Biotransformation of (4*E*,8*R*)-Caryophyll-4(5)-en-8-ol by *Botrytis cinerea*

Rosa Durán-Patrón, Josefina Aleu, Rosario Hernández-Galán, and Isidro G. Collado*

Departamento de Química Orgánica, Facultad de Ciencias, Universidad de Cádiz, Apdo. 40, 11510 Puerto Real, Cádiz, Spain

Received June 21, 1999

Biotransformation of (4*E*,8*R*)-caryophyll-4(5)-en-8-ol (**1**) with *Botrytis cinerea* afforded 14 products (**3**−**16**). Thirteen of these (**4**−**16**) are described here for the first time. The main reaction paths involved the isomerization of the double bond at C-4/C-5 and hydroxylation of methyl groups.

Botrytis species are potent pathogens implicated in many diseases of flowers, fruits, and vegetables. In particular, *Botrytis cinerea* attacks economically important crops such as carrots, grapes, lettuce, strawberries, and tobacco.[1] The rapid development of tolerance to commercial fungicides by *B. cinerea* has led to an increase in the quantities of these compounds that must be used, their persistence in the environment, and serious economic damage arising from the decreased quality of wines produced from treated grapes.[2]

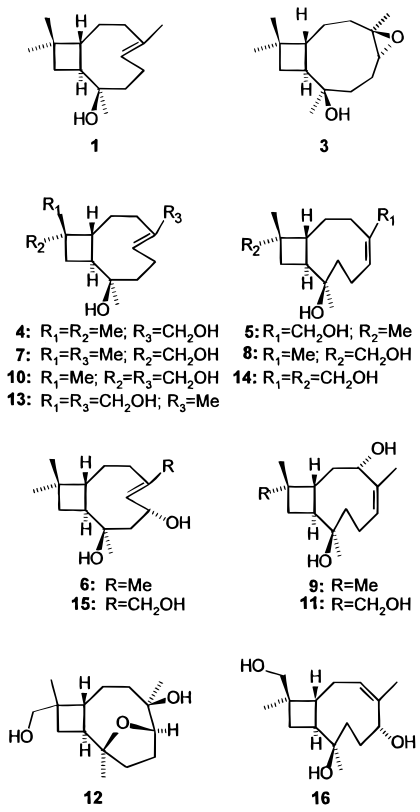
Over the past few years we have undertaken a research program directed toward the rational design of fungicides for *Botrytis* infections of commercial crops based on biosynthetic principles. Botrydial and structurally related compounds are characteristic metabolites of *Botrytis* species. Botrydial is a bicyclic nonisoprenoid sesquiterpene, which was isolated from cultures of *B. cinerea*,[3] and its biosynthesis has been investigated.[4] The first stages involve formation and cyclization of the caryophyllene cation at C-8. In the context of our studies on the fate of compounds having the caryophyllene skeleton in the metabolism of *B. cinerea*,[5] we have undertaken the biotransformation of the potential intermediate, (4*E*,8*R*)-caryophyll-4(5)-en-8-ol (**1**).

Results and Discussion

Compound **1** was prepared in 23% overall yield from caryophyllene oxide (**2**), as outlined in Scheme 1, by a modification of the method of Kaiser and Lamparsky.[6]

Compound **1** was incubated with *B. cinerea* following methodology previously described by our group.[5] Fourteen metabolites (**3**−**16**), which were not present in a control fermentation, were detected by TLC. The metabolites were extracted from the medium with pentane and ethyl acetate. Both extracts were purified by chromatography to yield these biotransformation products; seven (**3**−**9**) were obtained from the pentane extract and another seven (**10**−**16**) from the ethyl acetate extract.

Epoxidation at the double bond gave **3**, a product that was previously obtained in the biotransformation of caryophyllene oxide by *B. cinerea*.[5] Compounds **4** and **5**, both of which are monohydroxylated at the C-4 methyl group, were isolated from the pentane extract. The absence of the methyl group signals assigned to C-12 in the ^{1}H and ^{13}C NMR spectra and the appearance of new hydroxymethyl resonances (δ_H 3.51 and 4.41; δ_C 61.4 for **4** and δ_H 3.97; δ_C 68.1 for **5**) suggested that both compounds are hydroxylated at C-12. However, the ^{1}H NMR spectra were significantly different; while the ^{1}H NMR spectrum for compound **5** was perfectly resolved at room temperature, the spectrum of compound **4** had to be acquired at −50 °C. These differences are observed in the spectra of caryophyllene and isocaryophyllene, indicating that compounds **4** and **5** are the trans and cis derivatives, respectively. The stereochemistry of the double bonds was confirmed when signals corresponding to the hydroxymethyl groups were irradiated and an NOE effect was observed in the signal assigned to the vinylic proton in compound **5**.



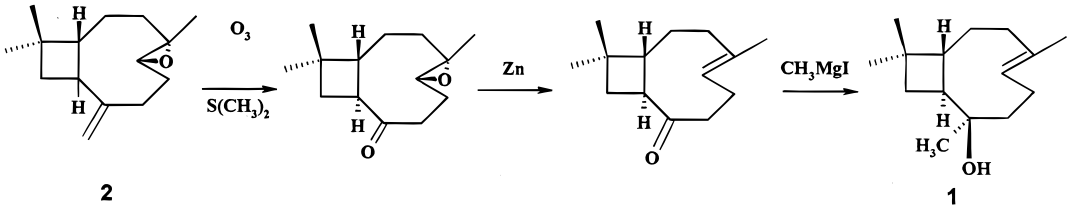
Further hydroxylation of compound **4** gave **10** and **13**. The stereochemistry of the hydroxymethyl group at C-11 in **13** was determined by NOE experiments, which showed an enhancement for the signal corresponding to H-9 when the signal assigned to the methyl group at C-11 was irradiated. This enhancement is only possible if the hydroxymethyl group has β stereochemistry in compound **13**, and, therefore, α stereochemistry was assigned for **10**.

