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Sesquiterpenes from *Leontopodium alpinum*

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Abstract

An essential oil produced by the roots of *Leontopodium alpinum* afforded isocomene and two of its derivatives, together with novel acetoxy derivatives of modhephene and caryophyllene. The structures were elucidated on the basis of spectral analysis. © 1999 Elsevier Science Ltd. All rights reserved.

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1. Introduction

The European alpine plant *Leontopodium alpinum* Cass., commonly known as Edelweiss, has been the subject of research by this group for some time. Initial work on this species has concentrated on the growth of various strains of *L. alpinum* (Hook, 1993). A limited chemical study resulted in the isolation of the hydroxycinnamic acid esters chlorogenic acid and 4,5-dicaffeoylquinic acid from the aerial structures of the plant (Hennessy, Hook, Mc. Gee, & Sheridan, 1989). More recently the production of an essential oil by the normal and cultivated 'hairy roots' of *L. alpinum* has been observed (Comey, Hook, & Sheridan, 1992; Hook, 1994). This oil has been shown to be a complex mixture of secondary metabolites from which a novel benzopyran-4-one has been isolated (Comey, Hook, Sheridan, Walsh, & James, 1997). Further investigation into the composition of the essential oil produced by *L. alpinum* has now led to the isolation and characterisation of a series of sesquiterpenes (**1**–**4**) related to isocomene (**1**) (Zalkow, Harris, Van Derveer, & Bertand, 1977) and modhephene (**5**) (Zalkow, Harris, & Van Derveer, 1988). A caryophyllene derivative (**6**) has also been isolated. The structural elucidation of these metabolites is reported in this paper and assignments are based on 2D NMR spectroscopy, especially long range ^{1}H–^{13}C correlations.

2. Results and discussion

The essential oil produced by normal and hairy roots of *L. alpinum* was subjected to repeated CC on silica gel impregnated with $AgNO_3$ using a petrol–EtOAc gradient as eluant. Compound **1** was recovered from the 15% EtOAc fraction. The $[M]^+$ at m/z 204 coupled with 15 resonances in the ^{13}C NMR spectrum of **1** yielded a molecular formula of $C_{15}H_{24}$ for this isolate. The IR spectrum contained absorptions at 3020, 1670 and 840 cm^{-1}. With the exception of an alkene proton in the ^{1}H NMR and ^{13}C NMR spectra (Table 1) the molecule is saturated which suggests a tricyclic structure for the isolate. The ^{1}H and ^{13}C NMR spectra Table 1 also indicated the presence of four methyls, five methylenes, two methines and four quaternary carbons. 2D COSY experiments were carried out which yielded some information on alkene and methyl couplings but methylene couplings overlapped too much for a definitive result. The results obtained from COLOC and HMBC experiments confirmed that isolate **1** is the sesquiterpene isocomene, previously isolated from *Callilepis salicifolia* (Compositae) (Bohlmann & Zedro, 1982).

Compound **2** was recovered from the 15% EtOAc fraction. Its IR spectrum revealed characteristic absorptions of an acetoxy carbonyl at 1743 cm^{-1}. Mass spectral data gave an $[M]^+$ at m/z 262.25 which in conjunction with ^{13}C NMR data yielded a formula of $C_{17}H_{26}O_2$. The ^{1}H and ^{13}C NMR spectra of **2** were almost identical to those of isocomene (**1**) and suggested that **2** was a derivative (Table 1). The most significant differences between the

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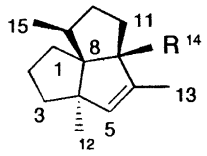
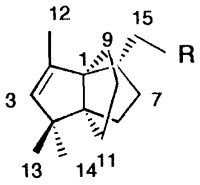
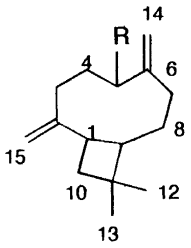


