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TERPENOIDS AND BIBENZYLS OF 25 LIVERWORT *FRULLANIA* SPECIES*

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Key Word Index—Jungermanniales; Hepaticae; sesquiterpene lactones; bibenzyls; cyclocolorenone; mono- and sesquiterpene hydrocarbons; normal- and *ent*-sesquiterpenes; allergy; chemotypes; chemosystematics.

Abstract—Twenty-five *Frullania* species (liverworts) were chemically investigated. Fourteen species produce allergy-inducing sesquiterpene lactones. Eighteen species contain bibenzyls. The sesquiterpene lactones and bibenzyls are obtained as the major components and they are valuable chemosystematic markers of *Frullania* species. On the basis of their chemical constituents, *Frullania* species can be divided into five chemotypes: sesquiterpene lactone–bibenzyl type; sesquiterpene lactone type; bibenzyl type; monoterpene type and cyclocolorenone type.

INTRODUCTION

The chemical analyses of 169 species of Japanese, European and South American liverworts have shown that members of the Hepaticae contain components which are of chemosystematic value [1–12]. Frullaniaceae belong to the Jungermanniales and more than 1000 species are known in the world; from Japan 40 species are recorded. *Frullania* species are very interesting from the viewpoint of medicinal chemistry since they are a rich source of sesquiterpene lactones which can produce intense allergenic contact dermatitis [13–15], antitumoral and plant growth regulatory activity [16]. In the present paper, we report the distribution of terpenoids, bibenzyls and sterols in 25 *Frullania* species and discuss some aspects of the chemosystematics of Frullaniaceae.

RESULTS AND DISCUSSION

Each *Frullania*, after being air-dried and ground, was extracted with ether. The crude extracts were monitored by TLC and GC to check whether or not each species contains major components useful as chemical markers. The crude extracts were also directly analysed by GC/MS. The mass spectra obtained by a computerized GC/MS were identified by direct comparison with those of authentic samples and/or published information. The main components were further isolated by a combination of column chromatography, preparative TLC and preparative GC and their chemical structures were confirmed by spectral evidence and some chemical degradation. Table 1 summarizes the distribution of terpenoids, bibenzyls and sterols in each species. Tables 2–5 show the sesquiterpene lactones, sesquiterpene hydrocarbons, ketone, alcohols, bibenzyls and monoterpenes isolated or detected in each *Frullania* species. By their chemical constitution, *Frullania* species fit into five chemotypes (see Table 1). The crude extracts of types I and II showed characteristic intense blue or pale red spots on TLC after 30% H_2SO_4 was sprayed and the plate heated at 100°. Types III–V did not show such characteristic colours. The IR spectra of the crude extracts belonging to types I and II indicated the presence of an absorption band at 1750–1770 cm^{-1}, assignable to a lactone group. On the other hand, the above absorption band did not appear in the crude extracts belonging to types III–V. This suggested that types I and II may contain lactones, revealed by aqueous H_2SO_4. Indeed, various sesquiterpene lactones were isolated or detected in types I and II. Each sesquiterpene lactone isolated from types I and II showed the above characteristic intense colour on TLC [17]. For example, costunolide (**1**), 4-epi-arbusculin A (**6**) and (+)- and (−)-frullanolides (**7**) gave deep blue spots. The sesquiterpene lactones (**2–5**, **8**, **9**, **12–18**) gave pale red colours and then gradually turned to brown. Thus, TLC is a valuable method for recognizing the presence of sesquiterpene lactones.

The allergenic sesquiterpene lactone (−)-frullanolide (**7**) has been isolated from European *F. tamarisci* together with costunolide (**1**) and cyclocostunolides (**3**, **5**) [13–15, 18]. *F. tamarisci* subsp. *obscura* grown in Japan is morphologically quite similar to *F. tamarisci*. *F. tamarisci* subsp. *obscura* elaborates 4-epi-arbusculin A (= 4-hydroxycyclocostunolide) (**6**) and eudesmanal (**27**) as major components [19]. However, frullanolide (**7**), dihydrofrullanolide (**8**) and α- and γ-cyclocostunolides (**3**, **5**) have not been detected in any specimens of *F. tamarisci* subsp. *obscura* collected in Hokkaido, Honshu, Shikoku and Kyushu at different times. Thus, the chemical constitution of *F. tamarisci* subsp. *obscura* is different from that of European *F. tamarisci*, although both taxa produce sesquiterpene lactones.