Comparison of the spectroscopic data of compounds **14** and **5**, which showed a similar relationship to that observed between **10** and **13** with **4**, indicates that **14** is the hydroxymethyl derivative of **5**. NOE experiments revealed an enhancement of the signal corresponding to H-9 when both signals corresponding to the CH_2OH group (δ_H 3.30

* To whom correspondence should be addressed. Tel.: 34-956-016371. Fax: 34-956-016288. E-mail: isidro.gonzalez@uca.es.

10.1021/np990296q CCC: $19.00
Published on Web 12/31/1999

Scheme 1. Synthesis of (4*E*,8*R*)-Caryophyll-4(5)-en-8-ol (**1**)



2 **1**

and 3.36) were irradiated, confirming an α configuration for the hydroxymethyl group. The stereochemistry of the double bond was confirmed when an NOE effect was observed between the signal corresponding to H-5 and both H-12 signals.

Compounds **7** and **8**, products of monohydroxylation at the *gem*-dimethyl group, were also isolated. Their structures were assigned by comparison of their NMR spectra with that of the starting material. NOE interactions between the methyl group at C-11 and H-1 in compound **7**, indicated the *S* configuration for C-11. A qualitative analysis of the NOE experiments in compound **8** showed interactions between H-12,H-5 and both H-14 and H-9, supporting the proposed structure.

Another biotransformation path was noted by oxidation of allylic methylenes. Compounds **6** and **9** were hydroxylated at C-6 and C-3, respectively. The absolute configurations of the compounds were established through a full set of NOE experiments. The stereochemistry of the double bond in compound **9** was assigned as *Z* as a consequence of the NOE effect observed between the signals corresponding to H-5 and H-12. In addition, the enhancement of the H-1 signal when H-3 was irradiated indicated an α configuration for the hydroxyl group on C-3. NOE interactions observed between H-6,H-12 and H-5,H-9 for compound **6** indicated the *E* disposition for the double bond and the *R* configuration for C-6. The absence of a signal corresponding to one of the methyl groups indicated that **11** and **15** were further hydroxylated. The observation of an NOE effect between H-9 and both H-14's, together with the downfield shift observed for the signal assigned to H-9 when the compound was acetylated, confirmed the α orientation for hydroxymethyl group in **11**.

The ^{1}H NMR spectrum of compound **16**, the major metabolite isolated, showed a broad triplet at δ_H 5.41, which is characteristic of a vinylic proton and was assigned to H-3 following the correlations observed in the COSY experiment. The ^{13}C NMR spectrum confirmed the presence of a double bond whose disposition was assigned as *Z* as a consequence of the NOE that was observed for the ^{1}H NMR signal corresponding to H-12 when H-3 was irradiated. The location of the secondary hydroxyl group at C-5 followed from the correlation observed in the COSY experiment between the proton geminal to the hydroxyl group and H-3. The absence of a methyl group, together with the presence of a hydroxymethylene group (δ_H 3.26 and 3.30; δ_C 70.4), indicated that hydroxylation at a methyl group had occurred. NOE experiments revealed an enhancement of the signal corresponding to H-1 when H-15 was irradiated, indicating an *R* configuration for C-11. In addition, when H-1 was irradiated an enhancement of the signal corresponding to H-5 was produced, which is only possible in the case of an α disposition of the hydroxyl group.

Compound **3** could be the precursor of **12**, which can result from the intramolecular attack of the hydroxyl group at C-8 on the 4,5-epoxide and further hydroxylation of the *gem*-dimethyl group. Comparison of the signals due to H-9 and H-1 with those of other hydroxymethyl derivatives seems to indicate a β disposition for the hydroxymethyl group. This could not be confirmed by NOE experiments due to the instability of this compound.

In conclusion, the biotransformation of (4*E*,8*R*)-caryophyll-4(5)-en-8-ol (**1**) produced a number of hydroxylated compounds. The major biotransformation pathways involved double-bond isomerization and hydroxylation at methyl groups. A delay in the fungal growth and a decrease in the production of the major metabolite, dihydrobotrydial, was observed. These results may be put in context in terms of the slight fungistatic activity shown for **1** and related compounds against *B. cinerea*.[7,8]

Experimental Section

General Experimental Procedures. Melting points were measured with a Reicher−Jung Kofler apparatus and are uncorrected. Optical rotations were determined with a Perkin−Elmer 241 polarimeter; $[\alpha]_D$ values are given in 10 deg cm^{-2} mg^{-1}. IR spectra were recorded on a Perkin−Elmer 881 spectrophotometer. ^{1}H and ^{13}C NMR measurements were obtained on Varian Gemini 200 and Varian Unity 400 NMR spectrometers with $SiMe_4$ as the internal reference. *J* values are given in Hertz. Mass spectra were recorded using a VG 12-250 and a VG Autospec spectrometer at 70 eV. HPLC was performed with a Hitachi/Merck L-6270 apparatus equipped with a 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 9385) was used for column chromatography. Purification by HPLC was accomplished using a Si gel column (Hibar 60, 7 μm, 1 cm wide, 25 cm long).

