SESQUITERPENE LACTONES AND HETEROCYCLIC COMPOUNDS OF BRYOPHYTA[+]

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Abstract: A review of some recent works on the chemical constituents of bryophytes is presented.

Introduction

Bryophyta are situated between Chlorophyta and Tracheophyta and divided into three classes, Anthocerotae, Hepaticae and Musci. It is known by bryologist that some bryophytes contain sweet or bitter substances, and pungent components. Bryophytes often show some remarkable biological activities like allergenic contact dermatitis, anticancer, antimicrobial and plant growth inhibitory or accelelate effects. In 1905, Müller[1] suggested that the component of the oil body in the liverworts was composed of sesquiterpenes. In spite of the presence of the biologically active substances, the chemical constituents of bryophytes have not been well investigated because the collection, separation, and identification of bryophytes are time-consuming.

Recent development of analytical apparatus makes it easy to determine the structure of even a micro-sized sample, and many terpenoids and aromatic compounds have been obtained from bryophytes, particularly from the liverworts. We have been interested in the biologically active substances included in

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[+] This paper is dedicated with the best wishes to Emeritus Professor Shigehiko Sugasawa on the occasion of his 80th birthday.

bryophytes and have stadied the chemical constituents from the point of view of pharmacognosy and the application as the source of the medicinal drugs. In this paper, we are concerning with a review of the recent work on the chemical constituents, particularly, sesquiterpene lactones, and heterocyclic compounds, including indole alkaloids, furanoterpenoids and flavonoids found in Bryophyta.

Sesquiterpene lactones of liverworts

In Europe, it is known that epiphytic bryophytes, namely *Frullania* and *Radula* cause some occupational allergies associated with handling European woods. In a typical case, *Frullania* species stored for a half century caused the intense allergy. In 1969, Ourisson and his coworkers[2a-b] isolated the active substances from *Frullania tamarisci* and *F. dilatata*, and determined their structures to be eudesmanolide [1], named frullanolide, and its enantiomer [5], respectively. This is the first isolation of the biological active substances against human body from bryophytes. Later, it was confirmed that some unknown sesquiterpene lactones were also contained in *Frullania dilatata* and they caused the intense allergy. The extract of *F. dilatata* was further carefully investigated and the additional sesquiterpene lactones were isolated and their structures [6, 7, 8, 9, 10, 11] were determined (see Fig. 1).[3]

Twenty-three patients sensitive to *Frullania* were tested to the new sesquiterpene lactones.[3] As can be seen in Table 1, all the patients sensitive to *Frullania* were sensitive to at least one of the lactones isolated from the plant. However, most patients were sensitive to only some of these lactones, and none of the lactones were active on every one of the patients. Exocyclic α-methylene group on the γ-lactone ring appears to be responsible for the intense allergenic eczematous contact dermatitis.

Table 1. Allergenic test (Patch-test, EtOH solution 1°/₀₀)

Product	1-5	6-14	15	16	17	18-19	20	21-22	23
[5]	+	+	+	+	+	0	0	0	0
[10]	+	X	+	X	0	+	+	+	X
[8]	+	+	0	+	+	+	+	0	0
[9]	+	+	0	0	0	+	0	0	+

(+): positive (0): no effect (X): not tested

Since Knoche et al[2a] discovered frullanolides [1, 5], various sesquiterpene lactones were found in American[4], European[5] and Japanese *Frullania* species[6] as listed in Table 2.

At present more than 400 sesquiterpene lactones have been found in nature, and most of them have been isolated from higher plants, especially those of the Compositae. Biochemical systematics of Compositae are now developing by application of the variation in these sesquiterpene lactones. It is also certain that the sesquiterpene lactones, eudesmanolides and eremophilanolides are the important genetic markers in Frullaniaceae. More recently, Andersen et al[7] isolated the sesquiterpene lactones [16, 17] from the liverwort, *Diplophyllum albicans* and confirmed that Diplophyllin [16] displayed significant activity against human epidermoid carcinoma (KB cell culture, ED $\sim$ 8 μg/ml) and cytotoxicity of enantiomeric Diplophyllin series against Carcinoma cells showed the chiral specificity. In contrast, we have confirmed that allergenic intensity of frullanolide and its related sesquiterpene lactones showed no chiral specificity.[8] Benešová et al[9] have showed the isolation of diplophyllolide [18] from European *Diplophyllum albicans*. Diplophyllolide has also been detected in Japanese *D. serrulatum*.[10] *Marchantia*

