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NEW FARNESANE SESQUITERPENES FROM *HEBELOMA SENESCENS*[1]

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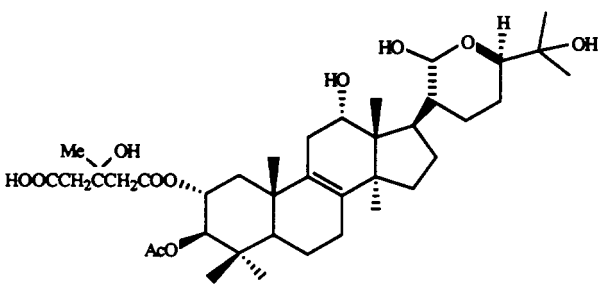
ABSTRACT.—Two farnesane derivatives have been isolated from the inedible mushroom *Hebeloma senescens* and have been identified as (*E*)-2,3-epoxy-2,6-dimethyl-10-methylene-6,11-dodecadiene [**2**] and (3*S*)-(*E*)-2,6-dimethyl-10-methylene-1,6,11-dodecatrien-3-ol [**3**]. This is the first report of farnesane sesquiterpenes in the Basidiomycetes.

The genus *Hebeloma* (Basidiomycetes, family Cortinariaceae) is a relatively large group of mushrooms comprising many inedible or toxic species growing in Italian woods during late summer and autumn. Some years ago we isolated a new cytotoxic triterpene, hebelomic acid A [**1**], from *Hebeloma crustuliniforme* and *Hebeloma sinapizans* (1). Japanese researchers have isolated numerous cucurbitane triterpene glycosides, called hebevinosides, from the poisonous mushroom *Hebeloma vinosophyllum* (2,3).

In the course of studies on biologically active substances from Basidiomycetes (4), we have found that a crude extract of *Hebeloma senescens* (Fr.) Berk. et Br. (syn. *Hebeloma edurum* Metr. ex Bon), growing in the forests of the Italian Apennines, exhibited moderate antibacterial activity against *Bacillus subtilis* and *Staphylococcus aureus* var. *oxford*. In addition, *H. senescens* has an unpleasant bitter taste and causes, if ingested, severe gastrointestinal diseases.

An EtOAc extract of *H. senescens* was separated by chromatography into nonpolar and polar fractions. The latter contained large quantities of hebelomic acid A [**1**] and



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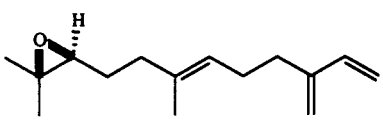

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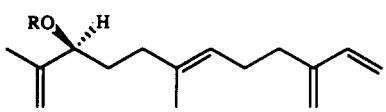

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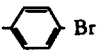
4 R=-OC-  Br

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other as yet unidentified polyhydroxy lanostane triterpenes having free carboxylic acid functions. These compounds show weak antibacterial activity against Gram-positive bacteria. The more lipophilic fractions were further purified by repeated cc to yield a mixture of triglycerides, saturated and unsaturated free fatty acids, and the two sesquiterpenes **2** and **3** as a colorless oil.

Sesquiterpenoid **2**, $[\alpha]^{20}D$ +1.87, had a molecular formula $C_{15}H_{24}O$ (ms and ^{13}C nmr) that required four sites of unsaturation. From the ^{13}C-nmr data and ir spectrum of **2**, which exhibited no band for hydroxyl or carbonyl groups, the four sites were assigned to one ether ring and three double bonds. From the ^{1}H-nmr spectrum it was evident that the latter comprised one trisubstituted double bond carrying a methyl group (δ 1.62), a vinyl group (ABX pattern at δ 5.05, 5.23, and 6.37) and one terminal methylene group (broad singlets at δ 4.98 and 5.00). These features coupled with the ir (1630, 1590, 985, and 890 cm^{-1}) and uv (223 nm) absorption data, comparable with those previously reported for (*E*)-β-farnesene (5,6), indicated a conjugated diene structure for **2**. The remaining ^{1}H-nmr signals and ^{13}C-nmr data of **2** were assigned to three allylic and one homoallylic methylene groups, two geminal methyls, and one proton on an oxirane ring (δ 2.70). These data were fully consistent with the structural features of a farnesane skeleton and allowed placement of the epoxide ring at C-2. Moreover, spin-decoupling experiments showed that the trisubstituted double bond was between C-6 and C-7 rather than between C-5 and C-6. In fact, irradiation of the multiplet attributed to the allylic methylene groups (δ 2.0–2.25) collapsed the signal of the olefinic proton (δ 5.20) but not the H-3 triplet which, instead, was collapsed to a singlet by irradiation of the multiplet assigned to the H-4 protons (δ 1.55–1.7). The stereochemistry of the double bond was determined to be *E* from the chemical shifts of the olefinic methyl group in the ^{1}H nmr (δ 1.62) (7) and ^{13}C nmr (δ 16.06) (8) spectra of compound **2**. Therefore **2** is shown to be (*E*)-β-10,11-dihydro-10,11-epoxyfarnesene.

