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INHIBITION OF *BOTRYTIS CINEREA* BY NEW SESQUITERPENOID COMPOUNDS OBTAINED FROM THE REARRANGEMENT OF ISOCARYOPHYLLENE

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ABSTRACT.—A careful study of the acid-catalyzed cyclization of isocaryophyllene has provided new information on the reaction mechanism. A novel sesquiterpene skeletal type has resulted from unusual rearrangements of isocaryophyllene. The alcohols ginsenol [**9**] and 4β,8α,10,10-tetramethyl-11-hydroxytricyclo[6,2,1,$0^{4,11}$]undecane [**10**] were found to inhibit the growth of *Botrytis cinerea* in vitro.

Botrytis species are serious pathogens of a number of commercial crops (1,2). For example, *Botrytis cinerea* attacks a wide range of plants, producing various leaf spot diseases and grey powdery mildews on lettuces, tomatoes, and grapes. In the past, we have undertaken a project to study the selective inhibition of the biosynthesis of sesquiterpenoid metabolites of the fungus *Botrytis cinerea* as a rational means of controlling this fungus and its pathogenicity.

Biosynthetic studies carried out by Hanson *et al.* have suggested that the sesquiterpenes botrydial [**1**] and dihydrobotrydial [**2**] are formed from farnesyl pyrophosphate (1). The first stages in the cyclization involve formation and cyclization of the caryophyllene cation at C-8. Accordingly, we have investigated the acid-catalyzed cyclization of caryophyllenes [**4**, **5**] in order to enhance our knowledge of reactions that could lead to the synthesis of compounds analogous to those proposed as metabolic intermediates.

