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Synthesis of Chaetomellic Acid A: A Potent Inhibitor of Ras Farnesyl-Protein Transferase

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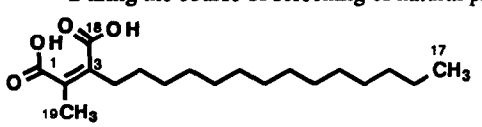
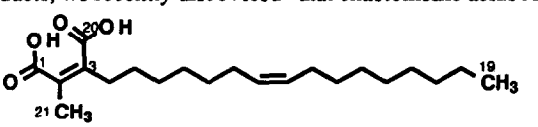
Abstract: *Chaetomellic acids are alkyl dicarboxylic acids, isolated from Chaetomella acutiseta and are potent and highly specific farnesyl-pyrophosphate (FPP) mimic inhibitors of Ras farnesyl-protein transferase. The first efficient and biogenetic type total synthesis of chaetomellic acid A has been described herein.*

The *ras* oncogene is found mutated in about 25% of human tumors.[1] These genes encode 21kDa proteins that are associated with the plasma membrane. Ras proteins are biologically active only when they are GTP bound and are inactivated when bound to GDP. This is controlled by a Ras intrinsic GTPase that can be stimulated by GAP or NF-1. Several potential points of intervention have become apparent from the analysis of Ras function.[2] One of these areas is the post-translational modification of Ras, which is required for its membrane association and oncogenic activity. Farnesylation by farnesyl-protein transferase (FPTase) is the first and the obligatory step in Ras processing.[3] Genetic experiments have shown that farnesylation is required for Ras cell-transforming activity. FPTase, therefore, appears to be a good 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). Selected isoprenoid pyrophosphates and CaaX tetrapeptides have been shown to inhibit FPTase.[4]

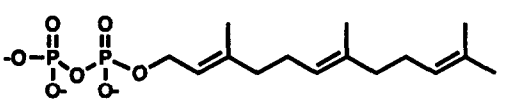
During the course of screening of natural products, we recently discovered[5] that chaetomellic acids A and



