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BRYOPHYTES: BIO- AND CHEMICAL DIVERSITY, BIOACTIVITY AND CHEMOSYSTEMATICS

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Abstract - The bryophytes contain the Marchantiophyta (liverwort), Bryophyta (moss) and Anthocerotophyta (hornwort) among which the Marchantiophyta contain cellular oil body and they produce a number of mono-, sesqui- and diterpenoids, aromatic compounds like bibenzyl, bis(bibenzyls) and acetogenins. Several of these compounds show interesting biological activity such as insecticide, insect antifeedant, cytotoxic, piscicidal, muscle relaxing, allergenic contact dermatitis, anti-HIV, DNA polymerase β, 5-lipoxygenase, calmodium inhibitory, anti-obesity, neurotrophic, cyclooxygenase, hyaluronidase and NO production inhibitory, antimicrobial and antifungal activities. Each liverwort biosynthesizes peculiar components, which are valuable for classification of liverworts. The typical chemical structures and bioactivity of the selected liverwort constituents as well as chemosystematics of several species of the Marchantiophyta are surveyed.

This paper is dedicated to Professor Emeritus Keiichiro Fukumoto for his 75th birthday.

INTRODUCTION

The bryophytes are found everywhere in the world except in the sea. They grow on the tree (Figures 1 and 2), on soil, lake, river even in Antarctic island. The bryophytes are placed taxonomically between algae and pteridophytes; there are about 24,000 species in the world. They are further divided into three phyla, Bryophyta (mosses 14,000 species), Marchantiophyta (liverworts 6,000 species) and Anthocerotophyta (hornworts 300 species). They are considered to be the oldest terrestrial plants, although no strong scientific evidence for this has appeared in the literature. This hypothesis was mainly based on the resemblance of the present-day liverworts to the first land plant fossils, the spores of which date back almost 500 million years.

Figure 1. Habitat of liverworts (*Mastigophora* sp.)

Figure 2. *Ptychantus striatus* (Lejeuneaceae)

Among the bryophytes almost all liverworts possess beautiful cellular oil bodies (Figure 3) which are peculiar, membrane-bound cell organelles that consist of ethereal terpenoids and aromatic oils suspended in carbohydrates- or protein-rich matrix, while the other two phyla do not. These oil bodies are very important marker for the classification in the Marchantiophyta.

Figure 3. Oil bodies of *Frullania vethii*

Phytochemistry of bryophytes has been neglected for a long time because they are morphologically very small and difficult to collect a large amount as pure samples, their identification is also difficult and they are considered to be nutritionally useless to humans. However, a number of bryophytes, in particular, mosses have been widely used as medicinal plants in China, to cure burns, bruises, external wounds, snake bite, pulmonary tuberculosis, neurasthenia, fractures, convulsions, scald, uropathy, pneumonia, neurasthenia *etc*.[1-6]

Many species of the Marchantiophyta show characteristic fragrant odors and an intense hot and bitter taste. Some bryophytes species produce sweet tasting secondary metabolites. Generally, bryophytes are not damaged by microorganisms, insects, snails, slugs, and other small mammals. Furthermore, some liverworts cause intense allergenic contact dermatitis and allelopathy. We have been interested in these biologically active substances found in bryophytes and have studied about 1000 species of bryophytes collected in North and South America, Australia, Europe, French Polynesia, India, Japan, Madagascar, Malaysia, Nepal, New Zealand, Pakistan, Taiwan and Turkey with respect to their chemistry, pharmacology, and application as sources of cosmetics, and medicinal or agricultural drugs. The biological activities of liverworts are due to terpenoids and aromatic compounds.[7-14a-c]

Bio- and chemical diversity of the Marchantiophyta and the chemical structures of heterocyclic terpenoids, aromatic and related compounds found in several liverworts and some biological activity including characteristic odor and taste are surveyed in this paper. Hemisynthesis of bioactive compounds from liverwort constituents and chemosystematics of several Marchantiophyta species are also discussed.

1. Biodiversity of Bryophytes

The Marchantiophyta (liverworts) includes two subclasses, the Jungermanniidae and Marchantiidae, and six orders, 49 families, 130 genera (Table 1) and 6,000 species. These small plant groups are distributed everywhere in the world. Still many new species have been recorded in the literatures. There are 54 endemic genera in southern hemispheric countries, such as New Zealand and Argentina (Figure 4).[14d] In Asia including Japan, relatively a large number of endemic genera (21) has been recorded, however, South Africa, Madagascar and both North America and Europe are very poor regions of endemic genera as shown in Figure 4. The richness of endemic genera of bryophytes in southern hemisphere suggests that the bryophytes might originate from the past Antarctic islands since 350,000,000 years ago and developed to the northern hemisphere with a long range evolutionary process. In Japan, Yaku Island is the most important place to watch many species of the Marchatiophyta. In the southern hemisphere, New Zealand is the most charming country to see many different species of the Marchantiophyta which are totally different from those found in Japan.

Table 1. Classification of Marchantiophyta (Liverworts)

Subclass: Jungermanniidae
Order: Metzgeriales
Suborder: Metzgeriineae
Family: Metzgeriaceae: *Metzgeria*, *Apometzgeria*
Phyllothalliaceae: *Phyllothalia*
Aneuraceae (= Riccardiaceae): *Aneura*, *Cryptophallus*, *Riccardia*
Pelliaceae (= Dilaenaceae): *Pellia*, *Makinoa*
Pallaviciniaceae: *Jensenia*, *Pallavicinia*, *Moerckia*
Blasiaceae: *Blasia*, *Cavicularia*
Suborder: Codoniaceae (= Fossombroniaceae)
Family: Treubiaceae: *Treubia*
Codoniaceae: *Fossombronia*, *Petalophyllum*, *Noteroclada*
Hymenophytaceae: *Verdoonia*, *Symphyogyna*, *Allisonia*, *Hymenophyton*, *Podomitrium*, *Xenothullus*
Order: Calobryales
Family: Haplomitriaceae: *Haplomitrium*
Order: Jungermanniales
Suborder: Jungermanniineae
Family: Jungermanniaceae
Subfamily: Lophozioideae: *Chandonanthus*, *Barbilophozia*, *Anastrepta*, *Lophozia*, *Gymnocolea*, *Sphenolobopsis*, *Sphenobus*, *Anastrophyllum*, *Tritomaria*
Jamesoniellaceae: *Jamesoniella*
Myliioideae: *Mylia*
Jungermannioideae: *Jungermannia*, *Nardia*
Family: Gymnomitriaceae: *Marsupella*, *Gymnomitrion*, *Prasanthus*
Acrobolbaceae: *Acrobolbus*, *Marsupidium*, *Tylimanthus*
Arnelliaceae: *Arnellia*, *Southbya*, *Gongylanthus*
Plagiochilaceae: *Pedinophyllum*, *Plagiochila*
Lophocoleaceae: *Heteroscyphus*, *Leptoscyphus*, *Lophocolea*, *Chiloscyphus*, *Clasmatocolea*
Geocalycaceae: *Geocalyx*, *Harpanthus*, *Saccogyna*
Scapaniaceae: *Douinia*, *Diplophyllum*, *Scapania*
Balantiopsidaceae: *Balantiopsis*
Schistochilaceae: *Schistochila*
Suborder: Lepidoziineae
Family: Adelanthaceae
Subfamily: Adelanthoideae: *Adelanthus*
Odontoschismatoideae: *Odontoschisma*, *Jackiella*
Family: Cephaloziellaceae: *Cephaloziella*
Cephaloziaceae
Subfamily: Cephalozioideae: *Cephalozia*, *Nowellia*, *Cladopodiella*, *Pleuroclada*
Hygrobielloideae: *Hygrobiella*
Family: Antheliaceae: *Anthelia*
Lepidoziaceae

Subfamily: Lepidoziodeae: *Telaranea*, *Kurzia*, *Lepidozia*
Acromastigoideae (= Bazzaioideae): *Bazzania*
Zoopsidoideae: *Zoopsis*, *Bonneria*, *Paracromastigum*
Family: Calypogeiaceae: *Calypogeia*, *Metacalypogeia*
Suborder: Ptilidiineae
Family: Isotachidaceae: *Isotachis*, *Neesioscyphus*, *Eoisotachis*
Pseudolepicoleaceae
Subfamily: Pseudolepicoleidae: *Pseudolepicolea*
Blepharostomatoidae: *Blepharostoma*
Family: Trichocoleaceae: *Trichocolea*, *Trichocoleopsis*, *Neotrichocolea*
Ptilidiaceae: *Ptilidium*
Lepicoleaceae
Subfamily: Mastigophoroideae: *Mastigophora*
Family: Herbertaceae: *Herbertus*, *Triandrophyllum*
Suborder: Radulineae
Family: Radulaceae: *Radula*
Suborder: Pleuroziineae
Family: Pleuroziaceae: *Pleurozia*
Suborder: Porellineae
Family: Porellaceae: *Porella*
Suborder: Jubulineae
Family: Frullaniaceae (= Jubulaceae): *Frullania*, *Jubula*, *Schusterella*
Lejeuneaceae
Subfamily: Ptychanthoideae: *Archilejeunea*, *Dicronolejeunea*, *Lopholejeunea*
Brachiolejeunea:*Achrolejeunea*, *Brachiolejeunea*,*Trocholejeunea*
Lejeuneodieae: *Anoplolejeunea*, *Cheilolejeunea*, *Drepanolejeunea*, *Lejeunea*, *Leptolejeunea*, *Leucolejeunea*, *Omphalanthus*
Cololejeuneoideae: *Colura*, *Cololejeunea*, *Diplasiolejeunea*
Subclass: Marchantiidae
Order: Sphaerocarpales
Family: Riellaceae: *Riella*
Sphaerocarpaceae: *Sphaerocarpos*
Order: Monocleales
Family: Monocleaceae: *Monoclea*
Order: Marchantiales
Suborder: Marchantiineae
Family: Targioniaceae: *Targionia*
Aytoniaceae (= Grimaldiaceae)
Subfamily: Aytonioideae: *Plagiochasma*
Reboulioideae: *Reboulia*, *Mannia*, *Asterella*
Family: Conocephalaceae: *Conocephalum*, *Wiesnerella*
Lunulariaceae: *Lunularia*
Cleveaceae: *Peltolepsis*, *Sauteria*, *Athalamia*
Marchntiaceae: *Bucegia*, *Preissia*, *Marchantia*, *Dumortiera*, *Neohodgsonia*
Exormothecaceae: *Exormotheca*
Corsiniaceae: *Corsinia*
Suborder: Ricciineae
Family: Oxymitriaceae: *Oxymitria*
Ricciaceae: *Ricciocarpus*, *Riccia*

In the tropical regions, such as Borneo, Sumatra and Papua New Guinea, there are rain forests where so many liverworts species have been found, but many different species like the Lejeuneaceae species are intermingled each other and it is time consuming work to purify all of them. In Ecuador and Columbia, the Marchantiophyta species grow in the high mountains, over 2,000 m where people live, not in the lower level of their lands. In Table 1, each subclass, order, family and genus of the Marchantiophyta is shown. Each underlined genus is that chemically already studied in our laboratory.

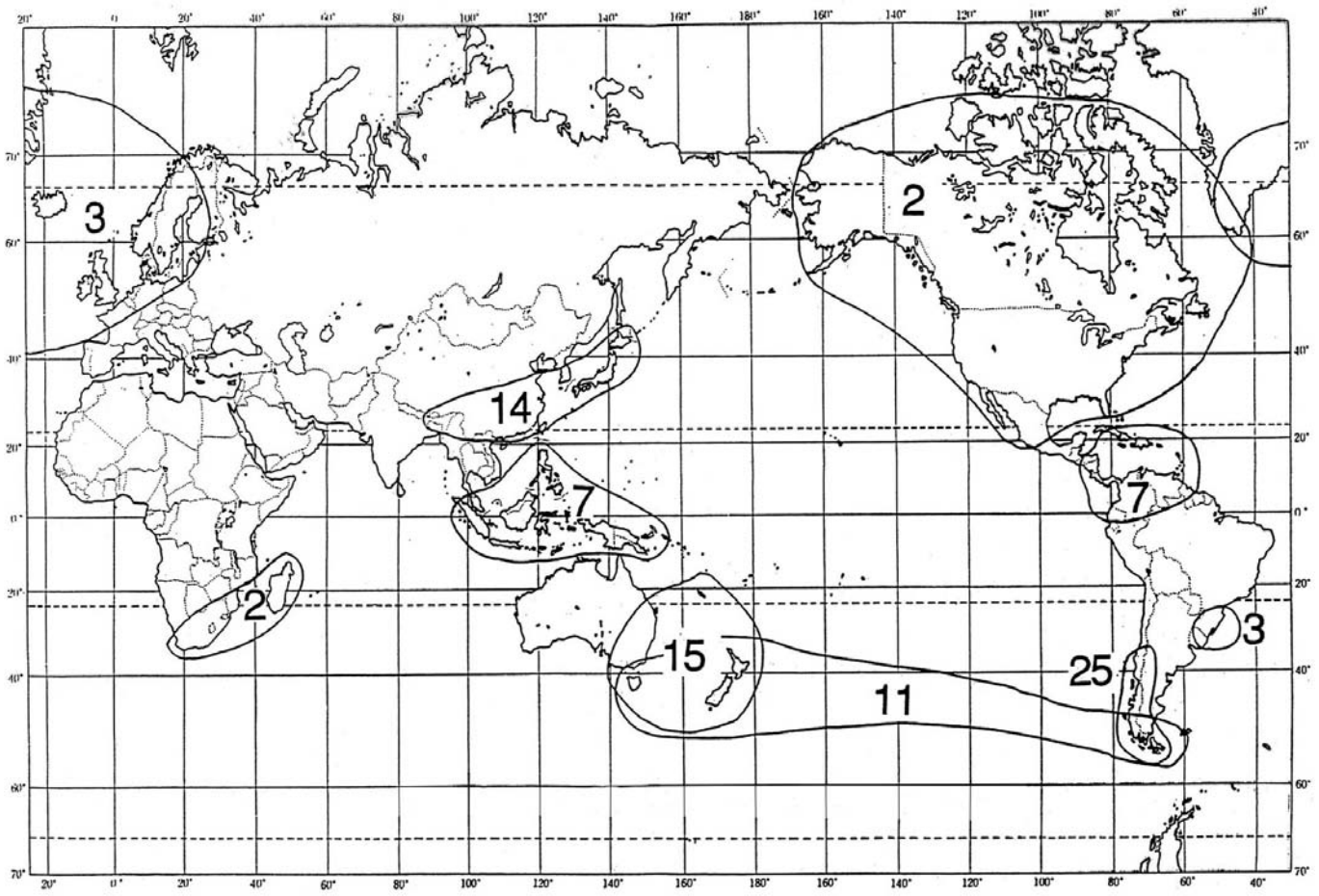


Figure 4. The distribution of endemic genera of bryophytes in the world[14d]

2. Chemical Diversity of Bryophytes

The extraction of oil bodies with organic solvent using ultrasonic apparatus is very easy for stem-leafy liverworts to give a large amount of crude extract. In case of thalloid liverworts the specimens are ground mechanically and then extracted with non-polar solvents. At present, over several hundred new compounds have been isolated from bryophytes and their structures were elucidated.[6-14a] Most of compounds found in liverworts are composed of lipophilic mono-, sesqui- and diterpenoids and aromatic compounds, such as bibenzyls and bis(bibenzyls) which have been isolated from the Marchantiaceae and Aytoniaceae in the Marchantiales, Lejeuneaceae, Lepidoziaceae and Plagiochilaceae in the Jungermanniales and Blasiaceae, Pelliaceae and Riccardiaceae in the Metzgeriales.

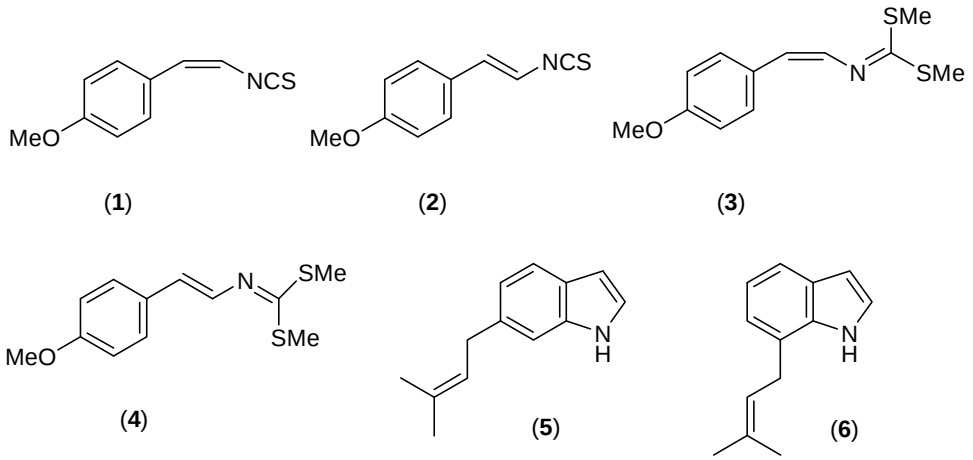


Chart 1. Nitrogen-containing aromatic compounds from liverworts

However, the presence of nitrogen or sulfur-containing compounds in bryophytes was very rare; recently several nitrogen-containing compounds have been isolated from the Mediterranean liverwort, *Corsinia coriandrina* belonging to the Corsiniaceae (Marchantiales).[14e]

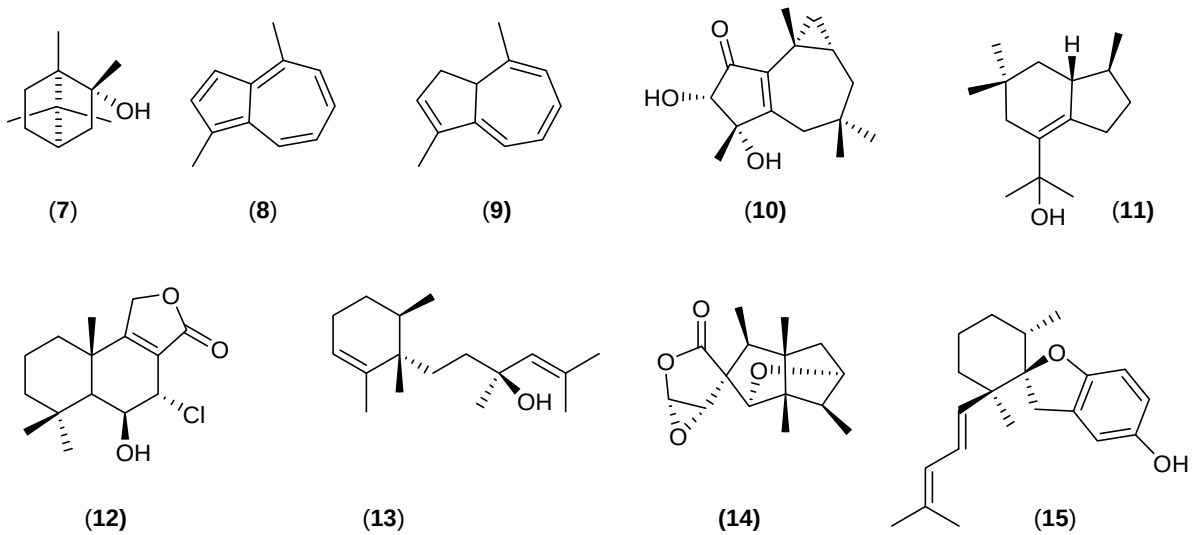


Chart 2. Typical terpenoids from liverworts

The most characteristic chemical phenomenon of liverworts is that most of sesqui- and diterpenoids are enantiomers of those found in higher plants although there are a few exceptions such as drimane, germacrane and guaianes. It is very noteworthy that the different species of the same genera, like *Frullania tamarisci* and *F. dilatata* (Frullaniaceae) each produces different sesquiterpene enatiomers.

