Tetrahedron Vol. 49, No. 27, pp. 5917-5926, 1993
Printed in Great Britain

0040-4020/93 $6.00+.00
© 1993 Pergamon Press Ltd

Isolation and Structure of Chaetomellic Acids A and B from *Chaetomella acutiseta*: Farnesyl Pyrophosphate Mimic Inhibitors of Ras Farnesyl-Protein Transferase

Sheo B. Singh*, Deborah L. Zink, Jerrold M. Liesch, Michael A. Goetz, Rosalind G. Jenkins, Mary Nallin-Omstead, Keith C. Silverman, Gerald F. Bills, Ralph T. Mosley, Jackson B. Gibbs, Georg Albers-Schonberg and Russell B. Lingham

Merck Research Laboratories, P. O. Box 2000, Rahway, New Jersey 07065 (U S A)

(*Received in USA* 24 *March* 1993; *accepted* 3 *May* 1993)

Keywords: Chaetomellic acids, Ras FPTase inhibitors, FPP mimic, *Chaetomella acutiseta*

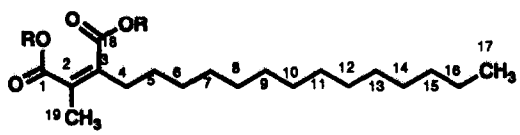
Abstract: *Farnesyl-Protein transferase catalyses a post-translational modification of Ras that is obligatory for the cell transforming activity of this oncogene protein. The screening of natural products to identify inhibitors of this enzyme as a potential anticancer agents, has led to the isolation of two novel dicarboxylic acids, named chaetomellic acids from Chaetomella acutiseta , as potent and selective inhibitors which appear to be the first examples of non-phosphorous containing FPP mimics.*

INTRODUCTION

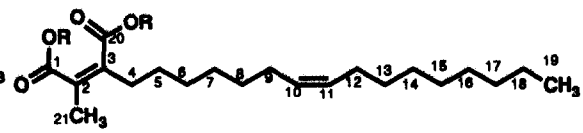
Mutated forms of the *ras* oncogenes are associated with about 25% of human tumors.[1] The *ras* genes encode 21kDa proteins, called p21 or Ras, that are associated with the plasma membrane. Ras proteins function by binding GTP to interact with effector molecules, and this interaction is terminated by GTPase activity that converts Ras into a complex with GDP. Inhibitors directed toward Ras function may have advantages over existing chemotherapeutic agents. The biochemical analysis of Ras function has revealed potential points of intervention.[2] One of these areas is the post-translational processing that is required to appropriately modify Ras prior to membrane association. The first and the obligatory step in Ras processing is farnesylation by farnesyl-protein transferase (FPTase).[3] Genetic experiments have shown that farnesylation is required for Ras cell-transforming activity. FPTase may, therefore, represent a target for chemotherapeutic intervention of human tumors having mutated *ras* genes.

FPTase utilizes farnesyl pyrophosphate (FPP) to modify the Cys residue at the C-terminus of Ras known as a CaaX box (C, Cys; a, usually an aliphatic amino acid; X, another amino acid). Analyses of the substrate

requirements for FPTase have shown that the enzyme binds with selective isoprenoid pyrophosphates and CaaX tetrapeptides.[4] Potential peptide inhibitors [e.g. CVLS (IC_{50} =2 μM), CVIM (0.1 μM), and CVFM (0.02 μM)][2] of FPTase can be designed from the substrate. However, peptides are not the most desired compounds to be considered as a therapeutic agents, and therefore, we were interested to find inhibitors of FPTase other than peptides. For this purpose, microbial fermentations were evaluated as sources for natural product inhibitors of FPTase. Among the compounds identified were two novel dicarboxylic acids, named here, chaetomellic acid A (**1a**) and B (**2a**)—isolated from the coelomycete *Chaetomella acutiseta* —which inhibited FPTase (recombinant human enzyme purified to homogeneity, see ref 5 for details) with IC_{50} values of 55 and 185 nM respectively.[5a] Further characterization showed that inhibition by Chaetomellic acid A was competitive with respect to FPP (Ki = 3.5 ± 0.2 nM) and noncompetitive with respect to Ras. Thus, chaetomellic acids appears to mimic the FPP at the active site. The chaetomellic acids have much poorer activity against other enzymes that bind isoprenoid pyrophosphates: geranylgeranyl-protein transferase (GGTase, IC_{50} values > 50 μM) and squalene synthetase (inactive up to 150 μM).