Microorganism. The culture of *Botrytis cinerea* employed in this work, *B. cinerea* (2100), was obtained from the "Centro Español de Cultivos Tipos (CECT)", Universidad de Valencia, Facultad de Biologia, Spain, where a culture of this strain is deposited.

Synthesis of (4*E*,8R)-caryophyll-4(5)-en-8-ol (1). Kobusone. Caryophyllene oxide (**2**, 984 mg) was dissolved in 5 mL of dichloromethane, and O_3 was passed through the solution until the reaction was complete (15 min). Me_2S (0.26 mL) was added; the mixture was stirred for 1 h. Removal of the solvent, followed by column chromatography, furnished 593 mg of kobusone[9] (60%).

(4*E*)-13-Norcaryophyll-4(5)-en-8-one. Kobusone (593 mg) dissolved in 15 mL of EtOH was treated with Zn previously activated with a 10% solution of HCl. The reaction mixture was refluxed for 15 min and allowed to cool to room temperature. The solution was filtered, and removal of the solvent, followed by column chromatography, led to (4*E*)-13-norcaryophyll-4(5)-en-8-one[10] (427 mg, 78%).

(4*E*,8*R*)-Caryophyll-4(5)-en-8-ol. A solution of (4*E*)-13-norcaryophyll-4(5)-en-8-one (427 mg) in Et_2O (15 mL) was treated, under a N_2 atmosphere, with 0.7 mL of a 3 M solution of CH_3MgBr in Et_2O. The reaction mixture was stirred at room temperature for 5 h. Then, water was added, and the product was extracted with Et_2O. The organic layer was washed with brine and dried over Na_2SO_4. Removal of the solvent, followed by HPLC, furnished (4*E*,8*R*)-caryophyll-4(5)-en-8-ol[6] (336 mg, 73%).

Biotransformation Experiment. *Botrytis cinerea* (2100) was cultivated at 25 °C and 250 rpm in 500-mL Erlenmeyer flasks containing 200 mL of Czapek−Dox medium.[5] The

Table 1. ^{13}C NMR Data of Compounds **4**, **6**, **7**, and **10** (100 MHz, $CDCl_3$, −50 °C)

position	4	6	7	10
1	48.3 d	47.6 d	50.4 d	48.3 d
2	29.8 t	29.5 t	29.6 t	27.7 t
3	31.6 t	34.2 t	39.4 t	31.6 t
4	135.7 s	136.9 s	136.2 s	134.4 s
5	132.8 d	129.6 d	122.1 d	132.8 d
6	24.5 t	65.5 d	22.3 t	24.2 t
7	44.3 t	52.7 t	40.7 t	43.9 t
8	73.8 s	72.6 s	74.4 s	73.5 s
9	52.7 d	52.1 d	38.4 d	52.1 d
10	37.9 t	37.0 t	29.6 t	32.7 t
11	32.4 s	32.2 s	35.8 s	37.1 s
12	61.4 t	21.1 q	16.1 q	61.3 t
13	30.9 q	29.3 q	30.0 q	30.6 q
14	29.4 q	29.2 q	68.8 t	66.8 t
15	22.1 q	21.7 q	19.1 q	22.1 q

organism was grown in 35 500-mL Erlenmeyer flasks, and the mycelia was transferred, after 72 h, into 30 500-mL flasks containing 200 mL of Czapek−Dox medium (without glucose) and the substrate (100 ppm). An additional five flasks were used as control. After 6 days, the mycelium was filtered, the broth (6 L) was saturated with sodium chloride and extracted with 2 × 3 L of pentane and then with 2 × 3 L of ethyl acetate. Extracts were dried over anhydrous sodium sulfate, and the solvents were evaporated under reduced pressure. The residues were purified by chromatography on a Si gel column and then by HPLC. From the pentane fraction (117 mg) the following compounds were isolated: (4*R*,5*R*,8*R*)-4,5-epoxycaryophyllan-8-ol (**3**, 4.0 mg, 1.1%),[5] (4*Z*,8*R*)-caryophyll-4(5)-ene-8,12-diol (**4**, 6 mg, 1.6%); (4*E*,8*R*)-caryophyll-4(5)-ene-8,12-diol (**5**, 4.5 mg, 1.2%); (4*E*,6*R*,8*R*)-caryophyll-4(5)-ene-6,8-diol (**6**, 5 mg, 1.4%); (4*E*,8*R*,11*S*)-caryophyll-4(5)-ene-8,14-diol (**7**, 7.8 mg, 2.1%); (4*Z*,8*R*,11*S*)-caryophyll-4(5)-ene-8,14-diol (**8**, 5 mg, 1.4%); and (4*Z*,3*S*,8*R*)-caryophyll-4(5)-ene-3,8-diol (**9**, 1 mg, 0.3%). From the ethyl acetate fraction (250 mg) the following compounds were isolated: (4*Z*,8*R*,11*S*)-caryophyll-4(5)-ene-8,-12,14-triol (**10**, 1 mg, 0.3%); (4*Z*,3*S*,8*R*,11*S*)-caryophyll-4(5)-ene-3,8,14-triol (**11**, 1.5 mg, 0.4%); (4*R*,5*S*,8*R*)-5,8-epoxycaryophyllane (**12**, 3 mg, 0.8%); (4*Z*,8*R*,11*R*)-caryophyll-4(5)-ene-8,12,15-triol (**13**, 2.5 mg, 0.7%); (4*E*,8*R*,11*S*)-caryophyll-4(5)-ene-8,12,14-triol (**14**, 1.5 mg, 0.4%); (4*Z*,6*R*,8*R*)-caryophyll-4(5)-ene-6,8,12-triol (**15**, 1 mg, 0.3%); and (3*Z*,5*R*,8*R*,11*R*)-caryophyll-3-ene-5,8,15-triol (**16**, 41 mg, 11.2%).

