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CHIRALITY OF TERPENOIDS ISOLATED FROM THE LIVERWORT *CONOCEPHALUM CONICUM*

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Key Word Index—*Conocephalum conicum*; Hepaticae; (+) - bornyl ferulate; bornyl 2 - methoxy - 4 - hydroxycinnamate; monoterpenes; *ent*-sesquiterpenes; 1 - octen - 3 - ol; 1 - octen - 3 - yl acetate; mushroom-like odour.

Abstract—Reinvestigation of a Japanese sample of the thalloid liverwort *Conocephalum conicum* afforded (+)-bornyl ferulate and bornyl 2 - methoxy - 4 - hydroxycinnamate together with (−) - limonene, (−) - β - sabinene, (+) - bicycloelemene, (+) - β - elemene, (−) - bicyclogermacrene, lunularin, and the two compounds, 1 - octen - 3 - ol and 1 - octen - 3 - yl acetate, responsible for the mushroom-like odour of the crushed thallus. The chirality of the compounds isolated is discussed.

INTRODUCTION

Higher plants produce mainly normal sesquiterpenoids [1] and very few examples of the occurrence of their enantiomers have been reported [2]. In contrast, the two optical antipodes of monoterpenoids generally occur in higher plants; they are often produced as a mixture but some species synthesize only one of the two isomers[3]. In general, the sesquiterpenoids isolated from the liverworts are enantiomeric to those found in higher plants [4]. Three monoterpene hydrocarbons [5] and a monoterpene ester [6] have so far been isolated from the liverworts and in the two investigated species, only one antipode is produced.

Some 30 terpenoids and aliphatic or aromatic compounds have been reported from European [7–12] or Japanese samples [6, 12–16] of *Conocephalum conicum* (L.) Lindb. (Marchantiales). Many of them have asymmetrical carbons but the chirality of only seven terpene derivatives have been reported. The sesquiterpene lactones zaluzanin C and D, 8α-acetoxyzaluzanin C and D and tulipinolide have the normal 6α, 7β configuration [13]. On the other hand, an enantiomeric δ-cadinene has been isolated from American *C. conicum* [17]. From both female and male thalli of Japanese *C. conicum*, (+)-bornyl acetate (**5**) has been isolated as a pure compound [6], the chirality of which showed unambiguously that the laevo-rotatory form is not produced.

We have reinvestigated a sterile sample of *C. conicum* collected in the Tokushima prefecture (Shikoku, SW Japan), in order to obtain more information on the chirality of the compounds produced. In the course of the work, we have identified the compounds responsible for the mushroom-like odour of the crushed thalli.

RESULTS AND DISCUSSION

Fractionation of the ether extract by a combination of CC, prep. TLC and prep. GLC afforded a new monoterpene ester, bornyl 2 - methoxy - 4 - hydroxycinnamate (**2**), together with (+)-bornyl ferulate (**1**), (−)-limonene (**3**), (−)-β-sabinene (**4**), (+)-bornyl acetate (**5**), *ent*-sesquiterpenes, (+)-bicycloelemene (**7**), (+) - β - elemene (**8**), (−)-bicyclogermacrene (**9**), lunularin (=3,4′-dihydroxybibenzyl) (**10**), 1 - octen - 3 - ol, 1 - octen - 3 - yl acetate and phytol. The isolation from a liverwort and the chirality of the compounds **1**, **2**, **4**, **7** and 1 - octen-3 - yl acetate are reported here for the first time. Compound **9** and phytol have not previously been reported from *C. conicum*. Compound **8** and 1 - octen-3 - ol have been isolated from a French sample [8] but their chiralities have not been reported. Table 1 shows the chiral properties of the terpenoids isolated from *C. conicum*. The structure of (+)-bornyl ferulate (**1**) was established by spectroscopic (UV, IR, ^{1}H NMR and MS) and chemical methods, the hydrolysis of **1** giving (+)-borneol (**5**) and ferulic acid. (−)-Bornyl ferulate, the enantiomer of **1**, has been isolated from *Verbesina rupestris* (Compositae) [18]. The structure of a new bornyl ester (**2**) was deduced from its ^{1}H NMR and MS spectra. The MS and signal patterns in the high field of the ^{1}H NMR spectrum were very close to those of **1** and (+)-bornyl acetate (**5**), indicating that **2** might be the isomer of **1**. The splitting patterns of the three benzylic protons one of which resonated at lower field (δ7.66), and the observation that the chemical shifts of the *trans*-ethylenic protons were higher than those of **1**, suggested that **2** might be bornyl 2 - hydroxy - 4 - methoxy or 2 - methoxy - 4 - hydroxy cinnamate. In the ^{1}H NMR spectrum of **11**, isolated from the leafy liverworts *Bazzania* species [19, 20], three benzylic protons, H-3, H-5 and H-6, appeared at δ7.60, 6.69 and 7.16 with the same splitting patterns as compound

