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Volatile components of selected species of the liverwort genera *Frullania* and *Schusterella* (Frullaniaceae) from New Zealand, Australia and South America: a chemosystematic approach

Yoshinori Asakawa[a,*], Masao Toyota[a], Matt von Konrat[b], John E. Braggins[c]

[a]Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Yamashiro-cho, Tokushima 770-8514, Japan
[b]Department of Botany, The Field Museum, 1400 South Lake Shore Drive, Chicago, IL 60605-2496, USA
[c]School of Biological Sciences, The University of Auckland, Private Bag, 92019, Auckland, New Zealand

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Dedicated to the celebration of the 70th birthday of Professor Dr. Meinhart H. Zenk

Abstract

The volatile components of 25 taxa of the liverwort family Frullaniaceae from New Zealand, Australia and South America have been analyzed by GC–MS. The present *Frullania* species are chemically divided into five major types: (1) sesquiterpene lactones, (2) sesquiterpene lactones-bibenzyls, (3) bibenzyls, (4) 2-alkanone and (5) triterpene types; the latter two chemo-types are newly proposed for the genus. *Schusterella chevalierii,* belonging to the Frullaniaceae, is closely related chemically to the sesquiterpene lactone type of the *Frullania* species since it elaborates two eudesmanolides, β-cyclocostunolide and dihydro-β-cyclocostunolide as major components.

Keywords: *Frullania* species; *Schusterella chevalierii*; Sesquiterpene lactones; Striatane; Drimane; Bibenzyls, 2-Alkanones; Taraxerol; Hepaticae; Chemosystematics; Allergenic contact dermatitis

1. Introduction

The bryophytes are taxonomically placed between algae and pteridophytes, approximately 18,000 species or more in the world (Crandall-Stotler and Stotler, 2000; Buck and Goffinet, 2000). Bryophytes have been hypothesized to represent several quite separate evolutionary lines and classified into three coordinate phyla: Anthocerotophyta (hornworts), Bryophyta (mosses), and Marchantiophyta (liverworts), e.g., Buck and Goffinet (2000); Crandall-Stotler (1983); Crandall-Stotler and Stotler (2000); Kenrick and Crane (1997). For liverworts, all major classification schemes recognise at least two distinct lineages. Recently, Crandall-Stotler and Stotler (2000) recognized these at the rank of class (Jungermanniopsida and Marchantiopsida) and identified 13 orders, including Porellales, to which the family Frullaniaceae is subscribed. Many sesqui-, diterpenoids and phenolic compounds isolated from liverworts so far show interesting biological activity, such as anti-micorbial, anti-fungal, cytotoxic, insect antifeedant, muscle relaxing, some enzyme inhibitory and apoptosis inducing activity (Asakawa, 1982, 1990a,b, 1993, 1995; Nagashima et al., 2002).

Frullania is a very large and complex genus with over 1000 described taxa (Yuzawa, 1991) and the subgeneric and even generic boundaries remain unresolved (von Konrat and Braggins, 2001a,b). Hattori (1982, 1984, 1986), and Hattori and Mizutani (1982) recognised three genera in Frullaniaceae, including *Frullania* and *Schusterella* (Schust.) Hatt. The most recent treatise discussing the generic and subgeneric boundaries of *Frullania* and its allies was by Schuster (1992) who remained uncommitted and proposed two alternative classification systems: (1) *Schusterella* was retained at the generic level; or (2) an alternative scheme tentatively proposed that *Schusterella* be incorporated into *Frullania* subg. *Microfrullania* and recognized a total of 12 subgenera. Recently, Schuster (2000) in his Austral Hepaticae retained *Schusterella* as distinct from *Frullania*. On the other hand, Crandall-Stotler and Stotler (2000), in a classification scheme of the Marchantio-

* Corresponding author. Tel.: +81-88-622-9611; fax: +81-88-655-3051.

E-mail address: asakawa@ph.bunri-u.ac.jp (Y. Asakawa).

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phyta, recognised only the single genus *Frullania* and treated*Schusterella* as a synonym.

Frullania species are rich sources of sesquiterpene lactones, diterpenoids and bibenzyl derivatives (Asakawa, 1995, 1997; Asakawa et al., 2001; Bardon et al., 2002; Nagashima et al., 1994, 1997; Toyota et al., 1998, 2000), which cause potent allergenic contact dermatitis and the extracts possess piscicidal activity as well as cytotoxicity against certain cancer cell lines (Asakawa et al., 1976; Asakawa, 1982, 1988, 1990a, b, 1995, 1999).

Prior to this study, 57 taxa of *Frullania* have been studied chemically and divided into six chemo-types: sesquiterpene lactone-bibenzyl (Type 1), sesquiterpene lactone; (Type 2); bibenzyl (Type 3); monoterpene (Type 4); cyclocolorenone (aromadendrane-type sesquiterpene ketone) (Type 5); and labdane-diterpenoid (Type 6) (Asakawa 1995; Asakawa et al., 1981, 1996; Nagashima et al., 1994, 1997; Toyota et al., 1998). Thus far from these 57 species, including *F. incumbens* (#NZ 9611-01) and an unidentified *Frullania* species (#9611-02) collected in New Zealand (Asakawa et al., 1996), 119 compounds have been identified. The latter species produces bazzanane-type sesquiterpenoids which have not been detected in any other *Frullania* species so far examined.

The present paper forms a continuation of our study of the chemical constituents of the genus *Frullania* with a focus on biologically active substances and their chemosystematics.

Here, we report the identification of the chemical constituents of a further 23 taxa of *Frullania* and *Schusterella chevalierii* (Frullaniaceae) collected in New Zealand, Australia, and South America and discuss aspects related to their chemosystematics.

2. Results and discussion

Each species was ground mechanically and extracted with diethyl ether. Each extract was filtered through a small glass column packed with silica gel using ether to give a green oil which was analyzed by TLC and GC–MS. Each component appearing on GC–MS was identified by comparison of each retention time and mass spectrum with those of authentic samples, reference (Joulin and König, 1998) and our library databases.

2.1. Chemical constituents (Charts 1–5)

2.1.1. Frullania (subg. Australes) anomala (bibenzyl-type)

The chemical constituents of this species are very complex. At least 50 peaks resulted from the GC analysis; five of these appear as predominant constituents. The major compounds are 3-methoxy-3′,4′-methylenedioxybibenzyl (**1**) [12% on total ion chromatogram (TIC)], tetradecanoic (18%) and 9,12-octadecadienoic acid (18%). Interestingly, this species elaborates two stilbene derivatives, 3-methoxy-3′,4′-methylenedioxy-*cis*-stilbene (**2**) and its *trans* isomer (**3**) as very minor components (Asakawa et al., 1987a). Both stilbene derivatives might be the precursors of dihydro derivative (**1**) found in the same species. Previously, 3,4-dihydroxy-3′-methoxystilbene has been isolated from the neotropic liverwort, *Marchesinia bongardiana* belonging to the Lejeuneaceae (Speicher and Schoeneborn, 1997). This represents the second identification of stilbene derivatives in bryophytes. *F. anomala* also produces α-copaene (**20**), β-barbatene (**21**), γ-cadinene (**22**), α-guaiene (**25**), eremophila-1(10),8,11-triene (**26**) and oleic acid as minor components.

2.1.2. Frullania (subg. Microfrullania) aterrima var. aterrima (sesquiterpene lactone-type)

The major component is an unknown sesquiterpene lactone [M^+ 230 (215)] (30%). β-Caryophyllene (**28**), selina-4,11-diene (**30**), α-selinene (**31**), bicyclogermacrene (**33**), with two unknown sesquiterpene hydrocarbons [M^+ 204 (133), M^+ 204 (148)], two unknown sesquiterpene alcohols [M^+ 220 (119), M^+ 222 (109)], a diterpene hydrocarbon, and kaurene (**66**) (10%) have been detected in *F. aterrima* as minor terpenoids. In addition, two unknown sesquiterpene lactones, [M^+ 232 (217)] (2%) and [M^+ 232 (217)] (5%) were also detected as minor components.

