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Studies on the Biosynthesis of Phomoidride B (CP-263,114): Evidence for a Decarboxylative Homodimerization Pathway

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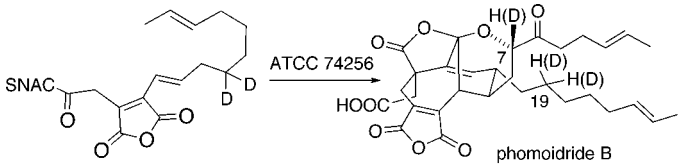
ABSTRACT

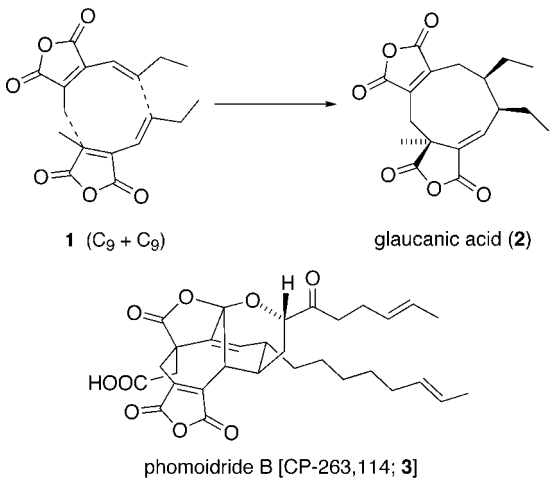


Feeding experiments using a deuterium labeled C_{16} maleic anhydride and whole cell culture of ATCC 74256 led to the isolation of phomoidride B with deuterium incorporation at C(7) and C(19) as determined by ^2H NMR and electrospray mass spectrometry. This result is in accord with a decarboxylative homodimerization of the C_{16} maleic anhydride as a key biosynthetic step leading to phomoidride B.

During the course of producing complex secondary metabolites, nature occasionally utilizes a dimerization step within a biosynthetic pathway.[1] One remarkable set of examples is illustrated in the nonadride group of fungal secondary metabolites whereby dimerization of unsaturated anhydrides is hypothesized to lead to the production of a series of nine-membered carbocyclic ring containing natural products.[2] For example, glaucanic acid is presumably produced by the dimerization of a nine-carbon anhydride unit (Scheme 1).[3]

In 1966 Sutherland and co-workers investigated the biosynthesis of glaucanic acid in *Penicillium purpurogenum* and demonstrated efficient incorporation of a ^{14}C-labeled deriva-

Scheme 1



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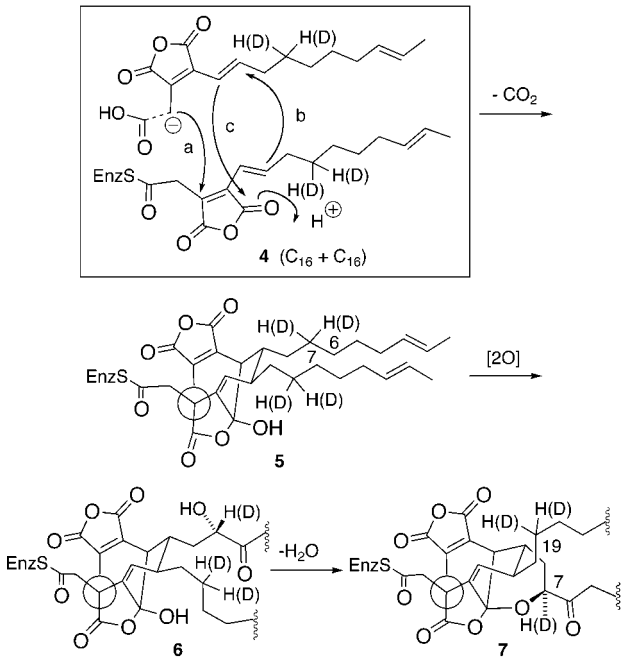
Scheme 2

