Biosynthetic Studies on the Fungal Secondary Metabolites CP-225,917 and CP-263,114

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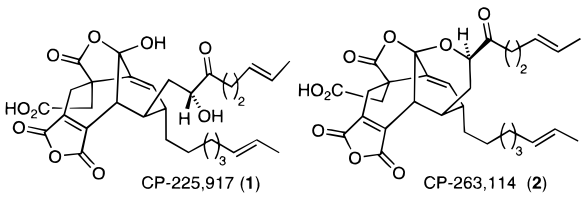
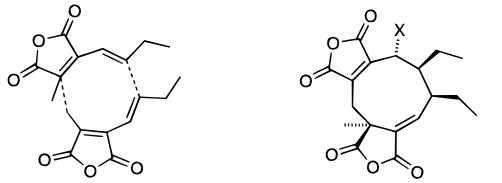
During the course of screening for farnesyl transferase (Ftase) and squalene synthase (SQS) inhibitors, workers at Pfizer Central Research identified two new natural products [CP-225,917 (**1**) and CP-263,114 (**2**)] produced by an unidentified fungus (ATCC 74256).[1] Isolated from the same fungus was zaragozic acid A (aka squalestatin I) which had been previously isolated from a variety of fungi by workers at Merck and Glaxo.[2] The CP producing fungus was in turn isolated from twigs of *Juniperus ashei* Bachh collected in a juniper-scrub oak forest located in Dripping Springs, Texas.[3] The assigned structures of CP-225,-917 (**1**) and CP-263,114 (**2**) were based on extensive NMR analysis and recently confirmed by total synthesis.[4]





The unique structure and potentially useful biological activity of **1** and **2** has generated considerable interest in the total synthesis of these natural products.[4,5] In contrast, no reports related to the biosynthesis of these metabolites have appeared following the initial suggestions of the Pfizer group who accurately classified these compounds as belonging to the nonadride group of natural products.[6] In the mid-1960s Barton and co-workers first suggested the name nonadrides be applied to the mold metabolites glaucanic (**4**) and glauconic (**5**) acids.[7] These early studies were followed by a series of papers related to the biosynthesis of **4** and **5** by Sutherland and co-workers.[8] One conclusion of these investigations was that glaucanic acid is produced by the head-to-head dimerization of a C_9 anhydride (cf. **3**). The biogenesis of the C_9 anhydride invoked the condensation of oxaloacetic acid and hexanoic acid.[7−9] Parallel reasoning leads to the suggestion that CP-225,917 (**1**) is the product of the dimerization of a C_{16} anhydride (cf. **10**) which in turn is produced from the condensation of oxaloacetyl-CoA (**7**) and a C_{12} carboxylic acid derivative **9** (Scheme 1).[1b] Oxaloacetic acid is a product of the citric acid cycle, succinic acid (**6**) being an intermediate further upstream in this important metabolic pathway. The production of the long chain carboxylic acid **9** can be achieved by either a fatty acid or a polyketide synthase pathway, both pathways utilizing acetyl-CoA (**8**) as a starter unit.[10] A most intriguing step in the proposed biosynthesis of CP-225,917 (**2**) is the dimerization of a C_{16} anhydride (**10**) leading to intermediate **11**.[11] In this step, three new carbon−carbon bonds are formed and the unique bicyclic core structure common to **1** and **2** is established. Subsequent decarboxylation and side-chain oxidation would then account for the production of CP-225,917 (**1**). Finally, cyclodehydration of CP-225,917 (**1**) to CP-263,114 (**2**) may or may not require enzyme assistance.[12]

The biosynthetic pathway outlined in Scheme 1 shows carbons C(12), C(13), C(14), and C(28) originate from the C(2) and C(3) carbons of succinic acid (**6**). The C(1) and C(4) carboxylic acid groups of succinic acid correlate with the C(27), C(29), and C(30) carbons of the CP molecules (the fourth carboxylate group being lost as CO_2). The remaining carbons of the CP molecules are traced back to acetyl-CoA (**8**). Using ^{13}C-labeled intermediates and suspended cells (ATCC74256) we have established the validity of these predictions providing, for the first time, experimental support for the biosynthetic pathway outlined in Scheme 1.

