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Investigations into the Production and Interconversion of Phomoidrides A–D

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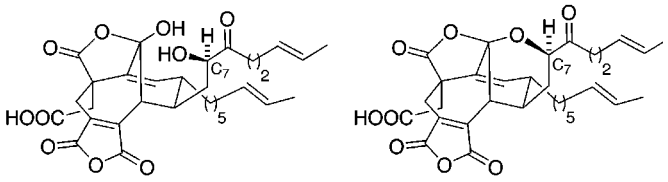
ABSTRACT



phomoidride C phomoidride D

Fermentation of ATCC 74256 led to the isolation and identification of C7 epimers of phomoidride A (CP-225,917) and phomoidride B (CP-263,114). We suggest the names phomoidrides C and D for these new fermentation products. Studies on the effect of pH on the distribution of phomoidrides A–D suggest phomoidride B (CP-263,114) is the first-formed secondary metabolite and the source of the remaining three phomoidrides.

Due primarily to their unique structure and dense array of functionality, the fungal secondary metabolites phomoidride A (CP-225,917, **1**) and phomoidride B (CP-263,114, **2**) have attracted the attention of many synthetic chemists.[1,2] In terms of biological activity, phomoidrides A and B are modest inhibitors of ras farnesyl transferase (IC_{50} values of 6 and 20 μM, respectively) and squalene synthase (IC_{50} values of 43 and 160 μM, respectively).[1b] Investigations of four research groups have culminated in the total synthesis of **1** and/or **2**.[3] As in all synthetic endeavors, a critical aspect of these investigations was the comparison of synthetic material to samples obtained from the natural source, in this case a nonsporulating fungus (ATCC 74256) described by workers at Pfizer. During the course of their comparison analysis, Danishefsky and co-workers noted the identification of variable (trace) amounts of the 7*R*-isomer (**4**) of phomoidride B in samples of **2** obtained from fermentation of ATCC 74256 supplied by workers at Pfizer.[1a,2n,4] The Sloan group

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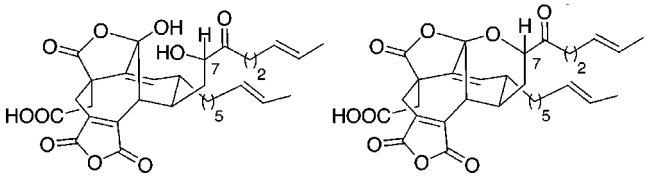
also characterized the 7*R*-isomer (**3**) of phomoidride A, in this case produced by chemical synthesis and/or hydroxide-induced epimerization of phomoidride A (**1**). As part of our investigations into the biosynthesis of these fascinating molecules, we discovered the coproduction of **3** by fermentation as well as the previously described 7*R*-isomer (**4**) of phomoidride B and suggest the names phomoidrides C and D for **3** and **4**, respectively. *The effect of pH and fermentation time on the distribution of these four products suggests that phomoidride B (**2**) is the first-formed secondary metabolite and the source of the remaining three components (**1**, **3**, and **4**).*



phomoidride A [β-H, α-OH; **1**]
phomoidride C [α-H, β-OH; **3**]

phomoidride B [β-H, α-O; **2**]
phomoidride D [α-H, β-O; **4**]

In earlier studies, we determined the production of phomoidride A (**1**) and phomoidride B (**2**) by ATCC 74256 to depend on the pH of the fermentation medium, with a pH range from 3.2 to 5 favoring production of these secondary metabolites.[5] Careful examination of the crude extracts of these cultures led to the isolation of phomoidride C (**3**) and phomoidride D (**4**). The ^{1}H NMR spectrum of the former compound correlated with a spectrum of synthetic **3** provided by the Danishefsky group.[6]

Next we examined the effect of pH and fermentation time on the production of these four compounds (Figure 1).

