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SESQUITERPENOIDS OF FOURTEEN *PLAGIOCHILA* SPECIES*†

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Key Word Index—*Plagiochila acanthophylla* subsp. *japonica*; *P. arbuscula*; *P. asplenioides*; *P. dendroides*; *P. fruticosa*; *P. hattoriana*; *P. magna*; *P. ovalifolia*; *P. orbicularis*; *P. porelloides*; *P. pulcherrima*; *P. semidecurrens*; *P. trabeculata*; *P. yokogurensis*; Plagiochilaceae; Hepaticae; *ent*-2,3-secoaromadendranes; *ent*-aromadendranes; *ent*-bicyclogermacranes; *ent*-maalianes; chemosystematics.

Abstract—The distribution of *ent*-2,3-secoaromadendrane-, *ent*-aromadendrane-, *ent*-bicyclogermacrane- and *ent*-maaliane-type sesquiterpenoids in fourteen *Plagiochila* species is described. These sesquiterpenes are the significant chemosystematic markers of *Plagiochila*. The intense pungent substance of some *Plagiochila* species is due to an *ent*-2,3-secoaromadendrane-type sesquiterpene hemiacetal, plagiochiline A.

INTRODUCTION

As a part of our programme of chemosystematic investigation of bryophytes, we are continuing to study their chemical constituents. In the previous papers [1–5], we reported the distribution of mono-, sesqui- and diterpenoids, and lipophilic aromatic compounds from 91 species of Japanese and European liverworts. We also indicated that each liverwort produces some characteristic terpenoids and aromatic compounds which are very important chemosystematic markers in each genus.

The Plagiochilaceae belongs to Jungermanniales and more than 1500 species are known in the world; from Japan 20 species have been reported. *Plagiochila* species are very interesting from the view-point of agricultural chemistry, since they are a rich source of *ent*-2,3-secoaromadendrane-type sesquiterpenoids, which show plant-growth inhibition and very intense insect antifeedant effects [1, 2, 6–12]. In the present communication, we wish to report the distribution of *ent*-2,3-secoaromadendrane-, *ent*-aromadendrane-, *ent*-bicyclogermacrane- and *ent*-maaliane-type sesquiterpenoids in fourteen *Plagiochila* species.

RESULTS AND DISCUSSION

Each *Plagiochila* species after being air-dried and ground was extracted with ether. The crude extracts were directly analysed by GC–MS. The mass spectra obtained by GC–MS equipped with a computer were identified by direct comparison with those of authentic specimens and/or published information. The major components were further isolated by preparative TLC and GLC and their stereostructures were confirmed by spectral evidence described in the preceding papers [6, 8–12]. Table 1 shows species, sampling months and locations, and compounds detected in each species.

It is known that there are two chemical types of *Plagiochila* species, one with the characteristic sharp pungent substance, plagiochiline A (**4**), and the other without this pungent substance. *P. asplenioides*, *P. hattoriana*, *P. fruticosa*, *P. ovalifolia*, *P. porelloides*, *P. pulcherrima*, *P. semidecurrens*, *P. magna* and *P. yokogurensis* belong to the former type (type A); *P. acanthophylla* subsp. *japonica*, *P. arbuscula*, *P. dendroides* and *P. trabeculata* belong to the latter type (type B). As shown in Table 1, *ent*-bicyclogermacrene (**1**) [13, 14], which might be one of the precursors of *ent*-2,3-secoaromadendrane-type sesquiterpenoids (**3**–**18**) and their related compounds (**19**–**26**) found in *Plagiochila* species, was identified in every specimen of type A and type B examined in the present study. Among type A, *P. fruticosa*, *P. hattoriana*, *P. pulcherrima* and *P. yokogurensis* display remarkably sharp pungent taste. This is the result of the difference in plagiochiline A content (**4**). These four species elaborate a large amount of plagiochiline A (**4**). In addition to plagiochiline A, the species belonging to type A contain various *ent*-2,3-secoaromadendrane- (**3**, **5**–**18**), *ent*-aromadendrane- (**19**–**23**) and *ent*-maaliane-type sesquiterpenoids (**24**–**26**). Among the species listed in type A, *P. magna* morphologically resembles *P. semidecurrens* and the chromatograms (TLC and GC) of *P. magna* were very similar to those of *P. semidecurrens*; in fact, almost all components found in *P. magna* could be detected in *P. semidecurrens*. *P. fruticosa* and *P. yokogurensis* are rich sources of a sesquiterpene lactone, plagiochilide (**3**), which has not been detected in any species except for European *P. asplenioides* collected in Alsace and in Switzerland [15]. However, *P. asplenioides* collected in south-west France did not contain plagiochilide (**3**). It is necessary to compare the chemical constituents of *P. asplenioides* collected in