(4*Z*,8*R*)-Caryophyll-4(5)-ene-8,12-diol (4): colorless solid; mp 60−61 °C; $[\alpha]^{27}_D$ −17° (*c* 3, EtOAc); IR (film) ν_{max} 3328, 1084, 1006 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, −50 °C) δ 0.85 (3H, s, H-14), 0.87 (3H, s, H-15), 1.00 (3H, s, H-13), 2.44 (1H, br d, J = 15.0 Hz, H-7β), 2.60 (1H, dq, J = 12.7, 4.2 Hz, H-6β), 3.51 (1H, d, $J_{12-12'}$ = 10.4 Hz, H-12), 4.41 (1H, d, $J_{12'-12}$ = 10.4 Hz, H-12′), 5.35 (1H, br d, $J_{5-6\beta}$ = 12.7 Hz, H-5); ^{13}C NMR data, Table 1; EIMS *m*/*z* 238 [M^+] (7), 220 [$M^+ - H_2O$] (7), 189 [$M^+ - H_2O - CH_2OH$] (21), 167 (36), 149 (67), 133 (100), 106 (55), 93 (71); HRMS *m*/*z* 238.1934 (calcd for $C_{15}H_{26}O_2$ 238.1933).

(4*E*,8*R*)-Caryophyll-4(5)-ene-8,12-diol (5): colorless solid, mp 113−114 °C, $[\alpha]^{27}_D$ −45° (*c* 1.5, EtOAc); IR (film) ν_{max} 3373, 1077, 1009 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.94 (3H, s, H-15), 0.96, 0.97 (3H each, s, H-13 and H-14), 1.64 (1H, br t, J = 10.2 Hz, H-10), 1.84 (1H, dt, J_{1-9} = 9.4 Hz, J_{1-2} = 2.5 Hz, H-1), 1.91 (1H, ddd, J = 14.6, 9.7, 5.0 Hz, H-6), 2.21 (1H, m, H-3), 2.22 (1H, m, H-6′), 2.00 (1H, br dd, J_{9-1} = 9.4 Hz, J = 8.9 Hz, H-9), 3.97 (2H, br s, H-12), 5.55 (1H, br t, $J_{5-6'}$ = 7.7 Hz, H-5);^{13}C NMR data, Table 2; EIMS *m*/*z* 220 [$M^+ - H_2O$] (81), 189 [$M^+ - H_2O - CH_2OH$] (26), 164 (29), 149 (57), 133 (75), 121 (51), 106 (72), 95 (89), 81 (100), 69 (64), 55 (85); HRMS *m*/*z* 220.1791 (calcd for $C_{15}H_{24}O$ 220.1827).

(4*E*,6*R*,8*R*)-Caryophyll-4(5)-ene-6,8-diol (6): colorless solid, mp 115−116 °C, $[\alpha]^{27}_D$ +17° (*c* 2, EtOAc); IR (film) ν_{max} 3404, 1656, 1163, 1002, 982, 875, 841 cm^{-1};^1H NMR (400 MHz, $CDCl_3$, −50 °C) δ 0.85 (3H, s, H-14), 0.88 (3H, s, H-15), 1.02 (3H, s, H-13), 1.20 (1H, br t, J_{1-9} = 9.5 Hz, H-1), 1.70 (1H, m, H-2), 1.80 (3H, s, H-12), 2.02 (1H, br dd, J_{9-1} = 9.5 Hz, J_{9-10} = 18.9 Hz, H-9), 2.29 (1H, dd, $J_{7'-6}$ = 5.2 Hz, $J_{7'-7}$ = 13.1 Hz, H-7), 2.49 (1H, br d, J = 15.2 Hz, H-3), 4.60 (1H, dt, J_{6-5} = J_{6-7} = 10.7 Hz, $J_{6-7'}$ = 5.2 Hz, H-6), 5.24 (1H, d, J_{5-6} = 10.7 Hz, H-5);^{13}C NMR data, Table 1; EIMS *m*/*z* 238 [M]$^+$ (3) , 220 [$M^+ - H_2O$] (6), 205 [$M^+ - H_2O - CH_3$] (8), 177 (15), 167 (33), 149 (60), 123 (42), 109 (52), 95 (64), 81 (100), 69 (70), 55 (44); HRMS *m*/*z* 238.1944 (calcd for $C_{15}H_{26}O_2$ 238.1933).

