Microbial Transformation of Sesquiterpenes, (−)-*Ambrox*® and (+)-Sclareolide

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The microbial transformation of (−)-*Ambrox*® (**1**), a perfumery sesquiterpene, by a number of fungi, by means of standard two-stage-fermentation technique, afforded ambrox-1α-ol (**2**), ambrox-1α,11α-diol (**3**), ambrox-1α,6α-diol (**4**), ambrox-1α,6α,11α-triol (**5**), ambrox-3-one (**6**), ambrox-3β-ol (**7**), ambrox-3β,6β-diol (**8**), 13,14,15,16-tetranorlabdane-3,8,12-triol (**9**), and sclareolide (**10**) (*Schemes 1* and *2*). Further incubation of compound **10** with *Cunninghamella elegans* afforded 3-oxosclareolide (**11**), 3β-hydroxysclareolide (**12**), 2α-hydroxysclareolide (**13**), 2α,3β-dihydroxysclareolide (**14**), 1α,3β-dihydroxysclareolide (**15**), and 3β-hydroxy-8-episclareolide (**16**) (*Scheme 3*). Metabolites **2**–**5**, **12**, **13**, and **16** were found to be new compounds. The major transformations include a reaction path involving hydroxylation, ether-bond cleavage and inversion of configuration. Metabolites **11**–**16** of sclareolide showed significant phytotoxicity (*Table 1*). The structures of the metabolites were characterized on the basis of spectroscopic techniques.

Introduction. – In continuation of our studies on biotransformation of bioactive natural and synthetic compounds [1–11], we have recently investigated the biotransformation of two sesquiterpenes, (−)-*Ambrox*® (**1**) and (+)-sclareolide (**10**), with various fungal strains. Ambergris, a metabolite of the sperm whale, is one of the most-valuable animal perfumes, ranking with civet and musk [12][13]. Ambrein is the major constituent of the ambergris. During exposure in the sea for many years, ambrein is oxidatively decomposed by the action of sea water, air, and sunlight to yield some other odorous compounds [14]. In these compounds, (−)-*Ambrox*® (**1**) has a very strong amber-like odor.

Fermentation of (−)-*Ambrox*® (**1**) with *Fusarium lini* (NRRL 68751) afforded the new mono-, di-, and trihydroxylated metabolites **2**–**5**, as a result of enantioselective α-hydroxylations at C(1), C(6), and C(11), while incubation of compound **1** with *Rhizopus stolonifer* (ATCC 10404) yielded metabolites **6**–**8** and ether-cleaved product **9**. The ether cleavage through biotransformation is a rare observation. Fermentation of compound **1** with *Curvularia lunata* (NRRL 2178) yielded metabolites **6** and **7**, while its biotransformation with *Cunninghamella elegans* (NRRL 1392) afforded compounds **6**, **7**, and (+)-sclareolide (**10**). Compounds **6**–**10** have already been reported as metabolites of **1** by *Cephalosporium aphidicola* (wild type), *Aspergillus niger* (IFO 4049), *Aspergillus cellulose* (IFO 4040), and *Botryatis cinerea* (AHU 9424) [15–19]. The oxygenated metabolites of compound **1** did not release any effective odor when compared to **1**, except for the ether-cleaved product **9**, which exhibited a strong sweet odor quit different from the amber-like odor.

(+)-Sclareolide (**10**), a constituent of *Arnica angustifolia* [20], *Sideritis nutans* [21], and *Kyllinga erecta* [22], has exhibited phytotoxic activity and cytotoxicity against

human cancer cell lines [6][23]. Compound **10** differs from **1** only in the presence of an oxo group at C(12). Due to the presence of the lactone moiety, compound **10**, on incubation with *Cunninghamella elegans*, yielded an entirely new set of oxidized metabolites **11**–**16**, resulting from the enantioselective hydroxylations at C(2) and C(1) and epimerization at C(8). The inversion of configuration is also a rare phenomenon in biotransformations. Metabolites **11**, **12**, and **15** have been reported earlier as fermented products of compound **10** by various fungi [11][15][24] and have shown cytotoxicity against various human cancer cell lines [17]. During this study, metabolites of compound **10** were subjected to phytotoxicity assay where metabolites **11**, **12**, and **15** showed significant phytotoxicity at higher dose against *Lemna minor* L. (*Table 1*).

Table 1. *Phytotoxicity against* Lemna minor L. *of (+)-Sclareolide* (**10**) *and Its Transformed Products* **11**–**16**

Concentration [μg/ml]	Growth inhibition [%][a])						
	10	**11**	**12**	**13**	**14**	**15**	**16**
100	62.5	100	87.5	72.3	50	82.7	75
10	29.7	31.2	50	54.1	43.7	65.4	31.2
1	17.4	25	18.7	24.6	18.7	32.9	31.2

[a]) Standard drug (paraquat) showed 100% growth inhibition at 7.5 μg/ml.

