Biotransformation of Caryophyllene Oxide by *Botrytis cinerea*

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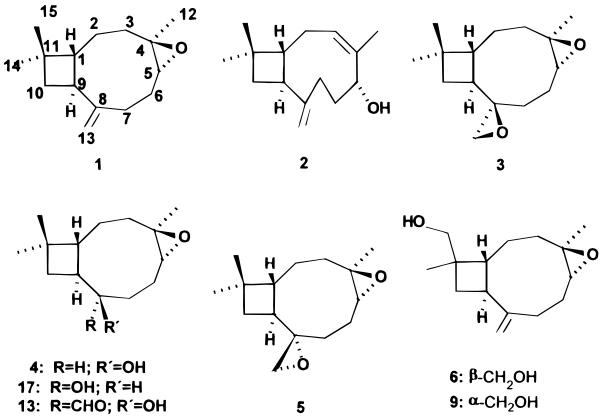
Biotransformation of caryophyllene oxide (**1**) with *B. cinerea* afforded 15 products (**2**−**16**). Ten of these (**3**−**5**, **7**, **9**−**11**, and **14**−**16**) are reported here for the first time. The main reaction paths involved stereoselective epoxidation at C-8/C-13 and hydroxylation at C-7. A rearranged compound was found, which was a cyclization product **16** possessing the caryolane skeleton.

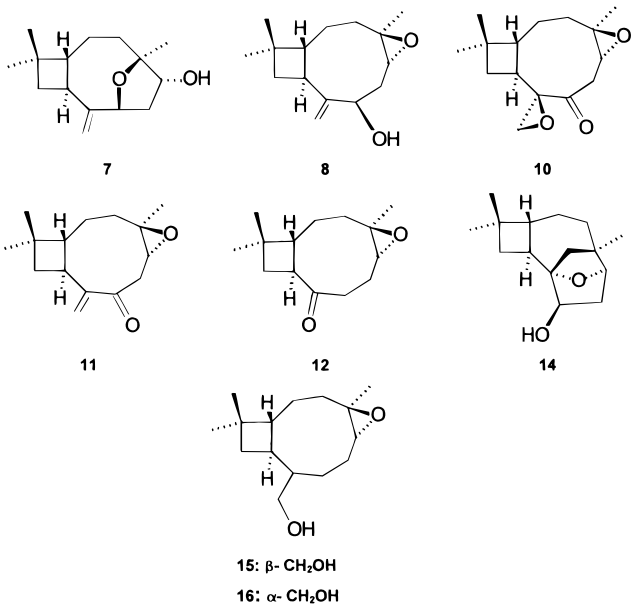
Botrytis species are serious pathogens toward a number of commercial plants.[1,2] In particular, *Botrytis cinerea* attacks a wide range of plants, producing various leaf spot diseases and gray powdery mildews on lettuce, tomatoes, and grapes.

Botrydial and structurally related compounds are characteristic metabolites of *Botrytis* sp.[3−5] Previous work[5] yielded evidence that botrydial played a role in the in vivo pathogenicity of *B. cinerea*. Biosynthetic studies suggest that the sesquiterpenes botrydial and dihydrobotrydial are formed from farnesyl pyrophosphate.[6] The first stage in the biosynthesis involves the formation and cyclization of a caryophyllene cation. Accordingly, we have investigated the biotransformation of caryophyllene oxide (**1**) in order to elucidate the fate of compounds having the caryophyllene skeleton.



Results and Discussion

The fermentation of **1** using *B. cinerea* and isolation of the biotransformation products was carried out as described in the Experimental Section. The extracts of the initial fermentation (2 days) contained products **2**−**9**. A second experiment was carried out under different conditions in an attempt to increase the yield. A culture of *B. cinerea* was grown for 3 days and then was filtered, and the mycelia was transferred to a new medium without glucose. The substrate was then added, and the mixture was incubated for 1 day. Under the latter conditions, 13 products were isolated; six of these (**3** and **5**−**9**) were previously obtained in the initial experiment along with the additional compounds **10**, **11**, kobusone (**12**), and **13**−**16**. Products **2**, **6**, **8**, and **12** were previously described in biotransformations of **1**,[7] while **12** and **13** were obtained from diepoxycaryophyllene.[7] Product **6** has also been obtained from caryophyllene using two different microorganisms, *Chaetomium cochiolides*[7] and *Diplodia gossypina*.[8]



Epoxidation at the double bond gave **3** and **5** as the main biotransformation products. The ratio of **3** to **5** indicated a selectivity toward diepoxide **3**. NOE difference experiments on **3** and **5** proved insufficient for the determination of stereochemistry at C-8. The diepoxides **3** and **5** were therefore reduced by treatment with $LiAlH_4$ to give alcohols **4** and **17**, respectively. Comparison of compounds **4** and **17** indicated that the C-8 sterochemistry of both **3** and **4** was *R*. Spectroscopic data (NMR, MS) of alcohol **4** were identical to those obtained for compound **4** isolated from the fermentation broth.

