Phytochemistry, 1979, Vol. 18. pp. 1007–1009. © Pergamon Press Ltd. Printed in England.

0031-9422/79/0601-1007 $02.00/0

PUNGENT SESQUITERPENE LACTONES OF THE EUROPEAN LIVERWORTS *CHILOSCYPHUS POLYANTHUS* AND *DIPLOPHYLLUM ALBICANS*

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(*Received* 14 *November* 1978)

Key Word Index—*Chiloscyphus polyanthus*; *Diplophyllum albicans*; Hepaticae; *ent*-eudesmanolides; pungency; plant growth inhibitory activity; sesquiterpene lactones.

Abstract—The characteristic pungency of the European liverwort, *Chiloscyphus polyanthus*, is due to a mixture of four sesquiterpene lactones, two new structures *ent*-5β-hydroxydiplophyllin and *ent*-3-oxodiplophyllin, and the previously known diplophyllin and diplophyllolide. A new dihydrodiplophyllin, together with the pungent diplophyllolide and diplophyllin, has been isolated from *Diplophyllum albicans*. *Ent*-dihydrodiplophyllolides derived from the above pungent diplophyllolides produce intense numbness of the tongue. All pungent sesquiterpene lactones showed inhibitory activity towards the germination and root elongation of rice husks.

INTRODUCTION

Some liverworts, *Porella*, *Trichocoleopsis*, *Plagiochila* and *Conocephalum* species, contain characteristic pungent substances. Recently we have reported that the pungency of these liverworts is due to sesquiterpene and diterpene dialdehydes, a secoaromadendrane type sesquiterpene hemiacetal and a germacranolide [1–5]. Pungent substances are also present in the European liverworts, *Chiloscyphus polyanthus* and *Diplophyllum albicans*. The pungency of the former species is exceedingly intense. The crude extracts of both species inhibit plant growth. In this paper, we wish to report the isolation and identification of the pungent substances of *C. polyanthus* and *D. albicans*.

