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Biologically active compounds from bryophytes*

Yoshinori Asakawa

Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Yamashiro-cho, Tokushima 770-8514, Japan

Abstract: Liverworts produce a great variety of lipophilic terpenoids, aromatic compounds, and acetogenins. Many of these constituents have characteristic scents, pungency, and bitterness, and display a quite extraordinary array of bioactivities and medicinal properties. These expressions of biological activity are summarized and discussed, and examples are given of the potential of certain lead compounds for structure–activity studies and synthesis.

Keywords: liverworts; lipophilic terpenoids; acetogenins; bryophytes; cytotoxic.

INTRODUCTION

The bryophytes are placed taxonomically between algae and pteridophytes; there are about 24 000 species in the world. They are further divided into three classes, Musci (mosses, 14 000 species), Hepaticae (liverworts, 6000 species) and Anthocerotae (hornworts, 300 species). The Hepaticae contain cellular oil bodies which are easily extracted with organic solvents, while the other two classes do not. A number of bryophytes (in particular, mosses) have widely been used as medicinal plants in China, to cure burns, bruises, external wounds, etc. [1–5]. The mosses and liverworts shown in Table 1 are medicinal plants and are said to possess certain biological activity and effect [1–5]. Some bryophytes show characteristic fragrant odors and an intense hot and bitter or saccharine-like taste. Generally, bryophytes are not damaged by insects, snails, slugs, and other small animals. 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 liverworts collected in North and South Africa, North and South America, Argentina, Australia, Europe, French Polynesia, India, Japan, Madagascar, Nepal, New Zealand, Pakistan, and Taiwan, with respect to their chemistry, pharmacology, and application as sources of cosmetics, and medicinal or agricultural drugs. It has been demonstrated that most of the Hepaticae contain mainly lipophilic mono-, sesqui-, and diterpenoids, aromatic compounds (bibenzyls, bis-bibenzyls, benzoates, cinnamates, long-chain alkyl phenols, naphthalenes, phthalides, isocoumarins), and acetogenins which constitute the oil bodies. The biological activities of liverworts are due to these substances [6–10]. At present, over 400 new compounds have been isolated and their structures elucidated [6,10]. 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 nitric oxide (NO) production inhibitory activity;

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‡E-mail: asakawa@ph.bunri-u.ac.jp

(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; (14) antiobesity activity; and (15) synthesis of bioactive compounds from liverwort constituents will be discussed.

Table 1 Medicinal bryophytes and their biological activity and effects [1–11a].

Musci	
Bryum argenteum	Antidotal, antipyretic, antirhinitic activity; for bacteriosis
Cratoneuron filicinum	For malum cordis (heart disease)
Ditrichum pallidum	For convulsions, particularly in infants
Fissidens japonicum	Diuretic activity; for growth of hair, burns, and choloplania (jaundice, icterus)
Funaria hygrometrica	For hemostatis, pulmonary tuberculosis, vomitus cruentus (hematemesis), bruises, and athlete's foot dermatophytosis (dermatomycosis, dermomycosis)
Haplocladium catillatum	Antidotal and antipyretic activity; for adenopharyngitis, pharyngitis, uropathy, mastitis, erysipelas (rose), pneumonia, urocystitis, and tympanitis
Leptodictyum riparium	Antipyretic; for choloplania and uropathy
Mnium cuspidatum	For hematostasis and nosebleed
Oreas martiana	For anodyne (pain), hemostasis, external wounds, epilepsy, menorrhagia, and neurasthenia (nervosism, nervous exhaustion)
Philonotis fontana	Antipyretic and antidotal activity; for adenopharyngitis
Plagiopus oederi	As a sedative; for epilepsy, apoplexy, and cardiopathy
Polytrichum species	Diuretic activity; for growth of hair
Polytrichum commune	Antipyretic and antidotal; for hemostasis, cuts, bleeding from gingivae, hematemesis, and pulmonary tuberculosis
Rhodobryum giganteum	Antipyretic, diuretic, and antihypertensive; for sedation, neurasthenia, psychosis, cuts, cardiopathy, and expansion of heart blood vessels
Rhodobryum roseum	As a sedative; for neurasthenia and cardiopathy
Taxiphyllum taxirameum	Antiphlogistic; for hemostasis and external wounds
Weissia viridula	Antipyretic and antidotal; for rhinitis
Hepaticae	
Conocephalum conicum	Antimicrobial, antifungal, antipyretic, antidotal activity; used to cure cuts, burns, scalds, fractures, swollen tissue, poisonous snake bites, and gallstones
Frullania tamarisci	Antiseptic activity
Marchantia polymorpha	Antipyretic, antihepatic, antidotal, diuretic activity; used to cure cuts, fractures, poisonous snake bites, burns, scalds, and open wounds
Reboulia hemisphaerica	For blotches, hemostasis, external wounds, and bruises

BIOLOGICAL ACTIVITY

Characteristic scents

Liverworts emit volatile terpenoids or simple aromatic compounds when crushed which are responsible for intense sweet-woody, intense turpentine, sweet-mossy, fungal-like, carrot-like, mushroomy, or seaweed-like scents [7,11a,b]. Table 2 lists some liverworts possessing such characteristic odors [7,11a,b].

Table 2 Characteristic odor of liverworts [7,11a].

Species	Odor
Asterella species	Indole- or skatole-like, and higher plant *Houttuynia cordata*-like
Bazzania japonica	Sweet, balsamic, tree moss-like
B. pompeana	Oak moss-like
Cheilolejeunea imbricata	Strong milky smell
Chiloscyphus pallidus	Intense smell reminiscent of stink bug
Conocephalum conicum	Camphoraceous, strong mushroomy, and lactone-like
Conocephalum japonicum	Higher plant *Houttuynia cordata*-like
Frullania davulica	Mossy
F. tamarisci subsp. *tarmarisci*	Oak moss-like
Jungermannia obobata (*Solenostoma obovata*)	Carrot-like
Leptolejeunea elliptica	Intense and sweet phenol-like Mixed smell of naphthalene, and dried bonito
Lophocolea heterophylla	Strong and distinct mossy
L. bidentata	Strong and distinct mossy
L. minor	Strong moss-like
Lophozia bicrenata	Pleasant odor like cedar oil
Makinoa crispata	Rooty, earthy, woody, and amber-like
Mannia fragrans	Strong sweet-mossy
Odontoschisma denudatum	Civet, animal-like
Pellia endiviifolia	Dried seaweed-like
Plagiochila sciophila (*P. acanthophylla* subsp. *japonica*)	Sweet-mossy and woody
Porella gracillima	Woody-earthy
P. vernicosa	Malty, earthy
Radula perrottetii	Castor-like, animal-like
Takakia lepidozioides	Mixed smell of cinnamon and burnt wheat powder
Targionia hypophylla	Sweet turpentine
Trichocolea tomentella	Sulfur-like
Wiesnerella denudata	Strong sweet-mushroomy, green, and citrus

Almost all liverworts that smell of mushrooms contain oct-1-en-3-ol and its acetate, which is generally more abundant than the free alcohol. A small thalloid unidentified liverwort, *Asterella* species emits an intense unpleasant odor which is due to skatole and composed of 20 % of the total extract [12]. The stink bug smell of the New Zealand *Cheilolejeunea pallidus* is attributable to (*E*)-dec-2-enal, (*Z*)-dec-2-enal, and (*E*)- and (*Z*)-pent-2-enals [13], although the major components of such insects are (*Z*)- and (*E*)-hex-2-enals. The characteristic fragrance of *Leptolejeunea elliptica* is due to *p*-ethylanisol, *p*-ethylphenol, and *p*-ethyl phenyl acetate [14]. The strong milk-like fragrance of *Cheilolejeunea imbricata* is due to a mixture of (*R*)-dodec-2-en-1,5-olide (**1**) and (*R*)-tetradec-2-en-1,5-olide (**2**) [14].

Bicyclohumulenone (**3**), 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 (**4**) 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 leaf, and seaweeds. Both compounds are important in commerce. They are used as perfumes as such or as perfume components of the pow-

dery floral-, oriental bouquet-, fantastic chypre-, fancy violet-, and white rose-types in various cosmetics. It is noteworthy that *Frullania* species producing tamariscol only grow in high mountains. Total synthesis of (±)-tamariscol (**4**) has been accomplished using commercially available *p*-methoxyacetophenone in 13 steps [15]. 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 (**4**), 13 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 (**5**) had a sweet-mossy aroma similar to that of tamariscol itself [16].