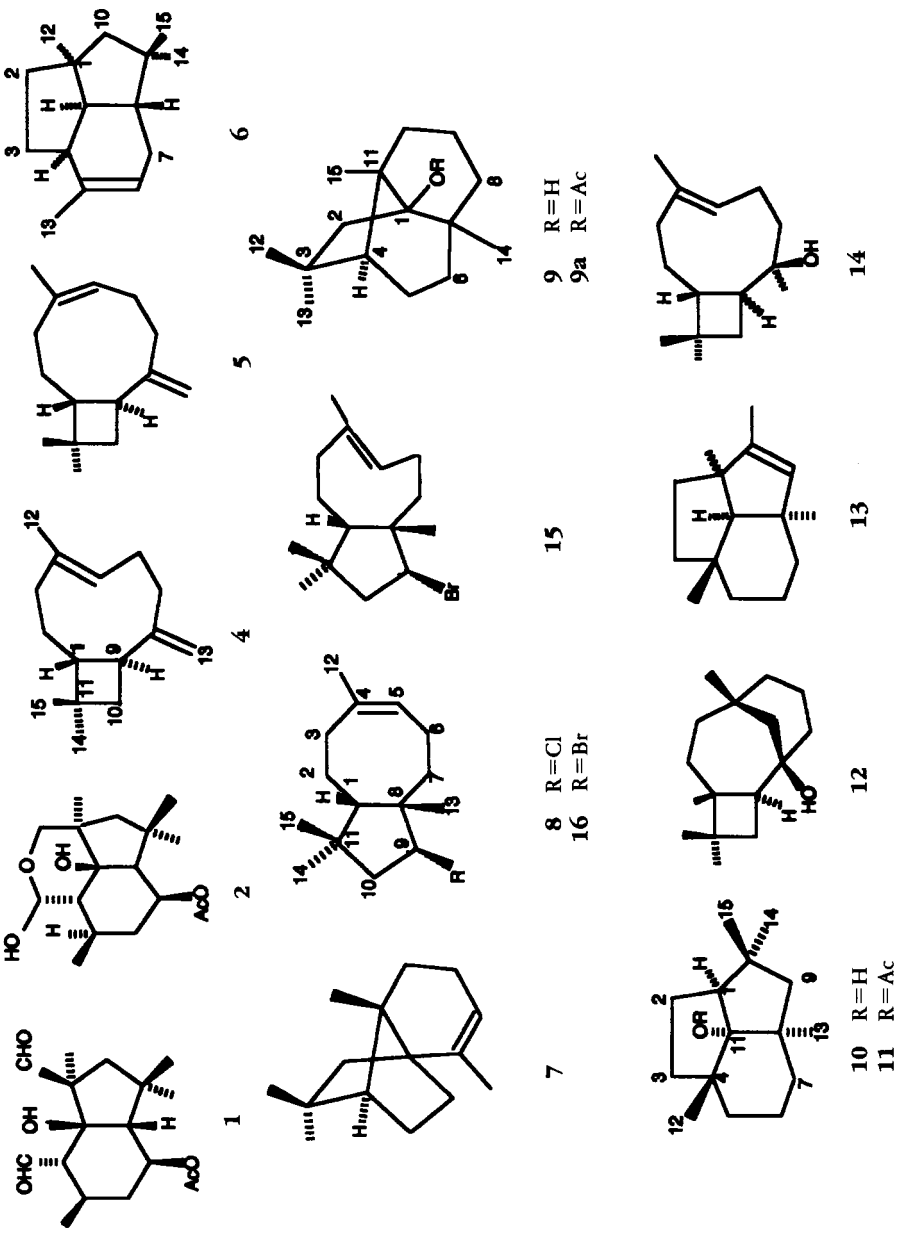
RESULTS AND DISCUSSION

The acid-catalyzed rearrangement of isocaryophyllene [**5**] has been described by Gollnick *et al.* (4). These authors found that pure isocaryophyllene [**5**], when treated with H_2SO_4 in Et_2O, yields a complex mixture of hydrocarbons from which two main components were isolated, neoclovene [**7**] (5–7) and the tricyclic sesquiterpene **6** (4). The mechanism by which **6** is formed from isocaryophyllene [**5**] (Scheme 1) is similar to the route proposed by Hanson (1), except for the cis-trans disposition of the C-4–C-5 double bond of the caryophyllene cation, **5a**.

To our knowledge, the tricyclic sesquiterpene **6** has been obtained only from isocaryophyllene [**5**] (4). Although the trans-isomer **4** has been treated with different reagents, neither tricyclic olefin **6** nor related compounds were detected (3).

We have re-examined the acid-catalyzed rearrangement of caryophyllene [**4**] and isocaryophyllene [**5**] under carefully controlled conditions. Our results were in general agreement with those previously reported (3,4), but when the reaction was carried out on isocaryophyllene [**5**] the chromatographic separations were tedious and the yields lower. An improved yield of **6**, was evident when the reaction was carried out by shaking isocaryophyllene [**5**] with $FeCl_3$ supported on SiO_2, with neoclovene [**7**] and **6** being obtained in 10% and 60% yields, respectively. In addition, a third compound [**8**] was obtained in small amounts.

Compound **8** had a molecular ion at *m/z* 240/242 and signals in the ^{13}C-nmr spectrum δ 71.78 (d, C-9), 123.9 (d, C-5), 136.4 (s, C-4) that were consistent with an unsaturated bicyclic sesquiterpene possessing a chlorine atom and with a molecular


1
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11 R=Ac
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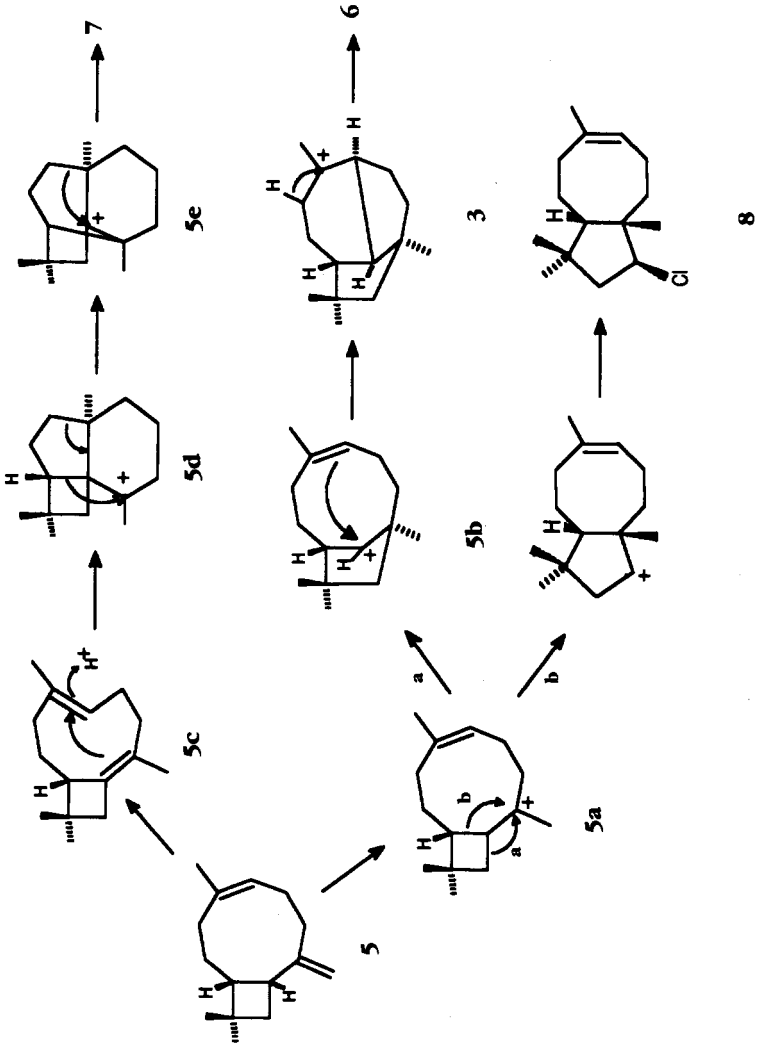


