Biotransformation of Citrus Aromatics Nootkatone and Valencene by Microorganisms

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Biotransformations of the sesquiterpene ketone nootkatone (1) from the crude drug Alpiniae Fructus and grapefruit oil, and the sesquiterpene hydrocarbon valencene (2) from Valencia orange oil were carried out with microorganisms such as *Aspergillus niger*, *Botryosphaeria dothidea*, and *Fusarium culmorum* to afford structurally interesting metabolites. Their stereostructures were established by a combination of high-resolution NMR spectral and X-ray crystallographic analysis and chemical reaction. Metabolic pathways of compounds 1 and 2 by *A. niger* are proposed.

Key words nootkatone; valencene; biotransformation; *Aspergillus niger*; *Fusarium culmorum*; *Botryosphaeria dothidea*

We are continuing to study the biotransformation of secondary metabolites such as terpenoids and aromatic compounds from crude drugs and liverworts by microorganisms[1—7] and mammals[8,9] to obtain some functional substances such as pheromones and aromatics. We reported the biotransformation of sesquiterpenoids such as dehydrocostuslactone, costunolide, α-, β-, and γ-cyclocostunolides, α-santonin, and atractylon from crude drugs, and an amber constituent, (−)-ambrox by microorganisms such as *Aspergillus niger*.[10] It has been clarified that a grapefruit essential oil containing nootkatone (**1**) decreases the somatic fat ratio[11] and its demand by cosmetic and fiber manufacturers has increased. Recently, we have found that the commercially available and cheap aromatic valencene (**2**) from Valencia orange oil is very efficiently converted into the expensive grapefruit aromatic, nootkatone (**1**) by biotransformations using *Chlorella* sp.[12] and *Mucor* sp.[13] In continuation of the biotransformation of the chemical constituents isolated from crude drugs into biologically active compounds, the biotransformations of nootkatone (**1**) and valencene (**2**) were examined using *Aspergillus niger*, *Fusarium culmorum*, and *Botryosphaeria dothidea*. This paper deals with the structural elucidation of metabolites of **1** and **2** converted by the three fungi.

Biotransformation of Nootkatone (1) by *Aspergillus niger* *A. niger* was inoculated and cultivated in a rotary (100 rpm) in Czapek-pepton medium (pH 7.0) at 30 °C for 7 d. (+)-Nootkatone (**1**) (80 mg/200 ml) was added to the medium and further cultivated for 7 d. The crude metabolites obtained from the culture broth by EtOAc extraction were chromatographed on silica gel (*n*-hexane–EtOAc gradient) to give two metabolites, 12-hydroxy-11,12-dihydronootkatone (**3**) (10.6%, isolated yield) and C-11 stereomixtures of 11,12-dihydroxy-11,12-dihydronootkatone (nootkatone-11,12-diol) (**4**, **5**) (51.5% isolated yield), respectively.

Compound (**3**) {$[\alpha]_D$ +133.3° ($CHCl_3$)} was obtained as a single isomer at C-11, for which the molecular formula $C_{15}H_{24}O_2$ was established in high-resolution electron-impact mass spectroscopy (HR-EI-MS) ($[M]^+$ *m/z* 236.1778). The FT-IR and UV spectra of **3** indicated the presence of a hydroxyl (3398 cm^{-1}) and a conjugated ketone (1657 cm^{-1}; λ_{max} 238 nm) group. The ^{1}H- (Table 1) and ^{13}C-NMR (Table 2) spectra of **3** showed the presence of a tertiary methyl [δ_H 1.09 (3H, s)], two secondary methyl [δ_H 0.93 (3H, d, J=7.1 Hz), 0.97 (3H, d, J=6.9 Hz)], a primary alcohol [δ_H 3.55 (1H, dd, J=6.3, 10.4 Hz), 3.63 (1H, dd, J=5.8, 10.4 Hz); δ_C 65.9 (t)], and a ketone [δ_C 199.7 (s)]. Compound **3** showed correlations between (i) H-12/C-7, C-11, and C-13; and (ii) H-13/C-7, C-11 and C-12 in the HMBC spectrum. On the basis of the above spectral data and careful analysis of its 2D NMR spectrum, the structure of metabolite **3** was established to be 12-hydroxy-11,12-dihydronootkatone. The relative configuration at C-11 remained to be clarified.

The HR-EI-MS of nootkatone-11,12-diol (a mixture of **4** and **5**) showed a peak for $[M^+]$ at *m/z* 252.1730, indicating the molecular formula of $C_{15}H_{24}O_3$. The FT-IR spectrum of the mixture indicated the presence of a hydroxyl (3358 cm^{-1}) and a conjugated ketone (1652 cm^{-1}) group. The ^{1}H- and ^{13}C-NMR spectra showed that **4** and **5** were a mixture of stereoisomers at C-11, the isolation of which was very difficult by any separation method. The structure of 11,12-diol was confirmed by the formation of a methyl ketone (**6**) on oxidation with $NaIO_4$. Treatment of the mixture (**4**, **5**) with 1,1′-thiocarbonyldiimidazole gave the thiocarbonates **7** and **8**, which were easily separated by HPLC in the ratio of 3 : 2. Teresa *et al.*[14] and Haines and Jenkins[15] reported that the configuration at C-11 of 11,12-diol (**9**) must be "*R*," because the thiocarbonate (**10**) used to prepare **9** showed a negative Cotton effect [308 nm ($\Delta\varepsilon$ −0.21)]. However, both thiocarbonates **7** and **8** showed negative Cotton effects [**7**: 319 nm ($\Delta\varepsilon$ −0.33); **8**: 310 nm ($\Delta\varepsilon$ −0.70)] in CD spectra, and thus the absolute configuration at C-11 of **4** and **5** could not be characterized by their CD spectra. Finally, the absolute structure of **4** was established based on X-ray crystallographic analysis of the thiocarbonate (**7**) as the *S* configuration at C-11, as shown in Fig. 1. Thus compounds **4** and **5** were determined to be (11*S*)- and (11*R*)-nootkatone-11,12-diol, respectively. 11,12-Epoxide (**11**) obtained by epoxydation of nootkatone (**1**) with *m*CPBA was biotransformed by *A. niger* for 1 d to 11,12-dihydroxy-11,12-dihydronootkatone (**4**, **5**) in good yield (81.4%). 1-Aminobenzotriazole, an inhibitor of cytochrome P450, inhibited the oxidation process of **1** into compounds **3**—**5**. From the above results, possible metabolic pathways of nootkatone (**1**) are depicted in Fig. 2.

