ORGANIC LETTERS

2006
Vol. 8, No. 26
6063−6065

Enzymatic Formation of Quinolone Alkaloids by a Plant Type III Polyketide Synthase

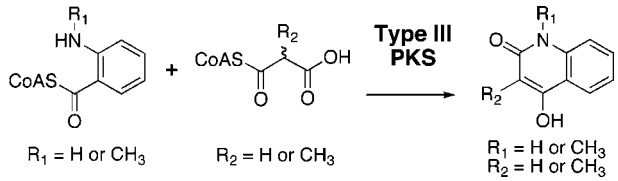
Ikuro Abe,*[,†,‡] Tsuyoshi Abe,[†] Kiyofumi Wanibuchi,[†] and Hiroshi Noguchi[†]

School of Pharmaceutical Sciences and the COE 21 Program, University of Shizuoka, Shizuoka 422-8526, Japan, and PRESTO, Japan Science and Technology Agency, Kawaguchi, Saitama 332-0012, Japan

abei@ys7.u-shizuoka-ken.ac.jp

Received October 13, 2006

ABSTRACT



Benzalacetone synthase from *Rheum palmatum* efficiently catalyzed condensation of *N*-methylanthraniloyl-CoA (or anthraniloyl-CoA) with malonyl-CoA (or methylmalonyl-CoA) to produce 4-hydroxy-2(1*H*)-quinolones, a novel alkaloidal scaffold produced by a type III polyketide synthase (PKS). Manipulation of the functionally divergent type III PKSs by a nonphysiological substrate thus provides an efficient method for production of pharmaceutically important quinolone alkaloids.

The functional diversity and catalytic potential of the chalcone synthase (CHS) (EC 2.3.1. 74) superfamily of type III polyketide synthases (PKSs) are remarkable.[1] The structurally simple homodimeric proteins catalyze iterative decarboxylative condensations of malonyl-CoA with a CoA-linked starter molecule to produce a variety of biologically active secondary metabolites. For example, CHS, a pivotal enzyme in flavonoid biosynthesis, catalyzes sequential condensation of 4-coumaroyl-CoA with three C_2 units from malonyl-CoA to produce a tetraketide naringenin chalcone (Scheme 1A),[2] whereas benzalacetone synthase (BAS) from *Rheum palmatum* (Polygonaceae) carries out a one-step decarboxylative condensation of 4-coumaroyl-CoA with malonyl-CoA to produce the C_6-C_4 skeleton of a diketide benzalacetone (Scheme 1B).[3] One of the most characteristic features is that plant type III PKSs exhibit unusually broad, promiscuous substrate specificities; the enzymes readily accept a variety of nonphysiological substrates, including aromatic and aliphatic CoA thioesters, to produce a vast array of chemically and structurally distinct unnatural polyketides.[4,5]

We now report that the diketide-producing *R. palmatum* BAS efficiently catalyzes condensation of *N*-methylanthraniloyl-CoA (or anthraniloyl-CoA) with malonyl-CoA (or methylmalonyl-CoA) to produce 4-hydroxy-2(1*H*)-quinolones, a

[†] University of Shizuoka.

[‡] PRESTO.

(1) For recent reviews, see: (a) Schröder, J. In *Comprehensive Natural Products Chemistry*; Elsevier: Oxford, 1999; Vol. 2, pp 749−771. (b) Austin, M. B.; Noel, J. P. *Nat. Prod. Rep.* **2003**, *20*, 79−110.