SCHEME 1

formula of $C_{15}H_{25}Cl$. The ^{1}H-nmr spectrum contained signals for four tertiary methyl groups (δ 0.83, 0.90, 0.97, 1.70) and a double doublet at δ 3.92 (J=6.1 and 12.4 Hz, H-9) characteristic of a proton on a carbon bearing a chlorine atom, and a double doublet at δ 5.26 (J=6.3 and 10.4 Hz), corresponding to H-5 of a cis-double bond. From these data, structure **8** was inferred. This structure was supported by homonuclear and heteronuclear 2D nmr correlation experiments. The β-orientations of the chlorine atom and the methyl group at C-8 were assigned on the basis of nOe experiments. Irradiation of the signal at δ 1.30 (H-1) led to the enhancement of those at δ 0.90 (H-13), 0.97 (H-15), while irradiation at δ 3.92 (H-9) enhanced the signals at δ 0.83 (H-14) and 1.88 (H-10α). Synthesis of the previously undescribed compound **8** can be explained by migration of the C-1–C-9 bond to C-8 of the caryophyllene cation [**5a**] (Scheme 1, route b).

The formation of **8** represents an alternative Wagner-Meerwein rearrangement that has not been detected previously in caryophyllene chemistry. In order to confirm structure **8** and the mechanism proposed, 8-hydroxycaryophyllene [**14**] was synthesized (8) and subjected to treatment with Lewis acids. Reaction of **14** with BCl_3 in $CHCl_3$ yielded the olefin **6**, and a compound with spectroscopic data identical to **8**. When **14** was treated with BBr_3, compound **6** and a mixture of **15** and **16** were obtained. Only isomer **16** was isolated from the mixture. However, it was possible to study compound **15** by means of a ^{1}H-nmr spectrum obtained from the mixture of isomers. The bromo derivatives **15** and **16** (*m/z* 285/287), gave ^{1}H-nmr signal patterns similar to **8**, with the exception of the olefinic proton. The coupling constants of these signals in **16** (δ 5.25, br dd, J=10.0 and 6.2 Hz) and **15** (δ 5.41, br t, J=6.0 Hz) clearly confirmed the cis-disposition assigned to the C-4–C-5 double bond of chloro-derivative **8**. All spectral data of **16** and **15** were consistent with the structures proposed.

On the other hand, when the acid-catalyzed reaction of isocaryophyllene [**5**] was carried out with H_2SO_4 in different solvents, followed by addition of H_2O to the reaction medium (see Experimental) in order to trap carbocation intermediates, compounds **6**, **7**, **9**, **10**, and **12** were obtained.

Compound **9** was isolated from the reaction mixture as an oil with a molecular ion at *m/z* 222 and its ^{13}C-nmr spectrum was consistent with a molecular formula of $C_{15}H_{26}O$. The ir absorption at 3504 cm^{-1} and a fragment ion peak at *m/z* 204 [M^+-18] in the ms, indicated the presence of a hydroxyl group. A quaternary carbon signal at δ 83.1 ppm in the ^{13}C-nmr spectrum revealed the hydroxyl group to be tertiary. The ^{1}H-nmr spectrum showed signals produced by four methyl groups (δ 0.80, 0.97, 1.13, and 1.19) and an AB quartet (J=14.1 Hz) (δ 1.62 and 1.92). These spectral data suggested that compound **9** was a saturated tricarbocyclic sesquiterpenoid possessing a tertiary hydroxyl group. This conclusion was confirmed by the spectral properties of its acetylated derivative [**9a**]. The spectroscopic data of **9** and **9a** were identical to those of a sesquiterpene alcohol isolated from the roots of *Panax ginseng* (9), which was named ginsenol [**9**].

The skeleton of ginsenol [**9**] corresponds to the hypothetically important bridgehead cation [**5e**] proposed in the route to neoclovene [**7**] (6) (Scheme 1), and its structure was confirmed by treating **9** with H_2SO_4/Et_2O affording neoclovene [**7**].