There are three chemo-types of *Conocephalum conicum*. 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 [17]. *Jungermannia obobata* contains a tris-normonoterpene ketone, 4-hydroxy-4-methylcyclohex-2-en-1-one (**6**), which possesses an intense carrot-like odor [18,19]. The strong and distinct mossy odor of *Lophocolea heterophylla* and *L. bidentata* is due to a mixture of (–)-2-methylisoborneol [20a] and geosmin (**7**) [20b]. Geosmin, possessing a strong earthy-musty odor, has also been found in in vitro cultured *Symphyogyna brongniartii* [21]. The strong sweet-mossy note of *Mannia fragrans* is attributable to the cuparene-type sesquiterpene ketone, grimaldone (**8**) [22]. The sweet turpentine-like odor of French *Targionia hypophyll*a is due to a mixture of *cis*- and *trans*-pinocarveyl acetates [23a].

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 [7]. 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 oct-1-en-3-ol (7 %) and its acetate (2 %) is lower than *C. conicum* belonging to the same genus of *Wiesnerella* [23b]. The structures of several characteristic odorants mentioned above are shown in Fig. 1.

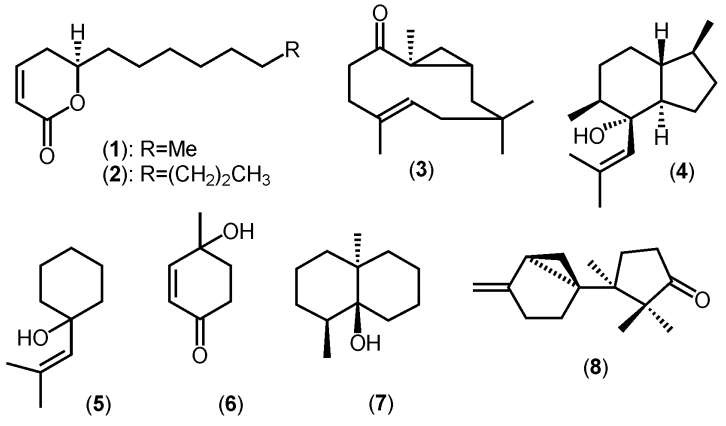


Fig. 1 Characteristic odorants from liverworts.

Pungency and bitterness

Some genera of the Hepaticae 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 [24a]. *Porella verni-*

cosa complex (*P. arboris-vitae*, *P. fauriei*, *P. gracillima*, *P. obtusata* subsp. *macroloba*, *P. roellii*, and *P. vernicosa*) contain potent pungent substances, and that *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 *P. vernicosa* complex is due to (–)-polygodial (**9a**) [6,11b] (Fig. 2). Polygodial is the major compound of the medicinal plant, *Polygonum hydropiper* (Polygonaceae). The Margash medicinal plant, *Cinnamosma fragrans* (Canellaceae) produces potent pungent component which is due to cinnamodial (**21**) [24b].

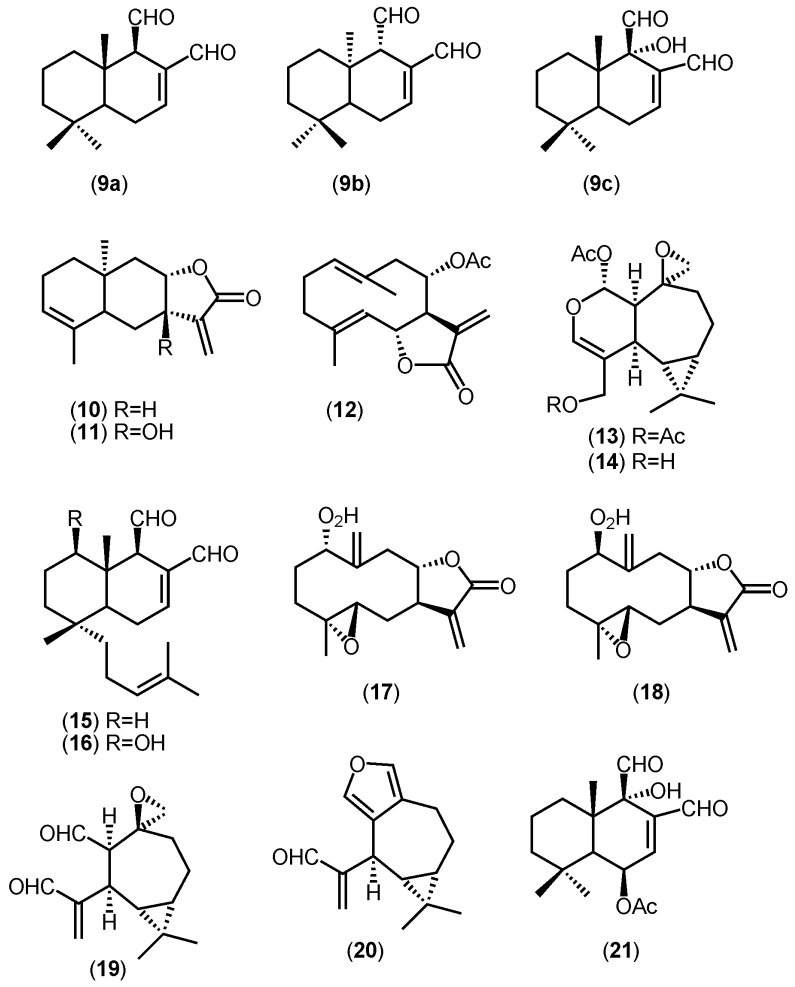


Fig. 2 Hot-tasting oxygenated sesqui- and diterpenoids (**9a–20**) from liverworts and **21** from *C. fragrans* (higher plant).

It is noteworthy that some ferns, *Blechnum fluviatile* collected in New Zealand and Argentinean *Thelypteris hispidula*, elabolate the pungent component, polygodial (**9a**), together with its related drimanes [24c,d].

We reported that two eudesmanolides, diplophyllolide (**10**), and *ent*-7α-hydroxydiplophyllolide (**11**), a germacranolide, tulipinolide (**12**), two 2,3-secoaromadendrane-type sesquiterpene hemiacetals, plagiochiline A (**13**), and plagiochiline I (**14**), and sacculatane-type diterpene dialdehyde, sacculatal (**15**) possessing an intense pungent taste had been isolated from some *Chiloscyphus*, *Wiesnerella*,

Plagiochila, *Pellia*, and *Trichocoleopsis* species, respectively [6]. Further fractionation of the ether extract of *Pellia endiviifolia* resulted in the isolation of a new pungent 1β-hydroxysacculatal (**16**), together with several sacculatane-type diterpenoids [25]. The hot taste of *Pallavicinia levieri* [10] and *Riccardia robata* var. *yakushimensis* [26] is also due to sacculatal (**15**). Polygodial and sacculatal have been obtained from cell suspension cultures from each liverwort [27,28]. *Porella acutifolia* subsp. *tosana* is a pungent stem-leafy liverwort. Its taste is due to the presence of hydroperoxysesquiterpene lactones, 1α- (**17**), and 1β-hydroperoxy-4α,5β-epoxygermacra-10(14),11(13)-dien-12,18α-olides (**18**) [29]. When one chews a whole plant of the stem-leafy liverwort, *Plagiochila asplenioides*, *P. fruticosa*, *P. ovalifolia*, and *P. yokogurensis*, which contain plagiochiline A (**13**), one feels a potent hot taste slowly. It is suggested that **13** might be converted into pungent unsaturated dialdehyde by human saliva. Enzymatic treatment of **13** with amylase in phosphate buffer or with human saliva produces two strong pungent 2,3-secoaromadendrane-type aldehydes, plagiochilal B (**19**), whose partial structure is similar to that of the pungent drimane-type sesquiterpene dialdehyde, polygodial (**9a**), and furanoplagiochilal (**20**) [30]. The New Zealand liverwort, *Hymenophyton flavellatum*, produces different pungent-tasting substance from the other aforementioned liverworts. Fractionation of the ether extract resulted in the isolation of several phenyl butanones (**22**–**27**) and their related compounds (**28–30**) (Fig. 3). Compound **22** is due to the hot tasting of the species [24c].

