Phytochemistry, 1979, Vol. 18, pp. 285–288. © Pergamon Press Ltd. Printed in England 0031-9422/79/0201-0285 $02.00/0

SESQUITERPENE LACTONES OF *CONOCEPHALUM CONICUM*

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(*Received* 25 *July* 1978)

Key Word Index—*Conocephalum conicum*; Hepaticae; sesquiterpene lactones; germacranolide; guaianolides; pungency; plant growth inhibitory activity.

Abstract—Investigation of the plant growth inhibitory activity of the extract of the liverwort, *Conocephalum conicum* afforded five sesquiterpene lactones, tulipinolide, zaluzanin C, zaluzanin D, 8α-acetoxyzaluzanin C and 8α-acetoxyzaluzanin D. The latter two compounds are reported for the first time. Tulipinolide is responsible for the characteristic pungency of the female gametophyte of *C. conicum*. The guaianolides showed inhibitory activity toward the germination and growth of roots of rice in the husk. The male gametophyte of *C. conicum* indicated no pungency, showing the lack of tulipinolide.

INTRODUCTION

The liverworts, *Porella*, *Trichocoleopsis* and *Plagiochila* contain unique pungent substances. Recently, we have reported that the pungency of these liverworts is due to a sesquiterpene- or diterpene dialdehyde and secoaromadendrane-type sesquiterpene hemiacetal [1–4]. The female and male gametophytes of *Conocephalum conicum* elaborate a large amount of (+)-bornyl acetate, which gives the characteristic fragrant odor of this liverwort [5], and related monoterpene hydrocarbons [6] and sesquiterpene hydrocarbons [7]. The female gametophyte, in particular, contains a unique pungent substance. The crude extract of both the female and male gametophyte further exhibits the plant growth inhibitory activity. In this paper, we wish to report the isolation and structures of a pungent sesquiterpene lactone and two new guaianolides, together with the previously known zaluzanin C and zaluzanin D, and the plant growth inhibitory activity of the guaianolides.

RESULTS AND DISCUSSION

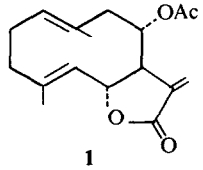
The crude ether extract of the air-dried material showed the unique pungency and inhibitory activity toward germination and growth of rice in the husk. Chromatography of the extract on Si gel using a *n*-hexane–EtOAc gradient afforded a germacranolide, tulipinolide (**1**), previously known guaianolides, zaluzanin C (**5**) and zaluzanin D (**6**), and two new guaianolides, named 8α-acetoxyzaluzanin D (**7**) and 8α-acetoxyzaluzanin C (**8**).

Tulipinolide (**1**), *zaluzanin C* (**5**) *and zaluzanin D* (**6**)

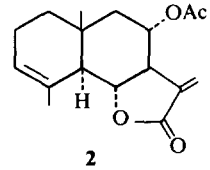
The structure of germacranolide (**1**) deduced from IR, NMR and MS, and from the chemical transformation of **1** to eudesmanolides **2**, **3** and **4**, was confirmed by the identity of the physical constants and spectral data with those of (+)-tulipinolide [8]. Tulipinolide is responsible for the characteristic pungency of the female gametophyte of *C. conicum*. The eudesmanolides **2** and **3** showed no pungency. The structures of two guaianolides **5** and **6** were established by the identity of the physical constants and spectral data with those of zaluzanin C and zaluzanin D, respectively [9–12].

8α-*Acetoxyzaluzanin D* (**7**)

The chemical ionization MS indicated $M^+ + 1$ at *m/e* 347 in agreement with a molecular formula $C_{19}H_{22}O_6$. The presence of acetoxyl and γ-lactone groups were confirmed by the intense IR bands at 1738 and 1765 cm^{-1}, respectively. The NMR spectrum included the signals due to two acetyl groups (δ 2.10 and 2.16 ppm, each *s*), two exocyclic methylene protons (5.00, 5.16, each *s* and 5.38, 5.56 each *bs*), typical exomethylene protons (5.66, 6.30 each *d*, $J = 3$ Hz) of an α,β-unsaturated γ-lactone. Two methine signals at *ca* 5.1 and at *ca* 5.4 overlapped by the signals of exomethylene protons, were assigned to protons on carbons bearing the acetoxyl group. The fragmentation pattern of the MS (EI) and the signal pattern of the NMR spectrum were quite similar to those of zaluzanin D (**6**), except for the presence of an additional acetoxyl group, indicating that compound **7** possessed the same guaianolide skeleton as that of zaluzanin D but an additional acetoxyl group was attached to a cyclopentane or cycloheptane ring. The positions of the two acetoxyl groups were established as follows; hydrolysis of **7** with K_2CO_3–MeOH gave