2.1.3. Frullania (subg. Microfrullania) aterrima var. lepida (sesquiterpene lactone-type)

Interestingly, this variety of *Frullania aterrima* appears to be chemically quite different from *F. aterrima sensu stricto*. It contained various sesquiterpene hydrocarbons, γ-cadinene (**22**), bicyclogermacrene (**33**), isobicyclogermacrene (**34**) (20%), β-elemene (**37**), β-longipinene (**40**) (15%), of which isobicyclogermacrene was the major component, together with two unknown sesquiterpene hydrocarbons [M^+ 204 (93), M^+ 204 (148)] and 1-octen-3-yl acetate. Three relatively large peaks attributable to three sesquiterpene lactones, [M^+ 232 (232)] (10%) and [M^+ 232 (232)] (17%) were also detected.

2.1.4. Frullania (subg. Diastaloba) congesta (sesquiterpene lactone-type)

This species is a rich source of sesquiterpene lactones. It produces dihydrofrullanolide (**10**) (14%), frullanolide (**11**) (5%) and three unknown sesquiterpene lactones [M^+ 234 (107)], [M^+ 232 (107)] (12%), [M^+ 232 (109)] (12%) as major components. Additionally, an unidentified compound [M^+ 382 (156)] (32%) and four unknown minor sesquiterpene lactones [M^+ 234 (234), M^+ 232 (91), M^+ 232 (135), M^+ 232 (109)] and hexadecanoic acid were detected. Three unknown aromatic compounds [M^+ 284 (199), M^+ 296 (175), M^+ 282

(256) (25%)] are present in the ether extract of this species. The sesquiterpene hydrocarbons, α-copaene (**20**), β-elemene (**37**), longifolene (**41**) (2%) and γ-cadinene (**22**), of which longifolene is predominant, are also found in this species.

2.1.5. *Frullania (sub. Trachycolea) deplanata (sesquiterpene lactone-type)*

Material representing Australia (AUST) and New Zealand (NZ) were analyzed. AUST: An unknown sesquiterpene lactone [M^+ 232 (232)] (33%) appeared as the major component together with two unidentified sesquiterpenoids [M^+ ? (94)] (16%) and [M^+ ? (96)] (17%). Minor components include sesquiterpene lactone, M^+ 232 (232)], β-bisabolene (**42**), *Z*-(α)-bisabolene (**43**) and cyclocolorenone (**44**). Furthermore, two unidentified sesquiterpene alcohols [M^+ 220 (79), M^+ 220 (67)] and three sesquiterpene alcohols [M^+ 234 (59), M^+ 246 (59), M^+ 250 (59)] possessing a 1,1-dimethyl carbinol (*m/z* 59) were present in the ether extract. NZ: The gas chromatographic profile of the crude extract was identical to that of the Australian collection except for the presence of striatane-type sesquiterpene alcohol, striatol (**50**), an unidentified aromatic compound [M^+ 314 (269)] (13%) having a methoxyl group and an oxygenated diterpene [M^+ 302 (122)].

2.1.6. *Frullania (subg. Trachycolea) falciloba (bibenzyl-type)*

Material representing both Australia and New Zealand was analyzed. AUST: It is noteworthy that this specimen biosynthesized a large amount of the striatane-type sesquiterpene hydrocarbon, striatene (**51**) (57%), along with an unknown diterpene alcohol (16%) [M^+ 290 (81)]. As minor components, 1-octen-3-yl acetate, β-barbatene (**21**), β-caryophyllene (**28**), β-elemene (**37**), thujopsene (**52**) (8%), β-acoradiene (**53**), β-bazzanene (**54**), cuparene (**56**), two unidentified sesquiterpene hydrocarbons (M^+ 204, (189), M^+ 204 (71), methyl β-orsellinate (**75**), an unidentified oxygenated sesquiterpene [M^+ 232, (232)], two fusicoccane-type diterpenoids 2(3),5(6)-fusicoccadiene (**67**) and fusicogigantepoxide (**69**) were identified, together with four unidentified diterpenoids [M^+ 272 (69), M^+ 272 (161), M^+ 290 (81), M^+ 304 (95)].

The sesquiterpene hydrocarbon β-caryophyllene is widespread in many *Frullania* species (Asakawa et al., 1981) and a previous chemical analysis of *F. falciloba* from Australia (Asakawa et al., 1987b). Two known fusicoccane-type epoxides, fusicogigantone A and fusicogigantone B had been isolated from *Frullania hamatiloba* (Hashimoto et al., 1998). Occurrence of fusicoccane-type diterpenoids in nature is rare. Several fusicoccanes have been isolated from the liverworts belonging to the Lophoziaceae, Pleuroziaceae, Plagiochilaceae, and Lejeuneaceae (Asakawa, 1995), and Riccardiaceae and Pallaviciniaceae (Spörle, 1990). It is also interesting to note that a minor peak corresponding to bibenzyl derivative [M^+ 330 (121)], which was identical to that found in *F. monocera* has been detected. This specimen is chemically quite different from the New Zealand material, since the present collection elaborated neither 3-methoxy-3′,4′-methylenedioxybibenzyl (**1**) nor 3,4′-dimethoxybibenzyl (**4**).

NZ: The major components of the New Zealand material are 3-methoxy-3′,4′-methylenedioxybibenzyl (**1**) (50%) and a monoterpene hydrocarbon, β-myrcene (11%). In addition, α-pinene, sesquisabinene A (**57**), methyl β-orsellinate (**75**), hexadecanoic acid and 3,4′-dimethoxybibenzyl (**4**) were detected as the minor components. Previously, chemical analysis of an Australian specimen of *F. falciloba* by Asakawa et al. (1987b) elaborated the same bibenzyl (**1**) and 3-hydroxy-4,3′-dimethoxybibenzyl (**5**) together with 3-[4′ - methoxybibenzyl]5,6 - dimethoxyphthalide (**6**), α-pinene, β-caryophyllene, β-barbatene, bicyclogermacrene, campesterol, stigmasterol and sitosterol. Unidentified diterpenoids [M^+ 274 (124), M^+ 304 (95), M^+ 304 (147), M^+ 304 (304), M^+ 304 (147), M^+ 304 (123)] which were detected in the Australian specimen in this analysis were also contained in the New Zealand material. The New Zealand specimen contained fusicogigantepoxide (**69**) (4%) as in the Australian sample, but also additional unidentified sesquiterpenoids [M^+ 204 (105), M^+ 220 (119)] and three diterpenoids [M^+ 272 (107), M^+ 304 (82), M^+ 304 (55)] as minor components. Further detailed analysis is required in order to investigate the chemical variation of *Frullania falciloba* and explore the possibility that more than one chemical race may exist.

2.1.7. *Frullania (subg. Australes) fugax (triterpene-type)*

F. fugax is chemically quite different from all the other species since it produces a triterpene alcohol, taraxerol (**71**) as major component (88%). It is the first instance to detect such a large amount of a triterpenoid in the a species of Hepaticae, although methyl 3α-hydroxy-18-oleanen-28-ate has been found in an unidentified *Frullania* species collected in Venezuela (Asakawa, 1995). Thus, we here propose a seventh broad major chemo-type for the genus *Frullania* i.e., triterpene-type (Type 7), to which *F. fugax* can be assgned. β-Elemene, labd-14-en-13,9-oxide (**70**) and methyl β-orsellinate (**75**) have been detected as minor components. Labdane-type diterpenoids are the most important chemical markers of *F. hamatiloba* (Hashimoto et al., 1998; Toyota et al., 1988).