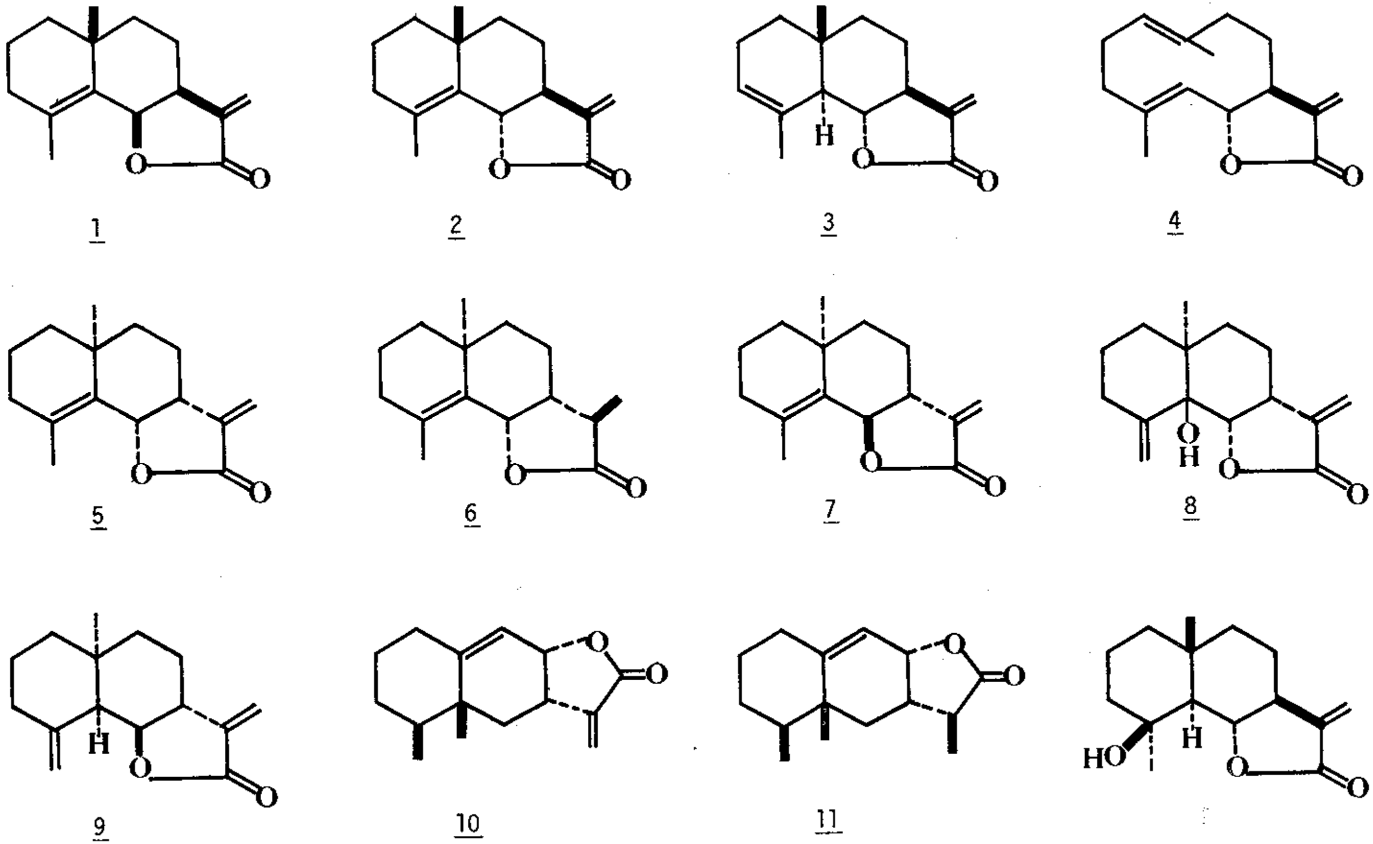
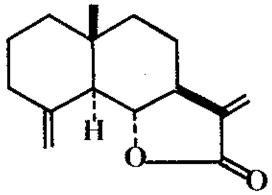


Fig. 1. Sesquiterpene lactones and the related compound isolated from the liv[illegible].