1



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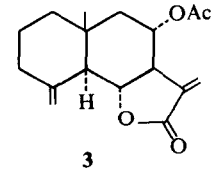

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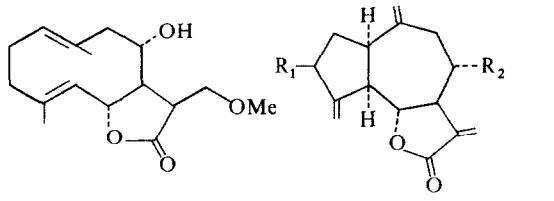
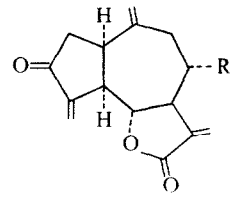
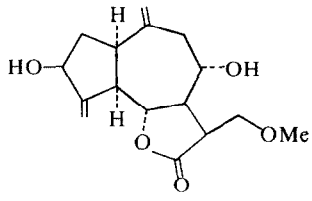


4

5 R_1 = OH; R_2 = H
6 R_1 = OAc; R_2 = H
7 R_1 = R_2 = OAc
8 R_1 = OH; R_2 = OAc



9 R = H
10 R = OAc



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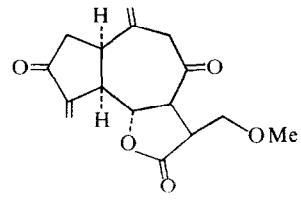

12

a diol (**11**), $C_{16}H_{22}O_5$ (M^+ *m/e* 294), mp 156–157°, followed by oxidation with CrO_3–Py to afford a diketone (**12**), $C_{16}H_{18}O_5$ (M^+ *m/e* 290), mp 155°–156°, whose UV and IR spectra showed bands at 210 nm and 1723 cm^{-1} respectively, characteristic of an α,β-unsaturated cyclopentenone [13], which confirmed the presence of a secondary hydroxyl group at C-3. No further absorption bands for an α,β-unsaturated carbonyl group in the UV and IR spectra supported the location of the additional carbonyl group at the C-8 position, hence the second acetoxyl group was at C-8. The stereochemistry of the ring junction at C-1 and C-5 was established by the coupling constant (J = 5 Hz) between H-1 and H-5. The lactone ring junction at C-6 and C-7 was confirmed to be *trans* by the large coupling constant (J = 3 Hz) of the α-methylene protons of the γ-lactone and a triplet signal (J = 9 Hz) for H-6. The stereochemistry of the two acetoxyl groups was confirmed as 3β and 8α on the basis of biogenetic considerations for the formation of 8-acetoxyguaianolide from tulipinolide (**1**) and by the coexistence of the known guaianolides (**5** and **6**). Thus, compound 7 was established to be $3\beta,8\alpha$-diacetoxyguaian-4(15), 10(14)-dien-6,12-olide.

8α-Acetoxyzaluzanin C (**8**)