Some liverworts, such as *Lepidozia* species (Lepidoziaceae), biosynthesize both enantiomers. Flavonoids are ubiquitous components in bryophytes and have been isolated from or detected both in the Marchantiophyta and the Bryophyta.

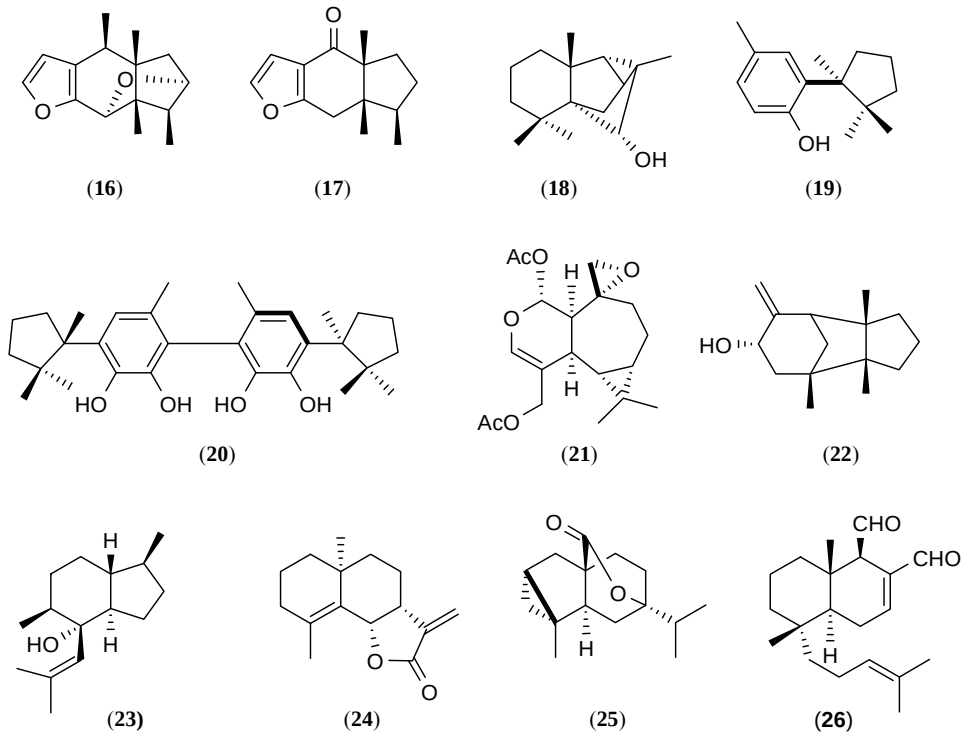


Chart 3. (continued) Typical terpenoids from liverworts

Almost all liverworts elaborate α-tocopherol, stigmasterol and squalene. The characteristic components of the Bryophyta are highly unsaturated fatty acids and alkanones, such as 5,8,11,14,17-eicosapentaenoic acid, 7,10,13,16,19-docosapentaenoic acid and 10,13,16-nonadecatrien-7-yn-2-one and triterpenoids. The neolignan is one of the most important chemical markers of the Anthocerotophyta.

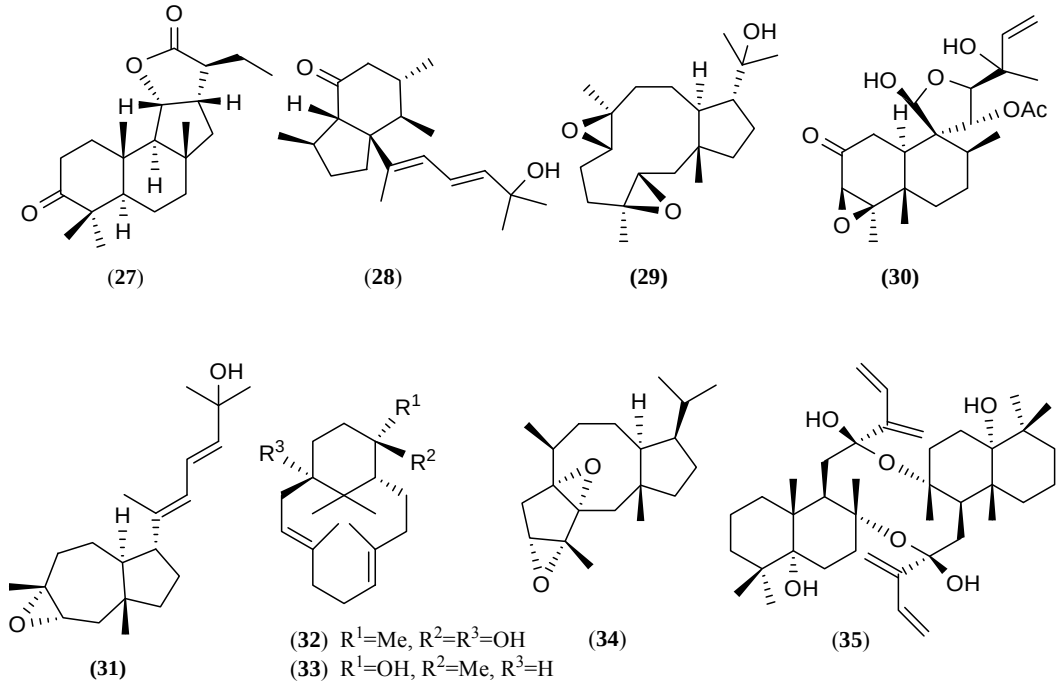


Chart 4. (continued) Typical terpenoids from liverworts

The presence of hydrophobic terpenoids is very rare in the Marchantiophyta. A few bitter kaurene glycosides have been found in the *Jungermannia* species (see later). However a numbers of flavonoid glycosides have been detected both in Marchantiophyta and Bryophyta. In the Charts 1-5, the typical terpenoids and aromatic compounds found in liverworts are shown.

3. Bioactive Compounds from Bryophytes

The biological characteristics of the terpenoids and aromatic compounds isolated from the liverworts in our laboratory are: 1) characteristic scents, 2) pungency and bitterness, 3) allergenic contact dermatitis, 4) cytotoxic, anti-HIV and DNA polymerase β inhibitory, 5) antimicrobial and antifungal activity, 6) insect antifeedant activity, mortality, and nematocidal activity, 7) superoxide anion radical release inhibitory activity, 8) 5-lipoxygenase, calmodulin, hyaluronidase, cyclooxygenase inhibitory activity and NO production inhibitory activity, 9) piscicidal and plant growth inhibitory activity, 10) neurotrophic activity, 11) muscle relaxing activity, 12) cathepsins B and L inhibitory activity, 13) cardiotonic and vasopressin antagonist activity and 14) anti-obesity activity.

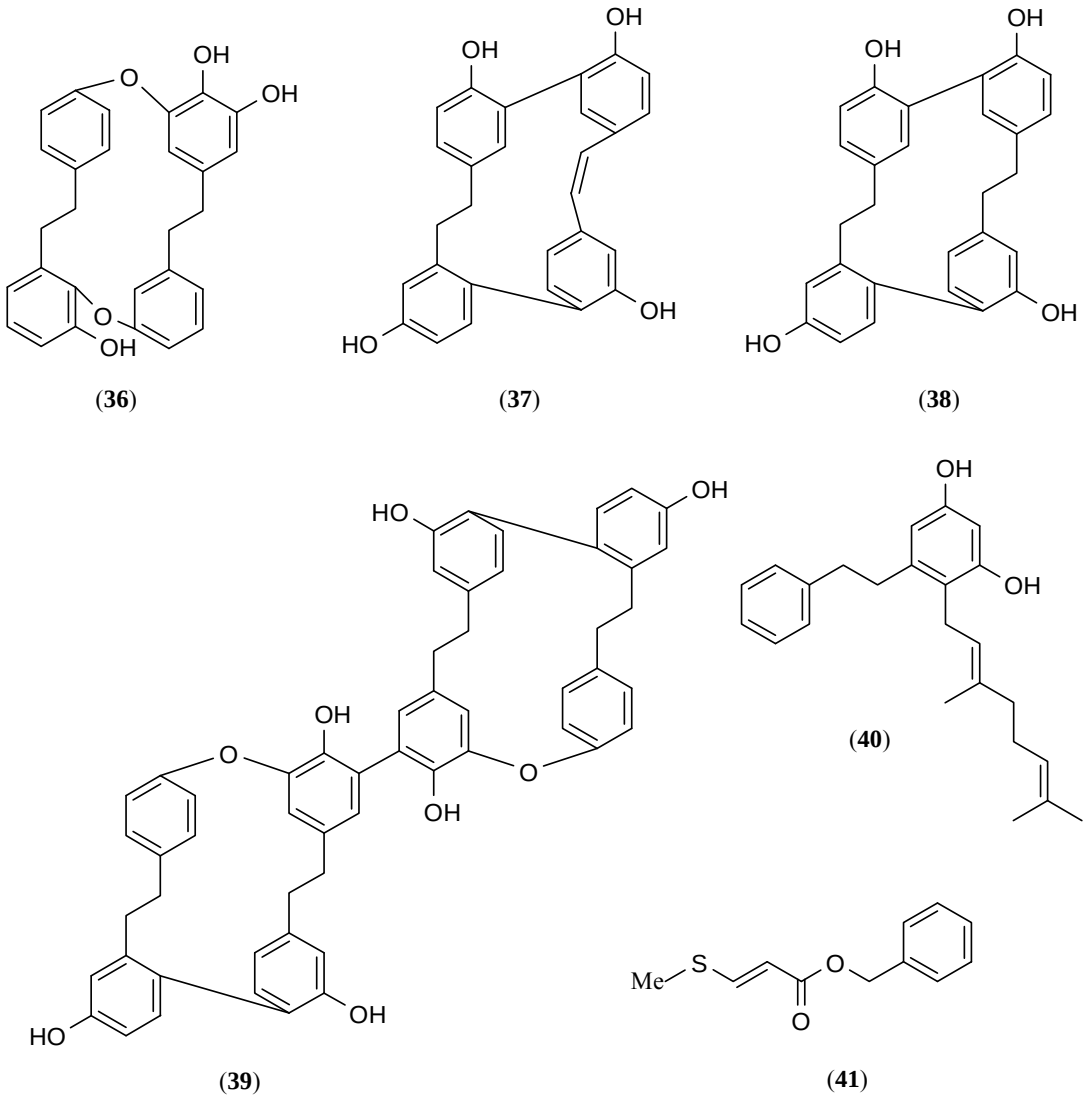
3.1. Characteristic Scents

A number of liverworts emit volatile terpenoids or simple aromatic compounds when crushed, which are responsible for intense sweet-woody, turpentine, sweet-mossy, fungal-like, carrot-like, mushroomy, or seaweed-like scents.[11,15,16]

Almost all liverworts which smell of mushrooms contain 1-octen-3-ol and its acetate, which is generally more abundant than the free alcohol. A small thalloid liverwort, *Asterella* species grown in Pulau Dayang Bunting island in Malaysia emits an intense unpleasant odor. Surprisingly, it produces two components: skatole which is responsible for this smell and is composes of 20% of the total extract, and 80% of 3,4-dimethoxystyrene.[17] The stink bug smell of the New Zealand *Cheilolejeunea pallidus* is attributable to (*E*)-dec-2-enal, (*Z*)-dec-2-enal, (*E*)- and (*Z*)-pent-2-enals,[18] although the major components of such insects are the *cis* and *trans*-2-hexenals. The characteristic mold like smell of *Leptolejeunea elliptica* is due to *p*-ethylanisol, *p*-ethylphenol, and *p*-ethylphenylacetate.[19] The strong milk-like fragrance of *Cheilolejeunea imbricata* is due to a mixture of (*R*)-dodec-2-en-1,5-olide (**42**) and (*R*)-tetradec-2-en-1,5-olide (**43**).[19]

Bicyclohumulenone (**44**) isolated from *Plagiochila sciophila* (=*P. acanthophylla* subsp. *japonica*) as a crystal possesses an aroma reminiscent of a variety of scents based on a strong woody note, resembling the odor of patchouli, vetiver, cedar wood, iris, moss, and carnations. Tamariscol (**23**) from European *Frullania tamarisci* subsp. *tamarisci*, Japanese *F. tamarisci* subsp. *obscura*, Taiwanese *F. nepalensis*, and East American *F. asagrayana* similarly possesses a remarkable aroma reminiscent of the woody and

powdery green notes of mosses, hay, costus, violet leaves and seaweeds. Both compounds are important in commerce. They are used as perfumes as such or as perfume components of the powdery floral-, oriental bouquet-, fantastic chypre-, fancy violet- and white rose-types in various cosmetics. It is noteworthy that *Frullania* species producing tamariscol grow in high mountains only.



(**36**) (**37**) (**38**)

(**39**) (**40**) (**41**)

Chart 5. (continued) Typical aromatic compounds terpenoids from liverworts

Total synthesis of (±)-tamariscol (**23**) has been accomplished using commercially available *p*-methoxyacetophenone in 13 steps.[20] After it had been shown that both the tertiary alcohol and the 2-methyl-1-propenyl group attached to the cyclohexane ring of tamariscol were necessary for the characteristic scent of (**23**), thirteen mini-tamariscols were synthesized by Grignard reactions of 2,7-dimethylcyclohexanone, 2-methylcyclohexanone, 4-methylcyclohexanone, cyclohexanone, and cyclopentanone with vinylmagnesium bromide, 2-methyl-1-propenylmagnesium bromide and

2-methyl-2-propenylmagnesium bromide, respectively. Among them, 1-hydroxy-1-(2-methyl-1-propenyl)cyclohexane (**45**) had a sweet mossy aroma similar to that of tamariscol itself.[21] There are three chemo-types of *Conocephalum conicum*. The types 1, 2 and 3 emit (-)-sabinene, (+)-bornyl acetate, and methyl cinnamate as the major components, respectively, which are responsible for the characteristic odor of each type.[22] *Jungermannia obovata* contains a tris-normonoterpene ketone, 4-hydroxy-4-methyl cyclohex-2-en-1-one (**46**) which possesses an intense carrot-like odor.[23,24]

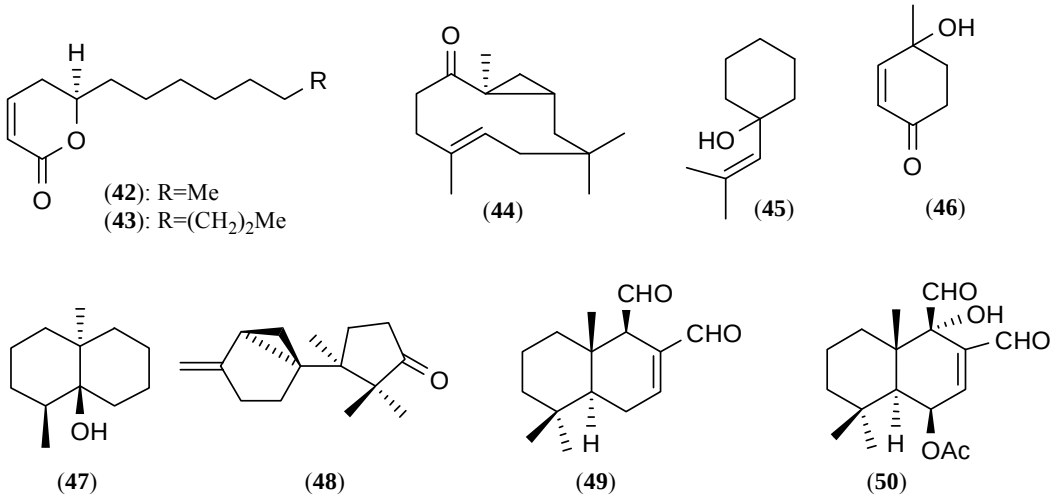


Chart 6. Scent components from liverworts

The strong and distinct mossy odor of *Lophocolea heterophylla* and *L. bidentata* is due to a mixture of (-)-2-methylisoborneol[25] and geosmin (**47**). Geosmin, possessing a strong earthy-musty odor, has also been found in *in vitro* cultured *Symphyogyna brongniartii.*[26] The strong sweet mossy note of *Mannia fragrans* is attributable to the cuparene-type sesquiterpene ketone, grimaldone (**48**).[27]

The sweet turpentine-like odor of the French *Targionia hypophyll*a is due to a mixture of *cis*- and *tans*-pinocarveyl acetates.[28] The strong sweet-mushroomy scent of the ether extract of *Wiesnerella denudata* is due to (+)-bornyl acetate and a mixture of the monoterpene hydrocarbons, α-terpinene, β-phellandrene, terpinolene, α-pinene, β-pinene, and camphene.[11] The odor of the steam distillate of *W. denudata* is weaker than that of its ether extract. The steam distillate contains nerol (14%), neryl acetate (27%), and γ-terpinene (31%), but the content of 1-octen-3-ol (7%) and its acetate (2%) is lower than that of *Conocephalum conicum* belonging to the same genus of *Wiesnerella.*

3.2. Pungency and Bitterness

Some genera of the Marchantiophyta produce intense pungent and bitter substances which exhibit interesting biological activities described in subsequent sections. Most North American liverworts contain

unpleasant substances, some of which taste like immature green pea seeds or pepper.[29] *Porella vernicosa* complex (*P. arboris-vitae, P. fauriei, P. gracillima, P. obtusata* subsp. *macroloba, P. roellii* and *P. vernicosa*) contain very pungent substances, and *Jamesoniella autumnalis* contains an intense bitter principle whose taste resembles that of the leaf of lilac and *Swertia japonica* or the root of *Gentiana scabra* var. *orientalis.* The strong hot taste of *Porella vernicosa* complex is due to (-)-polygodial (**49**).[7,16]