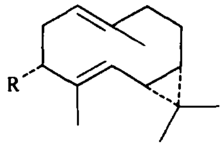
* Part of the present work has been reported at the 21st and 23rd Symposia of Chemistry of Terpenes, Essential Oils and Aromatics, Tokushima (1977) and Tottori (1979). Symposium papers pp. 225 and 211, respectively and International Congress of Bryology, Bordeaux, France (1977) [6].

† Part 6 in the series "Chemosystematics of Bryophytes". For Part 5, see (1980) *J. Hattori Bot. Lab.* **48**, 285.

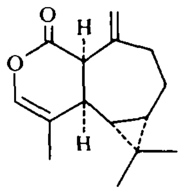
Table 1. *Ent*-2,3-secoaromadendrane-, *ent*-aromadendrane-, *ent*-bicyclo

Species		Date and site of collection	Compounds detected 1	2	3	4	5	6	7	8
P. acanthophylla subsp. *japonica*		Apr 1978, Kochi, Japan	+ +							
P acanthophylla subsp *japonica*		Nov. 1978, Tokushima, Japan	+ +							
P acanthophylla subsp *japonica*	[16]	Nov. 1978, Yabakei, Kyushu, Japan								
P arbuscula		Oct 1979, Yaku Island, Japan	+ + +							
P. asplenioides	[1, 10]	Apr. 1978, Puymartin, Dordogne, France	+ + +			+		+		
P asplenioides		Sept. 1979, Alfeld, Haut Rhin, France	+ + +	+	+ + + +	+	+	+		
P asplenioides		Aug 1979, Creux de l'Anvers, Jula, France	+ + +	+	+ + + +	+	+	+		
P asplenioides	[15]	Aug 1977, Brienz, Bernese, Alps, Switzerland			+					
P dendroides		Oct. 1979, Yaku Island, Japan	+ + +							
P fruticosa	[1, 2, 11, 12]	Sept. 1977, Tokushima, Japan	+	+	+ + + +	+ + + +	+			
P fruticosa		Dec. 1979, Miyazaki, Japan	+	+	+ + + +	+ + + +	+			
P hattoriana	[1, 2, 9]	Jan. 1977, Tokushima, Japan	+			+ + + +	+			
P hattoriana	[11]	Apr 1978, Tokushima, Japan	+			+ + + +	+			
P hattoriana		Apr 1978, Kochi, Japan	+			+ + + +	+			
P magna		Oct 1979, Yaku Island, Japan	+ + +			+	+ +			
P ovalifolia	[1, 2, 11, 12]	Apr 1978, Kochi, Japan	+	+		+ +	+		+	
P ovalifolia	[1, 2, 11, 12]	Nov 1978, Tokushima, Japan	+	+		+ +	+		+	
P ovalifolia		Nov 1979, Tokushima, Japan	+	+		+ +	+		+	
P ovalifolia	[17, 18, 20, 21]	Nov 1979, Kuju, Kyushu, Japan				+	+			
P orbicularis		Oct 1979, Yaku Island, Japan	+	+		+	+			
P porelloides		Aug 1979, Valee de Pont Nant, Switzerland	+ + +	+		+				
P pulcherrima		Oct 1979, Yaku Island, Japan	+ + +			+ + + +	+			
P semidecurrens	[1, 2, 10]	Apr 1977, Tokushima, Japan	+ + +			+	+			
P. semidecurrens		Aug 1978, Kochi, Japan	+ + +			+	+			
P semidecurrens		Oct 1979, Yaku Island, Japan	+ + +			+	+			
P semidecurrens	[17, 19, 20]	Nov 1977, Japan				+				
P trabeculata		Oct 1979, Yaku Island, Japan	+ + +	+						
P. yokogurensis	[1, 2, 6, 11]	Apr. 1976, Tokushima, Japan	+	+	+ + + +	+ + + +				+
P yokogurensis	[12]	May 1978, Tokushima, Japan	+	+	+ + + +	+ + + +				+
P yokogurensis		Apr 1979, Tokushima, Japan	+	+	+ + + + +	+ + + +				+

* The symbols, +, + +, + + +, etc. are the relative concentrations estimated by GC–MS.



