A Caryophyllene-Related Sesquiterpene and Two 6,7-Seco-caryophyllenes from Liquid Cultures of *Hebeloma longicaudum*

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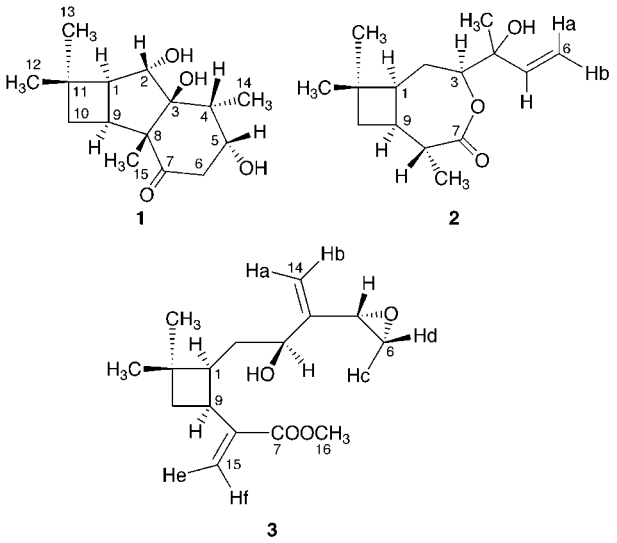
Three new caryophyllene-related sesquiterpenes, hebelophyllenes D (**1**), E (**2**), and F (**3**), were isolated from liquid cultures of the ectomycorrhizal fungus *Hebeloma longicaudum*. Their structures were determined by modern spectroscopic methods. Hebelophyllene D has a tricyclo[5.4.0.0^{2,5}]undecane skeleton, while hebelophyllenes E and F are the first naturally occurring 6,7-seco-caryophyllenes.

Hebeloma longicaudum (Pers.:Fr.) Kummer (Cortinariaceae) is an ectomycorrhizal fungus producing abundant ectomycorrhizae with conifer species providing increased host plant growth and survival.[1,2] Its potential for nursery inoculation prompted us to study the metabolites produced when the fungus was grown in liquid culture. In the present study we report the isolation and structure elucidation of three new caryophyllene-related sesquiterpenes, hebelophyllenes D−F (**1**−**3**), which are co-metabolites of the recently described *cis*-caryophyllenes, hebelophyllenes A−C.[3]

The fungus was grown as described previously.[3] The ethyl acetate extract of the filtered broth was subjected to flash column chromatography on Si gel and the crude fractions were further purified to provide pure products (**1**−**3**).

Hebelophyllene D (**1**) was obtained as a colorless solid. The molecular formula $C_{15}H_{24}O_4$ was derived from HRMS. The ^{1}H and ^{13}C NMR data of this compound are similar to those of the previously described hebelophyllenes, especially to those of hebelophyllene C. Unlike hebelophyllene C, however, **1** has only one unconjugated carbonyl group and four methyl groups (three singlets and one doublet in the ^{1}H NMR spectrum, Table 1). Another difference between hebelophyllene C and **1** is the presence of a C−OH group (singlet at δ 92.9 in the ^{13}C NMR) in **1**. All of the above information suggests that hebelophyllene D has structure **1**, derived from hebelophyllene C after a formal reduction of the double bond followed by transannular aldol reaction linking C-3 to C-8. This structure was further supported by the HMBC correlations (Table 2), notably those between C-3 and H-5 and H-14, and between C-8 and H-1, H-9, and H-10. The TROESY spectrum indicates a *cis*-juncture between the four- and five-membered rings (correlation between H-1 and H-9). It also requires a *cis*-juncture between the five- and the six-membered rings with β CH_3-15 and β hydroxyl groups at C-3 (correlations between CH_3-15 and H-4β, H-6β, H-9, and H-10β). This enables us to assign the α-orientation to both CH_3-14 and the hydroxyl group at C-5. The assumption of a biosynthetic relationship between hebelophyllene D (**1**) and hebelophyllene C leads to the absolute configuration 1*S*,2*S*,3*S*,4*R*,5*R*,8*R*,9*S* for **1**, as shown. The tricyclo[5.4.0.0^{2,5}]undecane skeleton of **1** has only two precedents, naematolins C and G, whose structures have been supported by X-ray analyses.[4]