2.1.8. *Frullania (Australes) incumbens (sesquiterpene lactone-type)*

In a previous chemical analysis of this species, 3-methoxy-3′,4′-methylenedioxybibenzyl (**1**) was identified together with bicylogermacrene (**33**), striatene (**51**), β-chamigrene and unidentified sesquiterpene lactones (Asakawa et al., 1996) and was classified to be of sesquiterpene lactone-bibenzyls type (Type 1). The major components of the present specimen are two sesquiterpene lactones, frullanolide (**11**) (34%) and dihyrofrullanolide (**10**) (15%), however, bibenzyls were not detected in the crude extract. It is suggested that there are at least two chemo-types in *F. incumbens*. The minor sesquiterpene hydrocarbons, β-elemene, γ-cadinene, bicyclogermacrene, an unidentified sesquiterpene hydrocarbon [M^+ 204 (107)], an unidentified sesquiterpene ketone [M^+ 218 (107)], four sesquiterpenoids [M^+ ? (109, 107, 95, 95)], tetradecanoic, octadecanoic, oleic, 9,12-octadecadienoic acids were also detected. This species is closely related chemically to *F. tamarisci* subp. *tamarsci* (Asakawa, 1982, 1995), although the pacifigorgiane sesquiterpenoid was absent in the present species.

2.1.9. *Frullania (subg. Amphijubula) lobulata (sesquiterpene lactone-type)*

The chemical constituents of this species are very complex. It elaborates four major sesquiterpene lactones, three of which are costunolide (**12**) (9%), eremofrullanolide (**14**) (1%) and dihydroeremofrullanolide (**15**) (15%). The other two lactones (32% and 12%) are unknown, possessing the molecular weight of M^+ 234 (234) and M^+ 232 (91), respectively. In addition, α-copaene (**20**), β-elemene, aromadendrene (**45**), spathulenol (**46**), tetradecanoic, octadecanoic, 9,12-octadecadienoic acids were identified, together with two unknown sesquiterpenoids [M^+ 204 (91), M^+ 220 (177)].

2.1.10. *Frullania (subg. Microfrullania) magellanica (sesquiterpene lactone-type)*

The gas chromatogram is very complex. The major compound is dihydrofrullanolide (**10**) (13%) and three unidentified aromatic compounds [each M^+ ? (m/z 105)] (31, 17 and 3%). Such aromatic components have not been seen in the other *Frullania* species analyzed. The sesquiterpene hydrocarbon, β-elemene (10%) was identified along with two unidentified oxygenated sesquiterpenoids [M^+ 220 (177), M^+ 218 (91)], oleic and octadecanoic acids and two unidentified sesquiterpene lactones [M^+ 250 (81), M^+ 248 (248)].

2.1.11. *Frullania (subg. Australes) media (sesquiterpene lactone-type)*

Four major peaks appeared on GC–MS, three of which are eremofrullanolide (**14**) (15%) and dihydroeremofrullanolide (**15**) (5%) and two unknown sesquiterpene lactones[M^+ 234 (91) (46%), M^+ 232 (232) (8%)] and the sesquiterpene hydrocarbon β-bazzanene (**54**) (8%). β-Elemene, spathulenol (**46**), α-humulene (**58**) and bicyclogermacrene were identified, along with 2(3),5(6)-fusicoccadiene (**67**) and oxgenated sesquiterpenoids [M^+ 232 (152), M^+ 232 (232), M^+ 234 (161), M^+ 232 (217)] as very minor terpenoid components.

2.1.12. *Frullania (subg. Amphijubla) microcaulis*

This species is chemically quite different from all the other *Frullania* species treated. It contains an unidentified aromatic compound (80%) having the molecular ion at M^+ 286 (159).

2.1.13. *Frullania (subg. Trachycolea) monocera (bibenzyl-type)*

Chemically, the New Zealand specimen is very characteristic since it produced a drimane-type sesquiterpene alcohol, albicanol (**45**) (39%) as the major component. Drimenol, the double bond isomer of albicanol has been detected in the Australian *F. clavata* (Asakawa et al., 1983), *F. osumiensis*, and Japanese material of *F. monocera* (Asakawa et al., 1981). Occurrence of drimane-type sesquiterpenoids has rarely been recorded in the Frullaniaceae; of over 50 species analysed to date this is only the fourth record of drimane-type sesquiterpene alcohol.

The sesquiterpene hydrocarbons β-elemene, β-caryophyllene and bicyclogermacrene were identified as the minor constituents, together with 1-octene-3-yl acetate, two unidentified sesquiterpene hydrocarbons, [M^+ 204 (105), M^+ 204 (189)], a sesquiterpene ketone [M^+ 218, (107)] and a sesquiterpene alcohol with 1,1-dimethylcarbinol [M^+ ?, (59)]. As minor constituents, 2(3),5(6)-fusicoccadiene (**67**) and fusicogigantepoxide (**69**) (8%) have been detected. In addition to the terpenoid constituents, two bibenzyl derivatives [M^+ 314 (151)] (11%) and [M^+ 330 (121)] (12%) which possess a 3-hydroxy-4,5-methylenedioxybenzyl and 3-methoxybenzyl moiety, respectively were detected.

2.1.14. *Frullania (subg. Trachycolea) patula (bibenzyl-type)*

The major component is the fusiccocane-type diterpene hydrocarbon, 2(3),5(6)-fusicoccadiene (**67**) (17%). 2(6),3(4)-Fusicoccadiene (**68**) (4%) and fusicogigantepoxide (**69**) (7%) were also detected as minor constituents. As minor constituents isobicylcogermacrene (**34**), β-longipinene (**40**) and three *n*-alkanes have been detected, together with three unknown sesquiterpenoids [M^+ 204 (93), M^+ 220 (99), M^+ 202 (119)] and an unknown diterpene hydrocarbon [M^+ 272 (229)]. An unknown bibenzyl (16%), possessing a trimethoxybibenzyl group [M^+ 360 (181)] is a characteristic chemical component in this species.

2.1.15. *Frullania (subg. Trachycolea) probosciphora (sesquiterpene lactone-type)*

Material from Australia and New Zealand was analyzed. The same major sesquiterpene hydrocarbons e.g., β-bisabolene and β-caryophyllene, were detected in material from both countries. AUST: The chemical constituents of the Australian specimen were very complex. At least 21 peaks appeared in the gas chromatogram among with alloaromadendrene (**47**) appearing as the most abundant (17%). Additionally, β-caryophyllene (7%), β-caryophyllene oxide (**29**) (3%) and β-bisabolene (**42**) (10%) were also detected. The three sesquiterpene lactones, frullanolide (**11**) (7%), eremofrullanolide (**14**) (8%) and dihydroeremofrullanolide (**15**) (9%) were identified along with an unidentified sesquiterpene lactone [M^+ 232 (217)]. Tetradecanoic acid, heneicosane, 9,12-octadecadienoic acid, docosane and two unidentified *n*-alkanes have been detected as minor components. The present species chemically resembles the European *F. dilatata* (Asakawa et al., 1976) since the latter species elaborates the same sesquiterpene lactones (**11, 14** and **15**).

NZ: The presence of three major sesquiterpenoids, frullanolide (**11**) (26%), β-caryophyllene (**28**) (13%) and β-bisabolene (**42**) (26%), which has also been identified in the Australian material was confirmed. In addition, dihydrofrullanolide (**10**), α-copaene (**20**), δ-elemene (**38**), bazzanene (**54**), cuparene (**56**), methyl β-orsellinate (**75**), tetradecanoic, 9,12-octadecadienoic and 9,12,15-octadecatrienoic acids have also been identified together with three sesquiterpenoids [M^+ 204 (93), M^+ 218 (132)]. It is interesting to note that although both specimens have the major sesquiterpene hydrocarbons e.g., β-bisabolene and β-caryophyllene in common, they also have different sesquiterpene lactones and sesquiterpene hydrocarbons.

