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VOLATILE COMPONENTS OF THE LIVERWORTS *ARCHILEJEUNEA OLIVACEA*, *CHEILOLEJEUNEA IMBRICATA* AND *LEPTOLEJEUNEA ELLIPTICA*

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Key Word Index—*Archilejeunea olivacea*; *Cheilolejeunea imbricata*; *Leptolejeunea elliptica*; Lejeuneaceae; Ptychantoideae; Lejeuneoideae; Hepaticae; olivacene; (*R*)-dodec-2-en-1,5-olide; (*R*)-tetradec-2-en-1,5-olide; 1-ethyl-4-methoxybenzene; 1-ethyl-4-hydroxybenzene; 1-ethyl-4-acetoxybenzene; β-monocyclonerolidol; (−)-spathulenol; volatile components; fragrance; chemosystematics.

Abstract—Three liverworts, *Archilejeunea olivacea*, *Cheilolejeunea imbricata* and *Leptolejeunea elliptica*, belonging to the Lejeuneaceae, have been investigated chemically. Olivacene, a new naturally occurring sesquiterpene hydrocarbon, β-monocyclonerolidol and (−)-spathulenol have been isolated from *A. olivacea*. The strong milky-like fragrance of *C. imbricata* is due to a mixture of (*R*)-dodec-2-en-1, 5-olide and (*R*)-tetradec-2-en-1,5-olide. *Leptolejeunea elliptica* produces an intensely fragrant odour which is due to a mixture of 1-ethyl-4-methoxy-, 1-ethyl-4-hydroxy- and 1-ethyl-4-acetoxybenene. There are no chemical affinities between the present three Lejeuneaceae species. Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

There are more than 3000 species belonging to the Lejeuneaceae family of the Hepaticae in the world; they are very difficult to classify because they are morphologically very small. Previous reports show that the species of the Lejeuneaceae are rich sources of striatane-, barbatane- and pinguisane-type sesquiterpenoids [1, 2]. We investigated the volatile components of three Lejeuneaceae species, *Archilejeunea olivacea*, *Cheilolejeunea imbricata* and *Leptolejeunea elliptica*, and isolated a new sesquiterpene hydrocarbon, olivacene (**1**), β-monocyclonerolidol (**2**) and (−)-*ent*-spathulenol (**3**) from *A. olivacea*, two known δ-lactones (**4** and **5**) from *C. imbricata* and 1-ethyl-4-methoxybenzene (**6**), 1-ethyl-4-hydroxybenzene (**7**) and 1-ethyl-4-acetoxybenzene (**8**) from *L. elliptica*.

RESULTS AND DISCUSSION

Archilejeunea olivacea was extracted with diethyl ether for one week. TLC analysis of the ether extract showed the presence of a major constituent with *ca* 80% of the total extract at the beginning of the extraction, although it was completely decomposed during the extraction. A combination of silica gel column chromatography and HPLC of the ether extract of *A. olivacea* resulted in the isolation of **1**, **2** and **3**.

The ^{1}H NMR spectrum of compound **1**, $C_{15}H_{22}$ ($[M]^+$ at *m/z* 202), contained two tertiary methyls [δ 1.26 (6H, *s*)], an isopropyl group [δ 1.23 (6H, *d*, $J = 7$ Hz) and 2.83 (1H, *sept*, $J = 7$ Hz)], three protons on a 1,2,4-trisubstituted benzene ring [δ 6.89 (*d*, $J = 2.2$ Hz), 7.01 (*dd*, $J = 8.3$, 2.2 Hz) and 7.24 (*d*, $J = 8.3$ Hz)]. The ^{13}C NMR and DEPT spectra exhibited three sp^3 methylenes (δ_c 19.8, 30.8 and 39.4). One sp^3 methine (δ_c 33.5), three sp^2 methine (*d*, 124.0, 126.5 and 126.8) and four quaternary (δ_c 33.5, 135.8, 143.1 and 145.5) carbons. The above spectral data indicated the presence of 22 protons. Hence, the molecular formula of **1** was found to be $C_{15}H_{22}$, confirming five degrees of unsaturation. The spectral data showed that **1** was a bicyclic sesquiterpene hydrocarbon with a 1,2,4-trisubstituted benzene ring. Analysis of the HMQC and HMBC spectra (summarized in Table 1) supports the structural assignment. In particular, the long-range ^{1}H–^{13}C correlation of H-11 with C-5, C-6, C-7, C-12 and C-13 supports the positioning of the isopropyl group at the benzene ring.

The synthesis of **1** has been reported by Eisfelder and Weyerstahl [3]. Compound **2** has been isolated from the present species and thus **1** may be formed by the biogenetic pathway shown in Scheme 1.

