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Cytotoxic and Antiviral Compounds from Bryophytes and Inedible Fungi

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Abstract

Over several hundred new compounds have been isolated from the bryophytes and more than 40 new carbon skeletal terpenoids and aromatic compounds found in this class. Most of liverworts elaborate characteristic odiferous, pungent and bitter tasting compounds many of which show, antimicrobial, antifungal, antiviral, allergenic contact dermatitis, cytotoxic, insecticidal, anti-HIV, superoxide anion radical release, plant growth regulatory, neurotrophic, NO production inhibitory, muscle relaxing, antiobesity, piscicidal and nematocidal activity. Several inedible mushrooms produce spider female pheromones, strong antioxidant, or cytotoxic compounds. The present paper concerns with the isolation of terpenoids, aromatic compounds and acetogenins from several bryophytes and inedible fungi and their cytotoxic and antiviral activity.

Key words

bryophytes, inedible fungi, terpenoids, bis-bibenzyls; cytotoxicity, antiviral activity

1. CHEMICAL CONSTITUENTS OF BRYOPHYTES

1.1 Introduction

The bryophytes are found everywhere in the world except in the sea. They grow on wet soil or rock, the trunk of trees, in lake, river and even in Antarctic island. The bryophytes are placed taxonomically between algae (Fig. 1) and pteridophytes (Fig. 2); there are approximately 24,000 species in the world. They are further divided into three phyla, Bryophyta (mosses 14,000 species, Fig. 3), Marchantiophyta (liverworts 6,000 species, Fig. 4a, 4b) and Anthocerotophyta (hornworts 300 species, Fig. 5). Although they are considered to be the oldest terrestrial plants, no strong scientific evidence for this has appeared in literatures. This hypothesis is 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. Among the bryophytes almost all liverworts possess beautiful cellular oil bodies (Fig. 6) which are peculiar membrane-bound cell organelles that consist of ethereal terpenoids and aromatic oils suspended

Figure 2. Fern

Figure 1. Green algae

Figure 3. Moss

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Figure 4a. Thalloid liverwort.

Figure 5. Hornwort

Figure 4b. Stem-leafy liverwort.

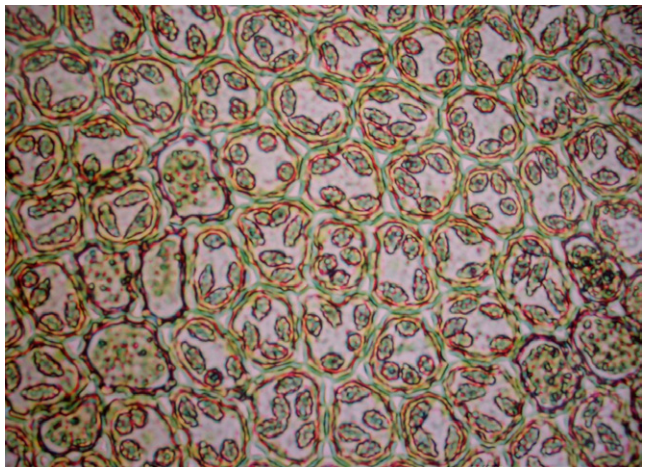


Figure 6. Oil bodies of the liverwort *Frullania vethii*

in carbohydrates- or protein-rich matrix, while the other two phyla do not. These oil bodies are very important biological marker for the taxonomy in the Marchantiophyta [1–8]. 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 sample, their identification is also very difficult even under the microscope. They are considered to be nutritionally useless to humans. In fact, nothing references concerning use as foods for humans have been seen. However, a number of bryophytes, especially, 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. as shown in Table 1 [9–11].

Many species of liverworts show characteristic fragrant odors and an intense pungent, sweet or bitter taste. Generally, bryophytes are not damaged by bacteria and fungi, insect larvae and adults, snails, slugs and other small mammals. Furthermore, some liverworts cause intense allergenic contact dermatitis and allelopathy. Although liverworts possess such pharmacologically interesting substances, their isolation and the structural elucidation were neglected for almost one century.

The paper concerns with the cytotoxic and antiviral activity of terpenoids, aromatic compounds and acetogenins from bryophytes [2–4, 8, 12, 13].

1.2 Bio- and Chemical Diversity of Bryophytes

1.2.1 *Biodiversity of bryophytes*

The Marchantiophyta includes two subclasses, the Jungermanniidae and Marchantiidae, and six orders, 49 families, 130 genera and 6,000 species. Still many new species have been recorded in the literatures. The southern hemisphere together with tropic region is characterized by extraordinarily high liverwort diversity [14]. This area has more endemic liverwort species than the northern hemisphere. As seen in Fig. 7, there are 54 endemic genera in southern hemispheric countries, such as New Zealand and Argentina [15]. In Asia including Japan, relatively large number of endemic genera (21) also has been recorded, however, South Africa, Madagascar and both North America and Europe are very poor regions of endemic liverworts. The richness of endemic genera of bryophytes in southern hemisphere suggests that the bryophytes might originate from the past Antarctic islands since 350 – 400 million 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 Marchantiophyta. 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 the northern Asia, including Japan.

In the tropical regions, such as south-east Asia, Borneo, Sumatra and Papua New Guinea and Colombia, Ecuador and Venezuela, there are rain forests where so many liverworts

Table 1. Medicinal bryophytes and their biological activity and effects

Musci:	**Biological activity and effects**
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, antidotal activity; for adenopharyngitis
Plagiopus oederi	As a sedative; for epilepsy, apoplexy, and cardiopathy
Polytrichum species	Diuretic activity; for hair growth
Polytrichum commune	Antipyretic, and antidotal; for hemostasis, cuts, bleeding from gingivae, hematemesis, and pulmonary tuberculosis
Rhodobryum giganteum	Antipyretic, diuretic, andantihypertensive; 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:	**Biological activity and effects**
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

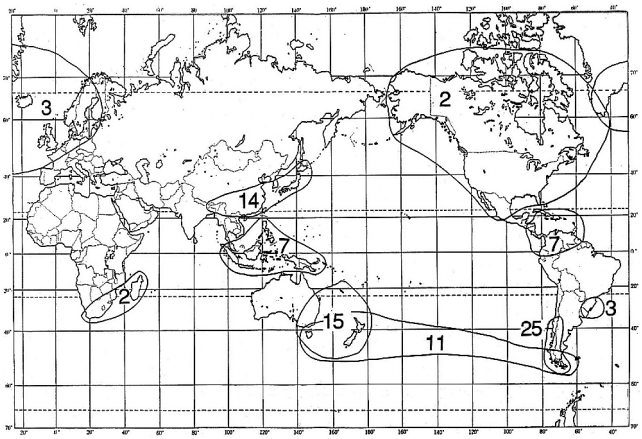


Figure 7. The distribution of endemic liverwort genera

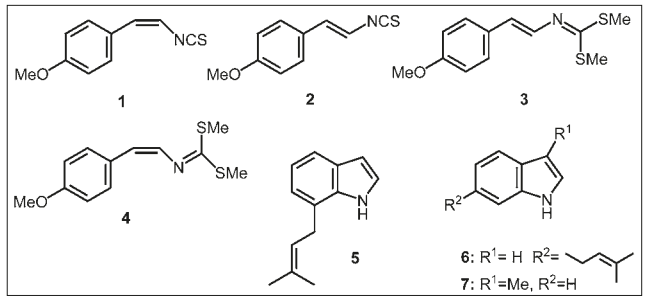
species have been found, but many different species like the Lejeuneaceae species are intermingled each other and thus it is thus time consuming work to purify all of them. In Ecuador and Columbia, the Marchantiophyta species grow in the high mountains, over 2,000m where people live, not in the lower level of their lands.

1.2.2. *Chemical Diversity of Bryophytes*

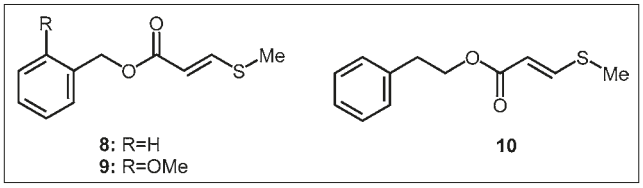
The extraction of oil bodies with *n*-hexane or ether, using ultrasonic apparatus is very easy for stem-leafy liverworts to give a large amount of crude extract. In case of thalloid liverworts, the specimens are ground mechanically and then extracted with non-polar solvents. At present, over several hundred new compounds have been isolated from bryophytes and their structures were elucidated [2, 3, 4]. Most of compounds found in liverworts are composed of lipophilic mono-, sesqui- and diterpenoids and aromatic compounds, such as typical bibenzyls and bis-bibenzyls.