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Table 1. Chiral properties of mono- and sesquiterpenes isolated from *C. conicum*

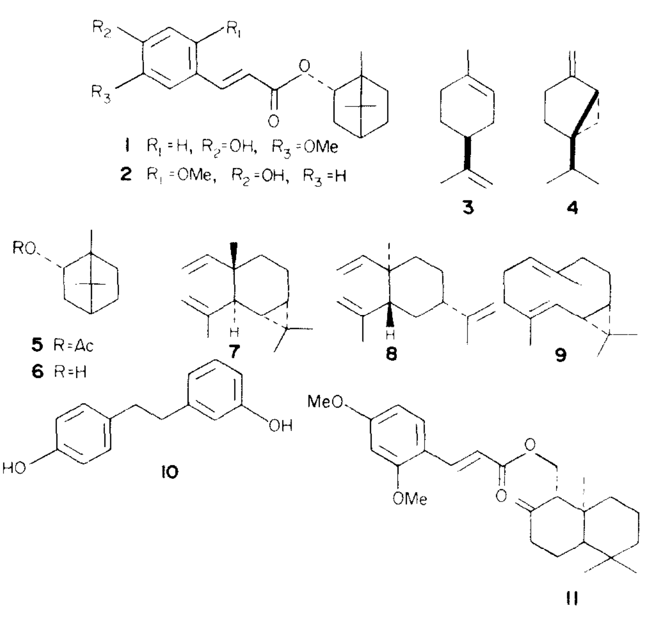
Compounds	$[\alpha]_D$	$[\alpha]_D^*$
Bornyl ferulate (**1**)	+ 10.5°	negative [18]
Bornyl 2-methoxy-4-hydroxycinnamate (**2**)		
Limonene (**3**)	− 111.5	± 123–126° [27]
β-Sabinene (**4**)	− 51.2	+ 89 [27]
Bornyl acetate (**5**)	+ 40.0 [6]	− 41.5†
Bicycloelemene (**7**)	+ 45.5	− 34.0 [29–31]
β-Elemene (**8**)	+ 14.0	− 16.9 [27]; − 16.6, + 18 [32]‡
Bicyclogermacrene (**9**)	− 63.5 [33, 34]	+ 61 [35]

*Optical rotations of the terpenoids isolated from higher plants.
†Prepared from (−)-borneol.
‡Isolated from insects.

2. However, the chemical shift of H-5 in **2** and in authentic methyl 3,4-dimethoxycinnamate [δ 6.97 and 6.83 (*d*, $J = 8$), respectively] indicated the present of a 2 - methoxy - 4 - hydroxycinnamate group in **2**. The suggested structure (**2**) was in agreement with the above spectral evidence. On the basis of the co-occurrence of (+)-bornyl esters (**1** and **5**), it is suggested that **2** should have the same chiroptical property as **1** and **5**. Four cinnamates whose alcohol moieties are not terpenoids have previously been reported from a leafy liverwort, *Isotachis japonica*, [21, 22] and from two Anthocerotes species [23].

