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The Chemical Constituents of Some Taiwanese Liverworts

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Abstract—Twelve species of Taiwanese liverworts from recent collection were examined by GC/MS and some of the major components were isolated and identified spectroscopically. Interesting findings from the view point of chemotaxonomy are discussed in the paper.

Keywords—Liverworts (Hepaticae)·chemotaxonomy·bicyclogermacrene·*Mylia nuda*·*Mastigophora diclados*·*Schistochila rigidula*·*Makinoa crispata*·*Pallavicinia longispina.*

Liverworts (Hepaticae) are taxonomically considered to be the lower terrestrial green plants, generally produce large amounts of terpenoids and/or aromatic compounds. The chemistry of liverworts is attractive in the following aspects:

1. New skeleton: Many sesquiterpenes and some diterpenes of novel skeleton (1~7)[1] have been found in liverwort oils, but not yet in any higher plants.

2. Enantiomeric character: It has been demonstrated in many reports that liverworts produce terpenoids mostly antipodal to those found in higher plants.[1]

3. Biological activity: Many oxygenated sesquiterpenes, diterpenes and isoprenyl bibenzyls of liverwort components have biological activities in the following categories: anticancer, tumor promoting, antifungal, antimicrobial, antifeedant, piscicidal, plant growth regulatory activity and ability to cause allergenic contact dermatitis.[1]

4. Chemotaxonomic significance: The rich data on liverwort components not only reveal the close chemical relationships of certain species among the Hepaticae, but also implies the chemical relationships between algae, bryophytes and pteridophytes.[1,2,17]

In this session, I will report our recent results on the examination of twelve Taiwanese liverworts and discuss some interesting findings from the chemotaxonomic view point. Among these species, seven of them had been investigated before from previous collections.[3] In general, the present results are parallel and comparable to what previously obtained except for one point which will be discussed in the following section. In Table 1, all data are from the present study, previous findings are not included here.

Results and Discussion

Bicyclogermacrene (8) has been reported to be a common constituent in liverworts and precursors for many other sesquiterpenes.[4,5] In our previous studies, however, bicyclogermacrene could not be detected or isolated in any of the ten Taiwanese species examined.[3] Nevertheless, the present results from twelve species which were collected in June of this year at Yuen-yang Lake showed that bicyclogermacrene (8) was present in six of these species. Among the six, four of them (*B. fauriana*, *P.kahsiana*, *S. rigidula* and *S. ornithopodioides*) had been exam-

Table I. Chemical constituents of liverworts

Species	Compounds detected
Bazzania fauriana (Steph.) Hatt.	α-copaene, anastreptene, β-patchoulene, α-barbatene,* isobazzanene, β-barbatene, α-selinene, β-chamigrene, bicyclogermacrene, α-bazzanene,* β-bazzanene,* δ-cuprenene,≠ β-eudesmol, many oxygenated sesquiterpenes (M^+ 218, 220, 222), a few diterpenes (272, 286), hopenes? (410)
B. angustifolia Horik.	α-copaene, α-barbatene, isobazzanene,* thujopsene, β-barbatene,* γ-cuprenene, β-chamigrene,* α-chamigrene, α-bazzanene, β-bazzanene, cuparene,* δ-cuprenene,*,≠ oxygenated sesquiterpenes (218, 220), diterpenes (272)
Makinoa crispata (Steph.) Miyake	β-barbatene, bicyclogermacrene, spathulenol, δ-cuparenol, other sesquiterpenes (204, 220), phytadienes, phytol, many diterpenes (272, 286, 288), certain class of compounds (300, 312, 314, 326, 328, 374), squalene, campesterol, stigmasterol, sitosterol, fern-9-ene?, hopanoids? (410, 426, 428)
Mastigophora diclados (Bird.) Nees ex Schhiffn.	β-barbatene, herbertene,* cuparene, calamenene, β-bazzanene, calacorene, guaiazulene, α-herbertenol, β-herbertenol, herbertenediol (or cuparenediol) ent-pimara-8 (14), 15-dien-19-oic acid,* methyl and ethyl fatty esters
Mylia nuda≠ Inoue et Yang	α-pinene, camphene, β-pinene, β-phellandrene, γ-terpinene, α-copaene? β-bourbonene, β-spathulene, ledene? γ-gurjunene, seline-4, 11-diene, γ-cadinene, δ-cadinene, α-longipinene? cuparene, calamenene, α-& β-calacorene, calorcorenol, hydroxycalamenene, other sesquiterpenes (204, 218, 220, 222), (+)-manoyl oxide,* (+)-labda-7, 14-dien-13-ol,* other diterpenes (272, 274, 290), marchantin X.*
Pallavicinia longispina Steph.	eudesmane-type ketone? (218), many diterpenes (272, 286, 288, 302, 362).
Plagiochila kahsiana Mitt.	anastreptene,* bicyclogermacrene, δ-cuprenene, oxygenated sesquiterpenes (216, 220, 222), diterpenes (286, 288, 290, 304), 2, 3-secoaromadendrane acetates (302, 304, 318, 344, 348, 360, 362), campesterol, stigmasterol, other sterols or triterpenes (402, 426, 430)
Pleurozia acinosa (Mitt.) Schiffn.	β-pinene, δ-elemene, β-elemene, (+)-α-ferulene,* α-elemene, β-patchoulene?, bicyclogermacrene, β-chamigrene, elemol, (−)-cleroda-3, 14-dien-13-ol*, 14-labden-8, 13-diol*, other diterpenes (272, 286, 288, 290, 304)
Scapania ornithopodioides (With.) Pears	anastreptene*, aromadendrene*, α-selinene, bicyclogermacrene, bicycloelemene, α-patchoulene, selina-4, 11-diene, sesquiterpene alcohol (220), phytadiene, phytol, sterols (430)
Schiffneria hyalina Steph.	spathulene, cyclocolorenone? other sesquiterpenes (202, 216, 234, 248), phytadienes, phytol, diterpene(286), diterpene acetate?(360), campesterol, stigmasterol, sitosterol.
Schistochila rigidula≠ Horik.	bicyclogermacrene, oxygenated sesquiterpene (220), many diterpenes (270, 272, 274, 286, 288, 290, 302, 304)
Trichocolea tomentella (Enrh.) Dum.	α-pinene?, phytadienes, phytols, tomentellin, isotomentellin, diterpene (288).