An early useful finding in our investigations was the dependence of fungal (ATCC 74256) secondary metabolism on the pH

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(1) (a) Dabrah, T. T.; Harwood, H. J.; Huang, L. H.; Jankovich, N. D.; Kaneko, T.; Li, J. C.; Lindsey, S.; Moshier, P. M.; Subashi, T. A.; Therrien, M.; Watts, P. C. *J. Antibiot.* **1997**, *50*, 1−7. (b) Dabrah, T. T.; Kaneko, T.; Massefski, W.; Whipple, E. B. *J. Am. Chem. Soc.* **1997**, *119*, 1594−1598.

(2) Bergstrom, J. D.; Dufresne, C.; Bills, G. F.; Nallin-Omstead, M.; Byrne, K. *Annu. Rev. Microbiol.* **1995**, *49*, 607−639 and references cited within.

(3) We have tentatively assigned the fungus to the *Ascomycete* order *Pleosporales* based on ITS sequence. Unpublished results by Nancy P. Keller, Paul Spencer, and Gary A. Sulikowski.

(4) Total Synthesis: (a) Nicolaou, K. C.; Baran, P. S.; Zhong, Y. L.; Choi, H. S.; Yoon, W. H.; He, Y.; Fong, K. C. *Angew. Chem., Int. Ed. Engl.* **1999**, *38*, 1669−1675. (b) Nicolaou, K. C.; Baran, P. S.; Zhong, Y. L.; Fong, K. C.; He, Y.; Yoon, W. H.; Choi, H. S. *Angew. Chem., Int. Ed. Engl.* **1999**, *38*, 1676−1678.

(5) (a) Bio, M. M.; Leighton, J. L. *J. Am. Chem. Soc.* **1999**, *121*, 890−891. (b) Clive, D. L. J.; Sun, S. Y.; He, X.; Zhang, J. H.; Gagliardini, V. *Tetrahedron Lett.* **1999**, *40*, 4605−4609. (c) Chen, C.; Layton, M. E.; Shair, M. D. *J. Am. Chem. Soc.* **1998**, *120*, 10784−10785. (d) Kwon, O. Y.; Su, D. S.; Meng, D. F.; Deng, W.; D'Amico, D. C.; Danishefsky, S. J. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 1877−1880. (e) Kwon, O. Y.; Su, D. S.; Meng, D. F.; Deng, W.; D'Amico, D. C.; Danishefsky, S. J. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 1880−1882. (f) Meng, D. F.; Danishefsky, S. J. *Angew. Chem., Int. Ed. Engl.* **1999**, *38*, 1485−1488. (g) Nicolaou, K. C.; Postema, M. H. D.; Miller, N. D.; Yang, G. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 2821−2823. (h) Nicolaou, K. C.; Harter, M. W.; Boulton, L.; Jandeleit, B. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 1194−1196. (i) Nicolaou, K. C.; Baran, P. S.; Jautelat, R.; He, Y.; Fong, K. C.; Choi, H. S.; Yoon, W. H.; Zhong, Y. L. *Angew. Chem., Int. Ed. Engl.* **1999**, *38*, 549−552. (j) Waizumi, N.; Itoh, T.; Fukuyama, T. *Tetrahedron Lett.* **1998**, *39*, 6015−6018.

(6) Biosynthetic studies on the zaragozic acids and squalestatins: (a) Byrne, K. M.; Arison, B. H.; Nallinomstead, M.; Kaplan, L. *J. Org. Chem.* **1993**, *58*, 1019−1024. (b) Jones, C. A.; Sidebottom, P. J.; Cannell, R. J. P.; Noble, D.; Rudd, B. A. M. *J. Antibiot.* **1992**, *45*, 1492−1498.

