Biotransformation of the Fungistatic Sesquiterpenoid Patchoulol by *Botrytis cinerea*

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Biotransformation of the fungistatic sesquiterpenoid patchoulol (**1**) by the fungus *Botrytis cinerea* affords the 5-, 7- and (8*R*)-hydroxy (**2**, **3**, and **5**) derivatives as the major metabolites, together with a number of minor metabolites (**4**, **6**−**9**) arising from hydroxylation at C-2, C-3, C-5, C-9, C-13, and C-14.

Botrytis cinerea is a gray powdery mould that causes diseases of many flowers, fruits, and vegetables. Economically important crops such as lettuces, carrots, tobacco, strawberries, and grapes are attacked by this fungus.[1] The widespread use of chemical fungicides to control pathogens has resulted in some serious problems arising from the appearance of highly resistant strains and the contamination of soil and water, causing significant economic damage because of the decreased quality of wines produced from treated grapes.[2]

Over the past few years we have undertaken research directed toward the rational design of fungicides, to control *Botrytis* in commercial crops, based on biosynthetic considerations. We have assessed the fungicidal activity of different natural products related to the sesquiterpenoid metabolites of *B. cinerea* in order to inhibit the formation of phytotoxic botrylane metabolites by the fungus. Subsequently, patchoulol (patchouli alcohol, **1**) was identified as a good fungistatic; however, the effect diminished after 6 days' incubation with the fungus. Our objective was to study the detoxification pathway of patchoulol by *B. cinerea*.

Results and Discussion

The antifungal properties of patchoulol (**1**) were established against the growth of *B. cinerea* using the "poisoned food" technique[3−5] (see Experimental Section), and the commercial fungicide Euparen was used as a standard. Patchoulol (**1**) displayed inhibitory activity at 40 and 60 ppm for 1 and 3 days, respectively, and total inhibition at 80 and 100 ppm for 4 days. Above 140 ppm, **1** exhibited total inhibition of the fungus for 6 days (Figure 1). The acetate of **1** was completely devoid of activity. However, *B. cinerea* showed that it had the ability to degrade patchoulol (**1**) as, depending on the concentration of **1** in the test, the fungus began to grow after 3−6 days, suggesting that a detoxification mechanism was present.

To study this mechanism, **1** was incubated with *B. cinerea* on surface culture for 3 days. Eight metabolites (**2**−**9**), which were not present in controls, were detected by TLC. These metabolites were extracted with EtOAc and separated by column chromatography. Three major metabolites, **2**−**4**, together with another five minor metabolites, **5**−**9**, were isolated. Compounds **2**, **3**, **5**, and **6** had been reported by Teisseire[6] using patchoulol (**1**) with other microorganisms. However, their IR and NMR data were not reported. Compounds **4**, **7**, **8**, and **9** are described here for the first time.

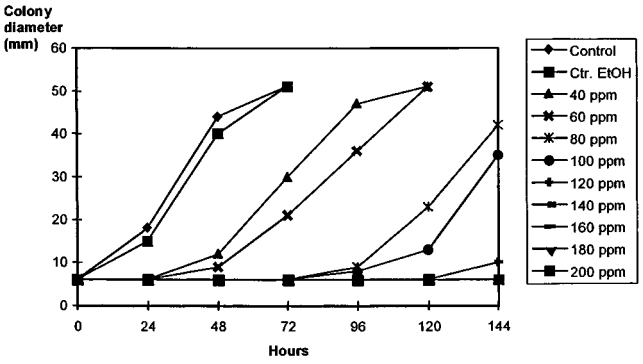

Figure 1. Effects of **1** on the growth of *B. cinerea*.

The locations of the additional oxygen functions were established by analysis of changes in the ^{13}C NMR spectra (Table 1).[7] Compounds **2** and **3**, both had the molecular formula $C_{15}H_{26}O_2$ based on their mass spectra (M^+ at *m*/*z* 238). Their ^{13}C NMR spectra contained signals indicating two tertiary hydroxyl groups (δ_C 75.6 and 76.4 for **2** and 72.9 and 75.9 for **3**).The downfield shift of neighboring carbon signals in both compounds, from the corresponding positions in **1** (C-4 and C-6 for **2**, C-6 and C-8 for **3**; Table 1), indicated that additional hydroxyl groups were located at C-5 in **2** and C-7 in **3**.[6] The proposed structures were supported by homonuclear and heteronuclear 2D correlation experiments.