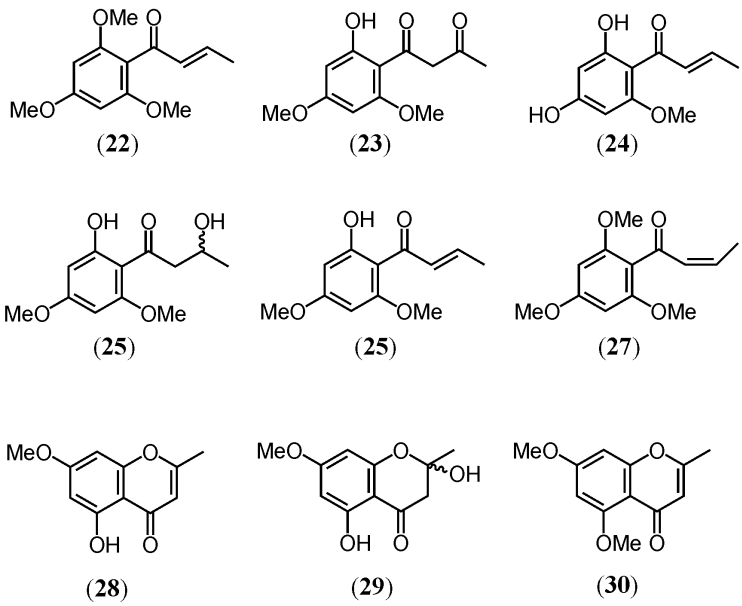


Fig. 3 Aromatic compounds, including pungent penyl butenone (**22**) isolated from the New Zealand liverwort *H. flabellatum* (Metzgeriales).

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 (**31**) (Fig. 4a) [6]. 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 (**32–36**), which are the first isolation of glycosides from liverworts (Figs. 4a,b) [31]. The bitterness of *Anastrepta orcadensis*, *Barbilophozia lycopodioides*, and *Scapania undulata* are attributable to highly oxygenated diterpenoids, anastreptin A (**37**) [18,32], barbilycopodin (**38**) [18,32], and scapanin A (**39**) [33a], respectively (Fig. 4b).

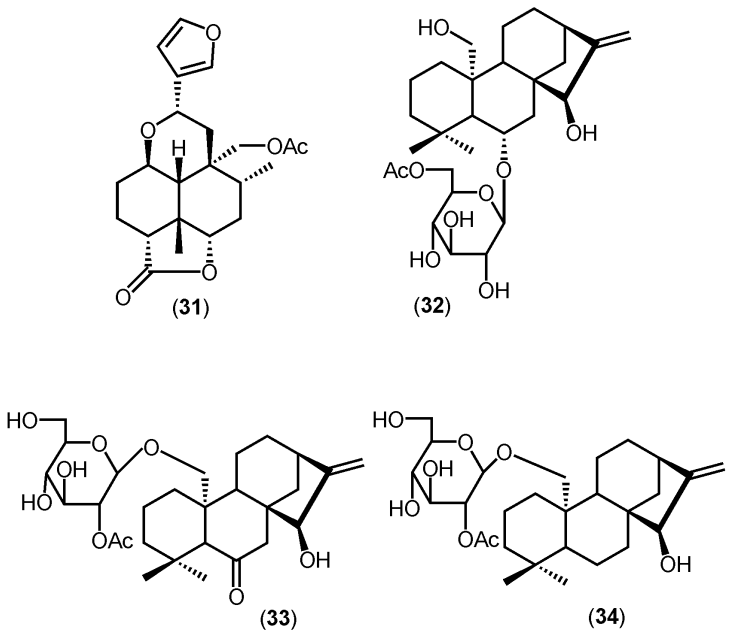


Fig. 4a Bitter diterpenoids from liverworts.

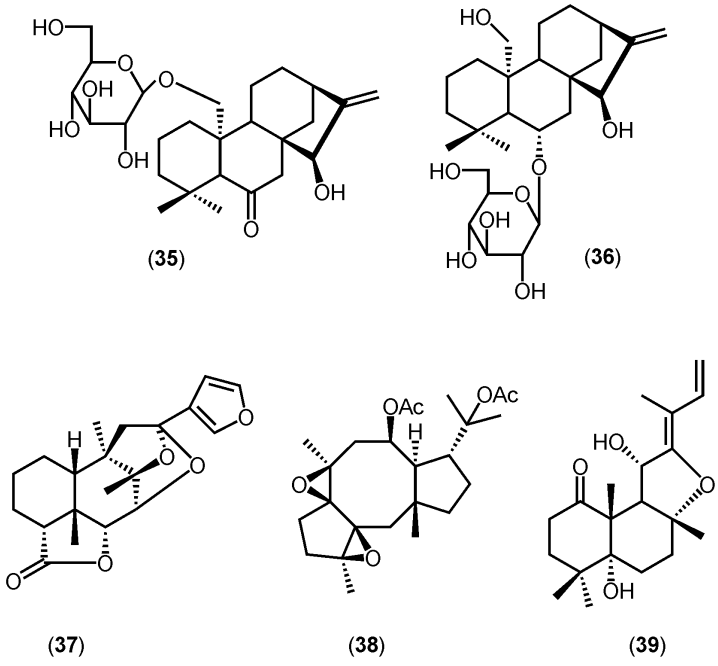


Fig. 4b Bitter diterpenoids from liverworts.

Allergenic contact dermatitis

Frullania species (Hepaticae) are notable as liverworts that cause very intense allergenic contact dermatitis [6,33b–f]. The allergy-inducing substances are sesquiterpene lactones, (+)-frullanolide (**40a**) and (–)-frullanolide (**40b**), which have been isolated from *Frullania dilatata* and *F. tamarisci* subsp. *tamarisci*, respectively (Fig. 5) [6]. Both dihydrofrullanolides (**40k**, **40l**) with an α-methyl-γ-butyrolactone isolated from the above mentioned liverworts does 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 (**40c–40j**) with α-methylene-γ-butyrolactones cause strong allergenic contact dermatitis as does *Schistochila appendiculata*. The allergens of the latter are long-chain alkylphenols, 3-undecyl- (**41**), 3-tridecyl (**42**), 3-pentadecyl (**43**), and 3-heptadecyl phenols (**44**), long-chain alkyl salicylic acids, 6-undecyl- (**45**), 6-tridecyl- (**46**), 6-pentadecyl salicylates (**47**), and their potassium salts, potassium 6-undecyl- (**48**), 6-tridecyl- (**49**), and 6-pentadecyl salicylates (**50**) as well as 6-undecyl catechol (**51**) (Fig. 6) [7]. 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.

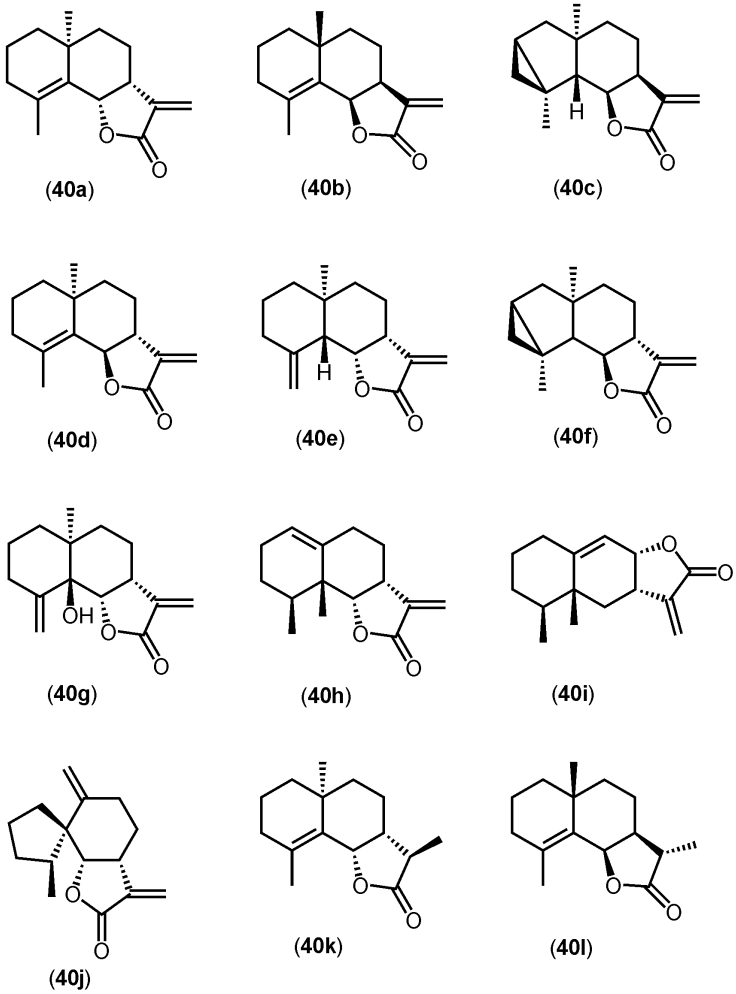


Fig. 5 Allergy-inducing sesquiterpene lactones (**40a–40j**) from liverworts *Frullania* species.