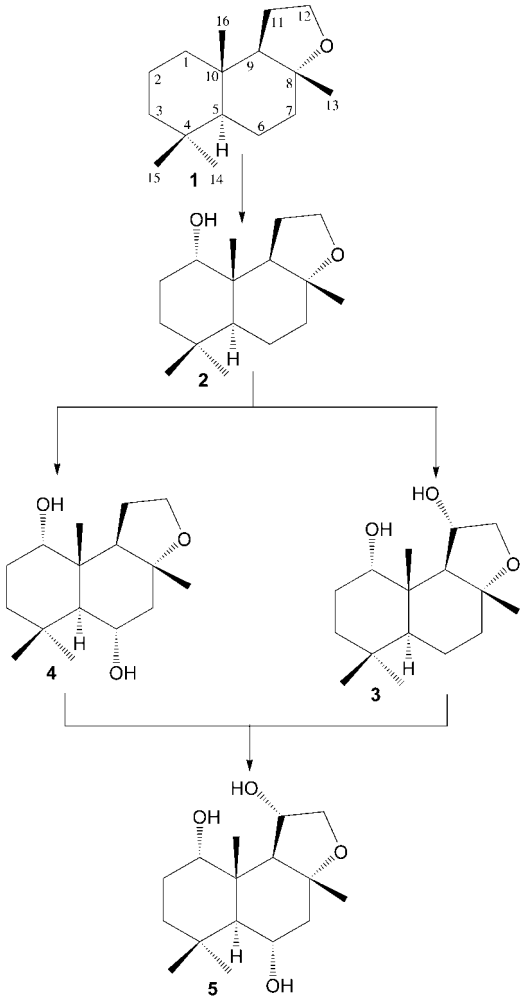
Results and Discussion. – Screening-scale experiments showed that all four fungal cultures were capable of converting (−)*Ambrox*® (**1**) to polar metabolites. Preparative-scale fermentation was thus carried out to produce sufficient quantities of new and known metabolites for structure elucidation and bioassay studies. Structures of known metabolites were elucidated through comparison of their reported data [15–19].

Incubation of (−)-*Ambrox*® (**1**) with *Fusarium lini* (NRRL 68751) yielded the new metabolites **2**–**5**, whose structures were elucidated through comparative spectroscopic spectral studies with substrate **1**. Metabolites **3** and **4** might be formed by monohydroxylation of compound **2** at C(11) and C(6), respectively, which could be monohydroxylated in turn to yield **5** (see *Scheme 1*).

The HR-EI-MS of **2** displayed the M^+ at m/z 252.2056, corresponding to the molecular formula $C_{16}H_{28}O_2$ (calc. 252.2089), indicating one more O-atom than **1**. Absorption at 3436 cm^{-1} in the IR spectrum of **2** indicated the presence of an OH group. The ^{1}H-NMR spectrum of **2** (*Table 2*) further confirmed the formation of a monohydroxy derivative through the geminal-proton signal at δ 3.46 (t, $J = 2.6$ Hz). The splitting pattern and coupling constant indicated that the OH group must be axially situated either at C(1), C(3), or C(7). The ^{13}C-NMR spectrum of **2** (*Table 3*) showed an additional CH signal at δ 72.4, along with the γ-upfield shifts of C(3), C(5), and C(9) as compared to **1**, suggesting that the location of the new OH group is C(1). H−C(1) (δ 3.46) showed homonuclear couplings with CH_2(2) (δ 2.10, 1.52), and HMBC interactions with C(3) (δ 35.3) and C(5) (δ 48.8). The configuration of the new OH group was deduced from the NOE experiment, which showed enhancement of the H−(1) signal (δ 3.46) on irradiation of the Me_β(16) signal. These interactions are only possible with α disposition (axial) of the OH−C(1).

The metabolite **3** showed an IR spectrum similar to that of **2**. Its HR-EI-MS exhibited the M^+ at m/z 268.2064, consistent with the molecular formula $C_{16}H_{28}O_3$ (calc. 268.2038), 32 amu greater than that of **1**. This suggested dihydroxylation of **1**. However, the ^{1}H-NMR spectrum of **3** (*Table 2*) was remarkably different from that of **1** in several respects: two downfield OH protons appeared at δ 3.69 (t, $J = 2.8$ Hz) and 4.51 (ddd, $J = 10.2$, 7.1, 3.9 Hz), while the CH_2(12) signals were slightly shifted downfield to δ 4.15 (dd, $J = 9.6$, 7.1 Hz) and 3.63 (dd, $J = 9.6$, 4.1 Hz), indicating the presence of an OH group at C(11). The ^{13}C-NMR spectrum of **3** (*Table 3*)

Scheme 1. *Metabolism of (–)*-Ambrox (**1**) *by* Fusarium lini



showed two new CH resonances at δ 72.0 and 70.1. The DEPT experiment revealed the presence of five CH_2 and four CH, indicating that two CH_2 moieties of **1** were converted to OH-bearing CH groups. The proton at δ 3.69 (t, $J = 2.8$ Hz) could be assigned to H_β–C(1), while the OH-bearing CH(11) appeared at δ 4.51. The configuration of OH–C(11) was deduced from an NOE experiment, which showed a 7.5% enhancement of the H–C(11) signal (δ 4.51) when Me_β(13) (δ 1.12) was irradiated. Similarly, irradiation of the H–C(11) signal caused a 3.6 and 2.1% enhancement of the signals at 1.12 (Me_β(13)) and 0.82 (Me_β(16)), respectively, supporting the β-position of H–C(11).

The structure of ambrox-1α,6α-diol (**4**) was deduced on the basis of the following considerations. HR-EI-MS showed the molecular ion at m/z 268.2041 ($C_{16}H_{28}O_3^+$; calc. 268.2038), revealing two new O-functions in the molecule as compared to **1**. These O-functions were deduced to be OH groups from the IR absorption at 3329 cm^{-1}. The ^{1}H-NMR spectrum of **4** (*Table 2*) showed additional signals for two protons geminal to the OH groups at δ 3.42 (t, $J = 2.7$ Hz) and 4.62 (dd, $J = 7.3$, 3.0 Hz). The splitting pattern and coupling constants suggested an axial orientation of the OH groups present at C(1) and C(6). The ^{13}C-NMR spectrum of **4**

Table 2. *^{1}H-NMR Data* (500 MHz, $CDCl_3$) *for Compounds* **2**–**5**, **13**, **14**, *and* **16**. δ in ppm and J in Hz.