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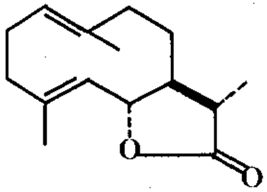

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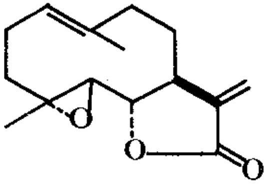

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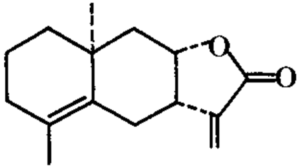

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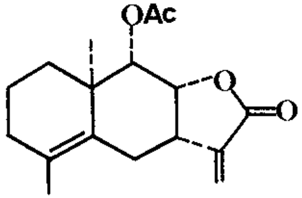

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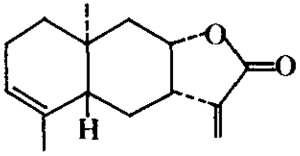

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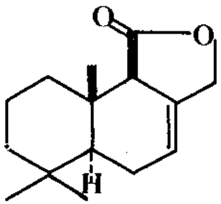

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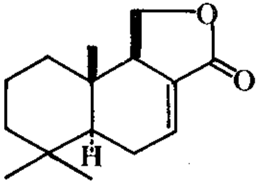

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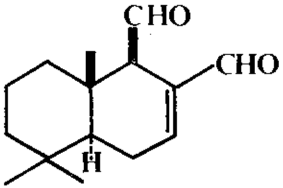

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Fig. 1 continued

polymorpha, some *Radula* and *Metzgeria* also cause allergenic contact dermatitis. We have confirmed that the allergenic agent in *M. polymorpha* was costunolide [4] and β-cyclocostunolide [13].[10]

Table 2. Sesquiterpene lactones found in liverworts.

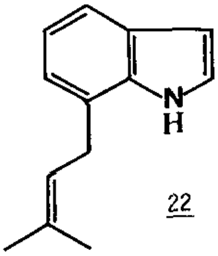
Species	Detected sesquiterpene lactones	References
Frullania tamarisci	[1, 2, 3, 4]	2a, 2b, 5
F. dilatata	[5, 6, 7, 8, 9, 10, 11]	2a, 2b, 3
F. tamarisci subsp. *obscura*	[12, 13]	6
F. nisquallensis	[1]	4
F. hampeana	[4, 14, 15]	10
F. jackii	[13]	10
F. yunnanensis	[1, 3]	10
F. pedicellata	[13]	10
Diplophyllum arbicans	[16, 17, 18]	7, 9
D. serrulatum	[18]	10
Marchantia polymorpha	[4, 13]	10

The liverworts, *Porella vernicosa* complex (Porellaceae): *Porella vernicosa, P. macroloba, P. gracillima* and *P. fauriei* display the intense pungent taste. We have isolated this unique pungent component from the above four species and determined its structure to be sesquiterpene dial [21][11], which is the same pungent component of the higher plant, *Polygonum hydropiper.*[12a,b] In addition to the sesquiterpene dial, two drimane sesquiterpene lactones, drimenin [19] and cinnamolide [20] have been found in the four *Porella* species cited above.[13] The compound [20] has also been isolated from the higher plant, *Cinnamosma fragrans.*[14] Cinnamolide possesses the activity against some dermatophyites.[14]

The liverworts very often elaborate the optical isomers of components found in the higher plants.[7, 15a-d] The seven sesquiterpene lactones isolated from *Frullania dilatata* and diplophyllin series found in *Diplophyllum arbicans* have all the unusual 7α-isopropyl configuration. It is interesting to note that the sesquiterpene lactones found in the other *Frullania* species except *F. dilatata* possess the usual 7β-isopropyl group and the drimane type sesquiterpenes found in *Porella vernicosa* complex do the usual 9β,10β-configuration.

Indole alkaloids, furanosesqui- and furanonorsesquiterpenes of liverworts.