Limonene (**3**) and β-sabinene (**4**) isolated from *C. conicum* showed negative optical rotation. It is worth noting that (+)-limonene, together with (+) - α - pinene and (+)-camphene have been isolated from a leafy liverwort, *Jungermannia exsertifolia* [6]. Thus, the two optical antipodes of limonene occur in liverworts as in higher plants but *C. conicum* and *J. exertifolia* produce only one of the two antipodes: no example for a mixture of antipodes has been so far reported for terpenoids or any other compounds from liverworts. The three sesquiterpene hydrocarbons, (+)-bicycloelemene (**7**), (+)-β-elemene (**8**) and (−)-bicyclogermacrene (**9**) belong to the *ent*-series and their co-occurrence with *n*-sesquiterpene lactones [13] clearly shows that *C. conicum* biosynthesizes both normal and *ent*-sesquiterpenoids, unlike most higher plants. δ-Cadinene previously reported from both European [7, 17] and Japanese samples [12, 14, 15] of *C. conicum* was surprisingly not found in the re-investigated sample.

The sterile, female and male thalli of *C. conicum* emit fragrant odours when they are crushed. The male thallus emits a strong mushroom-like odour which is very similar to that of the Japanese edible mushroom *Tricholoma matsutake* Sing. Careful chromatography of the odorous fractions from the crude extract resulted in the isolation of three fragrant compounds, (+)-bornyl acetate (**5**), 1-octen-3 - ol, and 1 - octen - 3 - yl acetate. 1 - Octen - 3 - ol is the main odorous substance of *T. matsutake* [24] and of clover plants [25]. 1-Octen-3-yl acetate emits a fragrant turpentine-like odour. Thus, it is clear that the turpentine- /mushroom-like odour of *C. conicum* is mainly due to the mixture of (+)-bornyl



acetate (**5**), 1 - octen- 3 - ol and its acetate. It is worth noting that three bornyl esters (two of which show dextrorotatory activity) and tulipinolide ($=8\alpha$-acetoxycostunolide) [13] occur in *C. conicum* whereas (+)-borneol (**6**) and costunolide have not been so far identified. On the other hand, 1 - octen - 3 - ol co-occurs with its acetate in the same way as zaluzanin C and zaluzanin D (3β-acetoxyzaluzanin C) co-occur with their corresponding 8α-acetoxy derivatives [13]. Such a comparison emphasizes the present lack of information on the biosynthetic pathways of terpenoids in liverworts. In addition, it emphasizes the large number of terpene acetates in *C. conicum*; a similar abundance also occurs in *Wiesnerella denudata* (Mitt.) Steph. (Marchantiales) [15] and in most *Plagiochila* species (Jungermanniales) which have been investigated [26]. On the other hand, the same sesquiterpene lactones having 6α, 7β configuratiuon and antipodes of some of the *ent*-terpenes identified in *C. conicum* and *W. denudata* [15] occur in Compositae species, although there is a considerable gap between the evolution of Marchantiales liverworts and the Compositae. Eugenol, so far isolated only from a French sample of *C. conicum* [12] was not found in the present species. The presence or absence of this compound is the most significant difference between Asiatic and European sterile samples from the standpoint of essential oil chemistry. Re-investigation of European sample might define more precisely the level of chemical differentiation between samples of different origin which show no consistent morphological differences.

EXPERIMENTAL

Plant material. A part of the analysed sample is preserved in the herbarium of Institute of Pharmacognosy, Tokushima Bunri University. UV, IR, ^{1}H NMR and MS spectra were performed as previously described [13]. $[\alpha]$ of the pure materials obtained by prep. GLC or prep. TLC were measured on ORD/UV spectrometer in $CHCl_3$.

