Phytochemistry, Vol. 20, No. 12, pp. 2695–2699, 1981.
Printed in Great Britain.

0031–9422/81/122695–05 $02.00/0

AROMATIC ESTERS AND TERPENOIDS OF THE LIVERWORTS IN THE GENERA *TRICHOCOLEA*, *NEOTRICHOCOLEA* AND *TRICHOCOLEOPSIS**

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(*Revised received* 30 *April* 1981)

Key Word Index—*Trichocolea tomentella*; *Neotrichocolea bissethii*; *Trichocoleopsis sacculata*; Trichocoleaceae; Hepaticae; trichocolein; tomentellin; isotomentellin; demethoxytomentellin; dehydrotomentellin; epoxytrichocolein-A; isoprenyl benzoates; sacculatane-type diterpenes; mono- and sesquiterpenes; Jungermanniales; Metzgeriales; chemosystematics.

Abstract—Three liverworts, *Trichocolea tomentella*, *Neotrichocolea bissethii* and *Trichocoleopsis sacculata* belonging to Jungermanniales were chemically investigated. Isoprenyl benzoates are the important chemical markers of *Trichocolea tomentella*. *Neotrichocolea bissethii* elaborates sesquiterpenes as the major components. The sacculatane-type diterpenes and pinguisane-type sesquiterpenes are the significant chemosystematic markers of *Trichocoleopsis sacculata*. These three species are chemically quite different. *Trichocoleopsis sacculata* is chemically rather close to *Porella* species. The present chemical results support the recent morphological classification of the above three species proposed by Schuster. Some species of Jungermanniales are chemically identical to those of Metzgeriales and these results also support the phylogenetic classification in which the two orders have been united within the subclass Jungermanniae.

INTRODUCTION

Most of the liverworts contain oil bodies which are composed of the mono-, sesqui- and diterpenoids and often lipophilic aromatic compounds. These substances are found as the main components in the species and hence they are valuable for the study of the chemosystematics of the Hepaticae. To date, we have reported the distribution of the terpenoids and aromatic compounds in 169 species of the Hepaticae and the Anthocerotae [1–11].

Only three species of the Trichocoleaceae [12], a family within the Jungermanniales, are known in Japan. These are *Trichocolea tomentella* (Ehrh.) Dum., *Neotrichocolea bissethii* (Mitt.) Hatt. and *Trichocoleopsis sacculata* (Mitt.) Okam. *T. tomentella* also grows in North America and Europe. These three species are morphologically fairly similar, in particular, *T. tomentella* is quite similar to *N. bissethii*. *T. sacculata* is somewhat smaller than the remaining two species and it contains characteristic pungent substances. *T. tomentella* and *N. bissethii* show no pungency. *T. tomentella* emits a weak phenolic odor when it is crushed or ground. When this species is handled for 1 or 2 days, the skin of the fingers gradually becomes rough. In this communication, we report the distribution of isoprenyl benzoates, mono-, sesqui- and diterpenes of these three species of Trichocoleaceae and the chemical interrelation among the above three species. We also note some chemical affinity between the species of the Jungermanniales and the Metzgeriales.

RESULTS AND DISCUSSION

Air-dried material of each species was ground and extracted with ether. The crude extracts were checked by TLC and GLC. Both the crude extracts of *T. tomentella* collected in Japan and Europe showed various blue spots on TLC under UV light (254 nm). The crude extracts of *T. sacculata* indicated the presence of five positive spots detected by 2,4-dinitrophenylhydrazine. Under UV light, no blue spots were detected in the crude extract of *N. bissethii*. The gas chromatogram of the crude extract of Japanese *T. tomentella* was very similar to that of the extract of European *T. tomentella*, except for the presence of one major peak and several minor peaks. The gas chromatogram of the crude extract of *N. bissethii* was considerably different from that of *T. tomentella*. The crude extract of *T. sacculata* showed a quite different gas chromatogram from those of *T. tomentella* and *N. bissethii*. Such preliminary chromatographic experiments imply that the chemical constituents of the three species may be quite different. Each crude extract was further analysed by GC/MS. The components were identified by GLC coinjection of standard compounds, analysis of each mass spectrum and direct comparison of mass spectra with those of the authentic samples. The major compounds were further isolated by preparative TLC and GLC, and their structures were confirmed by spectral evidence and some chemical degradations [13–15]. The species, sampling locations and months, and compounds detected in each species are listed in Table 1. The major components of Japanese *T. tomentella* are trichocolein (dimethylallyl-3,4-dimethoxybenzoate) (**14**), tomentellin (3,7-dimethyl-5-keto-2,6-octadienyl-3,4-dimethoxybenzoate) (**18**), isotomentellin (3,7-dimethyl-5-keto-3,6-

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* Part 9 in the series 'Chemosystematics of Bryophytes'. For Part 8, see (1981) *J. Hattori Bot. Lab.* **50**, 165.