Benešová et al[16] reported the first isolation of the indole alkaloides [22, 23] from *Riccardia sinuata*. Later, Huneck[17] have found the same compound [23] from *Riccardia chamedrifolia*. The unique furanosesquiterpene, pinguisone [24] was isolated from *Aneura pinguis*.[18] The structure was deduced from NMR and NMDR spectral analyses, and the formation of the adduct with maleic anhydride and of dimethyl ester [26] from the ozonolysis product of pinguisone also supported the structure (see Fig. 2). The location of the carbonyl group as well as the stereochemistry, however, had been ambiguous before the (Z)-p-bromobenzylidene derivative of [24] was established by the X-ray analysis.[19] One more furanosesquiterpene, deoxopinguisone [27] has been isolated from *Ptilidium ciliare*.[20] The structure was determined by the identity with the product derived from pinguisone by the Wolff-Kischner reduction. Except the pungent



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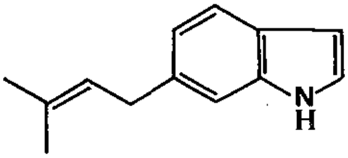

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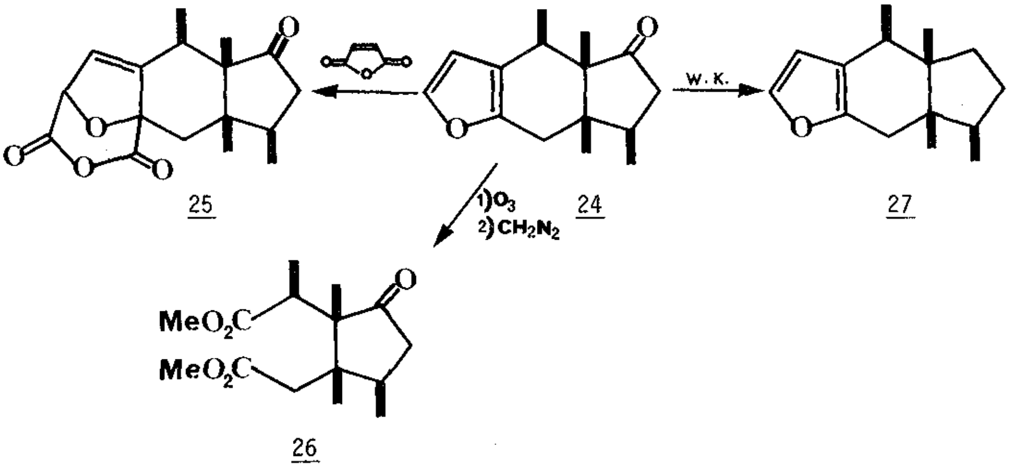
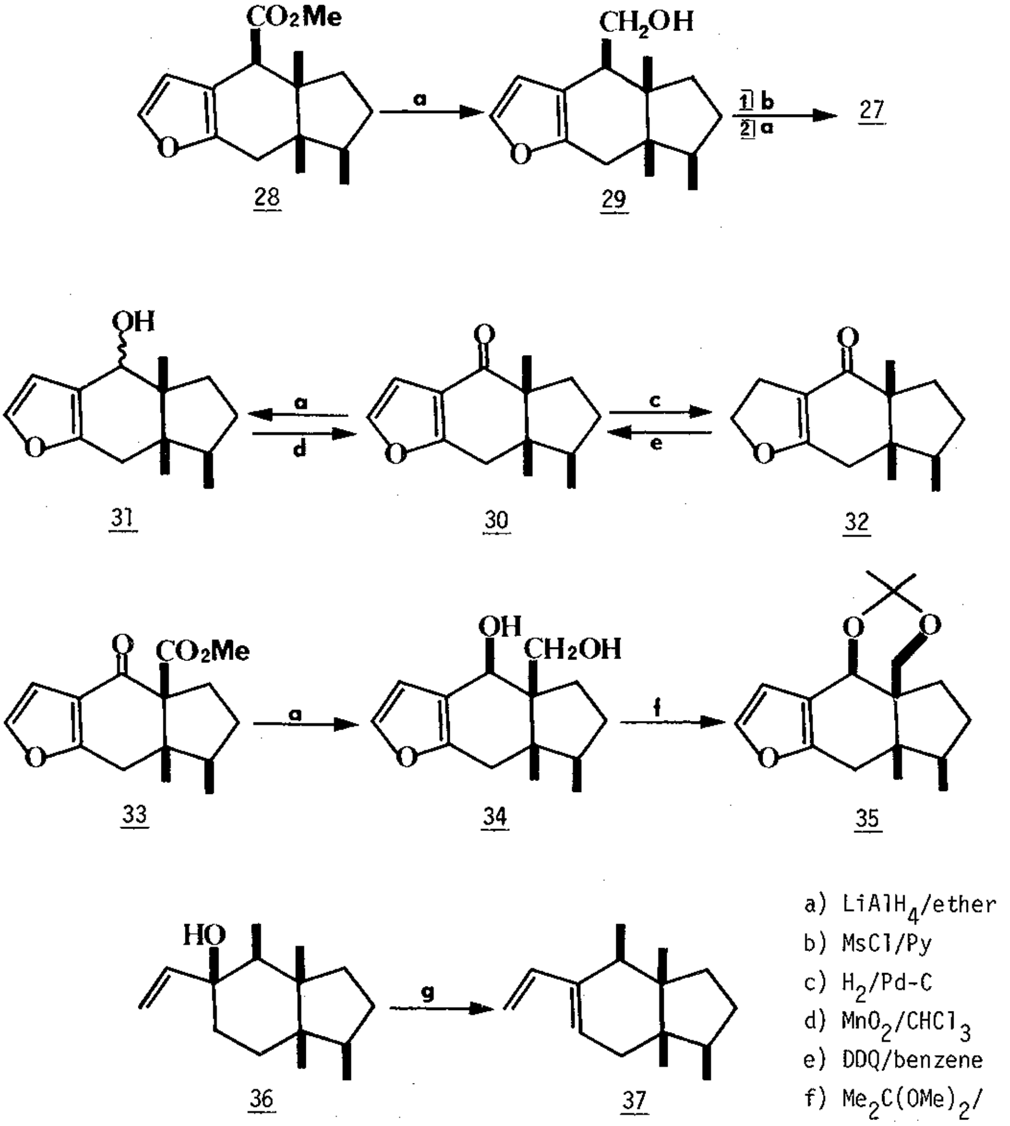