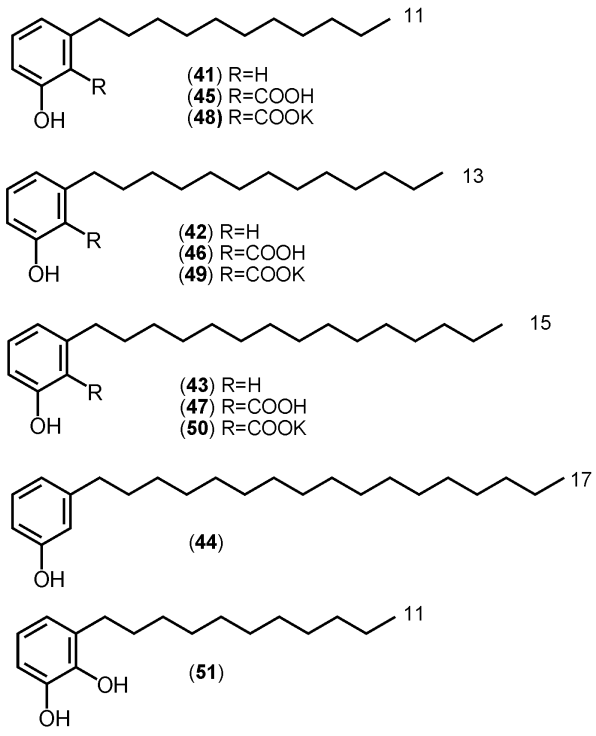


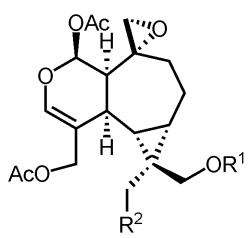
Fig. 6 Allergy-inducing long-chain alkyl phenols from liverworts.

Cytotoxic, anti-HIV-1, and DNA polymerase β inhibitory

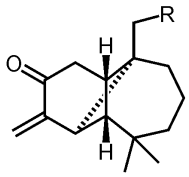
A few eudesmanolides and germacranolides possessing inhibitory activity against KB cells have been isolated from liverworts [7]. *C. conicum* and *W. denudata* contain guaianolides which exhibited cytotoxic activity against P-388 lymphocytic leukemia [7]. The crude ether extract (4–20 μg/ml) of the following liverworts showed cytotoxicity against P-388 in vitro [23b]: *Bazzania pompeana*, *Kurzia makinoana*, *L. heterophylla*, *Makinoa crispata*, *Marsupella emarginata*, *P. endiviifolia*, *P. 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*, *Pallavisinia subciliata*, *Plagiochila sciophila*, *Supraceanthus semirepandus*, and *Trocholejeunea sandvicensis* were not active against P-388.

2,3-Secoaromadendrane-type sesquiterpenoids, plagiochiline A (**13**), plagiochiline A 13-octanoate (**52**), and 12-hydroxyplagiochiline A 13-2*E*,4*E*-dodecadienoate (**53**) isolated from *P. ovalifolia* showed cytotoxic activity (ID_{50} 3, 0.05, 0.05 μg/ml, respectively) against P-388 (Fig. 7) [34]. Polygodial (**9a**) isolated from *P. vernicosa* complex, sacculatal (**15**) from *P. endiviifolia*, and two 2,3-secoaromadendrane-type sesquiterpene hemiacetals (**52**), and plagiochiline A 13-decanoate (**54**) from *P. ovalifolia* showed cytotoxic activity (2–4 μg/ml) against melanoma [35a]. Sacculatal (**15**) 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 [35b]. Marsupellone (**55**) and acetoxymarsupellone (**56**) from *M. emarginata* showed cytotoxicity (ID_{50} 1 μg/ml) against P388 [35a,36].

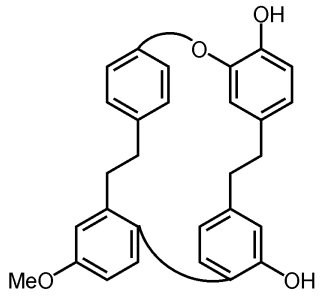
Riccardins A (**57**) and B (**58**) from *Riccardia multifida* subsp. *decrescens* inhibited KB cells at a concentration of 10 and 12 μg/ml, respectively. Many *Plagiochila* species and *R. perrottetii* contained cytotoxic plagiochiline A (**13**) (0.28 μg/ml) and perrottetin E (**59**) (12.5 μg/ml) against KB cell, respectively (Fig. 7).



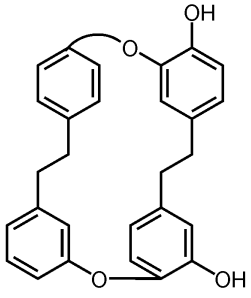
(**52**) R^1=Octanoyl, R^2=H
(**53**) R^1=2*E*,4*E*-Dodecadienyl R^2=OH
(**54**) R^1=Decanoyl, R^2=H



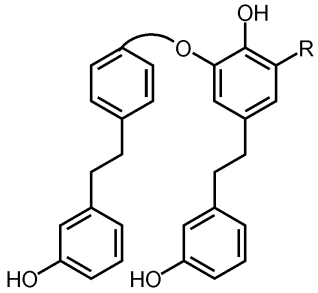
(**55**) R=H
(**56**) R=OAc



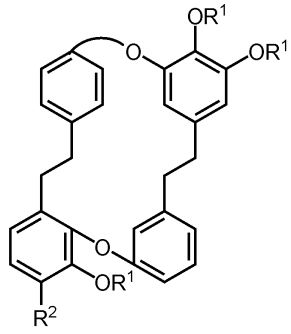
(**57**)



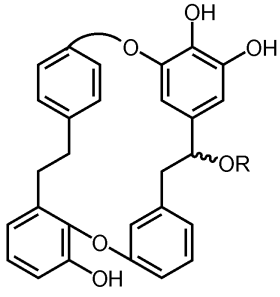
(**58**)



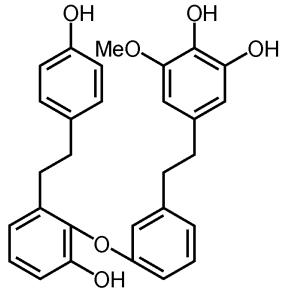
(**59**) R=H
(**60**) R=OH



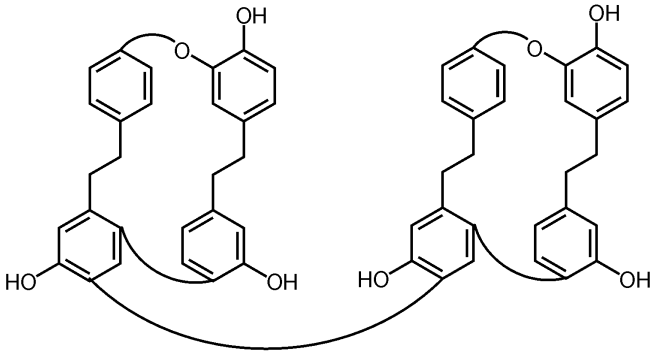
(**61**) R^1=R^2=H
(**62a**) R^1=Me, R^2=H
(**62b**) R^1=Me, R^2=OMe
(**63**) R^1=Ac, R^2=H
(**64**) R^1=H, R^2=OH



(**65**) R=H
(**66**) R=Me



(**67**)



(**68**)

Fig. 7 Sesquiterpenoids and cyclic and acyclic bis-bibenzyls possessing various biological activities.