(7) (a) Baldwin, J. E.; Barton, D. H. R.; Bloomer, J. L.; Jackman, L. M.; Rodriguez-Hahn, L.; Sutheland, J. *Experienta* **1962**, *18*, 345−388. (b) Barton, D. H. R.; Sutherland, J. K. *J. Chem. Soc.* **1965**, 1769−1771. (c) Barton, D. H. R.; Jackman, L. M.; Rodriguez-Hahn, L.; Sutherland, J. K. *J. Chem. Soc.* **1965**, 1772−1778. (d) Barton, D. H. R.; Godinho, L. D. S.; Sutherland, J. K. *J. Chem. Soc.* **1965**, 1779−1786.

(8) (a) Bloomer, J. L.; Moppett, C. E.; Sutherland, J. K. *J. Chem. Soc. C* **1968**, 588−591. (b) Huff, R. K.; Moppett, C. E.; Sutherland, J. K. *Chem. Commun.* **1968**, 1192−1193. (c) Huff, R. K.; Moppett, C. E.; Sutherland, J. K. *J. Chem. Soc., Perkin Trans. 1* **1972**, *20*, 2584−2590. (d) Sutherland, J. K. *Fortschr. Chem. Org. Naturst.* **1967**, *25*, 131−149.

(9) Baldwin, J. E.; Beyeler, A.; Cox, R. J.; Keats, G.; Pritchard, G. J.; Adlington, R. M.; Watkin, D. J. *Tetrahedron* **1999**, *55*, 7363−7374.

(10) Dewick, P. M. *The Acetate Pathway: Fatty Acids and Polyketides*, 1st ed.; John Wiley & Sons: New York, 1997; Chapter 3, pp 32−106.

(11) Further work is required to distinguish between a covalently bound intermediate (cf. **10** to **11**) and the possibility of a noncovalent Michael-type addition with concomitant decarboxylation. For a detailed discussion of the stereochemical aspects of the dimerization of **9** leading to the core structure of the CP molecules in favor of a covalently bound intermediate, see: Sulikowski, G. A.; Agnelli, F.; Corbett, R. M. *J. Org. Chem.* In press.

(12) The acid-catalyzed cyclodehydration of **1** to **2** has been noted. See ref 1b.

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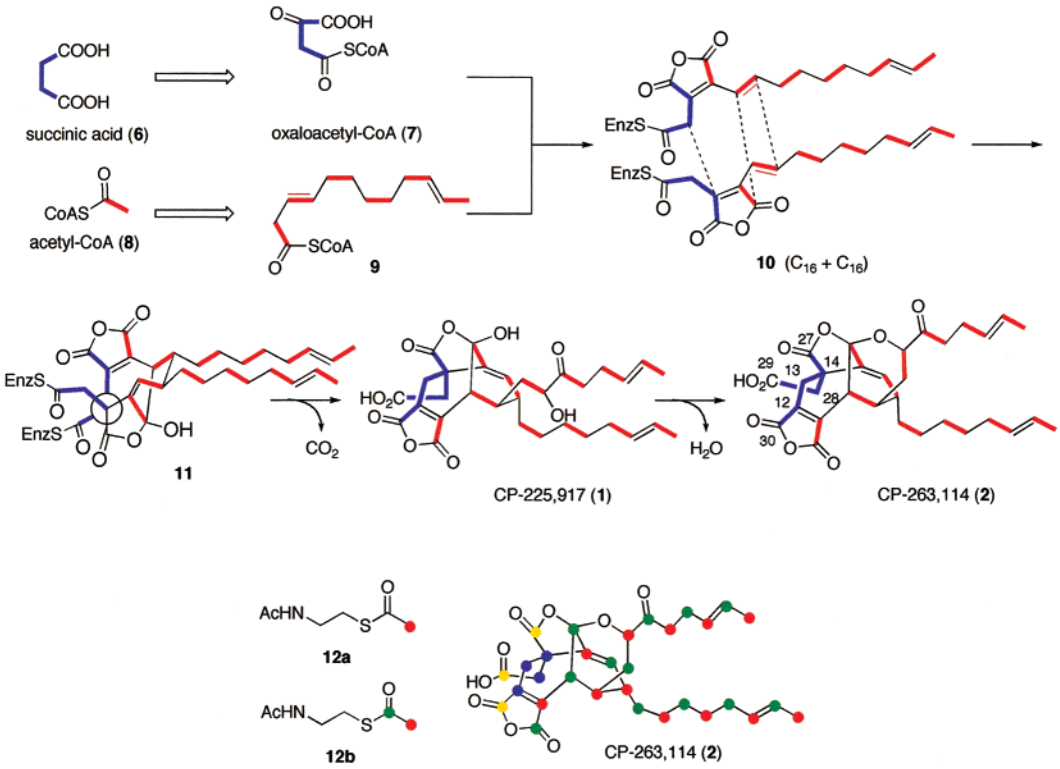
Scheme 1