F. dilatata var. *anomala*, which is morphologically quite close to *F. dilatata*, produces (+)-frullanolide

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*Part 10 in the series "Chemosystematics of Bryophytes". For Part 9, see (1981) *Phytochemistry* (to be submitted).

Table 1. Five chemotypes of *Frullania* species

Frullaniaceae		Chemical constituents					
Types	Species	M*	S*	SL*	D*	B*	St*
I	*F. densiloba*	+†	+	+ + + + +	+ +	+	+ + +
	F. dilatata	+	+ +	+ + + + +		+	+
	F. dilatata var. *anomala*	+	+	+ + + + +		+	+
	F. osumiensis		+ + +	+ + + + +		+ + +	+
	F. parvistipula		+	+ + + + +		+ + + +	+
	F. tamarisci	+	+ + +	+ + + + +		+	+
	F. tamarisci subsp. *obscura*		+ + + +	+ + + + +		+	+
	F. usamiensis	+	+ + + +	+ + + + +		+	+
	F. yunnanensis	+	+ +	+ + + + +		+	+
II	*F. hamatiloba*	+ + +	+ +	+ + +	+ + +		+
	F. inflata	+	+	+			+ + +
	F. motoyana		+ +	+ +	+ + +		+
	F. ramuligera		+ +	+ + + + +			+
	F. vethii		+ + +	+ + + + +			+
III	*F. amplicrania*		+ +		+ +	+ + + + +	+
	F. bonincola		+			+ + +	+
	F. brittoniae subsp. *truncatifolia*		+			+ + + + +	+
	F. davurica		+ + + + +			+ + + + +	+
	F. ericoides		+		+	+ + + + + +	+
	F. hampaena		+			+ + + + + +	+
	F. monocera		+			+ + +	+
	F. pedicellata		+ + + +		+ + + +	+ +	+
	F. taradakensis		+ +		+ + + + +	+ + + + +	+
IV	*F. fragilifolia*	+ + + + +	+				+
V	*F. diversitexta*		+ + + + + +		+ +		+

*M—monoterpenes; S—sesquiterpenes except sesquiterpene lactones; SL—sesquiterpene lactones; D—diterpenes; B—bibenzyls; St—sterols (campesterol, stigmasterol; sitosterol).

†The symbols +, + +, + + +, etc. are relative concentrations estimated by GC/MS.

Table 2. Sesquiterpene lactones of *Frullania* species*

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
F. brotheri									+	+	+							
F. densiloba							+	+										
F. dilatata [20]					+		+	+				+	+	+				+
F. dilatata var. *anomala*	+		+		+		+	+										
F. inflata	+	+																
F. nisquallensis [14]							+											
F. osumiensis			+		+		+											
F. parvistipula													+	+				
F. ramuligera	+	+							+	+								
F. tamarisci [13, 18]	+		+		+		+											
F. tamarisci subsp. [19] *obscura*	+			+		+												
F. usamiensis				+	+												+	
F. vethii															+	+		
F. yunnanensis							+	+										

**F. hamatiloba* and *F. motoyana* contain a few unidentified sesquiterpene lactones (see Table 1).

Table 3. Sesquiterpene hydrocarbons, ketone, aldehyde and alcohol of *Frullania* species*

Species	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
F. amplicrania		+					+								+						
F. densiloba			+								+										
F. dilatata							+†	+†		+	+										
F. dilatata var. *anomala*				+																	
F. diversitexta			+									+	+								
F. davurica		+			+	+				+	+				+		+	+	+		
F. ericoides	+														+						
F. hamatiloba	+											+			+						
F. hampeana	+	+													+					+	
F. inflata				+												+					
F. kagoshimensis						+					+			+	+						
F. monocera	+														+					+	
F. motoyana							+	+													
F. osumiensis										+					+	+				+	
F. parvistipula							+								+						
F. pedicellata											+			+				+			+
F. ramuligera				+								+			+						
F. tamarisci							+	+		+											
F. tamarisci subsp. *obscura*		+		+			+	+	+	+											
F. taradakensis				+						+					+			+			
F. usamiensis											+	+									
F. vethii							+	+							+						

* *F. bonincola*, *F. brittoniae* subsp. *truncatifolia* and *F. fragilifolia* contain several unidentified sesquiterpene hydrocarbons and alcohols.