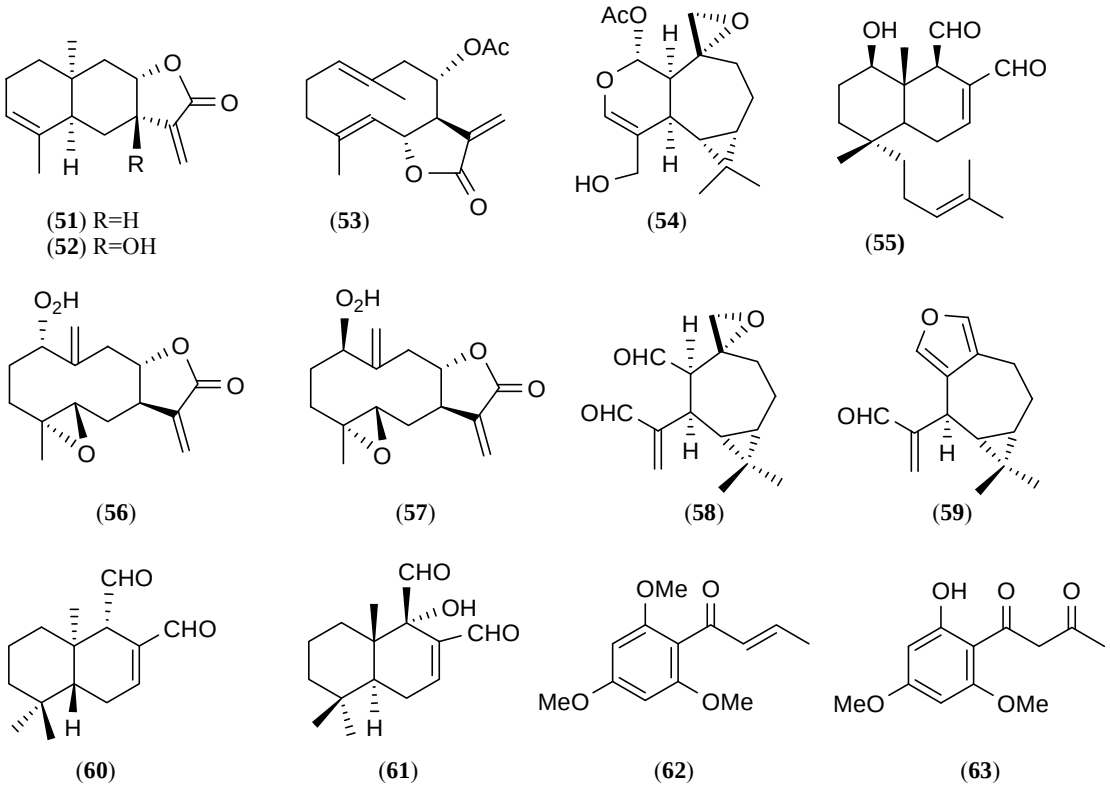


Chart 7. Pungent components from liverworts

Polygodial is the major component of the medicinal plant, *Polygonum hydropiper, P. minus* and *P. punctataum* var. *punctatum* (Polygonaceae). The Malagasy medicinal plant, *Cinnamosma fragrans* (Canellaceae) produces structurally similar pungent component, cinnamodial (=ugandensidial) (**50**).[30,31] It is noteworthy that some ferns, *Blechnum fluviatile* collected in New Zealand and Argentinean *Thelypteris hispidula* elabolate the pungent component, polygodial (**49**), together with its related drimanes.[32,33]

The sacculatane diterpenedialdehyde, sacculatal (**26**), two eudesmanolides, diplophyllolide (**51**) and *ent*-7α-hydroxydiplophyllolide (**52**), a germacranolide, tulipinolide (**53**) and two 2,3-secoaromadendrane-type sesquiterpene hemiacetals, plagiochiline A (**21**) and plagiochiline I (**54**) which possess potent pungency were isolated from *Pellia* and *Trichocoleopsis,* and *Chiloscyphus, Wiesnerella* and *Plagiochila,* species, respectively. An additional pungent 1β-hydroxysacculatal (**55**) was

obtained from *Pellia endiviifolia,* together with several sacculatane-type diterpenoids.[34] The hot taste of *Pallavicinia levieri* [14] and *Riccardia robata* var. *yakushimensis* [35] is also due to sacculatal (**26**).

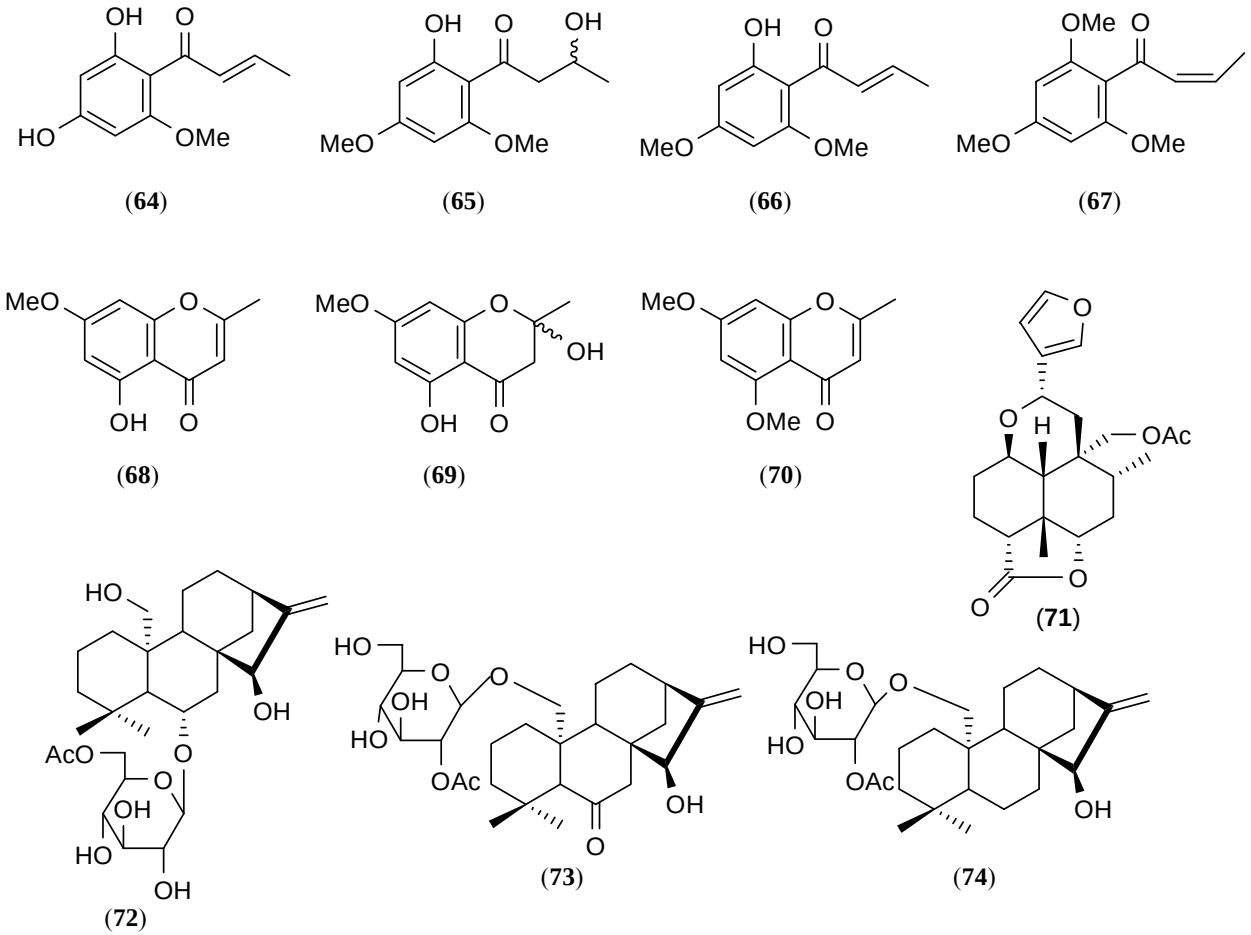


Chart 8. Bitter principles (**71-74**) from liverworts

Polygodial and sacculatal have been obtained from cell suspension cultures from each liverwort.[36,37] *Porella acutifolia* subsp. *tosana* is a pungent stem-leafy liverwort. Its taste is due to the presence of hydroperoxysesquiterpene lactones, 1α- (**56**), and 1β-hydroperoxy-4α,5β-epoxygermacra-10(14),11(13)-dien-12,18α-olides (**57**).[38] When one chews a whole plant of the stem-leafy liverwort, *Plagiochila asplenioides, P. fruticosa, P. ovalifolia,* and *P. yokogurensis* which contain plagiochiline A (**21**) one feels a potent hot taste slowly. It is suggested that (**21**) might be converted into pungent unsaturated dialdehyde by human saliva. Enzymatic treatment of (**21**) with amylase in phosphate buffer or with human saliva produces two strong pungent 2,3-secoaromadendrane-type aldehydes, plagiochilal B (**58**) whose partial structure is similar to that of the pungent drimane-type sesquiterpene dialdehyde, polygodial **(49**), and furanoplagiochilal (**59**).[39] The New Zealand liverwort, *Hymenophyton flabellatum* produces different pungent tasting substance from the other aforementioned liverworts. Fractionation of

the ether extract resulted in the isolation of several phenyl butanones (**62-67**) and their related compounds (**68-70**). The hot taste of the species is due to compound (**62**).[32]

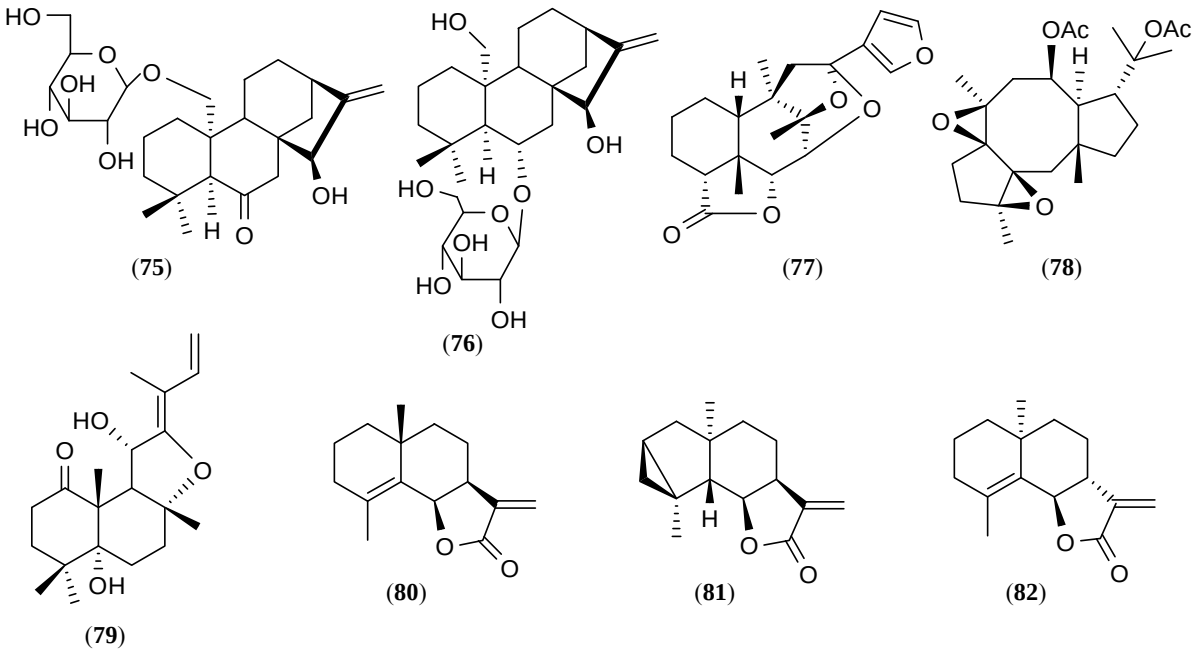


Chart 9. Bitter principles (**75-79**) and allergenic sesquiterpene lactones (**80-82**) from liverworts

Most of the species belonging to the Lophoziaceae produce surprisingly intense bitter substances. *Gymnocolea inflata* is persistently bitter and induces vomiting when one chews a few leaves for several seconds. The earlier review already mentioned that this is due to gymnocolin A (**71**).[11] It contains additional unknown minor bitter diterpenoids whose structures remains to be clarified. *Jungermannia infusca* has an intense bitter taste. This is due to the presence of the infuscasides A-E (**72-76**) which were the first reported isolation of glycosides from liverworts.[40] The bitterness of *Anastrepta orcadensis*, *Barbilophozia lycopodioides,* and *Scapania undulata* are attributable to highly oxygenated diterpenoids, anastreptin A (**77**) and barbilycopodin (**78**),[23,41] and scapanin A (**79**),[42] respectively.

3.3. Allergenic Contact Dermatitis

Frullania species are notable as liverworts that cause very intense allergenic contact dermatitis.[7,43-47] The allergy-inducing substances are sesquiterpene lactones, (+)-frullanolide (**24**) and (-)-frullanolide (**80**) which have been isolated from *Frullania dilatata* and *F. tamarisci* subsp. *tamarisci*, respectively.[7] Both dihydrofrullanolides (**89, 90**) with a α-methylene-γ-butyrolactones isolated from the above mentioned liverworts do not cause allergy. *F. asagrayana, F. bolanderi, F. brasiliensis, F. eboracensis, F.*

franciscana, F. inflata, F. kunzei, F. nisquallensis, F. riparia and the other *Frullania* species which contain sesquiterpenes (**81-88**) with α-methylene-γ-butyrolactones cause strong allergenic contact dermatitis as does *Schistochila appendiculata*.

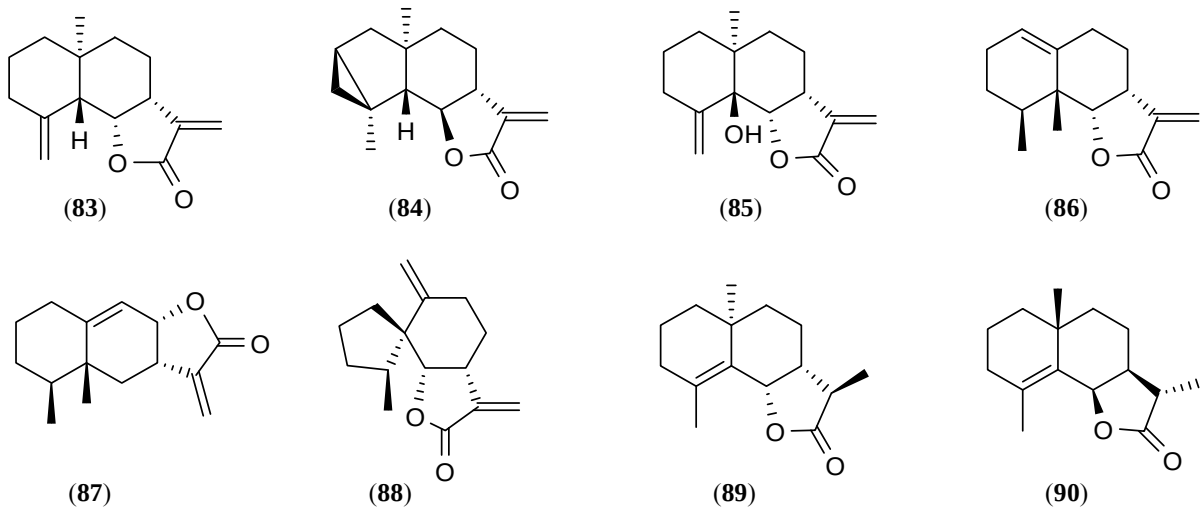


Chart 10. Allergenic sesquiterpene lactones (**83-88**) from liverworts

The allergens of the latter are long chain alkylphenols, 3-undecyl- (**91**), 3-tridecyl (**92**), 3-pentadecyl (**93**), and 3-heptadecyl phenols (**94**), long chain alkyl salicylic acids, 6-undecyl- (**95)**, 6-tridecyl- (**96**), 6-pentadecyl salicylates (**97**), and their potassium salts, potassium 6-undecyl- (**98**), 6-tridecyl- (**99**), and 6-pentadecyl salicylates (**100**) as well as 6-undecyl catechol (**101**).[11]

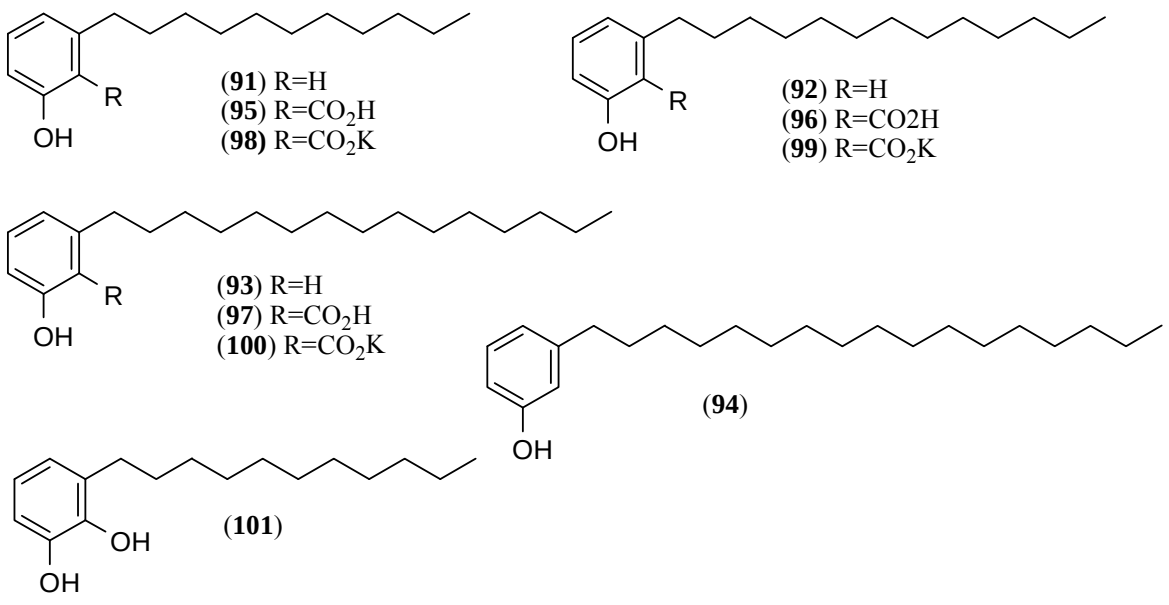


Chart 11. Allergenic alkylphenols from liverworts

Such dermatitis is similar to that caused by the long chain alkylphenols of the fruit of *Ginkgo biloba* and Anacardiaceae plants. *Marchantia polymorpha* and *Metzgeria furcata* also cause allergenic contact dermatitis but their allergens have not been isolated yet.

3.4. Cytotoxic, Anti-HIV-1 and DNA Polymarase β Inhibitory

A few eudesmanolides and germacranolide possessing inhibitory activity against KB cells have been isolated from liverworts.[11] *Conocephalum conicum* and *Wiesnerella denudata* contain guaianolides which exhibited cytotoxic activity against P-388 lymphocytic leukemia.[11] The crude ether extract (4-20 μg/mL) of the following liverworts showed cytotoxicity against P-388 *in vitro* [Asakawa Y. unpublished results]: *Bazzania pompeana, Kurzia makinoana, Lophocolea heterophylla, Makinoa crispata, Marsupella emarginata, Pellia endiviifolia, Plagiochila fruticosa, P. ovalifolia, Porella caespitans, P. japonica, P. perrottetiana, P. vernicosa*, and *Radula perrottetii.* On the other hand, *Frullania diversitexta, F. ericoides, F. muscicola, F. tamarisci* subsp. *obscura, Lepidozia vitrea, Pallavicinia subciliata, Plagiochila sciophila, Spruceanthus semirepandus,* and *Trocholejeunea sandvicensis* were not active against P-388.