1 R = H
2 R = OAc



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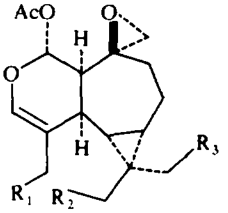
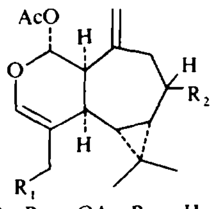
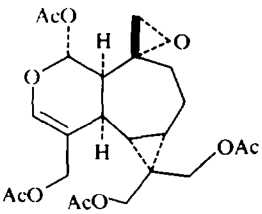


4 R_1 = OAc, R_2 = R_3 = H
5 R_1 = R_3 = OAc, R_2 = H
6 R_1 = R_2 = R_3 = OAc
7 R_1 = R_2 = OAc, R_3 = OH
8 R_1 = OH, R_2 = R_3 = H



9 R_1 = OAc, R_2 = H
10 R_1 = R_2 = H
11 R_1 = R_2 = OAc



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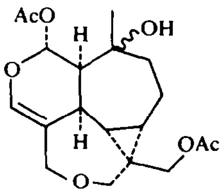

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germacrane- and *ent*-maaliane-type sesquiterpenes of *Plagiochila*

9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
												+ -					+ + +	
												+ -					+ + +	
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+	+		+	+				+						+				
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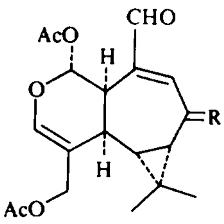
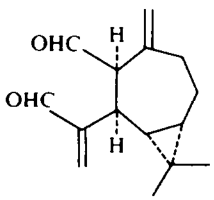


14 R = H_2
15 R = O



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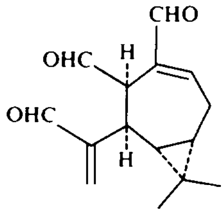

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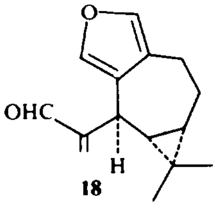

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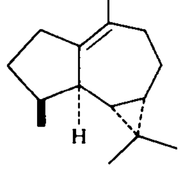

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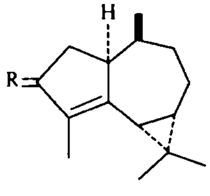

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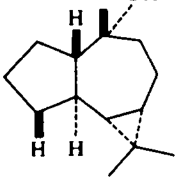
21 R = O



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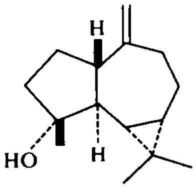

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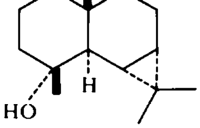

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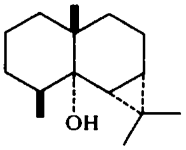

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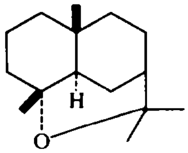

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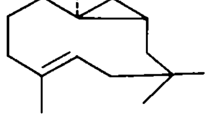