2.1.16. Frullania (subg. Trachycolea) pycnantha (bibenzyl-type)

The major constituents are 3-methoxy-3′,4′-methylenedioxybibenzyl (**1**) (22%), (*E*)-β-farnesene (**60**) (15%) and an unidentified sesquiterpene alcohol [M^+ 220 ((91)] (21%). The two monoterpene hydrocarbons, α-pinene and β-pinene were detected together with a characteristic known bibenzyl, brittonin B (**7**) as minor components. The other minor constituents are 1-octene-3-yl acetate, β-caryophyllene, valencene (**27**), δ-selinene (**32**), *trans*-β-bergamotene (**62**), 2(3),5(6)-fusicoccadiene (**67**), fusicogigantepoxide (**69**), tridecanoic acid, 9,12-octadecadienoic acid and an unidentified oxygenated diterpene[M^+ 304 (95)] having a 2,2,-dimethylallyl group.

2.1.17. Frullania (subg. Diastaloba) ptychantha (sesquiterpene lactone-bibenzyl-type)

One of the major peaks on GC is suggested to be a bibenzyl derivative (46%) possessing a methoxybenzyl group (*m*/*z* 121 and 193 (base). The second major compound is a sesquiterperpene hydrocarbon, ledene (**48**) (20%). Alloaromadendra-9-ene (**49**) (2%) was also identified as an aromadendrane-type sesquiterpene hydrocarbon. The distribution of aromadendrane-type sesquiterpenes is relatively rare, except for the presence of a large amount of cyclocolorenone (**44**) in *Frullania diversitexta* (Asakawa et al., 1981). Furthermore, two unidentified eudesmane-type sesquiterpene lactones [M? 232 (217), M^+ 234 (217)] were detected, along with a small amount of β-elemene, γ-cadinene, germacrene-D (**35**), germacra-(1*E*,5*E*)-dien-11-ol (**36**), an unidentified farnesene-type sesquiterpene hydrocarbon [M^+ 204 (69)] and a sesquiterpene alcohol [M^+ 222 (119)], a sesquiterpene ketone [M^+ 218 (107)] and two unidentified oxygenated sesquiterpenoids [M^+ ? (95), M^+ ? (95)].

2.1.18. Frullania (subg. Microfrullania) rostrata (sesquiterpene lactone-type)

The major component of *F. rostrata* is dehydrosaussurea lactone (**16**) (41%). It produces saussurea lactone (**17**) (11%), the dihydro derivative of **16** and two unidentified sesquiterpene hydrocarbons [M^+ 204 (base 108), M^+ 204 (105)] and squalene as minor constituents. Compounds **16** and **17** are easily obtained by Cope rearrangement from costunolide (**12**) and dihydrocostunolide (**13**). However, **16** and **17** are not artifacts since the crude extract shows two spots corresponding to **16** and **17** on a TLC plate. Interestingly, these sesquiterpene lactones costunolide and dihydrocostunolide are the precursors of β-cyclocostunolide and dihydro-β-cyclocostunolide, which were found in *Schusterella chevalieri* (see Section 2.1.24). Previously, Asakawa et al. (2001) reported four types of sesquiterpene lactones isolated from *Frullania*, including eromophilanolides, eudesmanolides, germacranolides, and guaianolides. The elemanolides isolated from *F. rostrata* represent a fifth type identified for *Frullania*.

2.1.19. Frullania (subg. Trachycolea) scandens (bibenzyl-type)

This species is closely related chemically to *F. falciloba* since it elaborates 3-methoxy-4′,5′-methylenedioxybibenzyl (**1**) (40%) as major component. 3,4′-Dimethoxybibenzyl (**4**) (1%) and 3-hydroxy-4,5-methylenedioxy-3′-methoxybibenzyl (**8**) (5%) were also detected. The other characteristic components are 1-docosene, 16-octadecenal and 17-octadecenal, which have not been found in any other *Frullania* species chemically analyzed. α-Copaene, β-barbatene (**21**), elema-1,3,7(11),8-tetraene (**39**), africa-1,5-diene (**63**), tetradecanoic, 9,12-octadecadienoic acids, docosane, tetracosane and pentacosane have been identified as minor metabolites. In addition, the species produces two unidentified sesquiterpene hydrocarbon isomers [M^+ 202 (119), M^+ 202 (119)].

2.1.20. Frullania (subg. Trachycolea) solanderiana (2-alkanone-type)

This species is very characteristic chemically since it elaborates 2-alkanones such as 2-undecanone (**72**) (9%), 2-tridecanone (**73**) (54%) and 2-pentadecanone (**74**)

(3%) as the main components. It also biosynthesizes γ-maaliene (**64**) (14%) along with bicylcogermacrene, hexadecanoic, 9,12-octadecadienoic acid and 1-tetradecanol. This is the first record of the identification of 2-alkanones in the genus *Frullania*. Thus this species is chemically quite distinct from the other *Frullania* species so far examined (e.g., Asakawa, 1982, 1995; Asakawa et al., 1981, 1996, 2001). We therefore propose an eighth broad chemo-type for the genus, 2-alkanone-type (Type 8) to which *F. solanderiana* can be chemically classified.

2.1.21. Frullania (subg. Trachycolea) spinifera (bibenzyl-type)

The GC pattern of the ether extract is very complex. At least 32 peaks appeared on GC. The major components are 3,3′,4,4′-dimethylenedioxybibenzyl (**9**) (25%). 3-Methoxy-3′,4′methylenedioxybibenzyl (**1**) (7%), *cis*-5-hydroxycalamenene (**23**) (8%) and an unidentified sesquiterpene hydrocarbon [M^+ 204 (107)] (15%)] and 9,12-octadecadienoic acid were also detected. Additionally, α-pinene and β-pinene, β-caryophyllene, δ-amorphene (**24**), bicyclogermacrene (**33**), β-longipinene (**40**), (*E*)-β-farnesene (**60**),(*E,Z*)-α-farnesene (**61**), tetradecanoic acid and methyl (*Z*)-5,11,14,17-eicosatetraenoate are detected as minor components, along with five unknown sesquiterpene hydrocarbons [M^+ 204 (105, 105, 197, 91, 107)] and an unidentified diterpene hydrocarbon [M^+ 272 (69)] (6%). Furthermore, two unknown bibenzyls [M^+ 234 (147), M^+ 280 (265)] are found in this species.

2.1.22. Frullania (subg. Trachycolea) squarrosula (sesquiterpene lactone-type ?)

Twenty peaks corresponding to sesquiterpenoids were confirmed by GC–MS. β-Barbatene (**21**) (15%), bazzanene (**54**), cuparene (**56**) and bazzanenol (**55**), β-cedrene (**65**), of which β-barbatene was the major component, have been identified along with sesquiterpenoids [M^+ 218 (163), M^+ 220 (109)]. It also elaborates 2(3),5(6)-fusicoccadiene (**67**), fusicogigantepoxide (**69**) (4%) as the minor components. The most abundant component of this species is one oxygenated sesquiterpene [M^+ 234 (163)], (28%) which might be a sesquiterpene lactone and one diterpene hydrocarbon [M^+ 272 (123)] (30%).

2.1.23. Frullania (sub. Microfrullania) "truncata"

This undescribed species is chemically quite distinct from the other *Frullania* species analyzed by TLC and GC–MS. It contains one major component [M^+? (179)] (71%), along with bicyclogermacrene (8%) and an unknown oxygenated sesquiterpene [M^+ 234 (179)].