of the growth medium.[13] When grown in media buffered from 6.0 to 5.0, the fungus produced little CP-225,917 (**1**) or CP-263,-114 (**2**); however, when the pH was lowered to pH 3.0 CP production increased significantly. In the first experiment, [2,3-$^{13}C_2$]-succinic acid was administered to a cell culture following a pH drop and incubation continued for a further 22 h; fermentation was then terminated and CP-263,114 (**2**) isolated.[14] When compared to a ^{13}C NMR spectrum of CP-263,114 (**2**) at natural abundance significant differences for the resonances at C(12), C(13), C(14), and C(28) were noted (blue solid circles in **2**), each signal now accompanied by flanking doublets (J = 35−55 Hz). A parallel experiment using [1,4-$^{13}C_2$]-succinic acid resulted in enhancement of signal intensities at the carbon resonances corresponding to C(27), C(29), and C(30) (yellow solid circles in **2**).[15]

The origin of the alkyl side-chain carbons extending into the bicyclic core structure (cf. **2** in Scheme 1) was examined using the *N*-acetylcysteamine thioesters of acetic acid as an acetyl-CoA (**7**) equivalent.[16] First, [2-^{13}C] acetyl NAC thioester (**12a**) led to significant enhancement of ^{13}C peaks at the expected alternating carbons (red solid circles in **2**).[17] An identical experiment this time using the [1,2-$^{13}C_2$] acetyl NAC thioester (**12b**) afforded the expected labeling pattern as determined by ^{13}C NMR analysis (red-green circles in **2**) determined in a ^{13}C COSY experiment.

In conclusion, we have determined the origin of all carbon atoms of CP-263,114 (**2**) using ^{13}C-labeled biosynthetic precursors. Further studies on the biosynthesis of the CP molecules including efforts to distinguish between a polyketide or fatty acid synthase biosynthetic pathway are currently under investigation.

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Supporting Information Available: Fermentation and product isolation procedures and a tabular summary of ^{13}C incorporation results and copies of ^{13}C NMR of enriched CP-263,114 samples (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(13) For a related pH-dependent secondary metabolism, see: Shah, A. J.; Tilburn, J.; Adlard, M. W.; Arst, H. N., Jr. *FEMS Microbio. Lett.* **1991**, *77*, 209−212.

(14) Incubation time of 36 h, following administration of [2,3-$^{13}C_2$]-succinic acid, resulted in exhausitive ^{13}C incorporation due to extensive catabolism of the labeled substrate. Shorter incubation times (i.e. less than 15−22 h) led to selective ^{13}C incorporation at C(12), C(13), C(14), and C(28), with only minor incorporation of ^{13}C at other carbons.

(15) The resonances corresponding to C(28) and C(29) variably broaden complicating comparison based on signal intensity to the natural abundance spectrum.

(16) Brobst, S. W.; Townsend, C. A. *Can. J. Chem.* **1994**, *72*, 200−207.

(17) During the course of our investigations we uncovered two minor misassignments of the ^{13}C NMR spectrum of CP-263,114 (**2**). Specifically, the resonances assigned to C(2) and C(3) should be interchanged as well as the resonances assigned to C(23) and C(24). The re-assignment is based on the ^{13}C−^{13}C COSY spectrum of labeled **2**.