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THE RACEMIZATION OF CRINITOL. RESOLUTION OF CRINITOL AND OF 3-NONEN-2-OL ENANTIOMERS BY RECYCLE-HPLC, VIA MTPA ESTERS

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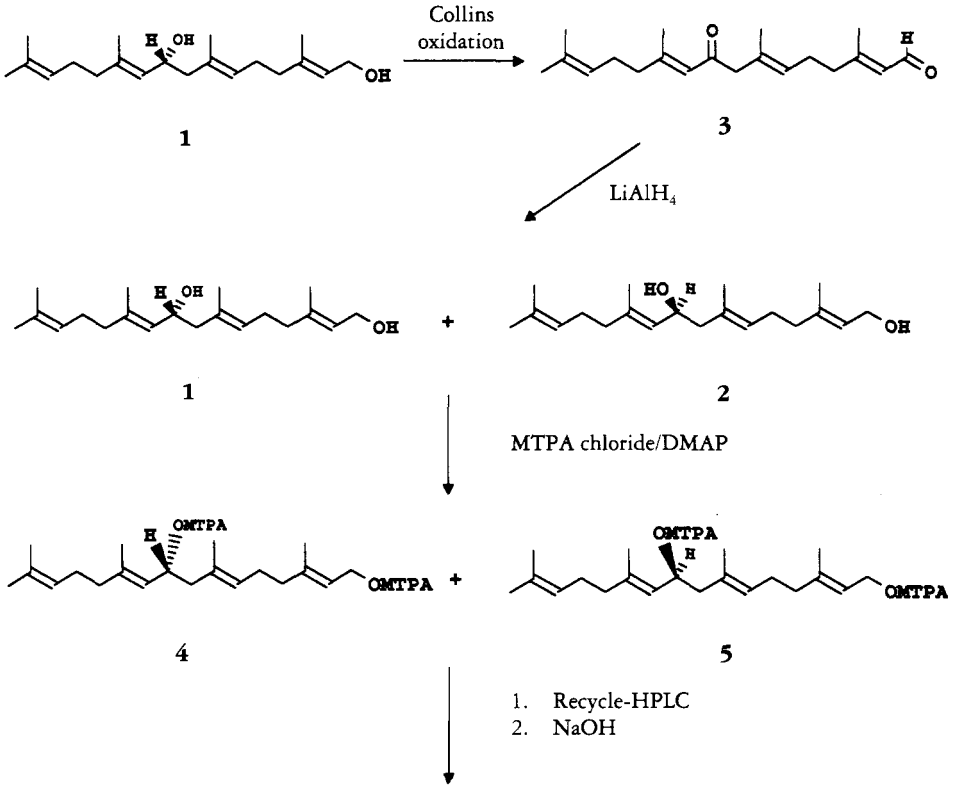
ABSTRACT.—The antimicrobial diterpene diol crinitol [**1**] is readily converted to its racemate by Collins oxidation, followed by lithium aluminum hydride reduction. Converting the racemate to the *bis*-*R*-(+)-α-methoxy-α-(trifluoromethyl)-phenylacetate (MTPA) ester, followed by recycle-hplc, separated the diastereomeric diesters. Hydrolysis then gave the "unnatural" (9*S*) crinitol enantiomer [**2**]. The derivatization sequence was also used to evaluate the enantiomeric purity of the naturally derived crinitol. Bioassays using Gram-positive bacteria showed that changing the C-9 configuration did not affect the antimicrobial activity of crinitol. In addition, we report a model sequence, the resolution of 3-nonen-2-ol ([**6**] and [**7**]), and nmr evidence for conformational change as a solution of crinitol was diluted.