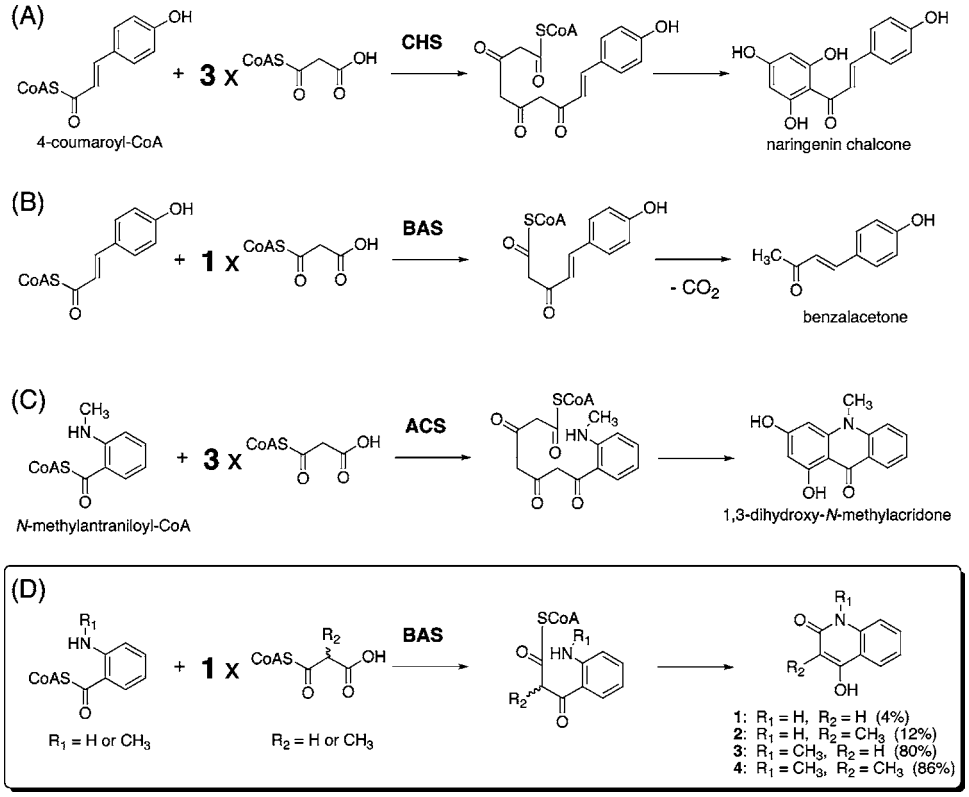
(2) (a) Ferrer, J. L.; Jez, J. M.; Bowman, M. E.; Dixon, R. A.; Noel, J. P. *Nat. Struct. Biol.* **1999**, *6*, 775−784. (b) Jez, J. M.; Ferrer, J. L.; Bowman, M. E.; Dixon, R. A.; Noel, J. P. *Biochemistry* **2000**, *39*, 890−902. (c) Jez, J. M.; Noel, J. P. *J. Biol. Chem.* **2000**, *275*, 39640−39646. (d) Jez, J. M.; Bowman, M. E.; Noel, J. P. *Biochemistry* **2001**, *40*, 14829−14838. (e) Tropf, S.; Kärcher, B.; Schröder, G.; Schröder, J. *J. Biol. Chem.* **1995**, *270*, 7922−7928. (f) Suh, D. Y.; Fukuma, K.; Kagami, J.; Yamazaki, Y.; Shibuya, M.; Ebizuka, Y.; Sankawa, U. *Biochem. J.* **2000**, *350*, 229−235. (g) Austin, M. B.; Bowman, M. E.; Ferrer, J.-L.; Schröder, J.; Noel, J. P. *Chem. Biol.* **2004**, *11*, 1179−1194. (h) Abe, I.; Watanabe, T.; Morita, H.; Kohno, T.; Noguchi, H. *Org. Lett.* **2006**, *8*, 499−502.

(3) (a) Abe, I.; Takahashi, Y.; Morita, H.; Noguchi, H. *Eur. J. Biochem.* **2001**, *268*, 3354−3359. (b) Abe, I.; Sano, Y.; Takahashi, Y.; Noguchi, H. *J. Biol. Chem.* **2003**, *278*, 25218−25226.

(4) (a) Abe, I.; Morita, H.; Nomura, A.; Noguchi, H. *J. Am. Chem. Soc.* **2000**, *122*, 11242−11243. (b) Morita, H.; Takahashi, Y.; Noguchi, H.; Abe, I. *Biochem. Biophys. Res. Commun.* **2000**, *279*, 190−195. (c) Morita, H.; Noguchi, H.; Schröder, J.; Abe, I. *Eur. J. Biochem.* **2001**, *268*, 3759−3766. (d) Abe, I.; Takahashi, Y.; Noguchi, H. *Org. Lett.* **2002**, *4*, 3623−3626. (e) Abe, I.; Takahashi, Y.; Lou, W.; Noguchi, H. *Org. Lett.* **2003**, *5*, 1277−1280. (f) Abe, I.; Watanabe, T.; Noguchi, H. *Phytochemistry* **2004**, *65*, 2447−2453. (g) Oguro, S.; Akashi, T.; Ayabe, S.; Noguchi, H.; Abe, I. *Biochem. Biophys. Res. Commun.* **2004**, *325*, 561−567. (h) Abe, I.; Utsumi, Y.; Oguro, S.; Morita, H.; Sano, Y.; Noguchi, H. *J. Am. Chem. Soc.* **2005**, *127*, 1362−1363. (i) Abe, I.; Oguro, S.; Utsumi, Y.; Sano, Y.; Noguchi, H. *J. Am. Chem. Soc.* **2005**, *127*, 12709−12716. (j) Abe, T.; Noma, H.; Noguchi, H.; Abe, I. *Tetrahedron Lett.* **2006**, *47*, 8727−8730.

