 334.2145, $C_{24}H_{38}O_7$ requires 334.2144; EI-MS: *m/z* 334 (M^+), 316, 301, 288 , 273; 203, 69 (100%); FT-IR (KBr) cm^{-1}: 3289 (OH), 1744 (CO); CD (EtOH): λ_{max} nm (Δε): 245 (+0.46), 194 (-1.10). UV (EtOH) λ_{max} nm (log ε): 205 (3.88), 220 (3.83); ^1H NMR($CDCl_3$): δ 3.50 (1H, *dd*, J=4.3, 10.9 Hz, H-1), 5.72 (1H, *d*, J=6.3 Hz, H-11).

10) **6** : mp 104-105°; $[\alpha]_D^{24}$ +49.9° (*c* 0.76, $CHCl_3$); HRMS: *m/z* 318.2213, $C_{20}H_{30}O_3$ requires 318.2195; EI-MS: *m/z* 318 (M^+), 300, 290, 272,, 69 (100%); FT-IR (KBr) cm^{-1}: 3420 (OH), 1701, 1686 (C=O); ^1H NMR ($CDCl_3$): δ 3.62 (1H, *dd*, J=4.6, 11.2 Hz, H-1), 9.39 (1H, *s*, H-12), 9.84 (1H, *d*, J=3.2 Hz, H-11).

11) **7** : $[\alpha]_D^{24}$ -71.1°(*c* 0.51, $CHCl_3$); HRMS: *m/z* 318.2208, $C_{20}H_{30}O_3$ requires 318.2195; EI-MS: *m/z* 318 (M^+), 300, 290, 272, 257, 218, 187 (100%); FT-IR (KBr) cm^{-1}: 3457 (OH), 1717, 1680 (C=O), 1028; ^1H NMR ($CDCl_3$): δ 3.50 (1H, *dd*, J=4.3, 10.9 Hz, H-1), 9.44 (1H, *s*, H-12), 9.88 (1H, *br. s*, H-11).

12) **8** : $[\alpha]_D^{24}$ -20.3° (*c* 0.34, $CHCl_3$); HRMS: *m/z* 318.2198, $C_{20}H_{30}O_3$ requires 318.2195; EI-MS: *m/z* 318 (M^+), 234, 125 (100%); FT-IR (KBr) m^{-1}: 3476 (OH), 1744 (C=O), 1231; ^1H NMR ($CDCl_3$): δ 3.38 (1H, *dd*, J=4.3, 10.9 Hz, H-1), 4.19 (1H, *dd*, J=9.5, 9.5 Hz, H-11β), 4.53 (1H, *dd*, J=9.5, 9.5 Hz, H-11α).

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