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OCCURRENCE OF A POTENT PISCICIDAL DITERPENEDIAL IN THE LIVERWORT *RICCARDIA LOBATA* VAR. *YAKUSHIMENSIS**

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Key Word Index—*Riccardia lobata* var. *yakushimensis*; Riccardiaceae; Metzgeriales; Hepaticae; sacculatal; isosacculatal; diterpenedials; piscicidal activity; chemosystematics.

Abstract—Sacculatal and isosacculatal, two diterpene dialdehydes, have been isolated from the liverwort *Riccardia lobata* var. *yakushimensis* as the major components. *Orizia latipes* (= killie fish) is killed within 2 hr by a 0.4 ppm solution of sacculatal. It is suggested that *R. lobata* var. *yakushimensis* and *Pellia endiviifolia* might have originated from a common ancestor since the latter produces the same sacculatals as the former species.

INTRODUCTION

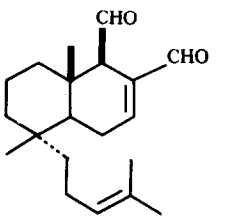
Terpenoids and lipophilic aromatic compounds are widely distributed in the Hepaticae. These chemical components are valuable chemosystematic markers of the Hepaticae [1]. Riccardiaceae (Metzgeriales) contains two genera, *Aneura* and *Riccardia*. The latter genus is further divided into two subgenera, *Lobatiriccardia* and *Riccardia*. Recently, we reported that there is no chemical similarity between *Aneura* and *Riccardia* [2, 3]. It is known that *Riccardia* is taxonomically one of the most difficult genera among the Hepaticae [4]. *Riccardia* elaborates many kinds of sesquiterpenes as well as aromatic compounds [1]. In Japan, there are 13 *Riccardia* species among which *R. multifida* produces two unique bis(bibenzyls) possessing cytotoxic activity against KB cell [5, 6].

In this paper, we report on the distribution of two sacculatane diterpenoids, one of which possesses piscicidal activity, in *Riccardia lobata* var. *yakushimensis* and discuss some aspects of the chemosystematics of Riccardiaceae and Dilaenaceae.

RESULTS AND DISCUSSION

Fresh *Riccardia lobata* var. *yakushimensis*, whose greyish oil bodies were seen in all tissue of the thallus (3–8 per cell), was homogenized with ether and, after removal of solvent, the crude extract was directly analysed by TLC, GC and GC/MS. The components were identified by direct comparison of their mass spectra with those of authentic samples. The main components were isolated by a combination of silica gel column chromatography and preparative TLC and their chemical structures were confirmed by spectroscopic methods. The major components were two isomeric diterpenes (**1** and **2**), $C_{20}H_{30}O_2$, the more polar of which, sacculatal (**1**), possessed a hot taste and killed *Orizia latipes* (= killie fish) within 2 hr at a concentration of 0.4 ppm.

The 400 MHz ^{1}H NMR, IR, mass and CD spectra of the dialdehydes were identical to those of sacculatal (**1**) and isosacculatal (**2**), which have been isolated from the liverwort *Pellia endiviifolia* belonging to the Dilaenaceae [7, 8]. The mono- and sesquiterpenoid content of *R. lobata* var. *yakushimensis* is very small and only limonene and an unidentified sesquiterpene hydrocarbon [M^+ 204 (base 161)] have been detected by GC/MS as very minor components. Thus, there is no obvious chemical affinity between *R. lobata* var. *yakushimensis* and the other *Riccardia* species examined so far [2, 3]. In the present classification, subgenus *Lobatiriccardia* is given to *R. lobata* var. *yakushimensis* and the other *Riccardia* species belong to subgenus *Riccardia* [4]. The above difference of terpenoid constituents may fit the reported classification of *Riccardia* genus. However, Hattori (private communication) suggests that *R. lobata* var. *yakushimensis* is morphologically similar to *Pellia endiviifolia* belonging to the Dilaenaceae and it should be placed in a different genus in Riccardiaceae. The present chemical results support Hattori's suggestion, since both *R. lobata* var. *yakushimensis* and *P. endiviifolia* synthesize sacculatal (**1**) and isosacculatal (**2**). This further suggests that *Riccardia* species belonging to subgenus *Lobatiriccardia* and *Pellia endiviifolia* (Dilaenaceae) might have originated from a common ancestor.



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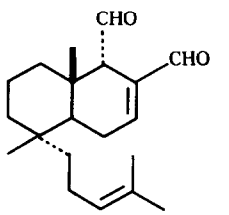

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*Part 17 in the series "Chemosystematics of Bryophytes". For Part 16 see Asakawa, Y., Toyota, M., Bischler, H., Campbell, E. O. and Hattori, S. (1984) *J. Hattori Bot. Lab.* **57**, 383.

EXPERIMENTAL

TLC, prep. TLC, GC and GC/MS were carried out as previously reported [9]. 400 MHz ^{1}H NMR spectra were measured in $CDCl_3$–TMS soln. The species identified by Dr. S. Hattori and Dr. M. Mizutani has been deposited at the Herbaria of the Hattori Botanical Laboratory, Miyazaki, Japan and Tokushima Bunri University.

Bioassay. Piscicidal activity was tested using a modified method of Kawazu method [10] in which EtOH was used as the solvent in place of MeOH.

Extraction and isolation of diterpenoids. Riccardia lobata Schiffn. var *yakushimensis* Hatt. was collected in Yakushima (Japan) in December 1983 and the fresh material was homogenized with Et_2O. The green extract after evapn of the solvent was directly analysed by TLC, GC and computerized GC/MS. Two major peaks corresponding to sacculatal (**1**) and isosacculatal (**2**) appeared on GC and GC/MS. The presence of limonene, an unidentified sesquiterpene hydrocarbon [M^+ 204 (base 161)], campesterol, stigmasterol and sitosterol was confirmed by GC/MS. The remaining pungent oil (300 mg) was chromatographed on silica gel using an *n*-hexane–EtOAc gradient. The fraction eluted by *n*-hexane–EtOAc (4:1) was further purified by prep. TLC to afford isosacculatal (**2**) (50 mg) and sacculatal (**1**) (140 mg), whose spectral data (400 MHz ^{1}H NMR, IR, MS and CD) were identical to those of authentic samples [7]. Sacculatal killed killie fish within 2 hr at a concn of 0.4 ppm. Isosacculatal, however, showed no piscicidal activity even at a concn of 10000 ppm.

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