The GC–MS showed the molecular ion at *m/e* 304, in agreement with a molecular formula $C_{17}H_{20}O_5$. The IR spectrum displayed the presence of a γ-lactone (1760 cm^{-1}), hydroxyl (3500 cm^{-1}) and acetoxyl groups (1730 cm^{-1}). The presence of α-methylene protons of an α,β-unsaturated lactone (δ 5.86 and 6.40 ppm, each *d*, J = 3 Hz), one acetoxy group (2.16 *s*) and two non-conjugated exomethylene groups (4.93, 5.06, 5.93, 6.36, each *bs*) were confirmed by the NMR and NMDR spectra. The fragmentation pattern of the MS and the NMR signals quite resembled those of zaluzanin C, except for the presence of an additional acetoxy group. Acetylation of **8** gave 8α-acetoxyzaluzanin D (**7**), indicating that **8** was 3β-acetoxy-8α-hydroxy or 3β-hydroxy-8α-acetoxyguaian-6,12-olide. Oxidation of **8** with CrO_3–Py afforded 3-oxo-8α-acetoxyguaianolide (**10**), $C_{17}H_{18}O_5$ (M^+ *m/e* 302), mp 133–134°, UV, 215 nm and IR, 1740 cm^{-1}, characteristic of an α,β-unsaturated cyclopentenone. The structure of the guaianolide (**8**) was thus established to be 3β-hydroxy-8α-acetoxyguaian-4(15), 10(14)-dien-6,12-olide.

Various guaianolides have been found in higher plants, particularly in Compositae. This is the first example of the isolation of the guaianolides from lower plants. The liverworts, *Frullania* species, contain a tremendous amount of eudesmanolides and eremophilanolides together with costunolide. In spite of careful observations using TLC and GC–MS, no eudesmanolides and eremophilanolides have been detected, except for the presence of tulipinolide (**1**) and the guaianolides (**5**–**7**) in the present species. It is known that the male gametophyte of *C. conicum* does not exhibit any pungency. This is due to the absence of tulipinolide in the male gametophyte. However, it elaborates the same common guaianolides as found in the female thallus [14]. The liverworts often elaborate the optical isomers of the sesqui- or diterpenes found in the higher plants. Like drimanes found in the liverworts, *Porella* species [2] and *Frullania tamarisci* [15, 16] and its subspecies [17], the present germacranolide and guaianolides are the same configuration of those found in higher plants.

The natural guaianolides (**5**–**7**) and deoxyzaluzanin C (**9**) and two eudesmanolides (**2** and **3**) derived from **1** showed inhibitory activity against the germination and growth of rice in husk (Table 1).

Table 1. Inhibitory activity toward germination and growth of rice in the husk

Sesquiterpene lactone	Complete germination inhibition (ppm)	Complete growth inhibition of root (ppm)
2	100	50
3	200	50
5	100	50
6	100	50
7	200	50
9	100	50

EXPERIMENTAL

All mps are uncorr. The solvents used for spectral determinations were: TMS–$CDCl_3$ (NMR); $CHCl_3$ (IR); 95% EtOH (UV); MeOH (CD and $[\alpha]_D$), unless otherwise stated. TLC: precoated Si gel (0.25 mesh) F_{254}, *n*-hexane–EtOAc (4:1) and C_6H_6–EtOAc (4:1 and 1:1). Spots were detected by UV light (254 nm) and by spraying with 50% H_2SO_4 or 2,4-DNP. GC–MS; 70 eV, 5% OV-17, 3 m × 2 mm, temp. programme: 180–250° at 5°/min, He 30 ml/min. CI–MS: 500 eV, reaction gas, isobutane.

Bioassay with rice in husk. Bioassay of each sesquiterpene lactone was carried out by Kato's method [18].

Extraction and isolation. Conocephalum conicum (female gametophyte) collected in Tokushima prefecture, Kamiyama-cho, May 1978 was washed with H_2O several times. After being air-dried for 5 days, the ground material (180 g) was extracted with Et_2O for 2 weeks and the crude green extract was directly chromatographed on Si gel using *n*-hexane–EtOAc gradient. The first fraction eluted with *n*-hexane contained mono- and sesquiterpene hydrocarbons (450 mg). The second fraction (*n*-hexane–EtOAc, 19:1) gave the mixture of triglycerides and sterols (120 mg). The third fraction (*n*-hexane–EtOAc, 4:1) gave the lactone mixtures (520 mg) which were rechromatographed on Si gel using C_6H_6–EtOAc gradient to afford tulipinolide (**1**) (355 mg) and zaluzanin D (6) (59 mg). Tulipinolide (**1**). mp 178° (decomp.), $[\alpha]_D$ + 244° (*c*, 6.5 in $CHCl_3$) (lit. [8] + 249°), $CD_{262\,nm}$ −4680 (lit. [8] 262 nm, −4780). All spectral data were completely identical to those of tulipinolide (**1**) [8]. Zaluzanin D (**6**). mp 103–104° (lit. [10] 104°), $[\alpha]_D$ ± 0°, monopyrazoline $[\alpha]_D$ + 274° (*c*, 5.1); $CD_{240\,nm}$ + 9382; $CD_{329\,nm}$ + 32352; IR ν_{max} cm^{-1}: 1777 (γ-lactone), 1732 (OAc), 1640 (C=C), 1245 (OAc). All spectral data of **6** were identical to those of zaluzanin D [10, 12].

