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Two 6,7-seco-caryophyllenes and an alloaromadendrane from liquid cultures of *Hebeloma longicaudum*

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Abstract

Two 6,7-seco-caryophyllene-related sesquiterpenes, hebelophyllenes G and H and an alloaromadendrane, hebelodendrol, were isolated from liquid cultures of the ectomycorrhizal fungus *Hebeloma longicaudum*. Their structures were determined by modern spectroscopic methods.

Keywords: *Hebeloma longicaudum*; Ectomycorrhizal fungi; Sesquiterpenes; Caryophyllenes; Hebelophyllenes; Alloaromadendrane; Hebelodendrol

1. Introduction

Recently we reported on the caryophyllenes, 6,7-seco-caryophyllenes and related sesquiterpenes isolated from liquid cultures of *Hebeloma longicaudum*, an ectomycorrhizal fungus associated with conifer species (Wichlacz, Ayer, Trifonov, Chakravarty & Khasa, 1999a, 1999b).

In this study we describe the isolation and structure elucidation of three minor metabolites, hebelohpyllenes G (**1**) and H (**2**), and hebelodendrol (**3**), obtained from the same extract after careful examination of the most polar chromatographic fractions (see Section 3).

2. Results and discussion

Hebelophyllene G (**1**) was obtained as a colorless oil. The molecular formula $C_{16}H_{26}O_5$ was determined by HR-EIMS in combination with NMR spectra, since the EIMS and CIMS did not provide a molecular ion. The ^{1}H- and ^{13}C-NMR spectral data for this compound were very similar to those of the previously reported hebelophyllene F (**4**) (Wichlacz et al., 1999b). Unlike the latter, however, hebelophyllene G does not have the characteristic signals of a monosubstituted oxirane ring. Instead, a CH(OH)–CH_2OH fragment was present as indicated by the ^{1}H- and ^{13}C-NMR spectral data (see Table 1). The remaining signals were virtually identical with those of hebelophyllene F.

All of the above information is consistent with the proposed structure **1** for hebelophyllene G, which is presumably formed from hebelophyllene F after epoxide ring opening.

Hebelophyllene H (**2**) was obtained as a colorless oil. The molecular formula $C_{15}H_{22}O_3$ was derived from the HR-MS spectrum. The ^{1}H- and ^{13}C-NMR spectra bear striking resemblance with those of the previously described 6,7-seco-caryophyllene lactone, hebelophyllene E (**5**) (Wichlacz et al., 1999b). Unlike the latter, however, hebelophyllene H has only three methyl groups. One additional double bond is present in hebelophyllene H, as indicated by the ^{1}H-NMR spectrum (Table 1; triplets at 5.39 and 5.71 ppm) and the ^{13}C-NMR spectrum (Table 2; triplet at 123.3 ppm and singlet at 144.1 ppm). This information is consist-

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Table 1
^{1}H-NMR spectral data[a] of **1**, **2**, and **3e**

No.	**1**[b]	**2**[c]	**3e**[d]
1	2.42 (*ddd*, 9.2, 9.0, 2.7)	2.28 (*dddd*, 12.5, 9.0, 6.0, 2.8)	1.96 (*dt*, 9.9, 5.7)
2	1.25 (*ddd*, 14.1, 8.4, 5.8)	1.86–1.95 (*m*)	0.01 (*t*, 9.7)
	1.66 (*ddd*, 14.1, 9.0, 5.0)		
3	3.83 (*dd*, 8.6, 5.0)	4.05 (*dd*, 9.5, 2.4)	
4			0.62 (*ddd*, 11.3, 9.4, 6.3)
5	4.12 (*dd*, 7.5, 3.6)	5.96 (*dd*, 17.4, 10.8)	1.60 (*dtd*, 14.0, 6.3, 2.3, α)
			1.48 (*dtd*, 14.0, 11.3, 2.2, β)
6	3.41 (*dd*, 11.5, 7.5)	5.38 (*dd*, 17.4, 1.1, a)	1.78 (*ddd*, 14.0, 11.2, 2.2, α)
	3.55 (*dd*, 11.5, 3.6)	5.24 (*dd*, 10.8, 1.1, b)	1.66 (*brdd*, 14.0, 6.0, β)
8			1.88 (*ddd*, 9.2, 5.7, 0.9)
9	3.52–3.60 (*m*)	3.30 (*dt*, 9.2, 8.7)	4.02 (*dd*, 9.2, 7.6)
10	1.74 (*ddd*, 11.2, 8.2, 2.7, α)	1.86–1.95 (*m*)	3.68 (*t*, 7.6)
	1.92 (*br. t*, 10.5, β)		
11			1.81 (*br. sext*., 7.2)
12	1.02 (*s*)	0.93 (*s*)	1.05 (*d*, 6.8)
13	1.27 (*s*)	1.24 (*s*)	1.33 (*s*)
14	5.11 (*br. s*, a)	1.35 (*s*)	1.01 (*s*)
	5.18 (*br. s*, b)		
15	5.60 (*t*, 1.2, c)	5.39 (*t*, 1.1, c)	0.97 (*s*)
	6.31 (*t*, 1.2, d)	5.71 (*t*, 1.1, d)	
16	3.73 (*s*)		

