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SESQUITERPENOIDS FROM *CHILOSCYPHUS, CLASMATOCOLEA* AND *FRULLANIA* SPECIES*

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Key Word Index—*Chiloscyphus polyanthos*; *Clasmatocolea vermicularis*; *Frullania apiculata*; *F. clavata*; *F. folciloba*; *F. gaudichaudii*; *F. serrata*; *F. ternatensis*; Jungermanniales; Hepaticae; *ent*-7α-hydroxydiplophyllolide; sesquiterpene lactones; chemosystematics; piscicidal activity.

Abstract—Eight liverworts, *Chiloscyphus polyanthos* and *Clasmatocolea vermicularis* (Lophocoleaceae), *Frullania apiculata*, *F. clavata*, *F. folciloba*, *F. gaudichaudii*, *F. serrata* and *F. ternatensis* (Frullaniaceae) were chemically investigated. *ent*-7,8-Eudesmanolides are important chemical markers of *C. polyanthos* and *C. vermicularis*. The latter species also produces 6,7-eudesmanolides which are the chemical markers of *Frullania* species. *C. polyanthos* and *C. vermicularis* are chemically quite close to some *Diplophyllum* species belonging to Scapaniaceae. The structure of 5β-hydroxydiplophyllolide previously isolated from *C. polyanthos* was revised to *ent*-7α-hydroxydiplophyllolide by analysis of its ^{1}H NMR spectrum. From the chemical constituents, the six *Frullania* species are classified to three chemotypes.

INTRODUCTION

Most liverworts contain terpenoids and/or aromatic compounds which are generally obtained as major components. These substances are important endogenous characteristics of species and can be used as chemosystematic markers [1–8]. Some *Chiloscyphus* and *Frullania* species contain large amounts of sesquiterpene lactones [2, 9, 10] which show intense cytotoxic, allergy inducing and plant growth regulatory activities [10–12].

In previous papers [2, 9], we reported the isolation and the structures of *ent*-7,8-eudesmanolides of French *C. polyanthos* and the distribution of sesquiterpene lactones amongst 25 species of *Frullania*. In the present communication, we report on the distribution of sesquiterpenoids in English *C. polyanthos* (L.) Corda and Colombian *Clasmatocolea vermicularis* (Lehm.) Grolle which belong to the Lophocoleaceae and six Asiatic *Frullania* species, *F. apiculata* (Nees) Dum., *F. clavata* (Hook. and Tayl.) Tayl., *F. folciloba* (Hook. and Tayl.) Lehm., *F. gaudichaudii* Nees and Mont., *F. serrata* Gott. and *F. ternatensis* Gott. var. *non-appendiculata* Hatt. belonging to the Frullaniaceae and the revised structure of 5β-hydroxydiplophyllolide previously isolated from French *C. polyanthos* [9]. We also discuss some aspects of the chemosystematics of Lophocoleaceae and Frullaniaceae.

RESULTS AND DISCUSSION

Each ground liverwort, after being air-dried, was extracted with ether and the crude extracts directly analysed by TLC, GC and computerized GC/MS. The mass spectra were identified by direct comparison with those of authentic samples. The main components were further isolated by prep. TLC or GC and their chemical structures were confirmed by spectral evidence. Table 1 summarizes the distribution of sesquiterpenoids in each species.

Previously, we reported that *Chiloscyphus polyanthos* produced the intensely pungent *ent*-7,8-eudesmanolides, diplophyllolide (**1**) and 5β-hydroxydiplophyllolide (**3b**), together with diplophyllin (**2**) and 3-oxodiplophyllin (**4**) [9]. From the spectral similarity between **1** and **3b** and biogenetic considerations we proposed structure **3b** for the pungent hydroxydiplophyllolide. However, structure **3b** must now be revised to *ent*-7α-hydroxydiplophyllolide (**3a**) on the basis of the following ^{1}H NMR spectral evidence. Thus the spectrum of **3a** showed the presence of α-methylene protons (δ5.85 and 6.21), which appeared as very sharp singlets, indicating the absence of an allylic coupling between the α-methylene protons and H-7. Shift reagent experiments indicated that one of the α-methylene protons and H-8 largely shifted to downfield, showing the presence of a hydroxyl group at C-7. The occurrence of sesquiterpene lactones with a hydroxyl group at C-7 in plants is quite rare [13].