10.1021/ol0625233 CCC: $33.50
Published on Web 11/21/2006

Scheme 1. Formation of Polyketides and Acridone/Quinolone Alkaloids by Plant Type III PKSs[a]



[a] Yield (%) of quinolone production under the standard assay conditions (see Supporting Information).

novel alkaloidal scaffold produced by a CHS superfamily type III PKS (Scheme 1D). The quinolone alkaloids[6] have been investigated as *N*-methyl-D-aspartate[7] (NMDA) and serotonin 5-HT_3[8] receptor antagonists. Moreover, they are also important intermediates in the chemical synthesis of alkaloids.[9]

Anthranilic acid has been postulated to be a key intermediate in the biosynthesis of quinolone and acridone alkaloids, which occur in the greatest abundance in plants from the family of Rutaceae (Figure 1).[10] In fact, acridone synthase (ACS) form *Ruta graveolens* is a plant-specific type III PKS that selects *N*-methylanthraniloyl-CoA as a starter and performs three condensations with malonyl-CoA to produce 1,3-dihydroxy-*N*-methylacridone (a tetraketide) (Scheme 1C);[11] however, the enzyme involved in the biosynthesis of quinolone alkaloids has not been identified yet. Interestingly, the chalcone-forming CHS and other type III PKSs, except ACS, do not accept the shorter and the bulkier *N*-methylanthraniloyl-CoA as a starter unit despite their promiscuous substrate specificity. Although the *Medicago sativa* CHS F215S mutant has been shown to accept *N*-methylanthraniloyl-CoA to produce an unnatural novel tetraketide lactone after three condensations with malonyl-CoA,[5c] enzymatic formation of a quinolone alkaloid (a diketide) by type III PKSs including mutants of *R. graveolens* ACS[11] has not been reported so far.

R. palmatum BAS is the enzyme that catalyzes one-step condensation with malonyl-CoA to produce a diketide benzalacetone.[3] When recombinant *R. palmatum* BAS functionally expressed in *Escherichia coli* was incubated with

(5) (a) Schüz, R.; Heller, W.; Hahlbrock, K. *J. Biol. Chem.* **1983**, *258*, 6730−6734. (b) Zuurbier, K. W. M.; Leser, J.; Berger, T.; Hofte, A. J. P.; Schröder, G.; Verpoorte, R.; Schröder, J. *Phytochemistry* **1998**, *49*, 1945−1951. (c) Jez, J. M.; Bowman, M. E.; Noel, J. P. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 5319−5324. (d) Samappito, S.; Page, J.; Schmidt, J.; De-Eknamkul, W.; Kutchan, T. M. *Planta* **2002**, *216*, 64−71. (e) Samappito, S.; Page, J. E.; Schmidt, J.; De-Eknamkul, W.; Kutchan, T. M. *Phytochemistry* **2003**, *62*, 313−323.

(6) Two-step synthesis of 4-hydroxyquinolinones from anthranilate derivatives by a tellurium-triggered cyclization reaction has been recently reported; see: Dittmer, D. C.; Li, Q.; Avilov, D. V. *J. Org. Chem.* **2005**, *70*, 4682−4686.

(7) (a) Rowley, M.; Kulagowski, J. J.; Watt, A. P.; Rathbone, D.; Stevenson, G. I.; Carling, R. W.; Baker, R.; Marshall, G. R.; Kemp, J. A.; Foster, A. C.; Grimwood, S.; Hargreaves, R.; Hurley, C.; Saywell, K. L.; Tricklebank, M. D.; Leeson, P. D. *J. Med. Chem.* **1997**, *40*, 4053−4068. (b) Zhou, Z.-L.; Navratil, J. M.; Cai, S. X.; Whittemore, E. R.; Espitia, S. A.; Hawkinson, J. E.; Tran, M.; Woodward, R. M.; Weber, E.; Keana, J. F. W. *Bioorg. Med. Chem.* **2001**, *9*, 2061−2071.

