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MONO- AND SESQUITERPENOIDS FROM *WIESNERELLA DENUDATA*

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Key Word Index—*Wiesnerella denudata*; Hepaticae; monoterpenoids; sesquiterpene lactones; guaianolides; germacranolides; pungency; plant growth inhibitory activity.

Abstract—The characteristic pungency of the liverwort *Wiesnerella denudata* is due to a germacranolide, tulipinolide. In addition to the previously known sesquiterpene lactones, tulipinolide, zaluzanin C, zaluzanin D, 8α-acetoxyzaluzanin C and 8α-acetoxyzaluzanin D, a novel germacranolide, dihydrotulipinolide with a pseudoaxial configuration of the C-11 methyl bond has been isolated. Inhibitory activity against the germination and growth of roots of rice in the husk is due to the guaianolides. The fragrant odor of *W. denudata* is responsible for the high content of monoterpenoids, particularly, (+)-bornyl acetate.

INTRODUCTION

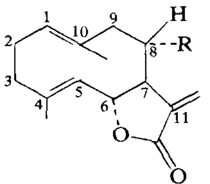
Some liverworts contain intensely pungent substances. Recently, we have reported that the pungency of the liverworts is responsible for terpenoids, like sesqui- or diterpene dialdehyde, secoaromadendrane-type sesquiterpene hemiacetal, germacranolide and eudesmanolides [1–6]. Pungent substances are also present in the liverwort *Wiesnerella denudata* (Conocephalaceae) and its crude extract also displays the unique fragrant odour and plant growth inhibitory activity. We investigated the chemical constituents of *W. denudata* which proved to be a rich source of sesquiterpene lactones and monoterpenoids. In the present communication, we wish to report the isolation and structures of the sesquiterpene lactones, including a new germacranolide, together with the previously known monoterpenoids and plant growth inhibitory active substances from *W. denudata*.

RESULTS AND DISCUSSION

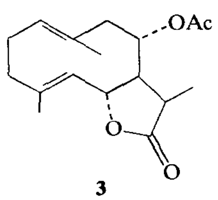
From the crude extract of the air-dried material which showed the unique pungency and inhibitory activity against germination and growth of rice in the husk, seven sesquiterpene lactones (**1–3**, **5–8**) could be isolated by combination of column and PLC, using a *n*-hexane–EtOAc gradient.

Costunolide (**1**), *tulipinolide* (**2**), *zaluzanin C* (**5**), *zaluzanin D* (**6**), *8α-acetoxyzaluzanin C*(**7**) *and 8α-acetoxyzaluzanin D* (**8**)

The stereostructures of the germacranolides (**1** and **2**) deduced from ^{1}H NMR, IR, CD and MS were confirmed by the identity of the physical constants and spectral data with those of (+)-costunolide and (+)-tulipinolide, respectively [7]. The unique pungency of this liverwort is due to the major component, tulipinolide **2** [5]. The stereostructures of four guaianolides (**5–8**) were established by the complete identity of the physical constants and spectral data with those of zaluzanin C, zaluzanin D, 8α-acetoxyzaluzanin C and 8α-acetoxyzaluzanin D, respectively [5].



1 R = H
2 R = OAc



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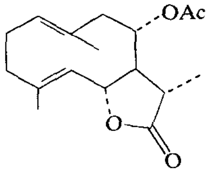

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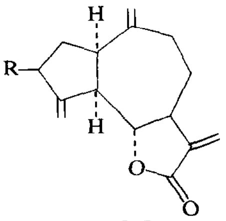
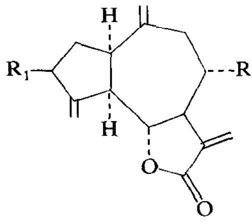
Dihydrotulipinolide (**3**)

A new germacranolide **3**, mp 160–161°, $[\alpha]_D + 59°$, showed the MS molecular ion at *m/e* 292, in agreement with molecular formula $C_{17}H_{24}O_4$. The presence of a γ-lactone and an acetoxyl group was confirmed by the intense IR bands at 1770 and 1750 cm^{-1}. The ^{1}H NMR spectrum included the signals attributable to



5 R = OH
6 R = OAc



7 R_1 = OH; R_2 = OAc
8 R_1 = R_2 = OAc

Table 1. Mono- and sesquiterpenes of *W. denudata* and *C. conicum*

	W. denudata	*C. conicum* (female)	*C. conicum* (male) [11]
α-Pinene	+*	+	+
Camphene	+	+	+
β-Pinene	+	+	+
α-Terpinene	++++	+++	+++
Sabinene	++++	++++++	++++++
γ-Terpinene	++++	++++	++++
Terpinolene	+	+	+
α-Terpineol		+	+
Bornyl acetate	+++	+++++	+++++
β-Elemene	+		
β-Barbatene	+		
δ-Cadinene		+	+
Costunolide (**1**)	+		
Tulipinolide (**2**)	++++++	+++	
Dihydrotulipinolide (**3**)	+		
Zaluzanin C (**5**)	+	+	
Zaluzanin D (**6**)	+++	+++	+
8α-Acetoxyzaluzanin C (**7**)	+	+	
8α-Acetoxyzaluzanin D (**8**)	+++	+++	+