† Enantiomers of **25** and **26**.

Table 4. Bibenzyls and flavone of *Frullania* species*

Species	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54
F. amplicrania						+	+								
F. brittoniae subsp. *truncatifolia*											+	+			
F. bonincola	+					+				+					
F. dilatata	+	[22]		+	[22]										
F. dilatata var. *anomala*		+													+
F. davurica		+	+		+										
F. ericoides						+			+						
F. osumiensis						+									
F. parvistipula						+		+	+		+	+	+		
F. pedicellata		+													
F. tamarisci	+	[22]		+	[22]										
F. tamarisci subsp. *obscura*		+													
F. usamiensis		+													
F. vethii														+	

* *F. densiloba*, *F. hampeana*, *F. monocera* and *F. taradekensis* contains a few unidentified highly oxygenated bibenzyls (see Table 1).

(enantiomer of **7**), (+)-dihydrofrullanolide (enantiomer of **8**), α- and β-cyclocostunolides (**3, 5**) and costunolide (**1**). The compounds (**1, 3, 5**) were detected only by TLC and GC/MS. From *F. dilatata*, the same eudesmanolides (**5, 7, 8**) isolated from *F. dilatata* var. *anomala*, oxyfrullanolide (**12**) and two eremophilanolides (**13**, **14**) have been isolated together with *cis*-β-cyclocostunolide (**18**) [13, 20]. Among them, (+)-frullanolide and (+)-eremophilanolide were obtained as the major components. However, neither eremophilanolides nor *cis*-β-cyclocostunolide were detected in *F. dilatata* var. *anomala* even by GC/MS. Thus, chemical differences were also clearly seen between *F. dilatata* and its subspecies.

A miniature *Frullania* species, *F. parvistipula*, is chemically similar to European *F. dilatata* since the former species elaborates the unique eremophilanolides (**13, 14**), although the constitution of bibenzyls is different (see later).

Indian *F. yunnanensis*, Japanese *F. densiloba*, *F. osumiensis* and American *F. nisquallensis* [14] are

Table 5. Monoterpenes of *Frullania* species

Species	55	56	57	58	59	60	61	62
F. amplicrania			+					
F. dilatata	+	+	+	+				
F. dilatata var. *anomala*			+					
F. diversitexta	+	+	+					
F. davurica	+	+	+					
*F. fragilifolia**		+	+	+	+	+	+	
F. hamatiloba	+			+	+	+	+	
F. inflata			+					
F. pedicellata	+	+	+			+		+
F. tamarisci [32]	+	+	+	+				+
F. tamarisci subsp. *obscura*	+	+	+			+	+	
F. usamiensis	+	+	+					
F. yunnanensis	+	+	+					

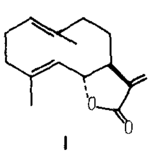
*The species contains a large amount of an unidentified monoterpene acetate (M^+ 194).

chemically quite similar to European *F. tamarisci*, since they produce (−)-frullanolide (**7**) as the major component. (+)-β-Frullanolide (**9**), a double-bond isomer of (+)-frullanolide, and its dihydro derivative (**10**), and brothenolide (**11**) were found in *F. ramuligera*. The lactone (**9**) and the cyclopropanoid eudesmanolide (**11**) have recently been isolated from *F. brotheri* [21]. Thus, these two species have identical sesquiterpene lactones. Chemically speaking, *F. inflata* may be more primitive than the other *Frullania* species belonging to types I and II since it elaborates only two germacranolides (**1**, **2**) which are the key precursors to eudesmanolides and guaianolides found in *Frullania* species classified in types I and II.