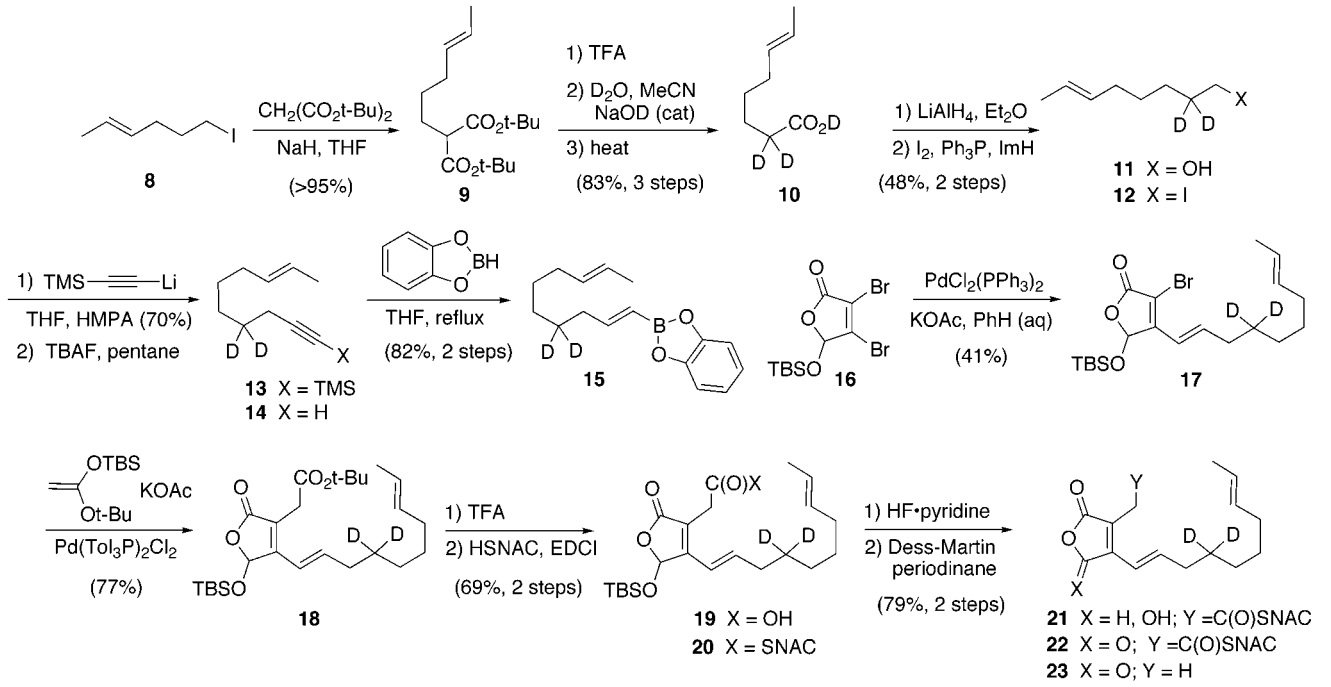


tive of the nine-carbon anhydride unit. In later work Tamm and co-workers studied the biosynthesis of the nonadride rubratoxin B in *Penicillium rubrum*.[4] In this case extensive randomization of the isotopic label was observed when potential precursors were fed to the rubratoxin-producing culture, leading to inconclusive results.

The most recent addition to the nonadride family of secondary metabolites is phomoidride B (CP-263,114, **3**, Scheme 1), which is produced by an unidentified fungus (ATCC 74256).[5] In this case, the biosynthetic pathway requires the decarboxylative condensation of two 16-carbon unsaturated anhydrides (Scheme 2). One possible scenario involves a cascade of bond-forming steps starting with a decarboxylative condensation between a Michael donor anhydride and an enzyme-bound Michael acceptor anhydride (bond **a** formation) (**4**, $C_{16} + C_{16}$).[6,7] In earlier work, using suspended cells and ^{13}C-labeled precursors, we demonstrated that seven carbons of phomoidride B are derived from succinic acid and the remaining carbons derived from acetic acid but left unproven the decarboxylative homodimerization step.[8] To establish support for this condensation (**4**, $C_{16} + C_{16}$) and side-chain oxidation (**5** → **6**) steps shown in Scheme 2, anhydrides **22**-d_2 and **23**-d_2 were synthesized and individually fed to cultures of ATCC 74256. *The results of this study are consistent with the biosynthetic pathway outlined in Scheme 2.*

Anhydride **22**-d_2 was synthesized following a procedure developed by our group for a biomimetic synthesis of the phomoidrides (Scheme 3).[9] Thus, alkylation of di-*tert*-butyl malonate with hexenyl iodide **8** gave malonate **9** in >95% crude yield. Removal of the *tert*-butyl groups with TFA followed by base-catalyzed deuterium exchange and thermal decarboxylation afforded carboxylic acid **10** in 83% for three steps. Reduction of **10** with $LiAlH_4$ followed by iodination afforded octenyl iodide **12**. Lithium acetylide displacement of iodide **12** followed by desilylation yielded alkyne **14**. The latter was hydroborated and subjected to a Suzuki reaction with mucobromic acid derivative **16** to provide bromide **17** exclusively. No coupling at the α bromide was observed during the Suzuki reaction. Palladium-mediated cross-

Scheme 3



coupling between bromide **17** and the silylketene acetal derived from *tert*-butyl acetate was promoted by KOAc and afforded **18** in 77% yield. *tert*-Butyl ester **18** was converted to *N*-acetyl cysteamine ester **20** using a two-step procedure.[10] Finally, anhydride **22**-d_2 was derived from **20** by desilylation and oxidation.[11]

A feeding experiment using ATCC 74256 with **22**-d_2 followed by isolation and purification of phomoidride B (**7**) revealed significant deuterium incorporation at C(7) and C(19) as observed by ^{2}H NMR spectroscopy (Figure 1).