Diepoxides **3** and **5** could be the precursors of epimeric alcohols **15** and **16**, which can be obtained through reductive opening of the 8,13-epoxide. Their structures were determined by comparison of their ^{13}C NMR spectra, which indicated the presence of a hydroxymethyl group in both cases. The downfield shift of the signal corresponding to H-9 in compound **16** indicated an α-orientation for the hydroxymethyl group at C-13. This was confirmed by the observation of an NOE effect between H-13 and H-9, which is only possible for a hydroxymethyl in the α-orientation.

Aldehyde **13**[7] probably arises from opening of the epoxide and further oxidation of compound **3**. Kobusone (**12**) was

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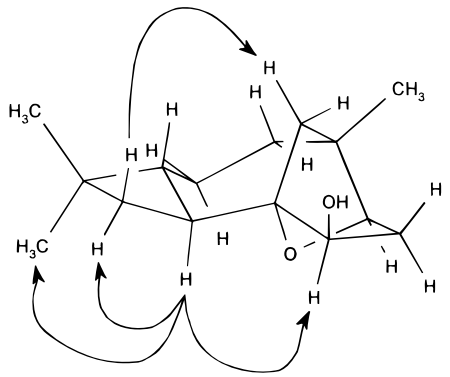

Figure 1. Selected NOE correlations observed for **14**.

also found in the extract and is probably derived from the 8,13-diol by diol cleavage.

Compounds **6** and **9**, products of hydroxylation at methyl groups, were also isolated. Spectroscopic data for **6** were the same as those described in the literature for the natural product 15β-(hydroxymethyl)-4,5-epoxycaryophyllene (**6**).[7] The structure of **9** was then assigned by comparison of their NMR spectra.

Another transformation of **1** by *B. cinerea* was oxidation at C-7. Compound **8**, already known from biotransformation with *C. Cochiolides*,[7] and compounds **7**, **10**, **11**, and **14** were also isolated from the fermentation broth. Compound **7** probably arises from **8** via an intramolecular reaction. A detailed study using 2D-NMR methods solved the structure of **7** unambiguously.

Compound **14** had a caryolane skeleton. In this case, a new bond had been formed between C-4 and C-13, which resulted in a caryolane derivative. Compound **14** could be formed by intramolecular attack of the exocyclic double bond of **1** by the 4,5-epoxide. The structure was assigned using 2D-NMR techniques, including long-range homo- and heteronuclear correlation. The stereochemistry of the compound was carefully established through NOE experiments (Figure 1).

Alcohol **8** was oxidized further to give the ketone **11**. Comparison of the ^{13}C NMR of **11** with that of **8** showed that one hydroxylated methine carbon signal had disappeared and was replaced by a ketone signal (δ = 214). An identical signal was observed in the ^{13}C NMR spectrum of compound **10**, which requires an additional epoxide at C-8/C-13. The stereochemistry at C-8 was determined by comparison of its NMR spectra with those of compounds **3** and **5**.

The biotransformation of caryophyllene oxide with *B. cinerea* produced a number of oxygenated compounds. The major biotransformation pathways involved stereoselective epoxidation at C-8/C-13 and hydroxylation at C-7. Interestingly, position C-7 on the caryophyllene skeleton corresponds with C-15 on a botryane derivative. This position usually bears an oxygen in the botryane skeleton. The presence of kobusone (**12**) and aldehyde **13** in the fermentation broth seems to indicate that another biotransformation path was hydrolysis of the 8,13-epoxide and a further diol oxidation process. The low recovery of products from the biotransformation may be related to their further degradation, which could explain why a shorter incubation (24 h) scheme yielded a much larger array of metabolites (13) than the longer one (48 h, eight metabolites).

Experimental Section

General Experimental Procedures. Melting points were measured using a Kofler block Reicher-Jung apparatus and are uncorrected. Optical rotations were determined using a Perkin-Elmer 241 polarimeter. IR spectra were recorded using a Perkin-Elmer 881 spectrophotometer. ^{1}H and ^{13}C NMR measurements (δ in ppm) were obtained using Varian Gemini 200 and Varian Unity 400 NMR spectrometers with $SiMe_4$ as the internal reference. Mass spectra were recorded using VG12-250 and VG Autospec. spectrometers. HPLC was performed using a Hitachi/Merck L6200 apparatus equipped with differential refractometer detector (RI-71). TLC was performed using Merck Kieselgel 60 F_{254}, 0.2 mm layer thickness. Silica gel (Merck) was used for column chromatography. Purification by HPLC was achieved using a Si gel column (LiChrospher Si 60 5 μm, 0.4 cm wide, 25 cm long).