	2	**3**	**4**	**5**	**13**	**14**	**16**
CH_2(1) or H–C(1)	3.46 ($t, J = 2.6$)	3.69 ($t, J = 2.8$)	3.42 ($t, J = 2.7$)	3.62 ($t, J = 2.2$)	1.81, 1.42 (2*m*)	1.78 (*dd*, $J = 12.9, 4.7$), 1.21 (*m*)	1.57, 1.22 (2*m*)
CH_2(2) or H–C(2)	2.10, 1.52 (2*m*)	2.02, 1.58 (2*m*)	2.17, 1.51 (2*m*)	2.14, 1.59 (2*m*)	3.90 (*m*, $w_{1/2} = 17.1$)	3.79 (*m*, $w_{1/2} = 18.6$)	1.61, 1.53 (2*m*)
CH_2(3) or H–C(3)	1.58 (*m*), 1.21 (*dd*, $J = 4.1, 2.6$)	1.26, 1.19 (2*m*)	1.60, 1.17 (2*m*)	1.69 (*m*), 1.14 (*ddd*, $J = 13.2, 6.9, 3.6$)	1.88, 1.15 (2*m*)	3.02 ($d, J = 9.1$)	3.22 (*dd*, $J = 11.9, 4.6$)
H–C(5)	1.42 (*m*)	1.50 (*m*)	1.58 ($d, J = 7.1$)	1.47 ($d, J = 7.3$)	1.37 (*m*)	1.21 (*m*)	1.14 (*m*)
CH_2(6) or H–C(6)	1.32, 1.17 (2*m*)	1.81, 1.33 (2*m*)	4.62 (*dd*, $J = 7.3, 3.0$)	4.63 (*dd*, $J = 7.4, 2.3$)	1.82, 1.54 (2*m*)	1.68, 1.46 (2*m*)	1.65, 1.38 (2*m*)
CH_2(7)	1.97, 1.38 (2*m*)	1.84, 1.56 (2*m*)	2.05 (*m*), 1.64 (*dd*, $J = 12.1, 3.8$)	2.01, 1.77 (2*m*)	2.11, 1.65 (2*m*)	2.07, 1.62 (2*m*)	1.81, 1.52 (2*m*)
H–C(9)	1.92 (*dd*, $J = 13.3, 2.6$)	2.11 ($d, J = 10.2$)	1.87 (*m*)	2.21 ($d, J = 10.1$)	1.98 (*dd*, $J = 14.7, 6.4$)	2.01 (*m*)	1.93 (*m*)
CH_2(11) or H–C(11)	1.46, 1.17 (2*m*)	4.51 (*ddd*, $J = 10.2, 7.1, 3.9$)	1.81, 1.47 (2*m*)	4.59 (*ddd*, $J = 9.9, 7.1, 4.0$)	2.42 (br. *t*, $J = 16.0$), 2.25 (*dd*, $J = 16.2, 6.4$)	2.59 (*dd*, $J = 15.6, 7.3$), 2.37 (*dd*, $J = 15.0, 3.9$)	2.52 (*dd*, $J = 16.1, 7.7$), 2.30 (*dd*, $J = 16.1, 4.2$)
CH_2(12)	3.88 (*m*, 2 H)	4.15 (*dd*, $J = 9.6, 7.1$), 3.63 (*dd*, $J = 9.6, 4.1$)	3.92 (*dd*, $J = 12.0, 7.8$), 3.88 (*dd*, $J = 16.0, 7.9$)	4.20 (*dd*, $J = 9.5, 7.1$), 3.61 (*dd*, $J = 9.4, 4.1$)			
Me(13)	1.07 (*s*)	1.12 (*s*)	1.31 (*s*)	1.36 (*s*)	1.31 (*s*)	1.31 (*s*)	1.18 (*s*)
Me(14)	0.90 (*s*)	0.93 (*s*)[a])	1.26 (*s*)	1.25 (*s*)	0.94 (*s*)[a])	0.94 (*s*)[a])	0.98 (*s*)
Me(15)	0.82 (*s*)	0.91 (*s*)[a])	1.16 (*s*)	1.20 (*s*)	0.93 (*s*)[a])	0.93 (*s*)[a])	0.78 (*s*)[a])
Me(16)	0.82 (*s*)	0.82 (*s*)	1.02 (*s*)	1.02 (*s*)	0.87 (*s*)	0.85 (*s*)	0.75 (*s*)[a])

[a]) Assignments are interchangeable.

Table 3. *^{13}C-NMR Data* (125 MHz, $CDCl_3$) *of Compounds* **1** *and* **10** *and of Their New Metabolites* **2**–**5** *and* **13**, **14**, *and* **16**, *respectively*. Multiplicities were determined by DEPT experiments.