The second new compound obtained, **10**, showed an ir absorption at 3474 cm^{-1}, a quaternary carbon signal at δ 76.8 in the ^{13}C-nmr spectrum, a molecular ion at *m/z* 222, and a fragment peak at *m/z* 204 [$M-18$]$^+$, that were also consistent with a saturated tricyclic sesquiterpene possessing a tertiary hydroxyl group and a molecular formula of $C_{15}H_{26}O$. When treated with Ac_2O, compound **10** formed a monoacetate **11** that lacked hydroxyl absorption in the ir spectrum. On analysis of its ^{1}H- and ^{13}C-nmr spectra, structure **10** was proposed, and supported by homonuclear and heteronuclear 2D nmr

correlation experiments. The stereochemistry of the 9α-OH group was assigned on the basis of the shift to a lower field of the H-8 signal of compound **10**. This shift correlates with the stereochemical course of the rearrangement discussed below to afford **10**. The previously undescribed compound **10** presents a structure isomeric to the olefin **6** that can be rationalized assuming that an alternative Wagner-Meerwein rearrangement from cation **5d** (Scheme 2) has taken place. Therefore, on the panasinsene cation [**5d**] two chair conformers that present an antiperiplanar disposition of bonds C-1–C-9 and C-10–C-9 on C-8, respectively, coexist. If the rearrangement involves migration of the C-1–C-9 bond to C-8, then ginsenol [**9**] and neoclovene [**7**] are obtained. On the other hand, migration of the C-10–C-9 bond affords **10** by trapping cation **5f** from the hydroxyl group present in the reaction medium. Cation **5f** has been proposed recently by Khomenko *et al.* (10) as an intermediate in the acid-catalyzed rearrangement of neoclovene [**7**] to olefin **13** in a clear "retrorearrangement" of neoclovene [**7**] to the panasinsene cation [**5d**]. In order to confirm the proposed structure [**10**] and its formation from caryophyllene [**4**], alcohol **10**, and neoclovene [**7**] were treated with H_2SO_4/1,4-dioxane. Both yielded olefin **13** (10). However, compound **10** was not formed from neoclovene [**7**] under these conditions, confirming the route and structure proposed by us for sesquiterpene alcohol **10**. The formation of this compound represents an unusual rearrangement that has not been reported previously. Furthermore, these results lend credence to the biogenetic pathway proposed by Roberts (11), by which isocomene and modhephene hydrocarbons are derived from a caryophyllene-type precursor via cation **5f** (11).

The isolation of caryolan-1-ol [**12**] in small amounts from the acid-catalyzed reaction of isocaryophyllene [**5**] was unexpected, because it is a known compound formed from the acid-catalyzed cyclization of caryophyllene [**4**] (5), and has never been observed during the cyclization of isocaryophyllene [**5**].

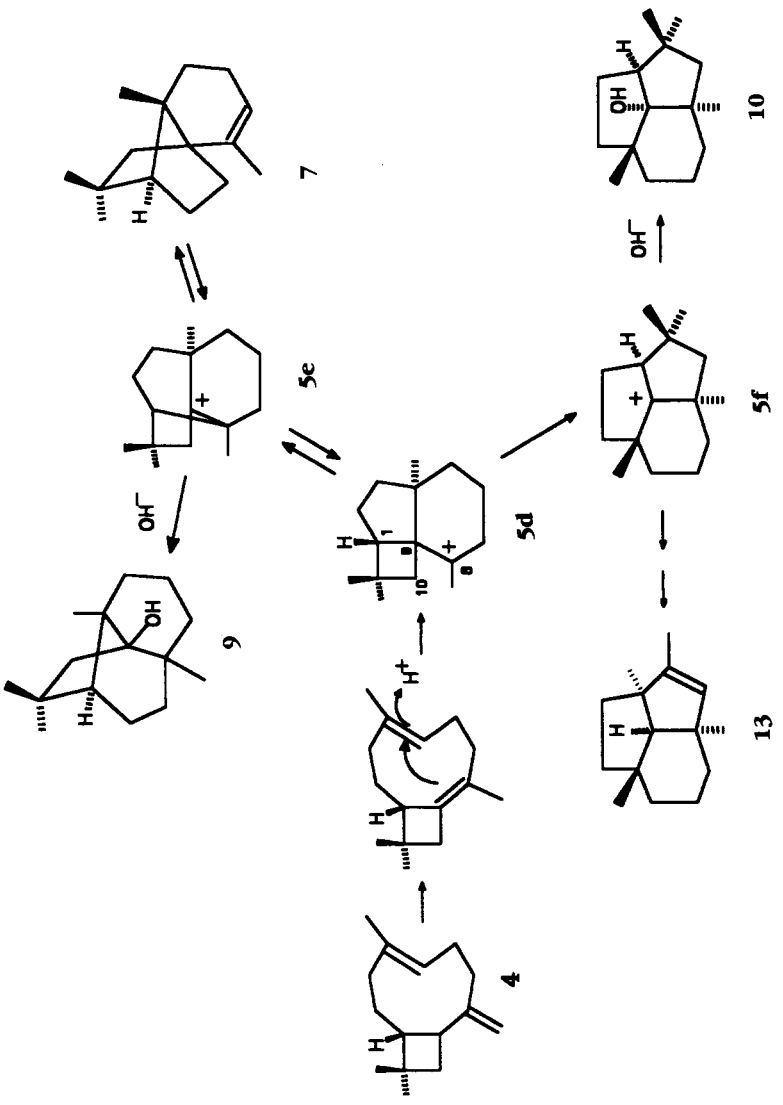
All compounds obtained were tested as fungicides against *B. cinerea*. The commercial fungicides, Ronilan® and Euparen®, were used as standards for comparison. Olefin **6** was inactive, as were the chloro- and bromo-derivatives **8** and **16**. However, ginsenol [**9**] and the alcohol **10** showed moderate activity. Ginsenol [**9**] showed activity inferior to that of **10**. Compound **9** produced zones of inhibition ranging from 6 mm to 8 mm at 130 ppm for 5 days and showed total inhibition at 200 ppm. Alcohol **10**, which possesses an isomeric structure to the proposed intermediate of the biosynthesis of botrydial [**1**] and its relatives (1), displayed activity in centerpoint inoculation disk assays from 20 mm to 25 mm at 17 ppm, and total inhibition at 100 ppm for 3 days.