The fourth fraction (hexane–EtOAc, 7:3) contained pure 8α-acetoxyzaluzanin D (**7**) (565 mg), liquid, $C_{19}H_{22}O_6$ [CI–MS (%) M^+ + 1, 347 (5), 227 (100)], $[\alpha]_D$ + 174° (*c*, 2.3); UV λ_{max} 206 nm (ε, 8933); IR ν_{max} cm^{-1}: 1765 (γ-lactone), 1738 (OAc), 1245 (OAc), 1140, 1024, 964, 915; GC–MS (%) 305 (4), 304 (21), 303 (26), 244 (27), 226 (39), 91 (29), 43 (100).

The fifth fraction (*n*-hexane–EtOAc, 1:1) gave the lactone mixture (224 mg) which was rechromatographed on Si gel using C_6H_6–EtOAc gradient to afford zaluzanin C (**5**) (98 mg) and 8α-acetoxyzaluzanin C (**8**) (87 mg). Zaluzanin C (**5**) mp 95–96° (lit. [10] 95°), $[\alpha]_D$ + 33° (*c*, 2.5) (lit. [10] + 37°). Deoxyzaluzanin C (**9**) was prepared from **5** by oxidation with CrO_3–Py, mp 134–135° (lit. [10] 134–135°), $CD_{344\,nm}$ + 8714, $CD_{265\,nm}$ −5809. All spectral data of compounds **5** and **9** were identical to those of zaluzanin C and deoxyzaluzanin C [10, 12]. 8α-Acetoxyzaluzanin C (**8**). $C_{17}H_{20}O_5$; IR ν_{max} cm^{-1}: 3500 (OH), 1760 (γ-lactone), 1730 (OAc); MS *m/e* (rel. int.) 304 (M^+, 2), 262 (9.4), 244 (M^+ − 60, 16), 91 (58), 43 (100).

Hydrolysis of **1**. To MeOH soln of **1** (120 mg) was added dry K_2CO_3 (50 mg) and the reaction mixture was then refluxed for 3 min. The product was filtered and the solvent evapd to give a methylated lactone (**4**) (94 mg). $C_{16}H_{24}O_4$; IR ν_{max} cm^{-1}: 3600 (OH), 3414 (OH), 1760 (γ-lactone), 1640 (C=C), 1600, 1183, 1128, 1104, 1036, 1006, 962, 900; NMR: δ 1.63 (6H, *bs*, =C(Me)$_2$), 3.46 (3H, *s*, OMe), 3.83–4.76 (complex *m*), 5.00 (1H, *bs*, =CH), 5.36 (1H, *bs*, =CH). MS *m/e* (rel. int.) 280 (M^+, 10), 159 (59), 109 (61), 108 (94), 45 ($MeOCH_2^+$, 100).

Cyclization of **1** *to eudesmanolides* (**2**) *and* (**3**). The eudesmanolides **2** and **3** were prepared by Doscotch's method [8]. The reaction mixture was directly chromatographed on Si gel impregnated with 5% $AgNO_3$ using hexane–EtOAc to afford pure eudesmanolides **2** and **3**, whose spectral data and physical constants were identical to those of 8α-acetoxy-α-cyclocostunolide and 8α-actoxy-β-cyclocostunolide, respectively.