2,3-Secoaromadendrane-type sesquiterpenoids, plagiochiline A (**21**), plagiochiline A 13-octanoate (**102**), and 12-hydroxyplagiochiline A 13-2*E*,4*E*-dodecadienoate (**103**) isolated from *P. ovalifolia* showed cytotoxic activity (ID_{50} 3, 0.05, 0.05 μg/mL, respectively) against P-388.[48] Polygodial (**49**) isolated from *P. vernicosa* complex, sacculatal (**26**) from *P. endiviifolia,* and two 2,3-secoaromadendrane-type sesquiterpene hemiacetals (**102**), and plagiochiline A 13-decanoate (**104**) from *P. ovalifolia* showed cytotoxic activity (2-4 μg/mL) against melanoma.[49] Sacculatal (**26**) also showed cytotoxic activity against Lu1 cell (IC_{50} 5.7 μg/mL), KB cell (3.2), LN Cap cell (7.6) and ZR-75-1 cell (7.6), respectively [Cordel GA, Pezzuto JM, Asakawa Y. unpublished results].

Marsupellone (**105**) and acetoxymarsupellone (**106**) from *Marsupella emarginata* showed cytotoxicity (ID_{50} 1 μg/mL) against P388.[49,50] Riccardins A (**107**) and B (**108**) which was the first bis-bibenzyls from the Japanese liverwort, *Riccardia multifida* subsp. *decrescens* inhibited KB cells at a concentration of 10 and 12 μg/mL, respectively. Many *Plagiochila* species and *Radula perrottetii* contained cytotoxic plagiochiline A (**21**) (0.28 μg/mL) and perrottetin E (**109**) (12.5 μg/mL) against KB cells, respectively.

The thalloid liverwort, *Marchantia polymorpha* which can cause allergenic contact dermatitis, shows inhibitory activity against Gram-positive bacteria, and has diuretic activity. The methanol extract (105 g) of Japanese *M. polymorpha* was chromatographed on silica gel and Sephadex LH-20 to give cyclic bis-bibenzyls, marchantin A (MA) (**36**, 30 g) and its analogues (MB-G). The yield of MA (**36**) is dependent upon *Marchantia* species. 80 to 120 g of pure MA has been isolated from 6.67kg of dried *M. paleacea* var. *diptera*. This thalloid liverwort elaborates not only the marchantin series, marchantin A (**36**), B (**114**), D (**115**) and E (**116**), but also the acyclic bis-bibenzyls, perrottetin F (**110**) and paleatin B (**117**).

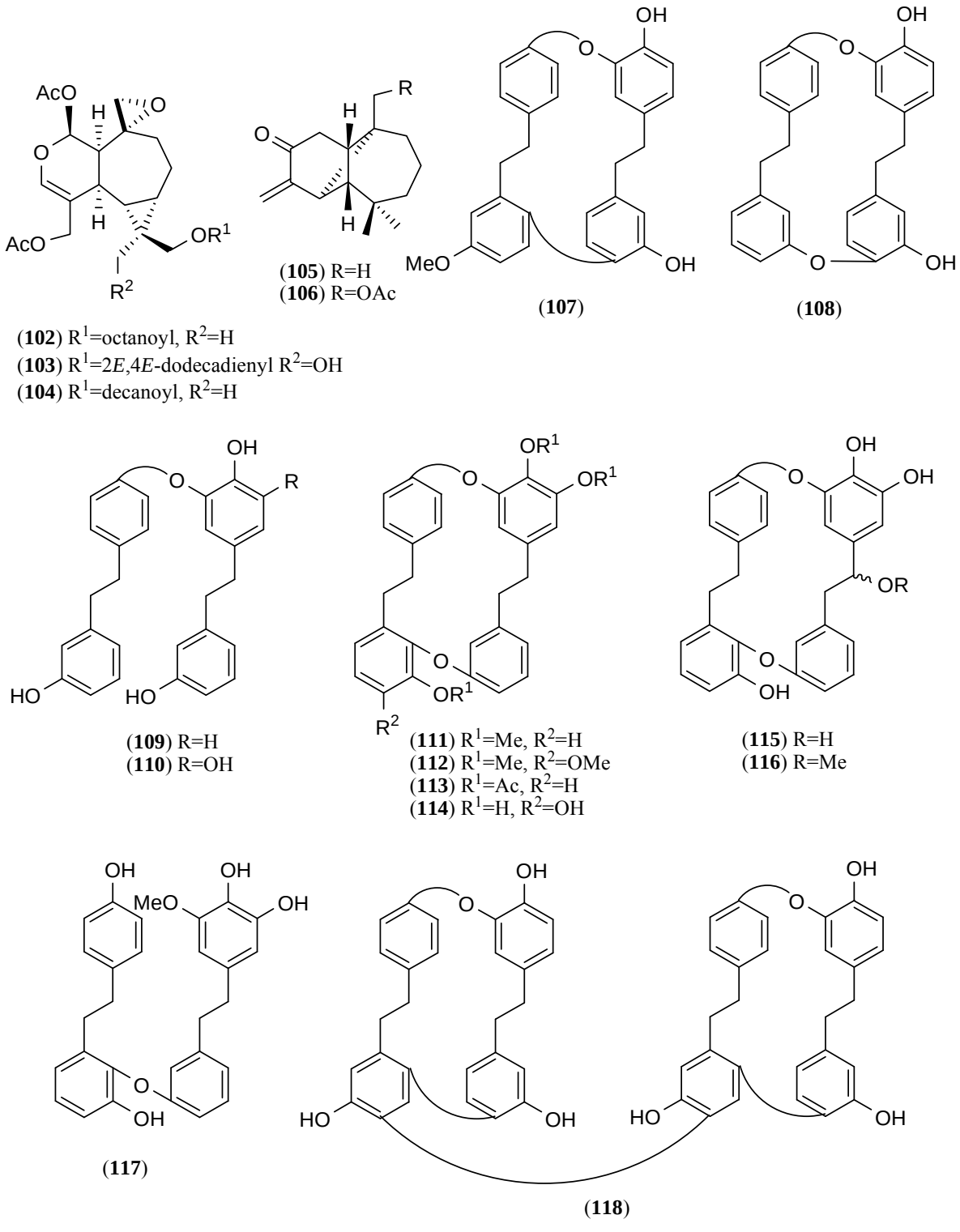


Chart 12. Cytotoxic, anti-HIV-1 and DNA polymarase β inhibitory activecomponents from liverworts

Marchantins A, B, D, paleatin B and perrottetin F show DNA polymelase β inhibitory (ID_{50} 14.4-97.5 μM), cytotoxic (3.7-20 μM against. KB cells) and anti-HIV-1 (5.30-23.7 μg/mL) activity.

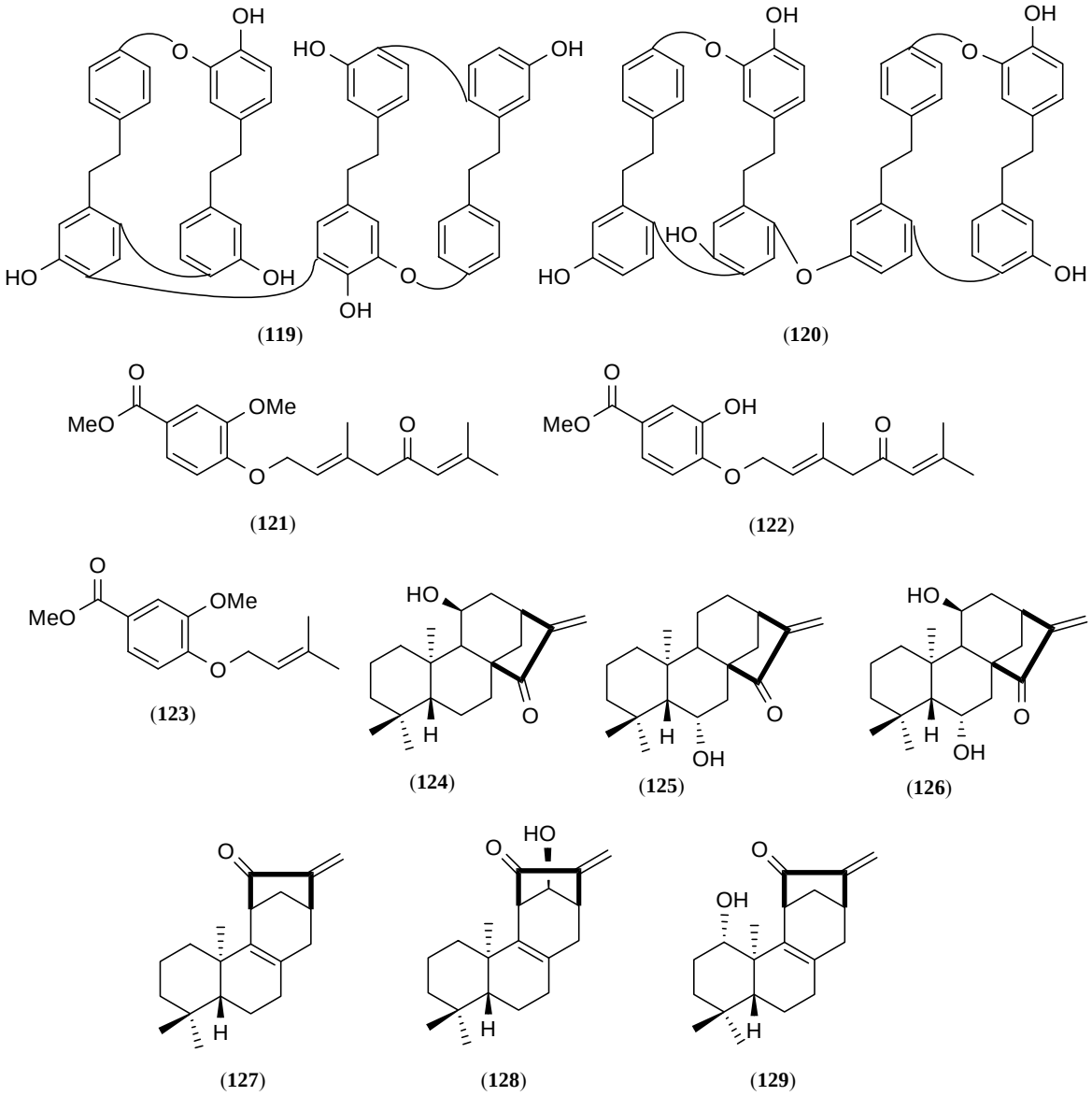


Chart 13. (Continued) Cytotoxic, anti-HIV-1 and DNA polymerase β inhibitory active components from liverworts

Marchantin A (**36**) also shows cytotoxicity (T/C 117) against P-388.[11] *Blasia pusilla* produces the bis(bibenzyl) dimers, pusilatin A (**118**), B (**39**), C (**119**) and D (**120**). Pusilatins B (**39**) and C (**119**) possess DNA polymerase β inhibitory activity (IC_{50} 13.0 and 5.16 μM), moderate cytotoxicity against KB cells (ED_{50} 13.1 and 13.0 μg/mL) and weak HIV-RT inhibitory activity.[51] *Trichocolea* species produce prenyl ethers, tomentellin (**121**), demethyltomentellin (**122**), and trichocolein (**123**).[52] Compound **121** is the major cytotoxic component of *T. mollissima*, active against BSC cells at 15 μg/disk.[52] Compound **122** isolated from *T. tomentella* also showed the same activity.[52] The *ent*-kaurenes and modified *ent*-kaurenes isolated from the New Zealand liverwort, an unidentified *Jungermannia* species showed cytotoxic activity against HL-60 cells. Compound (**124-129**) induced DNA fragmentation in HL-60 cells.[53-60]

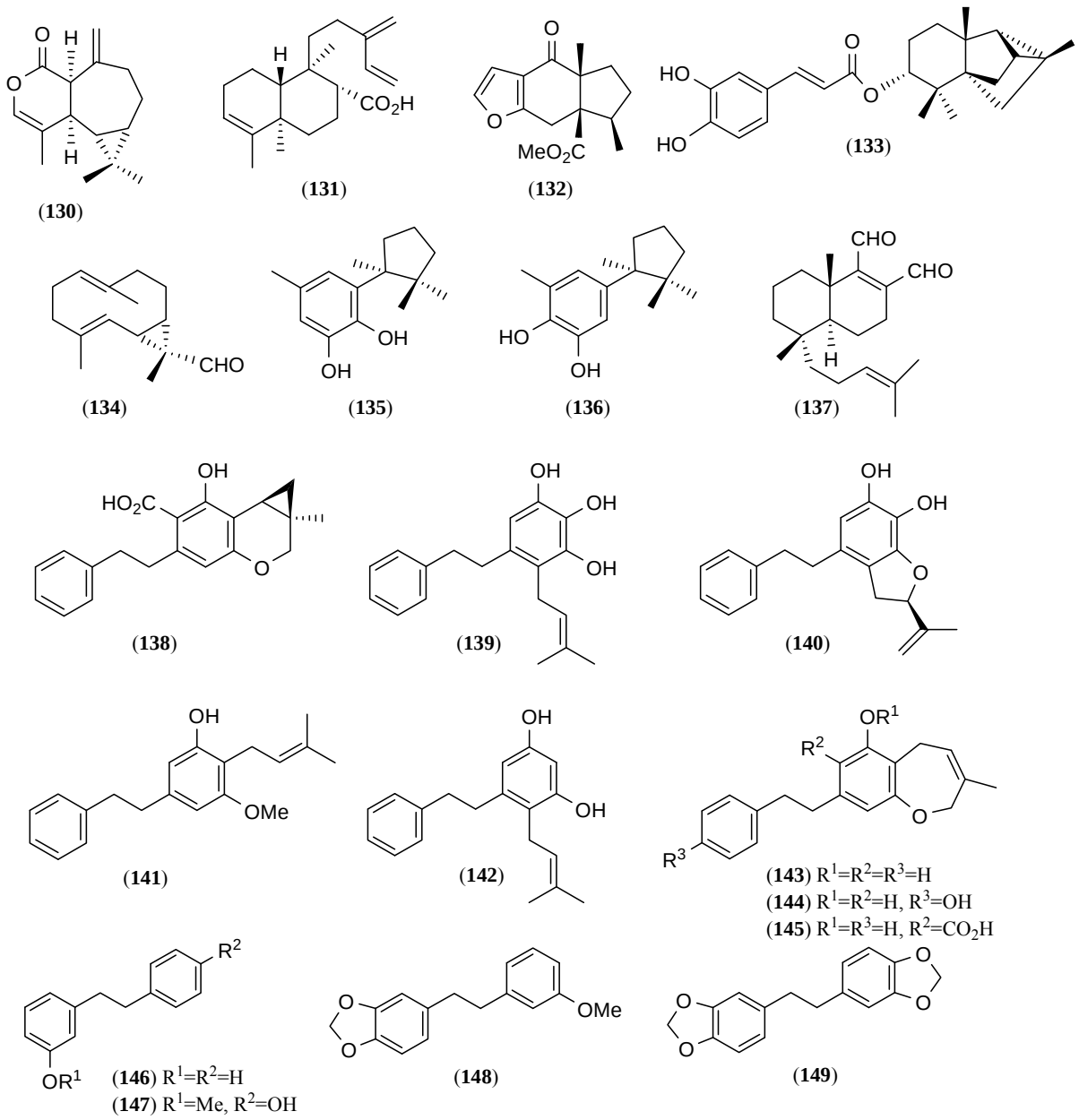


Chart 14. (Continued) Cytotoxic, anti-HIV-1 and DNA polymarase β inhibitory active components from liverworts

3.5. Antimicrobial and Antifungal Activity

Several liverworts, *Bazzania* species, *Conocephalum conicum*, *Dumortiera hirsuta*, *Marchantia polymorpha*, *Metzgeria furcata*, *Pellia endiviifolia*, *Plagiochila* species, *Porella vernicosa* complex, *P. platyphylla*, and *Radula* species show antimicrobial activity.[11] Several such as *Bazzania* species, *Conocephalum conicum*, *Diplophyllum albicans*, *Lunularia cruciata*, *Marchantia polymorpha*, *Plagiochila* species, *Porella vernicosa* complex, and *Radula* species display antifungal activity.[11]

Marchantin A (**36**) from many *Marchantia* species, *M. chenopoda*, *M. polymorpha*, *M. paleacea* var. *diptera*, *M. plicata,* and *M. tosana*, shows antibacterial activity against *Acinetobacter calcoaceticus* (MIC 6.25 μg/ml), *Alcaligenes faecalis* (100), *Bacillus cereus* (12.5), *B. megaterium* (25), *B. subtilis* (25),

Cryptococcus neoformans (12.5), *Enterobacter cloacae, Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella typhimurium* (100), and *Staphylococcus aureus* (3.13-25).[11] They also have antifungal activity against *Alternaria kikuchiana*, *Aspergillus fumigatus* (MIC 100 μg/mL), *A. niger* (25-100), *Candida albicans*, *Microsprorum gypseum*, *Penicillium chrysogenum* (100), *Piricularia oryzae* (12.5), *Rhizoctonia solani* (50), *Saccharomyces cerevisiae, Sporothrix schenckii* (100), and the dermatophytes *Trichophyton mentagrophytes* (3.13) and *T. rubrum* (100).[11]

The prenyl phenyl ethers (**121**, **122**) isolated from *Trichocolea mollissima* and *T. tomentella*, respectively were mildly antifungal against *T. mentagrophytes*.[52] Compound **123** isolated from *T. lanata* showed similar mild antifungal activity.[52] Sacculatal (**26**), isolated from *Pellia endiviifolia* showed strong antibacterial activity against *Streptococcus mutans* (dental caries) at LD_{50} 8 μg/mL, however, polygodial (**49**) is less active (100 μg/mL) than sacculatal.

3.6. Insect Antifeedant, Mortality and Nematocidal Activity

As mentioned earlier, plagiochiline A **(21**), found in several *Plagiochila* species, is a strong antifeedant against the African army worm (*Spodoptera exempta*).[7] Compound **21** shows nematocidal activity against *Caenorphabdiitis elegans* (111 μg/mL). The pungent sacculatal (**26**) kills tick species *Panonychus citri*. Compound **26**, eudesmanolides (**51, 52**) from *Chiloscyphus polyanthos,* and gymnocolin (**71**) from *Gymnocolea inflata* also have antifeedant activity against larvae of Japanese *Pieris* species.[11] A series of natural drimanes and related synthetic compounds was tested for antifeedant activity against aphids.[61] Polygodial (**49**) from the *Porella vernicosa* complex and warburganal (**61**) from the African tree *Warburgia ugandensis* were the most active substances. Natural (-)-polygodial (**49**) and the synthetic (+)-enantiomer (**60**) showed similar levels of activity as aphid antifeedants. (-)-Polygodial killed mosquito larvae at a concentration of 40 ppm and had mosquito repellent activity which is stronger than the commercially available DEET. Plagiochilide (**130**) isolated from *Plagiochila* species killed *Nilaparvata lugens* (Delphacidae) at 100 μg/mL.