[a] The assignments are based on ^{1}H–^{1}H COSY, HMQC, and TROESY spectra.
[b] 400 MHz, CD_3OD.
[c] 600 MHz, $CDCl_3$.
[d] 600 MHz, CD_3OD.

ent with the structure **2** proposed for hebelophyllene H. The characteristic signal for the H-3 lactone proton (4.05 ppm, *dd*) has coupling constants 9.5 and 2.5 Hz, notably different from those of hebelophyllene E (Wichlacz et al., 1999b). This indicates that the exocyclic double bond C-8 to C-15 forces the lactone ring to adopt a different conformation, in which the dihedral angle H2α-C2-C1-H1 is close to 90°. Further support for structure **2** was derived from the HMBC spectrum (Table 2) and especially from the TROESY spectrum (Fig. 1). The latter allowed us to assign the signals for all methyl groups, as well as to ascribe α orientation of H-3. As in the case of hebelophyllene E, the absolute configuration at C-4 of **2** remains undetermined. The absolute configuration at the remaining chiral centers is based on the biosynthetic relationship between hebelophyllene H and the previously described caryophyllenes isolated from *H. longicaudum*.

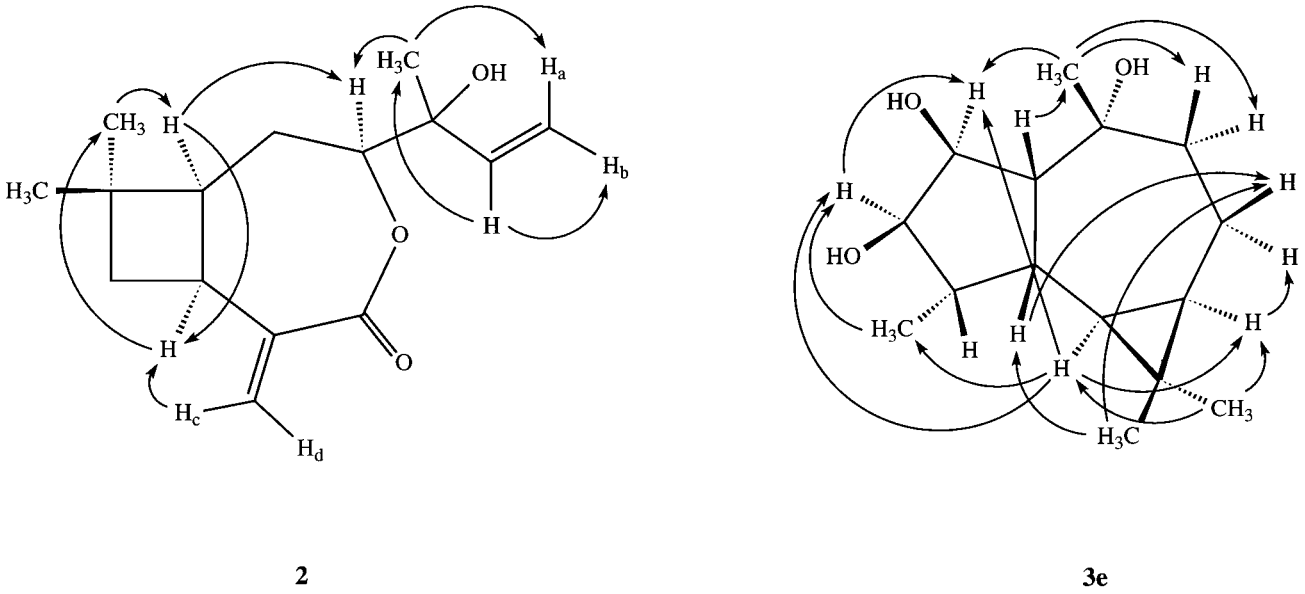
Table 2
^{13}C-NMR and HMBC (in parentheses) spectral data of **1**, **2**, and **3e**