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different locations and seasons in order to determine whether the presence or absence of plagiochilide is due to the geographical difference or a seasonal one. Except for the sesquiterpene lactone **3**, *P. asplenioides* elaborates three unique *ent*-2,3-secoaromadendrane-type sesquiterpene hemiacetals, plagiochilines D (**6**), E (**12**) and F (**13**) which have not been found in Japanese *Plagiochila* species so far examined. European *P. porelloides* is morphologically similar to *P. ovalifolia*. These two species elaborate a large amount of plagiochiline C (**9**), however, the GLC and TLC patterns of *P. porelloides* are considerably different from those of *P. ovalifolia*. The former species produces six additional unidentified sesquiterpene acetates as minor components. *P. porelloides* is also very similar to *P. asplenioides* morphologically, but the chemical compounds of both species are clearly different. The most important difference is that *P. porelloides* does not contain plagiochilide (**3**). *P. trabeculata* belonging to type B also elaborates *ent*-2,3-secoaromadendrane-type sesquiterpenes, an *ent*-aromadendrane and *ent*-bicyclogermacrenes. Thus, *P. trabeculata* is more closely related to type A. Among type B, *P. acanthophylla* subsp. *japonica* is a unique species since it elaborates *ent*-cyclocolorenone (**21**) and *ent*-maalioxide (**26**) as the major components [16]. The former compound has been found in a few species of type A as a minor component. Chemically speaking, *P. arbuscula* and *P. dendroides* are the most isolated species among the types A and B, since they elaborate neither *ent*-2,3-secoaromadendrane-, *ent*-aromadendrane-type sesquiterpenoids nor *ent*-maaliane-type sesquiterpenoids. Samples of *P. asplenioides*, *P. fruticosa*, *P. hattoriana*, *P. ovalifolia*, *P. semidecurrens* and *P. yokogurensis* (type A) and *P. acanthophylla* subsp. *japonica* were collected at more than one location. The results for these species show a considerable degree of intraspecific qualitative and quantitative similarity, except for the specimen of *P. asplenioides* collected in south-west France described above.

Since the isolation and structural determination of various *ent*-2,3-secoaromadendrane-type sesquiterpenoids from Japanese and European *Plagiochila* species [1, 2, 6, 8–12], Matsuo *et al.* [17–21] have recently confirmed the presence of the above unique *ent*-2,3-secoaromadendrane- and *ent*-maalian-type sesquiterpenes in *P. ovalifolia* and *P. semidecurrens*. *Ent*-2,3-secoaromadendrane-type sesquiterpenoids (**1**–**18**) have not been found in the other liverworts so far examined nor in higher plants. It seems quite clear that the present *ent*-secoaromadendrane-type sesquiterpenoids and their related *ent*-sesquiterpenoids are one of the significant chemosystematic markers in the Plagiochilaceae, and biochemical investigation of the metabolites of *Plagiochila* species may yield important information that will lead to a better understanding of the complex taxonomy of the Plagiochilaceae.

EXPERIMENTAL

GC–MS spectra were obtained under the following conditions: elec. energy 20 and 70 eV: trap current 60 μA: temp. 80–250°; GLC column: 1% SE-30, 3 m × 2 mm glass column: temp. 50–270° at 5°/min, inject. temp. 260°, He 30 ml/min. Analytical and prep. GLC: 1% SE-30, 3 m × 2 mm and 5% DEGS glass column, temp. programme 50–270° at 5°/min, inject. temp. 260°, N_2 30 ml/min. TLC and prep. TLC: precoated Si gel (0.25 mesh) F_{254}, *n*-hexane–EtOAc (4:1) and C_6H_6–EtOAc (4:1 and 1:1) as solvents. Spots were detected by 30% H_2SO_4 and UV light (254 nm).

Plant material. All *Plagiochila* species identified by H.I. are deposited in the Herbarium, National Science Museum of Tokyo (TNS).

Extraction and isolation of sesquiterpenoids from Plagiochila *species.* Each *Plagiochila* species (1.5–2253 g) after being air-dried was ground. Each ground material (1 g) was extracted with Et_2O (30 ml) for 5 days and the green extract was filtered through a short column packed with Si gel (70–270 mesh) and the solvent was evapd *in vacuo*. The green oil was diluted with Et_2O to a suitable concn and then analysed by a GC–MS apparatus equipped with computer. The remaining ground materials were also extracted with Et_2O in order to isolate sesquiterpenoids by prep. TLC and prep. GLC, after being chromatographed on Si gel (70–270 mesh) using an *n*-hexane–EtOAc or a C_6H_6–EtOAc gradient [6–12].

Acknowledgements – We are indebted to Dr. C. Suire (Université de Bordeaux I) for his collection and identification of European *Plagiochila* species

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