3.7. Superoxide Release Inhibitory Activity

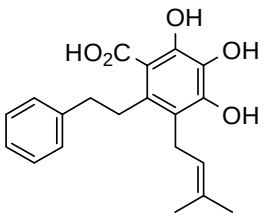
Excess superoxide anion radical (O_2^-) in organisms causes various angiopathies, such as cardiac infarction, and arterial sclerosis. Infuscaic acid (clerod-3,13(16)-14-trien-17-oic acid) (**131**) from *Jungermannia infusca* and plagiochilal B (**58**) inhibit the release of superoxide from rabbit PMN at IC_{50} 0.07 and 6.0 μg/mL, respectively and from guinea pig peritoneal macrophage induced by formyl methionyl leucyl phenylalanine (FMLP) at IC_{50} 40 μg /mL, and 25.0 μg/mL respectively.[11]

Norpinguisone methyl ether (**132**) from *Porella elegantula* exhibits 50% inhibition of the release of

superoxide from the guinea pig peritoneal macrophage at 35 μg/mL. The same activity (IC_{50} 7.5 μg/mL) has been found in cyclomyltaylyl-3-caffeate (**133**) from *Bazzania japonica*. Other sesquiterpenoids, plagiochilide (**96**) isolated from *Plagiochila fruticosa, P. ovalifolia* and *P. yokogurensis,* norpinguisone (**17**) from *Porella vernicosa*, bicyclogermacrenal (**134**) from *Conocephalum conicum,* herbertenediol (**135**) and isocuparene-3,4-diol (**136**) from *Mastigophola diclados,* the diterpenoids, infuscaside A (**72**) and infuscaside B (**73**) from *Jungermannia infusca*, and perrottetianal A (**137**) from *Porella perrottetiana* also inhibit superoxide release from guinea pig peritoneal macrophage (IC_{50} 12.5-50 μg/mL).[11] Radulanin K (**138**) from *Radula javanica* inhibits the release of superoxide anion radical from guinea pig macrophage (IC_{50} 6 μg/mL).[48] Polygodial (**49**) and sacculatal (**26**) also show superoxide anion radical release inhibition at 4.0 μg/ml from guinea pig peritoneal macrophage.

3.8. 5-Lipoxygenase, Calmodulin, Hyaluronidase Cyclooxygenase Inhibitory Activity and NO Production Inhibitory Activity

A macrocyclic bis-bibenzyl, marchantin A (**36**) isolated from several *Marchantia* species showed 5-lipoxygenase inhibitory activity [(89% at 10^{-5} mol, 94% at 10^{-6} mol, 45% at 10^{-7} mol, 16% at 10^{-8} mol) against LTB_3 (5*S*,12*R*-dihydroxy-6,8,10,14-eicosatetraenoic acid)], (99% at 10^{-5} mol, 97% at 10^{-6} mol, 70% at 10^{-7}mol, 40% at 10^{-8} mol) against 5-HETE (5-hydroxy-6,8,11,14-eicosatetraenoic acid)] and calmodulin inhibitory activity at ID_{50} 1.85 μg/mL.[11] Perrottetins A (**139**) and D (**140**) from *Radula perrottetii* and prenyl bibenzyls (**141-145**), also from *Radula* species, riccardin A (**107**) from *Riccardia multifida,* and marchantins D (**115**) and E (**116**) from *Marchantia* species had calmodulin inhibitory activity (ID_{50} 2.0-95.0 μg/mL).[11] The simple bibenzyls (**146-149**) from *Radula* and *Frullania* species also showed weak calmodulin inhibitory activity (ID_{50} 100 μg/mL) as did the labdane-type diterpene diol, labda-12,14-dien-7,8-diol (**151**) (ID_{50} 82 μg/mL) isolated from *Porella perrottetiana.*[11] Perrottetin A (**139**), prenylbibenzyls (**140, 148, 40, 150)**, marchantins D (**115**) and E (**116**), and riccardin A (**107**) also inhibited 5-lipoxygenase (76-4% at 10^{-6} mol).[7] The following phenolic compounds showed significant cyclooxygenase inhibitory activity: marchantin A (**36**) (IC_{50} 46.4 μM), marchantin B (**64**) (55.9), marchantin E (**116**) (58.0), paleatin B (**117**) (45.2), perrottetin D (**140**) ((26.2), radulanin H (**145**) (39.7), isoriccardin C (**152**) (50.8), and riccardin C (**153**) (53.5).[62]



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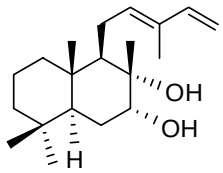

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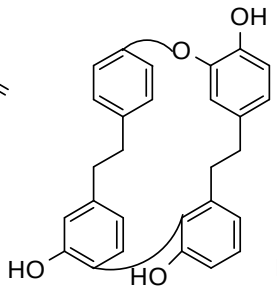

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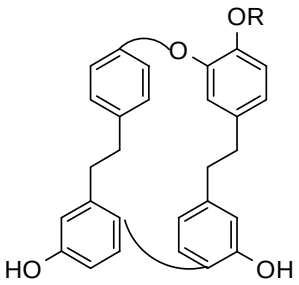
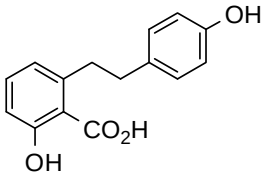


(**153**) R=H
(**154**) R=Me



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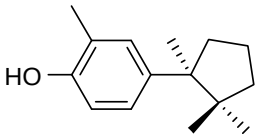

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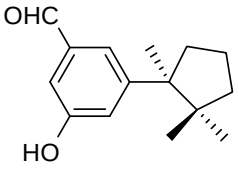

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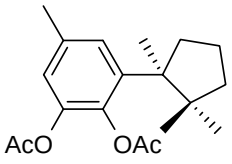

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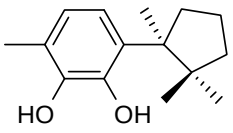

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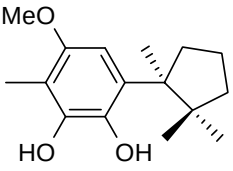

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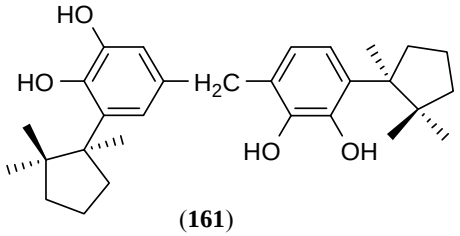

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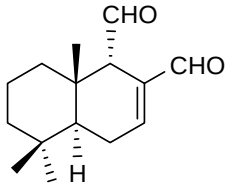

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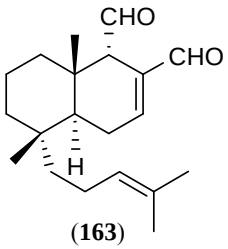

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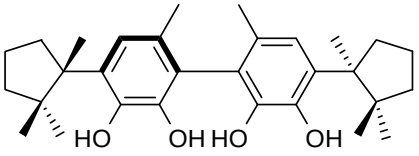

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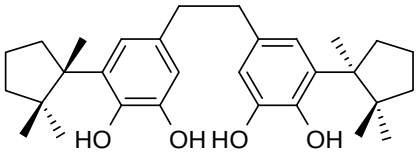

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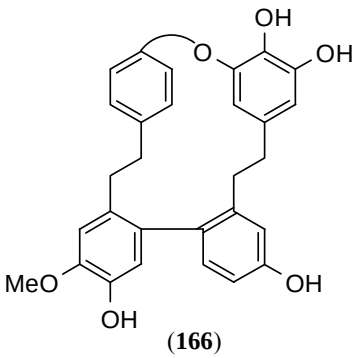


(**166**)

Chart 15. 5-Lipoxygenase, calmodulin, hyaluronidase cyclooxygenase inhibitory activity and NO production inhibitory activity

Lunularic acid (**155**) which is found in almost all liverworts as a minor component has anti-hyaluronidase activity (IC_{50} 0.13 nM). This activity is stronger than that of tranilast (*N*-3',4'-dimethoxy-cinnamoylanthranilic acid) which is an anti-allergenic agent developed in Japan for oral administration.

Lunularic acid (**155**) has been obtained from hydrangenol-β-glucoside *via* hydrangenol in good yield.[63] Perrottetin E (**109**) exhibited inhibitory activity for thrombin (IC_{50} 18 μM) which is associated with blood coagulation.[64]

Over production of nitric oxide (NO) is involved in inflammatory response-induced tissue injury and the formation of carcinogenic *N*-nitrosamines. Large amounts of NO were expressed and generated by induced iNOS on stimulation of endotoxins or cytokines involved in pathological responses. Thus inhibition of iNOS is very important to control inflammatory disease. The inhibition by macrocyclic bis-bibenzyls isolated from several liverworts of lipopolysaccharide-induced NO production in culture media on RWA 264.7 cells was tested and the IC_{50} values of each compound is reported in Table 2.[65]

Table 2. NO production inhibitory activity of bis-bibenzyls isolated from liverworts[8,65]

Compound	NO inhibition IC_{50} (μM)
Riccardin A (**107**)	2.50
Perrottetin F (**110**)	7.42
Marchantin A (**36**)	1.44
Marchantin B (**114**)	4.10
Marchantin D (**115**)	10.18
Marchantin E (**116**)	62.16
Riccardin C (**153**)	> 100
Riccardin F (**154**)	5.0
Marchantin A trimethyl ether (**111**)*	42.50
Marchantin B trimethyl ether (**112**)*	42.45

* Derivatives from marchantin A (**36**) and marchantin B (**114**)

The presence of 1-2' and 14-11' diaryl ether bonds is important for strong inhibition of NO production. The presence of phenolic hydroxyl groups also plays an important role in the inhibitory activity.

Compounds with 7,8-unsaturation dramatically decreased the inhibition of NO, while introduction of a hydroxyl group at C-7' resulted in slightly decreased activity. The methyl ether (**111, 112**) of marchantin A (**36**) and marchantin B (**114**) showed weaker activity than the original compounds. Herbertane monomers (**135, 156, 157**) and dimers **(161, 165**) and cuparenes (**159, 160**) isolated from *Mastigophora diclados* also showed inhibition of LPS-induced production of nitrite and their IC_{50} values were reported in Table 3.[66]

Table 3. NO production inhibitory activity of herbertane and its dimers and cuparenes isolated from *Mastigophora diclados*[66]

Compound	NO inhibition IC_{50} (μM)
α-Herbertenol (**19**)	76.0
Herbertenediol (**135**)	8.03
β-Herbertenol (**156**)	12.23
Herbertenal (**157**)	34
1,2-Diacetate (**158**) of **135**	7.01
Cuparenediol (**159**)	9.2
2-Hydroxy-4-methoxycuparene (**160**)	4.1
Mastigophorene C**(161**)	10.18
Mastigophorene D (**165**)	15.16
(L-N6-Iminoethyl)lysine	18.6

3.9. Piscicidal and Plant Growth Inhibitory Activity

The strongest piscicides are the pungent (-)-polygodial (**49**) from *Porella vernicosa* complex and sacculatal (**26**) from *Pellia endiviifolia, Pallavicinia levieri, Riccardia robata* var. *yakushimensis,* and *Trichocoleopsis sacculata.* Killie-fish (*Oryzia latipes*) are killed within 2 hr by 0.4 ppm solution of (**49**) and (**26**).[7,11] Sacculatal (**26**) and 1β-hydroxysacculatal (**55)** also kill killie-fish within 20 min at 1 ppm.[34] Killie-fish are also killed within 2 hr by a 0.4 ppm solution of synthetic pungent (+)-polygodial (**60**). Hence, piscicidal activity is not affected by the chirality of polygodial. Polygodial is also very toxic to fresh water bitterlings, which are killed within 3 min by a 0.4 ppm solution.[11] On the other hand, isopolygodial (**162**) from cultured cells of *Porella vernicosa* and the higher plant *Polygonum hydropiper* and isosacculatal (**163**) from *Pellia, Riccardia* and *Trichocoleopsis* species show neither piscicidal nor molluscicidal activity even at 10000 ppm.[11] Almost all crude extracts from liverworts which contain bitter or pungent substances show phytotoxic activity. (-)-Polygodial (**49**) inhibits the germination and root elongation of rice in husk at 100 ppm. At a concentration less than 25 ppm, it dramatically promotes root elongation of rice.[7,67]

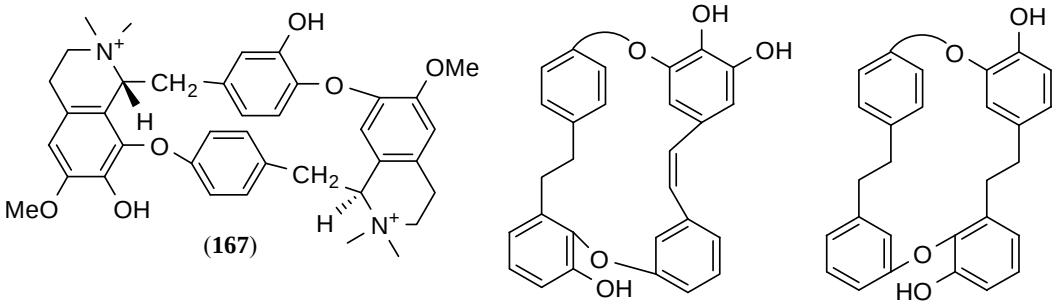
3.10. Neurotrophic Activity

Mastigophorenes A (**5**), B (**164**), and D (**165**) from *Mastigophora diclados* exhibit neurotrophic properties at 10^{-5}-10^{-7} M, greatly accelerating neuritic sprouting and network formation in the primary neuritic cell culture derived from the fetal rat hemisphere.[68] Plagiochilal B (**58**) and plagiochilide (**130**) from *Plagiochila fruticosa* show not only acceleration of neurite sprouting but also enhancement of

choline acetyl transferase activity in a neuronal cell culture of the fetal rat cerebral hemisphere at 10^{-5} M.[14] Plagiochin A (**166**) also shows the same activity at 10^{-6} M.[69] Two bitter diterpene glucosides, infuscaside A (**72**) and B (**73**) show neurite bundle formation at 10^{-7} M [Asakawa Y. unpublished results].

3.11. Muscle Relaxing Activity

Marchantin A (**36**) and the related cyclic bis-bibenzyls are structurally similar to bis-bibenzyl-isoquinoline alkaloids such as *d*-tubocurarine (**167**) which are pharmacologically important muscle relaxant active drugs. Surprisingly, marchantin A (**36**) and its trimethyl ether (**111**) also show muscle relaxing activity.[13,70] Nicotine in Ringer solution effects maximum contraction of rectus abdominus in frogs (RAF) at a concentration of 10^{-6} M. After pre-incubation of marchantin A trimethyl ether (**111**) (at a concentration of 2 x10^{-7}-2 x 10^{-4} M) in Ringer solution, nicotine (10^{-5}-10^{-4} M) was added. At a concentration of 10^{-6} M, the contraction of RAF decreased by about 30%. *d*-Tubocurarine chloride (**167**) exhibits similar effects as does (**111**) with acetyl choline.[13,70]



(**167**) (**168**) (**169**)

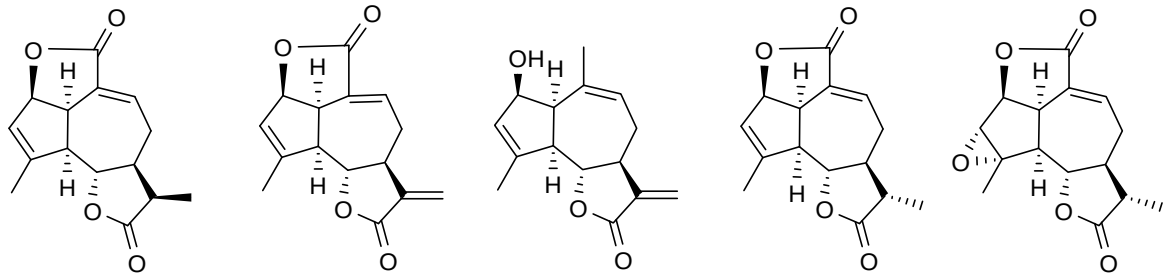
Chart 16. Muscle relaxing active *d*-tubocurarine (**167**)

Although the mechanism of action of marchantin A (**36**) and its methyl ether (**111**) in effecting muscle relaxation is still unknown, it is interesting that these cyclic bis-bibenzyls possessing no nitrogen atoms, cause concentration dependent decrease of contraction of RAF. Marchantin A and its trimethyl ether also had muscle relaxing activity *in vivo* in mice. MM2 calculations indicate that the conformation of marchantin A and its trimethyl ether and the presence of an *ortho* hydroxyl group in (**36**) and an *ortho* methoxyl group in (**111**) contribute to the muscle relaxing activity.[70] Marchantin triacetate (**113**) and 7',8'-dehydromarchantin A (**168**) and acyclic bis(bibenzyls), such as perrottetin E (**109**) and F (**110**) did not show any muscle relaxing activity.

3.12. Cathepsin L and Cathepsin B Inhibitory Activity

Cathepsin L is correlated with osteoporosis[71] and allergy.[72] We are currently searching for enzyme

inhibitors from natural products to develop chemopreventive drugs for these diseases. The marchantin series showed both cathepsins L and B inhibitory activity. Isomarchantin C (**169**) was the strongest inhibitor against both enzymes (95% for cathepsin L and 93% for cathepsin B at 10^{-5} M). Infuscaic acid (**131**) exhibited the same activity as mentioned above (63% and 32% at 10^{-5} M).



(**170**) (**171**) (**172**) (**173**) (**174**)

Chart 17.Cathepsin L and cathepsin B inhibitory activity

The crude extract of *Porella japonica* showed potent inhibition of cathepsins B and L. Biological activity guided fractionation gave three guaianolides, 11-epiporelladiolide (**170**), 11,13-dehydroporelladiolide (**171**), and porellaolide (**172**), together with porelladiolide (**173**) and its epoxide (**174**). Only compound (**171**) possessed a weak inhibitory activity against cathepsin B (13.4% at 10^{-5} M) and cathepsin L (24.7% at 10^{-5} M.[73]

3.13. Cardiotonic and Vasopressin (VP) Antagonist Activity

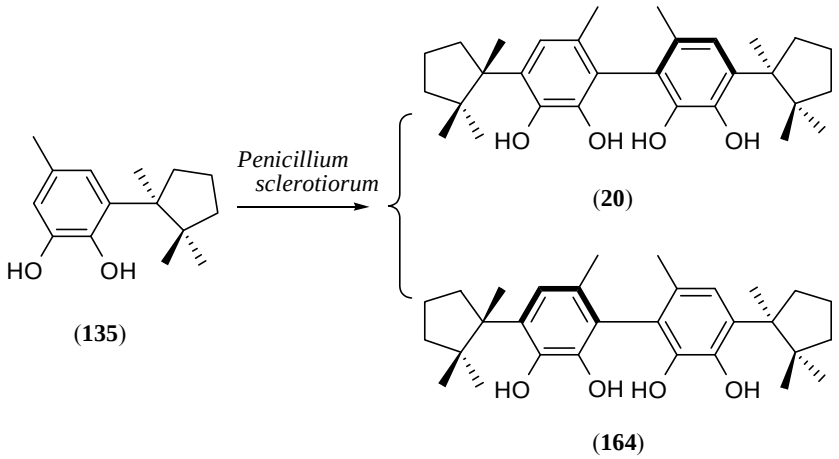
MA (**36**) shows cardiotonic activity [increase coronary blood flow (2.5ml/min. at 0.1mg)].[11] Prenyl bibenzyl (**142**) from *Radula perrottetii* indicates vasopressin antagonist activity (ID_{50} 27 μg/mL). However, 2-geranylbibenzyl (**40**) from the same liverwort did not show VP antagonist activity.[11]

3.14. Liver X-receptor (LXR)α agonist and LXRβ antagonist activity

Plasma high density lipoprotein (HDL) level is inversely related to the risk of atherosclerotic cardiovascular disease. In the research for agents that increases HDL-production, we found that riccardin C (**153**) and riccardin F (**154**) isolated from *Reboulia hemisphaerica* and *Blasia pusilla* functions as a liver X-receptor (LXR)α agonist and an (LXR)β antagonist. Riccardin C increases plasma HDL level without elevating triglyceride level in mice. This compound also enhances cholesterol efflux from THP-1 cells.[74] This compound may provide a novel tool for identifying subtype–function and drug development against obesity. From 1.25 kg of the dried liverwort *Blasia pusila*, 1 g of riccardin C (**153**) was obtained as a pure state.