___ Major components.
* Isolated either in the present study or in previous works from the same species.
≠ Endemic species.
≒ Named angustifolene in previous papers[3].
Figures in parentheses are molecular ions observed in GC/MS analyses.

ined before.[3] The discrepancy may be explained by the unstability of bicyclogermacrene, since in our previous studies, freshness of the oils was sometimes overlooked. The easy transformation of bicyclogermacrene into other sesquiterpenes had been observed in several occasions.[4-6] In the present work, the hexane extract of *S. rigidula* was checked again by GC/MS after two months' storage in refrigerator and found that indeed the bicyclogermacrene peak diminished substantially.

The major components of *Mylia nuda* were isolated and identified to be two labdane type diterpenes, (+)-7,14 labdadien-13-01(**9**), and (+)-manoyl oxide(**10**), as well as an unnamed bisbibenzyl compound **11**. Compound **11** was first found in an Indian liverwort *Marchantia polymorpha* by Asakawa and his coworkers.[7] Previously, two other *Mylia* species had been studied.[8,9] The maior components of *M. taylorii* are myliol (**12**), dihydromylione A (**13**) and taylorione (**14**), whereas those of *M. verrucosa* are a series of new type diterpene of verrucosane skeleton (**6**). Thus, it seems that these three *Mylia* species belong to three different chemotypes respectively since they all elaborate different types of terpenoids as their main constituents.

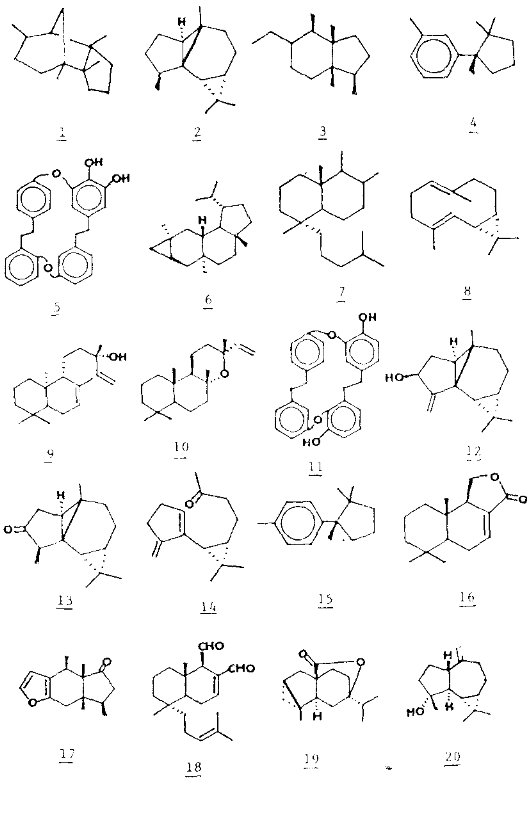
Mastigophora is a rather primitive genus with only a few species known. In the past, only the hydrophilic fraction of *M. woodsii* had been examined by Mues.[10] However, two *Ptilidium* species which belong to the same family *Ptilidiaceae* as *Mastigophora* had been studied chemically by Asakawa et al.[11,12] and pinguisane (**3**)-type compounds were identified. Now from our present and previous[13] studies on *M. diclados*, no trace amount of pinguisane-based compounds could be detected. On the other hand, sesquiterpenes of herbertene(**4**)-type appear to be the major components for *M. diclados*. Herbertene (**4**) is a structural isomer of cuparene (**15**), both herbertene and cuparene-type compounds had been identified as main components of *Herbertus*,[12,14,15] another primitive genus liverworts. Therefore, the liverwort *M. diclados* seems chemically quite distal from the family Ptilidiaceae, yet fairly close to the genus *Herbertus*.