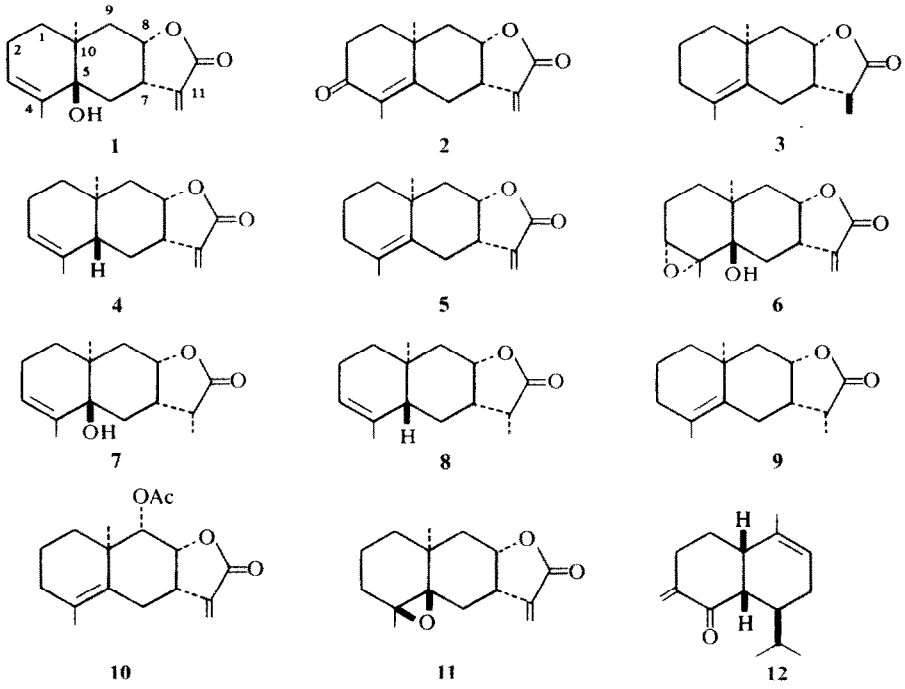
RESULTS AND DISCUSSION

Column chromatography and PLC on Si gel of the crude extract of *C. polyanthus* resulted in the isolation of two new eudesmanolides, *ent*-5β-hydroxydiplophyllolide (**1**) and *ent*-3-oxodiplophyllin (**2**), and the previously known diplophyllolide (**4**) and diplophyllin (**5**). The same treatment of the crude extract of *D. albicans* gave diplophyllin and diplophyllolide, together with a new *ent*-dihydrodiplophyllin (**3**). The structures of the eudesmanolides **4** and **5** were deduced from IR, ^{1}H NMR, ^{13}C NMR, mass and CD spectra and by chemical transformations. Identities were confirmed by comparison with literature data [6, 7].

ent-5β-Hydroxydiplophyllolide (**1**), the major component $C_{15}H_{20}O_3$ (M^+ 248) isolated from *C. polyanthus*, showed the presence of an OH group (3440 cm^{-1}) and a characteristic α-methylene-γ-lactone (1760 cm^{-1}; δ 5.85, 6.21 ppm, each, *s*). The ^{1}H NMR spectrum included the signals of one tertiary methyl (0.88 ppm), a vinylic methyl (1.63 ppm), a vinylic proton (5.43, *m*) and one proton on a carbon bearing a lactone oxygen (4.33 ppm, *m*). Treatment of **1** with *m*-chloroperbenzoic acid gave a monoepoxide (**6**), $C_{15}H_{20}O_4$ (M^+ 264). Reduction of **1** with $NaBH_4$ afforded a dihydro derivative (**7**), $C_{15}H_{22}O_3$ (M^+ 250). Resistance to acetylation and oxidation showed that the hydroxy group in **1** was tertiary; the presence of this OH group was further confirmed by the signal at 75.6 ppm (*s*) of the ^{13}C NMR spectrum. The ^{1}H NMR signal pattern and the fragmentation pattern of **1** were quite similar to those of **4**, indicating that **1** might be the 5-hydroxy derivative of **4**. The stereochemistry of **1** including the tertiary OH group was established by the positive Cotton effect ($\Delta\varepsilon_{283\,nm}$ +0.65) and by biogenetic considerations. Thus, the structure of the new eudesmanolide was established as **1**.

The minor component (**2**), $C_{15}H_{18}O_3$ (M^+ 246) showed the presence of an α,β-unsaturated carbonyl group (λ 245.5 nm; 1655 and 1620 cm^{-1}) and a typical α-methylene-γ-butyrolactone (1765 cm^{-1}; δ 5.70, 6.33 ppm, each *d*, J = 1.5). In the ^{1}H NMR spectrum, one tertiary methyl (1.26 ppm) and one vinylic methyl group (1.86 ppm), and a proton (4.61 ppm, *m*) on a carbon bearing a lactone oxygen could also be observed. The positions of the α,β-unsaturated carbonyl absorption bands in IR and UV spectra resembled those of 1,2-dihydro-α-santonine [8] and the signal pattern of the ^{1}H NMR spectrum was also quite similar to that of diplophyllin (**5**). The above spectral data indicated that **2** might be 3-oxodiplophyllin. This assumption was further confirmed by the arithmetical difference in frequencies ($\nu_{C=C} - \nu_{C=O}$, 35 cm^{-1} assigned transoid) between ν C=O and ν C=C in IR spectrum [9] and the lack of the signal of one methine group located between carbonyl group at C-6 and exomethylene group at C-11. The above results, coupled with its co-occurrence with diplophyllin and diplophyllolides and biogenetic considerations led us to structure **2** for the second new eudesmanolide.