sesquiterpene dial and its related sesquiterpene lactones, *Porella vernicosa, P. macroloba,* and *P. gracillima* contain Ehrlich-test positive components. Careful chromatography of each extract of these three *Porella* species on silica gel afforded one furanosesquiterpene [28], two furanonorsesquiterpene [30, 33] and pinguisane-type sesquiterpenes, α-pinguisene [37] and pinguisenol [36], together with deoxopinguisone [27]. The structures of these components have been deduced from UV, IR, NMR and mass spectra and the transformations described in Fig. 3.[10, 13, 21a-c] *Porella densifolia,* which is morphologically different from *Porella vernicosa* complex, contains no pungent substance, however, it does the same furanosesqui- and furanonorsesquiterpenes and pinguisane-type sesquiterpenes as those detected in *P. vernicosa* complex.[10,21a-c] The pinguisanes show the interesting structures from the point of their biosynthesis.

The biogenesis of pinguisanes is difficult to rationalise in terms of the isoprene rule, however, the structures of pinguisones [24, 27] have been regarded as sesquiterpenoid since mevalonic acid has sufficiently been incorporated into pinguisone, although the further mechanism of its biosynthesis has not been accurate.[22] The hydrocarbon, α-pinguisene [37] may be an intermediate to pinguisenol [36], from which various pinguisane derivatives would be formed.

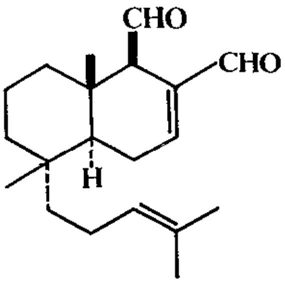
Wada et al[23] showed that pinguisone [24] displayed an antifeedant activity against the lavae of *Prodenia litura*. The norsesquiterpene [30] indicated the growth inhibitory activity against the seedling and the root of rice and wheat at ca. 50 ppm concentration.[10]



a) $LiAlH_4$/ether
b) MsCl/Py
c) H_2/Pd-C
d) $MnO_2/CHCl_3$
e) DDQ/benzene
f) $Me_2C(OMe)_2$/ p-TsOH
g) p-TsOH/benzene

Fig. 3. Pinguisane-type sesquiterpenes (27, 28, 30, 33, 36, 37) isolated from *Porella* species.