Not many *Schistochila* species have been chemically studied. The only literature report was by Asakawa and Inoue[16] on two Chilean *Schistochila* species. They found that the chemical constituents of these two species were rather simple. *S. laminigera* contained bicyclogermacrene(**8**) and cuparene(**15**). *S. reflexa* produced two unidentified sesquiterpene hydrocarbons and an oxygenated compounds (M^+ 302). Asakawa *et al.*[17] also investigated a New Zealand species *Schistochila appendiculata* and found that it did not elaborate terpenoids but synthesized several phenolic compounds. In our study on this Taiwanese endemic species *S. rigidula*, to the contrast, its constituent appears quite complex and diterpenes are the major component as shwon in Table 1.

Makinoa crispata and *Pallavicinia longispina* are morphologically similar. Both belong to the order of Metzgeriales, while the rest species in the present investigation belong to the order of Jungermanniales. Asakawa *et al.*[1] studied *M. crispata* before and found cinnamolide (M^+234, base 109) (**16**), some oxygenated pinguisanes (base 108) (e.g. **17**), many sacculatane—type diterpenes (M^+ 318) (e.g. **18**) and a new δ-lactone, named crispatanolide (M^+ 234, base 105) (**19**). From the GC/MS analysis of our Taiwanese species, nevertheless, none of the above mentioned were observed. Instead, we saw many sesquiterpenes of M^+ 204 & 220, phytadienes, phytol, a major diterpene of M^+ 272, common sterols and a certain class of compounds which all produced the strongest fragment with a loss of 75 mass units.

For the genus *Pallavicinia*, spathulenol (**20**) had been identified as major component in both *P. longispina* and *P. lyelii* in addition to other sesquiterpene hydrocarbons.[1] In *P.lyelii*, several diterpenoids, three of which were reported having typical sacculatane(**7**) skeleton, were also found in the preliminary study of Japanese species.[18] While *P. longispina*, on the other hand, was found synthesizing many triglycerides in place of diterpenes.[19] From the present study, again different results were observed. Very few sesquiterpenes and no spathulenol(**20**) could be detected in our species, yet many oxygenated diterpenes are the major constituents.

In conclusion, these preliminary chemical data do provide some interesting clues to the chemotaxonomy and further detailed studies are much encouraged. We see the chemical variation not only within the same genus such as *Mylia* & *Schistochila*, but also find the geographical difference chemically for the same species such as *M. crispata* & *P. longispina*. And sometimes the chemistry may even help the settlement between genera or families, such as the case of *Mastigophora diclados.*



Experimental

All liverworts listed in Table I were collected at Yuen-yang Lake, Ilan, in June, 1985 and identified by Dr. M.-J. Lai, Dept. of Landscape Architecture, Tunghai University.

GC, GC/MS and spectral analyses were perfomed in the Institute of Pharmacognosy, Tokushima Bunri University of Japan. Instrumental conditions were described in references 4 & 11.

A small air-dried (~1g) sample of each liverwort was crushed and extracted with n-hexane. After filtration through a very short column of Si-gel, the concentrated n-hexane extract was then analyzed by GC and GC/MS.

The n-hexane extracts of *M. nuda* (62g) and *P. acinosa* (13g) were each further chromatographed on Si-gel. Isolated components were purified by repeated C.C., prep. TLC or HPLC collection, if necessary. Identification was made by spectral comparsion with authentic sample or literature data. Spectral evidence will be reported elsewhere.

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