Hydrolysis of 8α-*acetoxyzaluzanin D* (**7**). The compound (**7**) (102 mg) in MeOH (8 ml) was refluxed with K_2CO_3 (50 mg) for 3 min. The reaction mixture was filtered through a short column packed with Si gel and evapn of the solvent to afford a viscous oil which was directly chromatographed on Si gel using a C_6H_6–EtOAc gradient to give pure diol (**11**) (87 mg). mp 156–157°; UV λ_{max} 203 nm (ε, 2359); IR ν_{max} cm^{-1}: 3560 (OH), 1760 (γ-lactone), 1636 (C=C), 1170, 1090, 1010, 910; NMR δ 3.50 (3H, *s*, OMe), 3.4–4.16 (complex *m*), 4.43 (1H, *bs*, CHOH), 5.02 (2H, *bs*, =CH$_2$), 5.38 (2H, *bs*, =CH$_2$); MS *m/e* (rel. int.) 294 (M^+, 14), 114 (100), 115 (23), 105 (64), 91 (74), 69 (64), 45 ($MeOCH_2^+$, 30).

Oxidation of the hydrolysed lactone (**11**). The diol (50 mg) in dry CH_2Cl_2 (1 ml) was added to CrO_3–Py complex (250 mg) in CH_2Cl_2 (3 ml) and stirred at room temp. for 2 hr. The reaction mixture was filtered and the solvent evapd to give a pale yellow oil, purified by PLC to afford pure diketone (**12**) (38 mg). mp 155–156°; $[\alpha]_D$ + 32° (*c*, 6.2 in $CHCl_3$); $CD_{376\,nm}$ + 109, $_{364\,nm}$ + 140, $_{338\,nm}$ − 514, $_{316\,nm}$ − 706, $_{307\,nm}$ − 545. UV λ_{max} 210 nm (ε, 28057); IR ν_{max} cm^{-1}: 1780 (γ-lactone), 1723 (C=O), 1635 (C=C), 1176, 1158, 1127, 1110, 1075, 1027, 960, 917; NMR δ 3.36 (3H, *s*, OMe), 3.3–3.96 (complex *m*), 4.18 (1H, *t*, *J* = 9 Hz, H-6), 5.00 (1H, *s*, =CH), 5.13 (1H, *s*, =CH), 5.81 (1H, *bd*, *J* = 2 Hz, =CH), 6.38 (1H, *bd*, *J* = 2, =CH); MS *m/e* (rel. int.) 290 (M^+, 8), 91 (39), 85 (100), 83 (63), 45 ($MeOCH_2^+$, 49).

Acetylation of 8α-*acetoxyzaluzanin C* (**8**). To a Py soln of **8** (25 mg) was added Ac_2O (1 ml) and the mixture allowed to stand overnight. Recovery of the product in the usual way gave monoacetylated compound (22 mg), whose physical and spectral data were in accordance with those of the natural 8α-acetoxyzaluzanin D (**7**).

Oxidation of 8α-*acetoxyzaluzanin C* (**8**). To a CH_2Cl_2 soln of Collins reagent (250 mg) was added a CH_2Cl_2 soln of compound **8** (53 mg) and stirred at room temp. for 2 hr. The recovered brown oil was purified by PLC to afford an α,β-unsaturated ketone (**10**) (24 mg). mp 133–134°; $[\alpha]_D$ + 25° (*c*, 0.6); UV λ_{max} 215 nm (ε, 19932); IR ν_{max} cm^{-1}: 1770 (γ-lactone), 1740 (C=O), 1730 (OAc), 1640 (C=C), 1230 (OAc), 1148, 1040, 1020, 970, 940, 815; NMR: δ 2.16 (3H, *s*, OAc), 4.06 (1H, *t*, *J* = 9 Hz, H-6), 4.93 (1*H*, *s*, =CH), 5.06 (1*H*, *s*, =CH), 5.86 (1H, *d*, *J* = 3 Hz, H-12), 5.93 (1H, *bs*, =CH), 6.36 (1H, *bs*, =CH), 6.40 (1H, *d*, *J* = 3 Hz, H-12'); MS *m/e* (rel. int.) 302 (M^+, 2), 242 (M^+ − 60, 100), 214 (68), 200 (55), 91 (45), 43 (70).

Acknowledgements—The authors are indebted to Dr. S. Hattori of Hattori Botanical Laboratory, Miyazaki, Japan for identification of *Conocephalum conicum*.

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