1 R = CH_3,
2 R = CH_2OCOCH_3
3 R = $COOCH_3$



4 R =$OCOCH_3$
5 R =H



6 R =$OCOCH_3$

Table 1
NMR data for compounds **1**–**4**

	1		**2**		**3**		**4**	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	33.1 t	H-a, 1.20–1.32 m; H-b, 1.27–1.65 m	31.8 t	H-a, 1.20–1.35; H-b, 1.37–1.70	32.7 t	H-a, 1.45–1.55 m; H-b, 2.40–2.50 m	72.5 s	–
2	24.0 t	1.15–1.25 m	24.3 t	1.35–1.45	23.6 t	1.30–1.40 m	140.0 s	–
3	42.4 t	H-a, 1.10–1.30 m; H-b, 1.60–1.72 m	42.7 t	H-a, 1.15–1.30; H-b, 1.50–1.65	42.1 t	H-a, 1.10–1.20 m; H-b, 1.60–1.65 m	136.5 t	4.88 m
4	56.7 q	–	57.4 s	–	57.6 q	–	46.2 s	–
5	137.4 d	4.90 d (J=1.6 Hz)	135.9 d	5.01 d (J=1.5 Hz)	137.2 d	5.06 d (J=1.5 Hz)	67.1 s	–
6	138.0 q	–	139.2 s	–	137.7 q	–	34.4 t	
7	60.1 q	–	63.4 s	–	73.8 q	–	31.7 t	1.20–1.40 m
8	64.0 q	–	64.6 s	–	69.3 q	–	48.2 d	1.90–2.00 m
9	33.4 d	1.98–2.05 m	40.1 d	1.95–2.05 m (J=7.2 Hz)	39.8 d	1.95–2.05 m (J=7.0 Hz)	30.7 t	1.20–1.25
10	35.5 t	H-a, 1.10–1.30 m; H-b, 1.60–1.72 m	34.0 t	H-a, 1.30–1.45; H-b, 1.62–1.75	33.1 t	H-a, 1.30–1.45 m	27.7 t	1.30–1.40
11	42.4 t	H-a, 1.10–1.30 m; H-b, 1.50–1.62 m	31.6 t	H-a, 1.20–1.35; H-b, 1.37–1.70	35.3 t	H-a, 1.30–1.40 m	38.2 t	–
12	23.9 q	1.05 s	23.7 q	1.05 s	23.5 q	1.05 s	14.2 q	1.58 d (J=1.6 Hz)
13	14.3 q	1.60 s	13.9 q	1.60 d (J=1.5 Hz)	14.1 q	1.62 d (J=1.5 Hz)	26.6 q	0.99 s
14	23.3 q	1.05 s	67.8 t	4.02–4.15 (J=11.5 Hz)	175.6 q	–	29.0 q	0.99 s
15	16.7 q	0.85 d	16.9 q	0.90 d (J=7.2 Hz)	16.4 d	0.94 d (J=7.0 Hz)	66.4 q	4.01–4.24 dd (J=11 Hz)
16	–	–	171.4 s	–	51.3 (OCH_3)	3.70 s	170.5 s	–
17	–	–	21.3 q	2.05	–	–	21.3 q	2.07 s

spectra lie in the presence of a carbonyl at δ 171.4 and a deshielded methylene group at δ 4.13/δ 4.02. These data suggest a methylene group linked to the acetoxy function. Another difference between the spectra of **1** and **2** lies in the absence of one of the methyl resonances at δ 1.05 which suggests that the acetoxymethyl group replaces one of the methyl groups at C-4 or C-7. HMBC results allow the acetoxy group to be placed on the quaternary at δ 63.4 (C-7) due to a strong NOE (7.3%) to the methyl at δ 1.60. Compound **2** was thus identified as the 12-acetoxyisocomene. The isovaltrate corresponding to this compound has been isolated from *C. salicifolia* (Bohlmann & Zedro, 1982).

Compound **3** was found to be spectroscopically similar to **1** and **2**. A combination of mass spectral ($[M]^+$ at m/z 248) and ^{13}C NMR data yielded a molecular formula of $C_{16}H_{24}O_2$. The spectral data (Table 1) showed the presence of four methyl groups including a methoxy of a methyl ester (1724 cm^{-1}) at δ 3.70. An isolated vinylic proton was again observed at δ 5.06 consistent with H-8 of **1** and **2**. Close comparison of the ^1H and ^{13}C NMR spectra (Table 1) of **3** with isocomenes **1** and **2** revealed that the only difference in the structures of the isolates was at the C-6 position, in that the methyl group in **1** and the acetoxymethyl of **2** is replaced by a carboxymethyl in **3**. By using HMBC and COLOC correlations the positions of the various carbons in the molecule were established and the structure was shown to be 12-*carboxymethylisocomene* (**3**) (Bohlmann & Zedro, 1982) also isolated from *C. salicifolia*.

