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(—)-*ENT*-SPATHULENOL ISOLATED FROM LIVERWORTS IS AN ARTEFACT

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Abstract—During the investigation on the constituents of liverworts, (−)-*ent*-spathulenol and (−)-*ent*-bicyclogermacrene were frequently found in many species. While studying the liverwort *Dicranolejeunea yoshinagana*, it was observed by chance that (−)-*ent*-bicyclogermacrene is easily converted to (−)-*ent*-spathulenol. A pure sample of (−)-*ent*-bicyclogermacrene was allowed to stand at room temperature and was found to have changed completely to (−)-*ent*-spathulenol. The conversion was accomplished quantitatively and the enantiomeric purity of (−)-*ent*-spathulenol was equivalent to that of (−)-*ent*-bicyclogermacrene. This transformation also occurred during the extraction of liverworts.

INTRODUCTION

The liverworts, including the Jungermanniales, are rich sources of terpenoids, particularly sesquiterpenoids, some of which are enantiomeric to those found in higher plants [1, 2]. During the investigation of liverwort constituents, it was found that (−)-*ent*-spathulenol and (−)-*ent*-bicyclogermacrene were encountered in many species. A number of authors have postulated that bicyclogermacrene is the biogenetic precursor of other sesquiterpenoids with a 1,1′-dimethyl cyclopropane ring, e.g. maaliane- [3, 4], aristolane- [5], 2,3-secoaromadendrane- [6, 7] and aromadendrane-type [3] sesquiterpenoids. All these skeletal compounds isolated from liverworts are enantiometric to those found in higher plants, except for 2,3 secoaromadendrane-type sesquiterpenoids which have not been found in higher plants, fungi or marine organisms.

During the investigation of volatile constituents from liverworts by GC-mass spectrometry, (−)-*ent*-spathulenol was often encountered, particularly in old liverwort extracts. Here we report on the result of a comparative GC-mass spectral analysis of fresh and old liverwort extracts.

RESULTS AND DISCUSSION

The GC-mass spectral analysis of 264 samples, containing 87 species of liverworts, showed the presence of (−)-*ent*-bicyclogermacrene (**1**) in 41 species. (−)-*ent*-Bicyclogermacrene (**1**) was therefore found to be quite a common constituent in the liverworts as well as β-barbatene (**2**), 1-octen-3-yl acetate (**3**) and *ent*-spathulenol (**4**). A determination of the enantiomeric purity of (−)-*ent*-bicyclogermacrene (**1**), found in liverworts, by GC-mass spectral analysis using a chiral capillary column was performed. This established the enantiomeric purity as almost 100:1 for (−)- to (+)-bicyclogermacrene. Recent work on the enantiomeric composition of bicyclogermacrene in the liverworts *Scapania uliginosa* and *Jamesoniella autumnalis* provided further evidence for this enantiomeric ratio in which (−) exceeds (+) [8]. The optical rotation of (−)-*ent*-bicyclogermacrene (**1**) isolated from many liverworts also confirmed an enantiometrically pure state by comparison with that of (+)-bicyclogermacrene isolated from the higher plant *Citrus junos* [9]. While higher plants produce (+)-bicyclogermacrene, liverworts and certain species of marine organisms elaborate (−)-*ent*-bicyclogermacrene (**1**). This difference interested us from the viewpoint of studying the phylogeny of liverworts.