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 germacrane- and guaiane-type sesquiterpenoids. It is very noteworthy that the different species of the same genera, like *Frullania tamarisci* and *F. dilatata* (Frullaniaceae) each produces different sesquiterpene lactone enatiomers. Some liverworts, such as *Lepidozia* species (Lepidoziaceae), biosynthesize both enantiomers. Flavonoids, fatty acids and phytosterols are ubiquitous components in bryophytes and have been isolated from or detected both in liverworts and mosses.

However, the presence of nitrogen or sulfur or nitrogen and sulfur containing compounds in bryophytes was very rare. Recently several nitrogen-sulfur containing compounds (**1**–**4**) have been isolated from the Mediterranean liverwort,



Corsinia coriandrina (Corsiniaceae: Marchantiales) [16], two prenyl indole derivatives (**5,6**) from the *Riccardia* species (Aneuraceae) [3], skatole (**7**) from the *Asterella* or *Mannia* (Aytoniaceae) [3] and the Tahitian *Cyathodium foetidissimum* (Cyathodiaceae) [8] and benzyl- (**8,9**) and β-phenethyl β-methylthioacrylates (**10**) from the Isotachidaceae [3].



Highly evolved liverworts belonging to the Marchantiaceae produce phytosterols, such as campe-, stigma- and sitosterols. Almost all liverworts elaborate α-tocopherol 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 [3].

The presence of hydrophobic terpenoids is very rare in the Marchantiophyta. A few bitter kaurene glycosides have been found in the *Jungermannia* species. However a numbers of flavonoid glycosides have been detected both in liverwort and mosses [2, 3, 4].

1.3. 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, 5) antimicrobial, antifungal and antiviral, 6) insect antifeedant, mortality, and nematocidal, 7) superoxide anion radical release inhibitory, 8) 5-lipoxygenase, calmodulin, hyaluronidase, cyclooxygenase, DNA polymerase β and α-glucosidase inhibitory, 9) antioxidant 10) piscicidal 11) neurotrophic, 12) muscle relaxing and calcium inhibitory, 13) cardiotonic and vasopressin antagonist, 14) liver X-receptor (LXR)α agonist and LXRβ antagonist, 15) cathepsins B and L inhibitory, antithrombin, 16) farnesoid X-receptor (FXR) activation, 17) nitric oxide production inhibitory, 18) plant growth inhibitory, 19) tublin polymerization inhibitory, 20) sex pheromones and 21) antiplatelet and brine shrimp lethal activity.

1.3.1 *Cytotoxic compounds from bryophytes*

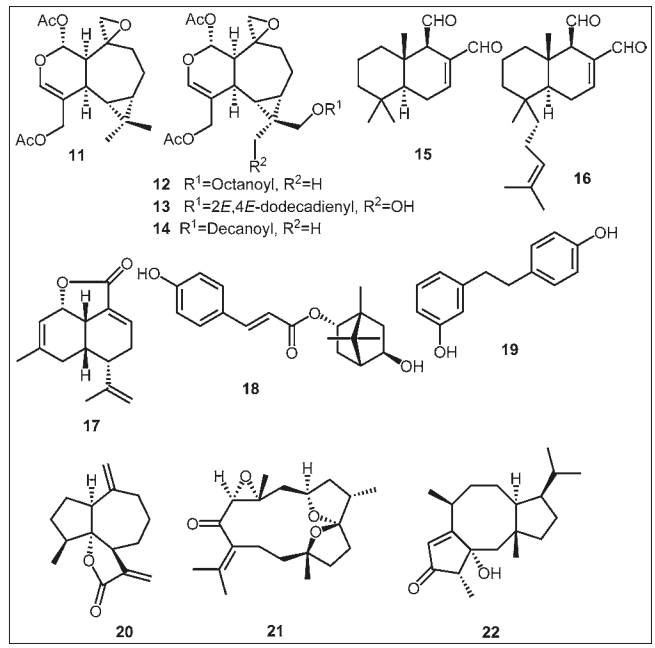
Several sesquiterpene lactones, such as eudesmanolides, germacranolides, and guaianolides isolated from liverworts exhibit cytotoxic activity against KB nasopharyngeal and P-388 lymphocytic leukemia cells (Asakawa 1995). The crude ether extracts of the liverworts *Bazzania pompeana, Kurzia makinoana, Lophocolea heterophylla, Makinoa crispata, Marsupella emarginata, Pellia endiviifolia, Plagiochila fruticosa, P. ovalifolia, Porella caespitans, P. japonica, P. perrottetiana, P. vernicosa*, and *Radula perrottetii* showed cytotoxicity against P-388 cells (IC_{50} value range 4–20 μg/mL). In contrast, the crude extracts of *Frullania diversitexta*, *F. ericoides*, *F. muscicola*, *F. tamarisci* subsp. *obscura, Lepidozia vitrea*, *Pallavicinia subciliata*, *Plagiochila sciophila*, *Spruceanthus semirepandus*, and *Trocholejeunea sandvicensis* were inactive against this same cell line (IC_{50} values >20 μg/ml) (Asakawa, unpublished results).

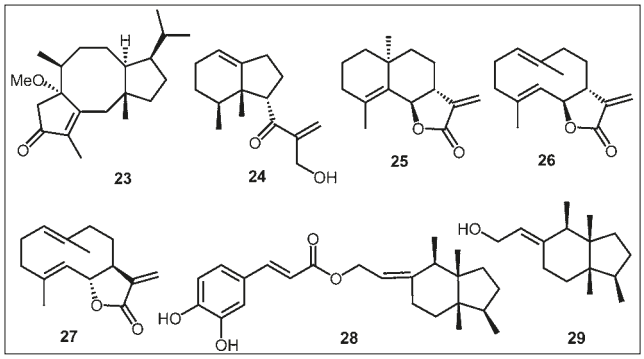
Many *Plagiochila* species contained cytotoxic plagiochiline A (**11**) (0.28μg/mL) against KB cell [12]. The ether extract of *Plagiochila ovalifolia* showed inhibitory activity against P-388 murine leukemia cells, and its constituents, plagiochiline A (**11**), plagiochiline A-15-yl octanoate (**12**), and 14-hydroxyplagiochiline A-15-yl (2*E*,4*E*)-dodecadienoate (**13**) exhibited IC_{50} values of 3.0, 0.05, and 0.05 μg/mL, respectively [17]. Compound **12** and plagiochiline A-15-yl decanoate (**14**) from *P. ovalifolia*, polygodial (**15**) from *P. vernicosa* complex, as well as sacculatal (**16**) from *P. endiviifolia* showed cytotoxic activity against a human melanoma cell line (IC_{50} value range 2–4 μg/mL). Compound **16** was cytotoxic also for Lu1 (IC_{50} 5.7 μg/mL), KB (3.2), LNCaP (7.6), and ZR-75–1 cells (7.6) (Cordell, Pezzuto, Asakawa, unpublished results). Lepidozenolide (**17**) showed potent cytotoxicity when evaluated in the P-388 murine leukemia cell line (IC_{50} 2.1 μg/mL) [18].

2α,5β-Dihydroxybornane-2-cinnamate (**18**) from *Conocephalum conicum* and lunularin (**19**) from *Dumortiera hirsuta*, exhibited cytotoxic activity against human HepG2 cells, with IC_{50} values of 4.5 and 7.4 μg/mL respectively [19].

Chandonanthus hirtellus produces a new sesquiterpene lactone, chandolide (**20**) [20], 13,18,20-tri-*epi*-chandonanthone (**21**) [21] and anadensin (**22**) which were evaluated for cytotoxic activity against the HL-60 leukemia cell line, and exhibited IC_{50} values, in turn, of 5.3, 18.1, and 17.0 μg/mL. 6α-Methoxyfusicoauritone (**23**) isolated from the same liverwort showed some cytotoxicity against KB cells (IC_{50} 11.2 μg/mL), although compounds **21** and **22** were inactive [20,22].

13-Hydroxychiloscyphone (**24**) from *Chilosyphus rivularis*, was tested against the RS322, RS188N, and RS321 yeast strains. It showed IC_{12} values of 75 and 88 μg/mL for strains RS321 and RS322. These data are characteristic of a selective DNA-damaging agent that does not act as a topoisomerase I or II inhibitor. Compound **24** also showed cytotoxic





activity against lung carcinoma A-549 cells (IC_{50} value 2.0 μg/mL) [23].