ent-Dihydrodipolophyllin (**3**), the minor component isolated from *C. albicans*, $C_{15}H_{22}O_2$ (M^+ 234), showed



the presence of a γ-lactone (1170 cm^{-1}). The ^1H NMR spectrum included the signals attributable to a tertiary methyl (1.12 ppm) ,a vinylic methyl (1.67 ppm), and a secondary methyl group (1.23 ppm) characteristic of an α-methyl group of a γ-lactone. The ^1H NMR and mass spectra were almost identical to those of dihydrodiplophyllin with pseudoequatorial α-methyl group [6, 10]; however the optical rotation was different. This suggested that **3** might be dihydrodiplophyllin with a pseudoaxial α-methyl group. The structure of the natural dihydrodiplophyllin was identical in all respects to the minor product **3**, $C_{15}H_{22}O_2$ (M^+ 234) obtained by $NaBH_4$ reduction of **5**. The assumption that **3** has pseudoaxial C-11 bond was confirmed by applying Narayanan's ^1H NMR solvent shift method [11]. The difference ($\delta_{C_6H_6}-\delta_{CDCl_3}$) for the C-11 methyl resonance between **3** and **9** was 0.25 and 0.16 ppm, respectively. Therefore, the structure of new *ent*-dihydroeudesmanolide could be established as **3**.

In addition to the above *ent*-eudesmanolides, α-selinene and β-chamigrene were detected in *C. polyanthus* by GC–MS. α-Elemene, α-selinene, calamenene and the previously known 9α-acetoxydiplophyllin (**10**) were also detected in *D. albicans* by GC–MS.

The characteristic pungency of the two species are due to the *ent*-eudesmanolides. *Ent*-5β-hydroxydiplophyllolide (**1**) and *ent*-diplophyllolide (**4**) are intensely pungent. 3-Oxodiplophyllin (**1**), diplophyllin (**5**), epoxides **6** and **11** are slightly pungent. It is interesting that the stereoisomeric *dihydro* diplophyllins **3** and **9** lack pungency; however, dihydrodiplophyllolides **7** and **8** produce intense numbness of the tongue. *C. polyanthus* is more pungent than *D. albicans*. This is due to differences in content of the pungent lactone **4** and the presence of the second pungent lactone (**1**) in *C. polyanthus*. In *C. polyanthus* **4** and **1** make up 15 and 9% of the total extract, whereas in *D. albicans* **1** makes up about 6% of the total extract.

Recently American [6] and Czechoslovakian groups [7] independently reported the isolation of the *ent*-eudesmanolides **4** and **5** from *D. albicans*. The American race elaborates diplophyllin (**5**) as the major component; the Czechoslovakian race produces diplophyllolide (**4**) and an unknown diplophyllolide B (M^+ 234). The present *D. albicans* collected in south-west France contains **5** as the major component and is similar to the American species. The liverworts, *Frullania* species elaborate various C-6/C-7 eudesmanolides and C-7/C-8 eremophilanolides [12]. In the present two species, neither C-6/C-7 eudesmanolides nor eremophilanolides could be detected by GC–MS.

Chiloscyphone (**12**), a cadalenic ketone, has been isolated from Japanese *C. polyanthus* [13]. In the present European species, the major components are *ent*-eudesmanolides and no chiloscyphone could be detected, even after GC–MS.

The pungent *ent*-eudesmanolides (**1**, **2**, **4**–**6**, **11**) inhibited the germination and root elongation of rice husk at *ca* 100–200 ppm, just as do the guaianolides and germacranolide isolated from *Conocephalum conicum* [5].

EXPERIMENTAL

All mps are uncorr. The solvent used for spectral determination were: TMS–$CDCl_3$, TMS–C_6H_6 (^1H NMR and ^{13}C NMR); $CHCl_3$ (IR and $[\alpha]_D$); 95% EtOH (UV); dioxane (CD), unless otherwise stated. TLC: precoated Si gel (0.25 mm) F_{254}, solvent system: *n*-hexane–EtOAc (4:1) and C_6H_6–EtOAc (4:1). Spots were detected in UV light (254 and 360 nm) and by spraying with 50% H_2SO_4. GC–MS: 70 eV, column OV-1 5% or OV-17 5%, 3 m × 2 mm, temp. programme, 50–250° at 5°/min, He 30 ml/min.