The alcohol **3**, $[\alpha]^{20}D$ −7.31, which the ms and nmr indicated was an isomer of **2**, contained the same conjugated diene structure (λ max 224 nm). Inspection of the ^{1}H- and ^{13}C-nmr spectra of **3** showed that, with the exception of the signals near C-3, the data were almost identical with those of compound **2**, suggesting that only modification of the oxirane ring had occurred. The signal of an additional olefinic methyl (δ 1.73), coupled with those of a secondary allylic alcohol (δ 4.05) and a vinylic methylene group (broad singlets at δ 4.84 and 4.94), clearly indicated that **3** was (*E*)-2,6-dimethyl-10-methylene-1,6,11-dodecatrien-3-ol.

Treatment of epoxide **2** with aluminum isopropoxide in boiling toluene (9) smoothly gave isomeric allylic alcohol **3** which was identical with the natural compound. Finally, the absolute stereochemistry at C-3 was established by applying the Nakanishi and Sharpless cd exciton chirality method for determining absolute configurations of acyclic allylic alcohols (10). The cd spectrum of the *p*-Br-benzoate **4** exhibited a positive Cotton effect (Δε + 1.53) at 237 nm, the sign of which showed that the absolute configuration of compound **3** must be *S*.

To the best of our knowledge this is the first report of the isolation of sesquiterpenoids **1** and **2** from a natural source. Racemic **3** was obtained during the synthesis of β-sinensal (11), but spectral data have not been reported. Farnesane sesquiterpenes have not yet been found in any other species of Basidiomycetes. Furthermore, this is the first finding of sesquiterpenoid compounds in *Hebeloma*. Their occurrence in *H. senescens*, which has triterpenes as major constituents, suggests a farnesyl or nerolidyl intermediate in the biosynthesis of these C_{30} metabolites.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The ir spectra were recorded with a Perkin-Elmer

TABLE 1. ^{1}H-nmr[a] and ^{13}C-nmr[b] Spectral Data for Compounds **2** and **3**.

Proton	Compound		Carbon	Compound	
	2[c]	**3**[d]		**2**	**3**
H-1	1.30 s	4.84 qu[e] 4.94 se[f]	C-1	24.9 (3)	111.1 (2)
			C-2	58.4 (0)	147.5 (0)
H-3	2.70 t	4.05 t	C-3	64.2 (1)	75.7 (1)
H-4	1.55–1.7 m	1.62–1.70 m	C-4	27.5[h] (2)	33.2[h] (2)
H-5	2.0–2.25 m	1.95–2.11 m	C-5	36.3 (2)	35.7[h] (2)
H-7	5.20 m	5.20 m	C-6	134.5 (0)	135.2 (0)
H-8	2.15–2.25 m	2.15–2.29 m	C-7	124.7 (1)	124.5 (1)
H-9			C-8	26.6[h] (2)	26.6 (2)
H-11	6.37 dd	6.37 dd	C-9	31.4 (2)	31.4 (2)
H-12	5.05 d[g]	5.06 d[g]	C-10	146.0 (0)	146.1 (0)
H-12′	5.23 dd	5.24 dd	C-11	138.9 (1)	139.0 (1)
H-13	4.98 bs	4.99 bs	C-12	115.6 (2)	115.8 (2)
H-13′	5.00 bs	5.02 bs	C-13	113.1 (2)	113.1 (2)
H-14	1.62 bs	1.62 d	C-14	16.06 (3)	16.06 (3)
H-15	1.26 s	1.73 t	C-15	18.8 (3)	17.6 (3)

[a]250 MHz. δ_H values in ppm, relative to $\delta_H = 0.00$ for TMS in $CDCl_3$ solutions.