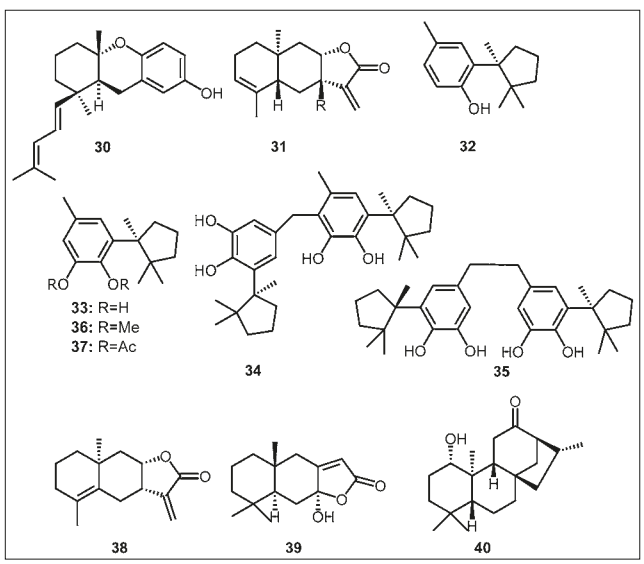
(-)-*ent*-Arbusculin B (**25**) and (-)-*ent*-costunolide (**26**) from *Hepatostolonophora paucistipula*, showed cytotoxic activity against P388 murine leukemia cells, with IC_{50} values of 1.1 and 0.7 μg/mL [24]. Costunolide (**27**) isolated from *Frullania nisqualensis* showed growth inhibitory activity against the A-549 human lung carcinoma cell line with an IC_{50} value of 12 μg/mL and moderate, but selective, DNA-damaging activity against the RS321N, RS322YK, and RS167K mutant yeast strains, with IC_{12} values of 50, 150, and 330 μg/mL [25]. Naviculyl caffeate (**28**), from *Bazzania novae-zelandiae*, demonstrated growth inhibitory effects against P-388 murine leukemia cells with a GI_{50} value of 0.8–1.1 μg/mL, although naviculol (**29**) was inactive [26]. Riccardiphenol C (**30**), from *Riccardia crassa* showed slight cytotoxicity against BSC-1 (African green monkey kidney epithelial) cells at 60 μg/disk [27].

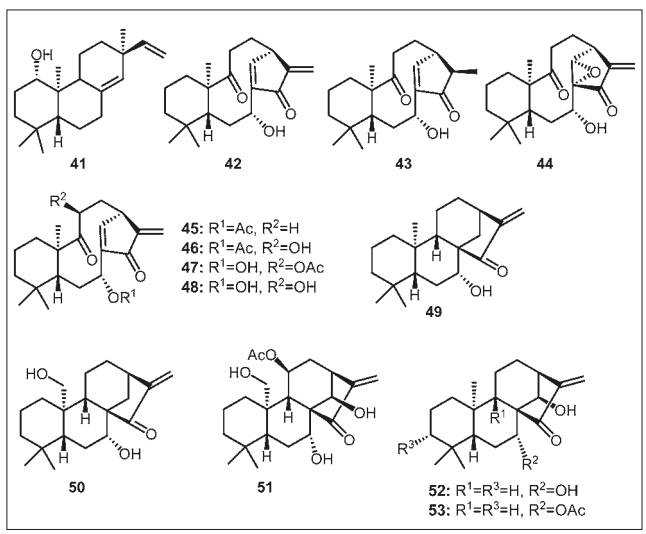
The ether and methanol extracts of the Tahitian *Mastigophora diclados* showed cytotoxic activity against HL 60 cells at IC_{50} 2.4 and 13.1 μg/mL and KB cells at 14.6 and 32.5 μg/mL, respectively [28]. (-)-Diplophyllolide (**31**), α-herbertenol (**32**), (-)-herbertene-1,2-diol (**33**), mastigophorene C (**34**) and mastigophorene D (**35**) isolated from both extracts were cytotoxic against HL 60 cells with IC_{50} values of 2.5, 1.4, 12.8, 1.4, and 2.4 μg/mL. They also showed cytotoxicity against KB cells (IC_{50} values of 14.2, 3.3, 12.5, 11.8 and 14.8 μg/mL). 2-Methoxy (**36**) and diacetoxy derivatives (**37**) of herbertane-1,2-diol (**33**) showed evidence of having less potent cytotoxicity than the parent compound against both HL 60 and KB cells. However, (-)-diplophyllin (**38**) did not indicate cytotoxicity against either of these cell lines [28].

Glaucescenolide (**39**) from *Schistochila glaucescens* showed cytotoxic activity against P-388 mouse leukemia cells (IC_{50} 2.3 μg/mL) [29]. *ent*-1β-Hydroxykauran-12-one (**40**), isolated from *Paraschistochila pinnatifolia* and 1α-hydroxy-*ent*-sandaracopimara-8(14),15-diene (**41**) from *Trichocolea mollissima*, showed IC_{50} values of 15 and >25 μg/mL, when evaluated against this same cell line [30].

The ethanol-soluble extract of *Lepidolaena taylorii* which showed cytotoxicity against P-388 cell line, (IC_{50} 1.3 μg/mL), was purified to give the 8,9-secokaurane diterpenoids, rabdoumbrosanin (**43**), 16,17-dihydrorabdoumbrosanin (**43**), 8,14-epoxyrabdoumbrosanin (**44**) and their related compounds **45–48** and the *ent*-kaur-16-en-15-ones (**49–53**). In turn, *L. palpebrifolia* also elaborated the 8,9-*seco*kauranes (**42**, **44**). The cytotoxicity of these *ent*-8,9-*seco* and *ent*-kaurenes was tested against the mouse P-388 leukemia and several human tumor cell lines, inclusive of six leukemia and a range of organ-specific cancer cell lines. Compounds **42** and **44** showed the most potent cytotoxic activities (mean IC_{50} values of 0.006 and 0.27 μg/mL; GI_{50} values of 0.10 and 1.2 μ*M*, respectively). Compound **44** also showed cytotoxicity against P-388 cell at 0.80 μ*M*.). Compounds **42** (including 10% of **43**) and **44** showed differential cytotoxicity in *vitro* when tested against five further leukemia cell lines with **41** showing an average IC_{50} value of 0.4 μ*M*; however, cell growth was not inhibited by **44** (IC_{50} >50 μ*M*). The growth of seven colon cancer cell lines was inhibited also by **42** (mean IC_{50} value, 6 μ*M*) [31,32]. Compounds **42** and **44** were tested in an *in vivo* hollow fiber model system, in which neither compound was active at the doses tested (18 and 12 μg/kg for **42** and 150 and 100 μg/kg for **44**). Compound **48** was most active against several leukemia cell lines (mean GI_{50} 0.3 μ*M*) and least active against various central nervous system cancer cell lines (mean GI_{50} 6 μ*M*) [32].

Ent-kaurene (**54**) and (**55**) from the New Zealand *Jungermannia* species showed weak cytotoxic activity against P-388 at 0.48 and 25μg/mL, respectively [33] Among the isolated compounds, 8,9-secokaurenes (**42, 44, 48**) showed selective toxicity amongst human tumor cell lines at a concentration of 1.2, 2.5 and 1.5 μM, respectively.

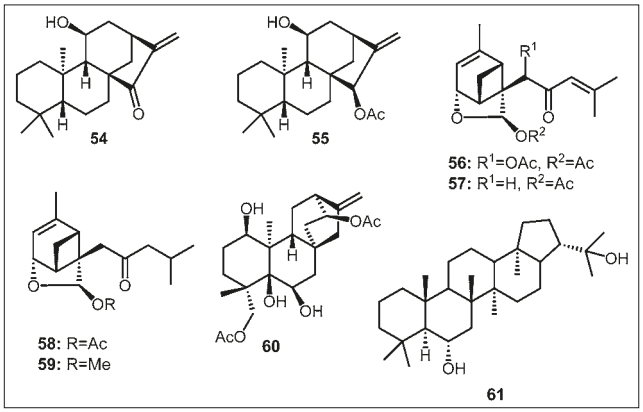




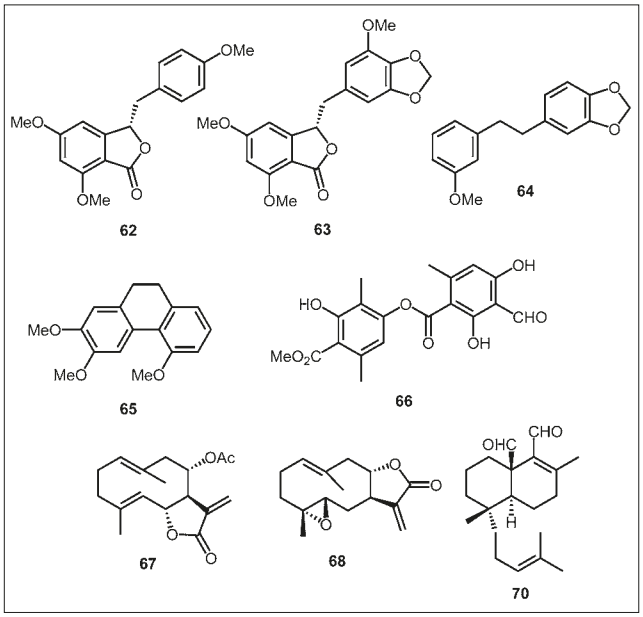
The mode of action for the cytotoxicity of the *ent*-8,9-*seco*kaur-16-en-15-one and *ent*-kaur-16-en-15-one series was supported by *Michael* addition of a thiol to the C-16–C-17 double bond of **42**, but the C-8–C-14 double bond of **43** was relatively unreactive [31,32].