In conclusion, we have showed unusual rearrangements of isocaryophyllene and panasinsene cations [**5a** and **5d**] (Schemes 1 and 2) that lead to new and interesting sesquiterpene skeletal types. Alcohols **9** and **10** displayed antifungal activity against *B. cinerea*. These results shed further light on the acid-catalyzed rearrangement of isocaryophyllene [**5**].

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps were measured with a Kofler block Reichert-Jung apparatus and are uncorrected. Optical rotations were determined with a Perkin-Elmer 241 polarimeter. Ir spectra were recorded on a Perkin-Elmer 881 spectrophotometer. ^{1}H-Nmr measurements were obtained on Varian Gemini 200 and Varian Unity 400 nmr spectrometers with $SiMe_4$ as internal reference. Mass spectra were recorded on VG 12-250 spectrometer at 70 eV. Hplc was performed with a Hitachi system, model L-6270 pump, LC injector and a model RI-71 differential refractometer detector. Tlc was performed on MN Alugram SIL G/UV 254 plates, 0.25 mm thick. Merck Si gel was used for cc. Purification by hplc was accomplished using a Si gel column (Hibar 60, 7 μm, 1×25 cm).

MICROORGANISM AND ANTIFUNGAL ASSAYS.—The culture of *Botrytis cinerea* employed in this work, *B. cinerea* (UCA 992), was obtained from grapes of Domecq vineyard, Jerez de la Frontera, Cádiz, Spain. This



SCHEME 2

culture of *B. cinerea* is deposited in the Universidad de Cádiz, Facultad de Ciencias Mycological Herbarium Collection (UCA). Bioassays were performed by measuring inhibition of radial growth on agar medium in a Petri dish. Test compounds were dissolved in EtOH to give a final concentration of 1 to 225 mg liter^{-1}. Solutions of test compounds 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 control and treated cultures. The medium was poured into 6-cm diameter sterile plastic Petri dishes and a 5-mm diameter mycelial disc 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 six days.