Hebelophyllene E (**2**) was obtained as a colorless oil. The molecular formula $C_{15}H_{24}O_3$ was determined by CIMS in combination with the NMR spectra, inasmuch as the EIMS did not provide a molecular ion. Of the four unsaturations, one is accounted for by a carbonyl group (δ 176.4 in the ^{13}C NMR, Table 2) and one by a vinyl group (dd's at δ 5.20, 5.39, and 5.90 in the ^{1}H NMR spectrum, Table 1). Thus, **2** is bicyclic. The presence of a CH−O fragment (δ 4.13 in the ^{1}H NMR and a doublet at δ 86.1 in the ^{13}C NMR) suggests that the carbonyl group is part of a lactone ring. HMBC correlations between the carbonyl carbon atom and C*H*−O and C*H*−CH_3 (Table 2) place the carbonyl group between these two fragments. In addition to the above methyl group, three more methyl groups are present in **2**, as indicated by the ^{1}H NMR spectrum. HMBC correlations between the vinyl group CH carbon atom (C-5) and C*H*−O−CO and one of the methyl group's singlets (at δ 1.38) clearly indicate the presence of a side chain C(OH)(CH_3)−CH=CH_2 attached to *C*H−O−CO. A facile loss of this side chain under EIMS conditions accounts for the lack of a molecular ion in the EIMS spectrum of **2**. Several of the ^{1}H and ^{13}C signals of **2** bear striking resemblance to the

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Table 1. ^{1}H NMR Data of Sesquiterpenes **1**−**3** (δ, mult., J in Hz)

assignment[a]	**1**[b]	**2**[c]	**3**[c]
H-1	2.03 dd (8.2, 2.2)	2.08 ddt (13.5, 13.5, 2.5)	2.39 dddd (9.0, 8.8, 6.0, 2.7)
H-2	3.99 br s	1.74 ddd (13.5, 6.2, 2.5, α)	1.30 ddd (14.1, 8.2, 6.0)
		2.22 dd (13.5, 11.5, *β*)	1.67 ddd (14.1, 8.8, 5.4)
H-3		4.13 dd (11.5, 6.2)	3.85 br dd (8.2, 5.4)
H-4	2.37 dq (4.7, 7.2)		
H-5	3.99 ddd (5.5, 4.7, 2.0)	5.90 dd (17.2, 10.7)	3.32 br t (3.4)
H-6	2.30 dd (14.5, 2.0, α)	5.39 dd (17.2, 1.1, a)	2.54 dd (5.9, 2.7, c)
	3.06 dd (14.5, 5.5, *β*)	5.20 dd (10.7, 1.1, b)	2.87 dd (5.9, 4.1, d)
H-8		2.81 dq (12.2, 6.5)	
H-9	3.41 ddd (9.5, 8.2, 7.8)	2.32 ddt (13.5, 12.2, 8.5)	3.55 dtt (9.5, 8.7, 1.3)
H-10α	1.47 ddd (11.1, 9.5, 2.2)	1.87 ddd (11.3, 8.2, 2.5)	1.74 ddd (10.5, 8.3, 2.7)
H-10*β*	2.28 dd (11.1, 7.8)	1.52 dd (11.3, 9.0)	1.92 br t (10.5)
H-12	1.05 s	0.90 s	1.00 s
H-13	1.22 s	1.17 s	1.26 s
H-14	1.27 d (7.2)	1.38 s	5.02 t (1.1, a)
			5.06 t (1.1, b)
H-15	1.14 s	0.97 d (6.5)	5.60 t (1.3, e)
			6.31 t (1.3, f)
H-16			3.73 s

[a] Assignments are based on coupling constants and ^{1}H−^{1}H COSY spectra. [b] $CDCl_3$, 600 MHz. [c] CD_3OD, 300 MHz.