Clavigerins A-D (**56–59**) isolated from *Lepidolaena clavigera* showed a weak cytotoxicity (30μg/disk) against BSC cells [34].

A new atisane-2 derivative (**60**) from *Lepidolaena clavigera* exhibited weak inhibitory activity against mouse lymphocytic leukemia cells (P-388) with an IC_{50} value of 16 μg/mL [35] [35]. α-Zeorin (**61**) has been isolated from several liverworts and displayed cytotoxic activity against P-388 cells with an IC_{50} of 1.1 μg/ml [36,37].

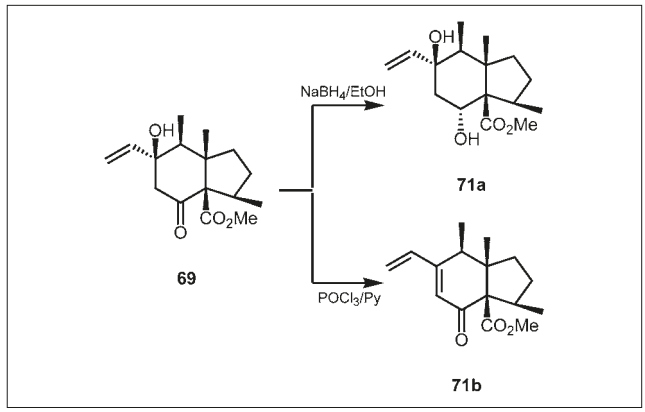


The crude ether extract of two unidentified Indonesian and Tahitian *Frullania* species exhibited cytotoxic activity against both the HL-60 and KB cell lines, with at EC_{50} values of 6.7 and 1.6 μg/mL (HL-60 cells) and 1.6 and 11.2 μg/mL (KB cells), respectively [38]. Bioactivity-guided fractionation of the Indonesian sample led to the isolation of (+)-3α-(4'-methoxybenzyl)-5,7-dimethoxyphthalide (**62**), (-)-3α-(3'-methoxy-4',5'-methylenedioxybenzyl)-5,7-dimethoxyphthalide (**63**), together with 3-methoxy-3',4'-methylenedioxybibenzyl (**64**), 2,3,5-trimethoxy-9,10-dihydrophenanthrene (**65**) and atranorin (**66**), among which



61 possessed the most potent cytotoxic activity against HL-60 and KB cells showing IC_{50} values of 0.92 and 0.96 μ*M*. The other compounds (**63–65**) and the 6'-nitro derivative of **63** indicated much less activity against both cell lines (HL-60 IC_{50} value range, 6.3–96.6 μ*M*; KB IC_{50} value range, 5.5–124.3 μ*M*). From the Tahitian sample, costunolide (**27**) and tulipinolide (**67**) were obtained and the former germacranolide shown to be cytotoxic against the HL-60 cell line (IC_{50} 4.6 μ*M*) [38].

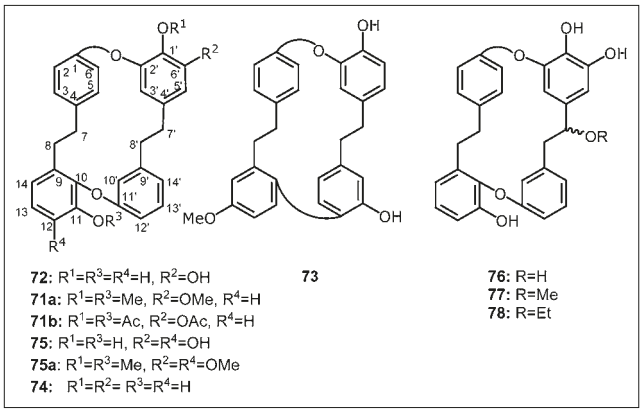
Porella perrottetiana produced cytotoxic compounds against both HL-60 and KB cell lines [38]. The same treatment as mentioned above gave 4α,5β-epoxy-8-*epi*-inunolide (**68**), 7-keto-8-carbomethoxypinguisenol (**69**) and perrottetianal A (**70**). The former two compounds exhibited moderate or weak cytotoxicity against HL-60 (IC_{50} 8.5 and 2.7 μ*M*) and KB cells (IC_{50} 52.4 and 46.3 μ*M*). 7α-Hydroxy-8-carbomethoxypinguisenol (**71a**) and acutifolone A (**71b**) prepared from **69** by reduction and dehydration were evaluated against HL-60 (IC_{50} 83.10 and >177 μ*M*) and KB cells (IC_{50} 2.7 and 46.6 μ*M*). It was suggested that the dienone group plays an important role in the mediation of cytotoxcity against HL-60 cells [38].



Macrocyclic bis-bibenzyls such as marchantin A (**72**) and riccardin A (**73**) were firstly isolated from liverworts by Asakawa et al.[1,2]. Up to now, more than 60 macrocyclic and acyclic bis-bibenzyls have been isolated from many liverworts and their stereostructures established [3,12,39]. The cyclic bis-bibenzyls such as marchantin (e.g.**72**) and riccardin series (e.g.**73**) might be biosynthesized from bibenzyls that correspond chemically to dihydrostilbenes [40]. This assumption was proved by feeding experiments using radioactive and ^{13}C-labelled precursors, like L-[*U*-^{14}C]-phenylalanine, [*U*-^{14}C]dihydro-*p*-coumaric acid, [2-^{13}C]acetate, and L-[^{13}COOH]phenylalanine. Marchantin C (**74**) was biosynthesized by coupling of two lunularic acid (**80**), followed by cytochrome P-450, named marchantin C hydroxylase to afford marchantin A (**72**) [4].

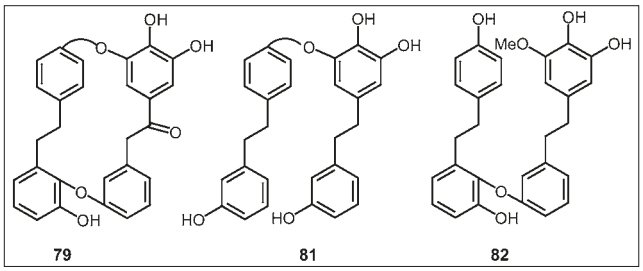
Macrocyclic bis-bibenzyls biosynthesized by liverworts possess various biological activities, like antimicrobial, antifungal, muscle relaxant, cytotoxicity against KB cells, inhibitory activity against DNA-polymerase β, cardiovascular activity, anti-HIV, and antitumor activity [2,3, 13]

The methanol extract (105 g) of a Japanese specimen of *M. polymorpha* was chromatographed over silica gel and Sephadex LH-20 to give the cyclic bis-bibenzyls, marchantin A (**72**) (30 g), and its analogues, marchantins B (**75**), C (**74**), D (**76**), E (**77**), G (**78**), J (**79**). The yield of **72** is dependent



upon the particular *Marchantia* species being investigated. Pure **72** (80 to 120 g) has been isolated from 6.67 kg of dried *M. paleacea* var. *diptera*[4].

This thalloid liverwort elaborates not only the marchantin series, including marchantins A (**72**), B (**75**), D (**76**), and E (**77**), but also the acyclic bis-bibenzyls, perrottetin F (**81**) and paleatin B (**82**). Marchantins A (**72**), B (**75**), D (**76**), perrottetin F (**81**), and paleatin B (**82**) showed cytotoxicity against KB cells (IC_{50} range 3.7–20 μ*M*) and P-388 (T/C 117) [13].

