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SESQUITERPENOIDS OF *RICCARDIA* AND *PALLAVICINIA* SPECIES

YOSHINORI ASAKAWA,* MASAO TOYOTA,* REIJI TAKEDA,† CLAUDE SUIRE* and TSUNEMATSU TAKEMOTO*

* Institute of Pharmacognosy, Tokushima Bunri University, Yamashiro-cho, 770 Tokushima, Japan;
† Suntry Institute for Bioorganic Chemistry, Wakayamadai, Shimamoto-cho, Mishima-gun, 618 Osaka, Japan

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Key Word Index—*Aneura pinguis*; *Riccardia multifida*; *R. jackii*; Riccardiaceae; *Pallavicinia longispina*; Dilaenaceae; Metzgeriales; Hepaticae; *ent*-sesquiterpenes; *ent*-selinanes; *ent*-bicyclogermacrane; *ent*-aromadendrane; (*R*)-cuprane; chemosystematics.

Abstract—*Riccardia* species (Metzgeriales) contain various types of sesquiterpenes. *R. jackii* produces *ent*-selinane-, *ent*-aromadendrane- and *ent*-bicyclogermacrane-type sesquiterpenes together with (*R*)-cuparene and α-barbatene. *Aneura pinguis* (= *Riccardia pinguis*) is chemically quite different from *R. multifida* and *R. jackii*. The former produces a large amount of pinguisone. *R. multifida* contains 6-(3-methyl-2-butenyl)-indole and (+)-β-elemene as the major components. *Pallavicinia longispina* (Dilaenaceae; Metzgeriales) produces mainly spathulenol. The chiral properties of the sesquiterpenes isolated from *R. jackii* are quite similar to those of red algae, *Laurencia* species.

INTRODUCTION

The results of the chemical analyses of ninety-eight species of Japanese and European liverworts have shown that members of the Hepaticae contain compounds which are of chemosystematic value [1–6]. The presence of carotenoids [7], lower carboxylic acids [8], carbohydrates [9, 10], indole derivatives [11, 12] and bibenzyl derivatives [13] has been reported for a few *Riccardia* species (Riccardiaceae; Metzgeriales). Recently, Benesova *et al.* [14] reported on the isolation of a unique sesquiterpene, pinguisone (**7**), from *Aneura pinguis* Dum. (= *Riccardia pinguis*). Except for the isolation of pinguisone, there are few reports concerning the presence of terpenoids in *Riccardia* species [15]. In Japan, five genera, *Makinoa*, *Pellia*, *Calycularia*, *Pallavicinia* and *Moerckia* constitute the Dilaenaceae, which is situated systematically near the Riccardiaceae. Recently, we reported that *Makinoa crispata* (Steph.) Miyake and *Pellia endiviifolia* (Dicks.) Dum. produce sesquiterpene lactones and diterpene dialdehydes as the major components, respectively [1, 16, 17]. In the present communication, we wish to report the distribution of sesquiterpenoids in three *Riccardia* species and *Pallavicinia longispina* Steph.

RESULTS AND DISCUSSION

Air-dried ground materials were extracted with Et_2O. Each crude extract was analysed by GC–MS to obtain an indication of the compounds present. The chemical structures of the terpenoids were confirmed by comparison of the MS spectra with those of authentic samples or with published data. The major components, which appeared to be of chemotaxonomic value were isolated by PLC or preparative GLC.

Riccardia multifida S. Gray contains a large amount of 6-[3-methyl-2-butenyl]-indole (**8**). This compound has already been found in European *R. incurvata* Lindb. and *R. sinuata* (With.) Grolle (= *R. chamedryfolia*) [11, 12]. Thus, Japanese *R. multifida* is chemically close to these two European species. In addition to the indole derivative, *R. multifida* contains the sesquiterpene hydrocarbon (+)-β-elemene (**1**). α-Elemene, γ-cadinene and calamenene were detected by GC–MS analysis.

A. pinguis (= *R. pinguis*) is chemically very specific, since it produces mainly a unique furanosesquiterpene, pinguisone (**7**), which has also been isolated from the same European species [14]. Recently, **7** and its related furanosesquiterpenes have been found in Jungermanniales: *Porella*, *Lejeunea*, *Ptilidium* and *Trichocoleopsis* [5, 18–22], although they are morphologically quite different from *Riccardia*.