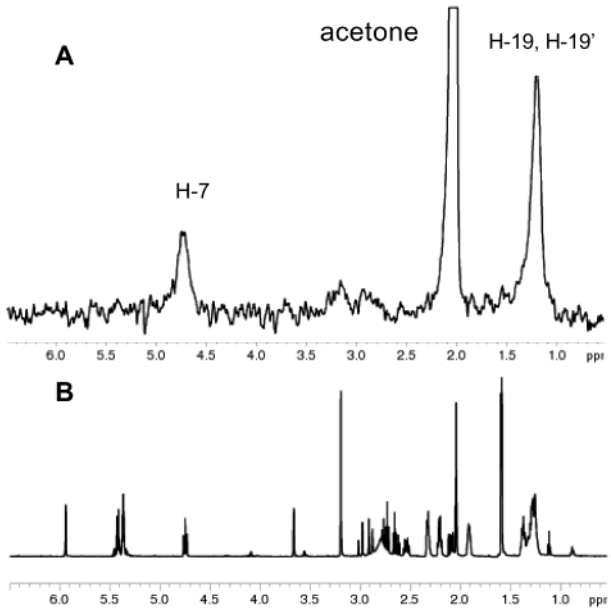
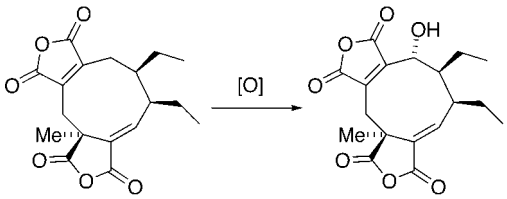


Figure 1. NMR spectra of phomoidride B: (A) 76.78 MHz ^{2}H NMR spectrum (acetone) of phomoidride B isolated from feeding experiment with **22**-d_2. (B) 500 MHz ^{1}H NMR spectrum of phomoidride B in acetone-d_6.

Analysis by electrospray mass spectrometry corroborated the NMR analysis by showing significant enhancement in isotopic masses M + 1 (d_1,+23.2%), M + 2 (d_2, +8.5%), and M + 3 (d_3, +4.2%).[12,13] In a second feeding experiment, labeled decarboxylated monomer **23**-d_2 failed to produce any significant label incorporation as determined within the limits of detection by electrospray mass spectrometry and ^{2}H NMR.

Since SNAC ester **22**-d_2 led to deuterium incorporation at C(7) and C(19) the proposed decarboxylative homodimerization step of a common 16-carbon anhydride as shown in Scheme 2 has been supported. Furthermore, the finding that C(7) effectively incorporates deuterium supports introduction of the C(6) and C(7) oxygen functionality by an oxygenase. In analogy to the biosynthesis of glauconic acid (**24**), which is known to be derived from glaucanic acid (**2**) (Scheme 4),

Scheme 4



oxidation of C(6) and C(7) *probably* occurs following the decarboxylative homodimerization step (Scheme 2, **5** → **6**).[14]

In conclusion, the experimental results presented support the decarboxylative homodimerization of a C_{16} anhydride (**3**) as a key step in the biosynthesis of phomoidride B. The results also support a dioxygenation to introduce the C(6) and C(7) oxygen functionalities following the homodimerization step. Finally, efficient decarboxylative homodimerization required both units to be introduced as SNAC esters. Efforts to elucidate further details of this and related decarboxylative dimerizations are currently under investigation.

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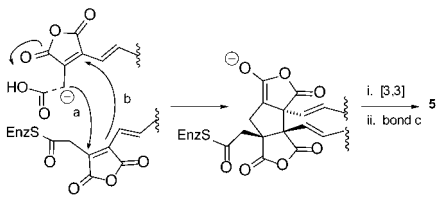
(6) The representation shown in **4** is one of four possilbilities. Alternatives include Michael acceptor and donor anhydrides *both* enzyme-bound or *neither* covalently attached within an enzyme active site.

(7) The order of bond formation applied in **4** (Scheme 2) is only one possibility. For example, intermediate **5** may be arrived by the [3,3]-sigmatropic route shown below; cf: Chen, C., Layton, M. E.; Shair, M. D. *J. Am. Chem. Soc.* **1998**, *120*, 10784−10785. We thank Professor Mathew Shair for bringing this possibility to our attention.



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(11) Analysis of SNAC ester **22**-d_2 by electrospray mass spectrometry showed the following isotopic mass distribution: M (d_0, 6.7%), M + 1 (d_1, 18.6%), and M + 2 (d_2, 74.7%).

(12) Values are reported as percentage of total ion signal. Peak areas (in counts) were integrated using TofMA software and corrected for ^{13}C contribution using the theoretical isotope profile generatd using Isopro 3.0.

(13) The uneven distribution of M + 1 and M + 2 in labeled phomoidride B *may* be related to uneven competition with exogenous monomer for two different enzyme sites occupied by the dimerizing C_{16} maleic anhydrides.

(14) See ref 3b.

Supporting Information Available: Full experimental procedures for the preparation of **22**-d_2 and fermentation, isolation, and spectral analysis of phomoidride B. This material is available free of charge via the Internet at http://pubs.acs.org.

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