[b]62.9 MHz ($CDCl_3$). Values in ppm, relative to $\delta_C = 76.9$ for $CDCl_3$. The number in parentheses indicates the number of hydrogens attached to the corresponding carbon and was determined from DEPT experiments.

[c]$J_{3,4} + J_{3,4'} = 12$; $J_{11,12} = 10.5$; $J_{11,12'} = 17.5$; $J_{12,12'} = 1.3$.

[d]$J_{3,4} + J_{3,4'} = 13$; $J_{11,12} = 10.5$; $J_{11,12'} = 17.5$; $J_{12,12'} = 1.3$; $J_{14,7} = 1.2$; $J_{15,1} = 1.2$.

[e]qu = *quintet* ($J = 1.6$).

[f]se = sextuplet ($J = 1.0$).

[g]Each line of the doublet is further split into a quartet by geminal and long range coupling constants ($J \simeq 1.1$).

[h]Assignments in the same vertical column may be interchanged.

Model 881 spectrophotometer; uv spectra were obtained with a Perkin-Elmer Lambda 5 spectrometer; ^{1}H-nmr and ^{13}C-nmr spectra were recorded on a Bruker 250 MHz instrument. Ms spectra were determined with a Finnigan MAT 8222 mass spectrometer at 70 eV using a direct inlet system. Specific optical rotations were recorded with a Perkin-Elmer model 241 digital polarimeter. Cd spectra were obtained with a Jasco L 500 A spectropolarimeter. Merck Kieselgel 60 (0.040–0.063 mm) was used for cc run at atmospheric pressure. Tlc was carried out on Si gel plates (GF_{254}, Merck, 0.25 mm). The spots were visualized by spraying the plates with 0.5% vanillin solution in H_2SO_4-EtOH (4:1) and then heating at 120° for 5 min.

FUNGAL MATERIAL.—*H. senescens* (15.6 kg) was collected at Pietragavina (Pavia) in November 1984, and was identified by Dr. Livio Quadraccia, University of Rome. A voucher specimen is preserved at the Herbarium of the Botanical Garden, University of Rome (ROHB).

EXTRACTION AND ISOLATION.—The fresh fruiting bodies of *H. senescens* were extracted with EtOAc (3 × 20 h) at room temperature, a few hours after collection. The extract was dried (Na_2SO_4) and concentrated to yield a solid brown residue (114.7 g). Part of the latter (85.3 g) was chromatographed on Si gel (1.1 kg) using a CH_2Cl_2/Me_2CO/HOAc gradient and collecting fractions of 300–400 ml each. Fractions were pooled into 16 groups (I-XVI) according to tlc analysis. Compounds **2** and **3** were present in fractions V and VI. The former (200 mg) was further purified by two consecutive Si gel cc's [A: hexane-Me_2CO (99:1), B: hexane-EtOAc (98:2)] to give sesquiterpene **2** (16 mg) as a colorless viscous oil, a mixture of triglycerides and an unidentified red pigment. Fraction VI (112 mg) was purified by three consecutive Si gel cc's [A: hexane-EtOAc (98:2→90:10), B: hexane-CH_2Cl_2-Me_2CO (59:39:2), C: hexane-CH_2Cl_2-Me_2CO (60:36:4)] to give more **2** (17.4 mg) and the alcohol **3** (9.1 mg) as a colorless viscous oil.