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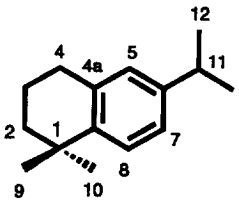
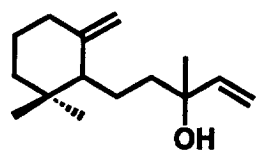


1



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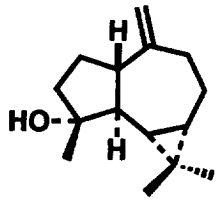

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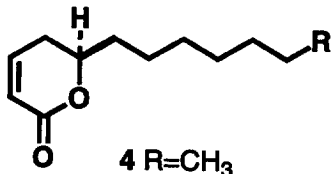
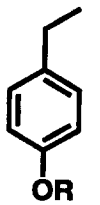


4 R=CH_3
5 R=$(CH_2)_2CH_3$



6 R=CH_3
7 R=H
8 R=Ac

The ether extract of *C. imbricata* was chromatographed on Sephadex LH-20 and the fractions obtained were purified by HPLC to give two milky-smelling lactones whose spectral data and specific rotation were identical to those of synthetic (*R*)-dodec-2-en-1,5-olide (**4**) and (*R*)-tetradec-2-en-1,5-olide (**5**) [4], respectively. The estimated enantio excess of more than 99%ee of **4** was examined by GC-mass spectral analysis using a chiral capillary column.

Leptolejeunea elliptica is a very small liverwort which grows on the leaves of some ferns or higher plants and emits an intensely fragrant odour when it is crushed. Nakayama *et al.* [5] reported that this smell is due to 1-ethyl-4-methoxybenzene (**6**). Further analysis by GC-mass spectrometry of a small amount of the ether extract of *L. elliptica* resulted in the detection not only of **6** but also 1-ethyl-4-hydroxybenzene (**7**) and 1-ethyl-4-acetoxybenzene (**8**). The presence of the latter two compounds were confirmed in the following manner. The commercially available **7** was methylated and acetylated to give **6** and **8**, respectively. The retention times and mass spectra of the peaks 1 and 3 which appeared in the mass spectral GC-total ion chromatogram (Fig. 1) were identical to those of the synthetic compounds **6** and **8**. The retention time and mass spectrum of peak 2 were in good agreement with those of **7**. In previous work on *L. elliptica*, α- and β-pinene and camphene were detected as the monoterpene hydrocarbons [5]. However, these compounds have not been detected in the plants analysed in this study (Fig. 1).

There are no chemical affinities between *A. olivacea*, *C. imbricata* and *L. elliptica*, which all belong to the Lejeuneaceae. *Cheiloleujeunea imbricata* and *L. elliptica* are very important species among the Hepaticae from the viewpoint of fragrance chemistry, because they produce chiroptical δ-lactones with a milky odour and simple alkylbenzenes with an intense phenol-like odour.