Biotransformation of Nootkatone (1) by *F. culmorum* and *B. dothidea* *F. culmorum* was inoculated and cultivated

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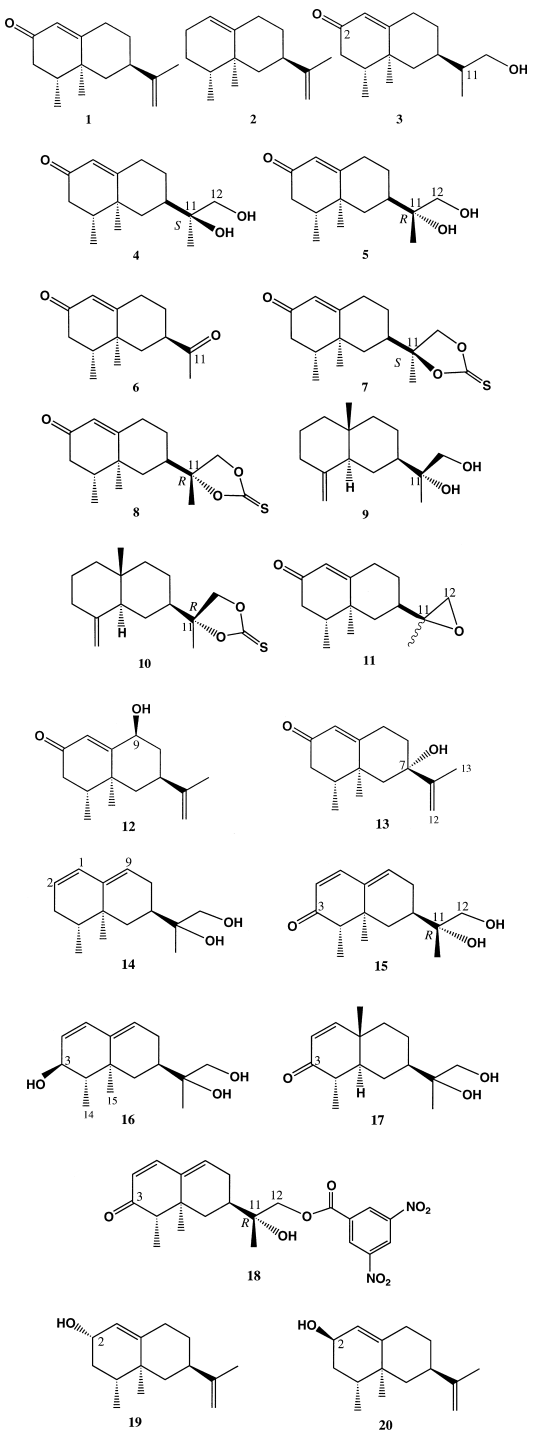

stationary in Czapek-pepton medium (pH 7.0) at 30 °C for 7 d. (+)-Nootkatone (**1**) was added to the medium and further cultivated for 7 d to afford (11*R*)-11,12-dihydroxy-11,12-dihydronootkatone (**5**) (47.2%) and 9β-hydroxynootkatone (**12**) (14.9%).

Compound **5** was stereospecifically obtained at C-11 by biotransformation of **1**. The purity of compound **5** was determined to be *ca*. 95% by HPLC analysis of the thiocarbonate (**8**).

Compound **12** {$[\alpha]_D$ +131.3° ($CHCl_3$)} was obtained as a colorless oil, for which the whose molecular formula $C_{15}H_{22}O_2$ was established using HR-EI-MS ([M]$^+$ *m/z* 234.1612). The FT-IR spectrum of **12** indicated the presence of a hydroxyl (3359 cm^{-1}) and a conjugated ketone (1652 cm^{-1}) group. The ^{1}H- (Table 1) and ^{13}C-NMR (Table 2) spectra of **12** showed the presence of a secondary alcohol [δ_H 4.47 (1H, ddd, J=1.9, 5.2, 12.1 Hz); δ_C 69.0 (d)] and a ketone [δ_C 199.8 (s)]. The carbon signals at the C-8, C-9, and C-10 positions of **12** appeared at a lower field (+9.2, +36.0, and +1.0 ppm) in comparison with those of nootkatone (**1**), as shown in Table 2. Compound **12** showed correlations between (i) H-9/C-1, C-5, and C-10 in the HMBC spectrum and NOEs between H-9/H-1, H-7, H-8α and H-14 in the NOESY, respectively. Thus the structure of **12** is 9β-hydroxynootkatone.

The biotransformation of nootkatone (**1**) was examined using the plant pathogenic fungus *B. dothidea* separated from the fungus which grows on the peach. (+)-Nootkatone (**1**) was cultivated with *B. dothidea* (peach PP8402) for 14 d to afford 11,12-dihydroxy-11,12-dihydronootkatone (**4**, **5**) (54.2%) and 7α-hydroxynootkatone (**13**) (20.9%). The ratio of compounds **4** and **5** was determined to be 3 : 2 by HPLC analysis of their thiocarbonates (**7**, **8**).

Compound **13** {$[\alpha]_D$ +118.8° ($CHCl_3$)} was obtained as a colorless oil, for which the molecular formula $C_{15}H_{22}O_2$ was established by HR-EI-MS ([M]$^+$ *m/z* 234.1610). The FT-IR and ^{1}H- and ^{13}C-NMR (Table 2) spectra of **13** resembled those of nootkatone except for the presence of a tertiary alcohol [δ_C 74.5 (s)]. The carbon signals at the C-6, C-7, and C-8 positions of **13** appeared at a lower field (+3.3, +34.2, and +4.3 ppm) in comparison with those of nootkatone (**1**), as shown in Table 2. The relative structure of compound **13** at C-7 was determined by NOEs between (i) H-12/H-8β; and (ii) H-13/H-6β in the NOESY spectrum.

The conversions of nootkatone (**1**) to 11*S*- and 11*R*-nootkatone-11,12-diol (**4**, **5**) by various microorganisms and rabbits are summarized in Table 3. The biotransformations of **1** by *A. niger* and *B. dothidea* resembled that after oral administration of **1** to rabbits[16] since the ratio of major metabolites (**4**, **5**) in rabbits was similar to that in the former two fungi. Recently, we have reported that the biotransformation of hinesol[5] and α-santonin[10] by *A. niger* is similar to that with their oral administration to mammals. It is noteworthy that the biotransformation of **1** by *F. culmorum* stereospecifically afforded 11*R*-nootkatone-11,12-diol (**5**).

Biotransformation of Valencene (2) by *A. niger* Valencene (**2**) from Valencia orange oil was cultivated with *A. niger* for 5 d to afford seven metabolites **3** (1.0%), **4** and **5** (13.5%), **14** (1.1%), **15** (1.5%), **16** (2.0%), and **17** (0.7%), respectively. The ratio of compounds **4** (11*S*) and **5** (11*R*) was determined to be 1 : 3 by HPLC analysis of their thiocarbonates (**7**, **8**).

Compound **14** {$[\alpha]_D$ −71.1° ($CHCl_3$)} was obtained as a single isomer at C-11, for which the molecular formula $C_{15}H_{24}O_2$ was established by HR-EI-MS ([M]$^+$ *m/z* 236.1749). The FT-IR and UV spectra of **14** indicated the presence of a hydroxyl (3350 cm^{-1}) and a conjugated diene (λ_{max} 235 nm) group. Its ^{1}H- (Table 4) and ^{13}C-NMR (Table 5) spectra showed the presence of a conjugated diene [δ_H 5.60 (m), 5.94 (1H, br d, J=9.6 Hz), 5.38 (m); δ_C 121.9 (d), 126.0 (d), 128.4 (d), 142.4 (s)] and 11,12-diol [δ_H 3.45, 3.60 (each 1H, d, J=11.0 Hz); δ_C 68.6 (t), 74.6 (s)]. The stereostructure of **14** was determined by the careful analysis of the 2D NMR spectra (HMBC, NOESY) and the above-men-

Table 1. 600 MHz ^{1}H-NMR Spectral Data of Compounds **1**, **3**, **5**, **12** and **13** in $CDCl_3$[a)]