1:Chaetomellic acid A



2:Chaetomellic acid B

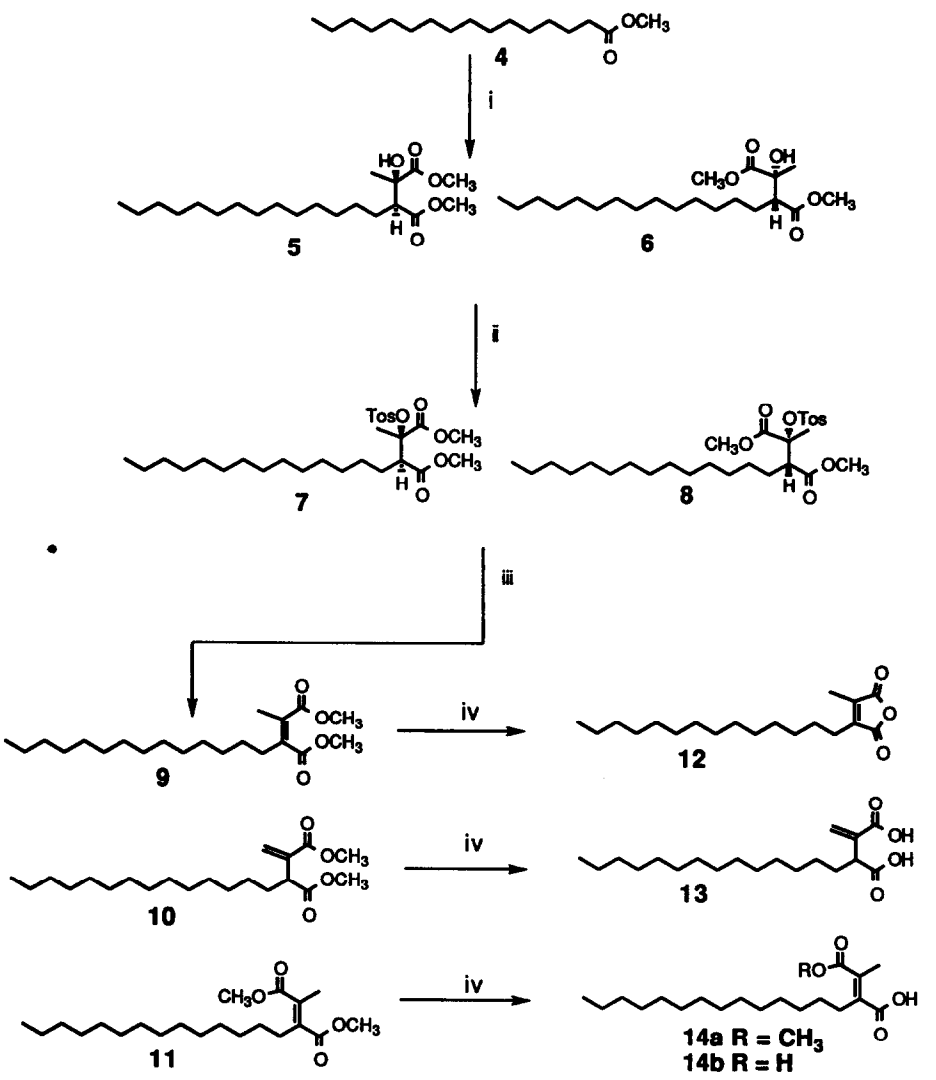


3: FPP

B(1, 2) are potent and highly specific inhibitor of FPTase. These acids were characterized as alkyl *cis*-dicarboxylic acids and appear to mimic FPP (3). Structurally, these acids have resemblance with FPP as demonstrated by molecular modelling.[5] Among the chaetomellic acids , A (IC_{50} 55 nM) is 3 times more active than B (IC_{50} 185 nM).[5] This class of natural products has propensity to cyclise and most members are isolated in their anhydride form. In order to gain access to larger quantities of material, prove the structure of chaetomellic acid A and develop preliminary structure activity relationship, a total synthesis was undertaken. This paper describes the first total synthesis of this class of compounds. The synthetic strategy is based on a biogenetic type approach which involves aldol reaction and subsequent double bond generation.

Aldol condensation (scheme 1) of methyl palmitate (4) with methyl pyruvate at -78°C proved difficult due to the insolubilty of the methyl palmitate at lower temperatures and under standard aldol conditions very poor yield of the reaction product was obtained. This problem could not be circumvented by raising the temperature due to polymerization of methyl pyruvate at room temperature in the presence of base. However, greater than 80% yield of the diastereomeric mixture (1:1) of the aldol products **5**[6] (less polar) and **6** was isolated when the methyl palmitate enolate (LDA, at -78 to -10°C) was added dropwise to a cooled (-78°C) solution of methyl pyruvate. Aldol products **5** (S^*R^*) and **6** (R^*R^*) were easily separated on silica gel and stereochemistry was assigned on the basis of ß-elimination reaction (*vide infra*). Attempted acid catalyzed dehydration failed due to the difficulty of formation of a carbonium ion α to the carboxymethyl group. Therefore, a base catalyzed β-elimination approach was undertaken. Tosylation of **5** and **6** using tosic anhydride (4 eq), 2,6-di-*tert*-butyl-4-methylpyridine (2 eq) and CH_2Cl_2 and pyridine (1.5:1) gave tosylate **7** and **8** respectively which were used in the next reaction without purification. Elimination reaction (DBU at 130°C, 6 h) of tosylate **7** *in situ* (only CH_2Cl_2 removed) gave predominantly the citraconate diester **9** (Z-isomer) and the itaconate diester **10** with a trace of the mesaconate diester **11** (E-isomer) in a ratio of 4 : 3 : 0.5, respectively, with a combined two step (**5** to **9**) yield of >85%. Similar reaction with tosylate **8** afforded predominantly E-isomer **11** with minor amounts of **9** and **10**. The elimination reaction of S^*,R^* isomer **7** was relatively slower than that of S^*,S^* isomer **8**. The stereochemistry of aldol products **5** and **6** was assigned based on the product distribution of these elimination reactions. The assumption was made that the *anti* periplanar elimination is the predominant pathway of the reaction and the minor products (E or Z) in both reactions were formed from *syn* eliminations. Formation of terminal olefin **10** was suppressed to less than 10% when the elimination reaction was performed in refluxing toluene with 2 equivalent of DBU using isolated (EtOAc extraction and acid/base work up, no chromatographic purification) tosylate. The mixture of aldol product could be directly used for preparation of desired olefins. Hydrolysis of methyl esters by refluxing 1N sodium hydroxide in methanol-THF mixture gave 90-95% yield of corresponding acid or anhydride. Hydrolysis of **9** was fast and gave chaetomellic acid anhydride **12** which can be converted to chaetomellic acid A (**1**) at pH 7.5 in solution. Hydrolysis of **12** was slower and after 30 minutes at 50°C mono methyl ester **14a** (structure was assigned based on LR ^1H-^{13}C COSY) was formed almost exclusively. Refluxing the reaction overnight gave diacid **14b**. Hydrolysis of **10** in identical conditions produced as expected, predominantly **13** with minor amounts of **12** and **14b**.