2.1.24. Schusterella chevalierii (sesquiterpene lactone type)

The ether extract showed two major peaks on GC–MS, retention times and mass spectra of which were identical to those of dihydro-β-costunolide (**19**) (6%) and β-cyclocostunolide (**18**) (50%), respectively. In addition, it produces β-barbatene (**21**), *n*-heptadecane, *n*-heptadecene and hexadecanoic acid as very minor components. This species could be classified to belong to the sesquiterpene lactone-type of the Frullaniaceae.

3. General discussion

The *Frullania* species analyzed in this study produced a vast number of sesquiterpenoids and bibenzyl derivatives as shown in Tables 2 and 3. Although there remain many unidentified terpenoids and aromatic compounds for each species, it is possible to classify most species into one of several broad chemical groups (Table 1). Formerly, Askawa et al. (1981) and Toyota et al. (1988) described a total of six different broad chemical groups for *Frullania*. Here, we have proposed two more chemotypes: the presence of triterpenoids in *F. fugax*, which is the first instance of such a large amount in liverworts required the establishment of a seventh chemical type (triterpene type, assigned Type VII); and justification for an eighth chemical type (2-alkanone type, assigned Type VIII) is based on the identification of 2-alkanones in *F. solanderiana*, which is the first record in the genus.

Table 1
Chemo-types of *Frullania* species and *Schusterella chevalierii*

Sesquiterpene lactone	Sesquiterpene lactones—bibenzyl	Bibenzyl	2-Alkanone	Triterpene	Others
F. aterrima	*F. ptychantha*	*F. anomala*	*S. solanderiana*	*F. fugax*	*F. microcaulis*
F. congesta		*F. deplanata*			*F. "truncata"*
F. deplanata		*F. falsiloba*			
F. incumbens		*F. monocera*			
F. lobulata		*F. pycnantha*			
F. magellanica		*F. patula*			
F. media		*F. scandens*			
F. probosciphora		*F. spinifera*			
F. rostrata					
F. squarrosula					
Schusterella chevalierii					

Table 2
The distribution of aromatic compounds in *Frullania*

Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**75**
F. anomala	+	+								
F. falciloba	+			+						+
F. incumbens			+							+
F. pycnantha	+									
F. scandes	+			+				+		
F. spinifera	+								+	

There are a host of other interesting chemical compounds that have been isolated from the present study, which have rarely been reported in bryophytes or within the genus *Frullania*, e.g., stilbene derivatives from *F. anomala* is only the second identification in bryophytes, and the identification of drimane-type sesquiterpene alcohols in New Zealand material of *F. monocera* represents only the fourth record in the genus. Conversely, several chemical compounds are shared between many species throughout the *Frullania*. Asakawa et al (1981) reported that the sesquiterpene hydrocarbon, β-caryophyllene, was the most widespread sesquiterpene hydrocarbon in *Frullania* species analysed to that date; in the present study β-caryophyllene was detected in a further seven morphologically diverse species. Similarly, compounds such as the sesquiterpene hydrocarbon, β-barbatene, are prevalent throughout the genus; in *Frullania squarrosula* this compound appeared as the major chemical constituent. However, Asakawa et al. (1981) stated that it was difficult to use these sesquiterpene hydrocarbons or alcohols as chemosystematic markers of *Frullania* species, since most other liverworts elaborate the same sesquiterpene hydrocarbons and alcohols.

Similarly, monoterpene hydrocarbons, e.g., α-pinene and β-pinene, have also been detected from several species in the current study. Previously, in a survey of 25 *Frullania* species, Asakawa et al. (1981) identified that most species produced these monoterpene hydrocarbons, but concluded that it was difficult to use monoterpene hydrocarbons as chemical markers for the genus because they are volatile and variable, according to whether fresh or dried tissue is examined. Frullanolide is a well known sesquiterpenoid lactone distributed throughout many species of the genus and is a major constituent of *Frullania tamarisci* (Connolly, 1990), *F. apiculata*, *F. serrata*, and *F. ternatensis* (Asakawa et al., 1983); in the present study frullanolide was identified in *F. probosciphora, F. congesta*, and *F. incumbens*.

The sesquiterpene lactones, dihydroeremofrullanolide and eromofrullanolide, were also significant chemical constituents isolated from three species; eromofrullanolide has previously been isolated from *F. dilatata* and showed cytotoxicity against KB cells or human epidermoid carcinoma cells (Asakawa, 1990a,b). Asakawa et al. (1976) reported for *F. dilatata* that it was the first isolation of both eromophilanolides and eudesmanolides from the same plant; later Kraut et al. (1994) stated that of the large number of *Frullania* species examined *F. muscicola* was only the second species in which eromophilanolides and eudesmanolides occur together. In the present study, both eromophilanolides (eremofrullanolide (**14**) and dihydroeremofrullanolide) and a eudesmanolide (frullanolide (**11**)) were also detected in *F. probosciphora* making this the third identification in the same plant; eremofrullanolide has showed strong allergenic contact dermatitis (Asakawa et al., 1976).

In several cases, terpenoids and aromatic compounds are valuable chemosystematic indicators that can help aid definition of species boundaries. For example, the identification of drimane-type sesquiterpene alcohols in both New Zealand and Japanese material of *F. monocera*, representing extremes in the range of its distribution, illustrates how chemical makers may help aid definition of species boundaries. Likewise, the almost identical chemical profiles between the Australian and New Zealand samples of *F. deplanata* exemplify the value of chemosystematics studies.

On the other hand, some species appear to be chemically very complex and difficult to interpret. The Australasian species, *F. falciloba*, had been chemically investigated on three prior occasions and assigned to three different chemotypes (Asakawa and Campbell, 1982; Asakawa et al., 1987b, 1983). In the present study, 3-methoxy-3′,4′-methylenedioxybibenzyl was idenfitied as a major component from the New Zealand material of *F. falciloba*, which was the same bibenzyl isolated as a major constituent from an Australian specimen by Asakawa et al. (1987b). The Australian specimen analyzed in the present study was chemically similar to that analyzed by Asakawa and Campbell (1982). A detailed study is required for this species in order to determine the chemical variation and to test for any chemical and geographical correlations, and the existence of chemical races. Previously, Asakawa et al. (1991) investigated the chemical constituents of the *F. tamarisci* species complex, and identified two distinct chemotypes for *F. tamarisci* subsp. *obscura* throughout Japan.

In most cases, intraspecific repetition is still lacking and it is difficult to determine characteristic patterns and at what taxonomic level various chemical differences may be applied. Therefore, it is difficult to assess if the eight chemical types, five of which are illustrated by Table 1 represent natural species groups, and how they relate to the current subgeneric classification scheme. To date, the chemical constituents of only about 5–10% of the *Frullania* species have been investigated and they appear as chemically diverse as they are morphologically. However, with over 70 *Frullania* species now chemically surveyed there are many suggestive relationships

Table 3
The distribution of terpenoids and alkanones in *Frullania* and *Schusterella chevalierii*

Species	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**
F. anomala											+	+	+			+	+					
F. aterrima s.str																			+		+	+
F. aterrima var. *lepida*													+									
F. congesta	+	+									+		+									
F. falciloba[a]												+							+			
F. falciloba[b]												+							+			
F. incumbens	+	+										+										
F. lobulata			+		+	+					+											
F. magellanica	+																					
F. media					+	+																
F. monocera																	+					
F. probosciphora[a]		+			+	+													+	+		
F. probosciphora[b]	+	+									+								+			
F. pycnantha																		+	+			
F. ptychanta													+									
F. rostrata							+	+														
F. scandens											+	+										
F. solanderiana																						
F. spinifera												+		+	+				+			
F. squarrosula												+										
Scusterella chevalierii									+	+												