Extraction and isolation. C. polyanthus collected in Balizac, Gironde, France in May, 1978 and *D. albicans* in Nassiet, Landes, France in May–June, 1978 were ground and air-dried for 5 days. The ground material (202 g, *C. polyanthus* and 230 g,

D. albicans) was extracted with Et_2O for 2 weeks. The pungent crude extract (12.603 g) of *C. polyanthus* was directly chromatographed on Si gel using *n*-hexane–EtOAc gradient. The first fraction eluted with *n*-hexane contained sesquiterpene hydrocarbons (120 mg) in which α-selinene and β-chamigrene were detected by GC–MS. The second fraction (*n*-hexane–EtOAc, 19:1) gave carotenoids (45 mg). The fourth fraction (9:1) gave a mixture of sesquiterpene lactones (3.012 g) which was rechromatographed on Si gel using the same solvent to afford pure diplophyllolide (**4**) (1.904 g) and diplophyllin (**5**) (0.188 g). All spectral data of **4** and **5** were identical to those reported [6, 7]. The fifth fraction (4:1) gave colorless crystals, recrystallization from *n*-hexane to give *ent*-5β-hydroxydiplophyllolide (**1**) (1.080 g), mp 72–74°; $[\alpha]_D$ −36.1° (*c*, 1.09); ν_{max} 3440, 1760, 1675, 820, 910 cm^{-1}; ^{13}C NMR 169.1 (*s*), 145.2 (*s*), 132.8 (*s*), 122.5 (*s*), 120.6 (*s*), 82.0 (*d*), 75.6 (*s*). MS: *m/e* (%) 248 (M^+, 1), 230 (25), 123 (26), 121 (base). The sixth fraction (7:3) contained a green mass (70 mg) which was rechromatographed on Si gel to afford colorless needles (**3**) (20 mg), mp 170–171°; $[\alpha]_D$ −40.2° (*c*, 0.4); λ_{max} 245.5 nm (ε 16870) [1,2-dihydro-α-santonin, λ 244 nm, ε, 16200 [8]]. ν_{max} 1765, 1655, 1620, 1260, 1130, 1000, 815 cm^{-1}. MS: *m/e* (%) 246 (M^+, $C_{15}H_{18}O_3$, base), 231 (M^+ −15, 32), 218 (52), 204 (74), 185 (46), 145 (67), 131 (35), 108 (73), 91 (68), $\Delta\varepsilon_{274\,nm}$ +21.7, $_{330}$ −3.9, $_{342}$ −5.2, $_{356}$ −4.7, $_{370}$ −1.9 [14]. The crude extract (2.760 g) of *D. albicans* was treated in the same manner as above. The first fraction contained sesquiterpene hydrocarbons (60 mg), analysed by GC–MS to detect α-elemene, α-selinene and calamenene. The second fraction (hexane–EtOAc, 19:1) gave carotenoids (25 mg). The third fraction (9:1) gave sesquiterpene lactones which were purified by PLC to afford *ent*-diplophyllolide (**4**) (180 mg), diplophyllin (**5**) (346 mg), and *ent*-dihydrodiplophyllin (**3**) (37 mg). $[\alpha]_D$ −43.8° (*c*, 1.8); ν_{max} 1770, 1160, 950 cm^{-1}; δ (C_6H_6) 1.01 (*d*, *J* = 7, 3H), 1.13 (*s*, 3H), 1.50 (*bs*, 3H), 3.90 (*bs*, $W_{\frac{1}{2}}$ = 10 Hz). MS: (%) *m/e* 234 (M^+, $C_{15}H_{22}O_2$, 21), 219 (M^+ −15, 65), 145 (base). The fourth fraction (4:1) gave a sesquiterpene lactone mixture (13 mg), analysed by GC–MS to detect 9α-acetoxydiplophyllin (**10**) [6]. MS: *m/e* (%) 290 (M^+, $C_{17}H_{22}O_4$, 10), 230 (M^+ −60, 80), 215 (100), 123 (45), 105 (45), 91 (40), 43 (80).