The diterpene diol, crinitol [(9*R*)-3,7,11,15-tetramethylhexadeca-2,6,10,14-tetraene-1,9-diol] [**1**] was first isolated from the brown marine alga *Cystoseira crinita* in 1976 (1). Its absolute configuration, 9*R*, was determined in 1985 after isolation from another marine alga, *Sargassum tortile* (2). Its biological properties include activity as an insect growth inhibitor (3) and as a Gram-positive antibacterial agent (4). As part of a study of the antibacterial activity of long-chain alcohols (5), it was of interest to test whether the two enantiomers of **1** exhibit the same or different activities, as a clue to whether the antimicrobial action might be due to some specific interaction beyond a hypothesized general lipophilic action on cell membranes. However, a source of the non-natural 9*S* isomer [**2**] was first required. The availability of natural **1** made a racemization/resolution route (Scheme 1) attractive. Racemization was accomplished by Collins oxidation (6) to the dicarbonyl compound **3**, followed by $LiAlH_4$ reduction to give the racemic mixture (**1** and **2**). Resolution via diastereomeric derivatives seemed plausible. Esterification with enantiomerically pure α-methoxy-α-(trifluoromethyl)-phenylacetic acid (MTPA) (7), followed by separation of diastereomers by recycle-hplc, had earlier been used to purify enantiomers of ipsenol and ipsdienol (8). We decided to follow this route with crinitol, using the *bis*-MTPA esters **4** and **5**.

As a model, we chose *E*-3-nonen-2-ol, a secondary allylic alcohol which has been found as a volatile constituent of Rooibos tea (9). This was easily prepared by $LiAlH_4$ reduction of *E*-3-nonen-2-one. The mixed MTPA esters **8** and **9** were prepared by reaction of the racemic alcohol (**6** and **7**) with the free acid in the presence of dicyclohexylcarbodiimide (DCC) and 4-pyrrolidinopyridine (4-PP). The diastereomeric esters were separated by recycle-hplc, then the free enantiomeric alcohols were regenerated by hydrolysis with NaOH in MeOH.

With **1**, the sequence was very similar, except that the MTPA/DCC/4-PP esterification gave monoesters along with the desired diesters **4** and **5**, which had to be separated by cc on Si gel to allow recycle-hplc separation of the diesters. Use of MTPA chloride/dimethylaminopyridine (7,10) gave the desired mixed diester cleanly and in high yield, how-

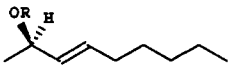


SCHEME 1

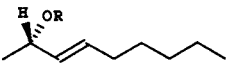
ever. It is of interest that recycle-hplc of the *bis*-MTPA ester of natural crinitol showed the presence of a small amount (13%) of the 9*S* isomer.

Absolute configurations were assigned to the 3-nonen-2-ol MTPA esters **8** and **9** by their ^{1}H-nmr spectra, using the Mosher method, as extended by Ohtani *et al.* (11). This method presumes a syn-periplanar arrangement of the C-H bond at the chiral center, the ester carbonyl bond, and the alpha *C*-trifluoromethyl bond of the ester moiety. In this geometry, the ester's phenyl and methoxy groups, respectively, shield and deshield nearby groups in the alcohol moiety.

Comparing spectra of diastereomers **8** and **9**, the relative shielding of the C-1 methyl of **8** (δ 1.34, vs. 1.40 in **9**), the deshielding of the C-3/C-4 olefinic H of **8** (δ 5.88–5.78 and 5.55–5.45, vs. 5.80–5.70 and 5.45–5.35 in **9**), and the deshielding of the C-5 methylene H of **8** (δ 2.03, vs. 2.00 in **9**), allowed assignment of the 2*R* configuration to **8**. Compound **9** was correspondingly assigned the 2*S* configuration. While the C-9 configurations of the crinitol *bis*-MTPA esters were already immediately assignable by comparison with material prepared from the known (2) 9*R* natural diol, the relative shifts of the clearly identifiable



6 R=H
8 R=MTPA



7 R=H
9 R=MTPA

C-8 methylene hydrogens (δ 2.37 and 2.17 in **4**, vs. 2.42 and 2.21 in **5**) are consistent with predictions by the same nmr method.

Diastereomeric excesses of the separated MTPA esters **4**, **5**, **8**, and **9** were measured by enlargement of selected portions of the ^{1}H-nmr spectra, followed by cut-and weigh measurements of corresponding non-overlapping peaks. On this basis, our de values were: **4**, >96%; **5**, >96%; **8**, 96%; **9**, 92%. Higher diastereomeric excesses could be obtained by further chromatography, but the values obtained sufficed for our current needs.

The "unnatural" 9*S* crinitol was assayed for antibacterial activity simultaneously with a sample of natural **1**, against the same battery of Gram-positive microorganisms originally used with the natural material alone (4). Against *Bacillus subtilis, Brevibacterium ammoniagenes, Staphylococcus aureus, Streptococcus mutans,* and *Propionobacterium acnes*, the natural and unnatural material showed identical activities, which also matched the results of earlier tests (4) on natural material alone. These data show that, for these test species at least, the configuration at C-9 appears irrelevant. This might indicate that antimicrobial activity is not due to interaction with a specific receptor, but rather perhaps to a more general lipophilic interaction affecting cell membranes (5).