Organism. The culture of *B. cinerea* 2100 employed was obtained from the "Centro Español de cultivos tipos" (CECT), Facultad de Biologia, Universidad de Valencia, Spain, where a culture of this strain is deposited.

Biotransformation Experiments. In procedure I, *B. cinerea* was cultivated at 25 °C and 250 rpm in a 500 mL Erlenmeyer flask containing Czapeck Dox medium. Biotransfomation experiments were carried out in two different ways. In the first experiment, a 48 h culture was used as the inoculum. The organism was grown in 14 × 500 mL Erlenmeyer flasks and transferred, after 48 h, into 50 × 500 mL flasks containing 160 mL of Czapeck Dox medium. These were then incubated for an additional 48 h. The culture medium and mycelia was separated by filtration. The mycelia was washed with 0.9% NaCl solution and distributed into 50 × 500 mL Erlenmeyer flasks, each containing 100 mL of Czapeck Dox medium without glucose, 100 mL of phosphate buffer, and the substrate (20 mg of **1** dissolved in 250 μL of EtOH).

After another 48 h, the culture medium and mycelia were separated by filtration, and the medium was extracted with pentane. The organic solvent was washed with water, dried over Na_2SO_4, and evaporated. The crude extracts were separated on Si-60 columns with a pentane/ethyl acetate mixture. Fractions collected were purified further by semipreparative HPLC when necessary.

In the second experiment (procedure II) 31 × 500 mL Erlenmeyer flasks, each containing 200 mL of Czapeck Dox medium, were inoculated with *B. cinerea* and incubated for 72 h. The medium and mycelia were then separated by filtration, and the mycelia was transferred to 31 × 500 mL flasks, each containing 200 mL of Czapeck Dox medium without glucose, and the substrate (20 mg of **1** dissolved in 250 μL of EtOH) was added and incubated for 24 h. The culture medium and mycelia were then separated, and the medium was extracted and purified as described previously for experiment one.

Biotransformation of Caryophyllene Oxide (1). Procedure I. Biotransformation of 1 g of compound **1** with *B. cinerea 2100* gave, after 48 h, **2**[7] (1 mg, 0.1%), **3** (10 mg, 1%), **4** (1 mg, 0.1%), **5** (1.5 mg, 0.15%), **6**[7,8] (2 mg, 0.2%), **7** (1 mg, 0.1%), **8**[7] (1.5 mg, 0.15%), and **9** (2 mg, 0.2%).

(4*R*,5*R*,8*R*)-4,5:8,13-Diepoxycaryophyllane (3): mp 72−73 °C, $[\alpha]^{19}_D$ −67° (*c* = 1, hexane); IR (film) ν_{max} 2953, 2860, 1458, 1386 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.07 (1H, dd, *J* = 10.2, 4.6 Hz, H-5), 2.66 (1H, dd, $J_{13'-13}$ = 5.2 Hz, *J* = 0.8 Hz, H-13), 2.55 (1H, d, $J_{13-13'}$ = 5.2 Hz, H-13'), 2.16 (1H, m, H-6β), 2.13−2.02 (3H, m, H-2, H-7″ and H-9), 1.76 (1H, t, *J* = 9.6 Hz, H-1), 1.65 (1H, ddd, $J_{3\alpha-3\beta}$ = 14.0 Hz, *J* = 4.1, 3.9 Hz, H-3α), 1.58−1.47 (3H, m, H-7β, H-10 and H-10'), 1.46−1.36 (1H, m, H-2'), 1.26 (s, 3H, H-12), 1.24 (1H, m, H-6α), 1.02 (1H, ddd, $J_{3\beta-3\alpha}$ = 14.0 Hz, *J* = 13.2, 4.4 Hz, H-3β), 0.94 (s, 3H, H-14), 0.92 (3H, s, H-15); ^{13}C NMR data, Table 1; EIMS *m*/*z* 236 $[M]^+$ (2), 221 $[M - CH_3]^+$ (5), 205 (4), 179 (6), 161 (6), 150 (8), 147 (14), 137 (26), 123 (32), 108 (100); HRMS 236.1769 (calcd for $C_{15}H_{24}O_2$ 236.1776).