	1	**2**	**3**	**4**	**5**	**10**	**13**	**14**	**16**
C(1)	40.0	72.4	72.0	74.7	73.2	38.9	48.4	49.9	37.4
C(2)	18.4	25.6	24.9	25.1	25.0	18.2	64.4	69.4	26.9
C(3)	42.5	35.3	35.5	38.8	37.6	42.3	51.5	84.6	79.4
C(4)	33.1	33.0	33.1	35.3	34.1	33.2	34.7	35.1	38.6
C(5)	57.3	48.8	49.0	52.1	51.0	56.6	56.2	56.0	56.3
C(6)	20.7	20.4	20.3	71.1	69.5	20.6	20.2	21.4	21.2
C(7)	39.8	39.5	39.9	48.5	48.2	39.6	38.5	39.3	44.7
C(8)	79.9	80.3	82.3	80.9	81.3	86.3	86.0	84.5	73.6
C(9)	60.2	52.5	59.0	54.0	59.1	59.2	58.9	59.1	58.5
C(10)	36.1	40.1	40.3	41.5	40.6	36.2	37.4	38.5	39.5
C(11)	22.6	22.3	70.1	27.1	71.0	28.7	28.7	32.7	28.6
C(12)	65.0	64.9	73.1	65.8	73.0	176.1	176.0	179.4	179.7
C(13)	21.2	21.3	21.9	23.9[a])	23.6	21.6	21.8	21.8	23.6
C(14)	33.6	33.3	33.3	25.1	32.8	33.2	33.2	33.3	27.7
C(15)	21.2	20.9	20.7	23.6[a]	27.7	21.0	21.7	21.0	21.1
C(16)	15.1	15.9	16.7	18.4	17.7	15.1	16.2	15.7	16.0

[a]) Assignments are interchangeable.

(*Table 3*) showed the appearance of two downfield CH signals at δ 74.7 and 71.1, as compared to **1**. The H–C(6) (δ 4.62) of **4** showed COSY-45° interactions with CH_2(7) (δ 2.05, 1.64) and H–C(5) (δ 1.58), and heteronuclear interactions with C(8) (δ 80.9) and C(10) (δ 41.5), which further supported the position of the new OH group at C(6). The configuration at C(1) and C(6) was further investigated by NOE experiments. An NOE between Me_β(16)/H–C(6) (24.5%) and H–C(1) (2.6%) was observed.

The metabolite **5** was isolated as a colorless crystalline compound. The HR-EI-MS showed the M^+ at m/z 284.1913 consistent with the molecular formula $C_{16}H_{28}O_4$ (calc. 284.1987), corresponding to a trihydroxy derivative. The IR spectrum displayed absorption at 3411 cm^{-1}, further indicating the presence of OH groups. The ^{1}H-NMR spectrum of **5** (*Table 2*) exhibited three additional downfield CH signals at δ 3.62 (*t*, J(1eq,2eq) = J(1eq,2ax) = 2.2 Hz), 4.63 (*dd*, J(6eq,5ax) = 7.4 Hz, J(6eq,7eq) = 2.3 Hz), and 4.59 (*ddd*, J(11,9ax) = 9.9 Hz, J(11,12a) = 7.1 Hz, J(11,12b) = 4 Hz) that could be assigned to H_β–C(1), H_β–C(6), and H_β–C(11), respectively. The ^{13}C-NMR spectrum of **5** (*Table 3*) showed three downfield CH signals at δ 73.2, 69.5, and 71.0 corresponding to the OH-bearing C(1), C(6), and C(11), respectively. The positions and configurations of the newly introduced OH groups were deduced by 2D-NMR techniques. The COSY-45° plot showed couplings between H–C(1) (δ 3.62)/CH_2(2) (δ 2.14, 1.59), H–C(6) (δ 4.63)/CH_2(7) (δ 2.01, 1.77), and H–C(5) (δ 1.47), H–(11) (δ 4.59)/CH_2(12) (δ 4.20, 3.61) and H–C(9) (δ 2.21), while the HMBC plot showed the heteronuclear interactions between H–C(6)/C(8) (δ 81.3) and C(10) (δ 40.6), H–C(1)/C(3) (δ 37.6), and C(5) (δ 51.0), and H–C(11)/C(12) (δ 73.0). The splitting patterns of H–C(1) and H–C(6) indicated their equatorial (β) and axial (β) orientations, respectively. The configuration at the newly created chiral centers was further deduced by the NOESY technique (*Fig. 1*), further supporting the structure as ambrox-1α,6α,11α-triol (**5**).

Fermentation of (–)-*Ambrox*® (**1**) with *Rhizopus stolonifer* (ATCC 10404) furnished the known metabolites **6**–**9** (*Scheme 2*), that with *Curvularia lunata* (NRRL 2178) **6** and **7**, and that with *Cunninghamella elegans* (NRRl 1392) **6**, **7**, and the known **10** (see *Exper. Part*).

Incubation of (+)-sclareolide (**10**) with *Cunninghamella elegans* (NRRL 1392) resulted in the formation of the new metabolites **13**, **14**, and **16** and the known metabolites **11**, **12**, and **15** (*Scheme 3*). The structures of these metabolites were elucidated on the basis of spectral data and comparative spectroscopic studies with