Species	**32**	**33**	**34**	**35**	**36**	**37**	**38**	**39**	**40**	**41**	**42**	**43**	**44**	**45**	**46**	**47**	**48**	**49**	**50**	**51**	**52**	**53**
F. aterrima var. *lepida*		+	+			+			+													
F. congesta						+				+	+											
F. deplanata											+	+	+									
F. deplanta[a]																			+			
F. falciloba[a]																				+	+	+
F. fugax						+																
F. incumbens						+																
F. lobulata						+								+	+							
F. magellanica						+																
F. media						+									+							
F. monocera						+												+				
F. patula		+							+													
F. probosciphora[a]											+					+						
F. probosciphora[b]							+				+											
F. pycnantha	+			+	+																	
F. ptychanta																	+	+				
F. scandens								+														
F. solanderiana		+							+													

Species	**54**	**55**	**56**	**57**	**58**	**59**	**60**	**61**	**62**	**63**	**64**	**65**	**66**	**67**	**68**	**69**	**70**	**71**	**72**	**73**	**74**
F. anomala													+								
F. falsiloba[a]			+											+		+					
F. falciloba[b]	+			+												+					
F. fugax																	+	+			
F. magellanica																					
F. media	+				+									+							
F. monocera														+	+	+					
F. patula														+		+					
F. probosciphora[b]	+		+																		
F. pycnantha		+							+					+		+					
F. scandens										+											
F. solanderiana																			+	+	+
F. spinifera						+	+	+													
F. squarrosula		+	+								+	+		+		+					

[a] Sample collected in Australia.
[b] Sample collected in New Zealand.

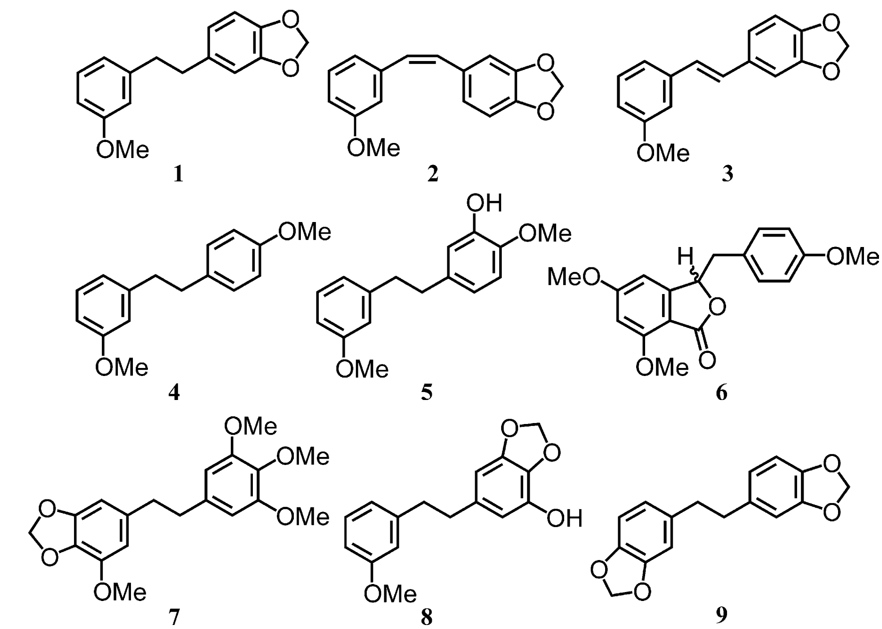


Chart 1. Bibenzyl and stilbene derivatives found in *Frullania* species.

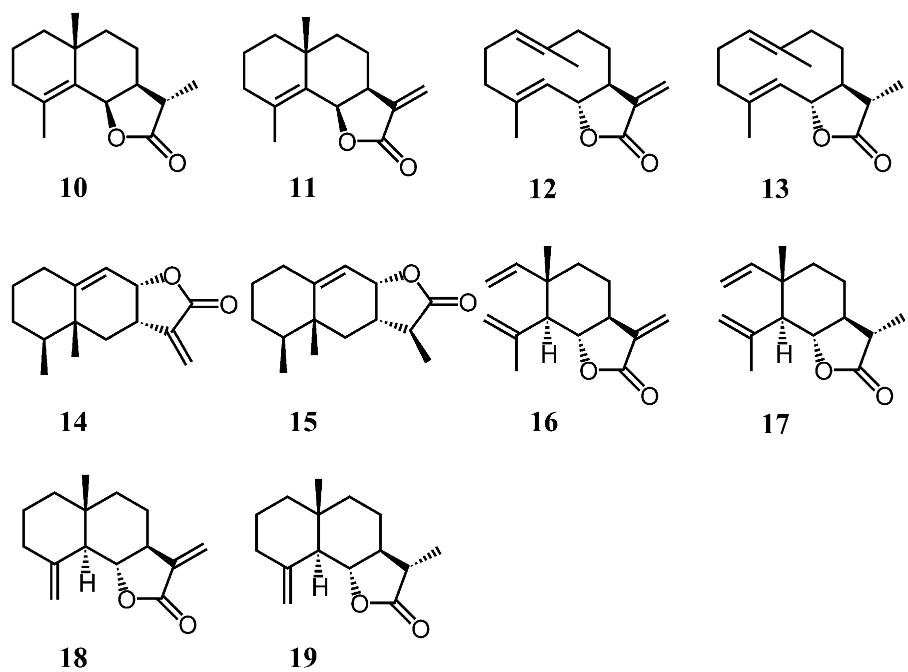


Chart 2. Sesquiterpene lactones from *Frullania* species and *Schusterella chevalierii*.

that may be implied, and there remains many more species to be analyzed before a more accurate interpretation of their chemical composition and systematic position can be made.

Mues and Zinsmeister (1988) stated that most of the bibenzyl producing *Frullania* species (type III) belonged to subgenus *Trachycolea*, and that further chemical analysis of other species was required to determine whether bibenzyls are chemical markers for the subgenus. To date, all the species except one (*F. anomala*) assigned to type III belong to subgenus *Trachycolea*. However, there are also a few species of subg. *Trachycolea* that have been assigned to other chemotypes e.g., *F. solanderiana* (type VIII) and *F. probosciphora* (type I). *Frullania falciloba* has been assigned to three chemotypes as discussed above.

It is generally accepted that the terpenoids, particularly the sesquiterpenoids, are located in the oil bodies of liverworts (Gradstein, 1990; Mues, 2000) and that there is a direct correlation between the abundance and