Table 1. Mono-, sesqui- and diterpenes and isoprenyl benzoates of *Trichocolea*, *Neotrichocolea* and *Trichocoleopsis* species*

Species	Weight (dried)	Site of collection and month	Compounds detected																														
			1	2	3	4†	5†	6	7	8	9	10†	11†	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	Sterols
Trichocolea tomentella	72	Todorokinotaki, Kaifu-gun, Tokushima, Japan, Feb. 1977	+	+	+	+	+									+				+	+		+		+	+	+	+					+
T. tomentella	170	Kitagawa-son, Aki-gun, Kochi, Japan, Apr. 1978	+	+	+	+	+									+				+	+		+		+	+	+	+					+
T. tomentella	55	Gotaki, Yada-cho, Tokushima, Japan, Nov. 1978	+	+	+	+	+									+				+	+		+		+	+	+	+					+
T. tomentella	15	Yamatake, Nichinan, Miyazaki, Japan, Dec. 1978	+	+	+	+	+									+				+	+		+		+	+	+	+					+
T. tomentella	12	Inohae-Valley, Nichinan, Miyazaki, Japan, Sept. 1979	+	+	+	+	+									+				+	+		+		+	+	+	+					+
T. tomentella	2	Near Brienz, Bernese, Alpes, Switzerland, May 1979	+	+	+			+†								+	+	+	+	+	+	+	+	+	+	+	+	+					+
T. tomentella	3	Near Klagenfurt, Austria, July 1980	+	+	+			+†								+	+	+	+	+	+	+	+	+	+	+	+	+					+
Neotrichocolea bissethii	29	Kitamata forest, Yoshino-gun, Nara, Japan, Oct. 1979						+	+	+	+																						+
Trichocoleopsis sacculata	188	Yamadani-mura, Kamiukena-gun, Ehime, Japan, Aug. 1978.				+				+		+	+	+	+														+	+	+	+	+

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* Three species contained several unidentified minor sesquiterpene hydrocarbons and alcohols. *N. bissethii* produced an unknown sesquiterpene alcohol as the major component.
† The absolute configurations were tentatively assigned.


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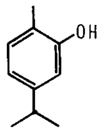
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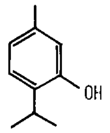
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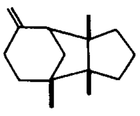
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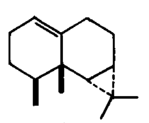
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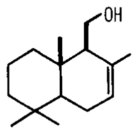
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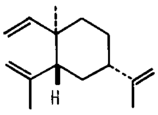
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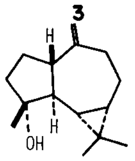
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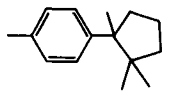
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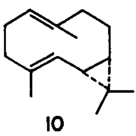
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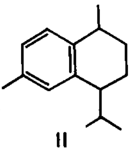
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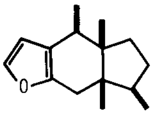
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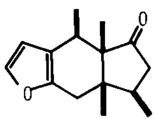
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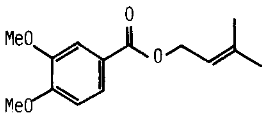
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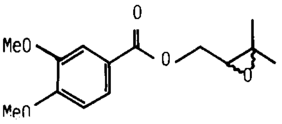
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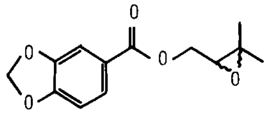
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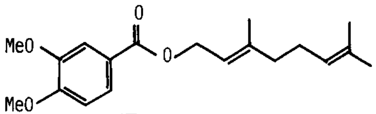
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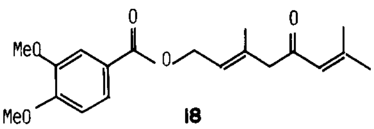
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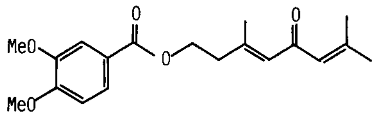
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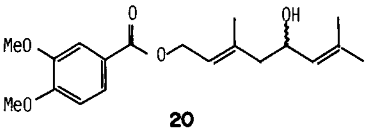
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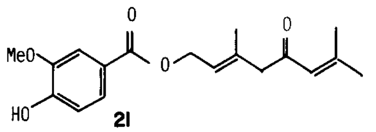
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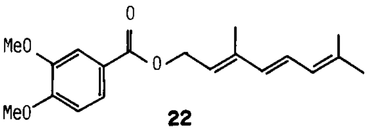
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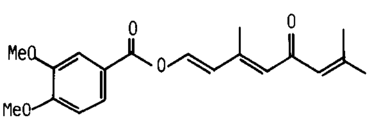
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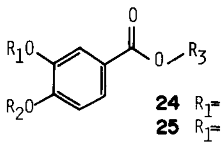
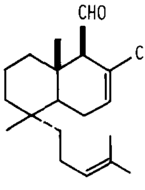