1a: R=H
1b: R=CH_3



2a: R=H
2b: R=CH_3

ISOLATION

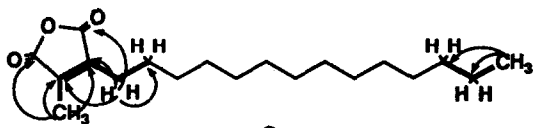
The methyl ethyl ketone extract of a one liter solid state fermentation[6] of *Chaetomella acutiseta* was chromatographed on Sephadex LH-20. The Ras FPTase active fractions were repeatedly chromatographed on reversed phase HPLC to give pure chaetomellic anhydride A (**3**) and anhydride B (**4**) in order of elution. These compounds were present in a ratio of 56:44 respectively. The acids **1a** and **2a** could not be isolated because of facile conversion to the corresponding anhydrides **3** and **4**. Therefore, the anhydrides were characterized.

STRUCTURE ELUCIDATION

Chaetomellic Acid A (1a) and Chaetomellic Anhydride A (3):

EIMS analysis of **1a/3** afforded the highest mass ions at m/z 308 and 309 corresponding to $[M\text{-}H_2O]^+$ and $[M\text{-}OH]^+$, respectively. The molecular weight , 326, of **1a** was indicated by EIMS of the bis-trimethylsilyl (TMS) derivative which by high resolution (HREIMS) measurement allowed assignment of the molecular formula as $C_{19}H_{34}O_4$. The carbon count was substantiated by C-13 NMR spectral analysis. IR spectra of a dried sample (as a thin film on ZnSe plate) exhibited a cyclic anhydride band at 1765 cm^{-1} and no hydroxy type absorption. Solution IR in CCl_4 also displayed a large anhydride band at 1767 cm^{-1} and a small acid band 1674 cm^{-1}. The UV

spectrum in $CHCl_3$ exhibited a λ_{max} 255 nm (ε=6762). The NMR analysis is based on the anhydride (**3**). ^{1}H-NMR spectrum (CD_2Cl_2) showed a methyl triplet (apparent, typically distorted) at (δ 0.88, J = ~6.9 Hz), degenerate methylene protons (22H, $11CH_2$), a degenerate methylene multiplet (δ 1.57), an olefinic methyl (δ 2.05, t = 0.8 Hz) and a degenerate deshielded methylene group (δ 2.45, tq = 7.5, 0.8 Hz). Proton spins were assigned (see **3**) on the basis of 2D ^{1}H-^{1}H COSY[7]. ^{13}C-NMR displayed 2 x CH_3, 14 x CH_2, 2 x olefinic quaternary and 2 x conjugated carbonyl type carbons. ^{1}H-^{13}C COSY[8] was used to assign the carbons bearing protons and LR ^{1}H-^{13}C COSY (J_{CH} = 8 Hz) was helpful in establishing (**3**) the connectivities of the maleate portion of the molecule. The latter method was also useful in assignment of C-15 and C-16 because of the two and three bond correlation with the methyl group, even though the corresponding proton shifts were degenerate.



3

—^{1}H-^{1}H COSY

LR ^{1}H ⌒ ^{13}C COSY

Reaction of **3** with diazomethane in a solution of CH_2Cl_2 and methanol gave a dimethyl ester **1b** which produced a molecular ion by EIMS at m/z 354. ^{1}H-NMR spectrum of **1b** showed an allylic coupling of 0.8 Hz between C-19 CH_3 and C-4 CH_2.

The MS fragmentation (Figure 1) fully supports the structure of chaetomellic acid A. Interestingly, the unusual loss of OH from the molecular ion in the EIMS suggests the presence of an ortho diacid-like structure. A similar fragmentation is observed for phthalic acid.[9a]