(4*E*,8*R*,11*S*)-Caryophyll-4(5)-ene-8,14-diol (7): colorless solid, mp 106−107 °C; $[\alpha]^{27}_D$ −28° (*c* 3, EtOAc); IR (film) ν_{max} 3308, 1026, 876 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, −50 °C) δ 0.85 (3H, s, H-15), 0.99 (3H, s, H-13), 1.44 (1H, m, H-10β), 1.59 (1H, m, H-7), 1.59 (3H, s, H-12), 1.71 (1H, br t, H-1), 2.06 (1H, br t, H-9), 3.16 (1H, d, $J_{14-14'}$ = 11.4 Hz, H-14), 3.26 (1H, d, $J_{14'-14}$ = 11.4 Hz, H-14′) 3.41 (1H, br s, −O*H*), 4.38 (1H, br s,−O*H*), 5.60 (1H, br t, J = 7.6 Hz, H-5);^{13}C NMR data, Table 1; EIMS *m*/*z* 238 [M^+], 220 [$M^+ - H_2O$] (5), 189 [$M^+ - H_2O - CH_2OH$] (21), 165 (22), 151 (79), 133 (61), 107 (77), 93 (95), 80 (100), 71 (50), 55 (46); HRMS *m*/*z* 238.1942 (calcd for $C_{15}H_{26}O_2$ 238.1933).

(4*Z*,8*R*,11*S*)-Caryophyll-4(5)-ene-8,14-diol (8): colorless solid, mp 105−106 °C; $[\alpha]^{27}_D$ −70° (*c* 2, EtOAc); IR (film) ν_{max} 3300, 1257, 1034, 853 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.95 (3H, s, H-15), 1.01 (3H, s, H-13), 1.69 (3H, s, H-12), 1.43−1.57 (4H, m, H-2β, H-3, H-7, H-10), 1.91−2.06 (4H, m, H-2α, H-3′, H-6, H-7′), 2.26−2.34 (2H, m, H-6′, H-10′), 2.15 (1H, dt, J = 8.1, 9.5 Hz, H-1), 2.42 (1H, br dd, J_{9-1} = 8.1 Hz, J = 14.9 Hz, H-9), 3.31 (1H, d, $J_{14-14'}$ = 10.5 Hz, H-14), 3.37 (1H, d, $J_{14'-14}$ = 10.5 Hz, H-14′), 5.20 (1H, br t, J = 7.3 Hz, H-5);^{13}C NMR data, Table 2; EIMS *m*/*z* [M^+], (238), 220 [$M^+ - H_2O$] (7), 189 [$M^+ - H_2O - CH_2OH$] (22), 148 (52), 133 (49), 107 (76), 93 (100), 81 (98), 71 (57), 55 (69); HRMS *m*/*z* 238.1939 (calcd for $C_{15}H_{26}O_2$ 238.1933).

(4*Z*,3*S*,8*R*)-Caryophyll-4(5)-ene-3,8-diol (9): colorless solid, mp 120−122 °C; $[\alpha]^{27}_D$ −30° (*c* 8.1, EtOAc); IR (film) ν_{max} 3389, 1064, 946, 760 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.96, 0.97 (3H each, s, H-14, H-15), 0.98 (3H, s, H-13), 1.46−1.57 (2H, m, H-10), 1.55 (1H, m, H-2β), 1.67 (1H, m, H-7α), 1.73 (3H, s, H-12), 1.77 (1H, m, H-7β), 2.00−2.09 (2H, m, H-9,

Table 2. ^{13}C NMR Data of Compounds **5**, **8**, **9**, **11**−**16** (100 MHz, $CDCl_3$, room temperature)

position	5	8	9	11	12	13	14	15	16
1	45.2 d	42.9 d	41.7 d	43.4 d	39.4 d	40.6 d	43.3 d	48.2 d	37.1 d
2	26.8 t	26.3 t	30.6 t	30.6 t	24.7 t	30.1 t	27.0 t	30.1 t	26.7 t
3	27.4 t	29.3 t	73.5 d	73.7 d	40.1 t	31.6 t	27.2 t	30.9 t	124.4 d
4	138.5 s	135.3 s	138.7 s	139.3 s	73.4 s	135.8 s	138.1 s	139.5 s	138.8 s
5	126.8 d	125.3 d	124.9 d	124.4 d	61.2 d	132.8 d	127.3 d	133.5 d	72.9 d
6	22.8 t	22.6 t	26.4 t	25.9 t	28.7 t	24.4 t	22.4 t	65.4 d	30.2 t
7	41.6 t	41.8 t	38.5 t	38.6 t	30.1 t	44.5 t	41.5 t	53.5 t	38.3 t
8	72.9 s	73.8 s	72.2 s	72.1 s	72.8 s	74.1 s	73.8 s	73.1 s	72.8 s
9	45.8 d	39.0 d	43.7 d	41.3 d	51.4 d	52.1 d	38.8 d	52.2 d	41.3 d
10	35.2 t	31.1 t	33.3 t	28.7 t	38.4 t	30.4 t	29.2 t	37.7 t	27.3 t
11	32.9 s	37.2 s	33.3 s	30.1 s	36.6 s	37.1 s	37.3 s	32.4 s	37.3 s
12	68.1 t	24.8 q	18.9 q	18.8 q	16.3 q	61.9 t	67.9 t	62.0 t	18.5 q
13	29.6 q	27.7 q	29.9 q	26.4 q	31.6 q	30.3 q	27.9 q	31.1 q	26.6 q
14	27.9 q	71.1 t	26.5 q	68.2 t	70.4 t	18.0 q	70.8 t	29.4 q	19.2 q
15	23.1 q	19.2 q	23.8 q	24.5 q	18.15 q	67.7 t	19.2 q	21.8 q	70.4 t

H-6α), 2.05 (1H, m, H-1), 2.15 (1H, m, H-6*β*), 4.43 (1H, dd, *J* = 8.4, 2.8 Hz, H-3), 5.41 (1H, br t, *J* = 8.2 Hz, H-5);^{13}C NMR data, Table 2; EIMS *m*/*z* 238 [M$^+$] (3), 220 [M$^+$ − H_2O] (6), 205 [M$^+$ − H_2O − CH_3] (8), 177 (15), 167 (33), 149 (60), 123 (42), 109 (52), 95 (64), 81 (100), 69 (70), 55 (44); HRMS *m*/*z* 238.1944 (calcd for $C_{15}H_{26}O_2$ 238.1933).