In verifying the identities of the resolved enantiomers, an interesting concentration effect was found in the 300 MHz proton ^{1}H-nmr spectrum of natural (9*R*)-crinitol. The C-9 hydroxyl results in a clearly separated C-9 proton (H-9) at δ 4.4, whose couplings provide clues to conformational preferences near the center of the molecule. In particular, the observed splitting goes from a quartet (J=7.4 Hz) at ca. 15% in $CDCl_3$, to a triplet (J=8.4 Hz) of doublets (J=4.5 Hz) in ca. 1% solution. Coupling to the C-10 olefinic proton (H-10, a doublet at δ 5.15) remains almost constant, but a clear differentiation appears in coupling to the diastereotopic methylene protons on C-8. A predominantly anti H-9/H-10 orientation throughout would be consistent with recent studies reported by Gung *et al.* (12). The spectra seem to indicate that there is no particular C-8/C-9 conformational preference at high concentration. On dilution, however, the different couplings that emerge would be more consistent with a predominantly extended C-8/C-9 conformation in which H-9 is anti to the pro-*S* proton (H-8*S*) on C-8, and therefore gauche to the pro-R proton (H-8*R*); H-8*S* and H-8*R* appear as a partially visible multiplet around δ 3.2. A "hairpin" conformation around C-8/C-9 might also rationalize the observed couplings, but would appear sterically unreasonable and at variance with reported studies on smaller analogues (12). Other concentration-related differences appear, for example in the C-1 methylene proton pattern at ca. δ 4.1, but interpretation is not so straightforward. A more thorough look at solvent effects on the spectrum of **1** may ultimately prove useful in studies of crinitol's mode of antibacterial action.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Infrared spectra were run between salt plates on a Perkin-Elmer model 1310 instrument. Nmr spectra were run on a Bruker AMX-300 instrument, with TMS as internal standard.

CONVERSION OF 3-NONEN-2-ONE TO 3-NONEN-2-OL (**6** AND **7**).—A 250-ml round-bottomed flask containing a magnetic stirbar was charged with 30 ml anhydrous Et_2O and 0.39 g (10 mmol) of $LiAlH_4$ (Aldrich). The flask was closed with a septum pierced with a syringe needle. With stirring and cooling (ice bath), 3.3 ml (2.80 g; 20 mmol) of 3-nonen-2-one (Aldrich) was injected (5 min). The ice bath was removed, and the mixture stirred at ca. 25° for 35 min. The ice bath was replaced, then 40 ml of H_2O-saturated Et_2O were injected over 3 min. The suspension was stirred for several min, then the septum was removed and 40 ml of 5% aqueous NaOH were added and mixed. The separated aqueous layer was extracted with two 30-ml portions of Et_2O, then was clarified by adding 3% aqueous HCl, then

extracted with 30 ml of Et_2O. The combined Et_2O extracts were dried over anhydrous Na_2SO_4, then decanted and evaporated to an oil. The oil was taken up in 15 ml of pentane, then dried again. Rotary evaporation, followed by evaporation at 5 Torr, gave 2.83 g (20 mmol; 100%) of almost colorless oil: ir (neat) ν max 3360, 3040, 2980, 2945, 2890, 2875, 1675, 1465, 1415, 1380, 1300, 1155, 1130, 1070, 975, 945, 875 cm^{-1}; ^1H nmr ($CDCl_3$, 300 MHz) δ 5.7–5.55 (1H, dt, J=16 and 6.5 Hz), 5.55–5.45 (1H, ddt, J=16, 7, and 1 Hz), 4.25 (1H, p, J=7 Hz), 2.0 (2H, q, J=7 Hz), 1.5 (1H, br s), 1.45–1.2 (9H, m, containing d at 1.25, J=7 Hz), 0.87 (3H, t, J=7 Hz); ^{13}C nmr ($CDCl_3$, 75.4 MHz) δ 134.4, 131.3, 69.1, 32.4, 31.7, 29.1, 23.7, 22.8, 14.3.