F. usamiensis contains β-cyclocostunolide (**4**) and γ-cyclocostunolide (**5**) as major components; however, 6,7-*cis*-eudesmanolides, 7,8-*cis*-eremophilanolides and 4β-hydroxycyclocostunolide have not been detected. In this respect, *F. usamiensis* differs from *F. tamarisci* subsp. *obscura* and the *Frullania* species which produce frullanolide (**7**) and γ-cyclocostunolide (**5**).

F. vethii produces guaianolides (**15**, **16**) which have not been detected in the other *Frullania* species so far examined. Thus, *F. vethii* is an isolated species in *Frullania*. This fact is further supported by the presence of a large quantity of a free flavone, apigenin 7,4′-dimethyl ether, in this species (see later).

In North America and Europe, it has been known that some occupational allergies are associated with handling wood upon which epiphytic liverworts grow [13]. The vectors of this allergy were the sesquiterpene lactones (e.g. **1**, **3**–**7**, **9**, **11**–**13**, **15**, **18**) possessing an α-methylene-γ-butyrolactone, contained in *Frullania tamarisci* [13], *F. nisquallensis* [14] and *F. dilatata* [20]. If patients sensitive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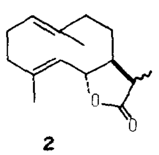
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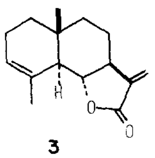
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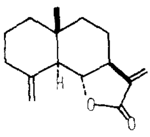
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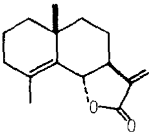
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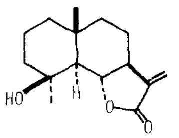
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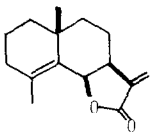
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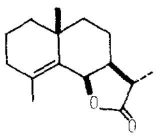
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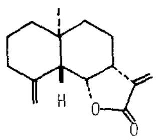
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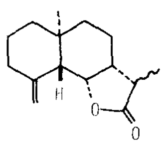
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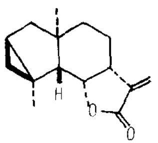
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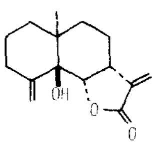
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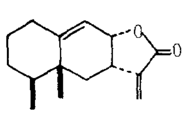
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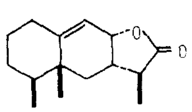
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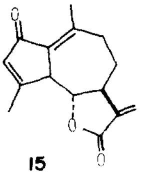
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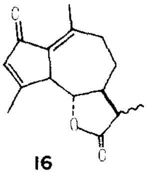
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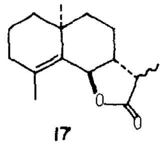
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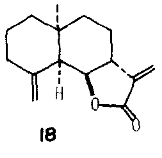
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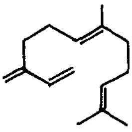
to the above *Frullania* touch the remaining *Frullania* species which contain sesquiterpene lactones, intense allergy will be induced because they produce the same allergenic α-methylene-γ-butyrolactones as those found in *F. tamarisci*, *F. nisquallenisis* and *F. dilatata*, or very similar potentially allergenic sesquiterpene lactones. The isomeric 6,7-*cis*- and *trans*-eudesmanolides and 7,8-*cis*-eremophilanolides have not detected in any other liverworts. Thus we can use them as chemosystematic markers of *Frullania* species.

In the course of the investigation of the sesquiterpene lactones of *Frullania*, we confirmed that some *Frullania* species elaborated various simple bibenzyl derivatives as major components. Tables 1 and 4 show the species which produce bibenzyls. As seen in Table 1, some species producing sesquiterpene lactones contain bibenzyls as minor component. These species are classified as type I. Type II produce a large quantity of sesquiterpene lactones but no bibenzyls. As major components, bibenzyls have been found in *Frullania* species which do not elaborate any sesquiterpene lactones. These species belong to type III.