Riccardia jackii Schiffn. produces various types of sesquiterpenoids, particularly hydrocarbons, e.g. (+)-α-selinene (**2**), (−)-β-selinene (**3**), (−)-bicyclogermacrene (**4**), (+)-cuparene (**5**) and (+)-α-barbatene (**9**) and alcohols, e.g. (−)-spathulenol (**6**). All these sesquiterpenes have been found in various species of Jungermanniales. *R. jackii* and *R. multifida* are morphologically different from *A. pinguis*. From the sesquiterpene constitution described above, it is obvious that *A. pinguis* is also chemically very different from *R. multifida* and *R. jackii*.

Makinoa crispata, *Pellia endiviifolia* and *Pallavicinia longispina* are morphologically similar and they are placed in the Dilaenaceae which is close to the Riccardiaceae. *M. crispata* chemically resembles *P. endiviifolia* since they both contain unique sacculatane-type diterpene dialdehydes [1, 16, 17]. *P. longispina* morphologically resembles *Pellia* species rather than *M. crispata*. It is clear that *P. longispina* is chemically quite different from *Makinoa* and *Pellia* species, since it elaborates a large amount of *ent*-spathulenol (**6**) together with much triglycerides in place of diterpene dialdehydes.

Most liverworts biosynthesize the enantiomers of the sesquiterpenoids found in higher plants, although there are several exceptions. The sesquiterpenes found in *R. jackii* are interesting from both a biogenetic and an evolutionary view point. The selinenes (**2** and **3**),

spathulenol (**6**) and bicyclogermacrene (**4**), which may be a precursor of **6**, are the enantiomers of the corresponding sesquiterpenes found in higher plants. The configuration of the cuparene series isolated from the liverworts so far examined is opposite to that isolated from the higher plants [23, 24]. In contrast, cuparene (**5**) isolated from *R. jackii* has the same configuration as that found in higher plants. Recently, many marine plants have been chemically investigated and various types of sesqui- and di-terpenoids have been isolated and their chemical structures established. As seen in Fig. 1, co-occurrence of *ent*-elemene (**1**), *ent*-selinenes (**2**, **3**) and (*R*)-(+)-cuparene (**5**) in *Riccardia* species has also been found in red algae, *Laurencia* species [25–27]. Roughly speaking, the Hepaticae are chemically quite different from the Musci. The former are similar to the higher plants and algae, whilst the latter resemble the pteridophytes [1, 3], although there is a large evolutionary distance between them. The morphological similarity of *Riccardia* species to some red algae and the chiral properties of the