(E)-2,3-Epoxy-2,6-dimethyl-10-methylene-6,11-dodecadiene [**2**].—R_f 0.25 in hexane-EtOAc (97:3); $[\alpha]^{20}D$ +1.87 (CH_2Cl_2, $c = 0.8$); ir ν max (film) 3092, 1632, 1594, 1460, 1376, 1323, 1247, 1123, 991, 898 cm^{-1}; uv λ max (ϵ) (hexane) 223.4 nm (10800); ^{1}H and ^{13}C nmr see Table 1; eims *m/z* (% rel. int.) $[M]^+$ 220 (0.3), 134 (33), 127 (19), 119 (19), 109 (19), 105 (10), 93 (100), 91 (18), 85 (27), 81 (38), 79 (24), 71 (50), 69 (14), 67 (15), 59 (22), 55 (17), 43 (67), 41 (30).

(3S)-(E)-2,6-dimethyl-10-methylene-1,6,11-dodecatrien-3-ol [**3**].—R_f 0.53 in hexane-CH_2Cl_2-Me_2CO (60:35:5); $[\alpha]^{20}D$ −7.31 (CH_2Cl_2, $c = 0.4$); ir ν max (film) 3364, 1594, 1466, 1385, 1159, 1059, 1018, 991, 896, 831; uv λ max (ϵ) (hexane) 224 nm (9858); ^1H and ^{13}C nmr see Table 1; eims *m/z* (% rel. int.) $[M]^+$ 220 (0.7), 205 (0.5), 202 (2.1), 187 (7), 159 (9), 146 (20), 133 (20), 120 (22), 119 (24), 109 (20), 107 (24), 105 (19), 93 (100), 91 (35), 81 (28), 79 (38), 77 (19), 71 (24), 69 (31), 67 (34), 55 (39), 43 (29), 41 (54).

ALUMINUM ISOPROPOXIDE ISOMERIZATION OF EPOXIDE **2** TO ALLYLIC ALCOHOL **3**.—A mixture of the epoxy compound **2** (10 mg, 0.045 mmol) and aluminum isopropoxide (260 µl of a 0.23 M solution in dry $C_6H_5CH_3$) in $C_6H_5CH_3$ (0.5 ml) was heated under reflux for 6 h under an Ar atmosphere. After cooling to room temperature, the reaction mixture was diluted with hexane and treated with 10% HCl to decompose the aluminum complex. The organic layer was separated, washed with 5% $NaHCO_3$, washed with brine, dried ($MgSO_4$), and evaporated in vacuo. The residue was chromatographed with hexane-EtOAc (12:1) on Si gel to give the desired allylic alcohol **3** (3.1 mg, yield 31%), identical (R_f, ir and ^1H nmr) with the natural compound.

p-Br-BENZOATE OF ALLYLIC ALCOHOL **3**.—A mixture of the allylic alcohol **3** (7.1 mg, 0.032 mmol), *p*-Br-C_6H_4COCl (10.5 mg, 0.048 mmol), $Et(iPr)_2N$ (8.5 µl, 0.084 mmol), and DMAP (1 mg) in dry CH_2Cl_2 (0.5 ml) was heated under reflux for 4 h under an Ar atmosphere. After cooling to room temperature, the reaction was quenched with H_2O (50 µl), and the mixture was passed with CH_2Cl_2 through activity III neutral Al_2O_3 (200 mg). After removal of solvent, the residue was chromatographed with hexane-EtOAc (98:2) on Si gel to give the *p*-Br-benzoate **4** (9.6 mg, 68.6%); uv λ max (ϵ) (MeOH) 234 nm (15224); cd λ ext ($\Delta\epsilon$) (MeOH) 237 nm (+1.53); cims (CH_4) *m/z* $[M+H]^+$ 405 and 403; ^1H nmr ($CDCl_3$, 300 MHz) 1.64 (3H, s), 1.79 (3H, s), 1.7–2.30 (8H, m), 4.94 (1H, bs), 4.98 (1H, bs), 5.02 (2H, bs), 5.05 (1H, d, $J = 10.8$ Hz), 5.16 (1H, m), 5.23 (1H, d, $J = 17.7$ Hz), 5.37 (1H, t, $J = 7.0$ Hz), 6.36 (1H, dd, $J = 10.8$ and 17.7 Hz), 7.75 (4H, AA'XX' system).

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