The diacids **13** and **14b** were 15 and 70 times less active[7] than diacid **1** against Ras FPTase.



SCHEME 1: (i) LDA, THF, -78 to -10°C, methyl pyruvate; (ii) CH_2Cl_2, pyridine, 40°C, 2,6-Di-*tert*-butyl-4-methylpyridine, p-toluenesulfonic anhydride; (iii) Toluene, DBU, reflux; (iv) a. 1N NaOH-CH_3OH-THF-H_2O, 80°C; b. 4N HCl

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6. All new compounds gave satisfactory HRMS and NMR data. ^{1}H-NMR in $CDCl_3$: **5**: 3.75 (3H, s), 3.67 (3H,s), 2.76 (1H, dd, J=10.5, 3.9 Hz), 1.68 (2H, m), 1.41 (3H,s), 1.25 (24H, brm), 0.87 (3H, t, J=6.3 Hz); **6**: 3.79 (3H, s), 3.71 (3H,s), 2.75 (1H, dd, J=11.7, 3.3 Hz), 1.83 (2H, m), 1.42 (3H,s), 1.23 (24H, m), 0.87 (3H, t, J=6.3 Hz); **7**: 7.76 (2H, d, J = 8.4 Hz), 7.32 (2H, d, J = 8.4 Hz), 3.83 (3H, s), 3.67 (3H, s), 2.95 (1H, dd, J = 11.7, 3.0 Hz), 2.44 (3H, s), 1.81 (3H, s), 1.60 (2H, m), 1.24 (24H, m), 0.88 (3H, t, J=6.6 Hz); **9**: 3.75 (3H, s), 3.74 (3H, s), 2.32 (2H, t, J = 7.2 Hz), 1.94 (3H, brs), 1.43 (2H, m), 1.24 (22H, m), 0.86 (3H, t, J = 6.6 Hz); **10**: 6.35 (1H, s), 5.74 (1H, s), 3.76 (3H, s), 3.67 (3H, s), 3.49 (1H, t, J = 7.2 Hz), 1.86 (1H, m), 1.65 (1H, m), 1.24 (24H, m), 0.87 (3H, t, J = 6.3 Hz); **11**: 3.78 (3H,s), 3.77 (3H, s), 2.43 (2H, t, J = 8.5 Hz), 1.99 (3H, brs), 1.40 (2H, m), 1.24 (22H, m), 0.87 (3H, t, J = 7.8 Hz); **13**: 6.52 (1H, s), 5.82 (1H, s), 3.37 (1H, t, J = 7 Hz), 1.93 (1H, m), 1.73 (1H, m), 1.25 (24H, m), 0.88 (3H, t, J = 6.6 Hz); **14a**: 3.81 (3H, s), 2.58 (2H, t, J = 7.5 Hz), 2.00 (3H, s), 1.43 (2H, m), 1.24 (22H, m), 0.88 (3H, t , J = 6.7 Hz); **14b**: 2.58 (2H, t, J = 7.8 Hz), 2.14 (3H, s), 1.52 (2H, m), 1.26 (22H, m), 0.89 (3H, t, J = 6.6 Hz).
7. Structure activity relationships displayed by these compounds and other analogs including chaetomellic acid B will be published elsewhere.

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