3-NONEN-2-OL MTPA ESTERS (**8** AND **9**).—A 25-ml round-bottomed flask with magnetic stirbar was charged with 258 mg (1.10 mmol) of *R*-(+)-MTPA (Aldrich), 23 mg (0.15 mmol) of 4-PP (Aldrich), 295 mg (1.43 mmol) of DCC (Aldrich), and 5 ml of CH_2Cl_2 (dried over molecular sieves). The flask was closed (septum), and 148 mg (1.04 mmol) of 3-nonen-2-ol were injected into the slurry. The mixture was stirred at ca. 25°; after 3 h, 2 ml of CH_2Cl_2 were injected to wash down the sides of the flask. After stirring 30 h, the mixture was filtered, using several ml of CH_2Cl_2 to rinse the flask and the filter cake. The filtrate was concentrated to an oil. Addition of several ml of pentane resulted in deposition of finely crystalline precipitate. The supernatant was filtered, then reconcentrated to an oil. The oil was purified by cc, using 15 g of EM Science Si Gel 60, 70–230 mesh; the column was packed in pentane, then eluted first with 200 ml pentane; then 100 ml of 5% Et_2O in pentane eluted 0.278 g (0.78 mmol, 75%) of faintly yellowish oil: ir (neat) ν max 3070, 3040, 2960, 2930, 2870, 2860, 1745, 1670, 1490, 1450, 1375, 1325, 1270, 1185, 1170, 1120, 1080, 1035, 1015, 990, 965, 870, 845, 765 cm^{-1}. Nmr data for the separated diastereomers appear below.

SEPARATION OF DIASTEREOMERIC 3-NONEN-2-OL MTPA ESTERS (**8** AND **9**) BY RECYCLE-HPLC.—Recycle-hplc was carried out using a 2.5 cm×25 cm Nomura Chemical Develosil™ column in a JAI LC-908 instrument, with a flow of 6 ml per min of 0.2% Me_2CO in hexane. The uv detector was set at 254 nm. Samples (typically ca. 10–30 mg) were injected in 1 ml of solvent; retention volumes were ca. 350 ml. Depending on loading, it took around five or more cycles to achieve complete separation. Ir spectra of the separated isomers were identical, but the proton nmr spectra showed differences which allowed assignments of configuration, as discussed in the text.

(2R)-Isomer [**8**].—^1H nmr ($CDCl_3$, 300 MHz) δ 7.55–7.49 (2H, m), 7.42–7.35 (3H, m), 5.88–5.78 (1H, m), 5.63–5.46 (2H, m), 3.55 (3H, s), 2.03 (2H, q, J=7.4 Hz), 1.41–1.2 (9H, m, containing d at 1.34, J=6.3 Hz), 0.88 (3H, t, J=7.2 Hz); ^{13}C nmr ($CDCl_3$, 75.4 MHz) δ 165.8, 135.7, 132.7, 129.5, 128.4, 128.3, 127.4, 125/122 (presumed center pair of quartet, J=280 Hz), 74.2, 55.4, 32.1, 31.3, 28.6, 22.5, 20.1, 14.0.

(2S)-Isomer [**9**].—^1H nmr ($CDCl_3$, 300 MHz) δ 7.55–7.49 (2H, m), 7.42–7.36 (3H, m), 5.80–5.70 (1H, m), 5.58 (1H, p, J=6.5 Hz), 5.45–5.35 (1H, ddt, J=15.7, 7.2, and 1.4 Hz), 3.55 (3H, s), 2.00 (2H, q, J=6.9 Hz), 1.42–1.20 (9H, m, containing d at 1.40), 0.88 (3H, t, J=6.8 Hz); ^{13}C nmr ($CDCl_3$, 75.4 MHz) δ 166, 135.4, 132.5, 129.5, 128.3, 128.1, 127.4, 125.5/122 (presumed center pair of quartet), 74.1, 55.5, 32.1, 31.3, 28.5, 22.5, 20.4, 14.0. To get diastereomeric compositions, the δ 5.7–5.9 regions of the proton nmr spectra were enlarged, then cut-and-weigh was used to compare areas of nonoverlapping corresponding peaks. Diastereomeric excess was 96% for **8** (2*R*), and 92% for **9** (2*S*).