CYCLIZATION OF ISOCARYOPHYLLENE [**5**] WITH $FeCl_3/SiO_2$.—Isocaryophyllene [**5**] (720 mg) was added dropwise, with strong shaking, for 5 min to $SiO_2/FeCl_3$ (65 g, 10% by weight of $FeCl_3$). The resulting mixture was packed in a chromatography column and was eluted with hexane to give 380 mg of a mixture that was purified by hplc to yield **6** (228 mg), **7** (38 mg), and **8** (12 mg). 1β,5α,9,9-Tetramethyltricyclo[6.2.1.0^{4,11}]undec-5-ene [**6**]: oil; ir (film) ν max 2914, 2881, 1646, 1474, 1382, 1079, 842 cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 0.81 (3H, s, H-14), 0.97 (3H, s, H-15), 1.07 (3H, s, H-12), 1.30 (1H, m, H-8), 1.47 (1H, m, H-2), 1.53 (2H, br s, H-10), 1.58–1.72 (4H, m, H-7, H-11, H-3, H-3′), 1.67 (3H, br s, H-13), 1.80 (1H, m, H-7′), 1.96 (1H, m, H-2′), 2.33 (1H, br dd, J=7.2 and 14.1 Hz, H-4), 5.40 (1H, m, H-6); ^{13}C nmr ($CDCl_3$) δ 22.30 (q, *C-14), 22.65 (q, C-13), 25.82 (t, †C-7), 28.66 (q, *C-15), 30.18 (q, C-12), 30.73 (t, C-2), 40.50 (s, ‡C-9), 42.91 (t, †C-3), 44.69 (d, C-4), 46.05 (s, ‡C-1), 48.75 (d, C-8), 57.57 (d, C-11), 58.90 (t, C-10), 122.06 (d, C-6), 138.64 (s, C-5) (*†‡=interchangeable signals); ms *m/z* 204 (3) [M$^+$], 189 (8), 175 (6), 161 (8), 149 (29), 135 (17), 119 (21), 109 (30), 95 (35), 81 (42), 69 (56), 57 (60), 55 (72), 44 (100). 9β-Chloro-4,8β,11,11-tetramethylbicyclo[6.3.0]undec-4-ene [**8**]: oil; ir (film) ν max 2924, 2862, 1640, 1464, 1372, 1079, 1045, 831 cm^{-1}; ^{1}H nmr ($CDCl_3$, 400 MHz) δ 0.83 (3H, s, H-14), 0.90 (3H, s, H-13), 0.97 (3H, s, H-15), 1.20–1.32 (2H, m, H-1 and H-7), 1.41 (1H, m, H-2), 1.56–1.74 (4H, m, H-6, H-7′, H-2′, and H-10β), 1.70 (3H, s, H-12), 1.79 (1H, ddd, $J_{3-3'}$=13.5 Hz, J_{3-2}=6.5 Hz, $J_{3-2'}$=1.5 Hz, H-3), 1.88 (1H, dd, $J_{10'-10}$=11.9 Hz, $J_{10'-9}$=6.3 Hz, H-10α), 2.11 (1H, ddd, $J_{6'-6}$=13.0 Hz, $J_{6'-5}$=10.3 Hz, $J_{6'-7}$=12.8 Hz, H-6′), 2.33 (1H, br t, J=13.0 Hz, H-3′), 3.92 (1H, dd, J_{9-10}=6.1 Hz, $J_{9-10'}$=12.4 Hz, H-9), 5.26 (1H, br dd, $J_{5-6'}$=6.3 Hz, J_{5-6}=10.4 Hz, H-5); ^{13}C nmr ($CDCl_3$, 400 MHz) δ 22.92 (q, C-14), 24.65 (t, C-2), 25.18 (q, C-12), 25.63 (t, C-6), 27.17 (q, C-15), 29.74 (q, C-13), 33.82 (t, C-3), 39.42 (q, C-11), 40.42 (t, C-7), 47.34 (s, C-8), 49.77 (t, C-10), 61.42 (d, C-1), 71.78 (d, C-9), 123.92 (d, C-5), 136.43 (s, C-4); ms *m/z* 242, 240 (77, 25) [M$^+$], 225 (40), 205 (59) [M$^+$−Cl], 189 (32), 149 (33), 135 (47), 123 (83), 109 (87), 107 (100), 95 (56), 81 (75), 67 (75).

TREATMENT OF **14** WITH LEWIS ACIDS.—(a) Compound **14** (8 mg) was dissolved in 2 ml of dry CH_2Cl_2 and BCl_3 (0.2 ml of 1 M solution in CH_2Cl_2) was added. The reaction mixture was stirred for 4 h at −80°, then poured over ice and extracted with CH_2Cl_2. After several purifications by cc and hplc, compounds **8** (4 mg) and **6** (2 mg) were obtained.

(b) When **14** (264 mg) in 3 ml of dry CH_2Cl_2 was treated with 0.11 ml of BBr_3 (1M in CH_2Cl_2) at −22° for 2 h, a mixture of 227 mg of **6**, **15**, and **16** was obtained. After several purifications by cc and hplc, only **6** (5%) and **16** (16%) could be isolated. 9β-Bromo-4,8,11,11-tetramethylbicyclo[6.3.0]undecen-4-ene [**16**]: oil, $[\alpha]^{25}D$ +34° (c=0.9, $CHCl_3$); ir (film) ν max 2965, 1469, 1365, 723 cm^{-1}; ^{1}H nmr ($CDCl_3$, 400 MHz) δ 0.81 (3H, s, H-15), 0.94 (3H, s, *H-13), 0.95 (3H, s, *H-14), 1.25 (1H, m, H-7), 1.29 (1H, dd, J=12.7 and 1.9 Hz, H-1), 1.42 (1H, m, H-2), 1.62 (1H, m, H-2′), 1.67 (1H, m, H-7′), 1.69 (4H, br s, H-12 and H-6), 1.79 (1H, m, H-3), 1.82 (1H, dd, $J_{10\alpha-10\beta}$=13.4 Hz, $J_{10\alpha-9}$=12.9 Hz, H-10α), 1.94 (1H, dd, $J_{10\beta-10\alpha}$=13.4 Hz, $J_{10\beta-9}$=6.2 Hz, H-10β), 2.11 (1H, ddd, J=12.9 Hz, 12.9 Hz, and 10.0 Hz, H-6′), 2.32 (1H, br t, J=13.1 Hz, H-3′), 4.06 (1H, dd, J=12.9 and 6.2 Hz, H-9), 5.25 (1H, br dd, J=10 and 6.2 Hz, H-5); ^{13}C nmr ($CDCl_3$, 200 MHz) δ 22.7 (q, C-15), 24.7 (t, C-2), 25.3 (q, C-12), 25.7 (t, C-6), 29.7 (q, *C-13), 29.8 (q, *C-14), 33.9 (t, C-3), 40.2 (t, C-7), 40.7 (s, C-11), 47.1 (s, C-8), 50.7 (t, C-10), 60.9 (d, C-1), 66.4 (d, C-9), 124.0 (d, C-5), 136.6 (s, C-4) (*=interchangeable signals); ms *m/z* 286, 284, (5,6) [M$^+$], 271, 269 (1,1) [M$^+$−15], 243, 241 (1:1), 205 (25) [M$^+$−Cl], 123 (50), 121 (35), 109 (64), 107 (56), 95 (67), 93 (48), 81 (95), 79 (43), 69 (67), 67 (53), 55 (54), 53 (27), 41 (100), 39 (28); hrms 286.1125, 284.1151 ($C_{15}H_{25}Br$ requires 286.1120, 284.1140).