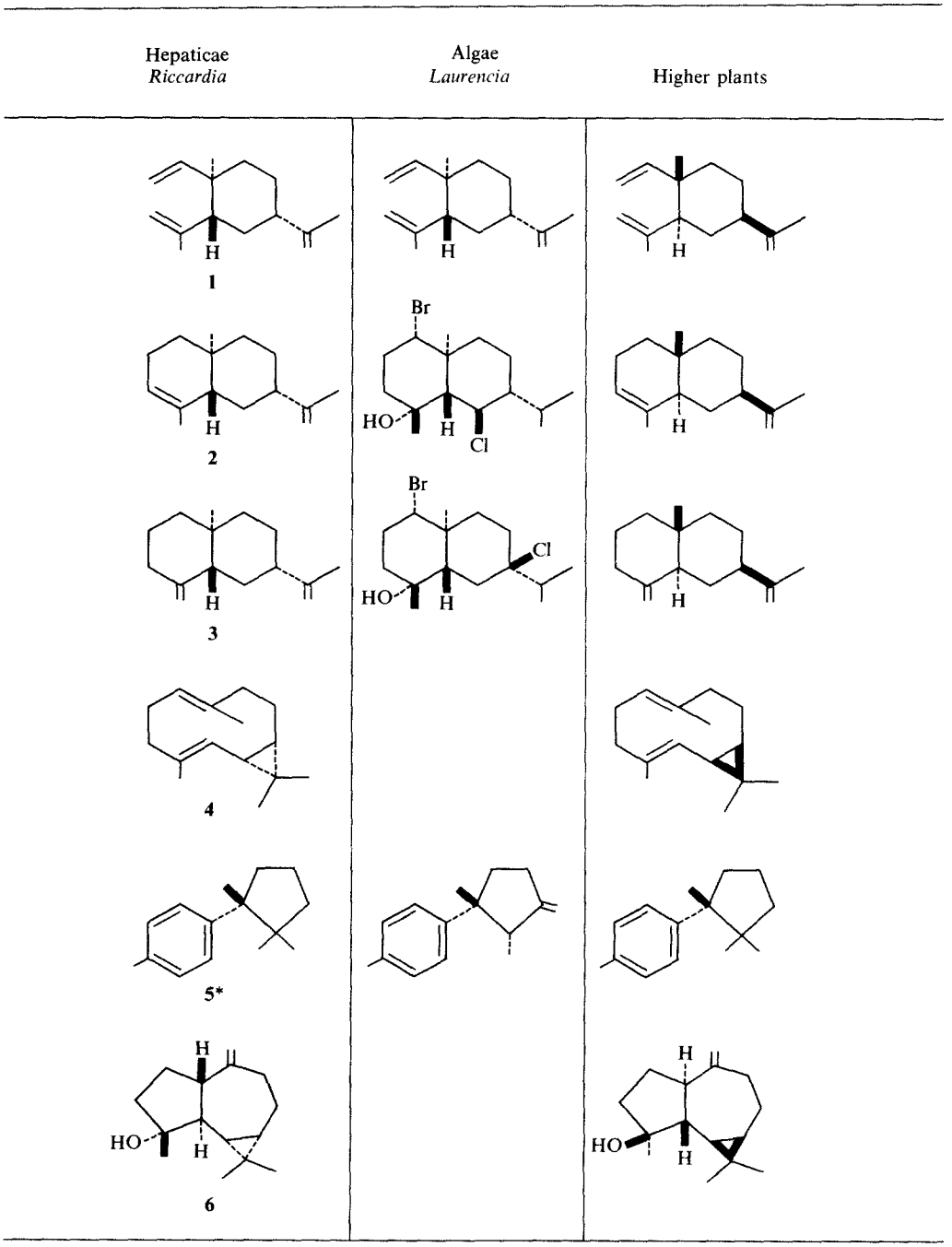
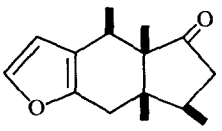


Fig. 1. Chiral properties of sesquiterpenoids among Hepaticae, algae and higher plants.* (*S*)-(−)-Cuparene has also been isolated from Jungermanniales.



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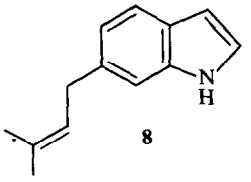

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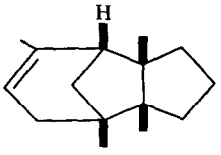

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sesquiterpenoids isolated from *R. jackii* led us to put forward the hypothesis that there is an evolutionary relationship between the Hepaticae and algae.

EXPERIMENTAL

^{1}H NMR: 60 MHz in $CDCl_3$; IR: film: MS (direct inlet system or GC–MS): 70 eV; Optical rotations: $CHCl_3$. GC–MS: glass column, SE-30 1%; 50–270°, 5°/min; He 30 ml/min. Preparative GLC: SE-30 10%, 3 m × 2 mm glass column; 80°–240°, 5°/min; N_2 30 ml/min. TLC: precoated Si gel (0.25 mesh) F_{254}, *n*-hexane–EtOAc (4:1) and C_6H_6–EtOAc (4:1). Spots were detected by spraying 30% H_2SO_4 and heating at 100°, and by UV light (254 nm).

Plant material. The species, identified by Drs. S. Hattori and M. Mizutani, are deposited in the Herbarium, Hattori Botanical Laboratory and Tokushima Bunri University, Inst. of Pharmacognosy.