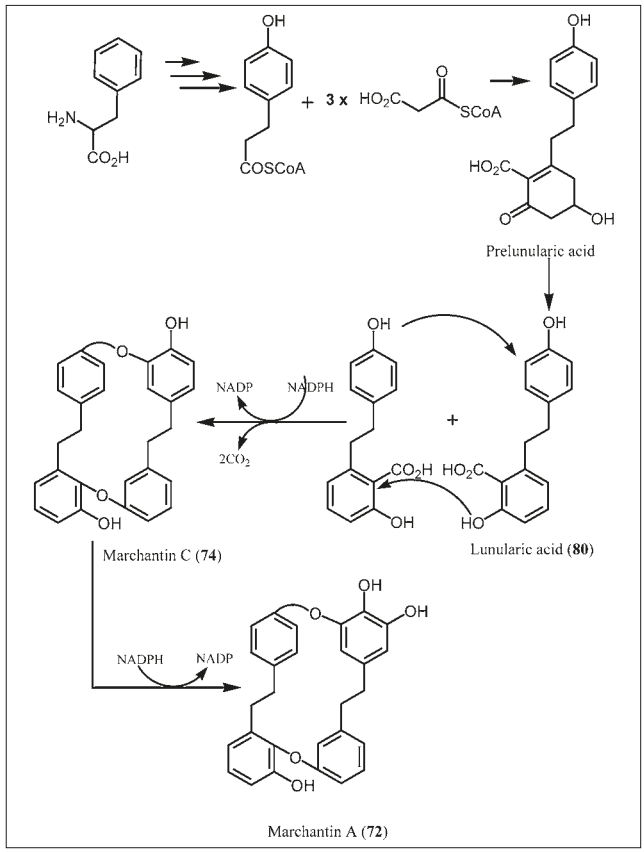


Marchantin A (**72**) induced cell growth inhibition in human MCF-7 breast cancer cells at IC_{50} 4.0 μg/mL. Fluorescence microscopic and a Western blot analysis indicated that compound **72** induced apoptosis of MCF-7 cells through a caspase-dependent pathway. The phenolic hydroxy groups at C-1' and C-6' are responsible for inducing cytotoxic and antioxidant activity [41].

Marchantin C (**74**) and its dimethyl ether, 7,8-dehydromarchantin C and its dimethyl ether were synthesized and their possible modulatory effects on P-glycoprotein in VCR-resistant KB/VCR cells were investigated [42]. The results indicated that **74** was the most potent inhibitor of cell proliferation in both KB and KB/VCR cells among these four synthetic compounds, while the three derivatives of **74** has little antiproliferative activity. Potent apoptosis in KB/VCR cells was induced by treatment with 16 μ*M* of dimethyl ether of marchantin C (**74**) and 0.2 μ*M* VCR for 48 hours [42]. Marchantin C also showed the induction of apoptosis of human glioma A172 cells at 8–16μ*M* [43].

Marchantin C (**74**), neomarchantins A (**83**) and B (**84**), and a mixture of sesquiterpene/bis-bibenzyl dimers, GBB A (**85**) and GBB B (**86**) from *Schistochila glaucescens* showed growth inhibitory activity against the P-388 cell line, with IC_{50} values of 18, 7.6, 8.5, and 10.3 μg/mL, respectively [29].

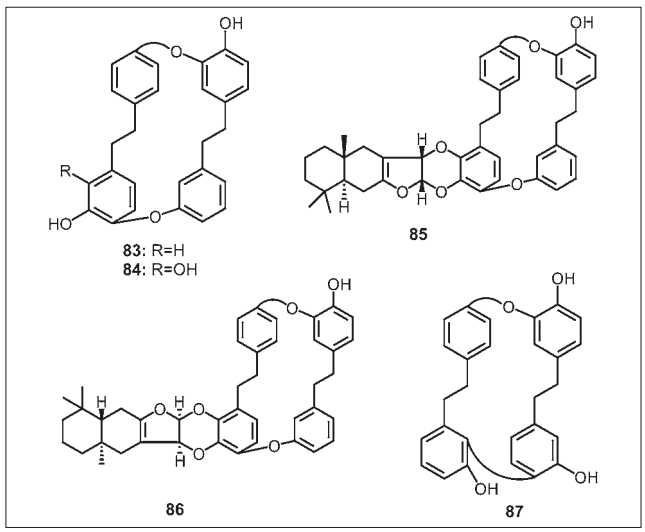
Riccardin D (**87**) from *Monocolea forsteri* [3] and *Marchantia polymorpha* [44, 45] indicated antiproliferative activity on human glioma A172 cells and induction of apoptosis at 16 μ*M*. Compound **87** also possesses potent



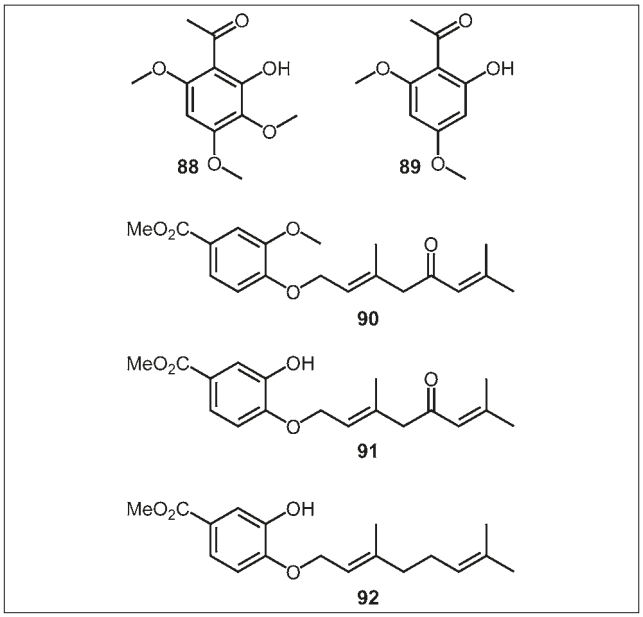
effects in reversing P-glycoprotein-mediated multidrug resistance [44].

2-Hydroxy-3,4,6-trimethoxyacetophenone (**88**) and 2-hydroxy-4,6-dimethoxyacetophenone (**89**) from *Plagiochila fasciculata*, were inactive against the P-388 cell line (IC_{50} values of >50 μg/mL) [46].

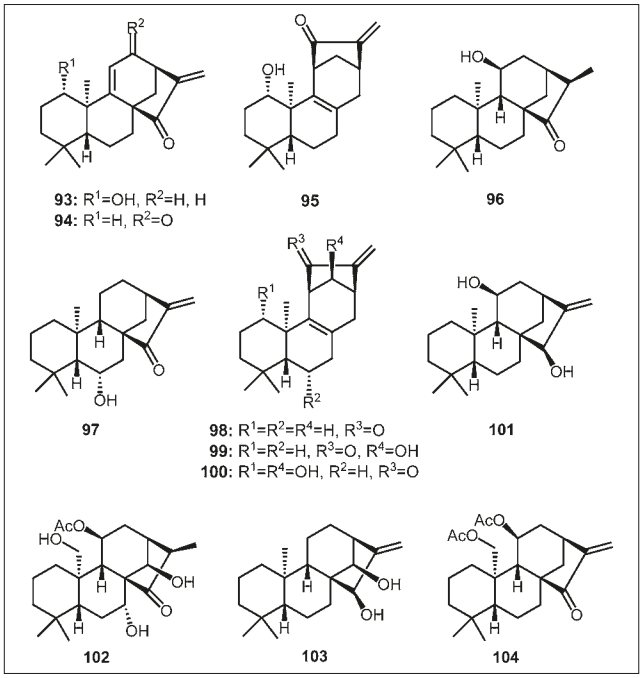
Trichocolea lanata and *T. tomentella* produced tomentellin (**90**), which showed inhibitory activity against African green monkey kidney epithelial (BSC-1) cells at 15 μg/mL, with no antiviral effects against herpes simplex or polio viruses. Demethoxytomentellin (**91**) from *T. tomentella* showed a similar cell growth inhibitory effect, indicating that both an allylic ether and a conjugated enone substructure are required for such activity [47]. Methyl-4-[(2*E*)-3,7-dimethyl-



2,6-octadienyl]oxy]3-hydroxybenzoate (**92**), isolated from *T. hatcheri*, showed a lack of cytotoxicity (IC_{50} >100 μ*M*) against both KB and SK-MEL-3 human melanoma cells, as well as NIT 3T3 fibroblasts [48]



The *ent*-kauranes and kaurenes (**54**, **93–95)** isolated from *Jungermannia* species inhibited HL-60 cells with IC_{50} values, in turn, of 0.49, 7.0, 0.59, and 0.28 μ*M*. Treatment of **54** and **93–95** caused proteolysis of poly(ADP-ribose) polymerase, a sign of activation of the apoptotic machinery, whereas the feature of cell death induced by treatment with compounds **93** and **94** was necrosis. Treatment with compound **95** induced apoptosis (see below) [49]. The *ent*-kaurane diterpenoids **96, 97** and **98–100** from a *Jungermannia* species showed cytotoxicity for HL-60 cells with IC_{50} values of 1.00, 0.40, 1.21, 1.28, and 0.78 μ*M*, respectively [50].