CYCLIZATION OF ISOCARYOPHYLLENE [**5**] WITH SULFURIC ACID.—A stirred solution of isocaryophyllene [**5**] (500 mg) in 4 ml of each of the following solvents: Et_2O, THF, dioxane, Me_2CO was treated respectively with H_2SO_4 96% (1 ml) for 20 min at room temperature. The reaction mixtures were neutralized with a solution of $NaHCO_3$ and extracted with EtOAc. The organic layers were washed with brine, dried over anhydrous Na_2SO_4 and evaporated to give (average yield) **7** (64%), **6** (12%), **10** (12.2%), **9** (5.4%), and **12** (2.7%). When the cyclization was carried out using THF as solvent, the following yields were obtained: **7** (50%), **6** (16%), **9** (22.2%), **10** (3.1%), and **12** (1.7%). 4β,8α,10,10-Tetramethyl-11-hydroxytricyclo[6,2,1,0^{4,11}]undecane [**10**]: mp 32–35° (hexane/EtOAc); $[\alpha]^{25}D$ +8° (c=0.9, $CHCl_3$); ir (KBr) ν max 3474, 2950, 1443, 1373 cm^{-1}; ^{1}H nmr ($CDCl_3$, 400 MHz) δ 0.98 (3H, d, J=0.9 Hz, H-13),

1.01 (3H, s, H-12), 1.02 (3H, s, H-15), 1.04 (3H, s, H-14), 1.32 (1H, m, H-6), 1.40 (2H, m, H-2), 1.60 (1H, m, H-5), 1.67 (1H, m, H-6′), 1.77 (2H, m, H-3), 1.88 (1H, ddd, J=14.1, 14.1, and 3.6 Hz, H-5′), 1.89 (1H, d, J=13.6 Hz, H-9α), 2.13 (1H, d, J=13.6 Hz, H-9β), 2.67 (1H, dd, J=12.4 and 8.0 Hz, H-1); ^{13}C nmr ($CDCl_3$, 200 MHz) δ 13.7 (q, C-13), 18.9 (t, C-2), 20.3 (t, C-6), 23.9 (q, C-12), 26.9 (q, C-15), 32.6 (s, C-10), 34.2 (q, C-14), 37.1 (t, C-5), 37.9 (s, C-4), 38.6 (t, C-7), 44.0 (t, C-3), 56.9 (s, C-8), 58.3 (d, C-1), 60.8 (t, C-9), 76.8 (s, C-11); ms *m/z* 222 (11) [M^+], 207 (3) [M^+-15], 204 (3) [M^+-18], 189 (2) [$M^+-15-18$], 180 (10), 165 (5), 151 (23), 125 (52), 40 (100); hrms 222.1988 ($C_{15}H_{26}O$ requires 222.1983).

ACETYLGINSENOL [**9a**].—To ginsenol [**9**] (92 mg), dissolved in 4 ml of Ac_2O, a catalytic amount of *p*-toluenesulfonic acid was added. The reaction mixture was stirred at room temperature overnight, then the reaction was quenched with H_2O, neutralized with $NaHCO_3$, and extracted with EtOAc. The organic layer was washed with brine and dried over anhydrous Na_2SO_4. The solvent was removed by distillation under reduced pressure to give 96 mg of a product, which was purified by hplc (hexane-EtOAc, 100:1) yielding 90 mg of acetylginsenol [**9a**]: oil, $[\alpha]^{25}D$ −4° (c=1.6, $CHCl_3$); ir (film) ν max 2939, 1731, 1452, 1362, 1244, 1034, 1020 cm^{-1}; ^{1}H nmr ($CDCl_3$, 400 MHz) δ 0.82 (3H, s, H-14), 1.05 (3H, s, H-13), 1.14 (3H, s, H-15), 1.24 (3H, s, H-12), 1.99 (3H, s, CH_3-COO-), 2.30 (1H, d, J=15.1 Hz, H-2β), 4.79 (1H, d, J=15.1 Hz, H-2α); ^{13}C nmr ($CDCl_3$, 400 MHz) δ 21.9 (t, C-9), 22.1 (q, CH_3COO-), 25.9 (t, C-5), 27.4 (q, C-14), 28.6 (q, C-13), 30.0 (q, C-15), 33.8 (t, C-10), 34.4 (q, C-12), 34.7 (t, C-6), 34.9 (t, C-8), 36.2 (s, C-3), 40.4 (s, C-7), 44.7 (t, C-2), 46.7 (s, C-11), 55.1 (d, C-4), 93.9 (s, C-1), 170.9 (s, C=O); ms *m/z* 264 (0.27) [M^+], 249 (3) [M^+-15], 222 (4), 207 (91) [$M^+-42-15$], 204 (82) [M^+-60], 189 (61) [$M^+-60-15$], 161 (73), 148 (24), 122 (85), 107 (81), 95 (74), 43 (100).