24 $R_1=R_2=R_3=H$
25 $R_1=R_3=Me$, $R_2=H$
26 $R_1=R_2=H$, $R_3=Me$

octadienyl-3,4-dimethoxybenzoate) (**19**) and demethoxytomentellin (3,7-dimethyl-5-keto-2,6-octadienyl-4-hydroxy-3-methoxybenzoate) (**21**) in which the compound (**14**) is the most abundant [14]. In addition to the above major isoprenyl benzoates, α-pinene (**1**), thymol (**2**), carvacrol (**3**) and two sesquiterpene hydrocarbons, β-barbatene (**4**) and β-gurjunene (**5**), and an additional isoprenyl benzoate, dehydrotomentellin (3,7-dimethyl-5-keto-1,3,6-octatrienyl-3,4-dimethoxybenzoate) (**23**) were detected as minor compounds. Japanese *T. tomentella* was collected at more than one location. The results for these specimens show a considerable degree of intraspecific qualitative and quantitative similarity as shown in Table 1. The chemical constituents of European *T. tomentella* are close to those of Japanese *T. tomentella*, except for the presence of four additional minor isoprenyl benzoates (**16**, **17**, **20**, **22**) and a sesquiterpene alcohol, drimenol (**6**) and for the absence of two sesquiterpene hydrocarbons (**4**, **5**). The major components are the isoprenyl benzoates, trichocholein (**14**), epoxytrichocolein-A (3-methyl-2,3-epoxybutyl-3,4-dimethoxybenzoate) (**15**), tomentellin (**18**), isotomentellin (**19**) and dehydrotomentellin (**23**) in which the compound (**23**) is the most abundant. European *T. tomentella* were collected in two different localities. Their chemical constituents were identical in TLC and GC/MS. From Japanese *T. tomentella*, the isoprenyl benzoates (**15**–**17**, **20**, **22**) and drimenol (**6**) have not been detected even by GC/MS. European and Japanese *T. tomentella* contain 3,4-dihydroxy benzoic acid (**24**) and its methyl ester (**26**) and methyl 3-hydroxy-4-methoxy benzoate (**25**). From the above results, it is quite clear that the isoprenyl benzoates and 3,4-disubstituted benzoic acid and its methyl esters are significant chemosystematic markers of *T. tomentella*.

Neotrichocolea bissethii produces (−)-drimenol (**6**), (+)-β-elemene (**7**), (−)-spathulenol (**8**) and (−)-cuparene (**9**). The major component is an unknown sesquiterpene alcohol; *m/z*: 222 $[M]^+$, 125 (100). Neither isoprenyl benzoates nor 3,4-disubstituted benzoic acids and their methyl esters found in *T. tomentella* have been detected in *N. bissethii.*

Trichocoleopsis sacculata is interesting in relation to medicinal chemistry since it elaborates some tumor-promoting substances [16]. We reported that *T. sacculata* produced four unique sacculatane-type diterpene dialdehydes (**27–30**) and pinguisane-type sesquiterpenes (**12**, **13**) [13, 15]. The major component is a pungent diterpene, sacculatal (**27**). In addition to the above diterpenes, *T. sacculata* contains bicyclogermacrene (**10**), calamenene (**11**), along with β-barbatene (**4**) and spathulenol (**8**) which are found in *T. tomentella* and *N. bissethii*, although their amounts are quite small. Thus, the chemical analysis indicated that pinguisane-type sesquiterpenes and sacculatane-type diterpenes are the significant chemosystematic markers of *T. sacculata.* On the basis of the above chemical evidence, it is quite clear that there is no chemical affinity among *T. tomentella*, *N. bissethii* and *T. sacculata*, although their morphologies are fairly similar. From the morphological background, Schuster [17, 18] recently proposed the phylogenetic classification of the Hepaticae in which *Trichocolea tomentella* belonging to Trichocoleaceae is classified into suborder Herbertineae and *Neotrichocolea bissethii* and *Trichocoleopsis sacculata* belonging to subfamilies Neotrichocoleoideae and Trichocoleopidoideae, respectively, are classified into suborder Lepidolaenineae, which is placed adjacent to the suborders Ptilidineae and Porellineae. The present chemical data essentially support Schuster's classification. However, it is suggestive from the chemical evidence that *T. sacculata* is in a completely different evolutionary line from *N. bissethii.* It is interesting to note that the chemical constituents of *T. sacculata* are rather similar to those of *Porella* species (Porellaceae). The sacculatane-type diterpenes (**31**, **32**) and pinguisane-type sesquiterpenes (**12**) and their closely related compounds are found in pungent or non-pungent *Porella* species [19–24]. It is suggested that *T. sacculata* may be in the same evolutionary line as some *Porella* species, although the former species are morphologically very far from the latter species. The chemical evidence also supports the arrangements of Porellineae and Lepidolaenineae proposed by Schuster [18].