Position	**1**	**3**	**5**	**12**	**13**
1	5.77 br s	5.75 br s	5.76 br s	6.25 br s	5.81 br s
3α	2.29 dd (13.7, 17.0)	2.29 dd (14.0, 17.3)	2.30 dd (14.0, 17.3)	2.31 dd (14.0, 16.8)	2.32 dd (14.0, 17.0)
3β	2.23 ddd (0.8, 4.1, 17.0)	2.22 ddd (0.8, 4.4, 17.3)	2.24 ddd (0.8, 4.4, 17.3)	2.25 ddd (0.8, 4.4, 16.8)	2.22 ddd (0.8, 4.4, 17.0)
4	2.20 m	1.99 m	2.02 m	2.06 m	1.33 m
6α	1.98 ddd (3.0, 3.0, 12.9)	1.91 ddd (2.7, 2.7, 13.2)	2.13 ddd (2.7, 2.7, 12.9)	1.97 ddd (2.5, 2.5, 13.2)	1.95 dd (3.0, 14.3)
6β	1.14 dd (12.9, 12.9)	0.96 dd (13.2, 13.2)	1.06 dd (12.9, 12.9)	1.14 dd (12.9, 12.9)	1.47 d (14.3)
7	2.33 m	1.83 m	1.97 m	2.41 m	
8α	1.92 m	1.87 m	1.87 m	2.23 m	1.85 m
8β	1.35 m	1.22 m	1.18 m	1.42 m	1.79 m
9α	2.52 m	2.48 m	2.50 m	4.47 ddd	2.24 m
9β	2.38 m	2.35 m	2.37 m	(1.9, 5.2, 12.1)	2.24 m
12	4.72 br s	3.55 dd (6.3, 10.4)	3.46 d (11.0)	4.76 br s	4.86 br s
	4.75 br s	3.63 dd (5.8, 10.4)	3.62 d (11.0)	4.78 br s	5.06 br s
13	1.74 br s	0.93 d (7.1)	1.09 s	1.75 s	1.81 br s
14	0.97 d (6.9)	0.97 d (6.9)	0.99 d (6.9)	0.98 d (6.9)	0.95 d (6.9)
15	1.12 s	1.09 s	1.10 s	1.13 s	1.31 s

a) Chemical shifts from TMS (multiplicity, *J* in Hz) in $CDCl_3$.

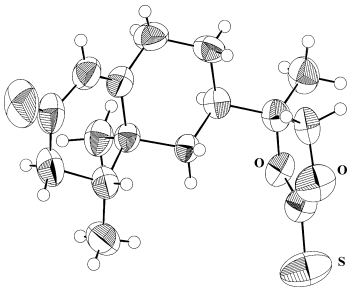

Fig. 1. ORTEP Drawing of Compound **7**

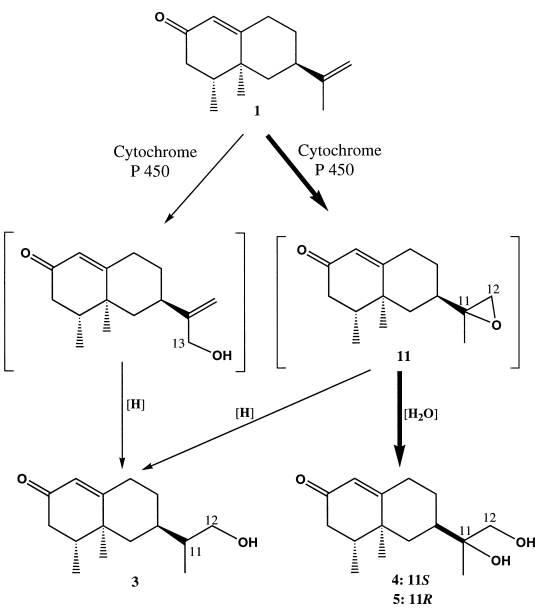

Fig. 2. Possible Metabolic Pathway of (+)-Nootkatone (**1**) by *Aspergillus niger*

Table 2. 150 MHz ^{13}C-NMR Spectral Data of Compounds **1**, **3**, **5**, **12** and **13** in $CDCl_3$[a)]

C	**1**	**3**	**5**	**12**	**13**
1	124.6	124.5	124.5	120.7	124.5
2	199.6	199.7	199.8	199.8	199.6
3	42.0	42.1	42.0	41.8	41.8
4	40.4	40.5	40.6	40.8	41.5
5	39.3	39.2	39.2	39.7	39.1
6	43.9	41.3	38.6	43.8	47.2
7	40.3	34.3	39.2	38.3	74.5
8	31.6	30.7	27.9	40.8	35.9
9	33.0	33.1	32.9	69.0	28.9
10	170.4	171.1	170.8	171.4	171.0
11	149.0	40.2	74.2	147.8	151.7
12	109.2	65.9	68.4	109.2	109.8
13	20.8	13.6	19.9	20.7	19.1
14	14.9	15.0	15.0	15.1	14.9
15	16.8	16.9	16.8	17.6	18.7

a) Chemical shifts from TMS in $CDCl_3$.

Table 3. Conversion of Nootkatone to 11*S* and 11*R*-11,12-Diol by Microorganisms and Rabbit

	Metabolites (ratio[a)] of **4** and **5**)	
	11*S*-11,12-diol (**4**)	11*R*-11,12-diol (**5**)
Aspergillus niger	3	2
Fusarium culmorum	1	20
Botryosphaeria dothidea	3	2
Rabbit	3	2

a) Product: Ratio was determined by HPLC of thiocarbonates of 11,12-diol.

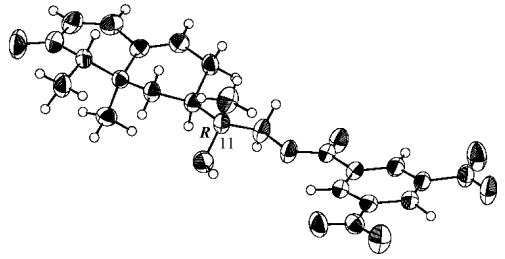

Fig. 3. ORTEP Drawing of **18**

tioned spectral data, except for the configuration at C-11.

Compound **15** {$[\alpha]_D$ −50.1° ($CHCl_3$)} was obtained as a single isomer at C-11, for which the molecular formula $C_{15}H_{22}O_3$ was established using HR-EI-MS ($[M]^+$ *m/z* 250.1562). The FT-IR and UV spectra of **15** indicated the presence of a hydroxyl (3417 cm^{-1}) and $\alpha,\beta,\gamma,\delta$-unsaturated ketone (1666 cm^{-1}; λ_{max} 289 nm) group which was confirmed by the ^{1}H- (Table 4) and ^{13}C-NMR (Table 5) spectral data, indicating the presence of an $\alpha,\beta,\gamma,\delta$-unsaturated ketone [$\delta_H$ 5.87 (1H, d, *J*=9.9 Hz), 5.99 (1H, dd, *J*=1.9, 5.2 Hz), 6.87 (1H, d, *J*=9.9 Hz); δ_C 125.1 (d), 132.5 (d), 141.3 (s), 145.2 (d), 201.5 (s)] and 11,12-diol [δ_H 3.48, 3.62 (each 1H, d, *J*=11.0 Hz); δ_C 68.5 (t), 74.6 (s)]. The stereochemistry at C-11 of **15** was established to be *R* configuration by the careful analysis of its 2D NMR spectra and the X-ray crystallographic analysis of 3,5-dinitrobenzoate (**18**) prepared from **15**, as shown in Fig. 3.