(4*R*,5*R*,8*R*)-4,5-Epoxycaryophyllan-8-ol (4): mp 99 °C; $[\alpha]^{20}_D$ −132° (*c* = 1, hexane); IR (film) ν_{max} 3476, 2952, 2858, 1456, 1367, 1263, 1119 cm^{-1}; ^{1}H NMR (C_6D_6, 400 MHz) δ 3.34 (1H, dd, $J_{5-6\alpha}$ = 9.3 Hz, $J_{5-6\beta}$ = 5.6 Hz, H-5), 2.28 (1H, dddd, 1H, $J_{6\beta-5}$ = 5.6 Hz, $J_{6\beta-6\alpha}$ = 12.9 Hz, $J_{6\beta-7\alpha}$ = 5.4 Hz, $J_{6\beta-7\beta}$ = 5.0 Hz, H-6β), 1.95 (1H, ddd, *J* = 12.3, 4.0, 2.5 Hz, H-3α), 1.83

Table 1. ^{13}C NMR Data (δ) of Compounds **3**−**5**, **7**, **9**−**11**, and **14**−**17** (100 MHz, $CDCl_3$, **4** in C_6D_6)

C	3	4	5	7	9	10	11	14	15	16	17
1	47.9	45.9	49.3	57.3	51.6	48.4	57.7[a]	50.8	46.1	42.4[a]	47.2
2	27.5	28.5	27.3	23.4	26.3	26.1	26.3	27.7	27.8	21.1	25.0
3	40.3	40.9	39.5	40.3	39.3	39.5	39.1	39.8[a]	40.1	38.4	40.5
4	58.4[a]	58.5	58.9[a]	84.8	59.5	59.3	59.3	59.0	59.3	60.3	58.4
5	61.8	60.7	62.6	79.2	63.5	55.0	57.8[b]	57.5	61.6	65.7	60.1
6	25.5	25.4	24.8	43.5	30.3	40.8	42.8	34.7	28.0	27.2	29.0
7	30.4	36.1	31.3	78.0	29.8	214.0	214.0	69.3	29.9	29.0	35.5
8	57.9[b]	71.7	59.8[b]	158.2	151.7	64.2	156.2	61.1	53.1	49.9	74.1
9	46.8	52.6	47.1	40.6	48.3	39.7	40.8	42.9	45.5	42.0[b]	52.5
10	35.1	38.6	35.5	35.4	35.2	33.4	37.5	33.8[b]	39.5	34.2	40.7
11	33.3	32.1	33.4	34.7	38.6	33.6	33.5	32.9	34.1	35.1	31.8
12	16.4	16.4	16.2	22.1	16.9	16.2	16.2	16.3	16.3	17.5	16.5
13	56.0	31.8	50.1	102.9	112.9	50.1	112.1	45.0	66.4	66.4	20.7
14	29.4[a]	29.5[a]	29.9[a]	29.9[a]	24.8	29.0[a]	29.7	30.0	29.8	29.9	23.3[a]
15	21.6[b]	22.6[b]	21.9[b]	21.8[b]	67.0	21.7[b]	22.1	22.7	21.8	21.3	30.1[b]

[a,b] Assignments may be interchanged.

(1H, dd, J_{1-9} = 8.3 Hz, J = 8.9 Hz, H-1), 1.57 (1H, dd, J_{9-1} = 8.3 Hz, J = 9.5 Hz, H-9), 1.54−1.42 (3H, m, H-7α, H-10 and H-10'), 1.45−1.39 (1H, m, H-2α), 1.29 (1H, ddd, $J_{7\beta-6\beta}$ = 5.0 Hz, J = 14.0, 13.0 Hz, H-7*β*), 1.24−1.16 (2H, m, H-6α and H-2*β*), 1.15 (3H, s, H-12), 1.04 (1H, m, H-3*β*), 0.88 (3H, s, H-15*β*), 0.85 (3H, s, H-14α), 0.81 (3H, s, H-13α); ^{13}C NMR data, Table 1; EIMS *m*/*z* 223 $[M - CH_3]^+$, 220 $[M - H_2O]^+$ (0.1), 205 $[M - H_2O - CH_3]^+$ (0.7), 177 (8), 149 (7), 141 (6), 121 (21), 109 (26), 95 (50) 43 (100); HRMS *m*/*z* 223.16675 (calcd for $C_{14}H_{23}O_2$ 223.1698).