EXPERIMENTAL

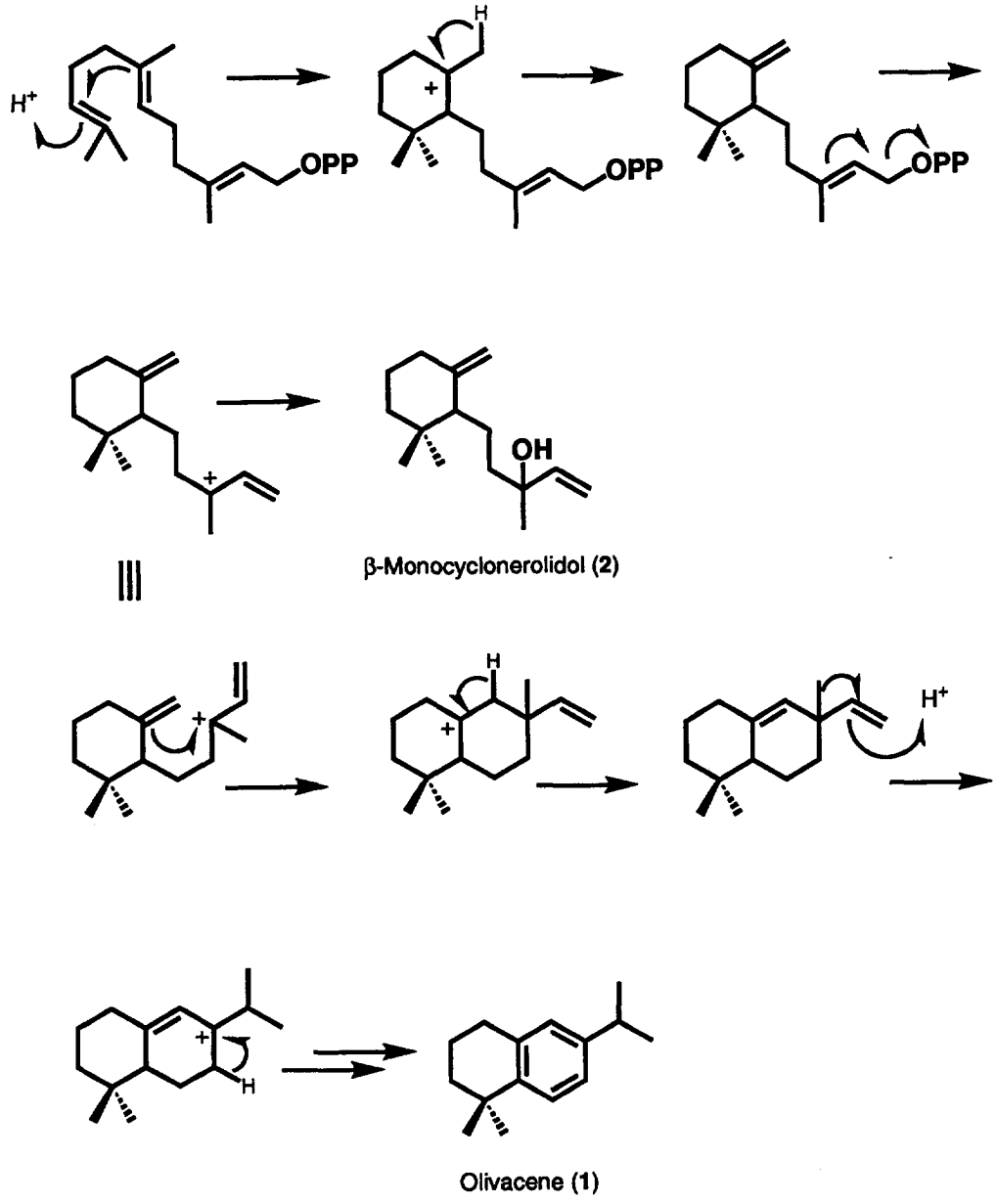
General. TLC was carried out on silica gel precoated glass plates with *n*-hexane–EtOAc (1:1 and 4:1). Detection was with Godin reagent [6]. For normal phase CC, silica gel 60 (40–63 μm) was used. CH_2Cl_2–MeOH (1:1) was used as solvent for CC on Sephadex LH-20.

Spectral data. NMR spectra were recorded at 100 or 50 MHz (^{13}C) and 400 or 200 MHz (^1H) EIMS were measured at 70 eV. The temp. programming for GC-MS was from 50° isothermal for 3 min, then 50–250° at 5° min^{-1}, and finally isothermal at 250° for 15 min. The injection temp. was 250°. A fused silica column

Table 1. The long-range ^1H–^{13}C correlations for olivacene (**1**)

^1H	^{13}C
5 [δ 6.89 (*d*, *J* = 2.2 Hz)]	4, 7, 11, 8a (δ 30.8, 124.0, 33.5, 143.1)
7 [δ 7.01 (*dd*, *J* = 8.3, 2.2 Hz)]	5, 11, 8a (δ 126.8, 33.5, 143.1)
8 [δ 7.24 (*d*, *J* = 8.3 Hz)]	1, 6, 4a (δ 33.5, 145.5, 135.8)
9, 10 [δ 1.26 (6H, *s*)]	1, 2, 8a (δ 33.5, 39.4, 143.1)
11 [δ 2.83 (*sept*, *J* = 7 Hz)]	5, 6, 7, 12, 13 [δ 24.0 (×2Me)]
12, 13 [δ 1.23 (6H, *d*, *J* = 7 Hz)]	6, 11, 12, 13 [δ 145.5, 33.5, 24.0 (×2Me)]

Measurement was carried out by HMBC spectrum; assignment was confirmed by HMQC spectrum.



Scheme 1. Possible biogenetic pathway of olivacene (**1**) and β-monocyclonerolidol (**2**).

coated with DB-17 (30 m × 0.25 mm i.d., film thickness 0.25 μm) was used. The temp. programming for GC-MS analysis of enantiomeric purity was from 50° for 3 min, then 50–230° at 3°C min^{-1}, and finally isothermal at 230° for 5 min. A fused-silica column coated with β-DEX 120 (30 m × 0.25 mm i.d., film thickness 0.25 μm) was used.