Compound **16** {$[\alpha]_D$ +17.4° (MeOH)} was obtained as a single isomer at C-11, for which the molecular formula $C_{15}H_{24}O_3$ was established using HR-EI-MS ($[M]^+$ *m/z* 252.1708). The FT-IR and UV spectra of **16** indicated the presence of a hydroxyl (3368 cm^{-1}) and a conjugated diene (λ_{max} 237 nm). The ^{1}H- (Table 4) and ^{13}C-NMR (Table 5) spectra of **16** showed the presence of a conjugated diene [δ_H 5.50 (1H, dd, *J*=2.7, 5.2 Hz) 5.59 (1H, br d, *J*=9.9 Hz), 6.00 (1H, br d, *J*=9.9 Hz); δ_C 124.2 (d), 128.6 (d), 129.4 (d), 141.3 (s)], a secondary alcohol [δ_H 3.91 (1H, br d, *J*=8.8 Hz); δ_C 71.6 (d)], and 11,12-diol [δ_H 3.42, 3.56 (each 1H, d, *J*=11.0 Hz); δ_C 68.6 (t), 74.6 (s)]. The relative structure of compound **16** at C-3 was determined based on NOEs between H-3/H-14 and H-15 in the NOESY spectrum.

Compound **17** {$[\alpha]_D$ +52.0° ($CHCl_3$)} was obtained as a single isomer at C-11, for which the molecular formula $C_{15}H_{24}O_3$ was established using HR-EI-MS ($[M]^+$ *m/z* 252.1736). The FT-IR and UV spectra of **17** indicated the presence of a hydroxyl (3410 cm^{-1}) and an α,β-unsaturated ketone (1653 cm^{-1}; λ_{max} 242 nm) group. The ^{1}H- (Table 4) and ^{13}C-NMR (Table 5) spectra of **17** showed the presence of an α,β-unsaturated ketone [δ_H 5.90 (1H, br s); δ_C 126.3 (d), 164.9 (s)] and 11,12-diol [δ_H 3.43, 3.55 (each 1H, d, *J*=11.0 Hz); δ_C 68.1 (t), 74.3 (s)]. The relative structure of **17** was established by HMBC and NOESY spectral analysis, as shown in Fig. 4. Compound **17** may be obtained by the rearrangement of the C-5 methyl group into C-10.

Compounds **14**—**17** could be biosynthesized by elimination of a hydroxyl group of 2-hydroxyvalencene (**19**, **20**). The reduction of nootkatone (**1**) with $NaBH_4$ and $CeCl_3$ gave 2α-hydroxyvalencene (**19**) in 87% yield, and the Mitsunobu reaction of **19** with *p*-nitrobenzoic acid, triphenylphosphine, and diethyl azodicarboxylate gave nootkatol (2β-hydroxyva-

Table 4. 600 MHz ^{1}H-NMR Spectral Data of Compounds **14**—**17** in $CDCl_3$[a)]

H	**14**	**15**	**16**	**17**
1	5.94 br d (9.6)	6.87 d (9.9)	6.00 br d (9.9)	2.10 d (16.2) 2.26 d (16.2)
2	5.60 m	5.87 d (9.9)	5.59 br d (9.9)	
3α	1.95 m		3.91 br d (8.8)	5.90 br s
3β	2.06 m			
4	1.53 m	2.42 q (6.9)	1.32 dq (8.8, 6.9)	
5				2.35 m
6α	1.86 br d (12.4)	1.99 m	1.92 m	1.21 m
6β	1.05 dd (12.4, 12.4)	1.33 dd (12.1, 12.1)	1.05 dd (12.4, 12.4)	1.23 m
7	2.03 m	1.99 m	1.96 m	1.68 m
8α	2.14 m	2.32 m	2.15 m	1.57 m
8β	1.83 m	1.99 m	1.81 m	1.33 m
9α	5.38 m	5.99 dd (1.9, 5.2)	5.50 dd (2.7, 5.2)	1.42 m
9β				1.59 m
12	3.45 d (11.0) 3.60 d (11.0)	3.48 d (11.0) 3.62 d (11.0)	3.42 d (11.0) 3.56 d (11.0)	3.43 d (11.0) 3.55 d (11.0)
13	1.12 s	1.14 s	1.09 s	1.11 s
14	0.90 d (7.4)	1.11 d (6.9)	1.07 d (6.9)	1.95 br s
15	0.89 s	1.01 s	0.92 s	0.87 s

a) Chemical shifts from TMS (multiplicity, *J* in Hz) in $CDCl_3$.

Table 5. 150 MHz ^{13}C-NMR Spectral Data of Compounds **14**—**17** in $CDCl_3$[a)]

C	**14**	**15**	**16**	**17**
1	128.4	145.2	129.4	54.0
2	126.0	125.1	128.6	200.3
3	32.2	201.5	71.6	126.3
4	39.0	52.6	48.0	164.9
5	36.0	39.4	37.0	47.7
6	34.9	35.1	34.9	22.8
7	36.5	35.6	35.1	44.7
8	27.2	27.7	27.0	21.9
9	121.9	132.5	124.2	39.7
10	142.4	141.3	141.3	37.5
11	74.6	74.1	74.3	74.3
12	68.6	68.5	68.3	68.1
13	19.4	19.6	19.1	19.2
14	14.8	7.2	10.6	21.8
15	17.1	19.2	17.9	16.5

a) Chemical shifts from TMS (multiplicity, *J* in Hz) in $CDCl_3$.

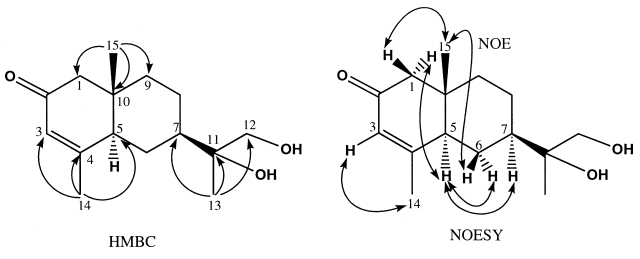

Fig. 4. Important HMBC and NOESY Spectra of Compound **17**

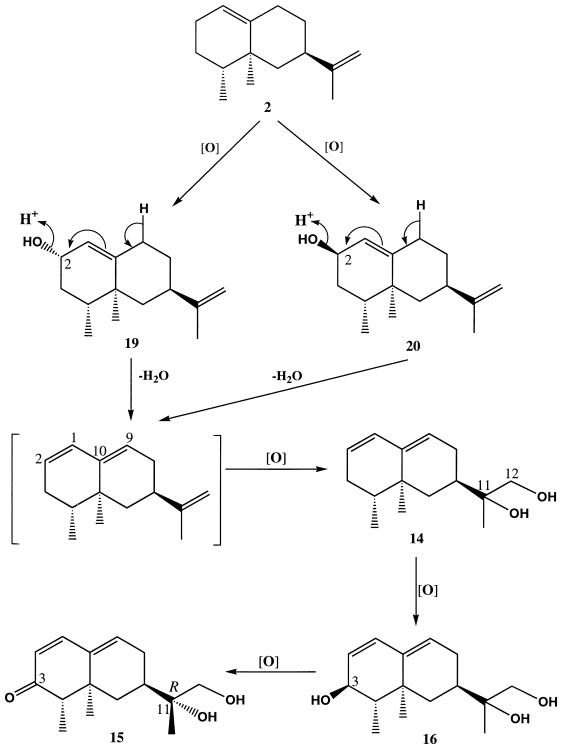

Fig. 5. Possible Metabolic Pathway of (+)-Valencene (**2**) into **14**—**16** by *Aspergillus niger*

lencene) (**20**) obtained in 42% yield. Nootkatol with calcium antagonistic activity was isolated from *Alpinia oxyphylla* Miq.[17] Compound **19** was biotransformed for 5 d by *A. niger* to give three metabolites, **4** and **5** (6.4%), **14** (34.6%), and **15** (5.5%), respectively. Compound **20** was biotransformed for 5 d by *A. niger* to give three metabolites, **4** and **5** (21.8%), **15** (5.5%), and **16** (10.4%), respectively. Both ratios of **4** (11*S*) and **5** (11*R*) obtained from **19** and **20** were 1 : 3. From the above results, plausible metabolic pathways of valencene (**2**) and 2-hydroxyvalencene (**19**, **20**) by *A. niger* are shown in Fig. 5.