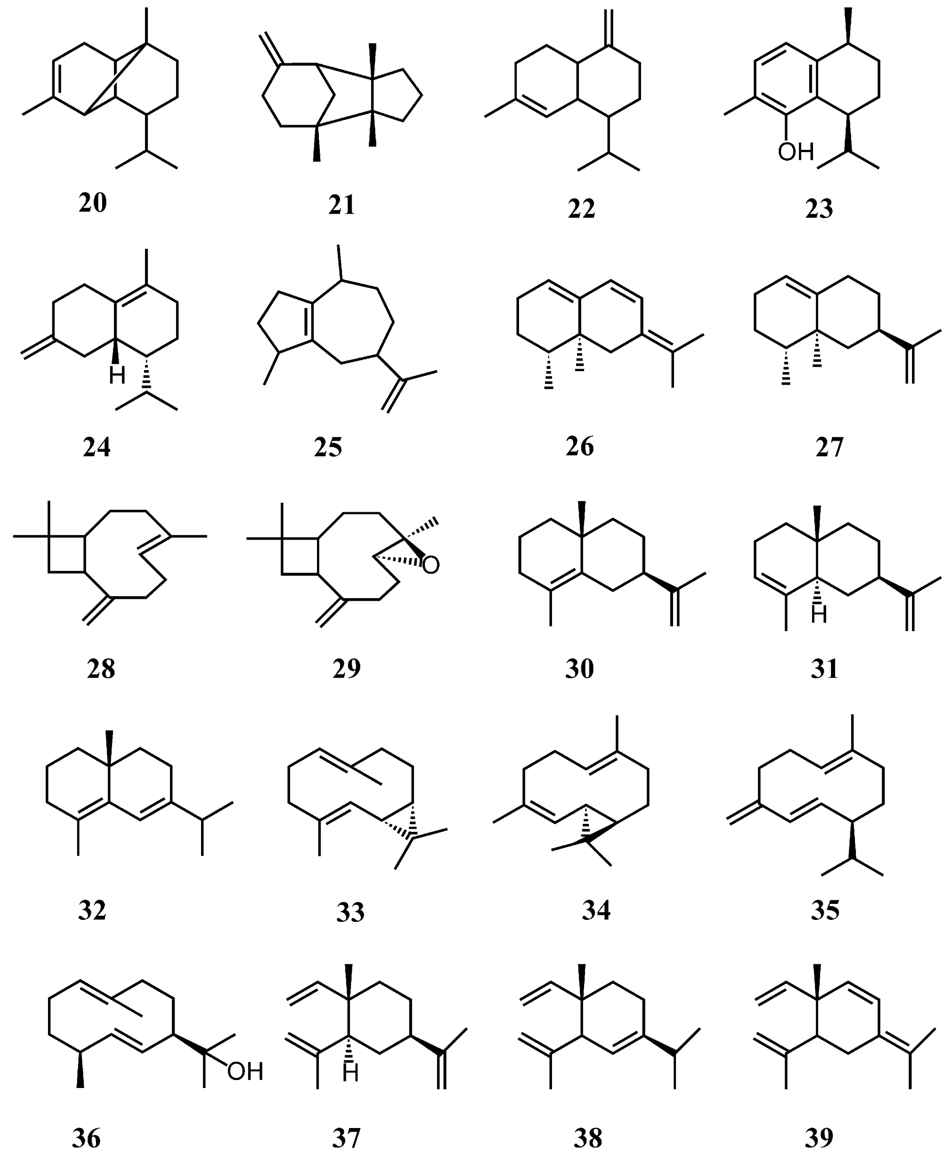


Chart 3. Sesquiterpenoids found in *Frullania* species.

size of the oil bodies and the amount of oils obtained from different species (Huneck, 1983). It is also noteworthy that study of the oil bodies from the New Zealand material of *F. monocera*, were similar to those described and illustrated by Hattori (1951) for material from Japan; likewise the chemical profiles are very similar between the New Zealand and Japanese material. There would also appear a correlation between the oil bodies of most members of subg. *Microfrullania*, which lack any significant ornamentation and frequently appearing as almost homogeneous oil droplets, and their chemical constituents; all but two species are of the sesquiterpene lactone chemotype (type II). Furthermore, some of the more chemically complex species e.g., *F. anomala* with over 50 peaks observed by GC and species such as *F. falciloba* have oil bodies that almost occupy the entire cell lumen.

It is significant that *Schusterella chevalierii* can be assigned to the sesquiterpene lactone-type because the status of this species and its allies remain controversial. The chemical affinity coupled with shared morphological characteristics, e.g., similar initial branching appendages (von Konrat and Braggins, 2001a,b) and oil body morphology, supports Schuster's (1992) supposition that *Schusterella* be assigned to *Frullania* subg. *Microfrullania*.

In conclusion, this study illustrates the vast number of sesquiterpenoids and bibenzyl derivatives produced in *Frullania* and provides a valuable guideline in the chemosystematic assignment of species in this large liverwort genus. Further investigation is required on a broader geographical scale coupled with an expansion of species analysed to explore the extent of chemical variation and aid our taxonomic understanding of the genus.

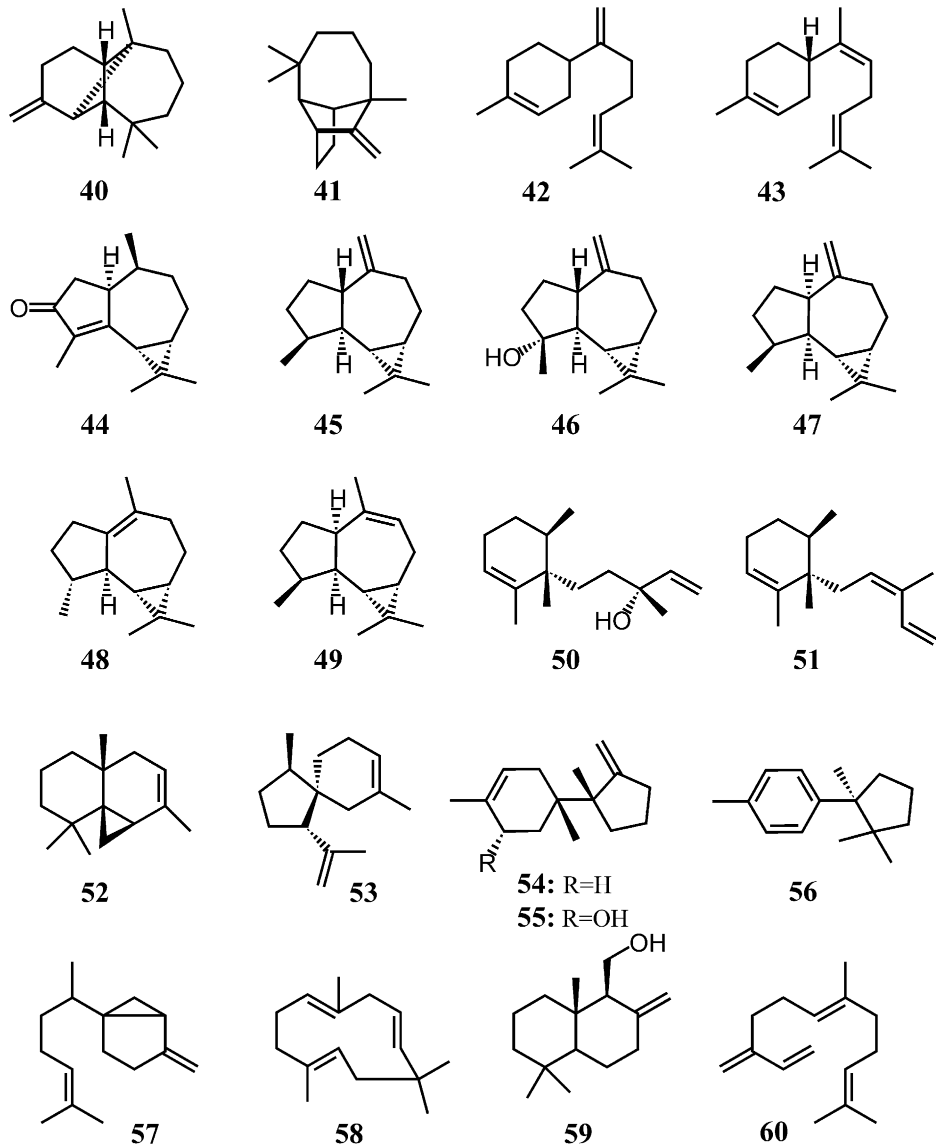


Chart 4. Sesquiterpenoids found in *Frullania* species.

4. Experimental

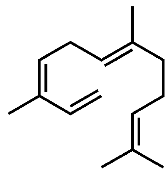
4.1. General

TLC was carried out on silica gel precoated glass plates with *n*-hexane–EtOAc (4:1). Spots were visualized by UV light, followed by spraying Godin reagent and the plates were heated at 100 °C. The temperature programming of GC–MS analysis performed from 50 °C isothermal for 3 min, then 50–250 °C at 5 °C min^{-1}, and finally isothermal at 250 °C for 15 min. Injection temperature was 250 °C. A fused silica column coated with DB-17 (30 m × 0.25 mm id, film thickness 0.25 μm was used.