Plant materials. Archilejeunea olivacea (Hook. & Tay) Schiffn. (no. F9502) was collected in Tom Bowring Bay, New Zealand, in December 1993 and identified by Dr J. Braggins. *Cheilolejeunea imbricata* (Nees) Hatt. (no. 95016) and *L. elliptica* (Lehm. et Lindenb.) Schiffn. (no. 95145) were collected in Sanagouchi-son, Myodo-gun, Tokushima, Japan, in January 1995 and at Aioi-cho, Naka-gun, Tokushima, in March 1995 and identified by Dr M. Mizutani. Voucher specimens are deposited at the Faculty of Pharmaceutical Sciences, TBU.

Extraction and isolation. Air-dried *A. olivacea* (16.0 g) was ground mechanically and extracted with Et_2O for 1 week. Filtration and solvent evapn gave a green oil (1.06 g) which was subjected to CC on silica gel using a *n*-hexane–EtOAc gradient to provide 2 frs. The 1st fr. was purified by HPLC to give **1** (6.3 mg). The 2nd fr. was also purified by HPLC to give **2** (23 mg) and **3** (3 mg).

Powder (4.0 g) of *C. imbricata* was treated in the same manner described above to give a green oil which emitted a strong milky odour. The crude extract was analysed by GC-MS to detect 2 major components.

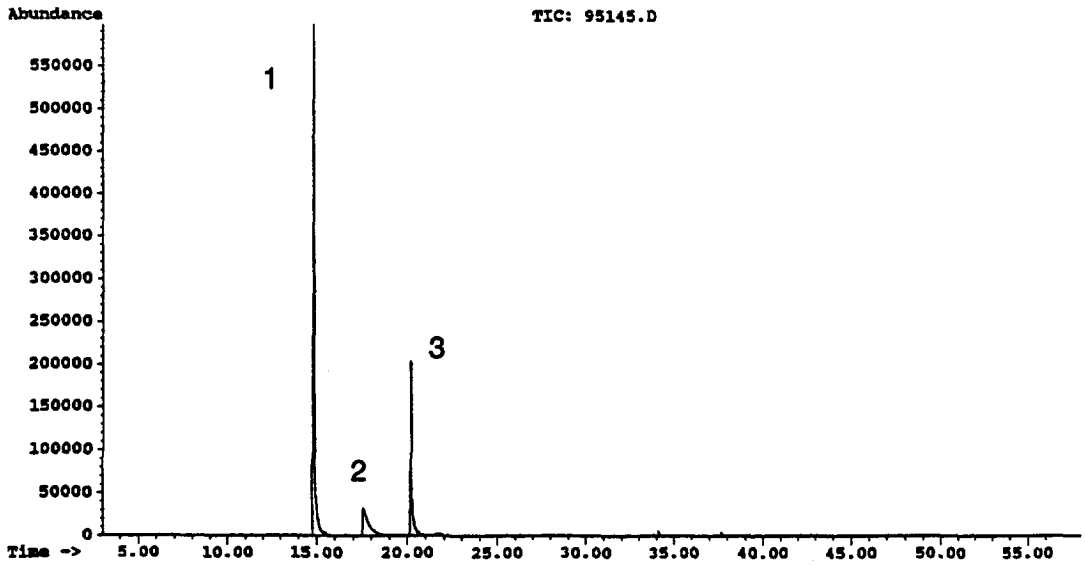


Fig. 1. Total ion chromatogram of the ether extract of *Leptolejeunea elliptica*.

A combination of Sephadex LH-20 CC and HPLC resulted in isolation of **4** (34 mg) and **5** (3.1 mg). Compound **4**: $[\alpha]_D$ −90.6 {Ref. [4] (*S*); +69.5, (*R*); −78}.

Leptolejeunea elliptica (<0.1 g) was washed with H_2O, impurities removed and the plant material ground in a mortar with Et_2O, then filtered through a short column (20 × 5 mm i.d.) of celite. The filtrate was concd under a stream of N_2. The green soln was analysed by GC-MS to detect **6**, **7** and **8**. Their spectral data and R_ts were identical to those of authentic samples.

Methylation and acetylation of 1-*ethyl*-4-*hydroxybenzene* (**7**). Compound **7** (52 mg) in dry Me_2CO (10 ml) was methylated with MeI (2 ml) in the presence of dry K_2CO_3 (150 mg) under reflux. The reaction mixt. was filtered and the solvent evapd. The resulting product was purified through a small column packed with silica gel using *n*-hexane–EtOAc (19:1) as solvent to give the monomethyl ether **6**. The R_t and mass spectrum of **6** were identical to those of the natural product which appeared in GC-MS of the crude extract of *L. elliptica*.

Compound **7** (45 mg) in pyridine (10 ml) was acetylated with Ac_2O (2 ml) for 24 hr at room temp. Work-up as usual gave **8** whose R_t and mass spectrum were identical to those of the natural product which appeared in GC-MS of the crude extract of *L. elliptica*.

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