Recently, (−)-*ent*-bicyclogermacrene (**1**) was isolated from *Dicranolejeunea yoshinagana* (Lejeuneaceae) by preparative HPLC. A pure sample of (−)-*ent*-bicyclogermacrene (**1**) was allowed to stand at room temperature and after 4 days was found to have changed completely to (−)-*ent*-spathulenol (**4**). The conversion occurred quantitatively and the enantiomeric purity of (−)-*ent*-spathulenol (**4**) was equivalent to that of (−)-*ent*-bicyclogermacrene (**1**). Thus, by chance it was observed that (−)-*ent*-bicyclogermacrene (**1**) is easily converted to (−)-*ent*-spathulenol (**4**). This discovery prompted us to reexamine by GC-mass spectrometry, 10 selected old extracts of liverwort species. Previous GC-mass spectral analysis of the fresh extract confirmed that all of these

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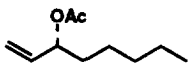

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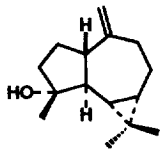

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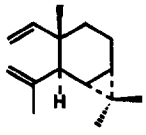

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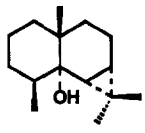
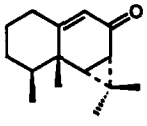
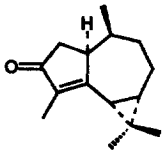


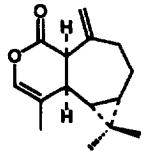
maalian-5-ol
(maaliane-type)



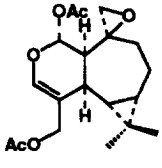
aristolone
(aristolane-type)



cyclocolorenone
(aromadendrane-type)



plagiochilide



plagiochiline A

(2, 3-secoaromadendrane-type)

species contained (−)-*ent*-bicyclogermacrene (**1**). The GC-mass spectra of these ether extracts 7 to 8 months after they were prepared, not only showed the absence of (−)-*ent*-bicyclogermacrene (**1**), but also displayed the appearance of (−)-*ent*-spathulenol (**4**), as shown in Table 1. This indicated that (−)-*ent*-bicyclogermacrene (**1**) was completely transformed to (−)-*ent*-spathulenol (**4**). This conversion also happened during the extraction of liverworts, as shown in Fig. 1 which displays the total ion chromatogram (TIC) of the GC-mass spectrum after 1 day and 4 months of extraction of *Scapania stephanii* (#95051) (Scapaniaceae) with distilled diethyl ether.

The GC-mass spectrum of bicyclogermacrene displays two peaks, even if pure bicyclogermacrene is injected, because at 200° Cope rearrangement of bicyclogermacrene gives bicycloelemene (**5**) [9, 10]. The proportion of (−)-*ent*-bicyclogermacrene (**1**) detected in each of the extracts was thus calculated as a total abundance value of both peaks on the total ion chromatograph. A mechanism for the transformation of bicyclogermacrene into spathulenol is under investigation and Scheme 1 presents a possible reaction sequence which may explain the transformation by autoxidation.

Many species of liverworts have been reported to contain (−)-*ent*-spathulenol (**4**) and to date more than 400 papers concerning its detection and isolation from all plant species have appeared. In many cases, bicyclogermacrene has also been isolated together with spathulenol. The results presented here indicate that in many of these cases (if not all) spathulenol in an artefact produced from bicyclogermacrene.

EXPERIMENTAL

General. The GC-MS analysis of the Et_2O extracts was carried out at 70 eV and on a fused silica capillary column coated with DB-17 (30 m × 0.25 mm i.d., film thickness 0.25 μm) using He as the carrier gas (1ml min^{-1}). Temp. programming from 50° isothermal for

Table 1. The results of comparative GC-mass spectral analysis of fresh and old extracts of some selected liverworts

Herbarium no.	Species	Fresh		Old	
		BG(%)	SP(%)	BG(%)	SP(%)
94003	*Plagiochila fruticosa*	12.8	N	N	5.4
94004	*Plagiochila fruticosa*	22.0	N	N	11.9
94005	*Plagiochila fruticosa*	13.3	N	N	10.8
94008	*Plagiochila fruticosa*	15.5	N	N	15.4
94013	*Plagiochila fruticosa*	20.2	N	N	16.3
94015	*Porella japonica*	3.0	N	N	3.1
94023	*Porella acutifolia* subsp. *tosana*	4.3	N	N	1.6
94025	*Plagiochila sciophila*	4.3	N	N	3.0
94030	*Plagiochila sciophila*	3.0	N	N	2.8
94083A	*Porella japonica*	4.9	N	N	4.2

Abbreviations used: BG = (−)-*ent*-bicyclogermacrene, SP = (−)-*ent*-spathulenol and N = not detected.