Extraction and isolation. Fresh thalli of *C. conicum* (L.) Dum. collected in Sept. 1980, in Kito, Tokushima, Japan, were carefully separated from other minor plants and then washed with H_2O. Air-dried ground material (673 g) was extracted with Et_2O for 2 weeks. The crude extract (20.5 g) was chromatographed on Si gel using a *n*-hexane–EtOAc–MeOH gradient to give nine fractions: 1–4 (*n*-hexane), 5 (*n*-hexane–EtOAc, 9:1), 6 (7:1), 7 (1:1), 8 (EtOAc) and 9 (MeOH). Each fraction was analysed by GC/MS before further fractionation. Fraction 1 (0.40 g) gave a mixture of mono- and sesquiterpene hydrocarbons in which α-pinene, camphene, β-pinene, β-sabinene, β-caryophyllene and γ-cadinene were identified by comparison (MS) with authentic samples. Fraction 2 (5.60 g) gave a fragrant oil in which camphene, β-pinene, limonene, β-sabinene, α-terpinene, bornyl acetate, 1 - octen - 3 - yl acetate, bicycloelemene, β-caryophyllene, β-elemene and bicyclogermacrene were identified by GC/MS. The above mixture was chromatographed on Si gel using a *n*-hexane–EtOAc gradient to give 12 fractions [2(1)–2(12)]. Prep. GLC of fraction 2 (5) afforded (−)-β-sabinene (**4**) (101.7 mg), (−)-limonene (**3**) (23 mg) and an unidentified diterpene (0.1 mg). Prep. GLC of fraction 2 (7) afforded (+)-bicycloelemene (**7**) (3.3 mg), (+)-β-elemene (**8**) (11 mg) and (−)-bicyclogermacrene (**9**) (5 mg). Fraction 2 (12) contained carotenoids (3.932 g). Fraction 3 (1.30 g) was subjected to prep. GLC to afford (+)-bornyl acetate (**5**) (80 mg) and (+) - 1 - octen - 3 - yl acetate (7.1 mg): $[\alpha]_D$ 0.00° (*c*, 2.03), $[\alpha]_{400}+1.96°$, $[\alpha]_{350}+3.44°$, $[\alpha]_{300}+7.86°$, $[\alpha]_{250}+15.70°$. Fractions 4 and 5 (2.17 g) were composed of mixtures of bornyl acetate (**5**), sesquiterpene hydrocarbons and sesquiterpene alcohols in which cuparene, 1 - octen - 3 - ol and phytol were detected by GC/MS. Repeated chromatography on Si gel using an *n*-hexane–EtOAc gradient afforded phytol (550 mg), (+)-bornyl acetate (932 mg) and 1 - octen - 3 - ol (18 mg): $[\alpha]_D$ 0.00° (*c*, 2.2) [24, 25, 27]. Fractions 6 and 7 (6.42 g) were mixtures of terpenic esters, bibenzyls and sterols in which brassicasterol, campesterol, stigmasterol and sitosterol (1:2:4:1) were identified by GC/MS. The mixtures were chromatographed on Sephadex LH-20 ($CHCl_3$–MeOH, 19:1) to give 26 fractions [9(1)–9(26)]. Rechromatography of the combined fractions 9(9–16) on Si gel using a C_6H_6–EtOAc gradient afforded 36 fractions. Fraction 14 was purified by prep. TLC to give (+)-bornyl ferulate (**1**) (21 mg): MS *m/z* (rel. int.): (330 $[M]^+$ (16), 177 [3 - OMe–4–OH–C_6H_3–CH=CH–CO]$^+$ (100), 145 (10), 79 (16), and bornyl 2 - methoxy - 4 - hydroxy cinnamate (**2**) (3 mg): ^{1}H NMR: δ 0.81, 0.86, 0.91 (each 3H, *s*), 4.92 (1H, *br d*, $J=10$, –C$\underline{H}$–OCO–), 5.78 [1H, *s*(*br*), OH], 5.80, 6.76 (each, 1H, *d*, $J=16$ Hz, Ph–C$\underline{H}\overset{t}{=}C\underline{H}$–CO), 6.83 (1H, *d*, $J=8$ Hz, H-6), 6.97 (1H, *dd*, $J=8$, 2 Hz, H-5), 7.66 (1H, *d*, $J=2$ Hz, H-3); MS *m/z* (rel. int.): (330 $[M]^+$ (17), 177 (100), 145 (14), 79 (16). Repeated prep. TLC of fractions 18–26 (177 mg) gave lunularin (**10**) (18 mg) [28] and four unidentified bibenzyl derivatives as minor components.

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