In summary, the grapefruit fragrance, nootkatone (**1**) used in the modern cosmetic and fiber business was biotransformed by *A. niger* and *B. dothidea* to give 11*S*- and 11*R*-nootkatone-11,12-diol (**4**, **5**) in the ratio of 3 : 2. The biotransformation of **1** by *F. culmorum* stereospecifically afforded 11*R*-nootkatone-11,12-diol (**5**). Biotransformation of (+)-valencene (**2**), which can be cheaply obtained from Valencia oranges, afforded the structurally interesting metabolites **14**—**17** together with **3**—**5**. Because biotransformation of 2-hydroxyvalencene (**19**, **20**) by *A. niger* gave metabolites **14**—**16**, the biosynthetic pathway (Fig. 5) was confirmed. The metabolites **3**—**5**, **12**, and **13** of (+)-nootktone (**1**) and **3**—**5** and **14**—**17** of (+)-valencene (**2**) did not show an effective odor.

Experimental

General Experimental Procedures Gas chromatography (GC) was carried out using a Agilent 6890 GC system-5973 Network instrument equipped with a flame ionization detector on a capillary column (30 m× 250 μm×0.3 μm). The column temperature was programmed from 70 °C to 230 °C. IR spectra were measured on a JASCO FT-IR 500 spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were recorded on a Varian unitiy 600 (^{1}H; 600 MHz, ^{13}C; 150 MHz) spectrometer. The solvent used for NMR spectra was $CDCl_3$. MS spectra were measured on a JEOL JMS HX-100 or a JEOL AX-500 spectrometer. The specific rotation was recorded on a JASCO DIP-140 polarimeter. X-Ray crystallographic analysis was carried out using a Mac Science MXC18 diffractiotometer. Slica gel 60 for column chromatography was purchased from Merck. TLC was carried out on silica gel 60 F_{254}, precoated (layer thickness 0.25 mm, Merck) with *n*-hexane–EtOAc (1 : 1). High-performance liquid chromatography (HPLC) was carried out on a Shimadzu LC-6A Liquid Chromatograph.

Materials (+)-Valencene ($[\alpha]_D$ +84.6°, *c*=1.0) (**2**) and (+)-nootkatone ($[\alpha]_D$ +193.5°, *c*=1.0) (**1**) which were isolated from the essential oil of Valencia orange and grapefruit, respectively, were provided from Takasago International Co., Ltd. (Japan). Purity was checked based on the 600 MHz ^{1}H-NMR spectrum, GC-MS, and optical rotation. The absolute configurations of (+)-nootkatone (**1**) and (+)-valencene (**2**) were already determined to be **1** and **2**, respectively.[17,18]

Microorganisms and Media *A. niger* was isolated from soil in Osaka prefecture and was identified based on its physiologic and morphologic features. A Czapek-pepton medium [1.5% sucrose, 1.5% glucose, 0.5% polypepton, 0.1% K_2HPO_4, 0.05% KCl and 0.001%$FeSO_4 \cdot 7H_2O$ in distilled water (pH 7.0)] was used for the biotransformation of nootkatone by *A. niger*, *B. dothidea* and *B. theobromae*.

Biotransformation of Nootkatone (1) by *A. niger* An Erlenmeyer flask (500 ml) containing 200 ml of Czapek-pepton medium was inoculated with a suspension of *A. niger* and incubated at 30 °C for 2 d in a rotary shaker operating at 100 rpm. After full growth of the microorganisms, a solution of (+)-nootkatone (**1**) (80 mg×5; 400 mg) was added to the culture medium of *A. niger*. The incubation was then continued for a further 7 d at 30 °C. After the completion of the incubation time, the culture was filtered *in vacuo* and the broth was extracted twice with EtOAc (each 200 ml) under stirring. The EtOAc layers were dried over $MgSO_4$ and the solvent was evaporated *in vacuo* to give the crude extract (494 mg) which was chromatographed on silica gel (50 g) with a gradient solvent system of *n*-hexane–EtOAc by increasing the amount of EtOAc stepwise in 5% increments to afford 12-hydroxy-11,12-dihydronootkatone (**3**) (4.6 mg; 10.6%) from *n*-hexane–EtOAc (1 : 1) C-11 mixtures of 11,12-dihydroxy-11,12-dihydronootkatone (**4**, **5**) (238.2 mg; 51.5%) from *n*-hexane–EtOAc (1 : 4) fraction.

12-Hydroxy-11,12-dihydronootkatone (**3**): Colorless oil, $[\alpha]_D^{22}$ +133.3° (*c*=0.75, $CHCl_3$); EI-MS: *m/z* 236 (M^+), 218, 203, 175, (100), 161, 147, 121; HR-EI-MS: *m/z* 236.1778 (M^+), $C_{15}H_{24}O_2$ requires 236.1777; FT-IR (KBr) cm^{-1}: 3399 (OH), 2926, 1657 (C=O), 1298, 1040; UV (MeOH) λ_{max} nm (log ε): 238 (4.19); ^{1}H- and ^{13}C-NMR (600, 125 MHz) spectral data are shown in Tables 1 and 2, respectively.

11*S*- and 11*R*-nootkatone-11,12-diol (**4**, **5**) colorless oil, $[\alpha]_D^{22}$ +130.5° (*c*=0.81, $CHCl_3$); EI-MS: *m/z* 252 (M^+), 234, 221 (100%), 206, 178, 161, 121; HR-EI-MS: *m/z* 252.1730 (M^+), $C_{15}H_{24}O_3$ requires 252.1725; FT-IR (KBr) cm^{-1}: 3358 (OH), 2938, 2866, 1652 (C=O), 1063; UV (MeOH) λ_{max} nm (log ε): 238.5 (4.16); ^{1}H-NMR ($CDCl_3$): compound **4** (11*S*); δ 0.97 (3H, d, *J*=6.9 Hz, H-14), 1.11 (3H, s, H-13 and H-15), 3.46 (1H, d, *J*=11.0 Hz), 3.60 (1H, d, *J*=11.0 Hz), 5.76 (1H, *J*=0.8 Hz, H-1); ^{13}C-NMR ($CDCl_3$): δ 14.9 (q, C-14), 16.9 (q, C-15), 20.3 (q, C-13), 26.8 (t, C-8), 33.0 (t, C-9), 39.2 (t, C-6), 39.6 (s, C-5), 39.7 (d, C-7), 40.5 (d, C-4), δ 42.0 (t, C-3), 68.2 (t, C-12), 74.3 (s, C-11), 124.4 (d, C-1), 171.0 (s, C-10), 199.9 (s, C-2). Compound **5** (11*R*) NMR data: see Tables 1 and 2.