(8) Hayashi, H.; Miwa, Y.; Ichikawa, S.; Yoda, N.; Miki, I.; Ishii, A.; Kono, M.; Yasuzawa, T.; Suzuki, F. *J. Med. Chem.* **1993**, *36*, 617−626.

(9) (a) Watters, W. H.; Ramachandran, V. N. *J. Chem. Res. Synop.* **1997**, 184−185. (b) Majumdar, K. C.; Choudhury, P. K. *Synth. Commun.* **1993**, *23*, 1087−1100. (c) Grundon, M. F.; Ramachandran, V. N. *Tetrahedron Lett.* **1985**, *26*, 4253−4256. (d) Corral, R. A.; Orazi, O. O.; Autino, J. C. *Tetrahedron Lett.* **1983**, *24*, 2359−2360. (e) Reisch, J.; Mueller, M.; Mester, I. *Z. Naturforsch. B: Anorg. Chem., Org. Chem.* **1981**, *36B*, 1176−1179.

(10) Dewick, P. M. *Medicinal Natural Products, A Biosynthetic Approach*, 2nd ed.; Wiley: West Sussex, 2002.

(11) (a) Junghanns, K. T.; Kneusel, R. E.; Baumert, A.; Mainer, W.; Groger, D.; Matern, U. *Plant Mol. Biol.* **1995**, *27*, 681−692. (b) Lukacin, R.; Springob, K.; Urbanke, C.; Ernwein, C.; Schröder, G.; Schröder, J.; Matern, U. *FEBS Lett.* **1999**, *448*, 135−140. (c) Springob, K.; Lukacin, R.; Ernwein, C.; Groning, I.; Matern, U. *Eur. J. Biochem.* **2000**, *267*, 6552−6559. (d) Lukacin, R.; Schreiner, S.; Matern, U. *FEBS Lett.* **2001**, *508*, 413−417. (e) Lukacin, R.; Schreiner, S.; Silber, K.; Matern, U. *Phytochemistry* **2005**, *66*, 277−284.

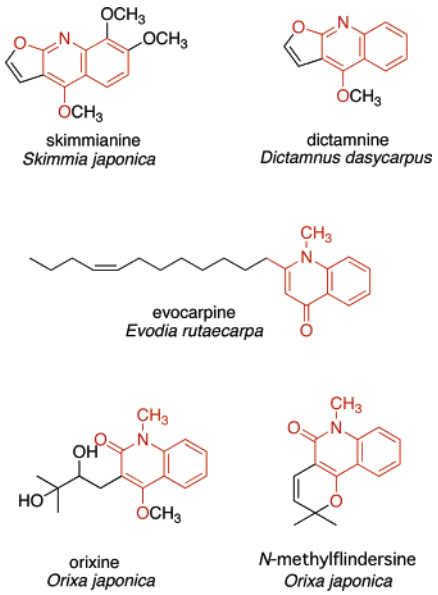


Figure 1. Naturally occurring quinoline alkaloids derived from 4-hydroxy-2(1*H*)-quinolones from Rutaceae plants.[12]

N-methylanthraniloyl-CoA (or anthraniloyl-CoA) and malonyl-CoA (or methylmalonyl-CoA) as substrates, the enzyme efficiently afforded 4-hydroxy-2(1*H*)-quinolones (**1**−**4**) as a single product (Scheme 1D). The condensation products were not detected in control experiments performed with a boiled enzyme preparation. The spectroscopic data (LC-ESIMS, UV, and ^{1}H NMR) of the compounds were characteristic of those of the quinolone alkaloids and completely identical with those of authentic compounds.[6] For example, the LC-ESIMS spectrum of 4-hydroxy-1-methyl-2(1*H*)-quinolone (**3**) gave a parent ion peak $[M + H]^+$ at *m*/*z* 176, indicating the structure of a diketide. The ^{1}H NMR of **3** obtained from a large-scale enzyme reaction (0.4 mg from 2 mg of *N*-methylanthraniloyl-CoA and 4 mg of malonyl-CoA) showed one methyl singlet (δ 3.52) in addition to one vinyl proton (δ 5.86) and four aromatic protons (δ 7.89−7.17), supporting that the heterocyclic system adopts the more stable 4-hydroxy-2(1*H*)-quinolone form. Confirmation of the structure was finally obtained by direct comparison with the commercially available authentic compound. These quinolone alkaloids have never been isolated from *R. palmatum* (Polygonaceae).