The thalloid liverwort, *M. polymorpha*, which can cause allergenic contact dermatitis, shows inhibitory activity against Gram-positive bacteria, and has diuretic activity. The methanol extract (100–150 g) of Japanese *M. polymorpha* was chromatographed on silica gel and Sephadex LH-20 to give cyclic bis-bibenzyls, marchantin A (MA) (**61**, 30–40 g) and its analogs (MB-G) [35a]. The yield of MA (**61**) is dependent upon *Marchantia* species. 80 to 120 g of pure MA has been isolated from 6.67 kg of dried *M. paleacea* var. *diptera*. This thalloid liverwort elaborates not only the marchantin series, marchantin A (**61**), B (**64**), D (**65**), and E (**66**), but also the acyclic bis-bibenzyls, perrottetin F (**60**) and paleatin B (**67**). 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 cell), and anti-HIV-1 (5.30–23.7 μg/ml) activity [36b]. Marchantin A (**51**) also shows cytotoxicity (T/C 117) against P-388 [7]. *Blasia pusilla* produces bis(bibenzyl) dimers, pusilatins A–D (**68–71**). Pusilatins B (**69**) and C (**70**) possess DNA polymerase β inhibitory activity (IC_{50} 13.0 and 5.16 μM), moderate cytotoxicity against KB cell (ED_{50} 13.1 and 13.0 μg/ml), and weak HIV-RT inhibitory activity [37]. *Trichocolea* species produce prenyl ethers, tomentellin (**72**), demethyltomentellin (**73**), and trichocolein (**74**) [38a]. Compound **72** is the major cytotoxic component of *T. mollissima*, active against BSC cells at 15 μg/disk [38a]. Compound **73** isolated from *T. tomentella* also showed the same activity (Figs. 7 and 8) [38a]. The *ent*-kaurenes and modified *ent*-kaurenes isolated from the New Zealand unidentified *Jungermannia* species

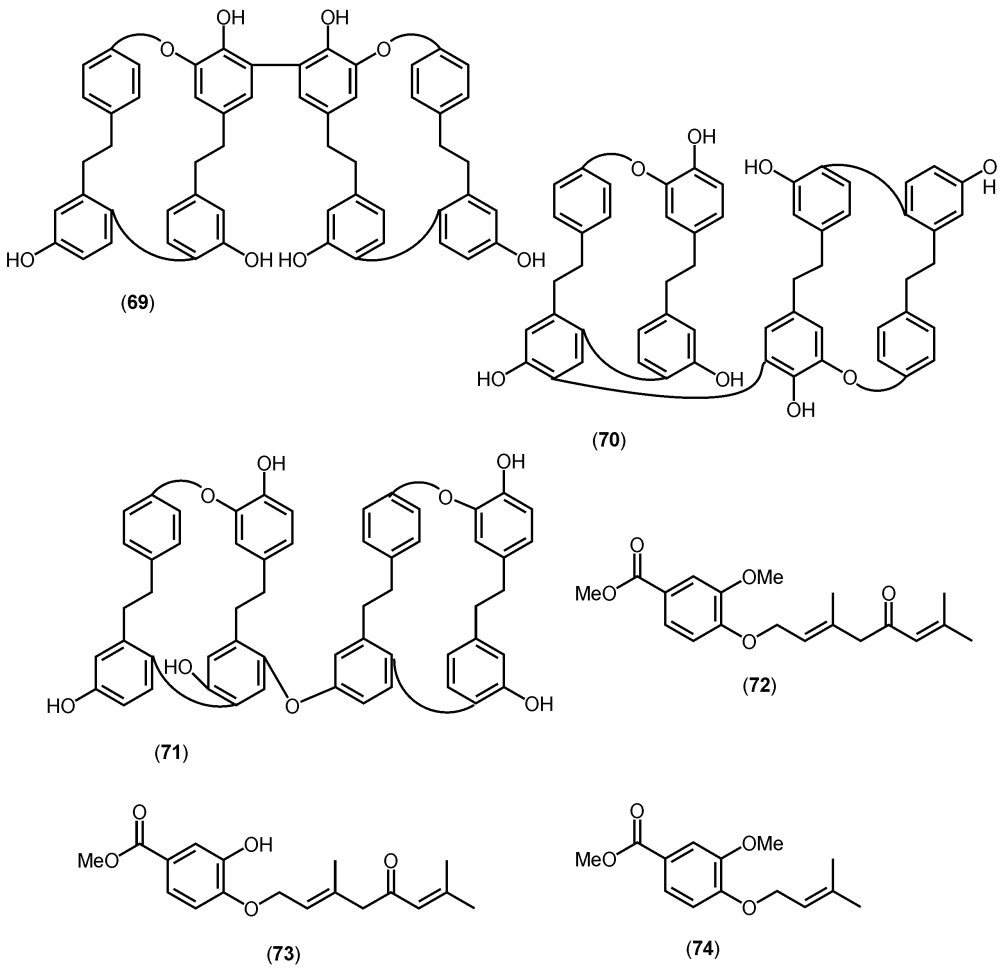


Fig. 8 Cyclic bis-bibenzyls and prenyl ethers from liverworts possessing various biological activities.

showed cytotoxic activity against HL-60 cells. Compounds **75–80** induced DNA fragmentation in HL-60 cells (Fig. 9) [38b–i].

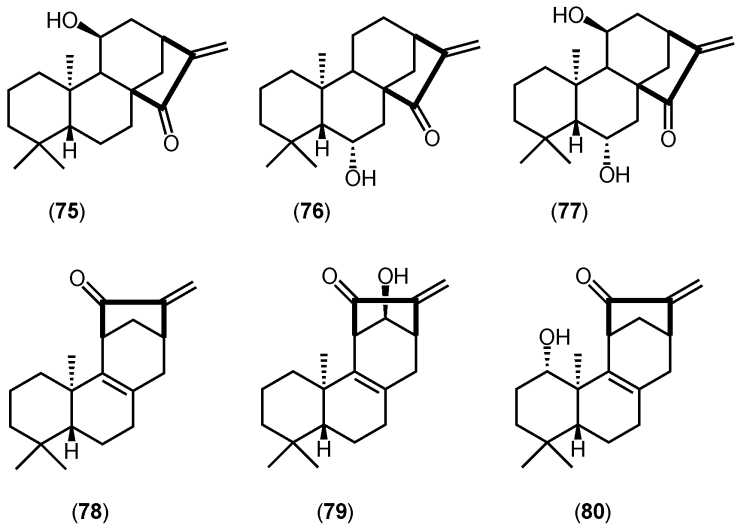


Fig. 9 Cytotoxic *ent*-kaurene- and rearranged *ent*-kaunenes from the liverwort *Jungermannia* species in HL-60 cells.

Antimicrobial and antifungal activity

Several liverworts, *Bazzania* species, *C. conicum*, *Dumortiera hirsuta*, *M. polymorpha*, *M. furcata*, *P. endiviifolia*, *Plagiochila* species, *P. vernicosa* complex, *P. platyphylla*, and *Radula* species show antimicrobial activity [7]. Several such as *Bazzania* species, *C. conicum*, *Diplophyllum albicans*, *Lunularia cruciata*, *M. polymorpha*, *Plagiochila* species, *P. vernicosa* complex, and *Radula* species display antifungal activity [7]. Marchantin A (**61**) 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*, *Salmonela typhimurium* (100), and *Staphylococcus aureus* (3.13-25) [7]. They also have antifungal activity against *Alternaria kikuchiana*, *Aspergillus fumigatus* (MIC 100 μg/ml), *A. niger* (25-100), *Candiada 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) [7,35a]. The prenyl phenyl ethers (**72** and **73**) isolated from *Trichocolea mollissima* and *T. tomentella*, respectively, were mildly antifungal against *T. mentagrophytes* [38a]. Compound **74** isolated from *T. lanata* showed similar mild antifungal activity [38a].

Sacculatal (**15**), isolated from *Pellia endeviifolia* showed strong antibacterial activity against *Streptococcus mutans* (dental caries) at LD_{50} 8 μg/ml, however, polygodial (**9a**) is less active (100 μg/ml) than sacculatal [23b].

Insect antifeedant, mortality, and nematocidal activity

As mentioned earlier, plagiochiline A (**13**) found in several *Plagiochila* species, is a strong antifeedant against the African army worm (*Spodoptera exempta*) [6]. Compound **13** shows nematocidal activity

against *Caenorphabdiitis elegans* (111 μg/ml) [23b]. The pungent sacculatal (**15**) kills tick species *Panonychus citri*. Compound **15**, eudesmanolides (**10**, **11**) from *Chiloscyphus polyanthos*, and gymnocolin (**31**) from *Gymnocolea inflata* also have antifeedant activity against larvae of Japanese *Pieris* species [7]. A series of natural drimanes and related synthetic compounds was tested for antifeedant activity against aphids [39]. Polygodial (**9a**) from the *P. vernicosa* complex and warburganal (**9c**) from the African tree *Warburgia ugandensis* were the most active substances. Natural (–)-polygodial (**9a**) and the synthetic (+)-enantiomer (**9b**) 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 (**81**), isolated from *Plagiochila* species, killed *Nilaparvata lugens* (Delphacidae) at 100 μg/ml [23b].