The fourth sesquiterpene isolated from *L. alpinum* was recovered from the column as a mixture and was further purified using a chromatotron. The molecular formula for this compound was found to be $C_{17}H_{26}O_2$. This compound was found to have some spectroscopic features in common with **1**–**3**. The IR spectrum showed the presence of a carbonyl group at 1740 cm^{-1}. The ^1H and ^{13}C NMR data (Table 1) showed the presence of four methyl groups including an acetoxymethyl and a *gem*-dimethyl group, six methylene groups, two methines and five quaternary carbons including a carbonyl group. An isolated vinylic proton was again observed at δ 4.88. Close comparison of the ^{13}C NMR spectrum of **4** with those of **1**–**3** (Table 1) revealed several differences between the structures and suggested that the carbon skeleton was that of the related tricyclic sesquiterpene modhephene (**5**) which has been shown to co-occur with isocomene (**1**) (Zalkow et al., 1988). The methyl group at δ 2.07 and the oxymethylene signal resonating at δ 4.01 and δ 4.24 supports the presence of an acetoxymethyl group as observed in **2**. HMBC correlations and a comparison with literature values for modhephene (**5**) were used to place this group at the C-8 position. Thus **4** was identified as 8-*acetoxymodhephene*.

The final compound isolated from this column in the pure form was identified as the caryophyllene (**6**), $[M]^+$ at m/z 262 corresponding to $C_{17}H_{26}O_2$. The nature of the carbon skeleton was deduced from the ^1H NMR and ^{13}C NMR spectra (Table 2). The IR spectrum showed the presence of an acetyl methyl (1726 cm^{-1}) which was supported by the ^{13}C NMR spectrum (δ 170.5). The ^1H NMR spectrum of **6** revealed the presence of three methyl groups including a *gem*-dimethyl group and the acetylmethyl. The ^{13}C NMR showed the presence of a further six methylene, three methine and three quaternary carbons. Two exoalkene groups were identified in the ^1H NMR spectrum at characteristic frequencies, the first a singlet at δ 4.80 integrating for two protons; the second resonated as two singlets at δ 5.02 and δ 5.08. A single proton signal at δ 5.15, was indicative of a carbinol proton. The positioning of the acetate group at C-5 was supported by the chemical shifts of H-14 as well as from the allylic coupling and HMBC results. In this way **6** was identified as 5-*acetoxy*-5,6-*dihydro*-6,14-*dehydrocaryophyllene*.

Table 2
NMR data for caryophyllene **6**

	δ_C(DEPT)	δ_H	HMBC correlations
1	44.2 d	2.50 ddd (J=9.2 Hz)	2, 7, 13
2	150.2 s	–	–
3	30.7 t	2.05 m, 2.15 m	–
4	32.8 t	1.8 m, 2.10 m (J=4.5, 12 Hz)	–
5	77.1 d	5.15 d (J=4.5, 12 Hz)	2, 3, 7, 14
6	147.5 s	–	–
7	34.8 t	hidden	5
8	30.8 t	hidden	–
9	54.8 d	2.15 dd (J=6, 9 Hz)	
10	37.1 t	1.74 dd, 2.04 dd (J=9.2, 11 Hz)	–
11	33.8 s	–	–
12	22.1 q	1.05 s	1, 2, 10, 13
13	30.1 q	1.05 s	1, 2, 10, 13
14	109.6 t	4.80 bs	3, 5, 6
15	115.7 t	5.02 s, 5.08 s	1, 6, 9
16	170.5 s	–	4, 6
17	21.6 q	2.05 s	–

This is the first report of the occurrence of sesquiterpenes related to isocomene and modhephene in *Leontopodium* species. Compounds **1**, **2** and **5** have been isolated from a number of taxa belonging to different tribes of the Compositae (Zalkow et al., 1977; Bohlmann & Zedro, 1981Bohlmann & Zedro, 1982; Zalkow et al., 1988). A number of caryophyllenes related to **6** have also been isolated (Bohlmann & Zedro, 1981). The isolation of these sesquiterpenes from *L. alpinum* is of taxonomic interest.