REGENERATION OF 2*S*-3-NONEN-2-OL [**7**] FROM THE MTPA ESTER [**9**].—41 mg (0.115 mmol) of **9** were dissolved in 1.0 ml of MeOH in a vial, and 0.097 g (2.4 mmol) of NaOH were added and broken up with a spatula. A stirbar was added, and the vial was closed and left to stir at ca. 25° for 21 h. The vial was then reopened, and 2 ml of Et_2O, 2 ml of pentane, and 2 ml of H_2O were added. After stirring for several min, the layers were allowed to separate. The lower aqueous MeOH layer was removed and placed in a separate vial. The organic phase in the original vial was washed with two 1-ml portions of H_2O, which were added to the second vial. The aqueous MeOH phase in the second vial was extracted with three 5-ml portions of 20% Et_2O/80% pentane. The combined organic phases were then washed with 1 ml of H_2O. Drying (anhydrous Na_2SO_4) and concentration (rotary evaporation, then 2 Torr) gave 12.7 mg (0.0894 mmol, 78%) of colorless oil, the ir and ^1H-nmr spectra of which matched those of 3-nonen-2-ol.

RACEMIZATION OF CRINITOL [**1**].—A 250-ml 4-necked flask was set up with thermometer, septum, stopper, drying tube, and magnetic stirbar. 250 ml of CH_2Cl_2 (dried over molecular sieves) were injected, followed by 9.8 ml of pyridine (120 mmol; Aldrich, dried over sieves). An ice bath was used to cool the solution to 10°, then (with rapid stirring) 6.0 g (60 mmol) of CrO_3 (Aldrich) were added. The mixture was stirred at ca. 10–15° for several min, then the ice bath was removed for 20 min. The ice bath was replaced, and the solution cooled to below 10°. A solution of 1.57 g (5.1 mmol) of natural (9*R*)-crinitol in 50 ml of CH_2Cl_2 was then injected, with stirring (3 min). After stirring at 10° for 20 min, the ice bath was

removed. Stirring continued for another 25 min, by which time the temperature reached 15°. The mixture was decanted into a separatory funnel; 80 ml of Et_2O were used to rinse the flask. The organic phase was washed with 3 100-ml portions of 5% aqueous NaOH, 1 100-ml portion of 3% aqueous HCl, 2 100-ml portions of 5% aqueous $NaHCO_3$, and 100 ml of saturated aqueous NaCl. The layers inverted at the final wash, at which time the organic phase was diluted with 50 ml of pentane and 50 ml of Et_2O, to aid separation. After drying over anhydrous Na_2SO_4, the product was concentrated by rotary evaporation, taken up in pentane, re-dried, and reconcentrated to give 1.30 g of yellow-brown liquid, the ir spectrum of which was consistent with that of compound **3**. In particular, OH (3400 cm^{-1}) was no longer evident, weak absorptions attributable to aldehyde were present (3770 and 3720 cm^{-1}), and an intense absorption at 1670 cm^{-1} indicated C=O. A 250-ml flask was charged with a magnetic stirbar and 0.86 g (20 mmol) of $LiAlH_4$ (Aldrich). The flask was closed with a septum, and 20 ml of anhydrous Et_2O were injected. The flask was chilled (ice bath), then the dicarbonyl compound, in 20 ml of anhydrous Et_2O, was injected with rapid stirring (2 min). After 15 min, the ice bath was removed and the mixture stirred at ca. 25° for 45 min. The ice bath was then replaced, and the reaction was quenched by addition (10 min) of 80 ml of H_2O-saturated Et_2O, followed by 80 ml of 5% aqueous NaOH. Fifty ml of pentane were also added. The layers were separated, and the aqueous phase was extracted with two 50-ml portions of Et_2O. The aqueous phase was then acidified with 3% aqueous HCl, and extracted once more with 50 ml of Et_2O. The combined organic phase was washed once with saturated aqueous NaCl, dried over Na_2SO_4, and evaporated (rotary evaporation, then 5 Torr) to give 1.13 g (72%) of slightly yellow oil (**1** and **2**) whose ir, ^{1}H-nmr, and ^{13}C-nmr spectra were satisfactory for a racemate of **1**.