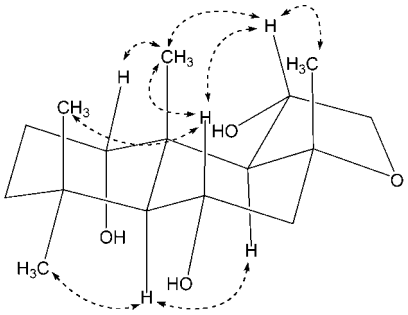
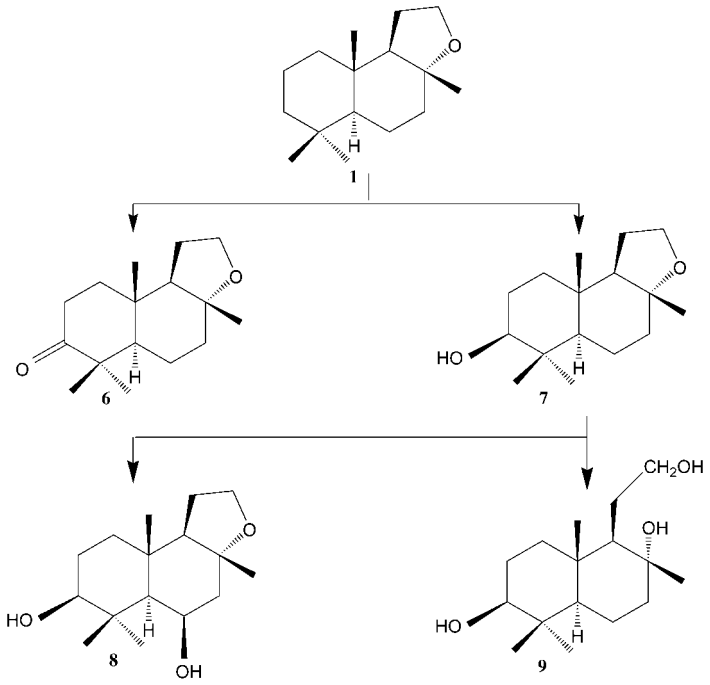


Fig. 1. *Selected NOESY correlations in compound* **5**

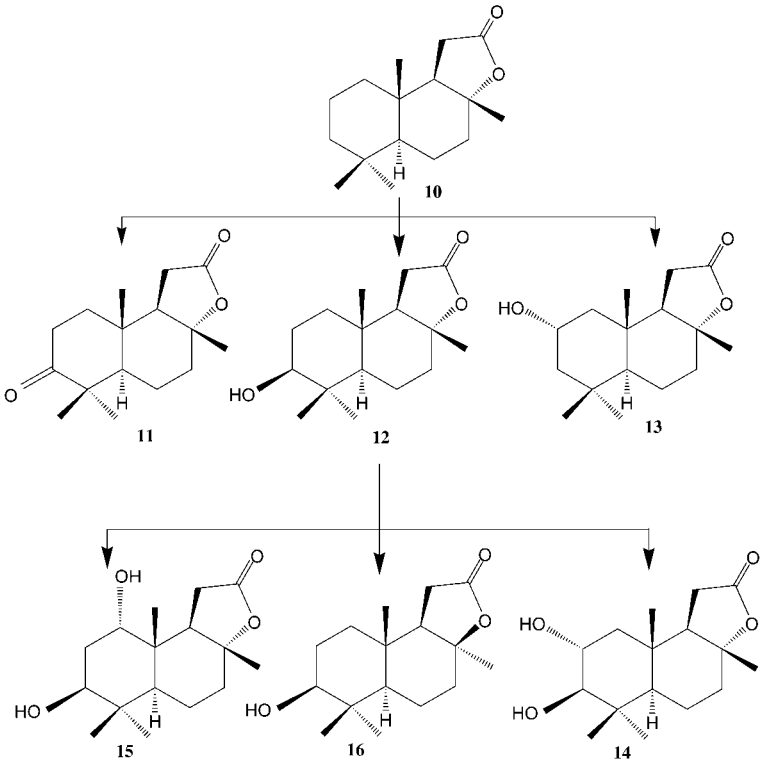
Scheme 2. *Metabolism of (−)-Ambrox* (**1**) *by* Rhizopus stolonifer



substrate **10**. The metabolite **14** could be formed by monohydroxylation at C(2) of **12** or at C(3) of **13** (see *Scheme 3*).

The HR-EI-MS of metabolite **13** showed the M^+ at m/z 266.1739, corresponding to the molecular formula $C_{16}H_{26}O_3$ (calc. 266.1884). The IR spectrum exhibited absorption at 3421 cm^{-1}, indicating the presence of an OH group. The ^{1}H-NMR spectrum of **13** (*Table 2*) showed the characteristic signals of a sclareolide derivative with an additional secondary OH group at a CH resonating at δ 3.90 (m, $w_{1/2} = 17.1$ Hz). The splitting pattern of this latter signal indicated that an axially oriented OH group could be present at H−C(2). The position of this OH group at C(2) was further ascertained by downfield shifts of the C(1) (δ 48.4) and C(3) (δ 51.5) signals in the ^{13}C-NMR spectrum of **13** (*Table 3*) when compared with **10**. H−C(2) of **13** showed homonuclear interactions

Scheme 3. *Metabolism of (+)-Sclareolide* (**10**) *by* Cunninghamella elegans



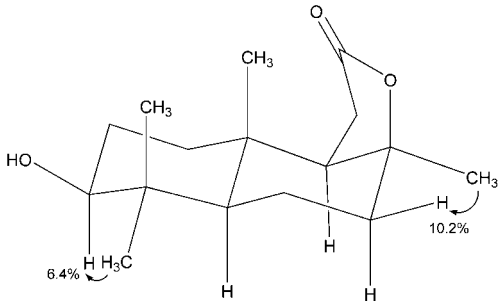


Fig. 2. *Selected NOE interactions in compound* **16**

with $CH_2(1)$ (δ 1.81, 1.42) and $CH_2(3)$ (δ 1.88, 1.15) in the COSY-45° plot. The configuration of OH–C(2) was further investigated by a NOESY experiment, which showed cross-peaks between H–C(2) (δ 3.90)/Me_β(15) (δ 0.93) and Me_β(16) (δ 0.87).