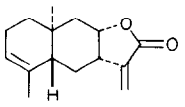
C. polyanthos collected in England had a hot taste. The major components are *ent*-diplophyllolide (**1**) and *ent*-7α-hydroxydiplophyllolide (**3a**). TLC and GC/MS showed that there is no remarkable degree of intraspecific qualitative and quantitative variation between French and English *C. polyanthos* except for the presence or absence of minor components. The major components of *C. vermicularis* are diplophyllin (**2**) and oxyfrullanolide (**7**). *ent*-7,8-Eudesmanolides found in *C. polyanthos* and *C. vermicularis* have also been isolated from *Diplophyllum albicans* (L.) Dum. and *D. taxifolium* (Wahl.) Dum.

* Part 14 in the series "Chemosystematics of Bryophytes". For Part 13 see Asakawa, Y. and Campbell, E. O. (1982) *Phytochemistry* **21**, 2663.

Table 1. Sesquiterpenoids of *Chiloscyphus*, *Clasmatocolea* and *Frullania* species

Family	Species	Date of collection and source	Compounds detected¶																
			1	**2**	**3a**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**
Lophocoleaceae	*Chiloscyphus polyanthos*	Oct. 1979, Wales, U.K.	+++++*	++	++++	+	—	—	—	—	—	—	—	—	+	—	+	—	+
	C. polyanthos [10]	May, 1978, Balizac, Gironde, France	+++++	++	++++	+	—	—	—	—	—	—	—	+	—	—	+	—	—
	Clasmatocolea vermicularis†	Aug. 1980, Paramode Gusca, Colombia	—	+++++	—	—	—	—	+++	+	—	—	—	—	+	—	+	—	+
Frullaniacea	*Frullania apiculata*	Oct. 1967, Pahang, Gunong Jasar, Cameron Highlands, Malaya	—	—	—	—	+++++	+	—	—	—	—	—	—	—	—	—	—	—
	F. clavata‡	Aug. 1981, Blue Mountains, New South Wales, Australia	—	—	—	—	—	—	—	—	—	+	+	—	+	—	—	—	—
	F. folciloba§	Aug. 1981, Blue Mountains, New South Wales, Australia	—	—	—	—	—	—	—	—	+	—	++	—	+	+	—	—	—
	F. gaudichaudii	Jan. 1979, Kalimantan Timur, Gunong Beratus, West of Balikpapan, Borneo	—	—	—	—	—	—	—	—	+	—	—	—	+++	-	—	+	—
	F. serrata‖	Oct. 1967, Pahang, Gunong, Brinchang, Cameron Highlands, Malaya	—	—	—	—	++	—	—	—	—	—	—	—	—	—	—	—	—
	F. ternatensis	Feb. 1979, Kalimantan Selatan, Gunong Besar, Borneo	—	—	—	—	+++++	—	—	—	—	—	—	—	—	—	—	—	—

*The symbols +, ++, +++, etc are relative concentrations estimated by GC/MS.
†This species produces two unidentified sesquiterpene lactones ($[M]^+$ 232 and 250) as the major components.
‡This species produces two unidentified sesquiterpene alcohols ($[M]^+$ 218 and 220) with a dimethylcarbinol group and a bibenzyl (M^+ 300) as the major components.
§This species produces two unidentified sesquiterpenes ($[M]^+$ 218 and 218) as the major components.
‖This species produces two unidentified sesquiterpene lactones ($[M]^+$ 232 and 248) as the major components.
¶The absolute configurations of the compounds (**5**–**10** and **12**–**17**) were tentatively assigned.