No.	**1**[a]	**2**[b]	**3e**[c]
1	44.5	44.5 (12, 13)	37.3 (8, 12)
2	35.3	26.7	26.4 (1, 14, 15)
3	72.1	85.7 (14)	20.2 (1, 14, 15)
4	154.1	74.7 (5, 6a, 14)	29.4 (6α, 6β, 14, 15)
5	73.8	140.3 (6a, 14)	19.8 (6α, 6β)
6	67.4	115.4	40.2 (5α, 5β, 8, 13)
7	169.7	171.0 (15d)	74.9 (5β, 8, 9)
8	142.3	144.1 (10, 15d)	62.4 (1, 10, 13)
9	34.1	35.5 (15c, 15d)	72.6 (1, 8)
10	35.7	38.0 (12, 13)	77.2 (1, 11, 12)
11	34.3	34.7 (2, 12, 13)	45.7 (1, 2, 12)
12	25.5	24.5 (13)	14.6 (10, 11)
13	30.8	29.2 (10, 12)	31.9
14	111.5	23.4	28.9 (15)
15	125.3	123.3 (9)	15.9 (14)
16	52.4		

[a] 100 MHz, CD_3OD.
[b] 100 MHz, $CDCl_3$.
[c] 100 MHz, CD_3OD.

Hebelophyllene H (**2**) was isolated from an inseparable mixture containing two compounds. Attempted acetylation of this mixture afforded the readily separable lactonic hebelophyllenes E and H (see Section 3). The ^{1}H-NMR spectrum of the crude mixture before acetylation indicated that none of these lactones was present (the characteristic H-3 signals at ca. 4.1 ppm of the lactones were not present). This leads to the conclusion that the parent compounds present in the initial mixture are most probably the corresponding carboxylic acids and the lactone rings are closed during attempted acetylation or during work up (Wichlacz et al., 1999b).

Hebelodendrol (**3**) was obtained as a colorless oil. The molecular formula $C_{15}H_{26}O_3$ was derived from the HR-MS spectrum. Since no evidence is available for the presence of double bonds or carbonyl groups,



2 **3e**

Fig. 1. Pertinent TROSEY correlations of hebelophyllene H (**2**) and hebelodendrol (**3e**) (all geminal correlations are omitted).

hebelodendrol is tricyclic. The presence of two signals at very high field (0.01 and 0.62 ppm) in the ^{1}H-NMR spectrum of hebelodendrol (**3**) (Table 1) distinguished this compound from the caryophyllenes and 6,7-seco-caryophyllenes isolated earlier from *H. longicaudum*. Such a pair of high field proton signals is strongly indicative of the presence of a cyclopropane ring in hebelodendrol. Four methyl groups are also present (three singlets and one doublet in the ^{1}H-NMR spectrum, Table 1), two of which are geminal (vide infra). The ^{13}C-NMR shifts of the latter are different from those of the geminal methyl groups of the caryophyllenes (Wichlacz et al., 1999a, 1999b) but very similar to those of the sesquiterpenes known as aromadendranes (such as globulol (**3a**) and its microbial transformation product **3b**) with *trans*-fused seven and five membered rings and alloaromadendranes (such as ledol (**3c**) and viridiflorol (**3d**)) with *cis*-fused seven and five membered rings. Aromadendranes and alloaromadendranes have been isolated previously from various plants, including liverworts. The NMR signals of compounds **3a**–**3d** have been recently assigned (Faure, Ramanoelina, Rakotonirainy, Bianchini & Gaydou, 1991; Abraham, Kieslich, Stumpf & Ernst, 1992) and revised, but the assignments remain even more confusing after the latest revision (Wu, Huang & Chen, 1996). In addition to the hydroxyl group at C-7 of these sesquiterpenes, hebelodendrol has two secondary hydroxyl groups (signals at 4.02 and 3.68 ppm in the ^{1}H-NMR, Table 1). The examination of the ^{1}H–^{1}H COSY spectrum immediately placed these hydroxyl groups at C-9 and C-10 (cross peaks between H-8 and H-9, and between H-9 and H-10). This information is consistent with the constitution of hebelodendrol provided by structure **3e** (without stereochemistry). This structure is further corroborated by the HMBC spectrum (Table 2).