(4*R*,5*R*,8*S*)-4,5:8,13-Diepoxycaryophyllane (5): mp 57−58 °C; $[\alpha]^{19}_D$ 46° (*c* = 1, hexane); IR (film) ν_{max} 2922, 2862, 1461, 1386, 1370 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.87 (1H, dd, $J_{5-6\alpha}$ = 10.5 Hz, $J_{5-6\beta}$ = 4.4 Hz, H-5), 2.67 (1H, d, $J_{13'-13}$ = 4.6 Hz, H-13), 2.65 (1H, d, $J_{13-13'}$ = 4.6 Hz, H-13'), 2.11 (1H, m, H-9), 2.05 (1H, dddd, $J_{6\beta-5}$ = 4.4 Hz, $J_{6\beta-6\alpha}$ = 13.9 Hz, $J_{6\beta-7}$ = 9.7 Hz, $J_{6\beta-7'}$ = 5.2 Hz, H-6*β*), 1.93 (1H, ddd, $J_{7'-6\alpha}$ = 5.8 Hz, $J_{7'-6\beta}$ = 5.2 Hz, $J_{7'-7}$ = 14.3 Hz, H-7), 1.76 (1H, ddd, $J_{7-6\alpha}$ = 4.9 Hz, $J_{7-6\beta}$ = 9.7 Hz, $J_{7-7'}$ = 14.3 Hz, H-7'), 1.65 (2H, m, H-1 and H-2), 1.56 (1H, dd, $J_{10'-9}$ = 9.5 Hz, $J_{10'-10}$ = 11.9 Hz, H-10) 1.43 (1H, dddd, 1H, $J_{6\alpha-5}$ = 10.5 Hz, $J_{6\alpha-6\beta}$ = 13.9 Hz, $J_{6\alpha-7}$ = 4.9 Hz, $J_{6\alpha-7'}$ = 5.8 Hz, H-6α), 1.25 (1H, dd, J_{10-9} = 11.0 Hz, $J_{10-10'}$ = 11.9 Hz, H-10'), 0.99 (1H, m, H-3*β*), 0.95 (3H, s, H-14*), 0.93 (3H, s, H-15*); ^{13}C NMR data, Table 1; EIMS *m*/*z* 236 $[M]^+$ (0.1), 221 $[M - CH_3]^+$ (1), 175 (2), 163 (7), 133 (11), 121 (29), 108 (43), 107 (3), 95 (42), 93 (77); HRMS *m*/*z* 236.1774 (calcd for $C_{15}H_{24}O_2$ 236.1776).

(4*R*,5*R*,7*S*)-4,7-Epoxycaryophyll-8(13)-en-5-ol (7): mp 129−130 °C; $[\alpha]^{25}_D$ −32° (*c* = 1, ethyl acetate); IR (film) ν_{max} 3410, 2926, 2864, 1462, 1105, 1068 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.90 (1H, dd, $J_{7-6\alpha}$ = 8.2 Hz, $J_{7-6\beta}$ = 9.0 Hz, H-7), 4.58 (2H, bs, H-13), 3.81 (1H, t, J = 5.1 Hz, H-5), 2.94 (1H, ddd, J = 11.0, 10.7, 10.0 Hz, H-9), 2.31 (1H, ddd, J = 14.0, 8.0, 7.8 Hz, H-6α), 2.06 (1H, ddd, J = 9.0, 4.4 Hz, H-6*β*), 1.87 (1H, ddd, J = 14.2, 7.6, 6.4 Hz, H-3α), 1.65−1.57 (2H, m, H-10), 1.47 (1H, m, H-2), 1.36 (1H, dddd, J = 13.7, 7.6, 1.9, 1.7 Hz, H-2), 1.27 (3H, s, H-15α), 0.97 (3H, s, H-14*β*); ^{13}C NMR data, Table 1; EIMS *m*/*z* 236 $[M]^+$ (18), 203 $[M - H_2O - CH_3]^+$ (2), 108 (58), 93 (49), 79 (68), 43 (100); HRMS *m*/*z* 236.1748 (calcd for $C_{15}H_{24}O_2$ 236.1776).

(4*R*,5*R*,11*S*)-4,5-Epoxycaryophyllan-8(13)-en-14-ol (9): colorless oil; $[\alpha]^{26}_D$ −65° (*c* = 1.7, ethyl acetate); IR ν_{max} 3432, 2925, 2863, 1631, 1457, 1387, 1257, 1041, 909, 863, 758, 734 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.00 (1H, bs, H-13), 4.89 (1H, bs, H-13'), 3.70 (1H, d, $J_{14'-14}$ = 10.8 Hz, H-14), 3.61 (1H, d, $J_{14-14'}$ = 10.8 Hz, H-14'), 2.85 (1H, dd, J = 10.5, 4.4 Hz, H-5), 2.66 (1H, dd, J = 9.6, 9.3 Hz, H-9), 2.35 (1H, ddd, $J_{7'-7}$ = 12.6 Hz, J = 7.4, 5.0 Hz, H-7), 2.23 (1H, m, H-6), 2.12−2.06 (2H, m, H-3 and H-7'), 1.94 (1H, m, H-1), 1.84 (1H, m, H-2*β*), 1.59 (1H, t, J = 11.0 Hz, H-10), 1.53 (1H, m, H-2α), 1.32 (1H, m, H-6'), 1.20 (3H, s, H-12), 1.07 (3H, s, H-15), 0.94 (1H, ddd, $J_{3-3'}$ = 13.4 Hz, J_{3-2} = 13.4, 5.0 Hz, H-3'); ^{13}C NMR data, Table 1; EIMS *m*/*z* 236 $[M]^+$ (0.5), 218 $[M - H_2O]^+$ (1), 205 $[M - CH_2OH]^+$ (11), 193 (11), 187 (18), 177 (17), 161 (34), 121 (35), 107 (46), 93 (100); HRMS *m*/*z* 236.1768 (calcd for $C_{15}H_{24}O_2$ 236.1776).