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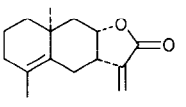

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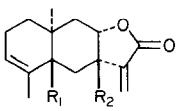
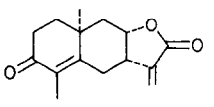


3a R_1=H, R_2=OH
3b R_1=OH, R_2=H



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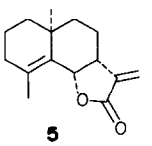

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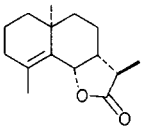

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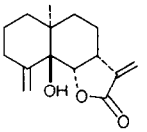

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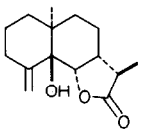

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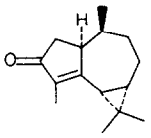

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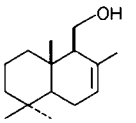

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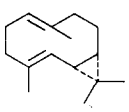

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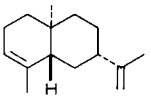

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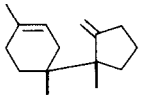

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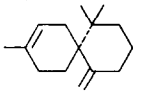

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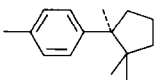

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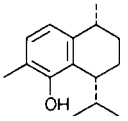

17

belonging to the Scapaniaceae [11, 14] and these sesquiterpene lactones have not been detected in the other species of the Lophocoleaceae, e.g. *Lophocolea* and *Heteroscyphus* species [5]. Thus, *Chiloscyphus polyanthos* is chemically similar to *Clasmatocolea vermicularis* and these two species also resemble *Diplophyllum* species. 6,7-Eudesmanolides (**7**, **8**) found in *C. vermicularis* are important chemical markers of Frullaniaceae (see below). Thus, *C. vermicularis* is also chemically similar to some *Frullania* species.

Frullania species are classified into five chemotypes: sesquiterpene lactone-bibenzyl type (type I), sesquiterpene lactone type (type II), bibenzyl type (type III), monoterpene type (type IV) and cyclocolorenone type (type V) [2]. *F. apiculata*, *F. serrata* and *F. ternatensis* belong to type II, since they produce frullanolide (**5**) as the major component. *F. clavata* contains an unidentified bibenzyl derivative (M^+ 330, base 121) and it belongs to type III. *F. folciloba* and *F. gaudichaudii* belong to type V, since they elaborate cyclocolorenone (**9**).

All of the sesquiterpene lactones isolated so far from liverworts show piscicidal activity. *Oryzia latipes* was killed within 2 hr by a 6.7 ppm solution of *ent*-diplophyllin (**2**) and within 4 hr by 0.4 ppm frullanolide (**5**) isolated from *Frullania dilatata* (L.) Dum. [10]. The detailed piscicidal activity of the other sesquiterpene lactones, and sesqui- and di-terpene dialdehydes will be reported elsewhere.

EXPERIMENTAL

GC/MS, TLC, prep. TLC and GC were carried out as indicated earlier [1]. All species identified by Drs. S. Hattori, S. R. Gradstein, C. Suire and J. G. Duckett were deposited in the Herbaria of the Hattori Botanical Laboratory, Miyazaki, Japan and the Institute of Pharmacognosy, Tokushima Bunri University.

Bioassay. Piscicidal activity was tested by Kawazu's method [15].

Extraction and isolation of terpenoids. Each liverwort (0.120–1.0) after being air-dried was ground and then extracted with Et_2O for 2 weeks. The green extracts were filtered through a short column packed with Si gel (30–70 mesh). Each extract was directly analysed by TLC, GC and computerized GC/MS. The components obtained by GC/MS were identified by direct comparison of the MS with those of authentic samples. The remaining materials of *C. polyanthos* (35 g) and *F. folciloba* (48 g) were extracted with Et_2O for 2 weeks. The intense pungent oil (2.02 g) of *C. polyanthos* was chromatographed on Si gel using a *n*-hexane–EtOAc gradient and each fraction was purified by prep. TLC to give *ent*-diplophyllolide (**1**) (290 mg), *ent*-diplophyllin (**2**) (17 mg), *ent*-7α hydroxydiplophyllolide (**3a**) (210 mg) and 3-oxodiplophyllin (**4**) (3 mg), together with phytosterols (45 mg) and fatty acids (22 mg). The green extract (2.60 g) of *F. folciloba* was also chromatographed on Sephadex LH-20 using $CHCl_3$–MeOH (1:1) and each fraction was purified by prep. TLC or GC to give (−)-bicyclogermacrene (**11**) (16 mg) and two very labile sesquiterpenes (M^+ 218, base 164) (50 mg) and (M^+ 218, base 95) (78 mg), together with phytosterols (38 mg), triglycerides (33 mg) and fatty acids (22 mg).

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