(4*Z*,8*R*,11*S*)-Caryophyll-4(5)-ene-8,12,14-triol (10): colorless oil, [α]$^{27}_D$ −18° (*c* 1.7, EtOAc); IR (film) ν_{max} 3365, 1657, 1524, 1043, 736 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$, −50 °C) *δ* 0.98 (3H, s, H-15), 1.03 (3H, s, H-13), 1.52 (1H, br t, *J* = 10.7 Hz, H-10), 1.61−1.72 (2H, m, H-2, H-7), 1.77−1.94 (5H, m, H-1, H-2′, H-3, H-7′, H-10′), 2.01−2.08 (2H, m, H-6, H-9), 2.44 (1H, br d, *J* = 15.3 Hz, H-3′), 2.58 (1H, tdd, $J_{6'-5} = J_{6'-6} = 12.5$ Hz, *J* = 12.5, 3.9 Hz, H-6′), 3.49 (1H, d, $J_{14-14} = 10.7$ Hz, H-14), 3.51 (1H, d, $J_{12-12'} = 10.3$ Hz, H-12), 3.70 (1H, d, $J_{14'-14} = 10.7$ Hz, H-14′), 4.42 (1H, d, $J_{12'-12} = 10.3$ Hz, H-12′), 5.34 (1H, br d, $J_{5-6'} = 12.5$ Hz, H-5);^{13}C NMR data, Table 1; EIMS *m*/*z* 254 [M$^+$] (2), 236 [M$^+$ − H_2O] (2), 223 [M$^+$ − CH_2OH] (3), 205 [M$^+$ − H_2O − CH_2OH] (16), 187 [M$^+$ − 2 × H_2O − CH_2OH] (17), 147 (32), 133 (72), 105 (63), 93 (96), 81 (37), 67 (65), 55 (100); HRMS *m*/*z* 205.1584 (calcd for $C_{14}H_{21}O$ requires 205.1592).

(4*Z*,3*S*,8*R*,11*S*)-Caryophyll-4(5)-ene-3,8,14-triol (11): colorless oil; [α]$^{25}_D$ −35° (*c* 1, ethyl acetate); IR (film) ν_{max} 3389, 1657, 786 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 0.99 (3H, s, H-13), 1.08 (3H, s, H-15), 1.09 (1H, m, H-7), 1.45−1.59 (3H, m, H-2, H-7′, H-10*β*), 1.64 (1H, dd, $J_{10\alpha-9} = 9.0$ Hz, $J_{10\alpha-10\beta} = 10.9$ Hz, H-10α), 1.70−1.83 (2H, m, H-2′, H-6*β*), 1.74 (3H, s, H-12), 2.09 (1H, br dd, $J_{9-10\alpha} = 9.0$ Hz, *J* = 18.2 Hz, H-9), 2.19 (1H, m, H-6α), 2.22 (1H, m, H-1), 3.59 (1H, d, $J_{14-14'} = 10.7$ Hz, H-14), 3.65 (1H, d, $J_{14'-14} = 10.7$ Hz, H-14′), 4.44 (1H, dd, *J* = 8.2, 2.1 Hz, H-3), 4.77 (1H, br s, O*H*), 5.42 (1H, br t, *J* = 6.7 Hz, H-5);^{13}C NMR data, Table 2.

3,14-Diacetate of 11: colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 0.98 (3H, s, H-13), 1.09 (3H, s, H-15), 1.62 (1H, dd, $J_{10\alpha-9} = 4.8$ Hz, $J_{10\alpha-10\beta} = 9.4$ Hz, H-10α), 1.69 (3H, s, H-12), 2.17 (1H, m, H-9), 2.04 and 2.06 (3H each, s, C*H*$_3$COO), 2.25−2.34 (2H, m, H-1, H-6α), 4.05 (2H, s, H-14), 5.36 (2H, m, H-3, H-5); EIMS *m*/*z* 320 [M$^+$ − H_2O] (4), 278 [M$^+$ − AcOH] (28), 260 [M$^+$ − H_2O − AcOH] (2), 218 [M$^+$ − 2 × AcOH] (25), 200 [M$^+$ − H_2O − 2 × AcOH] (7), 121 (69), 95 (98), 81 (100); HRMS *m*/*z* 320.2031 (calcd for $C_{19}H_{28}O_4$ requires 320.1988).