* The symbols, +, +++, etc. are the relative concentrations estimated by GLC.

two vinylic methyls (δ 1.43 and 1.71), an acetyl group (2.05) and a secondary methyl group (1.17, $J = 7$ Hz), characteristic of an α-methyl group of a γ-lactone. In the lower field region between 4.3 and 5.1, the complex signals of four protons could be observed. The ^{1}H NMR spectrum of **3** was similar to that of tulipinolide (**2**) except for the presence of the secondary methyl group and the absence of two pairs of doublets of an α-methylene group of the γ-lactone, indicating that the new germacranolide might be the dihydro derivative of **2**. This assumption was confirmed as follows; hydrogenation of **2** with $NaBH_4$ gave a dihydrotulipinolide (**4**), mp 121–122°, $C_{17}H_{24}O_4$ (M^+ 292), $[\alpha]_D + 52°$, whose MS was completely identical to that of **3**. However, the mp and the chemical shift of the C-11 methyl group were different from those of **3**, suggesting that the C-11 methyl group of the natural dihydrotulipinolide might possess a pseudoaxial configuration. This was further confirmed by applying Narayanan's ^{1}H NMR solvent shifts method [8]. The difference ($\delta_{CDCl_3} - \delta_{C_6D_6}$) for the C-11 methyl resonance between **3** and **4** is 0.11 and 0.01 ppm, respectively, giving further proof for a pseudoaxial configuration of the C-11 methyl bond in **3**. Thus, the structure of the new germacranolide was established to be **3**. In addition to the above sesquiterpene lactones, a large amount of mixtures of monoterpenoids was obtained and α- and γ-terpinene and (+)-bornyl acetate were isolated by GLC. The presence of monoterpene hydrocarbons, α-pinene, camphene, β-pinene, sabinene and terpinolene, and sesquiterpene hydrocarbons, β-elemene and β-barbatene (= β-pompene) was confirmed by GC–MS.

Conocephalaceae is divided into two genera, *Conocephalum* and *Wiesnerella*. In the former genus, two species (*C. conicum* and *C. supradecompositum*) are known in Japan. Only one species, *W. denudata* is known in the *Wiesnerella* genus. More recently, we have reported that the female gametophyte of *C. conicum* produced various sesquiterpene lactones [5]. The constitution of the mono- and sesquiterpenoids of the present *W. denudata* is closely related to those of *C. conicum* (Table 1). The crushed thallus of *W. denudata* emits the unique fragrant odour which is almost identical to that of *C. conicum*. The occurrence of this odour might be due to the high content of monoterpenoids. Among the detected monoterpenoids, the odour of (+)-bornyl acetate is the closest to that of the original crushed thallus of *W. denudata*.

The crude extract of *W. denudata* inhibits the germination and and root elongation of rice in the husk at *ca* 1000 ppm. The active substances are the guaianolides **5**–**8** which showed the above activity at *ca* 100–200 ppm. The bioassay of costunolide **1** and tulipinolide **2**, which may have potential plant growth inhibitory activity, has not been performed because of their instability in bioassay media. Liverworts often elaborate enantiomeric sesquiterpenoids to those found in higher plants [9]. It is now recognised that the occurrence of such enantiomeric sesquiterpenoids is an important biochemical property of Hepaticae. However, just like the sesquiterpene lactones found in *C. conicum*, the sesquiterpene lactones isolated from *W. denudata* have the usual 6α,7β-configurations found in the corresponding compounds of higher plants.

EXPERIMENTAL

All mps are uncorr. The solvents used for spectral determination were TMS–$CDCl_3$ and C_6D_6 (^{1}H NMR); $CHCl_3$ (IR and $[\alpha]_D$); 95% EtOH (UV); MeOH (CD). TLC: precoated Si gel (0.25 mesh) F_{254}, *n*-hexane–EtOAc (4:1) and C_6H_6 (4:1 and 1:1). Spots were detected by UV light (254 nm) and spraying 30% H_2SO_4. Prep. GC: 5% SE-30, 3 m × 2 mm, oven temp. 100–180°, He 30 ml/min. GC–MS: 70 eV, 1% SE-30, 3 m × 2 mm, temp. programme 60–270° at 5°/min, He 30 ml/min. DI-MS: 70 eV, trap current 60 μA, sepa. 250°.