Compound **14** ($C_{16}H_{26}O_4$) has two additional O-atoms as compared to **10**. The IR spectrum indicated the presence of OH groups at 3415 cm^{-1}. The ^{1}H-NMR data of **14** (*Table 2*) exhibited two additional downfield signals at δ 3.79 (m, $w_{1/2} = 18.6$ Hz) and 3.02 (d, $J = 9.1$ Hz). The multiplicity of these resonances indicated that OH groups could be present at C(2) (α position), C(3) (β position), or C(1) (β position). The ^{13}C-NMR (*Table 3*) also showed the appearance of two new CH signals at δ 84.6 (C(3)) and 69.4 (C(2)). The new CH

protons at δ 3.79 (H–C(2)) and 3.02 (H–C(3)) exhibited mutual coupling in the COSY-45° plot. Furthermore, H–C(5) (δ 1.21) also interacted with H–C(3), indicating the presence of OH groups at C(2) and C(3). H–C(2) showed heteronuclear interactions with C(1) (δ 49.9) and C(4) (δ 35.1), while H–C(3) interacted with C(5) (δ 56.0) and C(2) (δ 69.4). The configuration of the newly introduced OH groups was deduced to be α and β, respectively, based on the NOE interaction between H–C(2)/Me_β(15) and Me_β(16) and H–C(3)/Me_α(14).

The IR, mass and ^{1}H-NMR spectra (*Table 2*) of **16** were found to be completely identical with that of the known 3β-hydroxysclareolide (**12**) [24]. However analysis of the ^{13}C-NMR spectrum (*Table 3*) did not support the conclusion that compound **16** had the same structure as **12**. It showed an upfield shifted C(8) signal at δ 73.6. Furthermore, H–C(9) (δ 1.93) and 2 H–C(7) (δ 1.81, 1.52) exhibited heteronuclear interactions with C(8). The NOESY plot of **16** did not show any correlation between Me_β(16) and Me(13), which supported the structure of an 8-episclareolide. The inversion at the stereogenic center C(8) as compared to **12** was confirmed by a NOE differential experiment (see *Fig. 2*), which further supported the structure of 3β-hydroxy-8-episclareolide (**16**).

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Experimental Part

1. *General*. (–)-*Ambrox*® (**1**) and (+)-sclareolide (**10**) were purchased from *Sigma-Aldrich*. TLC: silica gel precoated plates (*Merck*, PF_{254}; 20 × 20, 0.25 mm); detection by vanillin spray reagent. Column chromatography (CC): silica gel (70–230 mesh, *Merck*). M.p.: *Büchi 535* melting-point apparatus. Optical rotations: MeOH solns.; *Jasco DIP–360* digital polarimeter. IR Spectra: $CHCl_3$ solns.; *FTIR-8900* spectrophotometer; in cm^{-1}. ^{1}H- and ^{13}C-NMR Spectra: $CDCl_3$ solns.; *Bruker Avance-500-NMR* at 500 and 125 MHz, resp.; 2D experiments with $CDCl_3$ solns. and the same instrument; chemical shifts δ in ppm rel. to $SiMe_4$ as an internal standard, coupling constants *J* in Hz. EI-MS and HR-EI-MS: *Jeol JMS-600 H* mass spectrometer; in *m*/*z* (rel. %).

2. *Organisms and Culture Media*. The fungal cultures were purchased from different sources such as *International Mycological Institute* (IMI), *American Type Culture Collection* (ATCC), *Northern Regional Research Laboratories* (NRRL), and *Institute of Fermentation*, Osaka, Japan (IFO). Stock cultures were maintained at 4^0 on agar slants (*Sabouraud* dextrose agar). The medium for *Fusarium lini* (NRRL 68751*)* was prepared by adding the following chemicals into dist. H_2O (3.0 l): glucose (30.0 g), glycerol (30.0 g), peptone (15.0 g), yeast extract (15.0 g), KH_2PO_4 (15.0 g), and NaCl (15.0 g), while the medium for *Rhizopus stolonifer* (ATCC 10404) (3.0 l, dist. H_2O) included glucose (60.0 g), glycerol (30.0 g), peptone (30.0 g), yeast extract (8.0 g), and KH_2PO_4 (15.0 g); the pH of the soln. was adjusted to 5.6 by adding few drops of 0.04N NaOH. The medium for *Cunninghamella elegans* (NRRL 1392) and *Curvularia lunata* (NRRL 2178) were prepared by mixing the following ingredients into distilled H_2O (3.0 l): glucose (30.0 g), peptone (15.0 g), yeast extract (15.0 g), KH_2PO_4 (15.0 g), and NaCl (15.0 g).

3. *General Fermentation and Extraction Conditions*. The fermentation medium was distributed among 30 flasks of 250-ml capacity (100 ml in each) and autoclaved at 121^0 for 20 min. The fermentation was carried out according to a standard, two-stage protocol [25]. The substrate was dissolved in acetone, and the resulting clear soln. was evenly distributed among the 30 flasks (20 mg/0.5 ml in each flask) containing 24-h-old stage-II cultures, and fermentation was carried out for further days on a rotatory shaker (200 rpm) at 29^0. During the fermentation period, aliquots from culture were taken out daily and analyzed by TLC to determine the degree of transformation of substrate. In all experiments, one control flask without fungus (for checking substrate stability) and another flask without exogenous substrate (for checking endogenous metabolites) were used. The culture media and mycelium were separated by filtration. The mycelium was washed with CH_2Cl_2 (1.5 l), and the filtrate was extracted with CH_2Cl_2 (3 × 2 l). The combined org. extract was dried (Na_2SO_4) and evaporated and the residue analyzed by TLC. Control flasks were also harvested and compared with the test by TLC to confirm the presence of biotransformed products.