Reaction of Compounds 4 and 5 with $NaIO_4$ To a solution of compounds **4** and **5** (10.0 mg) in ethanol (5 ml) was added $NaIO_4$ (40 mg) with stirring at room temperature. After stirring for 1 h, the mixture was extracted twice with Et_2O (50 ml). The organic phase was washed with brine, dried ($MgSO_4$), and evaporated to give a residue (13.4 mg). The residue was purified by a silica gel column chromatography with an *n*-hexane–AcOEt gradient to afford diketone (**6**) (7.9 mg, 78.8%).

Compound **6**: Colorless oil, $[\alpha]_D^{22}$ +163.5° (*c*=1.74, $CHCl_3$); EI-MS: *m/z* 220 (M^+; 100%), 205, 177, 150, 135; HR-EI-MS: *m/z* 220.1458 (M^+), $C_{14}H_{20}O_2$ requires 220.1463; FT-IR (KBr) cm^{-1}: 2942, 1709 (C=O), 1667 (C=O), 1298, 1200; UV (MeOH) λ_{max} nm (log ε): 236.5 (4.17); ^{1}H-NMR ($CDCl_3$): δ 0.99 (3H, d, *J*=6.9 Hz, H-14), 1.12 (3H, s, H-15), 2.20 (3H, s, H-12), 5.79 (1H, s, H-1); ^{13}C-NMR ($CDCl_3$): δ 14.9 (q, C-14), 16.7 (q, C-15), 28.2 (q, C-12), 28.5 (t, C-8), 32.1 (t, C-9), 38.9 (s, C-5), 40.0 (t, C-6), 40.2 (d, C-4), 42.1 (t, C-3), 46.8 (d, C-7), 125.3 (d, C-1), 168.5 (s, C-10), 199.2 (s, C-2), 210.4 (s, C-11).

Reaction of Compounds 4 and 5 with 1,1′-Thiocarbonyldiimidazole To a solution of compounds **4** and **5** (30.0 mg) in toluene (7 ml) was added 1,1′-thiocarbonyldiimidazole (45 mg) with stirring at room temperature. After stirring for 7 h, the mixture was purified by silica gel column chromatography with an *n*-hexane–AcOEt gradient to afford thiocarbonates (**7**,

8) (29.8 mg). The mixture of thiocarbonates was separated by HPLC on silica gel with *n*-hexane–EtOAc (1 : 1) to afford 11*S*-thiocarbonate (**7**) (13.1 mg, 37.4%) at Rt=67 min and 11*R*-thiocarbonate (**8**) (8.8 mg, 25.1%) at Rt=70 min.

Compound **7**: Colorless prisms; mp 182—183 °C; $[\alpha]_D^{22}$ +167.0° (c=0.83, $CHCl_3$); EI-MS: *m/z* 294 (M^+), 278, 217 (100%), 216, 201, 174, 161, 135, 91; HR-EI-MS: *m/z* 294.1269 (M^+), $C_{16}H_{22}O_3S$ requires 294.1290; FT-IR (KBr) cm^{-1}: 2969, 1662 (C=O), 1305 (C=S), 1197; UV (MeOH) λ_{max} nm (log ε): 237 (4.50); CD (MeOH) λ_{max} nm ($\Delta\varepsilon$): 319 nm (−0.33); ^{1}H-NMR ($CDCl_3$): δ 0.97 (3H, d, J=6.9 Hz, H-14), 1.11 (3H, s, H-15), 1.55 (3H, s, H-13), 4.28 (1H, d, J=8.8 Hz, H-12), 4.46 (1H, d, J=8.8 Hz, H-12), 5.79 (1H, d, J=1.4 Hz, H-1); ^{13}C-NMR ($CDCl_3$): δ 15.0 (q, C-14), 16.9 (q, C-15), 21.7 (q, C-13), 26.7 (t, C-8), 31.8 (t, C-9), 38.6 (t, C-6), 40.4 (d, C-4), 41.0 (d, C-7), 41.9 (t, C-3), 76.6 (t, C-12), 91.8 (s, C-11), 125.3 (d, C-1), 167.6 (s, C-10), 191.1 (s, C=S), 198.9 (s, C-2).

The crystal data for **7** are as follows: Monoclinic, space group $P2_1$, a=8.710 (0) Å, b=8.448 (0) Å, c=10.988 (0) Å, β=102.428, V=789.599976 (0) Å^3, Z=2, D_x=1.530 Mg m^{-3}, D_m=1.500 Mg m^{-3}, μ (Mo$K\alpha$)=2.09 mm^{-1}, Eta; +1.9. Final R and Rw were 0.044 and 0.074 for 1186 reflections. The supplementary materials have been deposited at the Cambridge Crystallographic Data Center.

Compound **8**: Colorless needles; mp 164—165 °C; $[\alpha]_D^{22}$ +167.0° (c= 0.83, $CHCl_3$); EI-MS: *m/z* 294 (M^+), 278, 217 (100%), 216, 201, 174, 161, 135, 91; HR-EI-MS: *m/z* 294.1272 (M^+), $C_{16}H_{22}O_3S$ requires 294.1290; FT-IR (KBr) cm^{-1}: 2941, 1662 (C=O), 1307 (C=S), 1200; UV (MeOH) λ_{max} nm (log ε): 237 (4.34); CD (MeOH) λ_{max} nm ($\Delta\varepsilon$): 310 nm (−0.70); ^{1}H-NMR ($CDCl_3$): δ 1.00 (3H, d, J=6.9 Hz, H-14), 1.12 (3H, s, H-15), 1.52 (3H, s, H-13), 4.39 (1H, d, J=8.8 Hz, H-12), 4.46 (1H, d, J=8.8 Hz, H-12), 5.78 (1H, d, J=1.4 Hz, H-1); ^{13}C-NMR ($CDCl_3$): δ 15.0 (q, C-14), 16.9 (q, C-15), 21.0 (q, C-13), 27.0 (t, C-8), 31.9 (t, C-9), 38.9 (t, C-6), 40.5 (d, C-4), 41.0 (d, C-7), 42.0 (t, C-3), 76.9 (t, C-12), 91.8 (s, C-11), 125.4 (d, C-1), 167.6 (s, C-10), 191.1 (s, C=S), 199.0 (s, C-2).

Epoxidation of Nootkatone (1) To a solution of nootkatone (**1**) (300.1 mg) in $CHCl_3$ (40 ml) was added *m*CPBA (687 mg) with stirring at 0 °C. After stirring for 3 h , water was added and the mixture was extracted with $CHCl_3$. The organic phase was washed with 5% $NaHCO_3$ and brine, dried ($MgSO_4$), and evaporated to give a residue (314.9 mg). The residue was purified by silica gel column chromatography with an *n*-hexane–AcOEt gradient to afford 11,12-epoxynootkatone (**11**) (116.1 mg, 82.7%).

Compound **11**: Colorless oil, $[\alpha]_D^{21}$ +173.9° (c=1.01, $CHCl_3$); EI-MS: *m/z* 234 (M^+), 216, 206, 176 (100%), 161, 134, 119, 105, 91; HR-EI-MS: *m/z* 234.1613 (M^+), $C_{15}H_{22}O_2$ requires 234.1620; FT-IR (KBr) cm^{-1}: 2934, 1667 (C=O), 1287, 1200; UV (MeOH) λ_{max} nm (log ε): 236.5 (4.13); ^{1}H-NMR ($CDCl_3$): δ 1.06 (3H, s, H-15), 1.08 (3H, d, J=7.1 Hz, H-14), 1.24, 1.26 (3H, S, H-13), 2.62 (2H, m, H-12), 6.08 (1H, d, J=1.4 Hz, H-1).