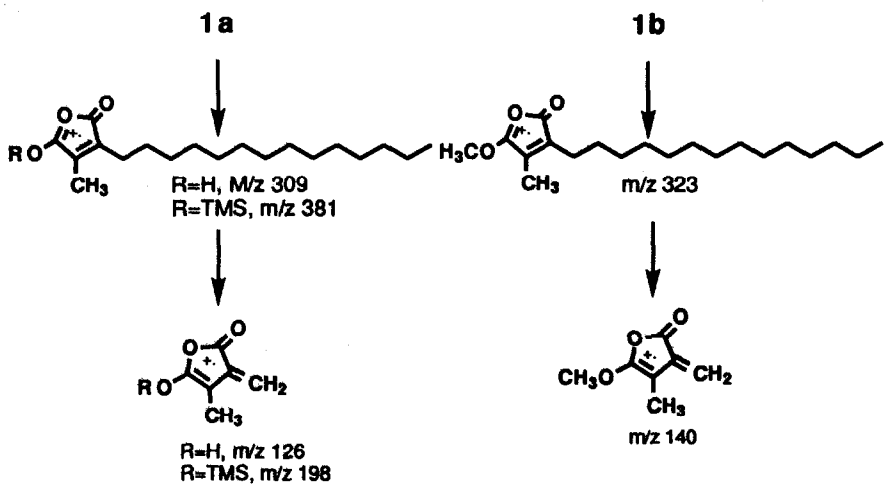
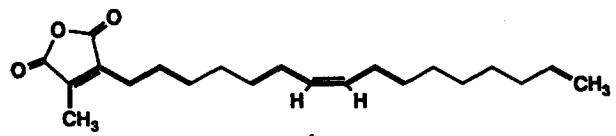


Figure 1: Critical EIMS fragmentations of 1a/3 and 1b

Chaetomellic Acid B (2a) and Anhydride (4):

As had been the case for 3 and **1a**, the EIMS of chaetomellic acid B (**2a**) showed highest mass corresponding to $[M-H_2O]^+$ and $[M-OH]^+$ ion (m/z 334/335) and ultimately afforded the formula $C_{21}H_{34}O_3$ for the anhydride (**4**). Chaetomellic acid B (**2a**) forms a bis-trimethylsilyl and dimethyl ester derivative (**2b**), indicating the parent molecular formula $C_{21}H_{36}O_4$. EIMS of **2a/4** and **2b** produced a fragment at m/z 126 and m/z 140 as was observed with **1a/3** and **1b**. Clearly, this compound contained two additional carbons in the form of an olefin. The Infra red spectrum of 4 showed a band for anhydride at 1767 cm^{-1} and the UV spectrum in $CHCl_3$ displayed a λ_{max} 254 nm (ε=7985). The ^{1}H-NMR spectrum (CD_2Cl_2) revealed similar peaks as in 3 with an additional multiplet at δ 5.3 for two protons and a four proton multiplet at δ 2.02 Applying the NMR methods described for 3, structure 4 could be established for chaetomellic acid B anhydride except the geometry and location of the double bond.



4

—^{1}H-^{1}H COSY

The location of the double bond in the side chain could not be determined either by MS or NMR analysis because of expected double bond migration under the conditions of mass spectrometry and degeneracy of the signals in ^{1}H NMR. Its location in the chain was established from the MS analysis (Figure 2) of the monoepoxide **5** (prepared by reacting **2a** with *meta*-chloroperbenzoic acid in CH_2Cl_2). As expected[9b], two critical fragment ions (Figure 2) were obtained, thus firmly locating the double bond between C-10 and C-11. The geometry of this double bond was established as follows: irradiation of H-9 and H-12 at δ 2.02 in ^{1}H-NMR spectrum collapsed the olefinic protons multiplet into two doublets with a coupling constant of 11 Hz (measured after resolution enhancement). This coupling constant is in full agreement with the Z-geometry.

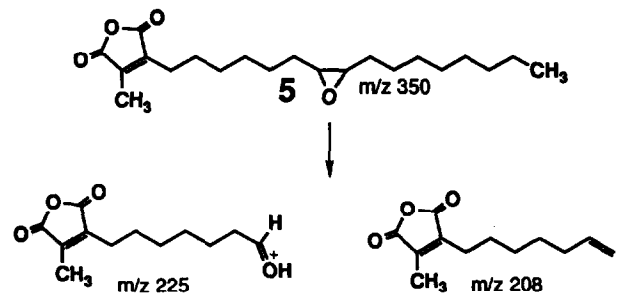


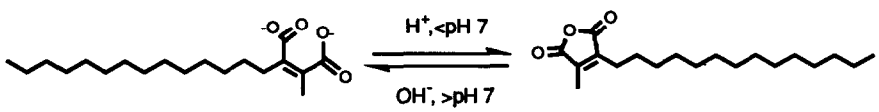
Figure 2: Critical EIMS fragmentation of epoxide 5.

Anhydride **4** has been isolated[10] previously from *Piptoporus australiensis* by vacuum distillation. While the location of the double bond was firmly established through ozonolysis, the geometry was assigned based solely on C-13 chemical shift comparison. The present method provides an alternative approach to determine both location and geometry of the double bond.