The *ent*-kaurenes **54**, **101–106,** isolated from the Japanese liverwort *Jungermannia truncata*, were evaluated for cytotoxicity against HL-60 human leukemia cells. Of these, *ent*-11α-hydroxy-16-kauren-15-one (**54**) induced apoptosis (programmed cell death) in this cell line partly through a caspase-8 dependent pathway [33,51]. The presence of an enone group in this class of molecule appears to be essential for the induction of apoptosis and the activation of caspases in human leukemia cell lines [49,52].

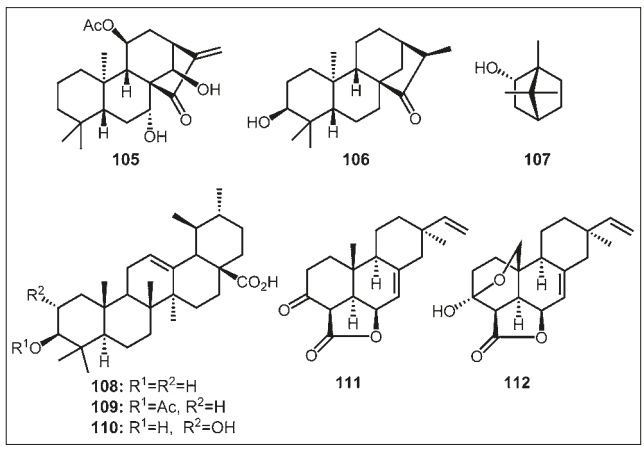
ent-Kaurenes **54**, **50** and **105** and *ent*-9(11),16-kauradien-12,15-dione (**93**), and the rearranged *ent*-kaurene, jungermannenone A (**95**), selectively inhibited nuclear factor-μB (NF-μB)-dependent gene expression due to treatment with TNF-α. Compound **54,** in combination with TNF-α caused a dramatic increase in apoptosis in human leukemia cells accompanied by activation of caspases. Compound **54**, when combined with camptothecin, also caused an increase in apoptosis [53,54]. Jungermanenones A-D (**95–100**), obtained from *Jungermannia* species, induced cytotoxicity against human leukemia HL-60 cells at 50% inhibitory concentrations of 1.3, 5.5, 7.8, and 2.7 μ*M*, respectively, and DNA fragmentation and nuclear condensation. Both are biochemical markers of apoptosis induction, and apoptosis was induced through a caspase-independent pathway. Compounds **95** and **100** showed inhibitory activity for NF-κB, which is a transcriptional factor of antiapoptotic factors. Thus, *ent*-kaurene diterpenoids from liverworts may be promising candidates as antitumor agents [55,56].

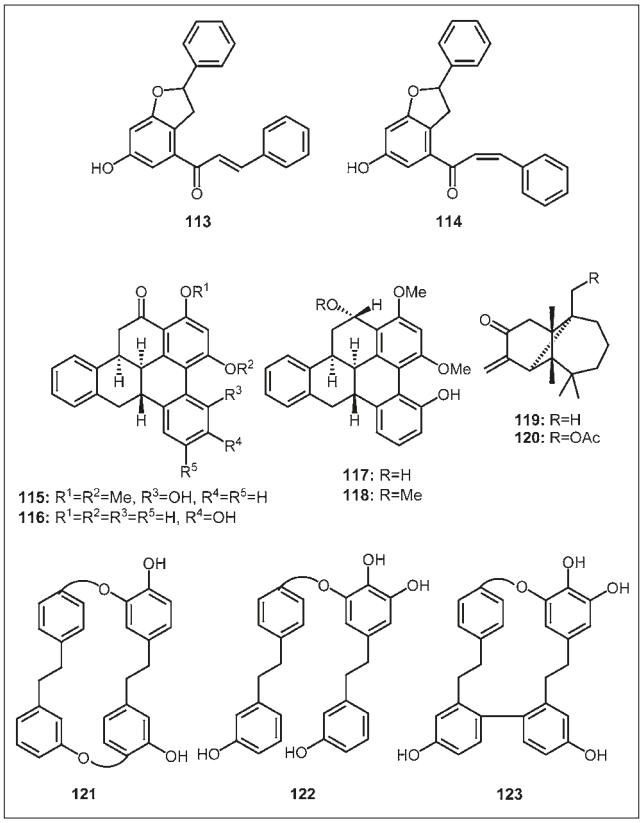
Some monoterpenoids, such as bornyl acetate (**107**) found in liverworts demonstrate potent apoptosis-inducing activities against the cultured cells of *Marchantia polymorpha*. Apoptosis induced by monoterpenoids occurs *via* the production of active oxygen species such as H_2O_2 [57].

The ursane triterpenoids from the liverwort *Ptilidium pulcherrimum*, ursolic acid (**108**), acetoxyursolic acid (**109**), and 2α,3β-dihydroxyurs-12-en-28-oic acid (**110**) showed inhibition of the growth of PC3 human prostate cancer cells, at concentrations between 10.1±1.00 and 39.7±2.98 μ*M* [58].

Previously, the two pimarane diterpenoids momilactones A (**111**) and B (**112**), which were identified as phytoalexins in rice, have been isolated from the moss *Hypnum plumaeforme* (Hypnaceae) [59]. Momilactone B (**112**) was shown to have cytotoxicity against human colon cancer HT-29 and SW620 cells at 1μ*M* [60].

Pallidisetin A (**113**) and pallidisetin B (**114**), isolated from the moss *Polytrichum pallidiscetum*, showed cytotoxicity against human melanoma (RPMI-7951) and human





glioblastoma multiforme (U-251 MG) cells, with ED_{50} values of 1.0 and 1.0 μg/mL and 2.0 and 2.0 μg/mL [61].

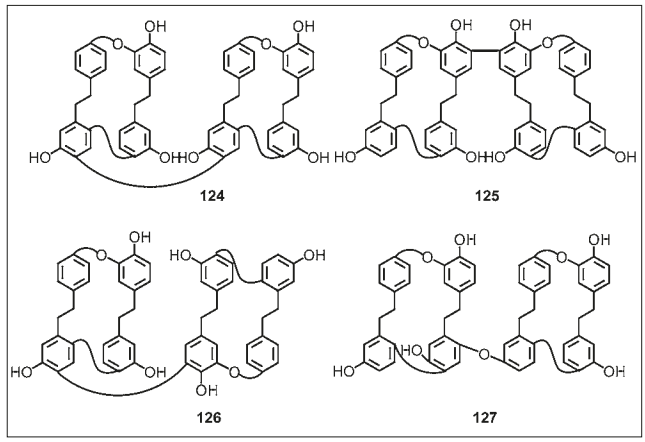
Three cytotoxic compounds, 1-*O*-methylohioensin B (**115**), 1-*O*-methyldihydroohioensin B (**116**) and 1,14-di-*O*-methyldihydroohioensin B (**118**) were also isolated from the moss *Polytrichum pallidisetum*. Compound **115** proved to be cytotoxic for human colon adenocarcinoma (HT-29), human melanoma (RPMI-7951), and human glioblastoma multiforme (U-251 MG) cells, with ED_{50} values of 1.0, 1.0, and 2.0 μg/mL. Compound **116** showed inhibitory activity only against U-251 cells (ED_{50} 0.8 mg/mL) while **118** inhibited the growth of the A549 lung carcinoma (A549) (ED_{50} 1.0 μg/mL) and RPMI-7951 melanoma (ED_{50} 1.0 μg/mL) cell lines [61]. Ohioensin H (**117**) from *Polytrichum commune* did not show any cytotoxicity against the five human cancer cell lines in which it was evaluated (IC_{50} in all cases >5 μg/mL) [62].

Marsupellone (**119**) and acetoxymarsupellone (**120**) from *Marsupella emarginata* showed cytotoxicity (ID_{50} 1μg/mL) against P388 [10]. Riccardins A (**73**) and B (**121**) which were 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. *Radula perrottetii* contained cytotoxic perrottetin E (**122**) (12.5 μg/mL) against KB cells [10].