4. *Fermentation of (–)*-Ambrox® (**1**) *with* Fusarium lini *(NRRL 68751)*. (–)-*Ambrox*® (**1**; 600 mg), dissolved in acetone (15 ml), was evenly distributed in 30 flasks containing 24-h-old stage-II culture. Fermentation was carried out for 8 days. After filtration, extraction, and evaporation, the obtained brown gum (1.63 gm) was subjected to repeated CC (petroleum ether/AcOEt gradient): **2** (16.4 mg, 2.7%; with petroleum ether/AcOEt 62 : 28), **3** (8.2 mg, 1.3%; with petroleum ether/AcOEt 61 : 39), **4** (19.1 mg, 3.2%; with petroleum ether/AcOEt 56 : 44), and **5** (27.8 mg, 4.6%; with petroleum ether/AcOEt 29 : 71).

Ambrox-2α-ol (=*(3a*R,*5a*S,*9s*,*9a*S,*9b*R*)-Dodecahydro-3a,6,6,9a-tetramethylnaphtho[2,1-*b*]furan-9-ol*; **2**): M.p. 101–102°. $[\alpha]_D^{25} = +1.72$ ($c = 0.1$, MeOH). IR ($CHCl_3$): 3436, 2938, 2870. ^{1}H-NMR ($CDCl_3$, 500 MHz): *Table 2*. ^{13}C-NMR ($CDCl_3$, 125 MHz): *Table 3*. EI-MS: 252 (17, M^+), 237 (100, $[M - Me]^+$), 219 (54, $[M - Me - H_2O]^+$), 163 (4), 152 (14), 111 (19), 97 (37), 55 (69). HR-EI-MS: 252.2056 (M^+, $C_{16}H_{28}O_2^+$; calc. 252.2089).

Ambrox-1α,11α-diol (=*(1S,3a*R,*5a*S,*9S,9a*S,*9b*S*)-Dodecahydro-3a,6,6,9a-tetramethylnaphtho[2,1-*b*]furan-1,9-diol*; **3**): M.p. 174–176°. $[\alpha]_D^{25} = -1.53$ ($c = 0.1$, MeOH). IR ($CHCl_3$): 3354, 2941, 2868, 1124. ^{1}H-NMR ($CDCl_3$, 500 MHz): *Table 2*. ^{13}C-NMR ($CDCl_3$, 125 MHz): *Table 3*. EI-MS: 268 (7, M^+), 253 (60, $[M - Me]^+$), 235 (87, $[M - Me - H_2O]^+$), 163 (4.6), 205 (24), 164 (12), 136 (79.2), 121 (52), 95 (55), 81 (100), 55 (95). HR-EI-MS: 268.2064 (M^+, $C_{16}H_{28}O_3^+$; calc. 268.2038).

Ambrox-1α,6α-diol (=*(3a*R,*5S,5a*S,*9S,9a*S,*9b*R*)-Dodecahydro-3a,6,6,9a-tetramethylnaphtho[2,1-*b*]furan-5,9-diol*; **4**): M.p. 119–120°. $[\alpha]_D^{25} = -18.7$ ($c = 0.1$, MeOH). IR ($CHCl_3$): 3329, 2931, 2877. ^{1}H-NMR ($CDCl_3$, 500 MHz): *Table 2*. ^{13}C-NMR ($CDCl_3$, 125 MHz): *Table 3*. EI-MS: 268 (7, M^+), 253 (100, $[M - Me]^+$), 235 (66, $[M - Me - H_2O]^+$), 163 (4), 217 (9), 191 (24), 167 (7), 139 (13), 111 (21), 81 (23), 55 (62). HR-EI-MS: 268.2041 (M^+, $C_{16}H_{28}O_3^+$; calc. 268.2038).

Ambrox-1α,6α,11α-triol (=*(1S,3a*R,*5S,5a*S,*9s,9a*S,*9b*S*)-Dodecahydro-3a,6,6,9a-tetramethylnaphtho[2,1-*b*]-furan-1,5,9-triol*; **5**): M.p. 191–192°. $[\alpha]_D^{25} = -26$ ($c = 0.1$, MeOH). IR ($CHCl_3$): 3411, 2930, 2874, 1217. ^{1}H-NMR ($CDCl_3$, 500 MHz): *Table 2*. ^{13}C-NMR ($CDCl_3$, 125 MHz): *Table 3*. EI-MS: 284 (2, M^+), 269 (32, $[M - Me]^+$), 251 (93, $[M - Me - H_2O]^+$), 218 (11), 215 (13), 175 (9), 139 (23), 109 (99), 81 (69), 55 (100). HR-EI-MS: 284.1913 (M^+, $C_{16}H_{28}O_4^+$; calc. 284.1987).

5. *Fermentation of (–)-*Ambrox® (**1**) *with* Rhizopus stolonifer *(ATCC 10404)*. Incubation of (–)-**1** (600 mg) with *Rhizopus stolonifer* (ATCC 10404) for 12 days yielded a brown oily matter (1.8 gm), which was subjected to CC (silica gel, AcOEt/petroleum ether gradient): **6** (7.9 mg, 1.3%; with 18% AcOEt), **7** (6.3 mg, 1%; with 31% AcOEt), **8** (11.4 mg, 1.9%; with 44% AcOEt), and **9** (28.1 mg, 4.7%; with 56% AcOEt).