The biogenesis of these compounds could be viewed as an aldol reaction of palmitate or oleate with pyruvate followed by dehydration.

FPTase Activity Due to Acid or Anhydride?

The FPTase active component is the diacid and not the anhydride based on the following evidence. The equilibrium between diacid and anhydride is pH dependent. The UV spectrum of **3** in CH_3CN showed a maximum for cyclic anhydride at 254 nm. The UV spectrum did not change by addition of 0.1N HCl, however, a hypsochromic shift to λ_{max} 243 nm and a significant hypochromic shift were observed upon addition of 0.1N NaOH. These shifts provide clear evidence that the anhydride opens into diacid anions.



The UV spectrum recorded in CH_3OH-HEPES buffer[11] solution (7 : 5, methanol was needed to maintain a clear solution) at pH 7.5 showed UV maximum at λ_{max} 243 nm. This indicates that under the assay conditions the compound exists in the **open dicarboxylate** form. This shift was not due to methyl ester formation as demonstrated by the following experiments. The UV spectrum in neat CH_3OH exhibited two maxima 211 and 253 nm. The latter band underwent a hypsochromic as well as hypochromic shift upon addition of 0.1N NaOH. The anhydride reacts slowly with CH_3OH (a methanolic solution of anhydride gave dimethyl ester after reacting with CH_2N_2). The mono methyl ester could not be isolated even after addition of triethylamine or *N,N*-diisopropylethylamine because of facile formation of anhydride during evaporation of the solvents. The open diacid form could be trapped as a *N,N*-diisopropylethylamine salt without any loss of FPTase activity.

Farnesyl Pyrophosphate Mimic:

FPTase activity[5a] of chaetomellic acid A is highly competitive with respect to FPP (**6**) and noncompetitive with respect to acceptor peptide Ras. Chaetomellic acid A (**1a**) and FPP (**6**) have structural similarity as they both possess a hydrophilic head group and hydrophobic tail group. The mechanism of inhibition of FPTase by the chaetomellic acids is most likely *via* mimicking the substrate (FPP) at the active site of the enzyme and thus replacing the substrate from the catalytic site. This could be explained to a greater degree by computer modeling of the chaetomellic acid A with FPP as explained below. The structural motif of hydrophilic head group and hydrophobic tail common to **1a** and FPP led us to question how they might superpose. Our primary interest was in the overlap of the head groups as, presumably, the flexible nature of the tail of **1a** (due to its lack of multiple

bonds) would permit it to fill the same space as the tail of FPP regardless of the conformation FPP takes upon binding. Single conformers of **1a** and FPP in which the hydrophobic tail is extended were created[12] and energy minimized.[13] The models were then aligned on the basis of their steric and electrostatic similarities;[14] this alignment is depicted in Figure 3. As shown, the carboxyl carbons which are 3.2Å apart map onto the phosphorous atoms which are 2.8Å apart reasonably well (the two least squares planes defined by the carboxylate units in **1a** have a dihedral angle of 77.48°). Any change in the geometry of the *cisoid* head unit or the chain length (as exemplified by **2a**) would reduce similarity to FPP and hence result in lowered FPTase activity (and probably concomitant increase in activity *Vs* GGTase, particularly in the case of compounds with longer chain length). The good fit of the Z-isomer diacid onto the pyrophosphate group also suggests that the E-isomer would be significantly less active.

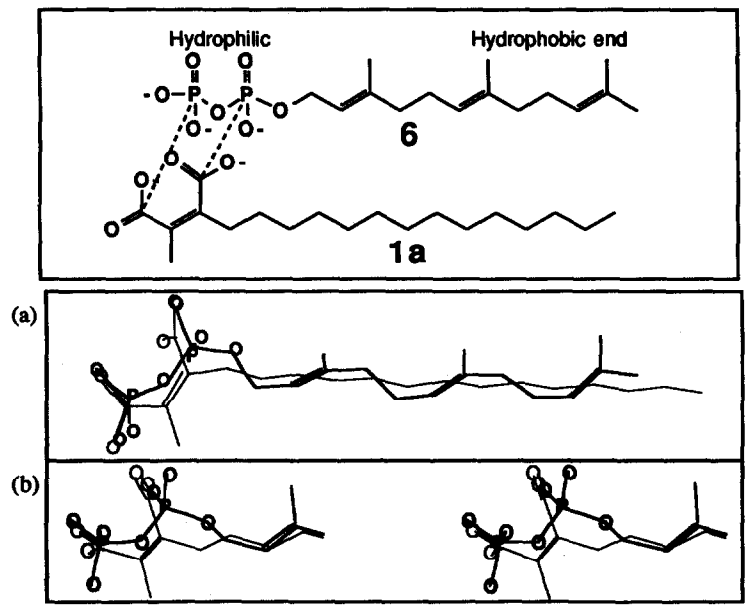


Figure 3. Visual and computer modeled superposition of chaetomellic acid A (1a) and FPP (6, in bold). (a) Full view of superposition, (b) Stereo view detailing head group overlap.