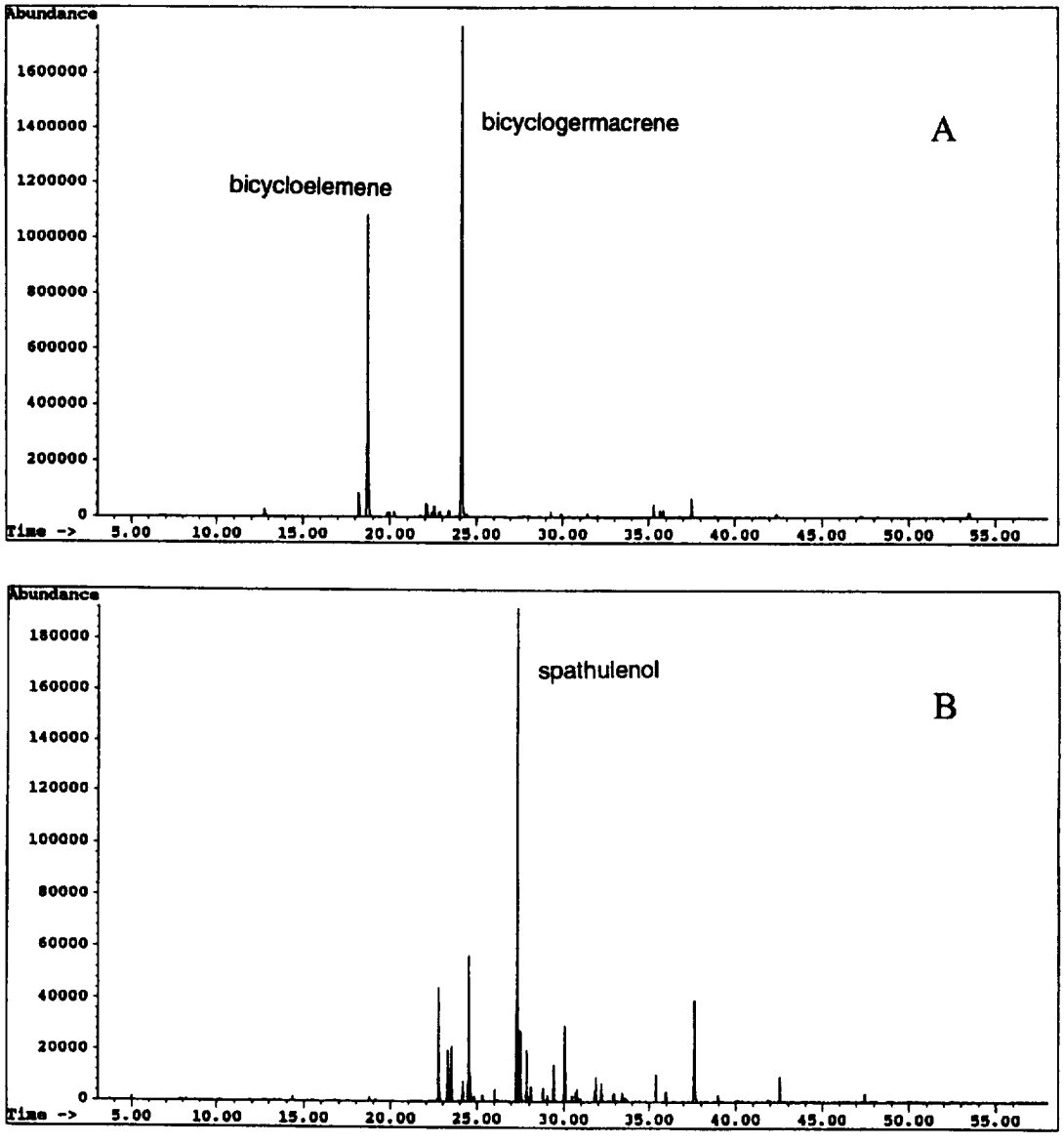
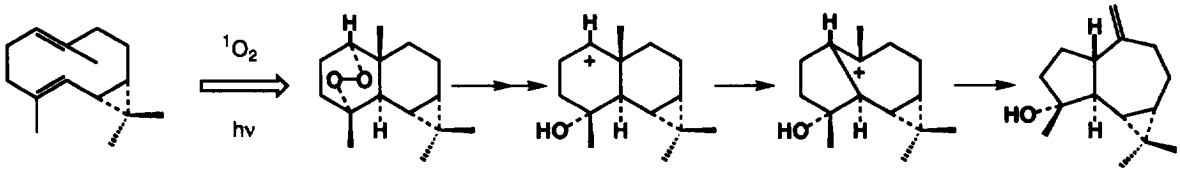