3.15. Synthesis of Bioactive Compounds from Liverwort Constituents

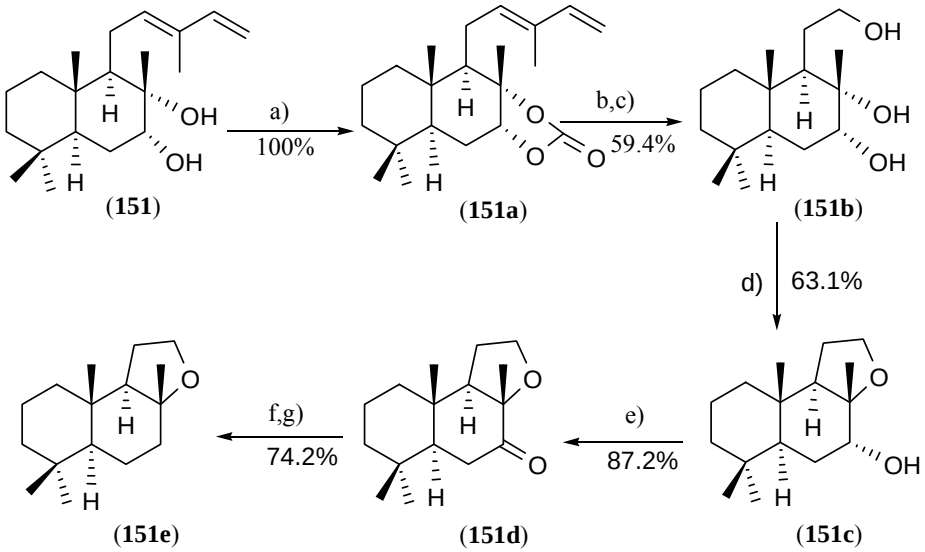
Mastigophola diclados collected in Borneo produces herbertane dimers (**20**) and (**164**) possessing neurotrophic activity. Both compounds were obtained by the biotransformation of herbertanediol (**135**) using *Penicillium sclerotiorum*.[75]



(**135**) (**20**) (**164**)

Chart 18. Synthesis of neurotrophic active mastigophorenes (**20**) and (**164**)

The large stem-leafy liverwort elaborates a large amount of labdanediol (**151**). We focused on this diterpene to transfer into ambrox (**151e**) which is an extremely expensive aroma originating from mammals. We succeeded in the hemisynthesis of this compound in seven steps.[76]



(**151**) (**151a**) (**151b**) (**151c**) (**151d**) (**151e**)

a) $MeCOCOOCCl_3$/Py/CH_2Cl_2 b) O_3/CH_2Cl_2 c) $LiAlH_4$ d) H^+/$MeNO_2$/*p*-TsOH
e) CrO_3-H_2SO_4 f) $TsNHNH_2$ g)$NaBH_3CN$

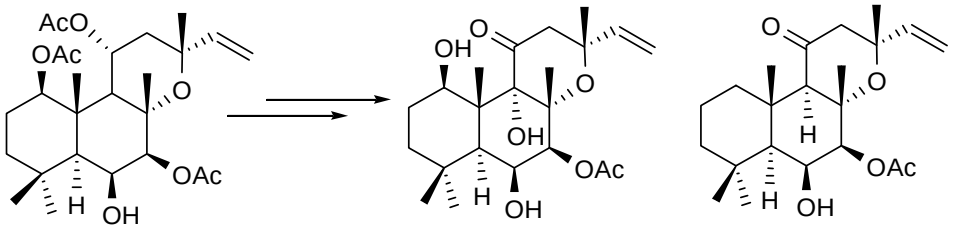
Chart 19. Chemical transformation of a labdane diterpene (**151**) from the liverwort to ambrox (**151e**)

The Indian medicinal plant, *Coleus forskolii* which has been used to treat disorders of the digestive organs, biosynthesized a highly oxygenated labdane diterpenoid, forskolin (**175a**) showing blood pressure lowering and cardio protective properties and to have therapeutic potential in glaucoma, congestive heart failure and bronchial asthma. On the other hand, very similar (**175**), to forskolin highly oxygenated labdanes, for example, folskolin (**175a**) and its congener (**175b**) were found in the Japanese liverwort *Ptychantus striatus* belonging to the Lejeuneaceae as the major component.

We also succeeded in the synthetic transformation of ptychantin A (**175**) to forskolin (**175a**) in 12 steps (12% overall yield) and 1,9-dideoxyfolskolin (**175b**) in 8 steps (37% overall yield).[77,78] The more expedient synthetic transformation to forskolin (**175a**) from ptychantins A (**175**) have been accomplished by our group.[79]

Gottsengen *et al*.[80] synthesized riccardin A-C (**108, 109, 153**) by using a combination of Ulmann, Wittig and Wurz reaction and Ni(0)-assisted intermolecular coupling reaction.

The total synthesis of riccardin B (**109**) and marchantin A (**36**) in twelve steps using the intramolecular Wadsworth-Emmons olefination and Wittig reaction was accomplished by Kodama's group.[81-83] Recently, Harrowven *et al*.[84] accomplished the shortest total synthesis of riccardin C (**153**).



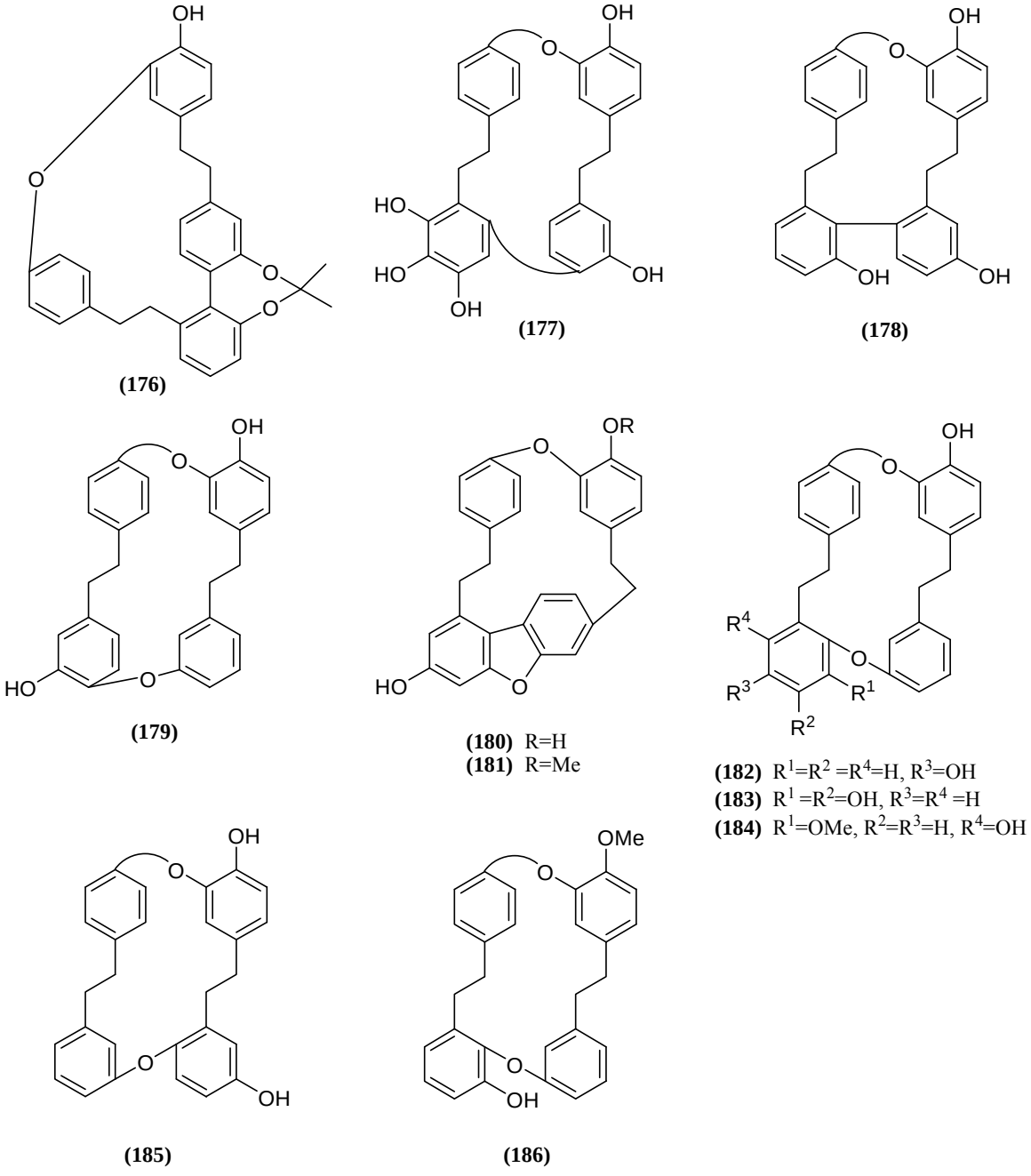
(**175**) (**175a**) (**175b**)

Chart 20. Chemical transformation of a labdane (**175**) to forskolins (**175a,b**)

3.16. Recent Reports of Antimicrobial, Cytotoxic and Insect Antifeedant Compounds from Liverworts

Bioactivity-guided fractionation of the crude ether extract of the Chinese *M. polymorpha* using TLC bioautography assay gave three new bis(bibenzyls), 13,13'-O-isopropylidenericcardin D (**176**), riccardin H (**177**) and plagiochin E (**178**), together with four known bis(benzyls), marchantin A (**36**), marchantin B (**114**), marchantin E (**116**) and neomarchantin A (**179**).[85] Significant antifungal activity against *C. albicans* was found for **178**, **179**, **176**, with relative MID values of 0.4, 0.2 and 0.25 μg/ml, respectively compared to that of 0.01 μg for positive control miconasole. Compounds (**36, 114, 116, 177**), showed the moderate activity against the same fungous at the concentration of 2.5, 4.0, 2.5 and 4.0 μg, respectively.[85]

The genus *Asterella* belongs to the Aytoniaceae and there are about 80 species in the world. Some species emits strong bad odor. The Chinese *A. angusta* produces two new bibenzofurane bis(bibenzyls), asterelin A (**180**) and asterelin B (**181**), 11-*O*-demethyl marchantin I (**182**) and dihydroptychantol A (**185**), together with known riccardin B (**17**), marchantin H (**183**), marchantin M (**184**), marchantin P (**186**), plagiochin E (**178**) and an acyclic bis(bibenzyls), perrottetin E (**109**).[86] Compounds (**109**, **180**-**186**) showed moderate antifungal activity against clinical pathogenic fungus *Candida albicans* with MIC values ranging from 16 μg/mL to 512 μg/mL.[86]



(176) **(177)** **(178)** **(179)**

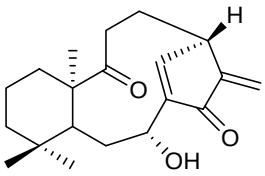
(180) R=H
(181) R=Me

(182) R^1=R^2 =R^4=H, R^3=OH
(183) R^1 =R^2=OH, R^3=R^4 =H
(184) R^1=OMe, R^2=R^3=H, R^4=OH

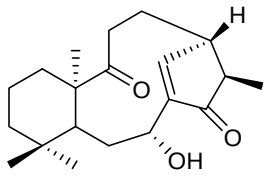
(185) **(186)**

Chart 21. Antifungal active compounds from liverworts

The New Zealand liverwort *Lepidolaena taylorii* elaborates 11-oxygenated 8,9-secokaurane diterpenoids (**187-193**) and their structurally related kaurene-15-ones (**194-199**) all of which were tested *in vitro* against mouse P388 leukemia and 60 human tumor cell lines including six leukemia cell lines and a range of organ-specific cancers. Compounds **187-189, 191-193** and **194-196, 198, 199** showed cytotoxic activity against P-388 at a concentration of 0.10-1.9 μg/mL.[87,88] *Ent*-kaurene (**124**) and (**200**) from the New Zealand *Jungermannia* species[53] showed weak cytotoxic activity against P-388 at 0.48 and 25 μg/mL, respectively. Among the isolated compounds, 8,9-secokaurenes (**187, 189, 193**) showed selective toxicity amongst human tumor cell lines at a concentration of 1.2, 2.5 and 1.5 μM, respectively. The mode action was explained by Michael acceptors which will act by alkylating cellular thiols for the α-methylene lactone, α,β-unsaturated ketone, α,β-epoxyketones or α,β-unsaturated dialdehyde like polygodial (**49**).[87,88]



(187)



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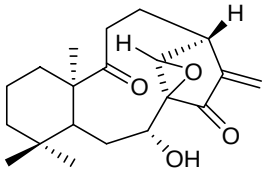
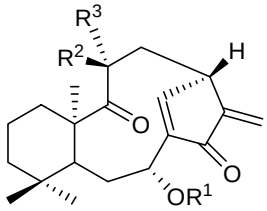
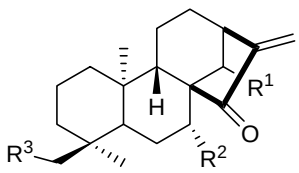


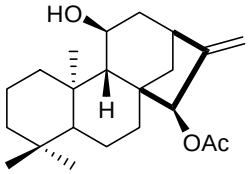
(189)



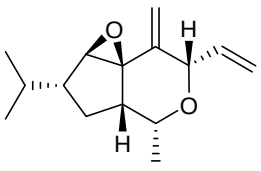
(190) R^1=Ac, R^2=R^3=H
(191) R^1=Ac, R^2=OH, R^3=H
(192) R^1=H, R^2=OAc, R^3=H
(193) R^1=H, R^2=OH, R^3=H



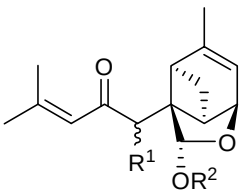
(194) R^1=R^2=OH, R^3=H
(195) R^1=R^2=R^3=H
(196) R^1=OH, R^2=OAc, R^3=H
(197) R^1=OAc, R^2=OH, R^3=H
(198) R^1= R^2=H, R^3= OH
(199) R^1=OH, R^2=R^3=H



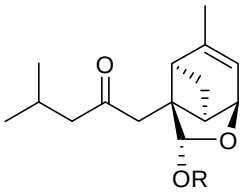
(200)



(201)



(202) R^1=OAc, R^2=Ac
(203) R^1=H, R^2 =Ac
(204) R^1=H, R^2=Me
(205) R^1=H, R^2=Et



(206) R=Ac
(207) R=Me

Chart 22. Cytotoxic and insect antifeedant active compounds from liverworts

Hodgsonox (**201**), a new class of sesquiterpene possessing cyclopenta[5,1]pyran ring system fused to an oxirane ring, was isolated with many other analogues.[89,90] Compound (**201**) exhibits toxicity (LC_{50} 0.27 mg/mL) against the larvae of the Australian green blowfly *Lucilia cuprina*. The activity of **201** was weaker than that of commercially available insecticide, diazinon (LD_{50} of 0.0016 mg/mL).[89]

The New Zealand *Lepidolaena clavigera* produces clavigerins A-C (**202, 203, 206**) and their derivatives (**204, 205, 207**) which are the artifacts formed by alcoholysis of the acetoxy acetal moiety of **203** and **206**. Compounds **203** and **206** showed insect antifeedant activities against webbing clothes moth larvae, *Tineola bisselliella* and Australian carpet beetle larvae *Anthrenocenrus australis*. Compounds **207** displayed significantly less activity against webbing cloth moss larvae than compounds **203** and **206**.[91] Compounds **202, 203, 206, 207** showed a weak cytotoxicity (30 μg/disk) against BSC cells. The insect antifeedant activity of compounds **203** and **206** might be due to their facile hydrolysis to corresponding aldehydes which act with biological nuclerophiles such as thiol or amine group. This mode of action was similar to plagiochiline A (**21**) from the liverwort, *Plagiochila* species which was easily hydrolyzed to give dialdehyde possessing 1,4-dicarbonyl group as mentioned earlier.

Recently, a number of terpenoids, aromatic compounds and acetogenins which may have some more interesting biological activities were isolated by our group.[92-112] The bioassays of several of new products are now in progress.

4. Chemosystematics

Although the morphological classification of liverworts is difficult, because of their small gametophytes, it has been demonstrated that the secondary metabolites, such as lipophilic terpenoids and aromatics in their cellular oil bodies can assist in their taxonomic differentiation. The pattern of terpenoids and aromatic compounds often depends not only on the developmental stage, season and altitudinal distribution, but also on sexual (male, female and sterile) forms of the same species and collection from different locations. Knowledge of chemical constituents of liverworts might serve to delineate not only chemical, but also evolutionary relationships within the Marchantiophyta at the genus or family level. At present, more than 1,000 species of liverworts were collected and investigated in the world.