EXPERIMENTAL

All the reagents and deuterated solvents were obtained from Aldrich Chemical Company and were used without any purification. E. Merck (Darmstadt) silica gel plates (0.25 mm) were used for TLC and developed either with 3% ceric sulfate in 3N H_2SO_4 spray and/or iodine vapors. Stationary phases used for column chromatography were E. Merck silica gel (70-230 mesh or 40-63 μ).

The IR absorption spectra were obtained with a model 1750 Infrared Fourier Transform Spectrophotometer using a multiple internal reflectance cell (MIR, ZnSe) on neat 10-20 μg samples. The UV

absorption spectra were measured with a DU-70 Spectrophotometer in $CHCl_3$ or MeOH solution. Mass spectral data were obtained on a Finnigan-MAT TSQ700 and MAT212 instrument by electron impact at 90 eV. Trimethylsilyl derivatives were prepared with a 1:1 mixture of BSTFA-pyridine at room temperature. Exact mass measurements were made on the same instrument at high resolution by the peak matching method using perfluorokerosene (PFK) as internal standard. The FAB spectra were obtained on a MAT731 instrument.

NMR data were obtained on a Bruker AM-250 with ASPECT 3000 computer or Varian XL-300 spectrometer at ambient temperature in $CDCl_3$ or CD_2Cl_2. ^1H NMR chemical shifts in $CDCl_3$ are given relative to the solvent peak at 7.256 ppm and CD_2Cl_2 at 5.32 ppm. ^{13}C NMR chemical shifts in $CDCl_3$ are given relative to the solvent peak at 77.05 ppm and CD_2Cl_2 at 53.8 ppm.

^1H-^1H COSY were recorded using the standard pulse sequence of Bax and Freeman[7]. The 1K-2K data set was accumulated in 512 increments with 32 transients respectively for each value of t_1 for full phase cycling. ^1H-^{13}C chemical shift correlation spectra (COSY) were recorded in $CDCl_3$ using the standard pulse sequence of Bax and Morris[8]. The 512 x 4K data set was accumulated in 128 increments with 512 transients for each value of t_1. The delay time between transients was 0.5 sec, and the experiment was optimized for $^1J_{CH}$ =150 Hz. The corresponding long-range experiment was optimized for a multiple bond carbon-proton coupling constant of 8 Hz.

Fermentation of *Chaetomella acutiseta:*

Chaetomella acutiseta (GB 1518 = ATCC 74113) was isolated from the internal tissue of a decayed basidioma of *Phellinus robiniae* (Murrill) A. Ames and was maintained on malt yeast extract agar slants at 4 °C. Malt yeast extract medium consisted of 1% (w/vol) malt extract, 2% (w/vol) yeast extract and 2% (w/vol) agar. Seed cultures were prepared by transferring a portion of the slant growth into 250 mL Erlenmeyer flasks containing 50 mL of seed medium consisting of (in g/L); corn steep liquor, 5.0; tomato paste, 40.0; oat flour, 10.0; glucose, 10.0; and trace elements solution, 10 mL/L (consisting of, in g/L: $FeSO_4.7H_2O$, 1.0; $MnSO_4.4H_2O$, 1.0; $CuCl_2.2H_2O$, 0.025; $CaCl_2.2H_2O$, 0.1; H_3BO_3, 0.056; $(NH_4)_6MoO_{24}.4H_2O$, 0.019; $ZnSO_4.7H_2O$, 0.2; dissolved in 0.6 N HCl). The pH of the medium was adjusted to 6.8 with NaOH before sterilization. Seed cultures were incubated at 25 °C on a gyratory shaker (220 rpm, 5.1 cm throw) for 48-72 hours prior to inoculation of fermentation flasks. Fermentations were performed on production media consisting of brown rice (5g), 0.01 g KH_2PO_4, 0.01 g sodium tartrate, distilled water (15 mL) and 0.02 g yeast extract (in 20 mL of water) in 250 mL Erlenmeyer flasks. Flasks were sterilized at 121 °C for 15 minutes, inoculated with 2.0 mL of the seed culture and incubated at 25 °C without agitation for 15 days. Twenty such fermentation flasks were pooled and one liter of fermentation was extracted with methyl ethyl ketone (MEK, 3.0 L) by shaking for 20 minute and processed as described below.