The *cis*-fusion of the seven and five membered rings is based on the coupling constant $J_{H\text{-}1/H\text{-}8} = 5.5$ Hz which is characteristic of alloaromadendranes. Aromadendranes, on the other hand, have $J_{H\text{-}1/H\text{-}8} =$ ca. 10.0 Hz (Faure et al., 1991; Abraham et al., 1992). The TROESY spectrum clearly indicates that both hydroxyl groups at C-9 and C-10 are in β position, while the methyl group at C-11 is in α position. The methyl group at C-7 has to be in β position in order to account for the cross peaks between CH_3-13 and H-8, and CH_3-13 and H-9.

The absolute configuration of hebelodendrol remains undetermined.

3. Experimental

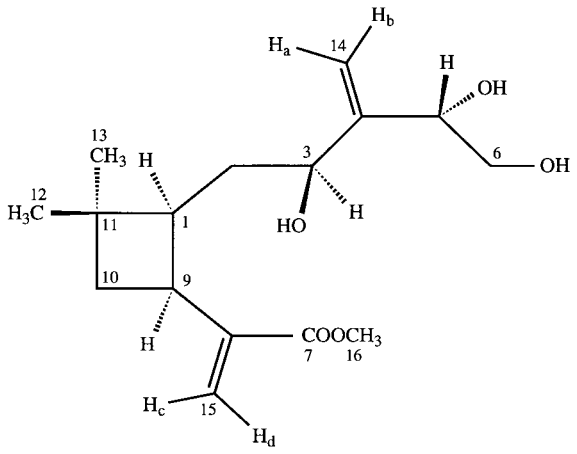
3.1. General experimental procedures (see Wichlacz et al., 1999a)

3.1.1. Sample collection

Hebeloma longicaudum (strain 16) was collected in August 1984 from a fruiting body associated with Norway spruce. A voucher specimen is deposited at the Northern Forestry Centre, Edmonton, Canada, as NOF 2298.

3.1.2. Isolation of sesquiterpenes

The fungus was grown as described (Wichlacz et al., 1999a). The filtered broth (5 l) was concentrated to 1 l



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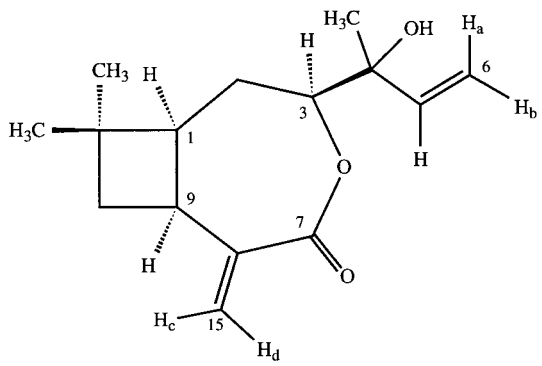

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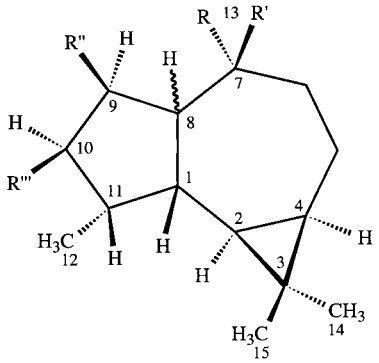
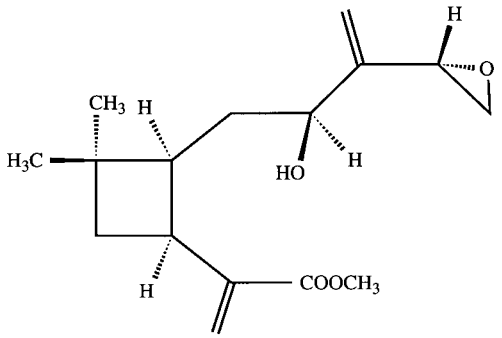


3a: H8α, R=OH, R'=CH_3, R''=R'''=H (globulol)
3b: H8α, R=OH, R'=CH_3, R''=OH, R'''=H
3c: H8β, R=OH, R'=CH_3, R''=R'''=H (ledol)
3d: H8β, R=CH_3, R'=OH, R''=R'''=H (viridiflorol)
3e: H8β, R'=CH_3, R=R''=R'''=OH (hebelodendrol)



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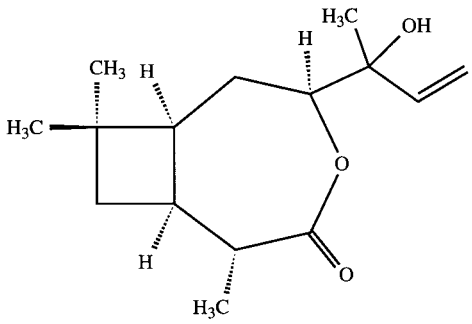