(4*R*,5*S*,8*R*)-5,8-Epoxycaryophyllane-4,14-diol (12): colorless oil; [α]$^{24}_D$ −33° (*c* 0.6, EtOAc); IR (film) ν_{max} 3426, 1126, 736 cm^{-1}; ^{1}H NMR((400 MHz, $CDCl_3$) *δ* 0.98 (3H, s, H-15), 0.99 (1H, m, H-2), 1.10 (3H, s, H-13), 1.23 (1H, m, H-6), 1.27 (3H, s, H-12), 1.44−1.63 (3H, m, H-2′, H-7, H-10), 1.84−1.98 (3H, m, H-7′, H-9, H-10′), 1.71 (1H, dt, $J_{3-3'} = 13.5$ Hz, *J* = 3.7 Hz, H-3), 2.05 (1H, dt, $J_{3'-3} = 13.5$ Hz, *J* = 3.5 Hz, H-3′), 2.21 (1H, br t, *J* = 9.1 Hz, H-1), 2.29 (1H, tt, *J* = 5.6, 13.4 Hz, H-6′), 3.23 (1H, dd, $J_{5-6} = 9.3$ Hz, $J_{5-6'} = 5.6$ Hz, H-5), 3.27 (1H, d, $J_{14-14'} = 10.7$ Hz, H-14), 3.31 (1H, d, $J_{14'-14} = 10.7$ Hz, H-14′), 4.79 (1H, br s, O*H*);^{13}C NMR data, Table 2; EIMS *m*/*z* 236 [M$^+$ − H_2O] (16), 161 (19), 135 (29), 123 (51), 109 (74), 95 (100), 81 (96), 67 (55), 55 (93); HRMS *m*/*z* 236.1839 (calcd for $C_{15}H_{24}O_2$ 236.1776).

(4*Z*,8*R*,11*R*)-Caryophyll-4(5)-ene-8,12,15-triol (13): colorless oil, [α]$^{25}_D$ −17° (*c* 2, EtOAc); IR (film) ν_{max} 3309, 1667, 1036, 734 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 0.87 (3H, s, H-14), 1.04 (3H, s, H-13), 1.33 (1H, m, H-10α), 1.51 (1H, br dd, $J_{2\alpha-3'} = 14.6$ Hz, *J* = 5.0 Hz, H-2α), 1.63 (1H, dt, $J_{7-6} = 5.0$ Hz, $J_{7-6'} = J_{7-7'} = 13.0$ Hz, H-7), 1.73 (1H, m, H-2*β*), 1.87−2.03 (4H, m, H-1, H-3, H-7′, H-10*β*), 1.99 (2H, m, H-6, H-9), 2.63 (1H, dq, $J_{6'-5} = J_{6'-6} = J_{6'-7} = 13.0$ Hz, $J_{6'-7'} = 3.8$ Hz, H-6′), 2.45 (1H, br d, $J_{3'-2\alpha} = 14.6$ Hz, H-3′), 3.12 (1H, d, $J_{15-15'} = 11.7$ Hz, H-15), 3.22 (1H, d, $J_{15'-15} = 11.7$ Hz, H-15′), 3.50 (1H, d, $J_{12-12'} = 11.1$ Hz, H-12), 4.39 (1H, d, $J_{12'-12} = 11.1$ Hz, H-12′), 5.36 (1H, br d, $J_{5-6'} = 13.0$ Hz, H-5); ^{13}C NMR data, Table 2; EIMS *m*/*z* 236 [M − H_2O]$^+$ (3), 218 [M$^+$ − 2 × H_2O] (3), 205 (M$^+$ − H_2O − CH_2OH] (25), 187 [M$^+$ − 2 × H_2O − CH_2OH] (16), 167 (26), 147 (34), 133 (91), 107 (64), 93 (100), 81 (100), 67 (69), 55 (91); HRMS *m*/*z* 218.1703 (calcd for $C_{15}H_{22}O$ 218.1671).

(4*E*,8*R*,11*S*)-Caryophyll-4(5)-ene-8,12,14-triol (14): colorless oil; [α]$^{25}_D$ −35° (*c* 1.2, ethyl acetate); IR (film) ν_{max} 3343, 1125, 1038, 734 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 0.93 (3 H, s, H-15), 1.01 (3H, s, H-13), 1.53−1.67 (3H, m, H_2-3, H-10*β*), 1.48 (1H, ddd, $J_{7-6} = 7.5$, 2.6 Hz, $J_{7-7'} = 14.3$ Hz, H-7), 1.97 (1H, ddd, $J_{7'-6} = 11.5$, 2.4 Hz, $J_{7'-7} = 14.3$ Hz, H-7′), 2.02−2.10 (3H, m, H-1, H-6α, H-10α), 2.16 (1H, m, H-2α), 2.37 (1H, m, H-6*β*), 2.29 (1H, m, H-2*β*), 2.45 (1H, m, H-9), 3.30 (1H, d, $J_{14-14'} = 10.7$ Hz, H-14), 3.36 (1H, d, $J_{14'-14} = 10.7$ Hz, H-14′), 4.00 (2H, s, H-12), 5.50 (1H, br t, *J* = 7.8 Hz, H-5);^{13}C NMR data, Table 2; EIMS *m*/*z* 236 [M$^+$ − H_2O] (5), 218 [M$^+$ − 2 × H_2O] (4), 205 [M$^+$ − H_2O − CH_2OH] (24), 187 [M$^+$ − 2 × H_2O − CH_2OH] (15), 165 (33), 133 (55), 106 (65), 93 (92), 81 (100), 69 (81), 55 (93); HRMS *m*/*z* 236.1801 (calcd for $C_{15}H_{24}O_2$ 236.1776).