The liverwort, *Trichocoleopsis sacculata* (Trichocoleaceae) is morphologically very different from *Porella*, however, contains Ehrlich-test positive substances and the intense pungent component. The two furanosesquiterpenes showing no pungency were isolated and these were consistent with pinguisone [24] and deoxopinguisone [27]. The pungent component was easily isolated and its structure was determined to be the exceptional diterpene dial [38], named sacculatal.[24] The C-9 epimer [39] of sacculatal was also found in the same liverwort.[24] Another liverwort, *Pellia endiviaefolia* (Dilaenaceae) contains the same diterpene dials as those found in *Trichocoleopsis sacculata.*[25]



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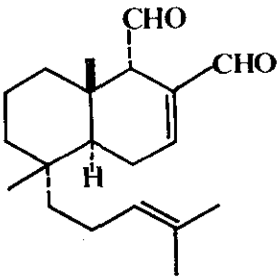

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It is known that the family Lophoziaceae includes an intense bitter principle. Huneck[26] has described the isolation of many furanoditerpenes displaying the bitter taste from Lophoziaceae and Scapaniaceae. Anastreptin ($C_{20}H_{24}O_5$) from *Anastorepta orcadensis*, barbilophozine ($C_{22}H_{32}O_5$) from *Barbilophozia barbata,* floerkein A and B (both $C_{20}H_{34}O_3$) from *Barbilophozia floerkei* and scapanin ($C_{20}H_{30}O_4$) from *Scapania undulata* (Scapaniaceae) have been isolated, however, their structures have not been elucidated.

Flavonoids

Although mono-, sesqui- and diterpenes, in general, have not been found in Musci, Musci and Hepaticae elaborate flavonoids. In most case, flavonoids are present as glycosides in cell wall of the bryophyte. It is known that Sphagnales and Bryales (Musci) contain red pigment. Bendz et al[27a,b] showed that the pigments of *Bryum cryophylum, B. rutilans* and *B. weigelii* were responsible for luteolinidin-5-monoglucoside [40] and luteolinidin-5-diglucoside [41]. The pigment of cell wall of *Sphagnum nemoreum* was also due to luteolidin type anthocyanidin.[28] Melchert et al[29] reported the presence of flavone C-glycoside and quercetin-3-diglucoside [62] in *Mnium affine* and *M. arizonicum*. *Corcinia coriandrina* contains quercetin [63] and kaempferol [61].[30] McCure et al[31] have investigated the distribution of flavonoids of seventy Musci by paper chromatography and spectrometric analysis and detected flavonoids and flavonoid like compounds in thirty-four species. Since flavone C- and O-glycosides were found in *Mnium* species, various flavonoid glycosides have been isolated from the various mosses and liverworts. The typical flavonoids and the plant sources are shown in Table 3.

The flavonoids have provided the important taxonomic characters. Thus, the flavonoid glycosides have been used in the biochemical systematics of higher plants. In chemosystematics in bryophyte field, the flavonoids are also valuable markers. The liverwort, *Conocephalum conicum* contains various monoterpenes; major component is (+)-bornyl acetate.[32] On the other hand, its water soluble fraction involves flavonoid glycosides. Markham[33] reported the flavonoid variation of American and Germany *C. conicum* and found that both population contained a common set of flavone glycosides. In European samples, a set of 7-4'-glucuronides of apigenin and luteolin are present and absent in American one, whereas a complex set of glycosides of luteolin which are not in European specimens are present in American one. From these differences, Markham et al concluded that *C. conicum* existed as two chemically distinguishable geographic races. Sphaerocarpaceae and Riellaceae have together, variously been placed in the orders Sphaerocarpales, Jungermanniales, and Marchantiales. Markham et al[34] have investigated the distribution of flavonoid glycosides of *Sphaerocarpos,Riella americana* and *R. affinis* and postulated that Sphaerocarpaceae and Riellaceae are included in the order Marchantiales rather than their separation into another order, since the flavone glycosides isolated from both *Riella* and *Sphaerocarpos* are all compounds which occur in the species of the order Marchantiales. The above population are consistent with the Groll's classification of the Hepaticae.[35] Markham[36] has also described that the genus *Hymenophyton* includes two species, *H. leptopodum* and *H. flabellatum* on the basis of the distribution of flavonoid constituents. A number of apigenin 6,8-di-C-pentosides and pentoside-hexosides are common to both species. However, *H. leptopodum* is distinguished from *H. flabellatum* in which kaempferol di- and triglycosides are present. Many bryologist believe that Hymenophytaceae is one of the most highly evolved family in Metzgeriales. Markham et al[36] indicated that the finding of flavonol glycosides could be explained as providing support for the above suggestion by the consideration of biosynthetic pathways of flavonol-3-glycosides and flavone glycosides and this significant biosynthetic difference is certainly consistent with the existence of two different species of liverworts.

