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SESQUITERPENE HYDROCARBONS OF THE LIVERWORT *SCAPANIA ORNITHOPODIOIDES*

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Key Word Index—*Scapania ornithopodioides*; Hepaticae; Jungermanniales; liverwort; sesquiterpenes; aromadendrene; cyclosativene; α-farnesene.

Abstract—The liverwort, *Scapania ornithopodioides*, was investigated for sesquiterpene hydrocarbons by utilizing GC and GC/MS and 17 components were identified. Among these, the major component, aromadendrene, and two minor ones, cyclosativene and α-farnesene are reported for the first time as constituents of liverworts. In addition, aromadendrene also occurred in three other Taiwanese liverworts examined.

INTRODUCTION

As part of a chemosystematic study [1, 2] of the liverworts grown in Taiwan, the oil of *Scapania ornithopodioides* was investigated. Due to the limited amounts of plant material available GC/MS has often been used for the analysis of the volatile constituents of liverworts. However, many sesquiterpene hydrocarbons which are steric or double bond isomers have very similar mass spectra with only a minor difference in peak intensities and these may vary with the analysis conditions used each time. Therefore, identification purely on the basis of mass spectral data sometimes appears doubtful. In the present work, in addition to GC/MS analysis, four different GC stationary phases were used to obtain four sets of Kovats' indices for comparison with established values from authentic sesquiterpenes [1–4]. In this way, the assignment to known compounds based on both GC and GC/MS data will be much more reliable. Moreover, by using Kovats' indices, the many authentic samples required for GC retention time comparison which are often difficult to store or to acquire, will usually no longer be necessary. By using this method, 17 sesquiterpene hydrocarbons were identified in the oil of *S. ornithopodioides*.

A GC survey of the oil of *S. ornithopodioides* was reported by Huneck *et al.* [5] and Andersen *et al.* [3] respectively. In the former paper sitosterol was reported in the oil. In the latter paper several Kovats' indices of six peaks were given and β-barbatene was assigned as the major constituent (30–60%). In the present work, however, β-barbatene was found to be only a minor constituent (5%). The major constituent (20%) found in the oil was aromadendrene. Aromadendrene has not been reported previously as a component in liverworts although other ent-aromadendrane-type sesquiterpenes such as ent-ledrene [6], ent-spathulenol [6] and tridensenone [7] have been found in liverworts. In addition, two minor components of the oil, cyclosativene and α-farnesene, are also reported for the first time as constituents of liverworts.

RESULTS AND DISCUSSION

The *n*-hexane extract of the powdered liverworts was first chromatographed over Si gel to remove polar fractions. The hydrocarbon oil was then further chromatographed to give various fractions in order to reveal more information about minor constituents. Both the total hydrocarbon oil and each of the fractions were examined by GC and GC/MS. Four different columns were used to acquire four sets of Kovats' indices for each peak of the hydrocarbon oil and at least two columns were used for GC analysis of each fraction. Table 1 lists the 17 components that were identified in the oil. Anastreptene, β-barbatene and β-bazzanene [8], the common constituents of liverworts, were detected in this oil.

Component 4 was suspected to be cyclosativene on the basis of its GC retention data. This assignment was supported by the GC/MS data obtained for one early fraction from CC. Its mass spectrum showed the eight most abundant fragments at *m/z* (rel. int.): 204(100), 161(90), 119(65), 189(63), 93(55), 95(48), 176(45) and 105(41) in accordance with the literature values [9, 10].

Due to its relatively high concentration (8%) in the oil, component 5 was obtained in a late fraction with 70% purity. The ^{1}H NMR spectrum [δ 1.6 (*s*, 3H), 1.64 (*s*, 3H), 1.68 (*s*, 3H), 1.76 (*s*, 3H), 2.82 (*t*, 2H), 4.9 (*d*, 2H), 5.22 (distorted *t*, 2H), 5.44 (*t*, 1H) and 6.35 (*dd*, 1H)] and the GC/MS data [*m/z* (rel. int.): 93 (100), 55(64), 41(55), 69(43) and 119(42)] of this material were all consistent with those of α-farnesene [11]. The GC Kovats' index of component 5 on an Apiezon L column further supported its identification as α-farnesene.

The identity of component 7 as aromadendrene was confirmed by comparison of the four sets of GC