The fresh liverworts were crushed and extracted with ether, followed by filtration through a Pasteur pipette packed with silica gel and evaporation of solvent to obtain the crude oil which was analyzed by TLC and GC/MS. The remaining crude extracts were chromatographed on silica gel or Sephadex LH-20 to isolate terpenoids and aromatic compounds and their structures elucidated by chemical and spectroscopic analyses. Many kinds of detected and isolated compounds could be used as taxonomic indicators of each species of the Marchantiophyta. Previously, the chemosystematics of 42 families, the Aneuraceae, Pelliaceae, Pallaviciniaceae, Blasilaceae, Fossombroniaceae, Hymenophytaceae and

Metzgeriaceae belonging to the order, Metzgeriales, Takakiaceae to the Takakiaceae, Haplomitriaceae to Calybryales, Jungermanniaceae, Lophocoleaceae, Gymnomitraceae (=Marsupellaceae), Arnelliaceae, Plagiochilaceae, Geocalyaceae, Acrobolbaceae, Scapaniaceae, Balantiopsilaceae, Adelantaceae, Lepidolaenaceae, Schistochilaceae, Antheliaceae, Lepidoziaceae, Calypogeiaceae, Cepharoziaceae, Isotachidaceae, Trichocoleaceae, Ptilidiaceae, Lepicoleaceae, Herbertaceae, Radulaceae, Pleuroziaceae, Porellaceae, Frullaniaceae, Jubulaceae and Lejeuneaceae to the Jungermanniales, Monocleaceae to the Monocleales, and Targioniaceae, Aytoniaceae (=Grimaldiaceae), Conocephalaceae, Lunulariaceae, Marchantiaceae and Ricciaceae to the Marchantiales were discussed.[9,10] Here we summarize the recent results of chemosystematics of several selected liverworts collected in Ecuador, Germany, Greece, Japan, and Mexico.[111]

4.1. Chemosystematics of the Jungermanniales

4.1.1. Frullaniaceae

4.1.1.1. *Frullania tamarisci* subsp. *obscura*

Frullania is a very large and complex genus with over 1000 taxa. *Frullania* species are divided chemically into five major groups: 1) sesquiterpene lactone-type, such as eudesmanolides and eremophilanolides, 2) bibenzyl-type, 3) sesquitepene lactone-bibenzyl-type, 4) aromadendorane-type and 5) monoterpene-type.[10]

Frullania tamarisci subsp. *obscura* is a rich source of sesquiterpenes, containing hinesene, β-caryophyllene, β-elemene, 4,5-di-*epi*-aristolochene, bicyclogermacrene (**208**), α-humulene and eremophilene, of which hinesene (35.5%) was the major component. This species is characterized by the presence of sesquiterpene lactones that cause allergic contact dermatitis. Significant amounts of 4-*epi*-arbusculin A (**209**) (11.8%), α-cyclocostunolide (**210**) (3.8%) and β-cyclocostunolide (**211**) (1.5%) have been detected. There are two district chemotypes of *F. tamarisci* subsp. *obscura* in Japan. The first, Type-T, contains tamariscol (**23**) which shows remarkable aroma reminiscent of woody note and powdery green note of moss and 5α,7β(H)-eudesma-4α,6α-diol as the major components, while the second, Type-O, produces eudesmane-type sesquiterpene lactones.[10] Because of the lack of the former two compounds, the present liverwort belongs to the Type O of *F. tamarisci* subsp. *obscura*, belonging to sesquiteperpene lactone-type of the *Frullania*.

4.1.2. Plagiochilaceae

4.1.2.1. *Plagiochila sciophila*

There are more than 3,000 species of *Plagiochila* which are divided mainly into two chemo-types; pungent and non-pungent species. The former type produces 2,3-secoaromadendrane-type sesquiterpene

hemiacetals like, plagiochiline A (**21**) which was degradated enzymatically to give two pungent components, plagiochilal (**58**) and furnoplagiochilal (**59**).[39] The chemical constitution of *P. sciophila* collected in Japan is totally different from that of the pungent group since it does not elaborate any plagiochiline series. Bicyclohumulenone (**44**), possessing a strong sweet mossy fragrance as mentioned earlier, β-barbatene (**212**) and (+)-cyclocolorenone (**213**) are main components occurring in the Japanese *P. sciophila*. Occurrence of a high amount of aromadendrane and humulane sesquiterpenoids in this Japanese species was previously reported.[10,14] In addition, the following constituents were also detected as minor components: 1-octen-3-yl acetate, bicyclogermacrene (**208**), cyclomyltaylane, α-barbatene, *allo*-aromadendrane, sesquisabinene A, and an unidentified sesquiterpene alcohol [M^+ 220 (59 base)] possessing a 1,1-dimethyl carbinol (*m/z* 59). The geographical and seasonal variation of the chemical constituents of the present liverwort has not been observed.

4.1.3. Radulaceae

4.1.3.1. *Radula perrottetii*

In Asia, there are ca 60 species of *Radula* which is divided taxonomically into three groups, *Radula, Cladoradula* and *Odotoradula*. Their chemical constituents are totally different from the other genera of the Marchantiophyta. Generally they contain a large amount of bibenzyls and prenyl bibenzyls. *R. perrottetii*, belonging to the subgenus *Cladoradula* is rich in heterocyclic bibenzyl derivatives.[10,14a] 2,2-Dimethyl-7,8-dihydroxy-5-(2-phenylethyl) chromene (**214**), perrottetin A (**139**) and 3,5-dihydroxy-2-(3-methyl-2-butenyl) bibenzyl (**142**) have been detected. This liverwort also produced a large amount of bisabola-2,6,11-triene (15.2%). The presence of this compound in the Japanese collection was previously confirmed by Tesso, König and Asakawa.[103] Other sesquiterpenoids, such as α- and β-chamigrene, eremophilene, cuparene, β-acoradiene, aristolene, thujopsene, isodauca-4,7(14)-diene and α-gurjunene have also been detected as minor components. Additionally, in the investigated Japanese material, as well as in the above-mentioned *Plagiochila sciophila*, bicyclohumulenone (**44**), cyclocolorenone (**213**) and β-barbatene (**212**) have been identified.

4.1.4. Lejeuneaceae

4.1.4.1. *Trocholejeunea sandvicensis*

There are more than 1,000 species of the Lejeuneaceae in the world. Almost all of the species belonging to the Lejeuneaceae is tiny except for *Ptychantus* species and their chemical constituents are very complex. One of the typical groups of this family is the genera which produce pinguisane-type sesquiterpenoids. *T. sandvicensis* is one of this groups to produce a large amount of pinguisane-type sesquiterpenoids, which are the most significant chemical markers of this species.[10] There are two

chemotypes of *T. sandvicensis*: type-1 contains pingusanin (**16**) and the other one produces dehydropinguisanin (**216**) as the major components. *T. sandvicensis* collected in Tokushima, Japan contains dehydropinguisenol (**215**) (39%) and an unidentified sesquiterpene lactone [M^+ 234 (124 base)] (10%) as the main component, and also pinguisenene, dehydropinguisanin (**216**), deoxopinguisone (**217**), ptychanolide (**218**) and *ent*-dihydrodiplophyllin. The present species also produced sesquiterpene hydrocarbons, such as bicyclogermacrene (**208**), isogermacrene D, β-caryophyllene, β-barbatene (**212**) and valencene.

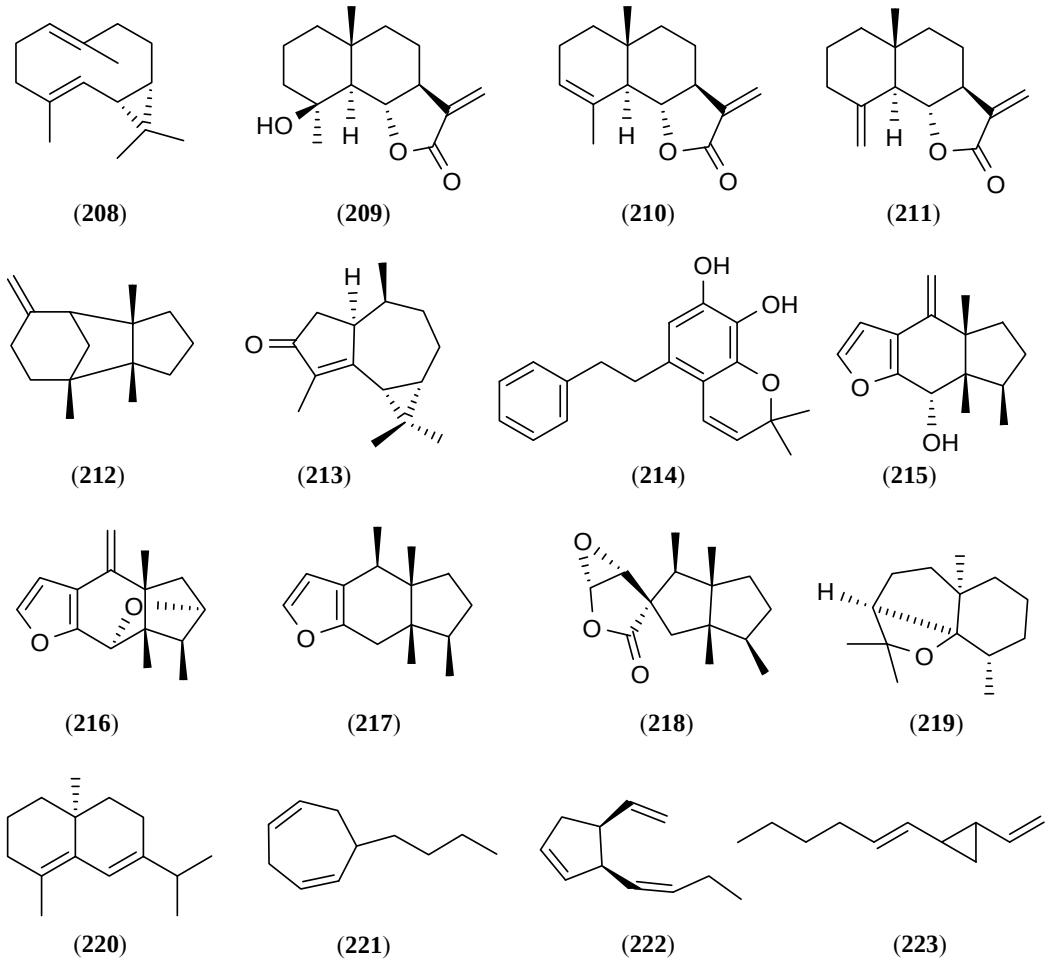


Chart 23. Chemical markers of some liverworts

4.2. Chemosystematics of the Metzgeriales

4.2.1. Pelliaceae

4.2.1.1. *Pellia endiviifolia* (Figure 5)

The Japanese *P. endiviifolia* possesses a persistent pungent taste that is due to the sacculatane-type diterpene dialdehyde. There are sacculatal (**26**) – the major component [11] and 1β-hydroxysacculatal (**55**)[34] – the minor one. In addition, the non-pungent isosacculatal, the C-9 isomer has been detected. In German

P. endiviifolia we also identified sacculatal as the main compound and two other sacculatanes, sacculatanolide and 7,17-sacculatadien-11,12-olide. The latter two compounds were isolated previously from axenic cultures of the liverwort *Fossombronia wondraczekii*, which is classified into the Fossombroniaceae (Metzgeriales).[113] Thus, *P. endiviifolia* is closely related chemically to *Fossombronia*. The Japanese and German collections also produced a large amount of bicyclogermacrene (**208**). The two collections differ from one another in the composition of the sesquiterpenes fraction. In Japanese material, β-acoradiene and (*E*)-β-farnesene have been identified, while in German samples, *allo*-aromadendrene, tritomarene, bourbon-11-ene, selina-4,7-diene and β-cubebene were detected.

Figure 5. *Pellia endiviifolia*

Figure 6. *Noteroclada confluens*

4.2.1.2. *Pellia epiphylla*

This German *Pellia epiphylla* is chemically similar to *P. endiviifolia* since it contains four sacculatane diterpenoids, sacculatal (**26**) (32.6%), isosacculatal (33.0%), sacculatanolide (1.3%) and its isomer 7,17-sacculatadien-11,12-olide (0.2%); these last two differ in the orientation of the γ-lactone moiety. Minor components include 1-octen-3-yl acetate, β-acoradiene, (*E*)-β-farnezene, zizaene, dioctyl ether which have been found in the other liverworts.

4.2.1.3. *Makinoa crispata*

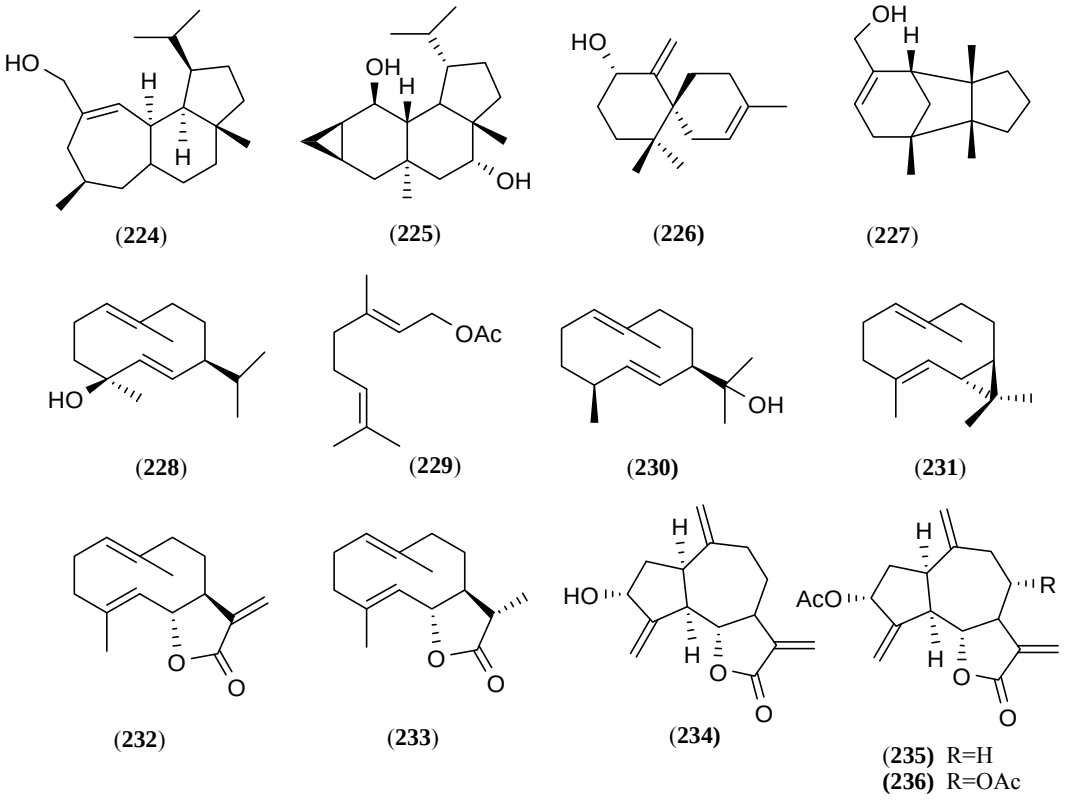
There are at least three chemotypes of *M. crispata*.[10] The present species collected in Japan belongs to the second chemotype, because this type produces dactylol and bicyclogermacrene (**208**) as major compounds. It is noteworthy that this species elaborated non-pungent sacculatane type diterpene dialdehyde, perrottetianal A (**137**) (7.5%), which is a characteristic compound of the first chemotype of *M. crispata*. A significant amount of β-barbatene (**212**) has also been detected. Thus, chemically, *M. crispata* is closely related to *Pellia endiviifolia*. In the volatile fraction of this liverwort many other minor compounds have been identified, for example α-himachalene, α-cubebene, α-copaene, β-bazzanene,

α-longipinene, isobazzanene, *cis*-calamenene, and cinnamolide which is also significant chemical marker of *Makinoa.* Previously a chlorine-containing drimane (**12**) as shown in Chart 2 was isolated from this species.[10]

4.2.1.4. *Noteroclada confluens* (Figure 6)

Noteroclada is classified in the order Metzgeriales and is a member of the family Pelliaceae within that order. Unlike *Pellia* and *Makinoa, Noteroclada* has a leafy appearance. The major component detected in this Ecuadorian species is an unknown sesquiterpene alcohol [M^+ 222 (108 base)] (49%). It produced also a large amount of bicyclogermacrene (**208**) and an unidentified bibenzyl derivative [M^+ 344 (249 base)]. Additionally, brasila-1,10-diene, brasila-5(10),6-diene, dactylol, sativene, α-isocomene, pacifigorgia-6,10-diene, pacifigorgia-1(6),10-diene, and another two unidentified compounds [M^+ 330 (139 base); M^+ 314 (base)] were detected.

It is noteworthy that *N. confluens* produces brasilane- and gorgonane-type sesquiterpenoids which have been found in *Conocephalum* belonging to the Marchantiales and *Frullania* species to the Jungermanniales, respectively.[10]



(**224**) (**225**) (**226**) (**227**)

(**228**) (**229**) (**230**) (**231**)

(**232**) (**233**) (**234**)

(**235**) R=H
(**236**) R=OAc

Chart 24. (continued) Chemical markers of some liverworts

4.2.2. Pallaviciniaceae

4.2.2.1. *Symphyogyna brasiliensis*

Previously, we placed this species in the Hymenophytaceae, however, it should be replaced in the Pallaviciniaceae.[114] The Pallaviciniaceae has thee genera, *Pallavicinia*, *Symphogyna* and *Allisonia* in New Zealand.[115] The Ecuadorian *S. brasiliensis* contains dihydroagarofurane (**219**) (36.3%) and δ-selinene (**220**) (20.6%) as the main compounds. Besides **220**, this species also produced other eudesmanes: cascarilladiene, selina-4,7-diene and eudesma-5,7(11)-diene. Bicyclogermacrene (**208**), thujopsene, calarene, γ- and δ-cuprenene, β-cubebene, bourbon-7(11)-ene and *trans*-dauca-4(11),7-diene were also present. The Venezuelan *S. brasiliensis* contains a unique labdane, symphyogynolide (**27**),[10] however it was not detected in the present Ecuadorian specimen.

4.2.3. Fossombroniaceae (Codoniaceae)

4.2.3.1. *Fossombronia angulosa*

The most interesting species belonging to the Metzgeriales order is *F. angulosa*. The species collected in Greece contained three compounds previously reported in brown algae.[116] These are dictyotene (**221**), the main compound of the volatile fraction (14.1%), (*Z*)-multifidene (**222**) and dictyopterene (**223**). When the species belonging to the Metzgeriales are dried, they immediately emit a sea-shore algae odor. The genuine component of this odor is dimethyl sulfide, which is the most important odor of almost all algal species. This may suggest that some liverworts originate from algae.[14] The marine algal components in *Fossombronia* species are extremely interesting since the species is morphologically quite similar to marine algae, for example *Ulva* species.

Besides dictyotene, *F. angulosa* also produced a large amount of an unknown compound [M^+ 300 (91 base)] (13.4%), 2-tridecanone (13.0%) and β-sabinene (11.7%). This species also biosynthesized cyathane-type diterpenoids; 5,18-dihydroxy-*epi*-homo-verrucosane (**224**) and (-)-2β,9α-dihydro-xyverrucosane (**225**) which are distributed in some Jungermanniales liverworts (see Table 3). This is the second record of such compounds in Metzgeriales; previously cyathane diterpenoids were isolated from the Arctic liverwort *F. alascana*.[117]

Some species belonging to the Metzgeriales biosynthesize the same or the similar specific terpenoids, such as drimanes, pinguisanes, sacculatanes or cyathanes and bis(bibenzyls) to those found in the Jungermanniales as shown in Table 4. In the modern classification of the Marchantiophyta the Jungermanniales and the Metzgeriales are united within the subclass Jungermannidae.[118-120] The chemical evidence that both *F. angulosa* and the *Jungermannia* genera produce typical cyatane diterpenoids supports the above classification.