More recently, the presence of the acetylated derivatives of apigenin 7-glucoside, apigenin-7,4'-diglucoside and isoscutellarein-7-glucoside have been found in the primitive New Zealand hepatic, *Haplomitrium gibbsiae.*[37]

It is known that there are more than 20,000 species in Bryophyta in the world, however, less than 200 species have been chemically investigated. We can not deeply study the evolutional process and the differenciation of the bryophytes, because of the lack of their fossils. When the chemical constituents of the bryophytes are further investigated systematically, the evolutional relationship between algae and bryophytes and that between Musci and Hepaticae will be gradually accurate. At that time, the biological active substances which prompt our interest will be isolated from various bryophytes.

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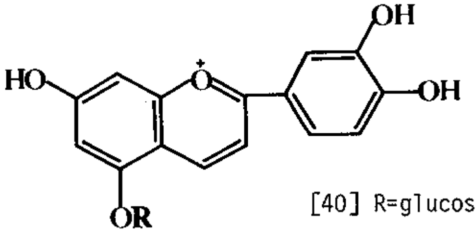
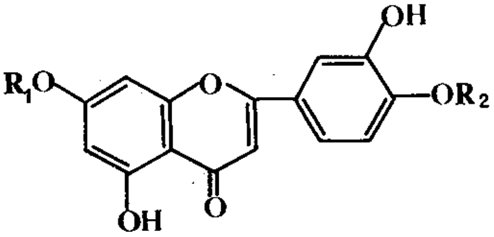
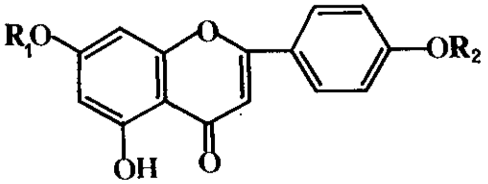
Table 3. Flavonoids found in Bryophytes.



	Species	References
[40] R=glucosyl	*Bryum cryophilum*	27a
[41] R=diglucosyl	*B. rutilans* *B. weigelii*	27b

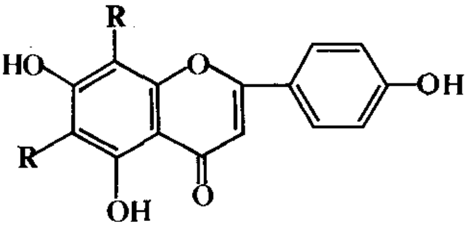


[42] R_1=rhamnosyl-glucosyl R_2=H	*Dicranum scoparium*	38
[43] R_1=R_2=glucuronyl	*Conocephalum conicum*	33
[44] R_1=glucuronyl R_2=Me [45] R_1=rhamnosyl-glucuronyl R_2=Me	*Marchantia foliacea* *M. beteroana*	44

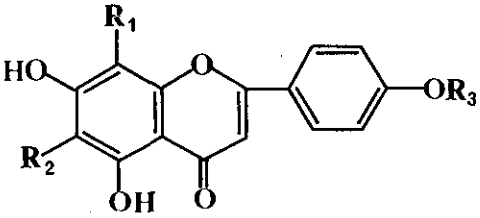


[46] R_1=R_2=Me	*Frullania jackii*	10
[47] R_1=2,4-dirhamnosyl-glucosyl R_2=H	*Dicranum scoparium*	38
[48] R_1=R_2=glucuronyl	*Conocephalum conicum*	33
[49] R_1=R_2=acetylated glucosyl	*Haplomitrium gibbsiae*	37
[50] R_1=glucuronyl R_2=H [51] R_1=rhamnosyl-glucuronyl R_2=H	*Marchantia foliacea* *M. beteroana*	44
[52] R_1=rhamnosyl-glucuronyl R_2=Me [53] R_1=rhamnosyl-galacturonyl R_2=Me	*Reboulia hemispherica*	46