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) (**82**) from *J. infusca* and plagiochilal B (**19**) 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 [7,35a]. Norpinguisone methyl ether (**83**) 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 (**85**) from *Bazzania japonica*. Other sesquiterpenoids, plagiochilide (**81**) isolated from *Plagiochila fruticosa, P. ovalifolia* and *P. yokogurensis*, norpinguisone (**84**) from *Porella vernicosa*, bicyclogermacrenal (**86**) from *C. conicum*, herbertenediol (**87**) and isocuparene-3,4-diol (**88**) from *Mastigophola diclados*, the diterpenoids, infuscaside A (**32**), and infuscaside B (**33**) from *J. infusca*, and perrottetianal A (**89**) from *Porella perrottetiana* also inhibit superoxide release from guinea pig peritoneal macrophage (IC_{50} 12.5–50 μg/ml) [7]. Radulanin K (**90**) from *Radula javanica* inhibits the release of superoxide anion radical from guinea pig macrophage (IC_{50} 6 μg/ml) (Fig. 10) [35a]. Polygodial (**9a**) and sacculatal (**15**) also show superoxide anion radical release inhibition at 4.0 μg/ml from guinea pig peritoneal macrophage [23b].

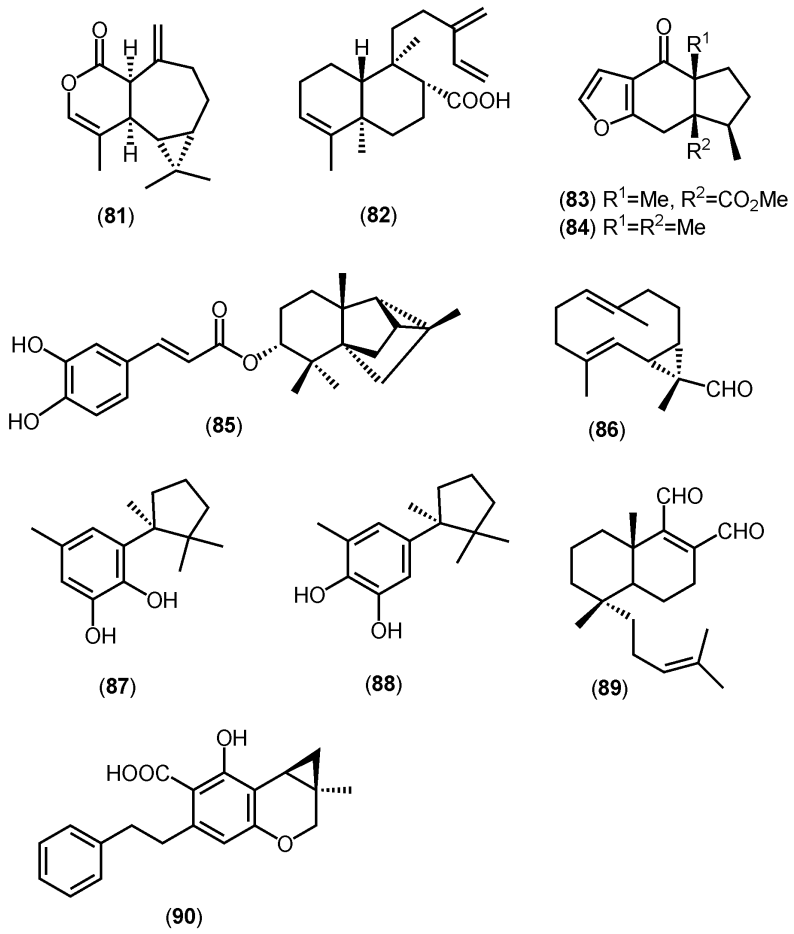


Fig. 10 Sesqui- and diterpenoids, and bibenzyls possessing superoxide anion radical release inhibitory activity.

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

Marchantin A (**61**) 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*-dihydroxyeicos-6,8,10,14-tetraenoic 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-hydroxyeicos-6,8,11,14-tetraenoic acid)] and calmodulin inhibitory activity at ID_{50} 1.85 μg/ml [7,35a]. Perrottetins A (**91**) and D (**92**) from *R. perrottetii* and prenyl bibenzyls (**93–97**) (Fig. 11), also from *Radula* species, riccardin A (**57**) from *R. multifida*, and marchantins D (**65**) and E (**66**) from *Marchantia* species had calmodulin inhibitory activity (ID_{50} 2.0–95.0 μg/ml) [7,35a]. The simple bibenzyls (**98–101**) (Fig. 11) 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 (**104**) (ID_{50} 82 μg/ml) isolated from *P. perrottetiana* [7,35a]. Perrottetin A (**91**), prenylbibenzyls (**92**, **100**, **102**, **103**), marchantins D (**65**) and E (**66**), and riccardin A (**57**) also inhibited 5-lipoxygenase (76–4 % at 10^{-6} mol) [7]. The following phenolic compounds showed significant cyclooxygenase inhibitory activity: marchantin A (**61**) (IC_{50} 46.4 μM), marchantin B (**64**) (55.9), marchantin E (**66**) (58.0), paleatin B (**67**) (45.2), perrottetin D (**92**) (26.2), radulanin H (**97**) (39.7), isoriccardin C (**105**) (50.8), and riccardin C (**106**) (53.5) [40].

Lunularic acid (**107**), 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'-dimethoxycinnamoylanthranilic acid) which is an anti-allergenic agent developed in Japan for oral administration. Lunularic acid (**107**) has been obtained from hydrangenol-β-glucoside via hydrangenol in good yield [41]. Perrottetin E (**59**) exhibited inhibitory activity for thrombin (IC_{50} 18 μM), which is associated with blood coagulation [42].

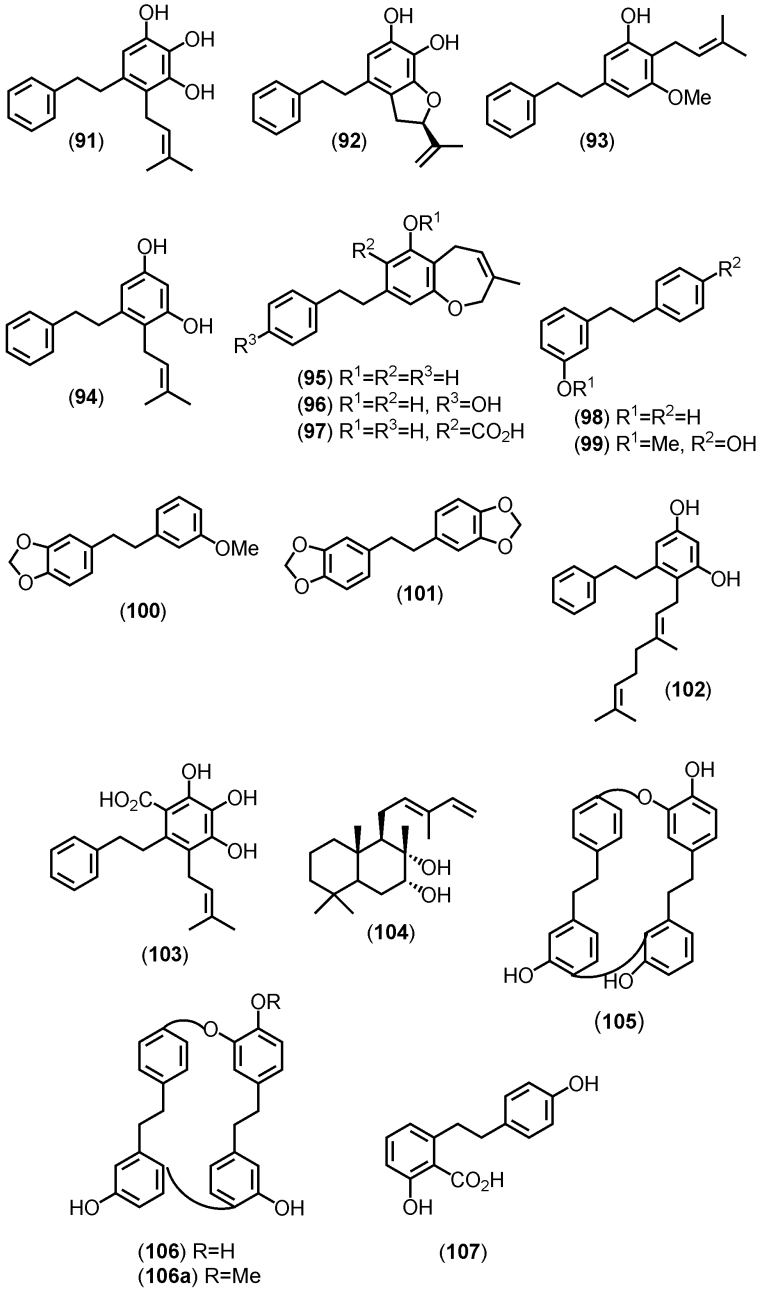


Fig. 11 Bibenzyls from liverworts possessing 5-lipoxygenase, calmodulin, cyclooxygenase, and hyaluronidase inhibitory activity.