4.2. Plant material

All *Frullania* species and *Schusterella chevalierii* were collected from either i) the North Island (NI), South Island (SI), or Stewart Island (StI) of New Zealand (NZ), ii) Australia (AUST), iii) Chile (CHILE) or iv) Argentina (ARG) by M. von Konrat (MVK), J. E. Braggins (JEB), John J. Engel (JJE), and Mike Ostafichuk (OST); specimens were identified by MVK. The species names and collection sites were as follows.

F. anomala Hodgs. (MVK98/45): Track to summit of Mt. Arthur, elev. c. 1050 m., Nelson District, SI, NZ. December 1998. *F. aterrima* (Hook. f. & Tayl.) Hook. f. & Tayl. var. *aterrima* (MVK 2000/44): At base of



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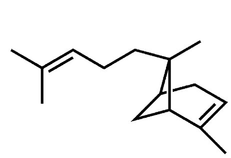

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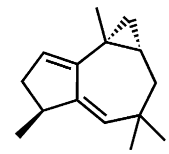

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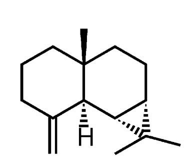

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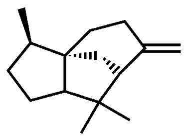

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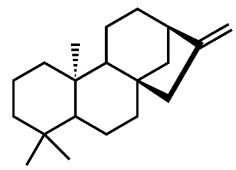

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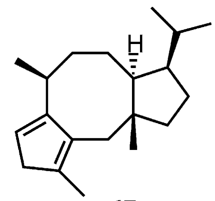

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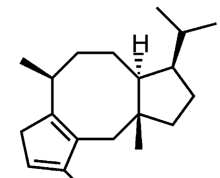

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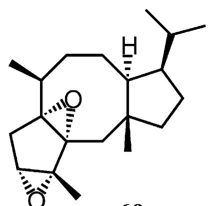

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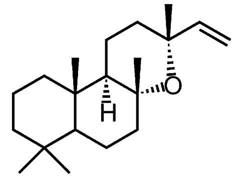

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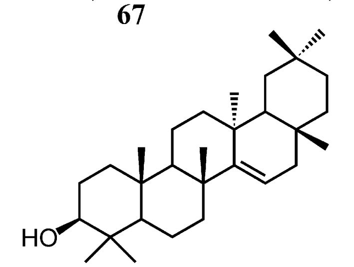

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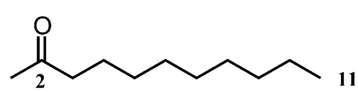

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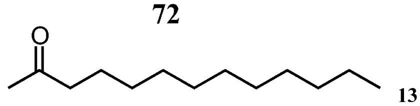

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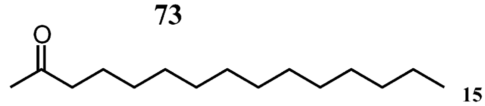

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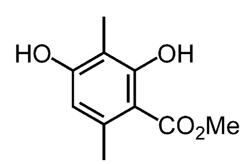

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Chart 5. Sesqui-, di- and triterpenoids, aliphatic ketones and aromatic compound found in *Frullania* species.

Okopeka, Northern Te Urewera, elev. c. 400 m., Te Urewera National Park, NI, NZ, June 2000. *F. aterrima* var. *lepida* Hodgs. (MVK2000/55): Waitakare Ranges, Auckland District, NI, NZ, May 2000. *F. congesta* (Hook. f. & Tayl.) Hook. f. & Tayl. (MVK99/211): Kaipipi Walk-Rakiura Tk to North Arm Hut, at an exposed bay of Paterson Inlet, StI. NZ, December 1999. *F. deplanata* Mitt. (JEB98/41): Roadside, route to Mt. Field, Tasmania, AUST, Febuary 1998. *F. deplanta* Mitt. (MVK99/01): Te Rangaakapua, Te Urewera National Park, NI, NZ, January 1999. *F. falciloba* Tayl. ex Lehm. (MVK2000/18): Otamatuna, Northern Te Urewera, Te Urewera National Park, NI, NZ, January 2000. *F. falciloba* Tayl. ex Lehm. (JEB 2000fal) Tongariro National Park, NI, NZ, April 2000. *F. fugax* (Hook. f. & Tayl.) Tayl. (MVK99/116B): Whakarewarewa, Rotorua, NI, NZ, Aug. 1999. *F. incumbens* Mitt. (MVK2000/12): Track to summit of Okopeka, elev. c 550 m., Northern Te Urewera, Te Urewera National Park, NI, NZ, March 2000. *F. lobulata* (Hook.) Dum. (JJE 2230): Brunswick Peninsula, CHILE, Dec. 1967. *F. magellanica* (Spreng.) Web and Nees (OST 1563): Bahia Buen Suceso, ARG, October 1971. *F. media* (Hodgs.) Hatt. (JEB98/343): Otago, StI, NZ, Nov. 1998.*F. microcaulis* Gola (JJE 6075): Prov. Magallanes, CHILE, Oct. 1969. *F. monocera* (Hook. f. & Tayl.) Tayl. (MVK

99/250): Forest margin, beginning of Ryans Creek Track, StI, NZ, Dec. 1999. *F. patula* Mitt. (JEB 2000pat): Tongariro National Park, NI, NZ, Apr. 2000. *F. probosciphora* Tayl. (JEB 98/81b): Tasmania, Australia, Feb. 1998. *F. probosciphora* Tayl. (MVK 99/228): Dobson Nature Walk, Arthurs Pass National Park, SI, NZ, Jan. 1997. *F. pycnantha* (Hook. f. & Tayl.) Tayl. (MVK 99/39): Track to summit of Okopeka, elev. c 550 m., Northern Te Urewera, Te Urewera National Park, NI, NZ, May 1999. *F. ptychanta* Mont. (MVK 99/249): Forest, Kaipipi Walk-Rakiura Tk to North Arm Hut, StI, NZ, Dec. 1999. *Frullania rostrata* (Hook. f. & Tayl.) Hook. f. & Tayl. (MVK 2000/08): Kaipipi Walk-Rakiura Tk to North Arm Hut, at an exposed bay of Paterson Inlet, StI, NZ, Dec. 1999. *Frullania scandens* Mont. (JEB 98/275): Mt. Egmont National Park, NI, NZ, Apr. 1999. *Frullania solanderiana* Col. (MVK 2000/73): Auckland University, Grounds, NI, NZ, July 2000. *Frullania spinifera* Tayl. (MVK 2000/78): Base of Okopeka, elev. c. 270 m., Northern Te Urewera, Te Urewera National Park, NI, NZ, May 2000. *Frullania squarrosula* (Hook. f. & Tayl.) Tayl. (JEB 2000squ): Tongariro National Park, NI, NZ, Apr. 2000 *Frullania* "*truncata*" (MVK 99/99): Kaipipi Walk-Rakiura Tk to North Arm Hut, forest margin of Paterson Inlet, StI, NZ, December 1999. *Schusterella chevalierii* (Schust.) Hatt. et al. (MVK 98/151): Forest interior. Radar Bush, Cape Reinga, NI, December 1999.]

Duplicates of all specimens are lodged at the Herbarium of the Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Japan.

4.3. Extraction

Each *Frullania* species was ground and the resulting powder was extracted with ether for 3 days. The extract was filtered through a small glass column packed with silica gel and the solvent evaporated to give a green oil. A small amount of each oil was analysed by TLC and GC–MS to detect the presence of mono-, sesqui- and diterpenoids and aromatic compounds whose structures were identified by comparison of the retention times of GC and mass spectra of authentic samples, reported mass spectra and library data bases.

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