Compounds **4** and **5**[6] were obtained as crystalline materials that showed molecular ions at *m*/*z* 238 and gave a ^{13}C NMR spectra consistent with the molecular formula C_{15} H_{26} O_2. The spectroscopic data, including the results of DEPT experiments, suggested that **4** and **5** were epimeric hydroxypatchoulol derivatives possessing a new secondary OH group. The ^1H NMR spectrum of **4** showed the characteristic pattern of signals of a patchoulol derivative with an additional secondary hydroxyl group (δ_H 4.24). Its ^{13}C NMR spectrum showed that signals corresponding to C-7 and C-9 (δ_C 45.6 and 40.7, respectively) were shifted downfield, compared to **1**, consistent with location of the hydroxyl group at C-8. Downfield shifts of the signals assigned to C-7 (δ_C 46.7) and C-9 (δ_C 39.4) in the ^{13}C NMR spectrum of **5**, together with the different multiplicity of the H-8 signal, indicated that this compound was the C-8 epimer of **4**. The study of coupling constants exhibited by the signals H-7, H-8, and H-9 in both compounds, the

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Table 1. ^{13}C NMR Data of Compounds **1**−**9** (100 MHz, $CDCl_3$)

C	1	2	3	4	4a	5	5a	6	6a	7	8	9
1	75.6 s	75.6 s	75.9 s	74.4 s	74.1 s	77.2 s		75.3 s	71.5 s	75.9 s		
2	32.7 t	31.7 t	32.8 t	32.8 t	32.8 t	32.5 t	32.6 t	33.4 t	33.1 t	42.6 t	32.8 t	72.3 d
3	28.6[a] t	22.8 t	28.3 t	28.6 t	28.6 t	28.5 t	28.5 t	28.1 t	28.0 t	72.4 d	28.6 t	35.8 t
4	28.1 d	34.6 d	27.9 d	28.0 d	28.0 d	27.7 d	27.8 d	27.5 d	27.4 d	37.2 d	27.5 d	24.5 d
5	43.7 d	76.4 s	43.3 d	42.8 d	42.3 d	42.8 d	42.4 d	39.2 d	39.0 d	43.7 d	41.1 d	42.3 d
6	24.6 t	34.5 t	32.1[a] t	15.5 t	16.4 t	24.1 t	25.0 t	24.4 t	24.2 t	25.0[a] t	22.9[a] t	24.6[a] t
7	39.1 d	39.0 d	72.9 s	45.6 d	42.8 t	46.7 d	43.3 d	35.3 d	33.8 d	38.6 d	38.9 d	36.8 d
8	24.3 t	23.5 t	32.0[a] t	66.1 d	70.6 d	72.5 d	74.5 d	36.0 t	36.4 t	25.9[a] t	23.6[a] t	23.2[a] t
9	28.8[a] t	29.6 t	29.9 t	40.7 t	37.1 t	39.4 t	35.9 t	69.1 d	72.8 d	28.9 t	24.4 t	30.1 t
10	37.7 s	43.4 s	37.4 s	38.8 s	38.4 s			43.4 s	42.8 s			
11	40.1 s	39.4 s	44.7 s	40.0 s	40.6 s	40.4 s		40.1 s	40.0 s			
12	18.5 q	14.0 q	18.5 q	18.4 q	18.2 q	18.8 q	18.7 q	18.6 q	18.2 q	15.0 q	18.4 q	18.2 q
13	20.6 q	14.8 q	20.4 q	20.2 q	20.0 q	20.1 q	20.0 q	15.9 q	15.7 q	20.3 q	68.5 t	18.9 q
14	26.8 q	27.0 q	18.3 q	26.1 q	26.1 q	28.1 q	27.6 q	27.2 q	27.1 q	26.3 q	26.8 q	71.2 t
15	24.3 q	24.3 q	21.7 q	24.6 q	24.3 q	25.4 q	23.5 q	24.3 q	24.2 q	24.1 q	24.1 q	21.5 q
CH_3CO					21.5 q		21.6 q		21.3 q			
CH_3CO					170.9 s		170.8 s		170.9 s			

[a] Assignments may be interchanged.