Overproduction of 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 cytokins involved in pathological response. Thus, inhibition of iNOS is very important to control inflammatory disease. The inhibition of 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 are reported in Table 3 [43a]. 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 important role in the inhibition 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 ethers (**62a**, **62b**) of marchantin A (**61**) and marchantin B (**64**) showed weaker activity than the original compounds.

Table 3 NO inhibition of bis-bibenzyls isolated from liverworts [43a].

Compound	NO inhibition IC_{50} (μM)
Riccardin A (**57**)	2.50
Perrottetin F (**60**)	7.42
Marchantin A (**61**)	1.44
Marchatnin B (**64**)	4.10
Marchantin D (**65**)	10.18
Marchantin E (**66**)	62.16
Riccardin C (**106**)	>100
Riccardin F (**106a**)	5.0
Marchantin A trimethyl ether (**62a**)[a]	42.50
Marchantin B trimethyl ether (**62b**)[a]	42.45

[a]Derivatives from marchantin A (**61**) and marchantin B (**64**).

Piscicidal and plant growth inhibitory activity

The strongest piscicides are the pungent (–)-polygodial (**9a**) from *P. vernicosa* complex and sacculatal (**15**) from *P. endiviifolia*, *P. levieri*, *R. robata* var. *yakushimensis*, and *Trichocoleopsis sacculata*. Killie-fish (*Oryzia latipes*) is killed within 2 h by 0.4 ppm solution of (**9a**) and (**15**) [6,7]. Sacculatal (**15**) and 1β-hydroxysacculatal (**16**) also kill killie-fish within 20 min at 1 ppm [25]. Killie-fish is also killed within 2 h by a 0.4 ppm solution of synthetic pungent (+)-polygodial (**9b**). 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 [7]. On the other hand, isopolygodial (**108**) from cultured cells of *P. vernicosa* and the higher plant *Polygonum hydropiper* and isosacculatal (**109**) from *Pellia*, *Riccardia*, and *Trichocoleopsis* species show neither piscicidal nor molluscicidal activity even at 10 000 ppm [7] .

Almost all crude extracts from liverworts which contain bitter or pungent substances show phytotoxic activity. (–)-Polygodial (**9a**) 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 [6,43b].

Neurotrophic activity

Mastigophorenes A (**110**), B (**111**), and D (**112**) 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 [35,44] (Fig. 12). Plagiochilal B (**19**) and pla-

giochilide (**81**) 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 [10,35a]. Plagiochin A (**113**) also shows the same activity at 10^{-6} M [45]. Two bitter diterpene glucosides, infuscaside A (**32**) and B (**33**), show neurite bundle formation at 10^{-7} M [23b].

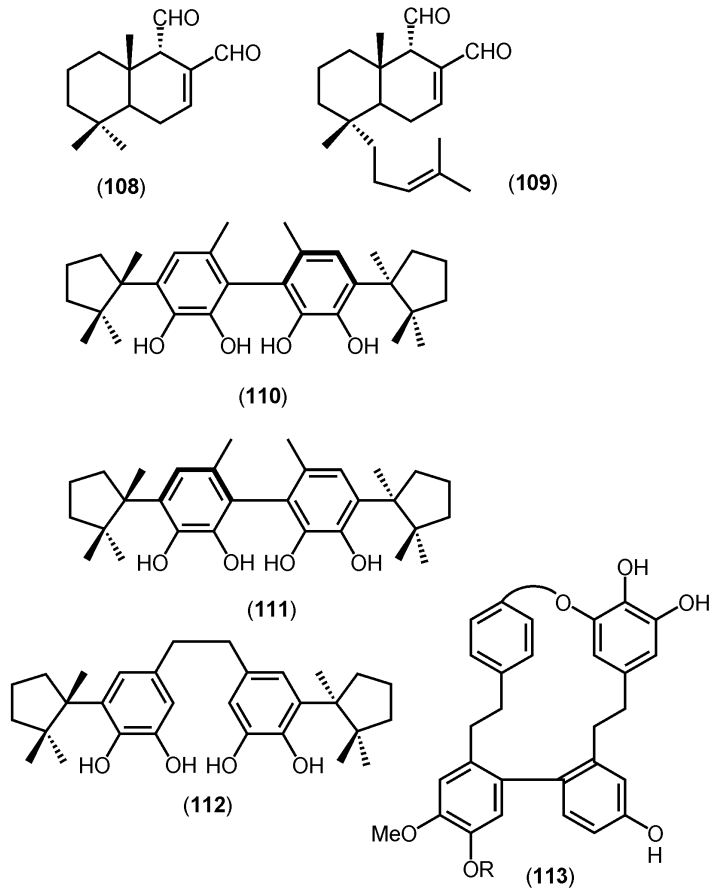


Fig. 12 Sesquiterpenoid and cyclic bis-bibenzyl from liverworts possessing neurotrophic activity.

Muscle relaxing activity

Marchantin A (**61**) and the related cyclic bis-bibenzyls are structurally similar to bis-bibenzyl-isoquinoline alkaloids such as *d*-tubocurarine (**114**), which are pharmacologically important muscle relaxing active drugs. Surprisingly, marchantin A (**61**) and its trimethyl ether (**62a**) also show muscle relaxing activity [9,46]. Nicotine in Ringer solution effects maximum contraction of rectus abdominis in frogs (RAF) at a concentration of 10^{-6} M. After preincubation of marchantin A trimethyl ether (**62a**) (at a concentration of 2×10^{-7}–2×10^{-4} M) in Ringer solution, nicotine (10^{-8}–10^{-4} M) was added. At a concentration of 10^{-6} M, the contraction of RAF decreased by about 30 %. *d*-Tubocurarine (**114**) exhibits similar effects as does (**62a**) with acetyl choline [9,35,46]. Although the mechanism of action of marchantin A (**61**) and its methyl ether (**62a**) 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 (**61**) and an *ortho* methoxyl group in (**62**) contribute to the muscle relaxing activity [9]. Marchantin A triacetate (**63**) and 7',8'-dehydromarchantin A (**115**) and acyclic bis(bibenzyls), such as perrottetin E (**59**) and F (**60**) did not show any muscle relaxing activity [23b] (Fig. 13).

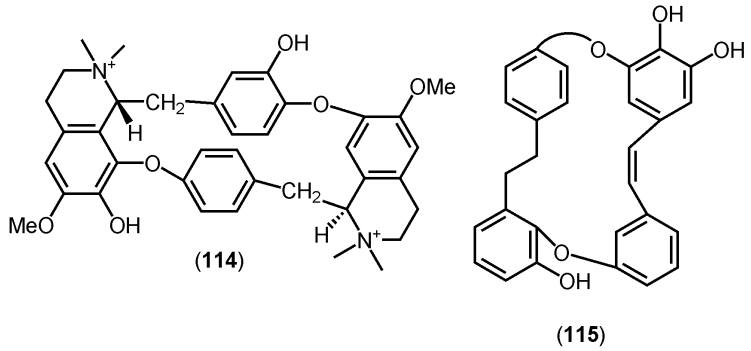


Fig. 13 *d*-Tubocurarine and 7',8'-dehydromarchantin A.

Cathepsin L and cathepsin B inhibitory activity

Cathepsin L is correlated with osteoporosis [47] and allergy [48]. 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 (**116**) was the strongest inhibitor against both enzymes (95 % for cathepsin L and 93 % for cathepsin B at 10^{-5} M). Infuscaic acid (**82**) exhibited the same activity as mentioned above (63 % and 32 % at 10^{-5} M) [23b]. The crude extract of *Porella japonica* showed potent inhibition of cathepsins B and L. Biological activity guided fractionation gave three guaianolides, 11-epiporelladiolide (**117**), 11,13-dehydroporelladiolide (**118**), and porellaolide (**119**), together with porelladiolide (**120**) and its epoxide (**121**). Only compound (**118**) possessed a weak inhibitory activity against cathepsin B (13.4 % at 10^{-5} M) and cathepsin L (24.7 % at 10^{-5} M) (Fig. 14) [49].