Extraction and isolation. R. multifida S. Gray collected in Gotaki, Tokushima prefecture in May 1979 was air-dried for 2 days and ground. The ground material (180 g) was extracted with Et_2O for 2 weeks. The crude extract (3.50 g) was chromatographed on Si gel using a *n*-hexane–EtOAc gradient. The first fraction (*n*-hexane) contained a mixture of sesquiterpene hydrocarbons (110 mg) in which α-elemene and β-elemene, γ-cadinene and calamenene were detected by GC–MS. The above mixture was rechromatographed on Si gel using *n*-hexane to afford (+)-β-elemene (**1**) (20 mg): $[\alpha]_D$ + 17.3° (*c*, 1.20) (lit. + 15.1° [28]). The second fraction (*n*-hexane–EtOAc, 9:1) gave a mixture of carotenoids (30 mg). The third fraction (4:1) (1.720 g) was rechromatographed on Si gel using C_6H_6–EtOAc gradient to afford 6-[3-methyl-2-butenyl]-indole (**8**) (720 mg) [11, 12], triglycerides (350 mg) and sterols (70 mg). The fourth fraction (1:1) gave fatty acids (530 mg).

A. pinguis Dum. (= *R. pinguis*) collected in Kochi prefecture in August 1978 and *R. jackii* Schiffn. in Kamikatsu-cho, Tokushima prefecture in May 1979 were treated in the same manner as *R. multifida*. Each crude extract (520 mg of *A. pinguis* and 2.330 g of *R. jackii*) was chromatographed on Si gel using the solvent system *n*-hexane–EtOAc. The first fraction (*n*-hexane) from *A. pinguis* (32 mg) contained at least six unidentified sesquiterpene hydrocarbons. The second fraction (*n*-hexane–EtOAc, 9:1) was a viscous fragrant oil which was rechromatographed on Si gel using C_6H_6 to afford carotenoids (10 mg), pinguisone (**7**) (80 mg) [14] and unidentified sesquiterpenes (20 mg). The third fraction (4:1) contained sterols (20 mg) and fourth fraction (1:1) contained fatty acids (45 mg). The first fraction (*n*-hexane) from *R. jackii* contained sesquiterpene hydrocarbons (220 mg) which were rechromatographed on Si gel impregnated with 5% $AgNO_3$ using *n*-hexane as a solvent and then divided into two fractions: the first fraction was purified by preparative GLC to afford: (+)-α-selinene (**2**) (25 mg), $[\alpha]_D$ +6.6° (*c*, 1.10) (lit. +7.8° [29]); (−)-β-selinene (**3**) (18 mg), $[\alpha]_D$ −32.5° (*c*, 0.90) (lit. +63° [30]); (+)-α-barbatene (**9**) (23 mg), $[\alpha]_D$ +44.7° (*c*, 1.30) (lit. +48° [31]). The second fraction was rechromatographed on Si gel using *n*-hexane to afford: (−)-bicyclogermacrene (**4**) (18 mg), $[\alpha]_D$ −66.0° (*c*, 0.80) (lit. +61° [32]); (*R*)-(+)-cuparene (**5**) (29 mg), $[\alpha]_D$ +63.0° (*c*, 0.88) (lit. +65° [33], −27.6° [24]). The second column fraction (*n*-hexane–EtOAc, 9:1) (134 mg) was rechromatographed on Si gel using a C_6H_6–EtOAc gradient to afford a mixture of carotenoids (14 mg), (−)-spathulenol (**6**) (48 mg): $[\alpha]_D$ −22.7° (*c*. 1.30) (lit. +56° [34]) and triglycerides (60 mg). The third fraction (4:1) (270 mg) was purified by PLC to give unidentified sesquiterpene alcohols (44 mg) and phytosterols (59 mg). The fourth fraction (1:1) gave fatty acids (82 mg).

P. longispina Steph. collected in Gotaki, Tokushima prefecture in May 1979 was treated as described above. The crude extract (7.5 g) was chromatographed on Si gel using an *n*-hexane–EtOAc gradient. The first fraction (*n*-hexane) contained a mixture of paraffins and sesquiterpenes (350 mg) in which calamenene and cuparene (**5**) were detected by GC–MS as minor components. The second fraction (*n*-hexane–EtOAc, 19:1) (500 mg) was rechromatographed on Si gel using C_6H_6 to give a mixture of carotenoids (50 mg) and a mixture of sesquiterpenoids (240 mg). The third fraction (9:1) gave triglycerides (2.805 g). The fourth fraction (9:1) gave (−)-spathulenol (**6**) (500 mg). The fifth fraction gave fatty acids (1.700 g).

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