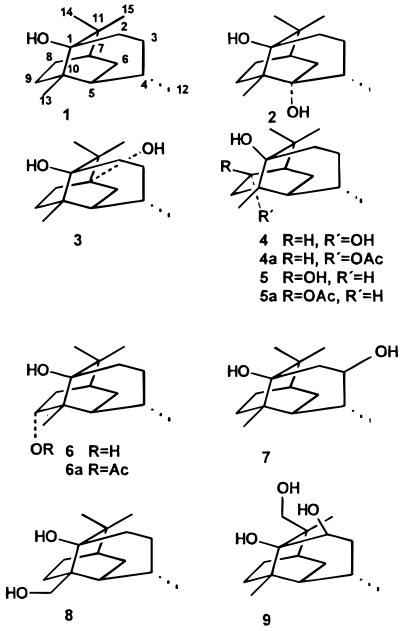
downfield shift of the signal assigned to H-14 in the ^1H NMR spectrum of **5** and the changes observed for their acetyl derivatives **4a** and **5a** indicated *R* stereochemistry at C-8 in **5** and *S* in **4**.

The presence of methine signals at δ_C 69.1 and 72.4 in **6** and **7**, respectively, suggested that both compounds were hydroxypatchoulol derivatives containing an additional secondary hydroxyl group. The downfield shifts of signals assigned to C-8 and C-10 in **6** and to C-2 and C-4 in **7** (Table 1) suggested that the hydroxyl groups were located at C-9 and C-3, respectively. The stereochemistry at C-9 was revealed by NOE effects between H-9 and H-13 (3%) and between H-9 and H-14 (3%). The ^1H NMR spectra of **6** and its acetate **6a** were consistent with (9*R*)-9-hydroxypatchoulol (**6**).[6] Compound **6** has also been obtained from biotransformation of patchoulol by *Mucor plumbeus*.[8] An NOE difference experiment established the *S* configuration at C-3 in **7**. In particular, irradiation at H-3 (δ_H 3.65) produced a 7% enhancement of the H-15 signal (δ_H 1.06), leading to the structure and stereochemistry given in **7**.

Compound **8** had a molecular formula $C_{15}H_{26}O_2$ (M^+ *m*/*z* 238). Its ^1H and ^{13}C NMR spectra revealed the presence of a primary OH group (δ_H 3.19 and 4.32; δ_C 68.5). The γ-gauche shielding of C-5 and C-9 suggested that the hydroxyl group was located at C-13. The marked difference in chemical shift between the two hydrogen atoms of the primary alcohol indicated that one of them was in the vicinity of the C-1 hydroxyl group of patchoulol (**1**).

The HREIMS and ^{13}C NMR spectra of compound **9** were consistent with the molecular formula, $C_{15}H_{26}O_3$, corresponding to a dihydroxypatchoulol. The absence of methyl group signals in the ^1H and ^{13}C NMR spectra assigned to C-14, and the appearance of new hydroxymethyl resonances (δ_H 4.32 and 3.10; δ_C 71.2) suggested that the compound was hydroxylated at C-14. The stereochemistry of the compound was confirmed by NOE experiments. In particular the *S* configuration at C-2 was established by the enhancement (5%) of the signal assigned to H-15 (δ_H 1.14) by irradiation of H-2.