Table 2. ^{13}C NMR Assignments and HMBC Data of Sesquiterpenes **1**−**3**

carbon[a]	**1**[b]	**2**[c]	**3**[d]
C-1	57.9 (9, 10α, 10*β*, 12, 13)	41.4 (2*β*, 10*β*, 12, 13)	44.6 (2α, 2*β*, 3, 9, 10α, 10*β*, 12, 13)
C-2	79.6 (1, 4, 9)	25.9 (1, 3, 9)	34.8 (3)
C-3	92.9 (4, 5, 9, 14, 15)	86.1 (2α, 14)	71.5 (1, 2α, 2*β*, 14a, 14b)
C-4	43.3 (5, 6α, 14)	76.2 (2α, 5, 6a, 6b, 14)	150.6 (2α, 2*β*, 3, 5, 6α, 6*β*, 14a, 14b)
C-5	71.0 (4, 6α, 6*β*, 14)	140.1 (3, 6α, 14)	51.7 (3, 6α, 6*β*, 14a, 14b)
C-6	47.1	114.9	50.0 (5)
C-7	214.1 (6α, 6*β*, 9, 15)	176.4 (3, 8, 15)	169.7 (15c, 15d, 16)
C-8	61.5 (2, 9, 10*β*, 15)	41.3 (9, 10α, 10*β*, 15)	142.3 (9, 10α, 10*β*, 15d)
C-9	36.6 (1, 2, 10*β*, 15)	34.9 (2α, 2*β*, 8, 10α, 10*β*, 15)	34.2 (1, 2*β*, 10α, 10*β*, 15c, 15d)
C-10	35.3 (1, 12, 13)	38.6 (8, 9, 12, 13)	35.8 (1, 9, 12, 13)
C-11	33.6 (1, 2, 12, 10*β*, 13)	33.4 (2*β*, 10α, 10*β*, 12, 13)	35.2 (1, 2α, 2*β*, 9, 10α, 10*β*, 12, 13)
C-12	25.0 (10*β*, 13)	24.3 (10α, 10*β*, 13)	25.4 (10α, 10*β*, 13)
C-13	33.9 (1, 10α, 10*β*, 12)	29.9 (10α, 10*β*, 12)	30.7 (1, 10α, 10*β*, 12)
C-14	12.0 (4)	25.5 (5)	111.0 (3,5)
C-15	16.4 (9)	12.9 (8, 9)	125.3 (9)
C-16			52.4

[a] Multiplicities were verified with APT and HMQC spectra. [b] In $CDCl_3$, 50 MHz. [c] In $CDCl_3$, 100 MHz. [d] In CD_3OD, 50 MHz.

signals of a four-membered ring of the previously described hebelophyllenes A−C isolated from the same fungus.[3]

All of the above information is consistent with the proposed structure **2** for hebelophyllene E. The *cis*-ring juncture is based on the cross peaks between H-1 and H-9 in the TROESY spectrum. The correlation between H-8 and H-2*β* confirms the *cis*-ring juncture and determines the α-position of CH_3-15. Finally, cross peaks between H-2α and H-1, and H-2α and H-3 ascribe the *β*-position to the side chain at C-3 and facilitate the assignments of these protons.

Hebelophyllene F (**3**) was isolated as a colorless oil. The molecular formula $C_{16}H_{24}O_4$was derived from the HRMS spectrum. The presence of a $H_2C{=}C{-}COOCH_3$ substructure was deduced from the ^{1}H NMR (singlet, 3H, at δ 3.73, and two triplets, at δ 5.60 and 6.31, Table 1) and ^{13}C NMR (quartet at δ 52.4, singlet at δ 169.7, singlet at δ 142.3, and a triplet at δ 125.3, Table 2), as well as from the IR (1720 cm^{-1}) and UV (max at 215 nm) spectra. A second $H_2C{=}C{-}$ group is also present, as indicated by a second set of triplets in the ^{1}H NMR spectrum (at δ 5.02 and 5.06) and ^{13}C signals at δ 150.6 (s) and 111.0 (t). The ^{1}H−^{1}H COSY spectrum clearly indicated that the fully substituted carbon of this $H_2C{=}C{-}$ group is directly attached to a C*H*−OH (at δ 3.85) and a C*H*−O (at δ 3.32) protons. The latter is also coupled to two geminal protons (dd's at δ 2.54 and 2.87). The small geminal coupling constant of 5.9 Hz is strong evidence for these three protons being part of an epoxide ring. In addition to the above NMR signals, all signals characteristic of a caryophyllene-type four-membered ring are also present. The TROESY spectrum of **3** indicates *cis*-substitution of the four-membered ring (cross peak between H-1 and H-9) and also allows us to assign the olefinic protons at C-14. All of the above information leads to structure **3** for hebelophyllene F. Assuming a biosynthetic relationship among the lactone **2**, the ester **3**, and the hebelophyllenes A−C,[3] one can expect the absolute configuration of **2** to be 1*S*,3*S*,8*R*,9*S* and that of **3** to be 1*S*,3*S*,9*S*. The absolute configuration of **2** at C(4) and of **3** at C(5), however, remains undetermined.

To the best of our knowledge, hebelophyllene E (**2**) and hebelophyllene F (**3**) are the first naturally occurring 6,7-seco-caryophyllenes. The only previously reported 6,7-seco-caryophyllene was a photoproduct produced by irradiation of caryophyllene.[5]

Hebelophyllenes D−F (**1**−**3**) are related to the previously described hebelophyllenes A−C, which are representatives of the small group of *cis*-caryophyllenes.[3,6,7]

Experimental Section

General Experimental Procedures. Procedures were followed as described previously.[3]

Organism. *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.