DIASTEREOMERIC CRINITOL *BIS*-MTPA ESTERS **4** AND **5**.—1.0 g (4.3 mmol) of *R*-(+)-MTPA was placed in a 25-ml round-bottomed flask with a magnetic stirbar. 2.5 ml of thionyl chloride were added, then the flask was fitted with a H_2O condenser topped by a $CaCl_2$ drying tube. The flask was heated to reflux, with stirring, for 46 h. The condenser was then replaced with a distillation head and receiver, and excess thionyl chloride was distilled out with the aid of a gentle stream of N_2, followed by brief vacuum treatment, giving a yellow oil (MTPA chloride). A separate 50-ml flask was charged with a stirbar, 0.59 g (1.93 mmol) of racemic crinitol, 0.53 g (4.3 mmol) of 4-*N*,*N*-dimethylaminopyridine, and 10 ml of dry CH_2Cl_2. The MTPA chloride, dissolved in 10 ml of CH_2Cl_2, was then injected. The closed mixture was left to stir at room temperature for 24 h. The mixture was then concentrated (rotary evaporator), then 40 ml of pentane and 5 ml of H_2O were added. After shaking, the aqueous layer was removed. After three more washes with 5-ml portions of H_2O, the pentane layer was dried over anhydrous Na_2SO_4 and evaporated to give 1.25 g (90%) of a faintly yellowish oil: ir (neat) ν max 3050, 3020, 2950, 2930, 2910, 2840, 1740, 1660, 1600, 1490, 1445, 1375, 1265, 1180, 1165, 1115, 1075, 1015, 990, 915, 765, 720, 695 cm^{-1}. Nmr data for the separated diastereomers appear below.

SEPARATION OF THE DIASTEREOMERIC *BIS*-MTPA ESTERS (**4** AND **5**) OF CRINITOL BY RECYCLE-HPLC.—Recycle-hplc was carried out using the 2.5 cm×25 cm Develosil™ column in the JAI LC-908 instrument, with a flow of 10 ml per min of 2% Me_2CO in hexane, and uv detection at 254 nm. Samples (typically 10–30 mg) were injected in 1 ml of solvent; retention volumes were ca. 400 ml. Depending on loading, five to eight cycles achieved separation. In addition to *bis*-MTPA esters from racemic crinitol, the *bis*-MTPA ester of the natural (9*R*) diol was also subjected to the separation, which showed that the natural material contained ca. 13% of the 9*S* isomer. Ir spectra of the separated isomers were identical to those of the mixed isomers, but the ^{1}H-nmr spectra showed differences consistent with the known configurations, as discussed above.

(9R)-Isomer [**4**].—^{1}H nmr ($CDCl_3$, 300 MHz) δ 7.55–7.48 (4H, m), 7.4–7.32 (6H, m), 5.91–5.82 (1H, m), 5.4–5.32 (1H, t, J=6.5 Hz), 5.21–5.16 (1H, d, J=8 Hz), 5.10–5.01 (2H, m), 4.84–4.76 (2H, m), 3.55 (3H, s), 3.54 (3H, s), 2.37 (1H, dd, J=13 and 8 Hz), 2.17 (1H, dd, partial overlap with m to right, J=13 and 5 Hz), 2.11–1.94 (8H, m, and methyl singlets at 1.77, 1.69, 1.66, 1.60, 1.56; ^{13}C nmr ($CDCl_3$, 75.4 MHz) δ 166.5, 165.8, 144.1, 142.1, 132.7, 132.5, 131.9, 130.4, 129.6, 129.4, 128.4, 128.2, 127.5, 127.3, 123.6, 122.4, 117.1, 72.7, 63.0, 55.4, 44.7, 39.5, 39.1, 26.3, 26.2, 25.7, 17.7, 16.9, 16.5, 16.2 (trifluoromethyl groups were lost in baseline noise).

(9S)-Isomer [**5**].—^{1}H nmr ($CDCl_3$, 300 MHz) δ 7.54–7.46 (4H, m), 7.4–7.32 (6H, m), 5.84–5.79 (1H, m), 5.40–5.34 (1H, t, J=7 Hz), 5.20–5.14 (1H, m), 5.07–5.00 (2H, d, J=8 Hz), 4.86–4.76 (2H, m), 3.55 (3H, narrow m, split at top with J=0.9 Hz), 3.52 (δ as with 3H, 3.55), 2.42 (1H, dd, J=13 and 8 Hz), 2.21 (1H, dd, J=13 and 5 Hz), 2.16–1.95 (8H, m), and methyl singlets at 1.77, 1.70, 1.64, 1.62, 1.58; ^{13}C nmr ($CDCl_3$, 75.4 MHz) δ 166.5, 165.8, 144.1, 141.9, 131.8, 130.6, 129.6, 129.4, 128.4, 128.2, 127.6, 127.5, 127.3, 123.6, 122.2, 117.2, 72.9, 63.0, 55.4, 44.7, 39.5, 39.1, 26.3, 26.3, 25.7, 17.7, 16.9, 16.5, 16.2. To get diastereomeric composi-

tions, the δ 2.3–2.5 regions of the ^{1}H-nmr spectra were enlarged, then cut-and-weigh was used to compare areas of non-overlapping corresponding peaks. Calculated diastereomeric excess was >96% for both **4** and **5**.