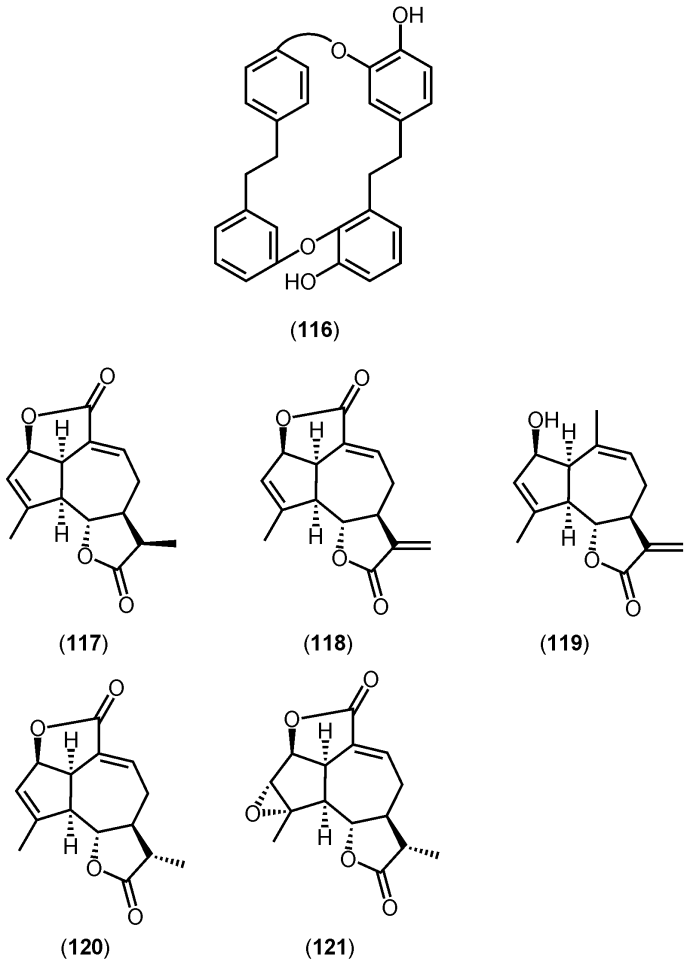


Fig. 14 Cyclic bis-bibenzyl, and guaiane sesquiterpene lactones from liverworts possessing cathepsins B and L inhibitory activity.

Cardiotonic and vasopressin (VP) antagonist activity

Marchantin A (**61**) shows cardiotonic activity [increase coronary blood flow (2.5 ml/min at 0.1 mg)] [7]. Prenyl bibenzyl (**94**) from *R. perrottetii* indicates vasopressin antagonist activity (ID_{50} 27 μg/ml). However, 2-geranylbibenzyl (**102**) from the same liverwort did not show VP antagonist activity [7].

Liver X-receptor (LXR)α agonist and (LXR)β antagonist activity

Liver X receptors (LXR)α agonist and (LXR)β share considerable sequence homology and several functions, respond to the same endogenous and synthetic ligands and play critical roles in maintaining lipid homeotasis. Riccardin C (**106**) and riccardin F (**106a**), isolated from the liverworts *Reboulia hemisphaerica* and *Blasia pusilla*, function as an LXRα agonist/LXRβ antagonist and an LXRα antagonist, respectively [50]. Riccardin C effectively enhances cholesterol efflux from THP-1 cells. This compound may provide a novel tool for identifying subtype function and drug development against antiobesity.

Synthesis of bioactive compounds from liverwort constituents

Mastigophora diclados, collected in Borneo, produces herbertane dimers (**110**) and (**111**) possesses neurotrophic activity. Both compounds were obtained by the biotransformation of α-herbertenol (**87**) using *Penicillium sclerotiorum* (Fig. 15) [51]. The stem-leafy liverwort *P. perrottetiana* elaborates a large amount of labdanediol (**104**). We focused on this diterpene to transfer into ambrox (**104e**), which is extremely expensive aroma originating from mammals. We succeeded in the hemisynthesis of this compound in seven steps as shown in Fig. 16 [52].

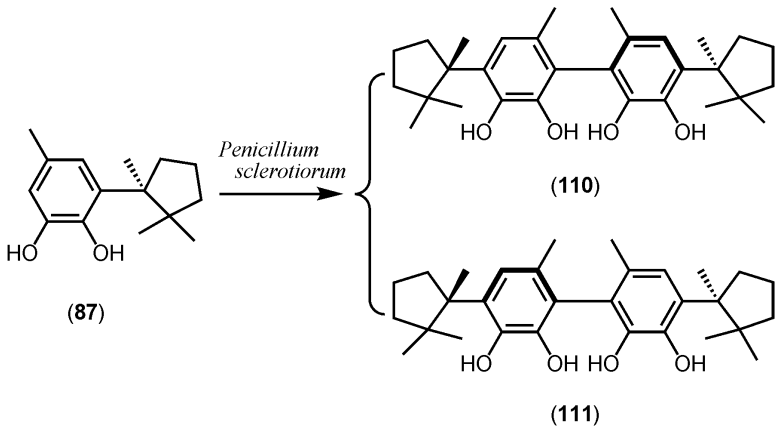


Fig. 15 α-Herbertenol (**87**), mastigophorenes A (**110**) and B (**111**) from the liverwort *M. diclados* and biotransformation of α-herbertenol by *P. sclerotiorum.*

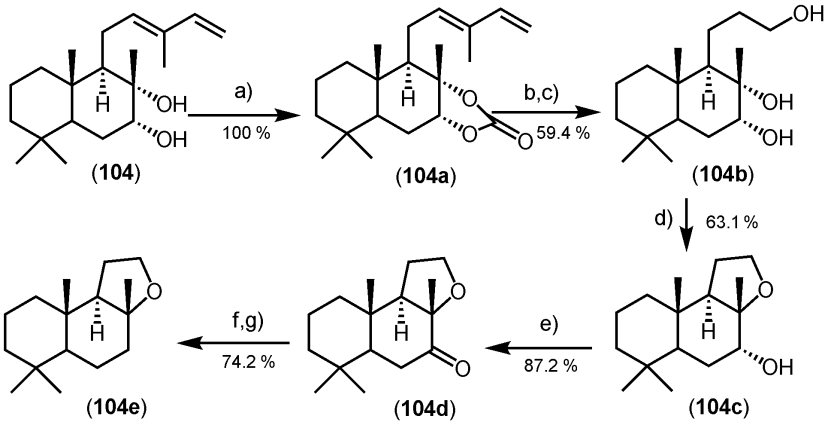
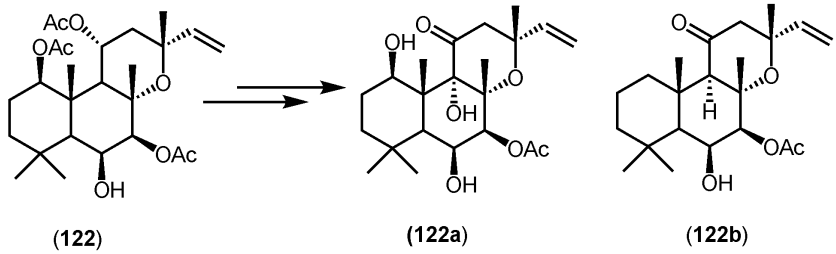


Fig. 16 Conversion of labda-12,14-diene (**104**) isolated from the liverwort *P. perrottetiana* to animal amoma, ambrox (**104e**).

The Indian medicinal plant *Coleus forskolii*, which has been used to treat disorders of the digestive organs, biosynthesized a highly oxygenated labdane diterpenoid, folskolin (**122a**), indicating blood pressure lowering and cardio protective properties and therapeutic potential in glaucoma, congestive heart failure, and bronchial asthmas. On the other hand, very similar highly oxygenated labdanes, for example, ptychantin A (**122**) to folskolin and its congener (**122b**), 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 (**122**) to folskolin (**122a**) in 12 steps and 12 % overall yield and 1,9-dideoxyfolskolin (**122b**) in 8 steps and 37 % overall yield (Fig. 17) [53,54].

Recently, we have isolated many new terpenoids and aromatic compounds whose structures were elucidated by 2D NMR spectroscopy and X-ray crystallographic analysis [55–73]. The bioassay of several of new products are now in progress.



(**122**) (**122a**) (**122b**)

Fig. 17 Synthetic transformation of ptychantin A (**122**) into folskolin (**122a**) and 1,9-dideoxyfolskolin (**122b**).

CONCLUSION

Most of the compounds isolated from or detected in the Hepaticae are lipophilic terpenoids (mono-, sesqui-, and diterpenoids) and aromatic compounds, of which only a few nitrogen- or sulfur-containing compounds have been found [6,10]. It is noteworthy that ca. 80 % of the sesqui- and diterpenoids found in liverworts are the enantiomers of those found in higher plants. Mono- and sesquiterpenoids are very rare in mosses and hornworts, but di- and triterpenoids have been isolated from certain mosses. At present, only 5 % of the total bryophytes have been studied chemically. Although liverworts are small plant groups, there are a number of new terpenoids and phenolic compounds, several of which show interesting biological activity.

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