The simplest bibenzyl isolated is 3-methoxybibenzyl (**54**) from *F. dilatata* var. *anomala*. 3-Methoxy-4′-hydroxybibenzyl (**41**) and 3,4-methylenedioxy-3′-methoxybibenzyl (**45**) are distributed in five species. Lunularic acid (**43**), an endogenous growth regulator, and its decarboxylated compound, lunularin (**40**), have been isolated from *F. dilatata* and *F. tamarisci* [22].

F. parvistipula belonging to type I contains several highly substituted bibenzyls, two of which have been isolated from *F. brittoniae* ssp. *truncatifolia* (type III) [23]. *F. parvistipula* is similar to *F. brittoniae* ssp. *truncatifolia* from the standpoint of bibenzyl constitution; however, the latter species elaborates no sesquiterpene lactones.

F. vethii produces a large quantity of 5-hydroxy-7,4′-dimethoxyflavone (**53**) along with guaianolides (**15**, **16**); however, no bibenzyls have been detected even by GC/MS. Lunularin (**40**) and lunularic acid (**43**) are widespread in Hepaticae [22]. However, except the above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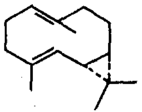
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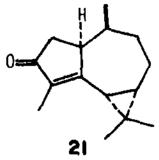

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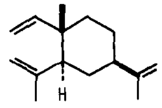

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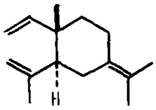

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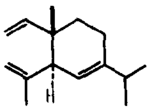

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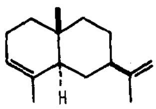

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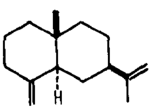

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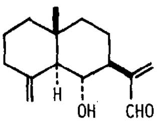

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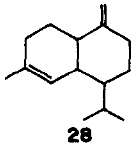
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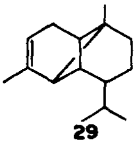
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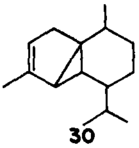
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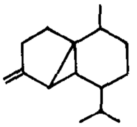
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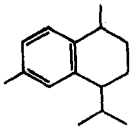
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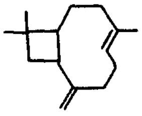
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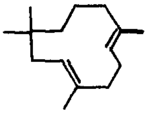
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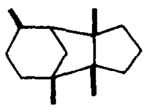
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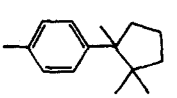
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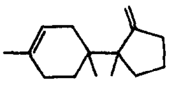
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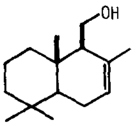

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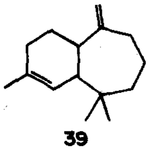
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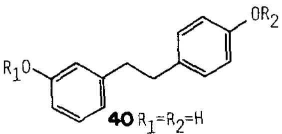
two bibenzyls (**40**, **43**), and bibenzyls (**44**, **45**), the remaining bibenzyls listed in Table 4 were newly isolated or detected. As can be seen in Tables 1 and 4, the present bibenzyls are also valuable chemical markers of *Frullania* species.

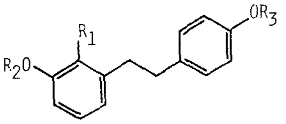
There are two more chemotypes of *Frullania* species which are chemically quite isolated. *F. fragilifolia* grown in Europe produces a large amount of monoterpene hydrocarbons and an unknown monoterpene acetate. This species does not contain any sesquiterpene lactones and bibenzyls. Such a species is classified as type IV.

F. diversitexta produces a large amount of (+)-cyclocolorenone (**21**), an *ent*-aromadendrane-type sesquiterpene ketone, together with a few diterpene acetates. (+)-Cyclocolorenone (**21**) is the main component of several *Porella* species belonging to Porellaceae (Jungermanniales) [24–27]. Some *Frullania* species are morphologically close to *Porella* species. Chemically, *F. diversitexta* is rather similar to *Porella vernicosa* complex, although the former species does not contain the intense pungent drimane-type sesquiterpene dial, which is one of the important chemosystematic markers of the *P. vernicosa* complex [24–27]. GC/MS showed that neither sesquiterpene lactones nor bibenzyls were present in *F. diversitexta*. Thus, *F. diversitexta* is isolated as type V. *F. densiloba* also contains cyclocolorenone (**21**); however, its content is negligible and the major components are the eudesmanolides. Hence, *F. densiloba* is chemically closer to *F. tamarisci*.