Isolation of Chaetomellic Acids-Anhydrides A and B:

The extract was filtered through celite and the filtrate was concentrated to dryness under reduced pressure. The residual semi-solid was suspended in methanol (55 mL) and filtered. The filtrate (55 mL) was chromatographed on a 2.0 L Sephadex LH-20 column in methanol. The Ras farnesyl transferase active fractions (eluted in 1250-1750 ml of MeOH) were subsequently chromatographed on a Whatman C-8 (22 x 250mm) column using CH_3CN (75%) and H_2O (25% containing 0.25% H_3PO_4) at a flow rate of 10 ml/min. At this time it

became clear that the biological activity was due to two different compounds having retention times of 13.2 and 14.2 minutes [Whatman C-8 (4.6 x 250 mm), CH_3CN (80%)-H_2O (20% containing 0.25% H_3PO_4) at a flow rate of 1.5 mL/min] in a ratio of 56:44 (integrated at 255 nm). The preparative HPLC was repeated seven times. Fractions containing chaetomellic acids/anhydrides were repeatedly chromatographed on the preparative HPLC and were concentrated to remove most of the CH_3CN, then extracted with ethyl acetate. The ethyl acetate extract was washed once with an equal volume of H_2O and concentrated to dryness to give a first fraction (100 mg) enriched in anhydride **3** and a second fraction (70 mg) enriched in anhydride **4**. These fractions were chromatographed repeatedly using the same HPLC conditions to give pure **3** (40 mg, ret time 13.2 min) and **4** (29 mg, ret time 14.2 min), as waxy solids. HPLC analysis of **3** and **4** at *pH* 7 indicated the presence of acids **1a** and **2a**. However, at acidic pH the acids were not detected. Following are the physical data of the thoroughly vacuum dried sample over KOH. **3** : UV: λ_{max} ($CHCl_3$): 255 (ε = 6762) nm., IR of dried sample ν_{max} (ZnSe): 2924, 2854, 1767, 1467, 1277, 1118, 923, 735 cm^{-1}, ^{1}H-NMR (300 MHz, CD_2Cl_2): 0.88 (t, 3H, J = ~6.9 Hz, H-17), 1.27 (very brs, 22H, 11xCH_2), 1.56 (m, 2H, H-5), 2.05 (brs, 3H, H-19), 2.45 (tq, 2H, J = 7.5, 0.8 Hz, H-4) and ^{13}C NMR (75 MHz, CD_2Cl_2): 9.7 (C-19), 14.3 (C-17), 23.09 (C-16), 24.7 (C-4), 27.93 (C-5), 29.6, 29.76, 29.78, 29.85, 29.98, 30.03, 30.05 (x2), 30.08 (each CH_2 C-6 to C-14), 32.3 (C-15), 141.0 (C-3), 145.1 (C-2), 166.4 (C-18) 166.8 (C-1); HREIMS (m/z): 308.2350 (M^+, calcd. for $C_{19}H_{32}O_3$: 308.2351, **1a**: M-H_2O). **4**: UV: λ_{max} ($CHCl_3$): 254 (ε=7985) nm., IR: ν_{max} (ZnSe): 2925, 2854, 1857, 1821, 1765, 1741, 1673, 1465, 1367, 1270, 1230, 1218, 1118, 1016, 922, 735 cm^{-1}., ^{1}H-NMR (300 MHz, CD_2Cl_2): 0.88 (t, 3H, J = ~6.7 Hz, H-19), 1.34-1.26 (m, 18H, 9xCH_2), 1.57 (m, 2H, H-5), 2.02 (m, 4H, H-9, 12), 2.05 (brs, 3H, H-21), 2.45 (t, 2H, J = 8.1 Hz, H-4), 5.33 (m, 1H, H-10 or 11), 5.36 (m, 1H, H-11 or 10); ^{13}C NMR (75 MHz, CD_2Cl_2): 9.7 (C-21), 14.3 (C-19), 23.07 (C-18), 24.7 (C-4), 27.44 (C-9 or 12), 27.56 (C-12 or 9), 27.88 (C-5), 29.67, 29.71 (x3),, 29.90, 29.93, 30.15 (each CH_2 for C-6, 7, 8, 13, 14, 15, 16), 32.3 (C-17), 129.38 (C-10 or 11), 130.49 (C11 or 10), 141.0 (C-3), 145.1 (C-2), 166.3 (C-20), 166.8 (C1); HREIMS (m/z) 334.2506 (M^+, calcd. for $C_{21}H_{34}O_3$: 334.2508).