Biotransformation of 620 mg of Caryophyllene Oxide (1). Procedure II. Biotransformation of 620 mg of **1** with *B. cinerea 2100* gave, after 24 h, **3** (50 mg, 8%), **5** (4 mg, 0.6%), **7** (2 mg, 0.3%), **8**[7] (8 mg, 1.2%), **9** (12 mg, 1.8%), **10** (1 mg, 0.15%), **11** (1 mg, 0.15%), kobusone (**12**,[7] 2 mg, 0.3%), **13**[7] (1 mg, 0.15%), **14** (1.5 mg, 0.24%), **15** (8 mg, 1.2%), and **16** (8 mg, 1.2%).

(4*R*,5*R*,8*R*)-4,5:8,13-Diepoxycaryophyllan-7-one (10): colorless oil; $[\alpha]^{26}_D$ −16° (*c* = 1, hexane); IR (film) ν_{max} 2927, 2859, 1723, 1461, 1260 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.48 (1H, dd, J_{5-6} = 8.8 Hz, $J_{5-6'}$ = 6.6 Hz, H-5), 3.31 (1H, dd, $J_{6'-5}$ = 6.6 Hz, $J_{6'-6}$ = 13.0 Hz, H-6), 2.85 (1H, d, $J_{13'-13}$ = 4.6 Hz, H-13), 2.77 (1H, q, J_{9-1} = 9.6 Hz, H-9), 2.70 (1H, d, $J_{13-13'}$ = 4.6 Hz, H-13'), 2.13 (1H, ddd, $J_{3'-3}$ = 12.9 Hz, J = 4.2, 2.8 Hz, H-3), 2.05 (1H, dd, J_{6-5} = 8.8 Hz, $J_{6-6'}$ = 13.0 Hz, H-6), 1.80 (1H, t, J_{1-9} = 9.6 Hz, H-1), 1.59 (1H, m, H-2), 1.48 (1H, dd, $J_{10'-9}$ = 8.0 Hz, $J_{10'-10}$ = 10.3 Hz, H-10), 1.41 (1H, m, H-2), 1.17 (3H, s, H-12), 1.17 (1H, dd, J_{10-9} = 10.5 Hz, $J_{10-10'}$ = 10.3 Hz, H-10'), 1.00 (1H, m, H-3'), 0.97 (3H, s, H-14*), 0.92 (3H, s, H-15*); ^{13}C NMR data, Table 1; EIMS *m*/*z* 250 $[M]^+$ (0.2), 235 $[M - CH_3]^+$ (5), 221 (52), 149 (24), 123 (24), 43(100).

(4*R*,5*R*)-4,5-Epoxycaryophyll-8(13)-en-7-one (11): colorless oil; $[\alpha]^{25}_D$ −40° (*c* = 1, hexane); IR (film) ν_{max} 2931, 2859, 1709, 1457, 1261 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.40 (1H, bs, H-13), 5.24 (1H, bs, H-13'), 3.35 (1H, dd, J_{5-6} = 14.1 Hz, $J_{5-6'}$ = 6.6 Hz, H-5), 3.06 (1H, dd, $J_{6'-5}$ = 6.6 Hz, $J_{6'-6}$ = 9.3 Hz, H-6), 2.78 (1H, ddd, J_{9-1} = 4.6 Hz, J_{9-10} = 8.7 Hz, $J_{9-10'}$ = 9.6 Hz, H-9), 1.87 (1H, dd, $J_{10'-9}$ = 9.6 Hz, $J_{10-10'}$ = 11.1 Hz, H-10), 1.79 (1H, dd, J_{10-9} = 8.7 Hz, $J_{10-10'}$ = 11.1 Hz, H-10′), 1.61−1.31 (3H, m, H-1, H-2′ and H-3), 1.02 and 1.01 (3H each, s, H-14* and H-15*), 0.92 (1H, m, H-3'); ^{13}C NMR data, Table 1; EIMS *m*/*z* 219 $[M - CH_3]^+$ (2), 205 (10), 191 (11), 163 (10), 49 (11), 135 (13), 43(100); HRMS *m*/*z* 219.1388 (calcd for $C_{14}H_{19}O_2$ 219.1385).