In the course of these biotransformation experiments we observed some effects on the growth of the fungus, *B. cinerea*. First, growth of the mycelium was inhibited when the substrate was added to the broth. Second, examination of the broth extracts showed that botrydial and its derivatives were not present in the early stages of the fermentation when **1** was still present. The low recovery of products from the biotransformation may be related to their further degradation. The tertiary hydroxyl group of patchoulol may play an important part in this. Hydroxylation at C-5 or C-7 might enable biodegradation via the biological equivalent of a retro-Prins reaction, while hydroxylation at the secondary centers could be the prelude to further oxidation to a ketone followed by a Baeyer−Villiger or retro-aldol cleavage of the ring system. The fact that the acetate of **1** had no effect on mycelial growth indicates the importance of the tertiary alcohol for fungistatic activity.



Experimental Section

General Experimental Procedures. Melting points were measured with a Reichert−Jung Kofler block (uncorrected). Optical rotations were determined with a Perkin−Elmer 241 polarimeter in $CHCl_3$. IR spectra were recorded on a Perkin−Elmer 881 spectrophotometer. ^1H and ^{13}C NMR measurements were obtained on Varian Gemini 200 and Varian Unity 400 NMR spectrometers with $SiMe_4$ as internal reference. MS were recorded on VG 12−250 spectrometer at 70 eV. HPLC was performed with a Hitachi/Merck L-6270 apparatus equipped with an UV/vis detector (L 4250) and a differential refractometer detector (RI-71). TLC was performed on Merck Kiesegel 60 F_{254}, 0.2 mm thick. Si gel (Merck) type 9385 was used for column chromatography. Purification by HPLC was accomplished using a Si gel column (Hibar 60, 7 m, 1 cm wide, 25 cm long).

Microorganism and Antifungal Assays. *B. cinerea* (UCA 992) was obtained from grapes of Domecq vineyard, Jerez de la Frontera, Cádiz, Spain. This culture is deposited in the Universidad de Cadiz, Facultad de Ciencias Mycological Herbarium Collection (UCA). Compound **1** was tested on *B. cinerea* by the "poisoned food technique". Test compound was dissolved in EtOH to give a final compound concentration of 50−200 ppm. Solutions of test compound were added to glucose−malt−peptone−agar medium (61 g of glucose−malt−peptone−agar per liter, pH 6.5−7.0). The final EtOH concentration was identical in controls and treatment. The medium was poured in 6-cm diameter sterile plastic Petri dishes and a 5-mm diameter mycelial disk of *B. cinerea* cut from an actively growing culture was placed in the center of the agar plate. Inhibition of radial growth was measured for 6 days.

General Culture Conditions. *Botrytis cinerea* (UCA 992) was grown on surface culture in Roux bottles at 25° C for 3 days on a Czapek−Dox medium (150 mL per flask) comprising (per liter of distilled H_2O) glucose (40 g), yeast extract (1 g), potassium dihydrogen phosphate (5 g), sodium nitrate (2 g), magnesium sulfate (0.5 g), ferrous sulfate (10 mg), and zinc sulfate (5 mg). The substrate dissolved in EtOH (250 μL) was added to each flask and the fermentation continued for a further period of 3 days. The mycelium was filtered and washed with brine and EtOAc. The broth was saturated with NaCl, acidified (pH 2), and extracted with EtOAc. The extracts were separated into acidic and neutral fractions with aqueous sodium bicarbonate. The acid fraction was recovered in EtOAc. The extracts were dried over Na_2SO_4, the solvent was evaporated, and the residues were chromatographed on Si gel 60 (70−230 mesh) in an increasing gradient of EtOAc to petroleum ether.

Biotransformation of Patchoulol (1) by *B. cinerea*. Compound **1** (150 ppm per flask) was distributed over 10 flasks of *B. cinerea* and the fermentation continued for 3 days. Following the general isolation procedure, a TLC of the acidic fraction revealed no differences between the treatment and control. It was not studied further. From the neutral fraction, nine compounds were isolated: recovered patchoulol (**1**) (62 mg); (5*R*)-5-hydroxypatchoulol (**2**) (11 mg); (7*S*)-7-hydroxypatchoulol (**3**) (47 mg); (8*R*)- and (8*S*)-8-hydroxypatchoulol (**4**, **5**) (28 and 3.6 mg); (9*R*)-9-hydroxypatchoulol (**6**) (4.3 mg); (3*R*)-3-hydroxypatchoulol (**7**) (1 mg); 13-hydroxypatchoulol (**8**) (1 mg); (2*S*)-2,14-dihydroxypatchoulol (**9**) (1.5 mg).