ACETYLATION OF **10**.—Compound **10** (50 mg) was dissolved in 3 ml of dry Ac_2O and a catalytic amount of *p*-toluenesulfonic acid was added. The reaction mixture was subjected to the same treatment above described for **9** to yield 40 mg of **11** (67%). 4β,8α,10,10-Tetramethyl-11-acetyloxiytricyclo[6,2,1,$0^{4,11}$]decane [**11**]: mp 39–41°; $[\alpha]^{25}D$ +7° (c=1.9, $CHCl_3$); ir (KBr) ν max 2975, 1710, 1433, 1361, 1249, 1232 cm^{-1}; ^{1}H nmr ($CDCl_3$, 200 MHz) δ 0.98 (3H, s, H-13), 1.00 (3H, s, H-12), 1.02 (3H, s, H-15), 1.03 (3H, s, H-14), 1.30 (1H, m, H-7), 1.33 (2H, m, H-6), 1.38 (2H, m, H-2), 1.40 (1H, m, H-7′), 1.61 (1H, m, H-5β), 1.77 (2H, m, H-3), 1.96 (3H, s, CH_3COO-), 2.30 (1H, m, H-5α), 2.31 (1H, d, J=14.6 Hz, H-9β), 2.53 (1H, d, J=14.6 Hz, H-9α), 2.59 (1H, m, H-1); ^{13}C nmr ($CDCl_3$, 200 MHz) δ 14.8 (q, C-13), 19.3 (t, C-2), 20.7 (t, C-6), 22.8 (q, CH_3COO-), 24.3 (q, C-12), 27.2 (q, C-14), 32.0 (t, C-5), 33.2 (s, C-10), 34.7 (q, C-15), 38.3 (s, C-4), 38.7 (t, C-7), 44.4 (t, C-3), 56.4 (d, C-1), 57.5 (t, C-9), 58.0 (s, C-8), 85.8 (s, C-11), 169.5 (s, C=O); ms *m/z* 264 (0.1) [M^+], 222 (2), 207 (5) [$M^+-42-15$], 204 (19) [M^+-60], 189 (33) [$M^+-60-15$], 125 (47), 122 (94), 121 (54), 107 (21), 55 (33), 43 (100).

TREATMENT OF GINSENOL [**9**] WITH SULFURIC ACID.—Ginsenol [**9**] (31 mg) dissolved in 0.5 ml of Et_2O was added dropwise to 1 ml of H_2SO_4 (96%) in 3.5 ml of Et_2O. The mixture was stirred at 0° for 30 min and allowed to warm to room temperature for another 30 min. Then it was neutralized with NaOH and extracted with Et_2O, and the organic layer was washed with brine and dried over anhydrous Na_2SO_4. Evaporation of the solvent afforded an oily residue which was studied by gc yielding neoclovene (**7**, 32%) and starting material (**9**, 64%).

TREATMENT OF NEOCLOVENE [**7**] WITH SULFURIC ACID.—Compound **7** (2.43 g) was dissolved in 55 ml of 1,4-dioxane and 11 ml of H_2SO_4 (95%) were added. The mixture was refluxed for 3 h, then it was cooled and H_2O was added. The reaction mixture was neutralized and extracted as indicated previously for isocaryophyllene [**5**]. The crude material obtained (2.35 g) was purified using SiO_2-$AgNO_3$ (80:20) for column chromatography, yielding olefin **13** (10% by glc).

TREATMENT OF ALCOHOL [**10**] WITH SULFURIC ACID.—Compound **10** (12 mg) was dissolved in 1 ml of 1,4-dioxane and 0.5 ml of H_2SO_4 (96%) were added. The reaction mixture was refluxed for 90 min and then extracted as described for isocaryophyllene [**5**] to give compound **13** (5 mg).

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