Isolation of Hebelophyllenes D−F. The fungus was grown as described previously.[3] The filtered broth (5 L) was concentrated under vacuum to 1 L and extracted with EtOAc (5 × 400 mL) to provide 1.06 g of crude extract. The latter was subjected to flash chromatography on Si gel 60 (230−400 mesh) with hexane−EtOAc (gradient, 25−100%). The fraction eluted with hexane−EtOAc, 75:25, was further purified by preparative TLC (hexane−$CHCl_3$−*i*-PrOH, 48.5:48.5:3, twofold development, R_f = 0.47) to afford pure **2** (9.2 mg). The fraction eluted with hexane−EtOAc, 60:40, preceding hebelophyllenes A−C was purified by preparative TLC (C_6H_6−Me_2CO 90:10, twofold development, R_f = 0.35) to give pure **3** (7.8 mg). The fraction eluted with hexane−EtOAc (34:66) was concentrated under vacuum to ca. 1 mL and left at room temperature overnight. The crystals were filtered and washed with hexane−EtOAc, 80:20 to provide pure **1** (19.2 mg).

Hebelophyllene D (1): colorless needles; mp 205.0−206.0 °C; $[\alpha]^{25}_D$ +110.4° (*c* 0.23, MeOH); UV (MeOH) λ_{max} (log ϵ) 273 nm (2.25); CD: $\Delta\epsilon_{292}$ +2.17 (*c* 0.23, MeOH); IR (Nic-Plan IR

MICROSCOPE) ν_{max} 3308 (OH), 3003, 2988, 2951, 2935, 2923, 2894, 2878, 2860, 1710 (>C=O), 1475, 1407, 1371, 1054, 846 cm $^{-1}$; ^{1}H NMR, Table 1; ^{13}C NMR, Table 2; CIMS (NH_3) 286 $[M + NH_4]^+$ (5), 268 $[M]^+$ (6); HREIMS *m/z* 268.1682 $[M]^+$ (2) (calcd for $C_{15}H_{24}O_4$, 268.1674), 253 $[M - CH_3]^+$ (11), 250 $[M - H_2O]^+$ (14), 235 $[M - CH_3 - H_2O]^+$ (33), 232 $[M - 2H_2O]^+$ (3).

Hebelophyllene E (2): colorless oil; $[\alpha]^{25}_D$ −63.3° (*c* 0.12, MeOH); CD $\Delta\epsilon_{218}$ −2.99 (*c* 0.12, MeOH); IR (Nic-Plan IR MICROSCOPE) ν_{max} 3446 (OH), 2950, 2865, 1712 (>C=O), 1458, 1336, 1290, 710 cm $^{-1}$; ^{1}H NMR, Table 1; ^{13}C NMR, Table 2; CIMS (NH_3) *m/z* 270 $[M + NH_4]^+$ (52), 253 $[M + H]^+$ (20), 252 $[M]^+$ (36); HREIMS *m/z* $[M - C_4H_7O]^+$ 181.1225 (28) (calcd for $C_{11}H_{17}O_2$, 181.1228), 71.0497 (19) (calcd for C_4H_7O, 71.0497), 109 (100).

Hebelophyllene F (3): colorless oil; $[\alpha]^{25}_D$ +30.0° (*c* 0.14, MeOH); UV (MeOH) λ_{max} (log ϵ) 215 nm (sh) (3.62) (*c* 0.028, MeOH); CD $\Delta\epsilon_{217}$ +10.24 (*c* 0.034, MeOH); IR (Nic-Plan IR MICROSCOPE) ν_{max} 3468 (OH), 2952, 2866, 1720 (>C=O), 1627 (>C=C<), 1438, 1383, 1071 cm $^{-1}$; ^{1}H NMR, Table 1; ^{13}C NMR, Table 2; CIMS (NH_3) *m/z* 298 $[M + NH_4]^+$ (80), 281 $[M + H]^+$ (29), 280 $[M]^+$ (6); HREIMS, *m/z* 280.1655 $[M]^+$ (0.04) (calcd for $C_{16}H_{24}O_4$, 280.1674), 265 $[M - CH_3]^+$ (0.1), 262 $[M - H_2O]^+$ (0.4).

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Supporting Information Available: Pertinent TROESY correlations for sesquiterpenes **1**−**3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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