(5R)-5-Hydroxypatchoulol (2): white solid; mp 105−107 °C; $[\alpha]^{25}_D$ −60° (*c* 1, $CHCl_3$); IR ν_{max} (film) 3513, 2954, 1460, 1012, 983, 836 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.95 (1H, ddd, J = 14.8, 11.8 and 2.9 Hz, H-9), 1.81 (1H, br dd, J = 12.1, 6.4 Hz, H-4), 1.73 (1H, dd, J = 13.8, 6.2 Hz, H-6), 1.48 (1H, dd, J = 13.8, 6.8 Hz, H-6′),1.18 (1H, br dd, J = 6.8, 6.2 Hz, H-7), 1.11 (1H, br dd, J = 11.8, 3.7 Hz, H-9′), 1.09 (3H, s, H-14), 1.04 (3H, s, H-15), 0.85 (3H, d, J = 6.4 Hz, H-12), 0.84 (3H, s, H-13); ^{13}C NMR data, Table 1; EIMS *m/z* 238 [M^+] (2), 220 (4), 205 (2), 202 (1), 151 (9), 123 (20), 69 (100); HRMS *m/z* 238.1940 [$M]^+$ (calcd for $C_{15}H_{26}O_2$ 238.1933).

(7S)-7-Hydroxypatchoulol (3): white solid; mp 108−110 °C; $[\alpha]^{25}_D$ −90° (*c* 27.3, $CHCl_3$); IR ν_{max} (film) 3459, 2922, 1472, 1380, 1080, 1012, 737 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.91 (2H, m, H-9, H-4), 1.72 (2H, m, H-2, H-8), 1.04 (3H, s, H*-14), 1.02 (3H, s, H*-15), 0.83 (3H, s, H-13), 0.77 (3H, d, J = 6.7 Hz, H-12) (* = assignments may be interchanged); ^{13}C NMR data, Table 1; EIMS *m/z* 238 [M^+] (10), 221 (58), 205 (11), 203 (10), 196 (14), 195 (100), 125 (46), 111 (55); HREIMS *m/z* 238.1930 [M^+] (calcd for $C_{15}H_{26}O_2$, 238.1933).

(8S)-8-Hydroxypatchoulol (4): white solid; mp 107−109 °C; $[\alpha]^{25}_D$ −77.2° (*c* 13.6, $CHCl_3$); IR ν_{max} (film) 3408, 2953, 1474, 1051, 978, 735, 654 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 4.24 (1H, br dd, J = 9.0, 4.5 Hz, H-8), 2.30 (1H, dd, J = 15.5, 9.0 Hz, H-9), 1.96 (1H, m, H-4), 1.79 (1H, ddd, J = 14.0, 11.3, 3.4 Hz, H-2), 1.73 (1H, ddd, J = 14.1, 8.3, 5.8 Hz, H-6), 1.26 (1H, ddd, J = 14.0, 6.0, 1.5 Hz, H-2), 1.13 (3H, s, H-14), 1.03 (3H, s, H-15), 0.91 (1H, dd, J = 15.5 Hz, H-9′), 0.86 (3H, s, H-13), 0.80 (3H, d, J = 6.6 Hz, H-12); ^{13}C NMR data, Table 1; EIMS *m/z* 238 [M^+] (1), 220 (1), 205 (2), 202 (1), 138 (100), 125 (23), 107 (18); HREIMS *m/z* 238.1931 [M^+] (calcd for $C_{15}H_{26}O_2$, 238.1933).