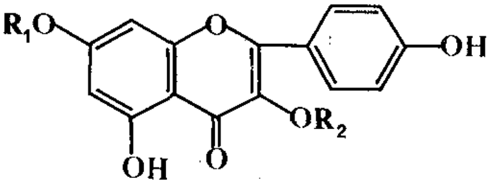
Table 3. continued



[54] R=glycosyl	*Porella platyphylla*	43
	Hymenophyton flabellatum	40
[55] R=glucosyl	*Marchantia foliacea*	44
	M. berteroana	

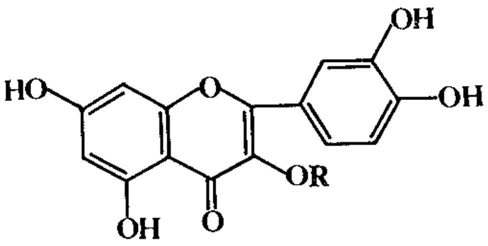


[56] R_1=R_2=pentosyl R_3=Me	*Hymenophyton leptopodum*	36
[57] R_1=R_2=pentosyl-hexosyl R_3=Me	*H. flabellatum*	36
[58] R_1=suger R_2=H R_3=Me	*Reboulia hemispherica*	46

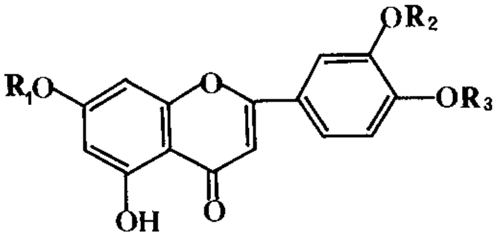


[59] R_1=rhamnosyl R_2=glucosyl	*Hymenophyton leptopodum*	36
[60] R_1=rhamnosyl R_2=rhamnosyl-glucosyl		
[61] R_1=R_2=H	*Corsinia coriandrina*	30

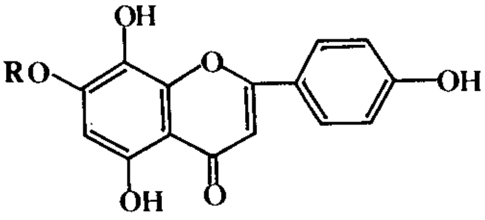
Table 3. continued



[62] R=diglucosyl	*Mnium arizonium*	29
[63] R=H	*Corsinia coriandrina*	30

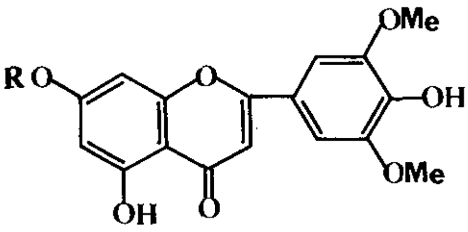


[64] R_1=R_2=glucuronyl R_3=H	*Conocephalum conicum*	33
[65] R_1=glucuronyl R_2=R_3=rhamnosyl + deriv.	*C. conicum*	

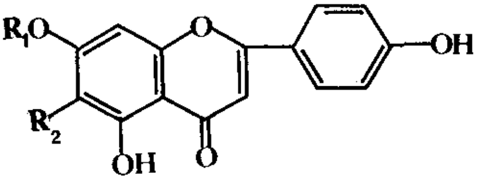


[66] R=acetylated glucosyl	*Haplomitrium gibbsiae*	37

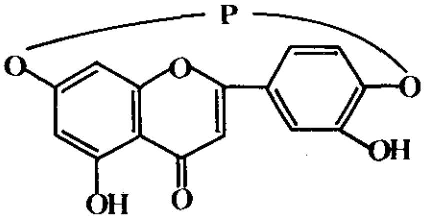
Table 3. continued



[67] R=glucuronyl	*Marchantia foliacea*	44
[68] R=rhamnosyl-glucuronyl	*M. beteroana*	



[69] R_1=glucosyl R_2=glucosyl	*Porella platyphylla*	41, 42
[70] R_1=H R_2=glucosyl		
[71] R_1=glucosyl R_2=OH	*Bryum weigelii*	39



[72] P=polysaccharide	*Monoclea forsteri*	45

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