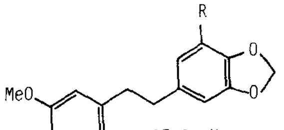
In addition to the sesquiterpene lactones, bibenzyls and cyclocolorenone indicated above, most *Frullania* species produce many sesquiterpene hydrocarbons and alcohols. Table 3 shows the components so far found in *Frullania* species. β-Caryophyllene (**33**) is the most widespread sesquiterpene hydrocarbon in *Frullania* species. β-Elemene (**22**), α-selinene (**25**), β-selinene (**26**), γ-cadinene (**28**) and α-copaene (**29**) are also distributed in the several species. *F. davurica* (type III) produces a large amount of bibenzyl (**41**) and sesquiterpene hydrocarbons in which eight known compounds have been detected, the major one being β-caryophyllene (**33**). *F. hampeana*, *F. monocera* and *F. osumiensis* produce a drimane-type sesquiterpene alcohol, drimenol (**38**). *F. tamarisci* and its subspecies produce α- and β-selinenes (**25**, **26**). The same hydrocarbons have been isolated from *F. dilatata*. However, their absolute configurations are opposite. The same phenomenon has been discussed in sesquiterpene lactones, (+)- and (−)-frullanolides (**7**, **8**). Except for the presence of (+)-cyclocolorenone (**21**) in *F. diversitexta* and β-caryophyllene (**33**) in the other species, it is somewhat difficult to use these sesquiterpene hydrocarbons or alcohols as chemosystematic markers of



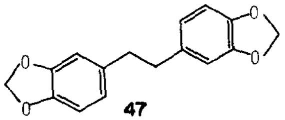
40 $R_1=R_2=H$
41 $R_1=Me$ $R_2=H$
42 $R_1=R_2=Me$



43 $R_1=COOH$ $R_2=R_3=H$
44 $R_1=OH$ $R_2=Me$ $R_3=H$



45 R = H
46 R = OH



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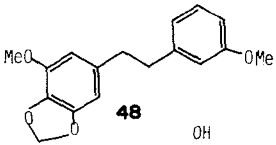

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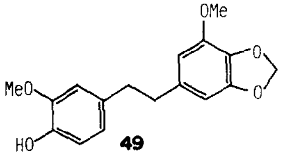

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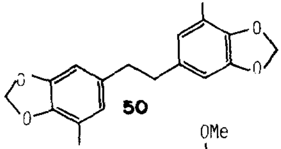

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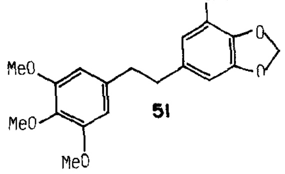

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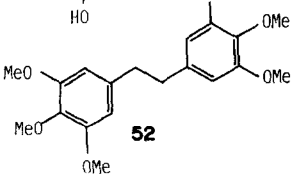

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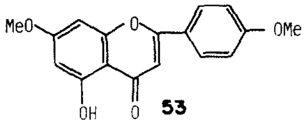

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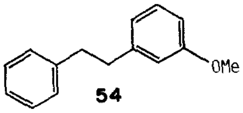

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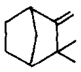

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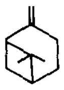

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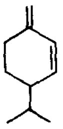

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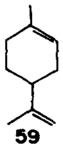

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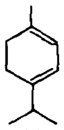

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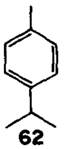

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Frullania species, since most other liverworts elaborate the same sesquiterpene hydrocarbons and alcohols.