Biotransformation of 11,12-Epoxynootkatone (11) by *A. niger* A solution of 11,12-epoxynootkatone (**11**) (20 mg) was added to the culture medium of *A. niger*. The incubation was then continued for 1 d at 30 °C. The culture was filtered *in vacuo* and the broth was extracted twice with EtOAc (each 200 ml) with stirring. The EtOAc layers were dried over $MgSO_4$ and the solvent was evaporated *in vacuo* to give the crude extract (30.5 mg) as an oil, which was chromatographed on silica gel with a gradient solvent system of *n*-hexane–EtOAc to afford 11*S*- and 11*R*-nootkatone-11,12-diol (**4** and **5**; ratio 1 : 1) (17.5 mg, 81.4%).

Biotransformation of Nootkatone (1) by *F. culmorum* An Erlenmeyer flask (500 ml) containing 200 ml of Czapek-pepton medium was inoculated with a suspension of *F. culmorum* and incubated at 30 °C for 5 d in a rotary shaker operating at 100 rpm. After full growth of the microorganisms, a solution of (+)-nootkatone (**1**) (20 mg) was added to the culture medium of *F. culmorum*. The incubation was then continued for a further 20 d at 30 °C. The culture was filtered *in vacuo* and the broth was extracted twice with EtOAc (each 100 ml) with stirring. The EtOAc layers were dried over $MgSO_4$ and the solvent was evaporated *in vacuo* to give the crude extract (494 mg) which was treated in the same manner as described above to give 9β-hydroxynootkatone (**12**) (3.2 mg; 14.9%) and (11*R*)-nootkatone-11,12-diol (**5**) (10.9 mg; 47.2%). The purity of compound **5** was determined to be *ca*. 95% by HPLC analysis of the thiocarbonate (**8**).

(11*R*)-Nootkatone-11,12-diol (**5**): Colorless oil, $[\alpha]_D^{24}$ +132.0° (c=0.82, $CHCl_3$); CI-MS: *m/z* 253 (M^++H; 100), 235, 221, 177; HR-CI-MS: *m/z* 253.1800 (M^++H), $C_{15}H_{25}O_3$ requires 253.1803; FT-IR (KBr) cm^{-1}: 3359 (OH), 2970, 1652 (C=O), 1615 (C=C), 1301, 1046; UV (MeOH) λ_{max} nm (log ε): 239 (4.13); NMR data: see Tables 1 and 2.

9β-Hydroxynootkatone (**12**): Colorless oil, $[\alpha]_D^{24}$ +131.3° (c=1.65, $CHCl_3$); EI-MS: *m/z* 234 (M^+), 216, 191, 166, 137 (100%), 109; HR-EI-MS: *m/z* 234.1612 (M^+), $C_{15}H_{22}O_2$ requires 234.1620; FT-IR (KBr) cm^{-1}: 3359 (OH), 2970, 1652 (C=O), 1615 (C=C), 1301, 1046; UV (MeOH) λ_{max} nm (log ε): 238 (4.06); NMR data: see Tables 1 and 2.

Biotransformation of (+)-Nootkatone (2) by *B. dothidea* An Erlenmeyer flask (500 ml) containing 200 ml of Czapek-pepton medium was inoculated with a suspension of *B. dothidea* (peach PP 8402) and incubated at 30 °C for 7 d in a rotary shaker operating at 100 rpm. (+)-Nootkatone (**2**) (20 mg×5; 100 mg) was added to the culture medium of *B. dothidea*. The inocubation was then continued for a further 14 d. The culture broth was treated in the same manner as described above give the crude extract (424 mg) which was chromatographed on silica gel with an *n*-hexane–ether gradient to afford 7α-hydroxynootkatone (**13**) (22.4 mg, 20.9%) and 11*S*- and 11*R*-nootkatone-11,12-diol (**4**, **5**) (62.7 mg, 54.2%). The ratio of compounds **4** and **5** was determined to be 3 : 2 by HPLC analysis of their thiocarbonates (**7**, **8**).

7α-Hydroxynootkatone (13): Colorless oil, $[\alpha]_D$ +118.8° ($CHCl_3$, c= 1.0); EI-MS: *m/z* 234 (M^+, 100), 219, 191, 161, 150, 136, 79, 69; HR-EI-MS; [M^+] 234.1610, $C_{15}H_{22}O_2$ requires 234.1619; IR (KBr) cm^{-1}: 3445 (OH): 3089, 2941, 1665 (α,β-unsaturated ketone), 1616, 1217; UV (MeOH) λ_{max} nm (log ε): 239.2 nm (4.17); NMR data: see Tables 1 and 2.

Biotransformation of (+)-Valencene (1) by *A. niger* *A. niger* was rotatory cultivated (100 rpm) in Czapek-pepton medium at 30 °C for 3 d. (+)-Valencene (**1**) (100 mg×15=1.5 g) was added to the medium and further cultivated for 5 d. The culture was worked up in a same manner as above. The EtOAc extract was chromatographed on silica gel (*n*-hexane–EtOAc gradient) to give seven metabolites: **3** (14.9 mg; 1.0%), **4** and **5** (202 mg; 13.5%), **14** (16.8 mg; 1.1%), **15** (22.6 mg; 1.5%), **16** (30.2 mg; 2.0%), and **17** (10.7 mg; 0.7%). The ratio of compounds **4** (11*S*) and **5** (11*R*) was determined to be 1 : 3 by HPLC analysis of their thiocarbonates (**7**, **8**).

Compound **14**: Colorless oil, $[\alpha]_D$ −71.1° ($CHCl_3$, c=1.0); EI-MS: *m/z* 236 (M^+), 218, 187 (100), 159 145, 105, 95; HR-EI-MS: [M^+] 236.1749, $C_{15}H_{24}O_2$ requires 236.1777; IR (KBr) cm^{-1}: 3350 (OH), 3019, 2968, 1460, 1380; UV (MeOH) λ_{max} nm (log ε): 235.0 (4.31), 228.0 0(4.29); NMR data: see Tables 4 and 5.

Compound **15**: Colorless oil, $[\alpha]_D$ −50.1° ($CHCl_3$, c=1.0); EI-MS: *m/z* 250 (M^+), 232, 201, 175 (100), 173, 105, 91, HR-EI-MS: [M^+] 250.1562, $C_{15}H_{22}O_3$ requires 250.1569; IR (KBr) cm^{-1}: 3417 (OH), 2940, 1666 (C=O), 1632, 1046; UV (MeOH) λ_{max} nm (log ε): 289.2 (4.31): NMR data: see Tables 4 and 5.

Compound **16**: Colorless oil, $[\alpha]_D$ +17.4° (MeOH, c=1.0); EI-MS: *m/z* 252 (M^+), 234, 203, 185, 159, 143, 105, 91 (100); HR-EI-MS: [M^+] 252.1708, $C_{15}H_{24}O_3$ requires 252.1726; IR (KBr) cm^{-1}: 3368 (OH), 2969, 2899, 1030, 755; UV (MeOH) λ_{max} nm (log ε): 237.0 (4.11); NMR data: see Tables 4 and 5.