Chaetomellic Acid A Dimethyl Ester (1a):

To a 5 mg solution of **3** in methylene chloride (1 mL) and methanol (0.2 mL) was added a 1 mL hexane solution of trimethylsilyl-diazomethane. The yellow solution was stored in a refrigerator overnight. Solvent was evaporated under a stream of nitrogen and residue was filtered through a pipette filled with silica gel. Elution with 2% methanol in methylene chloride yielded dimethyl ester **1b** (4.9 mg) as a wax. IR: ν_{max} (ZnSe): 2924, 2854, 1725, 1645, 1466, 1435, 1379, 1264, 1196, 1163, 1125, 1099, 1041, 952, 861, 770, 721 cm^{-1}, ^{1}H-NMR (300 MHz, $CDCl_3$): 0.86 (t, 3H, J = ~6.6 Hz), 1.24 (m, 22H), 1.43 (m, 2H), 1.94 (brs, 3H), 2.32 (t, 2H, J = 7.2 Hz), 3.74 (s, 3H), 3.75 (s, 3H); HREIMS (m/z) 354.2741 (M^+, calcd. for $C_{21}H_{38}O_4$: 354.2770), 323.2571 (calcd. for $C_{20}H_{35}O_3$: 323.2586), 140.0475 (calcd. for $C_7H_8O_3$: 140.0473),

Chaetomellic Acid B Dimethyl Ester (2a):

To a 5 mg solution of **4** in methylene chloride (1 mL) and methanol (0.2 mL) was added a 1 mL hexane solution of trimethylsilyl-diazomethane. Following the procedure described above afforded 5.0 mg of dimethyl ester **2b** as an oil . IR : ν_{max} (ZnSe): 2925, 2855, 1736, 1725, 1644, 1459, 1435, 1366, 1301, 1264, 1198, 1099, 1038, 950, 861, 770, 725 cm^{-1}. ^{1}H-NMR (300 MHz, $CDCl_3$): 0.88 (t, 3H, J = ~6.0 Hz), 1.31-1.24 (m,

18H), 1.42 (m, 2H), 1.57 (m, 2H), 1.94 (brs, 3H), 2.00 (m, 4H), 2.32 (t, 2H, J = 7.5 Hz), 3.74 (s, 3H), 3.76 (s, 3H), 5.34 (m, 2H); HREIMS: m/z 380.2928 (M^+, calcd. for $C_{23}H_{40}O_4$: 380.2927).

Preparation of Chaetomellic acid B Epoxide (5):

To a solution of anhydride 4 (2 mg) in CH_2Cl_2 (1 ml) was added *m*-chloroperbenzoic acid (4 mg) and solution was stirred at room temperature for 30 minutes. CH_2Cl_2 was removed under reduced pressure and the crude product was chromatographed on a Whatman C-8 (4.6 x 250 mm) column and eluted with 85% CH3CN -15% H2O containing 0.2% TFA at a flow rate of 1.5 ml /min. The polar peak eluting at 6 min was collected and freeze dried to give monoepoxide 5 (1 mg). HREIMS (m/z) 350.2446 (M^+, calcd. for $C_{21}H_{34}O_4$: 350.2457), 225.1126 (calcd. for $C_{12}H_{17}O_4$: 225.1127), 208.1096 (calcd. for $C_{12}H_{16}O_3$: 208.1099). No further characterization was done on this sample.

ACKNOWLEDGMENTS

The authors are thankful to Dr. A. Oliff for constant encouragements and support and Dr. L. Huang and Ms. M. Meinz for squalene synthetase data.