Some fresh *Frullania* species emit a pine-needle-like odour when they are crushed or ground. The GC/MS of the crude extracts showed that some species contained monoterpene hydrocarbons. Table 5 shows the species containing monoterpenes. Monoterpene hydrocarbons are present in 13 of 25 *Frullania* species. Most of the species contains α- and β-pinenes (**55**, **56**) and camphene (**56**). *F. hamatiloba* contains the same monoterpenes as those found in European *F. fragilifolia*. However, the other chemical constitution of the two species is completely different. It is difficult to use monoterpene hydrocarbons as chemical markers of *Frullania* species because they are volatile and variable, according to whether fresh or dried tissues are examined.

The liverworts so far examined in our laboratory contain phytosterols (campesterol, stigmasterol and sitosterol). This is true in *Frullania* species (Table 1) although the concentration of three sterols are irregular. In eight *Frullania* species, some diterpenes have been isolated. These include a few diterpene acetates whose structures remain to be clarified.

It is interesting from the standpoint of biogenesis that *Frullania* species elaborate normal or *ent*-sesquiterpenes. It is known that most of the liverworts produce sesquiterpenoids enantiomeric to those found in higher plants [28–31], with several exceptions. As far as we are aware, it is the first instance that either normal or enantiomeric sesquiterpenoids have been found in different species in the same genus, not only in the Hepaticae but also in more highly evolved plants. It is also interesting that sesquiterpene hydrocarbons and ketones as well as the sesquiterpene lactones found in the spore-forming *Frullania* species are the same or quite similar to those found in the highly evolved Compositae.

The present paper indicated the chemical constituents of only 2.5% of the total *Frullania* species. It is possible that other significant chemical properties will be found in the remaining *Frullania* species. Chemical study of the metabolites by a computerized GC/MS, TLC and GC may lead to a better understanding of the taxonomy of *Frullania* species and of the evolutional interrelationship between Frullaniaceae, Porellaceae and Lejeuneaceae.

EXPERIMENTAL

GC/MS, TLC, GC, prep. TLC and prep. GC were carried out as indicated earlier [6].

Plant materials. All *Frullania* species identified by S.H., G.O., Y.A., Drs. M. Mizutani, C. Suire, K. Yamada and S. Huneck were deposited in the Herbarium, The Hattori Botanical Laboratory, Miyazaki, Japan and Institute of Pharmacognosy, Tokushima Bunri University. The plant materials were collected in the following locations and months.