6. *Fermentation of (–)-*Ambrox® (**1**) *with* Cunninghamella elegans *(NRRL 1392) and* Curvularia lunata *(NRRL 2178)*. The fermentation of (–)-**1** (600 mg) with *Curvularia lunata* for 10 days afforded **6** (14 mg, 2.3%) and **7** (41 mg, 6.8%), while fermentation with *Cunninghamella elegans* for 12 days afforded **6** (3.2 mg, 0.53%), **7** (17.2 mg, 2.8%), and (+)-**10** (11.4 mg, 1.9%).

7. *Fermentation of (+)-Sclareolide* (**10**) *with* Cunninghamella elegans *(NRRL 1392)*. (+)-Sclareolide (**10**; 600 mg), dissolved in acetone (15 ml), was evenly distributed in 30 flasks containing stage-II cultures. Fermentation was stopped after 12 days, including control flasks. The org. metabolites were extracted from the medium. The extract was evaporated and the obtained brown gum (2.3 gm) subjected to CC (petroleum ether/AcOEt gradient): **11** (81 mg, 13.5%; with petroleum ether/AcOEt 76:24), an impure fraction containing **12**/**13** (with petroleum ether/AcOEt 63:37), which was purified by TLC (AcOEt/petroleum ether 3:7) to give **12** (21.7 mg, 2.1%) and **13** (31.7 mg, 5.3%), **14** (142 mg, 23.6%; with petroleum ether/AcOEt 53:47), **15** (7.9 mg, 1.3%; with petroleum ether/AcOEt 39:61), and **16** (16.5 mg, 2.7%; petroleum ether/AcOEt 9:91).

2α-Hydroxysclareolide (=*(3a*R,*5a*S,*8*S,*9a*S,*9b*R*)-Dodecahydro-8-hydroxy-3a,6,6,9a-tetramethylnaphtho[2,1-*b*]furan-2(1*H*)-one*; **13**): M.p. 154–155°. $[\alpha]_D^{25} = -100$ ($c = 0.006$, MeOH). IR ($CHCl_3$): 3421, 2927, 2869, 1227. ^{1}H-NMR ($CDCl_3$, 500 MHz): *Table 2*. ^{13}C-NMR ($CDCl_3$, 125 MHz): *Table 3*. EI-MS: 251 (8, $[M - Me]^+$), 233 (19, $[M - Me - H_2O]^+$), 221 (24), 204 (49), 165 (41), 139 (31), 107 (43), 81 (34), 55 (100). HR-EI-MS: 266.1739, (M^+, $C_{16}H_{26}O_3^+$; calc. 266.1884).

2α,3β-Dihydroxysclareolide (=*(3a*R,*5a*R,*7*R,*8*R,*9a*S,*9b*R*)-Decahydro-7,8-dihydroxy-3a,6,6,9a-tetramethylnaphtho[2,1-*b*]furan-2(1*H*)-one*; **14**): M.p. 189–190°. $[\alpha]_D^{25} = -136$ ($c = 0.08$, MeOH). IR ($CHCl_3$): 3415, 2933, 2873, 1757. ^{1}H-NMR ($CDCl_3$, 500 MHz): *Table 2*. ^{13}C-NMR ($CDCl_3$, 125 MHz): *Table 3*. EI-MS: 282 (2, M^+), 266 (3, $[M - H_2O]^+$), 238 (4), 195 (15), 135 (19), 123.1 (100), 81 (31), 71 (77), 55 (58). HR-EI-MS: 282.1884, (M^+, $C_{16}H_{26}O_4^+$; calc. 282.1831).

3β-Hydroxy-8-episclareolide (=*(3a*S,*5a*R,*7*S,*9a*S,*9b*R*)-Decahydro-7-hydroxy-3a,6,6,9a-tetramethylnaphtho[2,1-*b*]furan-2(1*H*)-one*; **16**): M.p. 181–182°. $[\alpha]_D^{25} = -136$ ($c = 0.08$, MeOH). IR ($CHCl_3$): 3330, 2925, 2856, 1712. ^{1}H-NMR ($CDCl_3$, 500 MHz): *Table 2*. ^{13}C-NMR ($CDCl_3$, 125 MHz): *Table 3*. EI-MS: 266 (3, M^+), 251 (10, $[M - Me]^+$), 233 (12, $[M - Me - H_2O]^+$), 218 (11), 204 (12), 167 (13), 139 (38), 109 (43), 81 (38), 55 (100). HR-EI-MS: 266.1891 (M^+, $C_{16}H_{26}O_3^+$; calc. 266.1884).

Phytotoxicity Assay. Phytotoxicity of the sesquiterpene lactones **10**–**16** was determined against *Lemna minor* L. according to the modified protocol of *McLaughlin* and co-workers [26]. The test compounds were incorporated with E-medium at different concentrations, *i.e.*, 1, 10, and 100 μg/ml in MeOH. Conical flasks were inoculated with compounds at the desired concentration prepared from the stock soln. and allowed to evaporate overnight. Each flask was inoculated with 2 ml of E-medium and ten *Lemna minor* L., each containing a rosette of three fronds. Other flasks were supplemented with MeOH serving as negative control and reference inhibitor,

i.e., paraquat serving as positive control. Treatments were replicated three times, and the flasks were incubated at 37° for 7 days. Growth of *Lemna minor* L. in a compound-containing flask was determined by counting the number of fronds per dose, and growth inhibition was calculated with reference to negative control.

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