(4*Z*,6*R*,8*R*)-Caryophyll-4(5)-ene-6,8,12-triol (15): colorless oil; [α]$^{25}_D$ −10° (*c* 1.4, EtOAc); IR (film) ν_{max} 3458, 1657, 1012, 735 cm^{-1}; ^{1}H NMR 400 MHz, $CDCl_3$) *δ* 0.90 (3H, s, H-14), 0.92 (3H, s, H-15), 1.08 (3H, s, H-13), 1.49−1.70 (6H, m, H-1, H_2-2, H-7, H_2-10), 1.92−2.03 (1H, m, H-3), 1.97 (1H, m, H-9), 2.31 (1H, dd, $J_{7'-6} = 5.3$ Hz, $J_{7'-7} = 13.5$ Hz, H-7′), 2.48 (1 H, br dd, *J* = 14.3, 5.4 Hz, H-3′), 3.62 (1H, d, $J_{12-12'} = 10.9$ Hz, H-12); 4.45 (1H, d, $J_{12'-12} = 10.9$ Hz, H-12′), 4.75 (1H, dt, $J_{6-5} = J_{6-7} = 10.6$ Hz, $J_{6-7'} = 5.3$ Hz, H-6), 5.41 (1H, dd, $J_{5-6} = 10.6$ Hz, *J* = 1.7 Hz, H-5); ^{13}C NMR data, Table 2; EIMS *m*/*z* 236 [M$^+$ − H_2O] (7), 223 [M$^+$ − CH_2OH] (14), 218 [M$^+$ − 2 × H_2O] (6), 205 [M$^+$ − H_2O − CH_2OH] (16), 147 (38), 123 (43), 109 (52), 95 (70), 81 (100), 69 (98), 55 (86); HRMS *m*/*z* 236.1781 (calcd for $C_{15}H_{24}O_2$ 236.1776).

(3*Z*,5*R*,8*R*,11*R*)-Caryophyll-3-ene-5,8,15-triol (16): colorless oil, IR (film) ν_{max} 3382, 1665, 914, 736 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 0.97 (3H, s, H-14), 0.98 (3H, s, H-13), 1.35 (1H, dd, *J* = 10.5, 9.5 Hz, H-10α), 1.47 (1H, m, H-6), 1.55−1.66 (2H, m, H_2-7), 1.71 (3H, s, H-12), 1.79−1.85 (1H, m, H-6′), 1.87 (1H, br t, *J* = 10.2 Hz, H-10*β*), 2.03 (1H, m, H-2α), 2.04 (1H, br dd, $J_{9-1} = 9.2$ Hz, *J* = 18.7 Hz, H-9), 2.19 (1H, m, H-2*β*), 2.49 (1H, dt, $J_{1-9} = 9.2$ Hz, $J_{1-2} = 6.5$ Hz, H-1), 3.26 (1H, d, $J_{15-15'} = 11.0$ Hz, H-15), 3.30 (1H, d, $J_{15'-15} = 11.0$ Hz, H-15′), 4.51 (1H, dd, *J* = 8.8, 3.3 Hz, H-5), 5.41 (1H, br t, *J* = 7.7 Hz, H-3); ^{13}C NMR data, Table 2; EIMS *m*/*z* 253 [M$^+$ − 1] (3), 237 [M$^+$ + 1 − H_2O] (31), 219 [M$^+$ + 1 − 2 × H_2O] (100), 201 [M$^+$ + 1 − 3 × H_2O] (100), 191 (23), 175 (16), 161 (67); HRMS *m*/*z* 253.1818 (calcd for $C_{15}H_{25}O_3$ 253.1804).

Acknowledgment. This work was supported by grants from CICYT AGF95-0779 and DGICYT PB95-1235-CO2-01.

References and Notes

(1) Coley Smith, J. R.; Verhoeff, K.; Jarvis, W. R., Eds. *The Biology of Botrytis*; Academic Press: London, 1980; pp 153−175.
(2) Staub, T. *Annu. Rev. Phytopathol.* **1991**, *29*, 421.
(3) Fehlhaber, H. W.; Geipel, R.; Mercker, H. J.; Tchesche, R.; Welmar, K. *Chem. Ber.* **1974**, *107*, 1720−1730.
(4) Hanson, J. R. *Pure Appl. Chem.* **1981**, *53*, 1155−1162.
(5) Duran, R.; Corrales, E.; Hernández-Galán, R.; Collado, I. G. *J. Nat. Prod.* **1999**, *62*, 41−44.
(6) Collado, I. G.; Aleu, J.; Macías-Sánchez, A. J.; Hernández-Galán, R. *J. Chem. Ecol.* **1994**, *20*, 2631−2644.
(7) Kaiser, R.; Lamparsky, D. *Helv. Chim. Acta* **1976**, *59*, 1803−1808.
(8) Collado, I. G.; Aleu, J.; Macías-Sánchez, A. J.; Hernández-Galán, R. *J. Nat. Prod.* **1994**, *57*, 738−746.
(9) Hikino, H.; Aota, K.; Takemoto, T. *Chem. Pharm. Bull.* **1969**, *17*, 1390.
(10) Tkachev, A. V.; Mamatyuk, V. I.; Dibovenko, G. V. *Izv. Sib. Otd. Akad. Nauk SSSR, Ser. Khim. Nauk* **1986**, 88.

NP990296Q