Table 4. The distribution of specific sesqui- and diterpenoids and bis(bibenzyls) in liverworts

Order / Genus	Drimanes	Pinguisanes	Sacculatanes	Cyathanes	Bis(bibenzyls)
METZGERIALES					
Aneurea		+			
Riccardia			+		+
Makinoa	+		+		
Pellia			+		+
Pallavicinia			+		
Blasia					+
Fossombronia			+	+	
JUNGERMANNIALES					
Bazzania	+				+
Ptychantus		+			+
Porella	+	+	+		
Trichocoleopsis		+	+		
Ptilidium	+	+			
Mylia				+	
Jamesoniella				+	
Herbertus			+		+
Plagiochila		+			+
Frullania					+
Schistochila					+
Lepidolaena		+	+		

4.3. Chemosystematics of Marchantiales

4.3.1. Aytoniaceae

4.3.1.1. *Reboulia hemisphaerica*

R. hemisphaerica has several chemotypes.[109] The Japanese *R. hemisphaerica*, collected in two different places in Tokushima, is characterized by totally different chemical compositions. The collection from Tokushima Bunri University Campus produced cyclomyltaylane and chamigrane sesquiterpenoids. Cyclomyltaylan-5α-ol (**18**) (43.5%) was the major component, which was present with β-chamigrenes, β-chamigrene-1β-ol and β-chamigrene-10α-ol (**226**). This collection belongs to the cyclomyltaylane-type. It is noteworthy that the separately investigated sporophytes and thalli of this species are characterized by very similar chemical composition. The second collection, from Momijigawa (Aioicho, Tokushima), 50km apart from the University campus contained mainly gymnomitranes, of which (+)-gymnomitr-8(12)-en-9α-ol (**47**) (31.3%) was the main compound. The other characteristic components were β-barbatene (**212**) (14.7%), gymnomitr-8-en-12-ol (**227**), gymnomitr-8(12)-en-9-one,

(8*R*)-(+)-gymnomitran-9-one and gymnomitrol. This collection also produced a significant amount of β-caryophyllene (16.8%).

4.3.1.2. *Asterella echinella* (Figure 7)

Reboulia and *Asterella* are both members of the Reboulioideae, subfamily Aytoniaceae. *Asterella* species are known to emit intense, characteristic scents, both pleasant and unpleasant.[121] The Mexican *A. echinella* produces mainly sesquiterpenes. It elaborated the sesquiterpene hydrocarbons: β-barbatene (**212**) (43%), β-caryophyllene (7.6%), β-acoradiene (2.9%), α-barbatene, isobazzanene, and δ-cuprenene, and also significant amounts of the sesquiterpene alcohols 4β-hydroxygermacra-1(10),5-diene (**228**) (4.2%), β-sabinene (3.5%), 3,7,11-trimethyl-2,6,10-dodecatrien-1-ol (5.3%) and its acetate (4.9%). The present species is chemically different from *A. venosa* since the major constituents of *A. venosa* are monoterpenoids.

Figure 7. *Asterella echinella*

Figure 8. *Asterella venosa*

4.3.1.3. *Asterella venosa* (Figure 8)

As mentioned above, monoterpenes were the most representative in *A. venosa* (77%). The main compound was geranyl acetate (**229**) (40%), together with β-myrcene (13.9%), α-pinene (9.7%) and myrtenyl acetate (9.2%). Besides monoterpenoids, β-barbatene (**212**), β-caryophyllene and squalene have been identified. This Mexican species is closely related to the Portuguese *A. africana*. Chemical analysis of the essential oils from this species indicated that they were dominated by the monoterpene fraction. Myrtenyl acetate and α-pinene were the major components of all the oil samples.[122]

4.3.2. Conocephalaceae

4.3.2.1. *Conocephalum conicum*

The Japanese *C. conicum* is chemically more advanced than *C. japonicum*, since the former elaborates not only monocyclic but also bicyclic monoterpenoids.[10] There are three chemo-types of *Conocephalum conicum*. The types 1, 2 and 3 emit (-)-sabinene, (+)-bornyl acetate, and methyl cinnamate as the major components, respectively, which are responsible for the characteristic odor of each type.[22] In *C. conicum* collected in Momijigawa, Tokushima, myrcene, limonene, neryl acetate, camphene, β-sabinene and β-pinene were identified. This *C. conicum*, like the German collection, belongs to the β-sabinene chemotype, because it also produced a large amount of this compound (38.4%).[10,22] β-Elemene, β-caryophyllene, germacrene D, cubebol and *ent*-1(10)*E*,5*E*-germacradien-11-ol **(230)** have also been identified.

4.3.2.2. *Conocephalum japonicum*

The Japanese *C. japonicum* produced only limonene, as a minor monoterpene metabolite. The major component is isolepidozene (**231**) (49%), the diastereomer of the widespread sesquiterpene hydrocarbon, bicyclogermacrene (**208**). In addition, β-sabinene (31.7%) and isolepidozene (**231**) (14.8%) are also the significant components of the German *C. conicum*. Bicyclogermacrene (**208**) and another two germacranes: bicyclogermacrene-14-al (**134**) and 4β-hydroxygermacra-1(10),5-diene (**228**) as well as costunolide (**232**) and dihydrocostunolide (**233**) and β-cyclocostunolide (**211**) have been detected as minor components. We reported that *C. japonicum* is chemically different from the Japanese *C. conicum* and rather similar to *Wiesnerella denudata* since *C. conicum* does not produce germacrane-type sesquiterpene lactones, while *W. denudata* elaborates the same lactones as those found in *C. japonicum.*[10] It is noteworthy that the German *C. conicum* contains the same monoterpene hydrocarbon, β-sabinene and isolepidozene (**231**) like *C. japonicum*.

4.3.2.3. *Wiesnerella denudata*

There are three different chemical races of *W. denudata*, the costunolide-guaianolide-type, the costunolide-type and the guaianolide-type.[123] Guaianolides, for example zaluzanin C (**234**), zaluzanin D (**235**) and 8α-acetoxyzaluzanin D (**236**), alongside a large amount of neryl acetate (40.7%), have been detected with tulipinolide (**53**), dihydrotulipinolie (**237**) and its cyclized β-cyclocostunolide (**211**) in the Japanese *W. denudata*. The present species belongs to the costunolide-guaianolide chemo-type. In addition, Δ^3-carene, 1-octen-3-yl acetate, nerol, 4β-hydroxygermacra-1(10),5-diene (**228**), together with two unidentified sesquiterpenoids [M^+ 230 (105 base); M^+ 230 (107 base)] were also detected as minor components. The presence of tulipinolide and guaianolides in *W. denudata* indicates that this liverwort is

more evolved chemically that *C. japonicum* since the latter species produces neither C-8 acetoxylated costunolide nor guaianolides.[10]

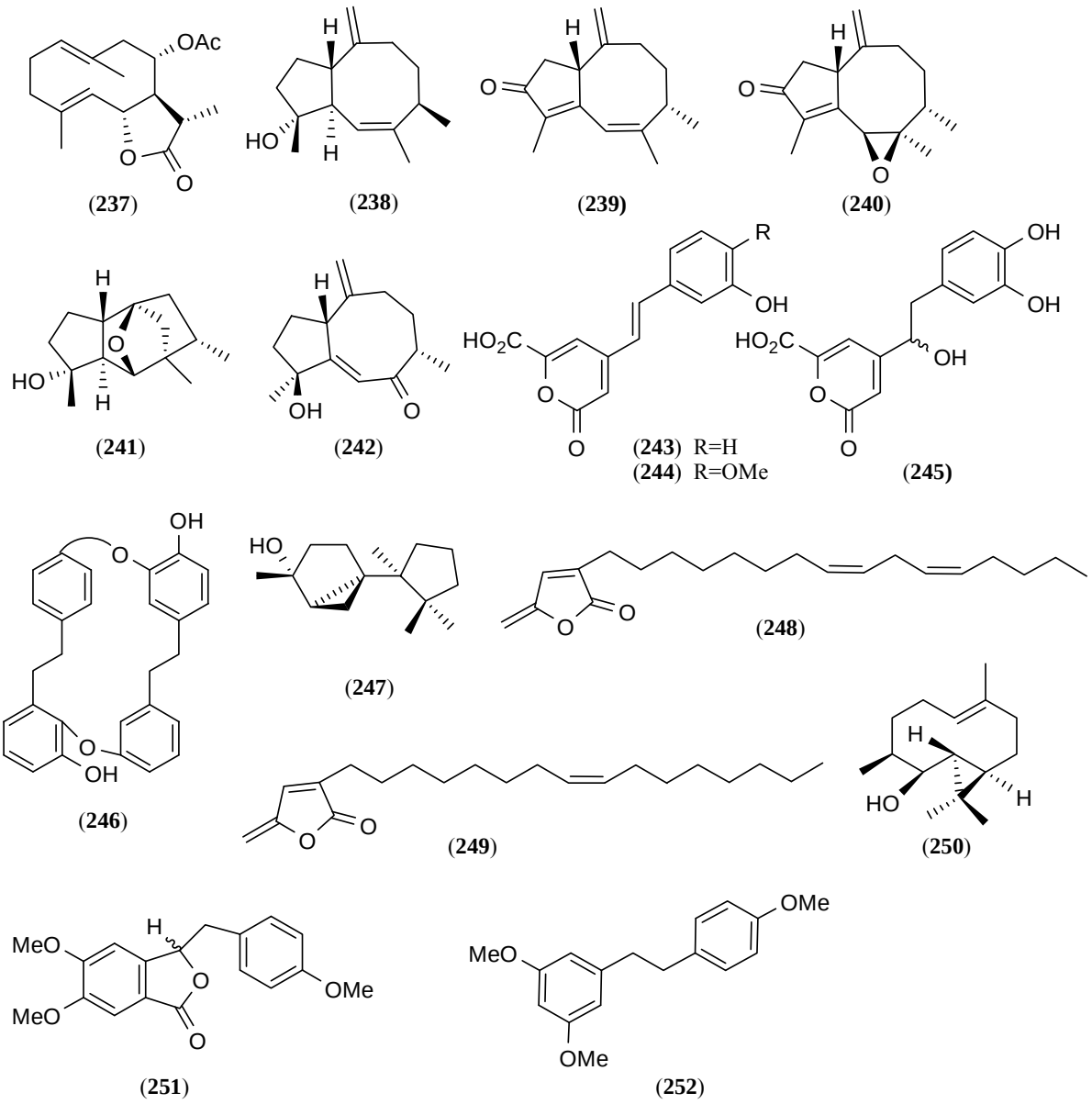


Chart 25. (continued) Chemical markers of some liverworts

4.3.3. Marchantiaceae

4.3.3.1. *Dumortiera hirsuta* (Figure 9)

There are a few chemo-types of *Dumortiera hirsuta*. The Argentinean *D. hirsut*a elaborates three dumortane sesquiterpenoids (**238-240**), a rearranged dumortane (**241**) and a nordumortane (**242**), along with marchantin C (**246**).[10] The Ecuadorian *D. hirsuta* elaborates the peculiar α-pyrone derivatives, dumortins A-C (**243-245**).[124] The Japanese and Argentinean species do not produce such aromatic

products. The major component detected in the Japanese *D. hirsuta* is an unknown sesquiterpenoid [M^+ 220 (43 base)] (33%). This species elaborates germacrane type sesquiterpenoids, especially *ent*-1(10)*E*,5*E*-germacradien-11-ol (**230**) (13.8%), germacrene D (8.7%) and 4β-hydroxygermacra-1(10),5-diene (**228**) (3.6%). These compounds were also detected in *Conocephalum conicum*; the first and second in the Japanese collection and the third in the German liverwort. Minor components include 1-octen-3-yl acetate, β- and δ-elemene, β-barbatene (**212**), γ-muurolene, (*Z*,*E*)-α-farnesene and cubebol.

4.3.3.2. *Marchantia polymorpha* (Figure 10)

The GC-MS pattern of the ether extract of *M. polymorpha* which were collected in Hatacho, Tokushima and Toyama in Japan is almost the same as that of the Polish specimen collected in Wroclaw.

Figure 9. *Dumortiera hirsuta*

Figure 10. *Marchantia polymorpha*

(–)-Cyclopropanecuparenol (**247**) is the main component identified in the Japanese *M. polymorpha* thalli and female sporophytes. This liverwort also produces other cuparane as well as thujopsane and chamigrane sesquiterpenoids. α-Cuprenene and also δ- and β-cuprenene, thujopsene, thujopsane-2β-ol, thujopsenone, α-chamigrene, *ent*-9-oxo-α-chami-grene have been identified. The sesquiterpenoid composition of *M. polymorpha* thalli was quite similar to that of female sporophytes. In contrast to thalli, female sporophytes elaborated hexadecanoic acid and its ethyl ester, (*Z*,*Z*) 9,12-octadecadienoic acid, oleic acid and also oxacycloheptadeca-2-one.

4.3.3.3. *Marchantia paleacea* var. *diptera*

The Japanese *M. paleacea* var. *diptera* showed at least 27 peaks on the gas chromatogram of the diethyl ether extract of among which β-caryophyllene (36.5%) and an unidentified diterpenoid [M^+ 290 (81

base)] (15.3%) were the most abundant components. Additionally, two monoterpenes, β-pinene and limonene, 1-octen-3-yl acetate, β-elemene, calarene, β-selinene and β-caryophyllene oxide have been identified. Fatty acids and long chain butenolides (**248, 249**) which are characteristic secondary metabolite of this species [10] have also been detected. All terpenoids detected are ubiquitous component not only in liverworts but also in higher plants, however, the presence of a great amount of marchantin-type macrocyclic compounds, for example, marchantin A (**36**) is the chemical marker of this species and *M. polymorpha.*[10]

4.3.3.4. *Marchantia tosana*

There are two chemotypes of *M. tosana.* Type 1 is closely related chemically to *M. polymorpha* and *M. paleaceae* var. *diptera* since these three *Marchantia* species produce marchantin type macrocyclic compounds with 2,5-dimethoxy-3-hydroxyphenthrene and type 2 produces 2,7-dimethoxy-3-hydroxyphenanthrene derivatives.[10] The present specimen collected in Momijigawa, Tokushima produced large amounts of isolepidozene (**231**) (45.2%) and β-barbatene (**212**) (23.1%). It is noteworthy that isolepidozene has been found as a predominant component in *Conocephalum japonicum* and the German *C. conicum* belonging to the Conocephalaceae as mentioned earlier. *M. tosana* also contained β-elemene, α-barbatene, isobazzanene, β-acoradiene, germacrene D, α- and γ-cuprenene and (4S*,5S*,6R*,7R*)-1(10)*E*-lepidozen-5-ol (**250**) as minor constituents. The present species contains marchantin A, thus it is classified as type 1.

4.3.4. Monosoleniaceae

4.3.4.1. *Monosolenium tenerum*

M. tenerum was studied chemically first time. In the German *M. tenerum* only two components have been detected in GC-MS. Analysis of MS suggested that the first one was a new bibenzyl derivative, 3,5,4'-trimethoxybibenzyl (**251**) and the second one a phthalide, identified as 3-(4'-methoxybenzyl)-5,6-dimethoxyphthalide (**252**). The latter compound was isolated previously from the Australian liverwort *Frullania falciloba*, belonging to the Jungermanniales.[125] The Chinese specimen was also analyzed by GC-MS. The GC-MS pattern was completely identical to that of the German collection.

The chemical constitution of *M. tenerum* is absolutely different from that of all Marchantiales species so far investigated chemically. It is quite interesting to note that the present species is closely related chemically to the *Frullania* (Jungermannales) chemotype II, which produce bibenzyls as the major product.

The volatiles of the liverwort species are characterized by a large number of sesquiterpenoids, as shown

in Table 5. Several acyclic, mono-, bi-, and tricyclic sesquiterpene systems are present, including many unique combinations. The investigated liverworts produced mainly sesquiterpene hydrocarbons, with bicyclogermacrene (**208**) and β-barbatene (**212**) being the most widespread in the present and previously analyzed species. Because sesquiterpene hydrocarbons are prevalent throughout liverworts and most liverwort species elaborate the same compounds, it is difficult to use these compounds as chemosystematic markers. However, other sesquiterpenoids, such as eudesmane, eremophilane, germacrane and guaiane sesquiterpene lactones, pinguisane, cuparane and gymnomitrane sesqui- and sacculatane and cyatane diterpenoids can be used as chemosystematic markers.

Table 5. The distribution of volatile components of the investigated liverwort species[111]

	Mono-terpenoids	Sesqui-terpenoids	Diterpenoids	Aromatics	Acetogenins
Metzgeriales					
*Pellia endiviifolia**		+	+		+
*Pellia endiviifolia***		+	+		+
Pellia epiphylla		+	+		+
Makinoa crispata		+	+		
Symphyogyna brasiliensis		+		+	
Noteroclada confluens		+		+	
Fossombronia angulosa		+	+		+
Jungermanniales					
Plagiochila sciophila		+			+
Frullania tamarisci subsp. *obscura*		+			
Radula perrottetii		+		+	+
Trocholejeunea sandvicensis		+			
Marchantiales					
Reboulia hemisphaerica		+			+
Asterella echinella	+	+			+
Asterella venosa	+	+			
*Conocephalum conicum**	+	+			
*Conocephalum conicum***	+	+			+
Conocephalum japonicum	+	+			+
Wiesnerella denudata	+	+			
Dumortiera hirsuta		+			+
Marchantia polymorpha		+			+[#]
Marchantia tosana		+			+
Marchantia paleacea var. *diptera*		+			+
Monoselenium tenerum	+			+	

* species collected in Japan; ** species collected in Germany; [#] only in sporophytes

Some specific monoterpenoids are also used as chemical markers. Monoterpenes amounted to 77% of all compounds present in the volatiles of *Asterella venosa*. The main component was geranyl acetate (**229**), a compound with a lemon-like odor. In contrast to the above mentioned species, which have a pleasant smell, *Asterella* species grown in Malaysia emit an intense, unpleasant odor, which is due to skatole, which composes about 20% of the total extract.[14a]

In several cases, aromatic compounds are valuable chemosystematic indicators, especially bibenzyls and bis(bibenzyl) derivatives.[10,14a] Among investigated liverworts, the presence of bibenzyls and prenyl bibenzyls (**139, 142, 214**) are characteristic of the Japanese *Radula perrottetii* (Jungermanniales). It is noteworthy that the German and Chinese *Monoselenium tenerum*, belonging to the Marchantiales, produced bibenzyls and phthalides, previously detected in the Jungermanniales.[125]

CONCLUSION

The bryophytes, in particular, the Marchantiophyta (liverworts) are charming plants as the sources of bioactive substances. Most of liverworts produce lipophilic terpenoids (mono-, sesqui-and diterpenoids) and aromatic compounds, of which only a few nitrogen or sulfur containing compounds have been found.[7,14a,e] It is noteworthy that ca, 80% of the sesqui- and diterpenoids found in liverworts are the enantiomers of those found in higher plants. Since the last century, 5% of the total bryophytes have been studied chemically. Although liverworts are tiny plant groups, they produce many peculiar terpenoids, such as homomonoterpenoids, 1,4-dimethylazulenenoids, sesquiterpenoids: africane, brasilane, drimane, eremophilane, eudesmane, germacrane, guaiane, gymnomitrane (=barbatane), herbertane, myltaylane, cyclomyltaylane, pacifigorgiane, pinguisane, 2,3-secoaromadendrane, striatane, trifarane and diterpenoids: cyatane, dolabellane, fusicoccane, sacculatane and aromatic compounds: bibenzyl, bi(bibenzyls), naphthalene, 4-mehylstyrenethiocyanate, β-methylthioacrylate etc, all of which are significant chemical markers of each liverwort genus and family.

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