(8S)-8-Acetoxypatchoulol (4a): white solid; mp 113−115 °C; $[\alpha]^{25}_D$ −61° (*c* 3.8, $CHCl_3$); IR ν_{max} (film) 3532, 2953, 1721, 1465, 1370, 1028 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.16 (1H, br dd, J = 9.4, 4.3 Hz, H-8), 2.33 (1H, dd, J = 15.6, 9.4 Hz, H-9), 2.03 (3H, s, CH_3CO−), 1.12 (3H, s, H-14), 1.09 (3H, s, H-15), 0.86 (3H, s, H-13), 0.81 (3H, d, J = 6.7 Hz, H-12); ^{13}C NMR data, Table 1.

(8R)-8-Hydroxypatchoulol (5): white solid; mp 179−181 °C; $[\alpha]^{25}_D$ −75.4° (*c* 1.1, $CHCl_3$); IR ν_{max} (film) 3410, 2908, 1462, 1381, 1048, 1000, 736, 615 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.90 (1H, br t, H-8), 1.94 (1H, m, H-4), 1.87 (1H, br dd, J = 13.8, 7.7 Hz, H-9), 1.74 (1H, ddd, J = 13.9, 6.3, 4.9 Hz, H-2), 1.60 (1H, dd, J = 13.8, 10.3 Hz, H-9′), 1.23 (3H, s, H-14), 1.11 (3H, s, H-15), 0.88 (3H, s, H-13), 0.79 (3H, d, J = 6.6 Hz, H-12); ^{13}C NMR data, Table 1; EIMS *m/z* 238 [M^+] (1), 220 (15), 205 (46), 202 (1), 177 (6), 125 (100), 96 (63).

(8R)-8-Acetoxypatchoulol (5a): colorless oil; $[\alpha]^{25}_D$ −77.3° (*c* 1.1, $CHCl_3$); IR ν_{max} (film) 3523, 2927, 1737, 1461, 1369, 1252, 1034 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 4.79 (1H, ddd, J = 10.2, 7.7, 1.6 Hz, H-8), 2.01 (3H, s, CH_3CO−), 1.95 (1H, dd, J = 14.0, 7.7 Hz, H-9′), 1.94 (1H, m, H-4), 1.14 (3H, s, H-14), 1.09 (3H, s, H-15), 0.89 (3H, s, H-13), 0.79 (3H, d, J = 6.7 Hz, H-12); ^{13}C NMR data, Table 1; EIMS *m/z* 238 [M^+] (1), 220 (15), 205 (46), 202 (1), 177 (6), 125 (100), 96 (63); HREIMS *m/z* 280.2036 [M^+] (calcd for $C_{17}H_{28}O_3$ 280.2038).

(9R)-9-Hydroxypatchoulol (6): white solid; mp 124−126 °C; $[\alpha]^{25}_D$ −92° (*c* 1, $CHCl_3$); IR ν_{max} (film) 3383, 2917, 1462, 1050, 988, 738, 641 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 4.12 (1H, br t, J = 7.1, 6.8 Hz, H-9). 2.51 (1H, m, H-8), 1.82 (1H, m, H-4), 1.72 (1H, ddd, J = 12.9, 7.7, 6.3 Hz, H-2), 1.55 (1H, m, H-2′), 1.48 (1H, m, H-3), 1.35 (1H, m, H-3′), 1.29 (1H, m, H-7), 1.11 (1H, m, H-8), 1.08 (3H, s, H-14), 1.02 (3H, s, H-15), 1.00 (3H, s, H-13), 0.80 (3H, d, J = 6.6 Hz, H-12); ^{13}C NMR data, Table 1; EIMS *m/z* 238 [M^+] (8), 220 (28), 205 (16), 202 (9), 177 (47), 125 (99), 93 (100).