1.3.2 *Antiviral compounds from bryophytes*

Marchantins A (**72**), B (**75**), D (**76**), perrottetin F (**81**), and paleatin B (**82**) showed anti-HIV-1 activity (IC_{50} range 5.3–23.7 μg/mL) [10,63] as shown in Table 2.

The H1N1 and H5N1 influenza A virus caused pandemics throughout the world in 2009. Influenza A possesses an endonuclease within its RNA polymerase comprised of PA, PB1, and PB2 subunits. In order to obtain potential new anti-influenza compounds, 33 different types of phytochemicals



were evaluated using a PA endonuclease inhibition assay *in vitro* [64]. Among them, the bis-bibenzyls, marchantins A (**72**), B (**75**), and E (**77**) and plagiochin A (**123**) inhibited influenza PA endonuclease activity at a concentration of 10 μ*M*. This was the first evidence that the phytochemicals derived from liverworts can inhibit influenza A endonuclease.

Blasia pusilla produces the bis-bibenzyl dimers, pusilatins A-D (**124–127**). Pusilatins B (**125**) and C (**126**) were found to possess DNA polymerase b inhibitory activity (IC_{50} values of 13.0 and 5.16 μ*M*, respectively) and showed weak HIV-RT inhibitory activity [65] as shown in Table 2.

Table 2. Cytotoxic and anti-viral bis-bibenzyls from bryophytes against KB cells, DNA polymerase β and HIV-1

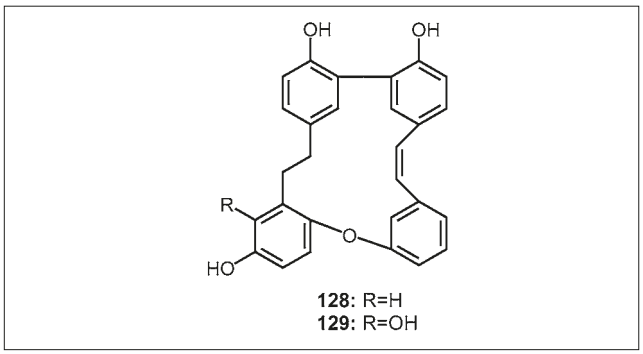
Bis-bibenzyls	KB (μ*M*)	KB+VLB* (μ*M*)	KB-VLB* (μ*M*)	DNA polymerase-b (μ*M*)	HIV-1 (μg/mL)
Marchantin A (**72**)	3.7	1.3	2.7	97.5	11.5
Marchantin B (**75**)	3.2	9.3	4.0	14.4	9.3
Marchantin D (**76**)	10.8	>20.0	>20.0	55.4	23.7
Marchantin E (**77**)	7.6	1.6	2.9	20.0	21.2
Perrottetin F (**81**)	>20.0	12.6	9.5	14.3	5.3
Paleatin B (**82**)	>20.0	>20.0	>20.0	18.5	22.1
Pusilatin B (**125**)	13.1	15.3	11.9	13.0	**
Pusilatin C (**126**)	13.8	7.1	11.7	5.16	**

* VLB: Vinblastine: ** not tested

1.3.3 *Tubulin polymerization inhibition*

Marchantin C (**74**) strongly inhibited the growth of human cervical tumor xenografts in nude mouse and decreased the quantity of microtubules in a time- and dose-dependent manner at the G2/M phase in human glioma tumor cells and HeLa (human cervical adenocarcinoma cell line) cells at 8–16 μ*M* [43, 66]. The same compound (**74**) decreased the polymerization rate of gross tubulin, similar to the microtubule depolymerizor, vincristine, at 8–24 μ*M*. These results indicated that **74** plays the same role in microtubule depolymerization in both its apoptotic effects in the cell and subsequent antitumor activity *in vivo*. Compound **74** is a novel microtubule inhibitor that induces mitotic arrest of tumor cells and suppresses tumor cell growth. The structure of marchantin C is distinct from classical microtubule inhibitors like colchicine, paclitaxel, vinblastine, and vincristine. However, this macrocyclic bis-bibenzyl may be regarded as a potential antitumor agent as a result of inhibiting microtubule polymerization [43,66].

Isoplagiochins A (**128**) and B (**129**) isolated from *Plagiochila fruticosa* inhibited the polymerization of tubulin with IC_{50} values of 50 and 25 μ*M*. The dihydro derivatives of both **128** and **129** were found to be inactive (IC_{50} >100 μ*M*), and, when compared with the parent compounds, indicated that a restricted biaryl ring system is favorable for tubulin binding. A Monte Carlo search showed that the presence of two aromatic rings connected by a two-carbon bridge with a double bond may serve to maintain the backbone conformation [67].



128: R=H
129: R=OH

Figure 8. *Cryptoporus volvatus* (Polyporaceae) on dead pine tree.

2. CHEMICAL CONSTITUENTS OF INEDIBLE FUNGI

2.1 Introduction

There are about 1500 identified fungi in Japan among which 300 species are edible, 1150 inedible and 50 toxic. The chemical constituents of toxic and edible fungi have been fully studied. Recently, many biologically interesting compounds were isolated from inedible mushrooms by our group and their structures and biological activity reported [68,69].

2.2 Bioactive compounds from a few inedible fungi

The biological properties of the terpenoids and aromatic compounds and acetogenins isolated from inedible fungi in our laboratory are: 1) cytotoxic, 2) anti-HIV-1, 3) anti-HSV, 4) antimicrobial and antifungal, 5) antitumor promotion, 6) insecticidal, 7) nematocidal, 8) antifeedant against snail and slug, 9) plant growth inhibitory, 10) anti-Alzheimer disease, 11) superoxide anion release inhibitory, 12) anticholestemic activity, and 13) spider female sex pheromone production.

2.2.1 *Cytotoxic compounds from Cryptoporus volvatus and Daldinia species*

The fungus *Cryptoporus volvatus* belonging to the Polyporaceae grows on decayed pine tree and its bruiting body emits resinous smell by which insects such as *Parabolitophagus felix* and *Ischnodactylus loripes* are attracted. The wet and dried fruiting bodies contain surprisingly strong bitter principles. The fractionation of the ethyl acetate extracts from 40kg of wet fungus gave 20g order of cryptoporic acids A (=CPA-A) (**130**), CPA-B (**131**), CPA-C (**132**), CPA-D (**133**), CPA-F (**135**), CPA-G (**136**), together with CPA-E (**134**, 700g) as the major component. Compounds **130–136** indicated CPA-F (**135**), CPA-G (**136**). Compounds **130–136** indicated very strong inhibition effect for superoxide anion radical from guinea-pig macrophage induced by O_2^- radical stimulant formyl methionyl leucyl phenylalanine (FMLP) at a concentration of 13, 25, 0.07, 0.1, 0.05, 0.3 and 0.15 μg/mL, respectively. CPA-C (**133**) also possessed the 88% of inhibitory effect for superoxide anion release from rabbit polymorphonuclear leucocyte induced by O_2^- radical stimulant FMLP at a concentration of 6 μg/mL [70]. Those bitter drimanes with isocitric acid moiety showed cytotoxicity against KB, Lui, LNCaP ad ZR-75–1 cancer cell lines as shown in Table 3. The cytotoxicity of the permethylated CPA series is more potent than that of their naturally occurring CPAs [Asakawa & Hahimoto, unpublished results].

The same CPA series (**130–136**) showed inhibitory effect at a concentration of >50, >50, 40, >50, >50, >50 and >50 μg/mL against lysosome enzyme release from rat peritoneal neutrophil cell stimulated by FMLP (10^{-6} *M*) and cytochalasin B (5 μg/ml) [70].