(9S)-Crinitol [**2**].—95 mg (0.129 mmol) of 9*S*-crinitol-*bis*-MTPA ester [**5**] were dissolved in 4.0 ml MeOH in a vial, and 0.24 g (6.0 mmol) NaOH were added and broken up with a spatula. A stirbar was added; the vial was closed and stirred for 22.5 h at ca. 25°. The vial was opened; 4 ml pentane, 4 ml Et_2O, and 4 ml H_2O were added, then stirred for several min. The lower layer was separated into a second vial. The upper layer was washed with 1 ml 5% aqueous NaOH and with 1 ml H_2O; washes were added to the second vial. The aqueous MeOH in the second vial was extracted with three 5-ml portions of Et_2O-pentane (1:4). The combined organic layer was washed with 1 ml 5% aqueous NaOH, then with 1 ml H_2O. Drying (Na_2SO_4) and evaporation (rotary evaporator, then 2 Torr) gave 37.2 mg (94%) of an almost colorless oil whose spectra matched those of natural **1**: ir (neat) ν max 3460, 3040 (sh), 2980, 2940, 2870, 1670, 1445, 1385, 1105, 1015, 840, 820 cm^{-1}; ^{1}H nmr ($CDCl_3$, 300 MHz) δ 5.4 (1H, t, J=6.2 Hz), 5.23–5.05 (3H, m), 4.44–4.36 (1H, m, discussed in text), 4.19–4.05 (2H, m), 2.3–1.93 (12H, m), 1.69–1.67 (12H, 2 br s), 1.60 (3H, s); ^{13}C nmr ($CDCl_3$, 75.4 MHz) δ 138.7, 138.1, 132.2, 131.5, 128.2, 127.0, 124.6, 123.9, 65.4, 59.1, 48.0, 39.5, 39.1, 26.3, 25.8, 25.6, 17.6, 16.5, 16.1, 15.8.

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LITERATURE CITED

1. E. Fattorusso, S. Magno, L. Santacroce, and D. Sica, *Tetrahedron Lett.*, 937 (1976).
2. I. Kubo, T. Matsumoto, and N. Ichikawa, *Chem. Lett.*, 249 (1985).
3. I. Kubo and J.A. Klocke, in: "Plant Resistance to Insects." Ed. by P. Hedin, ACS Symposium Series No. 208, American Chemical Society, Washington, DC, 1983, p. 329.
4. I. Kubo, M. Himejima, K. Tsujimoto, H. Muroi, and N. Ichikawa, *J. Nat. Prod.*, **55**, 780 (1992).
5. I. Kubo, H. Muroi, M. Himejima, and A. Kubo, *Bioorg. Med. Chem. Lett.*, **3**, 1305 (1993).
6. L.F. Fieser and K.L. Williamson, "Organic Experiments," D.C. Heath, Lexington, MA, 1979, 4th Ed., p. 148.
7. J.A. Dale, D.L. Dull, and H.S. Mosher, *J. Org. Chem.*, **34**, 2543 (1969).
8. I. Kubo, S. Komatsu, T. Iwagawa, and D.L. Wood, *J. Chromatogr.*, **363**, 309 (1986).
9. M. Kawakami, A. Kobayashi, and K. Kator, *J. Agric. Food Chem.*, **41**, 633 (1993).
10. H.-S. Byun and R. Bittman, *J. Org. Chem.*, **59**, 668 (1994).
11. I. Ohtani, T. Kusumi, Y. Kashman, and H. Kakisawa, *J. Am. Chem. Soc.*, **113**, 4092 (1991).
12. B.J. Gung, M.A. Wolf, K. Ohm, and A.J. Peat, *Tetrahedron Lett.*, **34**, 1417 (1993).

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