Epoxidation of ent-5β-*hydroxydiplophyllolide* (**1**). Compound **1** (20 mg) in $CHCl_3$ was treated with *m*-chloroperbenzoic acid (20 mg) for 1 hr. The reaction mixture was washed with dil. $NaHSO_3$ and worked up as usual to give epoxide (**6**) (18 mg), including a small amount of β-epoxide. Recrystallization from *n*-hexane afforded a pure epoxide, mp 108–109°; $[\alpha]_D$ −40° (*c*, 1.2); ν_{max} 3375 (OH), 1760 cm^{-1}; δ 0.88 (*s*, 3H), 1.23 (*s*, 3H), 2.97 (*bs*, 1H), 3.66 (*bd*, *J* = 4, 1H), 5.65 (*bs*, 1H), 5.70 (*s*, 1H), 6.10 (*s*, 1H); MS: *m/e* (%) 264 (M^+, $C_{15}H_{20}O_4$, 12), 249 (M^+ −15, 77), 139 (base).

Reduction of **1** *with* $NaBH_4$. To compound **1** (34 mg) in EtOAc (5 ml) was added $NaBH_4$ (40 mg) at room temp. for 3 hr with stirring. Work-up yielded a viscous oil which was purified by PLC to give dihydro derivative (**7**) (30 mg). $[\alpha]_D$ −36° (*c*, 1.1); ν_{max} 3440, 1760, 910, 800 cm^{-1}; δ 0.87 (*s*, 3H), 1.22 (*d*, *J* = 7, 3H), 1.68 (*bs*, 3H), 2.85 (*q*, *J* = 8, 1H), 4.40 (*bs*, 1H), 5.50 (*bs*, 1H); MS: *m/e* (%) 250 (M^+, $C_{15}H_{22}O_3$, 3), 232 (M^+ −18, base), 217 (53), 159 (53), 143 (53), 121 (68), 107 (50), 105 (59), 91 (56).

Reduction of **5** *with* $NaBH_4$. Compound **5** (40 mg) was hydrogenated with $NaBH_4$ as described above to afford the stereoisomeric dihydro derivatives which were purified by PLC to give dihydrodiplophyllin **3** (8 mg) and **9** (16 mg). The former lactone was identical to the natural dihydrodiplophyllin (**3**) in all respects. The major lactone, $[\alpha]_D$ −53° (*c*, 0.12); δ (C_6H_6) 1.10 (*d*, *J* = 7, 3H), 1.13 (*s*, 3H), 1.50 (*bs*, 3H), 4.00 (*m*, 1H), was identical in all respects to dihydrodiplophyllin (**9**) [6, 10].

Reduction of **4** *with* $NaBH_4$. Compound **4** (20 mg) was treated with $NaBH_4$ to give dihydro derivative, purified by PLC to afford pure lactone **8** (17 mg). ν_{max} 1760 cm^{-1}; δ 0.88 (*s*, 3H), 1.22 (*d*, *J* = 7, 3H), 1.65 (*bs*, 3H), 2.91 (*q*, *J* = 7, 1H), 4.58 (*bs*, 1H), 5.45 ppm (*bs*, 1H). MS: *m/e* (%) 234 (M^+, $C_{15}H_{22}O_2$, 19), 219 (M^+ −64), 145 (base).

Epoxidation of **5**. Compound **5** (102 mg) was treated with *m*-chloroperbenzoic acid. Work-up afforded a monoepoxide (**11**) (94 mg). $[\alpha]_D$ −94° (*c*, 6.1); ν_{max} 1770, 900, 820 cm^{-1}; δ 1.16 (*s*, 3H), 1.27 (*s*, 3H), 2.90–3.63 (*m*, 1H), 4.50 (*m*, 1H), 5.46 (*d*, *J* = 1.5, 1H). 6.03 (*d*, *J* = 1.5, 1H); ^{13}C NMR: 170.5 (*s*), 141.8 (*s*), 120.5 (*t*), 76.7 (*d*), 67.5 (*s*), 63.6 (*s*), $\Delta\varepsilon_{266\,nm}$ −7.4; MS: *m/e* (%) 248 (M^+, $C_{15}H_{20}O_3$, 7), 217 (62), 190 (52), 178 (base), 145 (61).

Acknowledgements—The authors are indebted to Dr. S. Hattori of Hattori Botanical Laboratory, Miyazaki, Japan, and to Dr. H. Inoue of National Museum, Tokyo, for the identification of the species and to Miss R. Matsuda for the bioassay of the sesquiterpene lactones. The authors thank all members of this Institute for partaking in the pungency tests.

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