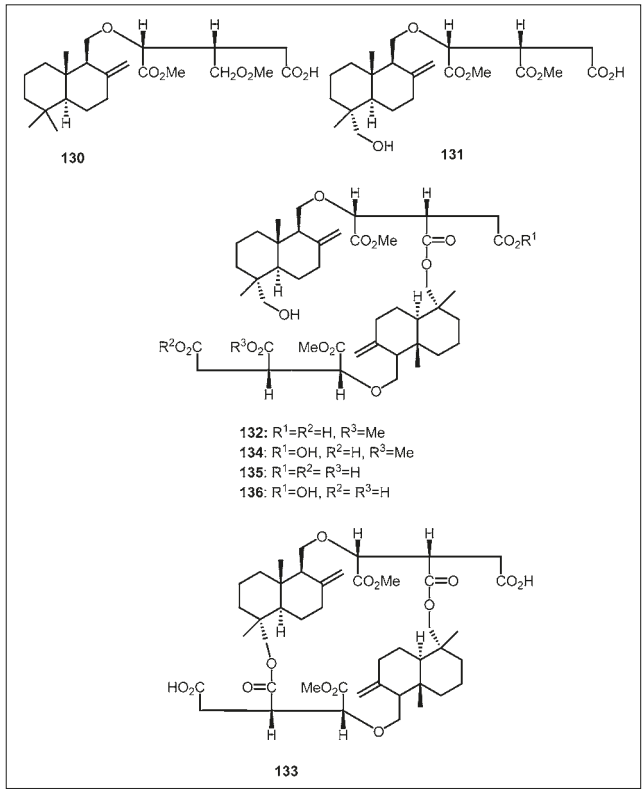


Table 3. Cytotoxicity testing (ED_{50}: μg/ml) of cryptoporic acids A-G and their methylated derivatives on KB, KB-V, Lu1, LNCaP and ZR-75–1 cell lines.

Samples	KB	KB-V (+VLB)[a]	KB-V (-VLB)[b]	Lu1	LNCaP	ZR-75-1
Cryptoporic acid-A (**130**)	>20	>20	>20	>20	>20	>20
CPA-A Me3	2.3	2.6	3.4	>20	>20	12.2
CPA-B(**131**)	>20	>20	>20	>20	>20	>20
CPA-B Me3	>20	3.8	>20	>20	>20	>20
CPA-C(**132**)	>20	>20	>20	>20	>20	12.9
CPA-C Me5	14.2	3.8	>20	>20	15.7	13.0
CPA-D(**133**)	>20	>20	>20	>20	>20	14.5
CPA-D Me4	>20	>20	>20	>20	>20	>20
CPA-E(**134**)	>20	>20	>20	>20	>20	>20
CPA-E Me5	>20	>20	>20	>20	>20	>20
CPA-F(**135**)	>20	>20	>20	>20	>20	>20
CPA-G(**136**)	>20	>20	>20	>20	>20	>20

[a]: +VLB = presence of vinblastine; [b]: -VLB = absence of vinblastine

An unidentified *Daldinia* species belonging to the Xylariaceae produces cytochalasin series belonging to 10-pheyl-(11) class of cytochalasins and 10-phenyl-22-oxa-(12)-cytochalasin [71]. Compounds **137–141** exhibited the cytotoxic activity against KB cell line as shown in Table 4 [Hashimto & Asakawa, unpublished results].

Table 4. Cytotoxic effect of cytochalasins against KB cells

Cytochalasins	Inhibition %	
	KB cell at $10^{-5}M$	KB cell at $10^{-6}M$
137	96	58
138	84	50
139	89	44
140	84	50
141	96	28

2.2.2 *Antiviral compounds from inedible fungi*

The cryptoporic acids isolated from *C. volvatus* showed not only cytotoxicity against cancer cell lines but also inhibitory activity against HIV 1 RT (p66) as shown in Table 5. Among which, two permethylated products, cryptoporic acid B (**131**) trimethyl ether and cryptoporic acid E (**134**) pentamethyl ether showed highest HIV-1 inhibitory activity [Hashimoto & Asakawa, unpublished results].

Table 5. Inhibitory effect of cryptoporic acids A-G and their derivatives on HIV-1 RT (p66).

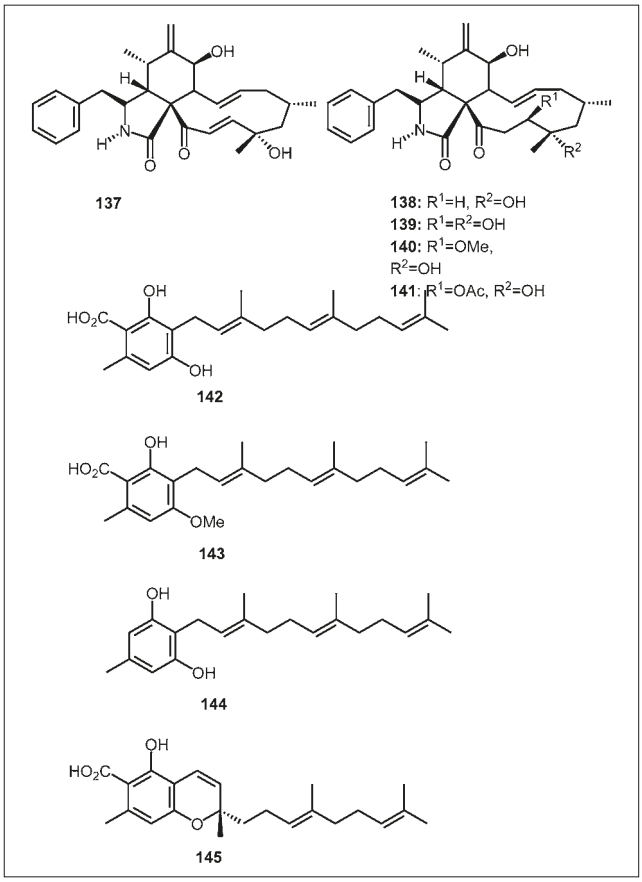
Samples	% Inhibitory at 20 μg/mL	IC_{50} (μg/mL), r^2
Cryptoporic acid A (=CPA-A) (**130**)	16.10	Inactive
CPA-A Me3	2.23	Inactive
CPA-B(**131**)	5.77	Inactive
CPA-B Me3	2.23	61.0 μg/mL, r^2=0.992
CPA-C (**132**)	99.6	Inactive
CPA-C Me5	0.00	Inactive
CPA-D (**133**)	25.40	Inactive
CPA-D Me4	0.00	Inactive
CPA-E (**134**)	19.00	Inactive
CPA-E Me5	3.75	42.2 μg/mL, r^2=0.889
CPA-F (**135**)	99.7	Inactive
CPA-G (**136**)	37.3	Inactive

Kashiwada et al. [72] reported that *Rododendron dauriaum* contained (-)-daurichromenic aicd (**145**) which showed potent anti-HIV activity as shown in **Table 6**. The inedible fungous *Albatrellus dispansus* produces a large amount of grifolic acid (**146**), along with grifolic acid methyl ether (**143**) and grifolin

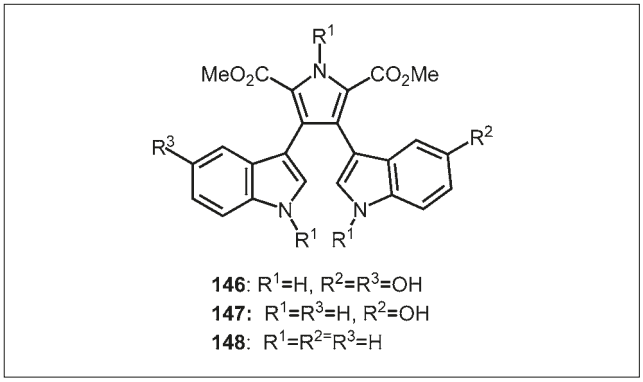
Table 6. Anti-HIV I activity of daurichomenic acid and grifolins

Compounds	ED_{50} μg/mL[a]	CC_{50} μg/mL[b]
Grifolic acid (**142**)	40	40
Grifolic acid methyl ether(**143**)	53	53
Grifolin (**144**)	38	38
(-)-Daurichromenic acid (**145**)	0.00056	557

α: 50% effective concentration; β: 50% cytotoxic concentration



(**144**). Acidic treatment of **142** gave racemate daurichromenic acids which was purified by HPLC using chiral column to give natural (-)-daurichromenic acid (**145**) [Hashimoto & Asakawa, unpublished results]. *Lycogala epidendrum*, a smile mould belonging to the Myxomycetes elaborates unique pyrroledicarboxylates attached to two indoles,, named lycogarubins A-C (**146–148**) of which compound **148** showed anti-HSV virus activity at a concentration of IC_{50} 17.2 μg/mL *in vitro* [68].



3. CONCLUSION

The bryophytes and inedible fungi are found in whole the world. They are generally very tiny organisms. However, they produce a great number of secondary metabolites, including pungent and bitter terpenoids and polyphenolic compounds or nitrogen containing compounds many of which show interesting biological activity such as cytotoxicity, antiviral activity. Only 5% of the total bryophytes have been chemically investigated since 20 century. Further search on the secondary metabolites of bryophytes and inedible fungi will results in the discovery of many different compounds with biologically and pharmaceutically interest.

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