Compound **17**: Colorless oil, $[\alpha]_D$ +52.0° ($CHCl_3$, c=1.0); EI-MS; *m/z* 252 (M^+), 221 (100), 203, 163, 161, 123, 121, 75; HR-EI-MS; [M^+] 252.1736, $C_{15}H_{24}O_3$ requires 252.1726; IR (KBr) cm^{-1}: 3410 (OH), 2941, 1653 (C=O), 1045; UV (MeOH) λ_{max} nm (log ε): 241.6 (3.94); NMR data: see Tables 4 and 5.

3,5-Dinitrobenzoylation of Compound 15 To a solution of **15** (10.5 mg) in pyridine (3 ml) was added 3,5-dinitrobenzoyl chloride (38.4 mg) and dimethylaminopyridine (10 mg). The mixture was stirred at room temperature overnight. Water was added and the mixture was extracted with $CHCl_3$. The organic phase was washed with 1 N HCl, 5% $NaHCO_3$, and brine, and dried ($MgSO_4$) and the solvent was evaporated to give a residue (15.0 mg) that was purified by a silica gel column chromatography with a *n*-hexane–AcOEt gradient to afford **18** (3.9 mg) as colorless prisms; mp 181—183 °C, $[\alpha]_D$ +34.6° ($CHCl_3$, c=1.0); CI-MS: *m/z* 445 ($M+H^+$, 55%), 233 (16%); HR-CI-MS; $[M+H]^+$ found 445.1607, $C_{22}H_{25}O_8N_2$ 445.1611; IR (KBr) cm^{-1}: 3451 (OH), 2973, 1733 (C=O), 1668 (C=O), 1632 (C=C), 1280; UV (MeOH) λ_{max} nm (log ε): 286.4 (4.10), 230.0 (4.45), 208.0 (4.55); CD (MeOH) λ_{max} nm ($\Delta\varepsilon$): 335.8 (+4.45), 288.7 (−7.21), 228.7 (−0.38), 211.7 (+2.36); ^{1}H-NMR ($CDCl_3$): δ 1.03 (3H, s, H-15), 1.12 (3H, d, J=6.9 Hz, H-14), 1.35 (3H, s, H-13), 4.43 (1H, d, J=11.5 Hz, H-12), 4.51 (1H, d, J=11.5 Hz, H-12), 5.89 (1H, d, J=9.9 Hz, H-2), 6.01 (1H, m, H-9), 6.89 (1H, d, J=9.9 Hz, H-1), 9.17 (2H, d, 2.2 Hz, aromatic protons), 9.26 (1H, t, 2.2 Hz, aromatic proton).

The crystal data for **18** (Table 3) are as follows: Triclinic, space group P_1, a=6.2170 (3) Å, b=9.0460 (5) Å, c=10.5030 (8) Å, V=525.56 (6) Å^2, α= 68.852 (3) Å, β=87.842 (3) Å, γ=73.086 (3) Å, Z=1, D_x=1.360 Mg m^{-3}, μ (Mo$K\alpha$)=0.105 mm^{-1}, λ=0.71073, final R was 0.0457 for 1883 reflections. The supplementary materials have been deposited at the Cambridge Crystallographic Data Center.

Reduction of Nootkatone (1) with $NaBH_4$–$CeCl_3$ $CeCl_3 \cdot 7H_2O$

(9.24 g) was added to a solution of nootkatone (**1**) (5.52 g) in EtOH (120 ml) and stirred at room temperature. $NaBH_4$ (1.45 g) was slowly added over 20 min. The mixture was stirred for 1 h and extracted with Et_2O (500 ml) and 1 N HCl (500 ml). The organic phase was washed with brine, dried ($MgSO_4$), and evaporated to give a residue (6.187 g). The residue was purified by a silica gel column chromatography with an *n*-hexane–AcOEt gradient to afford 2α-hydroxyvalencene (**19**) (4.846 g, 82.7%) as a colorless oil.

2α-Hydroxyvalencene (**19**): Colorless oil, $[\alpha]_D^{24}$ +94.2° (c=0.92, $CHCl_3$); EI-MS: *m/z* 220 (M^+), 202, 187, 161, 145, 119 (100%), 105; HR-EI-MS: *m/z* 220.1818 (M^+), $C_{15}H_{24}O$ requires 220.1827; FT-IR (KBr) cm^{-1}: 3320 (OH), 2928, 1644 (C=C), 1024.

Preparation of Nootkatol (20) by Mitsunobu Reaction of 2α-Hydroxyvalencene (19) To a solution of *p*-nitrobenzoic acid (485 mg) and triphenylphosphine (767 mg) in dry toluene (60 ml) at −30 °C was added 2α-hydroxyvalencene (**19**) (500 mg). Diethyl azodicarboxylate-toluene 40% solution (1.14 ml) was added dropwise for 15 min to the mixture. After stirring for 1 h at −30 °C, the mixture was extracted with Et_2O (300 ml) and a saturated $NaHCO_3$ solution (100 ml). The organic phase was washed with brine, dried ($MgSO_4$), and evaporated to give a residue (1.295 g). A solution of the residue in MeOH (30 ml) and H_2O (3 ml) was treated with KOH (1.50 g) and stirred for 12 h at room temperature. The mixture was extracted with Et_2O (200 ml) and brine (100 ml), dried ($MgSO_4$), and evaporated to give a residue (0.985 g) which was purified by a silica gel column chromatography with an *n*-hexane–AcOEt gradient to afford nootkatol (**20**)[19] (208 mg, 41.6%) as a colorless oil.

Nootkatol (**20**): $[\alpha]_D^{25}$ +213.3° (c=0.98, $CHCl_3$); EI-MS: *m/z* 220 (M^+), 202, 187, 161, 145, 119 (100%), 105, 91; HR-EI-MS: *m/z* 220.1857 (M^+), $C_{15}H_{24}O$ requires 220.1887; FT-IR (KBr) cm^{-1}: 3251 (OH), 2929, 1645 (C=C), 1011.

Biotransformation of 2α-Hydroxyvalencene (19) by *A. niger* *A. niger* was rotary cultivated (100 rpm) in Czapek-pepton medium at 30 °C for 3 d. 2α-Hydroxyvalencene (**19**) (100 mg) was added to the medium and further cultivated for 5 d. The culture was worked up in the same manner as above. The EtOAc extract was chromatographed on silica gel (*n*-hexane–EtOAc gradient) to give three metabolites: **4** and **5** (7.3 mg; 6.4%), **14** (37.1 mg; 34.6%), and **15** (6.3 mg; 5.5%).

Biotransformation of Nootkatol (20) by *A. niger* *A. niger* was rotary cultivated (100 rpm) in Czapek-pepton medium at 30 °C for 3 d. Nootkatol (**20**) (100 mg) was added to the medium and further cultivated for 5 d. The culture was worked up in the same manner as above. The EtOAc extract was chromatographed on silica gel (*n*-hexane–EtOAc gradient) to give three metabolites: **4** and **5** (25.0 mg; 21.8%), **15** (6.3 mg; 5.5%) and **16** (11.9 mg; 10.4%).

Acknowledgments We thank Dr. M. Tanaka, Mr. S. Takaoka, and Miss Y. Okamoto (TBU) for providing 600-MHz NMR, X-ray crystallographic analysis, and mass spectra, respectively. We also thank Takasago International Co., Ltd., Japan, for providing valencene and nootkatone.

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