Interestingly, the enzyme reaction with the anthraniloyl-CoA proceeds without the "decarboxylation" step, and the amide formation immediately follows the condensation reactions of *N*-methylanthraniloyl-CoA (or anthraniloyl-CoA) and malonyl-CoA (or methylmalonyl-CoA) (Scheme 1D). This was also the case for the conversion of benzoyl-CoA (i.e., without the methylamino group of *N*-methylanthraniloyl-CoA) to 6-phenyl-4-hydroxy-2-pyrone (a triketide) by *R. palmatum* BAS.[3b] Furthermore, the quinolone formation activity showed a broad pH optimum within a range of pH 6.0−8.5. Thus, the pH change did not affect the product profile; formation of triketides or other polyketides was not detected in the reaction mixture. In contrast, the pH optimum for the formation of benzalacetone was reported to be at maximum within a range of pH 8.0−8.5. It should be noted that *R. palmatum* BAS exhibited a dramatic change from benzalacetone to triketide pyrone production at acidic pH.[3b]

For the quinolone production, the best yield (86% under the standard assay conditions) was obtained with the combination of *N*-methylanthraniloyl-CoA and methylmalonyl-CoA. Notably, both esters are nonphysiological substrates for *R. palmatum* BAS. On the other hand, a combination of anthraniloyl-CoA and malonyl-CoA afforded only 4% yield. Steady-state enzyme kinetics for the formation of 4-hydroxy-1,3-dimethyl-2(1*H*)-quinolone (**4**) revealed K_M = 23.7 μM and k_{cat} = 1.48 min^{-1} for *N*-methylanthraniloyl-CoA, which was comparable with those for the formation of benzalacetone, the normal product of the enzyme; K_M = 10.0 μM and k_{cat} = 1.79 min^{-1} for 4-coumaroyl-CoA. This strongly suggests the presence of a not yet identified novel type III PKS that produces quinolone alkaloids[12] from the CoA thioester of anthranilic acid (Figure 1).

In *R. graveolens* ACS, the residues Ser132, Ala133, and Val265 (numbering in *M. sativa* CHS) have been proposed to play a critical role for the selection of *N*-methylanthraniloyl-CoA as a starter substrate and for the formation of the acridone alkaloid.[11d] Indeed, an ACS triple mutant (S132T/A133S/V265F) has been shown to yield an enzyme that was functionally identical with CHS.[11d] However, interestingly, two of the residues are not conserved in *R. palmatum* BAS (S132L/V265F) that accepted the anthraniloyl starter. On the other hand, the diketide-forming activity of the BAS enzyme is derived from the characteristic substitution of the active-site gatekeeper Phe215; the conformationally flexible residue conserved in all the known type III PKSs is uniquely replaced with nonaromatic Leu in BAS.[3] Although the BAS L215F mutant has been shown to recover chalcone-forming activity to produce chalcone after three condensations with malonyl-CoA,[3b] the mutant did not produce the tetraketide acridone from the anthraniloyl starter but instead just yielded the diketide quinolone. These results suggest subtle differences of the active-site structure between *R. palmatum* BAS and *R. graveolens* ACS.

In summary, the present work describes the first enzymatic synthesis of 4-hydroxy-2(1*H*)-quinolones. Manipulation of the functionally divergent CHS superfamily type III PKSs by nonphysiological substrates thus provides an efficient method for production of pharmaceutically important plant alkaloids.

Acknowledgment. This work was supported by the PRESTO program from the Japan Science and Technology Agency, a Grant-in-Aid for Scientific Research (Nos. 18510190 and 17310130), and the COE21 program from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

Supporting Information Available: Materials and methods. This material is available free of charge via the Internet at http://pubs.acs.org.

OL0625233

(12) Michael, J. P. *Nat. Prod. Rep.* **2004**, *21*, 650−668.