Recently, many kinds of mono-, sesqui-, and diterpenoids and aromatic compounds have been found in the Hepaticae. However, much less attention has been paid to the chemical inter-relationships between the orders of the Hepaticae. Recent analyses indicated that *Makinoa crispata* (Steph.) Miyake, *Pellia endiviifolia* (Dicks.) Dum. and *Aneura pinguis* (L.) Dum., belonging to the Metzgeriales, biosynthesise the same sacculatane-type diterpene dialdehydes (**27**, **28**, **31**) and their related diterpene lactones, pinguisane-type sesquiterpene (**13**) and its related compounds, and drimane-type sesquiterpene lactone as those isolated from *T. sacculata* or *Porella* species [7, 15, 25–27]. These chemical data strongly suggest that some species of the Jungermanniales and of the Metzgeriales possess the same enzymes producing the specific diterpene dialdehydes and pinguisane-type sesquiterpenes which have been found only in the Hepaticae, although the present species belonging to both orders are morphologically quite distinct. In the modern classification of the Hepaticae, the Jungermanniales and the Metzgeriales are united within the subclass Jungermanniae [18] and a relationship between these orders is also suggested by sporogenesis [28]. The chemical identity between some species of the Jungermanniales and of the Metzgeriales also supports the above phylogenetic classification of the two orders.



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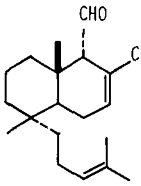

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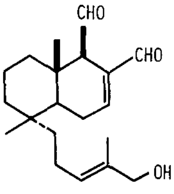

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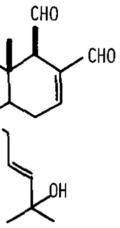

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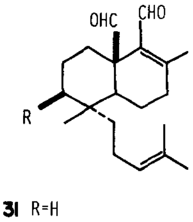

31 R=H
32 R=OH

EXPERIMENTAL

GC/MS, TLC, GLC, prep. TLC and prep. GLC were carried out in the manner described in the preceding papers [6, 15].

Plant materials. All the *Trichocolea tomentella*, *Neotrichocolea bissethii* and *Trichocoleopsis sacculata*, identified by R. M., Drs. S. Hattori, M. Mizutani, H. Inoue, T. Kodama and S. R. Gradstein, were deposited in the Herbarium, Institute of Pharmacognosy, Tokushima Bunri University.

Extraction and isolation. Each material (2–188 g) after being air-dried was ground. Each ground material (1 g) was extracted with Et_2O (30 ml) for 10 days. The pale green extracts were filtered through a short column packed with Si gel (230–400 mesh). The green oils were monitored by TLC under UV light (254 nm) and GLC. Each extract was directly analysed by a computerized GC/MS. The components obtained by GC/MS were identified by co-injection of the authentic samples and by the direct comparison of mass spectra with those of the authentic samples. The remaining materials were also extrd with Et_2O for 2 weeks and then re-extracted with MeOH for 2 weeks. Each extract was combined and the crude oils were chromatographed on Si gel and/or Sephadex LH-20 using *n*-hexane–EtOAc, C_6H_6–EtOAc gradients or MeOH–$CHCl_3$ (1 : 1). The chemical structures of the isolated compounds were established by UV, CD, IR, ^1H NMR, ^{13}C NMR and MS analyses and some chemical degradation and synthesis correlations. The structural elucidation and synthesis of some minor isoprenyl benzoates isolated from *T. tomentella* will be reported elsewhere.

Acknowledgements—We thank Drs. S. Hattori and M. Mizutani, The Hattori Botanical Laboratory, Miyazaki, Japan, Dr. H. Inoue, National Science Museum of Tokyo, Dr. T. Kodama, Ottemon Gakuin High School, and Dr. S. R. Gradstein, University of Utrecht, for their collection and identification of the liverworts and useful suggestions.

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