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and was extracted with ethyl acetate (5 × 400 ml). The crude extract (1.06 g) was subjected to flash chromatography on silica gel 60 (230–400 mesh) with hexane–EtOAc (gradient, 25–100%). The fraction (180 mg) eluted with EtOAc, was further purified by flash chromatography on silica gel with hexane–EtOAc–MeOH, 50 : 50 : 6. The first combined fraction (88 mg) was subjected to prep. TLC with the same solvent system (threefold development). The zone at $R_f = 0.39$ afforded pure hebelophyllene G (**1**) (9.0 mg); the zone

at $R_f = 0.46$ provided pure hebelodendrol (**3**) (5.6 mg). The crude product from the zone at $R_f = 0.64$ was dissolved in pyridine (0.5 ml) and acetic anhydride (0.5 ml), and was left at room temperature for 3 h. The solvent was removed under vacuum and the residue was dissolved in CH_2Cl_2 and washed with 1% aqueous ammonia. The organic layer was dried over $MgSO_4$, the solvent was removed under vacuum and the residue was subjected to prep. TLC on silica gel with hexane–EtOAc, 4 : 1. The zone at $R_f = 0.34$ afforded pure hebelophyllene E (**2**) (4.3 mg) while the zone at $R_f = 0.25$ gave hebelophyllene H (6.4 mg).

*3.1.2.1. Hebelophyllene G (**1**).* Colorless oil; $[\alpha]_D^{25}$ — 9.5° (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε): 215 nm (3.69); CD $\Delta\varepsilon_{240} + 7.22$ (*c* 0.05, MeOH); IR ($CHCl_3$) ν_{max} 3374 (OH), 2950, 2865, 1718 (>C=O), 1626 (>C=C<), 1439, 1383, 1365, 1277, 1195, 1158, 1065, 1034, 939, 920, 870, 818, 755 cm $^{-1}$; ^{1}H-NMR spectral data Table 1; ^{13}C-NMR spectral data Table 2; HR-EIMS *m/z* 267.1593 $[M–CH_2OH]^+$ (6) (calculated for $C_{15}H_{23}O_4$, 267.1596), 249 $[M–CH_2OH–H_2O]^+$ (4), 117 $[HOCH–C(=CH_2)CH(OH)CH_2OH]^+$ (62).

*3.1.2.2. Hebelophyllene H (**2**).* Colorless oil; $[\alpha]_D^{25}$ — 18.3° (*c* 0.24, MeOH); UV (MeOH) λ_{max} (log ε): 215 nm (sh) (3.60); CD: $\Delta\varepsilon_{270} - 0.47$ (*c* 0.08, MeOH); IR ($CHCl_3$) ν_{max} 3457 (OH), 3091, 2954, 2866, 1717 (>C=O), 1625 (>C=C<), 1304, 1260, 1219, 1196, 1166, 927 cm $^{-1}$; ^{1}H-NMR spectral data Table 1; ^{13}C-NMR spectral data Table 2; HR-EIMS *m/z* 250.1563 $[M]^+$ (0.3) (calculated for $C_{15}H_{22}O_3$, 250.1569), 235 $[M–CH_3]^+$ (0.7), 233 $[M–OH]^+$ (0.6), 232 $[M–H_2O]^+$ (0.6), 179.1070 $[M–C_4H_7O]^+$ (27) (calculated for $C_{11}H_{15}O_2$, 179.1072).

*3.1.2.3. Hebelodendrol (**3e**).* Colorless oil; $[\alpha]_D^{25}$ — 13.6° (*c* 0.22, MeOH); CD:$\Delta\varepsilon_{242} + 4.20$ (*c* 0.11, MeOH); IR ($CHCl_3$) ν_{max} 3362 (OH), 2957, 2927, 2869, 1710 (>C=O), 1456, 1375, 1231, 1154, 1095, 1073, 1044, 986, 942, 876, 805, 758, 701, 667 cm $^{-1}$; ^{1}H-NMR spectral data Table 1; ^{13}C-NMR spectral data Table 2; HR-EIMS *m/z* 254.1878 $[M]^+$ (2) (calculated for $C_{15}H_{26}O_3$, 254.1882), 236 $[M–H_2O]^+$ (11), 221 $[M–H_2O–CH_3]^+$ (9), 218 $[M–2H_2O]^+$ (23).

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