Table 1. Sesquiterpenes in the oil of *Scapania ornithopodioides*

Compound No.*	Sesquiterpenes	%	I_A^{190}†	I_B^{170}	I_C^{150}	I_D^{160}	MS ref.
1	Anastreptene	2	1404	1395	1568	1722	[8]
2	β-Elemene	3	1428	1410	1608		[11]
				—‡		—	
3	β-Farnesene	3	1436	1450	1664	1824	[11]
4	Cyclosativene	2	1436	1404	1532	1678	[9, 10]
			(1435)§		(1537)		
5	α-Farnesene	8	1484	1501	1747		[11]
			(1489)	—	—	—	
6	β-Maaliene?	0.5					[11]
			—	—	—	—	
7	Aromadendrene	20	1500	1463	1650	1824	[11, 17]
			(1501)	(1465)	(1650)	(1820)	
8	Ar-curcumene	4	1500	1481	1776	1994	[14]
			—				
9	Selina-4,11-diene	4	1515	1491	1704	1722	[14]
10	α-Patchoulene?	3	1536	1529	1763		[11]
			—	—	—	—	
11	β-Barbatene	5	1536	1473	1691	1896	[2, 24]
12	β-Selinene	11	1559	1505	1750	1966	[11, 14–17]
13	α-Selinene	12	1559	1512	1750	1966	[14–16]
						—	
14	β-Chamigrene	3	1559	1501	1740	1948	[14–17]
15	Cuparene	3	1559	1512	1841	2092	[17]
16	Calacorene	1	1594		1893	2194	[25, 26]
			(1590)	—	(1892)	(2191)	
17	β-Bazzanene	8	1594	1535	1790		[27]

*Listed according to the elution order from an Apiezon L column.

†GC liquid phase designations; the superscript is the temperature, the subscript is the column used (see the Experimental).

‡A dash line indicates that the reference data are not available.

§Indices in parentheses are reference data not included in refs. [3, 4].

indices, the mass spectrum and the ^{1}H NMR spectrum [δ 0.5–0.8 (*br m*, 2H), 0.945 (*d*, $J = 7$ Hz, 3H), 0.96 (*s*, 3H), 1.02 (*s*, 3H) and 4.57 (*s*, 2H)] of the isolated component with those obtained from a commercial sample of aromadendrene. It is surprising that many other ent-aromadendrane-type sesquiterpenes have been identified in liverworts, yet the presence of aromadendrene in liverwort oils is reported here for the first time. Furthermore, aromadendrene was also found in three other Taiwanese liverwort species as shown in Table 2. Several American and European *Scapania* species and Japanese *Plagiochila* species have been investigated by Andersen *et al.* [3, 12] and Asakawa *et al.* [13], respectively, but aromadendrene was not detected in any of them. The production of a reasonable amount of aromadentrene by liverworts grown in Taiwan may have some geographic significance for certain species.

The assignments of components 6 and 10 as β-maaliene and α-patchoulene, respectively, are considered tentative since their Kovats' indices were not available for comparison. Their major mass spectral fragments [*m/z* (rel. int.): 161(100), 189(97), 133(85), 91(83) for component 6 and 93(100), 135(72), 107(70)

Table 2. The occurrence of aromadendrene in Taiwanese liverworts

Liverworts	% aromadendrene in the hydrocarbon fraction of the oil	Ref.
Scapania robusta	5	[19]
Schistochila rigidula	12	[19, 20]
*Plagiochila kahsiana**	8	[19, 21]
Scapania ornithopodioides	20	†

*Reported as *P. gollani* in ref. [19].

†The present study.

for component 10] are quite compatible with the published data [11] for β-maaliene and α-patchoulene, respectively.

It is worth noting that bicyclogermacrene which has been claimed as a common constituent in liverworts [18] was not found in the oil of *S. ornithopodioides* in any significant amount. Bicyclogermacrene also has not been detected in other Taiwanese liverwort species [1, 2, 19–21].

EXPERIMENTAL

Plant materials. Liverworts were collected in July 1980 at Yuenyang Lake, Ilan. The voucher specimens were deposited in the Herbarium of Taiwan Museum. The sample was identified by Dr. M. C. Lai, Associate Curator, Department of Botany, Taiwan Museum.

GC. Columns used were: A, Apiezon L, 10%, 2 m × 2 mm; B, SF-96, 3%, 8 m × 2 mm or Golay SF-96, 45 m × 0.25 mm; C, Carbowax 20 M, 10%, 2 m × 2 mm; D, DEGS, 10%, 3 m × 2 mm. N_2 was used as carrier gas with the flow rate at 15 ml/min.

GC/MS. Most analyses were carried out at 20 or 70 eV. GC separations were performed on one of the following columns: 1% SE-30, 1 m × 3 mm; 10% OV-17, 3 m × 3 mm; or column B, see above. Two capillary columns (WCOT SE-30, 30 m and FSOT OV-101, 30 m) were also used on a GC/MS instrument linked to a computer.

NMR. ^{1}H NMR spectra were obtained in $CDCl_3$ soln.

GC method. All hydrocarbon fractions were analysed using longifolene and cuparene as standards according to the modified 'self-consistent Kovats' indices' method described previously [1, 22, 23].

Identification of the constituents. The air-dried plant material (256 g) was extracted with *n*-hexane for 2 weeks after freezing with liquid N_2. The crude extract (2 g) was chromatographed over Si gel (70–230 mesh) using a *n*-hexane–C_6H_6–EtOAc gradient. The *n*-hexane fraction afforded a hydrocarbon oil (350 mg). The oil (100 mg) was rechromatographed to give two fractions, I and II. The rest of the oil was again chromatographed on Si gel and 13 fractions were collected. The hydrocarbon oil, I, II and each of the 13 fractions were analysed by GC and GC/MS. The sesquiterpenes listed in Table 1 were identified by comparison of their Kovats' indices with those for authentic samples and comparison with reported mass spectra.

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