(1*S*,9*R*,11*R*)-1,9-Epoxycaryolan-11-ol (14): oil; $[\alpha]^{25}_D$ −30° (*c* = 1, ethyl acetate); IR (film) ν_{max} 3461, 2954, 2922, 2862, 1462, 1366, 1080, 1031, 903 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.89 (1H, b dd, J_{7-6} = 8.2, 7.8 Hz, H-7), 2.96 (1H, d, $J_{13'-13}$ = 4.1 Hz, H-13), 2.90 (1H, d, $J_{13-13'}$ = 4.1 Hz, H-13'), 2.77 (1H, d, J = 1.4 Hz, O*H*), 2.73 (1H, dd, J_{5-6} = 8.2, 7.1 Hz, H-5), 2.42 (1H, q, $J_{9-10\beta}$ = 9.5 Hz, H-9), 2.16 (1H, ddd, $J_{3\alpha-3\beta}$ = 13.0 Hz, J = 3.7, 3.1 Hz H-3α), 1.93 (2H, dd, J_{6-5} = 8.2 Hz, J_{6-7} = 7.8 Hz, H-6), 1.67−1.47 (3H, m, H-2, H-10α, H-2'), 1.34 (3H, s, H-12), 1.25 (1H, dd, J = 11.9, 9.5 Hz, H-10*β*), 0.98 (3H, s, H-14*), 0.94 (1H, m, H-3'), 0.93 (3H, s, H-15*); ^{13}C NMR data, Table 1; EIMS *m*/*z* 237 $[M + 1]^+$ (0.1), 149 (1), 135 (3), 119 (3), 109 (11), 93 (10), 81 (14), 69 (18), 55 (40), 43 (100); HRMS *m*/*z* 237.1882 (calcd for $C_{15}H_{25}O_2$ 237.18545).

(4*R*,5*R*,8*R*)-4,5-Epoxycaryophyllan-13-ol (15): mp 88 °C; $[\alpha]^{25}_D$ −50° (*c* = 3.5, ethyl acetate); IR (film) ν_{max} 3236, 2950, 2882, 1456, 1068, 1034 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.56 (1H, dd, $J_{13'-8}$ = 3.2 Hz, $J_{13'-13}$ = 10.5 Hz, H-13), 3.29 (1H, dd, J_{13-8} = 7.2 Hz, $J_{13-13'}$ = 10.5 Hz, H-13'), 3.01 (1H, dd, J = 9.6, 5.2 Hz, H-5), 2.11 (1H, ddd, $J_{3'-3}$ = 12.6 Hz, J = 4.3, 2.7 Hz, H-3), 1.78 (1H, m, H-9), 1.64 (1H, dt, $J_{2'-2}$ = 14.9 Hz, $J_{2'-3}$ = 3.6 Hz, H-2), 1.55 (1H, dd, J = 8.7, 9.4 Hz, H-1), 1.48

(1H, m, H-8), 1.42 (1H, m, H-2'), 1.35 (1H, dd, $J = 9.8$, 9.6 Hz, H-10), 1.28 (3H, s, H-12), 1.28 (2H, m, H-10′ and H-12), 1.00 (1H, ddd, $J_{3-2} = 13.5$ Hz, $J_{3-3'} = 12.6$ Hz, $J_{3-2'} = 3.6$ Hz, H-3'), 0.93 and 0.92 (3H each, s, H-14 and H-15); ^{13}C NMR data, Table 1; EIMS *m*/*z* 223 $[M - CH_3]^+$ (0.4), 207 $[M - CH_2OH]^+$ (0.5), 177 $[M - CH_2OH - 2 \times CH_3]^+$ (0.5), 167 (4), 151 (9), 133 (15), 121 (18), 93 (100); HRMS *m*/*z* 233.1702 (calcd for $C_{14}H_{23}O_2$ 223.1698).

(4*R*,5*R*,8*S*)-4,5-Epoxycaryophyllan-13-ol (16): mp 72−73 °C; $[\alpha]^{25}_D$ −31° (*c* = 1.6, hexane); IR (film) ν_{max} 3425, 2930, 2860, 1461, 1388, 1367, 1067, 1041 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.24 (2H, m, H-13), 2.92 (1H, dd, $J = 11.4$, 3.5 Hz, H-5), 2.29 (1H, m, H-9), 2.23 (1H, m, H-6β), 2.06 (1H, ddd,, $J_{3'-3} = 12.7$ Hz, $J_{3'-2} = 4.5$, 2.1 Hz, H-3), 1.79 (1H, t, $J = 9.8$ Hz, H-1), 1.70 (1H, ddd, $J_{2-2} = 14.5$ Hz, $J_{2-3} = 6.0$ Hz, $J_{2-3'} = 2.1$ Hz, H-2), 1.66 (1H, b dd, $J = 13.9$, 7.0 Hz, H-8) 1.45 (dd, $J_{10\beta-10\alpha} = 10.0$ Hz, $J_{10\beta-9} = 7.7$ Hz, H-10β), 1.32 (m, 1H, H-2'), 1.27 (3H, s, H-12), 1.26 (1H, dd, H-10α), 1.18 (1H, m, H-6α), 0.99 (1H, m, H-3'), 0.97 (3H, s, H-14α), 0.94 (3H, s, H-15β); ^{13}C NMR data, Table 1; EIMS *m*/*z* 223 $[M - CH_3]^+$ (0.5), 207 $[M - CH_2OH]^+$ (0.5), 151 (7), 123 (7), 121 (17), 107 (23), 43 (100); HRMS *m*/*z* 223.1687 (calcd for $C_{14}H_{23}O_2$ 223.1698).