(9R)-9-Acetoxypatchoulol (6a): colorless oil; $[\alpha]^{25}_D$ −75° (*c* 2.7, $CHCl_3$); IR ν_{max} (film) 3497, 2929, 1462, 1720, 1462, 1382, 1264, 1029, 982 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.13 (1H, dd, J = 9.9, 6.0 Hz, H-9). 2.59 (1H, m, H-8), 2.03(3H, s, CH_3CO−), 1.84 (1H, m, H-4), 1.73 (1H, ddd, J = 13.2, 7.9, 6.1 Hz, H-2), 1.55 (1H, m, H-2′), 1.48 (1H, m, H-3), 1.35 (1H, m, H-3′), 1.29 (1H, m, H-7), 1.10 (1H, m, H-8), 1.09 (3H, s, H-14), 1.06 (3H, s, H-15), 0.91 (3H, s, H-13), 0.81 (3H, d, J = 6.6 Hz, H-12); ^{13}C NMR data, Table 1; EIMS *m/z* 280 [M^+], 220, 205, 177, 125; HREIMS 280.2030 [M^+] (calcd for $C_{17}H_{28}O_3$ 280.2038).

(3R)-3-Hydroxypatchoulol (7): colorless oil; $[\alpha]^{25}_D$ −45° (*c* 0.4, $CHCl_3$); IR ν_{max} (film) 3382, 2931, 1461, 1379, 1041, 1011 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.65 (1H, ddd, J = 13.4, 11.2, 6.6 Hz, H-3), 2.16 (1H, dd, J = 13.4, 6.6 Hz, H-2), 1.74 (1H, m, H-4), 1.08 (3H, s, H-15), 1.06 (3H, s, H-14), 1.01 (3H, d, J = 6.6 Hz, H-12), 0.88 (3H, s, H-13); ^{13}C NMR data, Table 1; EIMS *m/z* 238 [M^+] (1), 220 (1), 205 (1), 202 (0.4), 177 (2), 121 (5), 100 (100); HREIMS *m/z* 238.1936 [M^+] (calcd for $C_{15}H_{26}O_2$ 238.1933).

13-Hydroxypatchoulol (8): colorless oil; $[\alpha]^{25}_D$ −36.7° (*c* 0.6, $CHCl_3$); IR ν_{max} (film) 3363, 2926, 1462, 1385, 1098 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 4.32 (1H, d, J = 11.1 Hz, H-13), 3.19 (1H, br d, J = 11.1 Hz, H-13′), 2.60 (1H, br s, OH), 2.28 (1H, ddd, J = 13.7, 7.3, 7.1 Hz, H-9), 1.90 (1H, m, H-8), 1.80 (1H, m, H-4), 1.08 (3H, s, H*-14), 1.07 (3H, s, H*-15), 0.77 (3H, d, J = 6.6 Hz, H-12) (* = assignments may be interchanged); ^{13}C NMR data, Table 1; EIMS *m/z* 238 [M^+] (3), 220 (4), 207 (21), 205 (4), 202 (1), 189 (8), 158 (2), 55 (90), 43 (100); HREIMS *m/z* 238.1934 [M^+] (calcd for $C_{15}H_{26}O_2$ 238.1933).

(2S)-2,14-Dihydroxypatchoulol (9): white solid; mp 96−98 °C; $[\alpha]^{25}_D$ −21° (*c* 1, $CHCl_3$); IR ν_{max} (film) 3390, 2490, 1463, 1380, 1031 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 4.32 (1H, dd, J = 10.5, 2.6 Hz, H-14), 3.85 (1H, dd, J = 3.0, 2.8 Hz, H-2), 3.60 (1H, s, OH), 3.10 (1H, dd, J = 10.5, 8.6 Hz, H-14′), 2.75 (1H, dd, J = 8.6, 2.6 Hz, OH), 2.18 (1H, m, H-4), 2.05 (1H, ddd, J = 13.9, 7.9, 7.5 Hz, H-9), 1.70 (2H, m, H-8 and H-3), 1.47 (1H, m, H-5), 1.17−1.34 (2H, m, H-8′ and H-7), 1.14 (3H, s, H-15), 1.04 (3H, s, H-13), 0.97 (1H, m, H-9′), 0.82 (3H, d, J = 6.8 Hz,

H-12); ^{13}C NMR data, Table 1; EIMS *m*/*z* 254 [M^+] (9), 236 (19), 218 (13), 200 (17), 179 (26), 140 (100), 123 (64), 107 (72); HREIMS *m*/*z* 254.1882 [M^+] (calcd for $C_{15}H_{26}O_3$ 254.1882).

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