3. Experimental

NMR: 300 and 400 MHz (^1H) and 100.6 MHz (^{13}C) $CDCl_3$, with TMS as int. standard. HMBC spectra were

measured at 7, 10 and 12 Hz. EIMS: 70 eV; FID-GC: Carbowax 20 M column (2 m × 1.75 mm i.d). Operating conditions; temperature programme, 100–200°C; ramp rate, 2°C min; N_2 flow rate, 30 ml/min.; injector temperature, 220°C; detector temperature, 220°C. Powdered root material from both natural and 'hairy' roots was subjected to steam distillation as described in Comey et al. (1997). This powdered material yielded an essential oil: natural roots ca. 2.0% and hairy roots ca. 0.6%.

The essential oil (800 mg) was chromatographed on silica gel impregnated with $AgNO_3$ (15%) gradient eluted with petrol-EtOAc and $CHCl_3$–MeOH mixtures. Samples were collected in 10 ml fractions and were analysed by TLC and GC; like fractions were combined.

3.1. Isocomene (*1*)

Isocomene (**1**) (3 mg) was isolated from fractions 9–16. GC R_t 13.35; $[\alpha]_D$ −52° (c=0.5, $CHCl_3$); IR_ν cm^{-1} (neat), 1615, 1462, 1445; EIMS (probe) m/z (rel. int): 204 $[M]^+$ (28), 189 (48), 162 (100), 147 (90), 134 (64), 119 (89), 91 (73); ^1H and ^{13}C NMR: Table 1.

3.2. 12-Acetoxyisocomene (*2*)

12-Acetoxyisocomene (**2**) (35 mg) was isolated from fractions 119–134. GC R_t 44.8; $[\alpha]_D$ −112° (c=0.98, $CHCl_3$); IR_ν cm^{-1} (neat) 1743, 1440, 1380; EIMS (probe) m/z (rel. int): 262 $[M]^+$ (4), 247 (1.5), 220 (13), 202 (7), 189 (50), 173 (3), 161 (16), 147 (16), 145 (44), 133 (55), 119 (28), 105 (22), 91 (23), 43 (100); ^1H and ^{13}C NMR: Table 1.

3.3. Methylisocomen-12-oate (*3*)

Methylisocomen-12-oate (**3**) (20 mg) was isolated from fractions 102–112. GC R_t 34.28; $[\alpha]_D$ −78° (c=0.89, $CHCl_3$); IR_ν cm^{-1} (neat) 1724, 14409 1375; EIMS (probe) m/z (rel. int): 248 $[M]^+$ (20), 206 (100), 189 (65), 147 (30), 119 (28), 91 (22), 43 (100); ^1H and ^{13}C NMR: Table 1.

3.4. 8-Acetoxymodhephene (*4*)

8-Acetoxymodhephene (**4**) (1 mg) was isolated as a mixture from fractions 130–145 of the parent column. This sample was further chromatographed on a chromatotron (Silica gel 7749 PF_{254}; toluene–EtOAc, 3:1; flow rate, 2 ml/min; N_2 flow, 4–8 ml/min). GC R_t 42.30; IR_ν cm^{-1} (neat), 1745, 1448; EIMS (probe) m/z (rel. int): 262 $[M]^+$ (1), 247 (1.5), 220 (20), 203 (18), 189 (50), 147 (8), 119 (28), 105 (25), 43 (100); ^1H and ^{13}C NMR: Table 1.

3.5. 5-Acetoxy-5,6-dihydro-6,14-dehydrocaryophyllene (*6*)

5-Acetoxy-5,6-dihydro-6,14-dehydrocaryophyllene (**6**) (5 mg) was isolated as an oil. GC R_t 48.23 min; IR_ν cm^{-1} (neat), 1726 cm^{-1}; EIMS (probe) m/z (rel. int): 262 $[M]^+$ (1), 247 (15), 220 (10), 187 (10), 159 (20), 147 (8), 136 (100); ^1H NMR and ^{13}C NMR: Table 2.

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