Epoxidation of Caryophyllene Oxide (1): Caryophyllene oxide (**1**) (77 mg) dissolved in Et_2O, was treated with 60 mg of *m*-chloroperbenzoic acid. The reaction mixture was stirred at room temperature for 7 h, a solution of Na_2SO_3 10% w/v was then added, and the mixture was extracted three times with Et_2O. The organic layer was washed with brine and dried over anhydrous Na_2SO_4, and the solvent was evaporated under reduced pressure. The reaction mixture was purified by column chromatography to give 43 mg of caryophyllene oxide (**1**) and 28 mg of a mixture of (4*R*,5*R*,8*R*)-4,5:8,13-diepoxycaryophyllane (**3**) and (4*R*,5*R*,8*S*)-4,5:8,13-diepoxycaryophyllane (**5**) (yield 71%). The epimeric compounds **3** and **5** were separated by HPLC using hexane/ethyl acetate (95:5) to give 11 mg (28%) of **3** and 5 mg (13%) of **5**.

Reduction of Diepoxides 3 and 5. Both compounds were treated with a 1 M solution of $LiAlH_4$ in Et_2O. In each case, the reaction mixture was stirred under an N_2 atmosphere for 2 h. Water was then added, and the reaction mixture was extracted in the usual way. Purification of the reaction mixture by column chromatography yielded 32 mg of starting material together with 43 mg of (4*R*,5*R*,8*R*)-4,5-epoxycaryophyllan-8-ol (**4**) and 10 mg of (4*R*,5*R*,8*S*)-4,5-epoxycaryophyllan-8-ol (**17**) (yield 98%).

(4*R*,5*R*,8*S*)-4,5-Epoxycaryophyllan-8-ol (17): mp 79−80 °C; $[\alpha]^{20}_D$ −91° (*c* = 1, hexane); IR (film) ν_{max} 3435, 2949, 1656, 1465, 1104, 1067 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.54 (1H, dd, $J_{5-6\alpha} = 9.5$ Hz, $J_{5-6\beta} = 5.6$ Hz, H-5), 1.96 (1H, dddd, $J_{6\beta-5} = J_{6\beta-7\beta} = 5.6$ Hz; $J_{6\beta-7\alpha} = 5.9$ Hz, H-6β), 1.90 (1H, ddd, $J_{3\alpha-2} = 3.6$ Hz, $J_{3\alpha-2'} = 3.3$ Hz, $J_{3\alpha-3\beta} = 12.8$ Hz, H-3α), 1.78 (1H, ddd, $J_{9-1} = 8.3$ Hz, $J_{9-10\alpha} = 9.2$ Hz, $J_{9-10\beta} = 10.2$ Hz, H-9), 1.68 (1H, ddd, $J_{7\beta-6\alpha} = 2.6$ Hz, $J_{7\beta-6\beta} = 5.6$ Hz, $J_{7\beta-7\alpha} = 13.8$ Hz, H-7β), 1.54 (1H, dd, $J_{10\alpha-9} = 9.2$ Hz, $J_{10-10\beta} = 11.2$ Hz, H-10α), 1.49 (1H, ddd, $J_{7\alpha-6\alpha} = 5.3$ Hz, $J_{7\alpha-6\beta} = 5.9$ Hz, $J_{7\alpha-7\beta} = 13.8$ Hz, H-7α), 1.47 (1H, dd, $J_{10\beta-9} = 10.2$ Hz, $J_{10\beta-10\alpha} = 11.2$ Hz, H-10β), 1.33 (1H, m, H-2α), 1.30 (1H, m, H-1), 1.23 (1H, m, H-6α), 1.15 (1H, m, H-2β), 1.11 (3H, s, H-12), 1.00 (3H, s, H-13), 0.86 (1H, m, H-3β), 0.83 (3H, s, H-14), 0.82 (3H, s, H-15); ^{13}C NMR data, Table 1; EIMS *m*/*z* 238 $[M]^+$ (0.1), 177 (4), 149 (6), 121 (20), 95 (30), 91 (11), 81 (52); HRMS 238.1939 (calcd for $C_{15}H_{26}O_2$ 238.1933).

References and Notes

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