Frullania amplicrania Steph.: Yamatake, Nichinan, Miyazaki, Japan, Dec. 1978; Izu, Miyake Island, Japan, Dec. 1978. *F. brittoniae* subsp. *truncatifolia* (Steph.) Schust. & Hatt.: Yamatake, Nichinan, Miyazaki, Japan, Dec. 1978. *F. davurica* Hampe: Kainan-cho, Kaifu-gun, Tokushima, Japan, April 1978; Urago-cho, Hidaka-gun, Hokkaodo, Japan, July 1978, Katsuura-cho, Katsuura-gun, Tokushima, Japan, May, 1979; Eba-mura, Yazu-gun, Tottori, Japan, Oct. 1979. *F. densiloba* Evans: Yamatake, Nichinan, Miyazaki, Japan, Dec. 1978. *F. dilatata* (L.) Dum.: Allas, Dordogne, France, April 1978, Nov. 1978. *F. dilatata* (L.) Dum. var. *anomala* Corb.: near Varna, Black Sea, Bulgaria, Sept. 1978. *F. diversitexta* Steph.: Yamatake, Nichinan, Miyazaki, Japan, Dec. 1978; Inohae Valley, Minaminaka-mura, Miyazaki, Japan, Feb. 1979. *F. ericoides* (Nees) Mont.: Kochi-Castle, Kochi-shi, Kochi, Japan, Aug. 1978; Yada-cho, Tokushimashi, Japan, April 1978. *F. fragilifolia* (Tayl.) Gott. *et al.*: Sewen, Haut-Rhin, France, Sept. 1979. *F. hampeana* Nees: Great Shrine of Ise, Ise-shi, Mie, Japan, Jan. 1979. *F. inflata* Gott.: Kouraibiro, Ise-shi, Mie, Japan, Jan. 1979. *F. kagoshimensis* Steph.: Yamatake, Nichinan, Miyazaki, Japan, Dec. 1978; Hachinosu, Nichinan, Miyazaki, Japan, Dec. 1978, Minaminaka-gun, Miyazaki, Japan, Feb. 1979. *F. monocera* Steph.: Ise-shi, Mie, Japan, June, 1978. *F. motoyana* Steph.: Yamatake, Nichinan, Moyazaki, Japan, Dec. 1978; Ise-shi, Mie, Japan, Jan. 1979. *F. osumiensis* (Hatt.) Hatt.: Inohae ravine, Nichinan, Miyazaki, Japan, Dec. 1978. *F. parvistipula* Steph.: Amanohashidate, Kyoto, Japan, March 1979. *F. pedicellata* Steph.: Ootsubo, Nikenya, Tokushima, Japan, Dec. 1978; Hachinosu, Nichinan, Miyazaki, Japan. Dec. 1978; Izu, Miyake Island, Japan, Dec. 1978. *F. ramuligera* (Nees) Mont.: Inohae and Inotani, Nichinan, Miyazaki, Japan, Dec. 1978. *F. tamarisci* (L. Dum.: Cote de Jor, Dordogne, France, April 1978, Creux de l'Anvers, Jura, France, Aug. 1979. *F. tamarisci* subsp. *obscura* (Verd.) Hatt.: Arataniyama, Numata-cho, Hiroshima, Japan, Mar. 1975; Koyu-gun, Miyazaki, Japan, Feb. 1976; Kizawa-son, Naka-gun Tokushima, Japan, June 1977; Urago-cho, Hidaka-gun, Hokkaido, Japan, July, 1978; Tengukogen, Yamadani-mura, Kamiukenagun, Ehime, Japan, Aug. 1978; Monobe-mura, Kami-gun, Kochi, Japan, Aug. 1978; Yamatake, Nichanan, Miyazaki, Japan, Dec. 1978; Kamikatsu-cho, Katsuura-gun, Tokushima, Japan, May, 1979; Kito-cho, Naka-gun, Tokushima, Japan, Sept. 1980. *F. taradakensis* Steph.: Kizawa-son, Naka-gun, Tokushima, Japan, Dec. 1978, Kamikatsu-cho, Katsuura-gun, Tokushima, Japan, May, 1979. *F. usamiensis* Steph.: Tengukogen, Yamadani-mura, Kamiukena-gun, Ehime, Japan, Aug. 1978; Izu, Miyake Island, Japan, Jan. 1979. *F. vethii* Sande Lacoste: Inohae, Nichinan, Miyazaki, Japan, Nov. 1978. *F. yunnanensis* Steph.: Darjeeling, India, June, 1976. *F. bonicola* Hatt.: Hahajima Island, Mt. Chibusa, Japan, Aug. 1980.

Extraction and isolation of terpenoids, bibenzyls and sterols. Each *Frullania* species (250–1250 g) after being air-dried for 5 days was ground and ground material (250–500 mg) was extrd with ether (10 ml). The green extract was filtered through a short column packed with Si gel (230–400 mesh) and the solvent was evapd *in vacuo* at 25°. After the green oils were monitored by TLC (sprayed by 30% H_2SO_4 and heated at 100°) and by GC, they were directly analysed by a computerized GC/MS apparatus. The remaining ground materials were also extrd with Et_2O for 2 weeks and then re-extrd with the same solvent for 2 weeks. The two extracts were combined and the crude oils were chromatographed on Si gel and/or Sephadex LH-20 using *n*-hexane–EtOAc, C_6H_6–EtOAc gradient or MeOH–$CHCl_3$ (1:1) as eluants. The chemical structures of the isolated compounds were established by the combination of the spectral data (UV, IR, ^1H NMR, ^{13}C NMR and MS) and the chemical degradation [20]. The structural elucidation of bibenzyls (**41**,**42**,**45**,**47**–**50**) and their syntheses will be reported elsewhere.

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