Fig. 1. A: Total ion chromatograph (TIC) of the extract of *Scapania stephanii* after 1 day's extraction, showing two major constituents. The two main peaks are due to the Cope rearrangement of (−)-*ent*-bicyclogermacrene. B: TIC of the extract of *Scapania stephanii* 4 months after extraction, showing how (−)-*ent*-bicyclogermacrene has changed to (−)-*ent*-spathulenol.



3 min, then 50–250° at 5° min^{-1}, and finally isothermal at 250° for 15 min. Injection temp. was 250°. The GC-MS analysis for the enantiomeric purity was carried out on a fused silica capillary column coated with CDX-B (30 m × 0.25 mm i.d., film thickness 0.25 μm). Temp. programming was performed from 50° isothermal for 3 min, then 50–230° at 3° min^{-1}, and finally isothermal at 230° for 5 min.

Plant materials. Plagiochila fruticose (#94003, 94004, 94005, 94008 and 94013), *Plagiochila sciophila* (#94025 and 94030), *Porella acutifolia* subsp. *tosana* (#94023) and *Porella japonica* (#94015) were collected in January 1994

at Kamikatsu-cho, Katsuura-gun (Tokushima, Japan). *Porella japonica* (#94083A) was collected in March 1994 at Kamiyama-cho, Myouzai-gun (Tokushima, Japan). *Dicranolejeunea yoshinagana* (#94210) was collected in July 1994 at Beppu-valley, Kami-gun (Kochi, Japan). *Scapania stephanii* (#95051) was collected in February 1995 at Katsuura-cho, Katsuura-gun (Tokushima, Japan). The voucher specimens are deposited at the Faculty of Pharmaceutical Sciences, Tokushima Bunri University. The liverworts were dried for 1 day, impurities removed and the plant material ground in a mortar with Et_2O then filtered through a short pad column on celite. Removal of the solvent was done under a stream of air.

Isolation of (−)-ent-*bicyclogermacrene* (**1**). The Et_2O extract of *Dicranolejeunea yoshinagana* (#94210) (0.68 g) was chromatographed on silica gel using *n*-hexane–EtOAc gradient, yielding a mixture containing bicyclogermacrene. The mixture was further purified by prep. HPLC using *n*-hexane to give (−)-*ent*-bicyclogermacrene (**1**) (14.5 mg; 2.1% of total extract). $[\alpha]_D$ − 63° (in acetone, *c* 0.46); ref. [9] $[\alpha]_D$ + 61° (in Me_2CO). The pure (−)-*ent*-bicyclogermacrene (**1**) (14.5 mg) was allowed to stand at room temp. free from solvent for 4 days. The ^{1}H NMR and GC-MS analyses of the sample after 4 days were identical to those of spathulenol (**4**) [11].

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