Bioassay with rice in the husk. Bioassay of each guaianolide was carried out by Kato's method [10].

Extraction and isolation. Wiesnerella denudata collected in Tokushima prefecture, Gotaki, April 1979 was washed with H_2O several times. After being air-dried for 5 days, the ground material (267 g) was extracted with Et_2O for 10 days and the crude fragrant extract (14.6 g) was directly chromatographed on Si gel using a *n*-hexane–EtOAc gradient. The first fraction (*n*-hexane 100%) gave a large amount of colourless oil (2.550 g) with pine needle-like odour. Prep. GLC of the fragrant oil gave α-terpinene and γ-terpinene. The presence of α-pinene, camphene, β-pinene, sabinene, terpinolene, β-elemene and β-barbatene (= β-pompene) was also confirmed by GC–MS. The second fraction (*n*-hexane–EtOAc, 9:1) gave fragrant oil containing carotenoids (520 mg). The third fraction (4:1) gave fragrant oil containing crystalline material (7.080 g) which was rechromatographed on Si gel using a C_6H_6–EtOAc gradient to give costunolide (**1**) (152 mg, mp 105–106°, $[\alpha]_D + 126°$ (*c* 1.5), dihydrotulipinolide (**3**) (35 mg), tulipinolide (**4**) 4.005 g), mp 178° (decomp), $[\alpha]_D + 248°$ (*c* 2.5), zaluzanin D (**6**) (680 mg), mp 103–104°, $[\alpha]_D$ 0° (*c* 2.0). All spectral data and the physical constants of **1**, **2** and **6** were completely identical to those of the authentic samples [5]. Dihydrotulipinolide (**3**), mp 160–161°, $[\alpha]_D + 59°$ (c1.2); IR$\nu_{max}^{CHCl_3}$ cm^{-1}: 1770 (γ-lactone), 1750, 1240 (OAc), 1050, 1020, 980, 950; ^1H NMR (C_6D_6): δ 1.06 (3H, *d*, $J = 7$, C-11 Me); MS *m/e* (rel. int.): 292 (M^+, 1, $C_{17}H_{24}O_4$), 232 ($M^+ - HOAc$, 63), 217 (29), 189 (26), 176 (45), 161 (33), 159 (35), 147 (37), 121 (83), 109 (42), 107 (34), 105 (33), 93 (64), 91 (36), 81 (38), 43 (100). The mother liquor, after costunolide and tulipinolide were filtered off, was combined and evapn of the solvent gave a dark green fragrant oil. Rechromatography of this oil on Si gel using C_6H_6–EtOAc gave (+)-bornyl acetate (750 mg), a sterol mixture (campesterol, stigmasterol and sitosterol) (120 mg) and triglycerides (200 mg). The fourth fraction (7:3) contained 8α-acetoxyzaluzanin D (**8**) (720 mg), $[\alpha]_D + 173°$ (*c* 3.0) [5]. The fifth fraction (1:1) (530 mg) gave a sesquiterpene lactone mixture and bibenzyls which were rechromatographed on Si gel using a C_6H_6–EtOAc gradient to afford zaluzanin C (**5**) (30 mg), $[\alpha]_D + 33°$ (*c* 0.5), 8α-acetoxyzaluzanin C (**7**) (11 mg) and an unidentified bibenzyl (476 mg). The sixth fraction (1:1) gave yellow oil containing hydroxylated carotenoids (350 mg) which were not identified.

Hydrogenation of tulipinolide (**2**). To an EtOAc soln of **2** (100 mg) was added $NaBH_4$ (43 mg) with stirring at 0° for 1 hr. The excess $NaBH_4$ was decomposed by 1% HOAc at 0°. The reaction mixture was filtered through a short column packed with Si gel and evapn of solvent afforded dihydrotulipinolide (**4**) (98 mg), mp 121–122°, $[\alpha]_D + 52°$ (*c* 2.7); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1770 (γ-lactone), 1740, 1245 (OAc), 1065, 1025, 990, 960, 680, 540; ^1H NMR ($CDCl_3$): δ 1.37 (3H, *d*, $J = 7$ Hz, C-11 Me), 1.51 (3H, *bs*), 1.67 (3H, *bs*), 2.06 (3H, *s*), 4.5–5.4 (4H, complex *m*), ^1H NMR (C_6D_6): δ 1.36 (3H, *d*, $J = 7$ Hz, C-11 Me); MS *m/e* (rel. int.): 292 (M^+, 1, $C_{17}H_{24}O_4$), 232 ($M^+ - HOAc$, 74), 217 (32), 189 (28), 176 (41), 161 (32), 159 (31), 147 (33), 121 (73), 109 (45), 107 (32), 105 (29), 93 (57), 91 (33), 81 (37), 43 (100).

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