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

Toshihiro HASHIMOTO, Yoko OKUMURA, Kumiko SUZUKI, Shigeru TAKAOKA, Yukiko KAN, Motoo TORI and Yoshinori ASAKAWA*

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

Eight new sacculatane-type diterpenoids, named 12-deoxy-1 β , 11 α -dihydroxy-sacculatanolide (**1**), 11 α -hydroxysacculatanolide (**2**), pellianolactones A, B (**3**, **4**), 1 β , 11 α -dihydroxysacculatanolide (**5**), 1 β -hydroxysacculatal (**6**), 1 β -hydroxy-isosacculatal (**7**), and 1 β -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 Et₂O 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-1 β , 11 α -dihydroxysacculatanolide (**1**)²⁾ was determined to be C₂₀H₃₂O₃ by HRMS. Acetylation of **1** afforded diacetate (**12**) [¹H NMR (CDCl₃): 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; ¹³C 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 analysis³⁾ as shown in Fig. 1. The CD spectra of *p*-bromodibenzoate (**14**) showed the negative first Cotton effect at 253 nm ($\Delta\epsilon$ -47.3) and the positive second Cotton effect at 235 nm ($\Delta\epsilon$ +20.4), indicating that the absolute configurations at C-1 and C-11 of **1** were represented as *R*, respectively.

The IR, and ¹H and ¹³C NMR spectra of 11 α -hydroxysacculatanolide (**2**)⁴⁾ (C₂₀H₃₀O₃) 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 spectra of **2** [λ_{max} 243 nm ($\Delta\epsilon$ +2.75)] and **15** [λ_{max} 239 nm ($\Delta\epsilon$ +1.53)], and the experimental result indicated that Jones oxidation (CrO₃-H₂SO₄/acetone/0-5°) of sacculatal (**9**) afforded **2**.

* To whom correspondence should be addressed.

The IR, and ^1H and ^{13}C NMR spectra of pellianolactone A (**3**)⁶⁾ ($\text{C}_{22}\text{H}_{32}\text{O}_6$) contained the absorption bands and the signals due to a secondary hydroxyl group [3375 cm^{-1} ; δ_{H} 3.76 (dd, $J=10.3, 4.4\text{ Hz}$)], 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 analysis⁷⁾ as shown in Fig. 2. The absolute structure of **3** was determined by the negative single Cotton effect [λ_{max} 235 nm ($\Delta\epsilon$ -5.03)] in its CD spectrum.

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 1 β -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 1 β -hydroxyl group. Piscicidal activity of compounds **1**~**11** was tested. Pungent-tasting 1 β -

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 9 β -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°; $[\alpha]_D^{24} +13.8^\circ$ (*c* 0.80, CHCl₃); HR-MS: *m/z* 320.2299, C₂₀H₃₂O₃ 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 (CDCl₃): 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 P2₁2₁2₁ with *a*=7.839 (2), *b*=35.506 (8), *c*=6.648 (2) Å, *V*=1850.1 (8) Å³, *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°; $[\alpha]_D^{25} -49.3^\circ$ (*c* 0.51, CHCl₃); HR-MS: *m/z* 318.2202, C₂₀H₃₀O₃ 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) $\lambda_{\max}$ nm ($\Delta\epsilon$): 245 (+1.61), 198 (-4.33); UV (EtOH) $\lambda_{\max}$ nm (log ϵ): 205 (4.04), 220 (3.85); ¹H NMR (CDCl₃): δ 5.68 (1H, *d*, *J*=5.9 Hz, H-11), ¹³C NMR (CDCl₃): δ 99.4 (*d*, C-11).
- 5) The crystal data for **2**: monoclinic; space group P2₁ with *a*=12.062 (3), *b*=9.234 (2), *c*=8.506 (3) Å, *V*=942.2 (4) Å³, *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°; $[\alpha]_D^{21} -1.7^\circ$ (*c* 0.64, CHCl₃); HR-MS: *m/z* 392.2217, C₂₂H₃₂O₆ 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) $\lambda_{\max}$ nm ($\Delta\epsilon$): 235 (-5.03); UV (EtOH) $\lambda_{\max}$ nm (log ϵ): 218 (3.94); ¹H NMR (CDCl₃): 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), ¹³C NMR (CDCl₃): δ 214.2 (*s*, C-17).
- 7) The crystal data for **3**: orthorhombic; space group P2₁2₁2₁ with *a*=10.539 (9), *b*=24.71 (2), *c*=8.155 (9) Å, *V*=942.2 (4) Å³, *Z*=2 and μ (Cu K- α)=6.37 cm⁻¹. Final *R* value was 0.063 for 1973 reflections.
- 8) **4**: $[\alpha]_D^{20} +14.1^\circ$ (*c* 0.39, CHCl₃); HR-MS: *m/z* 376.2251, C₂₂H₃₂O₅ 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): $\lambda_{\max}$ nm ($\Delta\epsilon$): 236 (-5.97). UV (EtOH) $\lambda_{\max}$ nm (log ϵ): 219 (3.85); ¹H NMR (CDCl₃): δ 2.06 (3H, *s*, -OAc), 5.01 (1H, *br. t*, *J*=5.9 Hz, H-17), ¹³C NMR (CDCl₃): δ 123.7 (*d*, C-17).
- 9) **5**: $[\alpha]_D^{21} -12.5^\circ$ (*c* 0.91, CHCl₃); HR-MS: *m/z* 334.2145, C₂₄H₃₈O₇ 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): $\lambda_{\max}$ nm ($\Delta\epsilon$): 245 (+0.46), 194 (-1.10). UV (EtOH) $\lambda_{\max}$ nm (log ϵ): 205 (3.88), 220 (3.83); ¹H NMR (CDCl₃): 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°; $[\alpha]_D^{24} +49.9^\circ$ (*c* 0.76, CHCl₃); HRMS: *m/z* 318.2213, C₂₀H₃₀O₃ 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 (CDCl₃): 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**: $[\alpha]_D^{24} -71.1^\circ$ (*c* 0.51, CHCl₃); HRMS: *m/z* 318.2208, C₂₀H₃₀O₃ 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 (CDCl₃): 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**: $[\alpha]_D^{24} -20.3^\circ$ (*c* 0.34, CHCl₃); HRMS: *m/z* 318.2198, C₂₀H₃₀O₃ 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 (CDCl₃): δ 3.38 (1H, *dd*, *J*=4.3, 10.9 Hz, H-1), 4.19 (1H, *dd*, *J*=9.5, 9.5 Hz, H-11 β), 4.53 (1H, *dd*, *J*=9.5, 9.5 Hz, H-11 α).

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