REFERENCES AND NOTES

1. (a) Barbacid, M. *Ann. Rev. Biochem.* **1987**, 56, 779. (b) Rodenhuis, S. *Semin. Can. Biol.* **1992**, 3, 241.
2. Gibbs, J. B. *Semin. Can. Biol.* **1992**, 3, 383.
3. (a) Gibbs, J. B. *Cell* **1991**, 65, 1 and references cited therein. (b) Der, C. J. and Cox, A. D. *Cancer Cells* **1991**, 3, 331.
4. (a) Reiss, Y.; Goldstein, J. L; Seabra, M. C.; Casey, P. J.; and Brown, M. S. *Cell* **1990**, 62, 81. (b) Schaber, M. D.; O'Hara, M. B.; Garsky, V. M.; Mosser, S. D.; Bergstrom, J. D.; Moores, S. L.; Marshall, M. S.; Friedman, P. A.; Dixon, R. A. F.; and Gibbs, J. B. *J. Biol. Chem.* **1990**, 265, 14701.
5. (a) Gibbs, J. B.; Pompliano, D. L.; Mosser, S. D.; Rands, E.; Lingham, R. B.; Singh, S. B.; Scolnick, E. M.; Kohl, N. E.; and Oliff, A. *J. Biol. Chem.* **1993**, 268 (11), 7617. (b) Omer, C. A.; Kral, A. M.; Diel, R. E.; Prendergast, G. C.; Powers, S.; Allen, C. M.; Gibbs, J. B.; and Kohl, N. E. *Biochemistry* **1993** (in press). (c) Moores, S. L.; Schaber, M. D.; Mosser, S. D.; Rands, E.; O'Hara, M. B.; Garsky, V. M.; Marshall, M. S.; Pompliano, D. L.; and Gibbs, J. B. *J. Biol. Chem.* **1991**, 266, 14603.
6. Lingham, R. B.; Silverman, K. C.; Bills, G. F.; Cascales, C.; Sanchez, M.; Jenkins, R. G.; Gartner, S. E.; Martin, I; Diez, M. T.; Pelaez, F.; Mochales, S.; Kong, Y. L.; Burg, R. W.; Meinz, M. S.; Huang, L.; Nallin-Omstead, M.; Mosser, S. D.; Schaber, M. D.; Omer, C. A.; Pompliano, D. L.; Gibbs, J. B.; and Singh, S. B. *Appl. Microbiol. Biotech.*. **1993** (submitted).
7. Bax., A.; Freeman, R. *J. Magn. Reson.* **1981**, 44, 542.
8. (a) Bodenhausen, G.; Freeman, R. *J. Magn. Reson.* **1971**, 28, 471. (b) Bax, A.; Morris, G. A. *J. Magn. Reson.* **1981**, 42, 501.

9. (a) Budzikiewicz, H.; Djerassi, C.; and Williams, D. H.; *Mass Spectrometry of Organic Compounds*-Holden-Day, Inc., San Francisco, CA. 1967, pp 221. (b) ibid pp 449-465
10. Gill, M. *Phytochemistry*, **1982**, 21, 1786.
11. HEPES (N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid]) buffer adjusted to pH 7.5.
12. "AMF - The Advanced Modeling Facility", Molecular Systems Department, Merck Research Laboratories, a division of Merck & Co., Inc., version 2.0, October, 1992.
13. The MM2X force field is derived in large part from the MM2 force field.[15] For intramolecular interactions, the force field includes all the type of interactions that are in MM2, and functional form of interaction is identical with that in MM2 with one exception. The electrostatic term is represented by Coulomb's law acting on atom-centered point charges. These atomic charges q are, however, derived from bond dipole moments q=1/4.803 Σ_i $\mu_i r_{i0}$ where μ is the bond dipole moment, r_0 is the reference bond length, and the sum extends over all the bonds for a given atom. MM2X does not use lone pairs on aliphatics amines, alcohol, and ether oxygens, and carboxylic acid and ester oxygens and the parameter differences are due primarily to the need to make up for the elimination of lone pairs on oxygen, reproduction of gas-phase dimerization energies of acids and amides, and incorporation of parameters for aromatic carbon based on the AMBER force field.[16] Parameters for the non-bonded, out-of-plane, and stretch-bend interactions are taken directly from MM2 without change.
14. Kearsley, S. K., Smith, G. S. *Tetrahedron Comput. Methodol.* **1990**, 3, 615.
15. Allinger, N. L.; Yuh, Y. H. *QCPE* **1981**, 13, 395. There are slight differences between the parameters found in this program and those reported in the original reference to MM2.[17] In addition, two modifications designed for C_{ar}-C_{ar} bonds are also used: r_0=1.3937Å and k_r =8.0667 mdyn Å^{-1}.
16. Weiner, S. J.; Kollman, P. A.; Case, D. A.; Singh, U.C.; Ghio, C.; Alagona, G.; Profeta, S., Jr.; Weiner, P. *J. Am. Chem. Soc.* **1984**, 106, 765.
17 Allinger, N. L. *J. Am. Chem. Soc.* **1977**, 99, 8127.