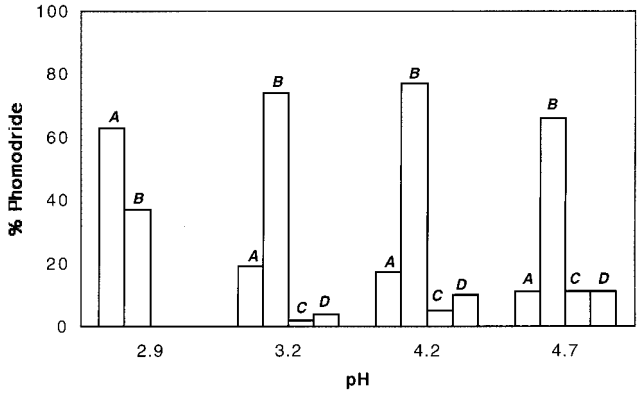
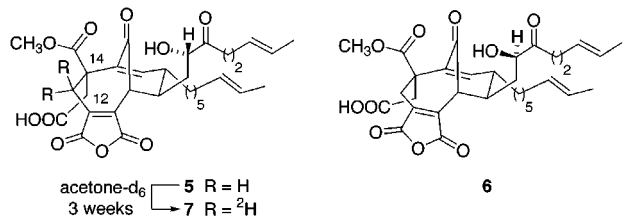


Figure 1. Distribution of phomoidrides A−D after a 7 day culture at the pH values shown.

Fermentation at pH 3 to 4 for 7 days favored production of phomoidride B (**2**, ca. 75%) with phomoidride A (15−20% **1**) produced as the second major product. Cultures maintained in a pH range of 4 to 5 for the same time period also produced **2** as the major isolate although at the higher end of this pH range minor quantities (ca. 10%) of **3** and **4** were observed. Cultures maintained at pH 2.9 for 7 days were the first to produce phomoidride A (**1**) as the major fermentation product. Above pH 3, similarly aged cultures consistently produced phomoidride B (**2**) as the major product. The rate of production of **2** by the fungus (ATCC 74356) decreases significantly below pH 3. This observation is consistent with the reversed distribution of **1** and **2** below pH 3 (i.e., production of **2** is slow relative to the rate of hydrolysis of **2** to **1** under the fermentation conditions). The most significant evidence for phomoidride A (**1**) to be produced by the hydrolysis of phomoidride B (**2**) was the observation that pure samples of the latter maintained at pH 7 or above were converted to phomoidride A (**1**) in a time-dependent manner. The converse experiment showed that **1** has no tendency to cyclodehydrate to **2** under identical conditions. These observations support the alkaline hydrolysis of phomoidride B to phomoidride A within a high pH compartment of the phomoidride producing fungus as a plausible biosynthetic route. Finally, during the course of these experiments we corroborated Danishefsky's finding that the C(7) epimers **3** and **4** are indeed thermodynamic products that do not show any tendency to revert to **1** or **2**, respectively.

In regard to the interconversion of the phomoidrides, the Pfizer group was the first to report the conversion of **1** to **2** by treatment of the former with methanesulfonic acid in dichloromethane.[1b] Conditions for effecting the reverse transformation (**2** to **1**) were first described by Nicolaou and co-workers [LiOH, THF (aq)].[3c] To shed some insight into the mechanism of these transformations, we examined the hydrolytic opening of phomoidride B (**2**) using aqueous lithium hydroxide in methanol (20 min) followed by an inverse quench (0.5% trifluoroacetic acid−ethyl acetate). Using these conditions we were able to isolate and fully characterize methyl ester **5** which slowly reverted back to phomoidride B (Scheme 1). Under identical hydrolytic

Scheme 1



conditions, phomoidride D (**4**) generated an unstable methyl ester (presumably **6**) that rapidly reverted back to **4** (Scheme

(4) The Danishefsky publication appeared prior to the assignment of absolute stereochemistry of the phomoidrides and for the purpose of discussion arbitrarily assigned the absolute stereochemistry with the natural epimer appearing as 7*R*. Subsequently, the absolute stereochemistry of the phomoidrides has been determined and correctly depicted as **1a** and **2a** where the natural stereochemistry is 7*S*.

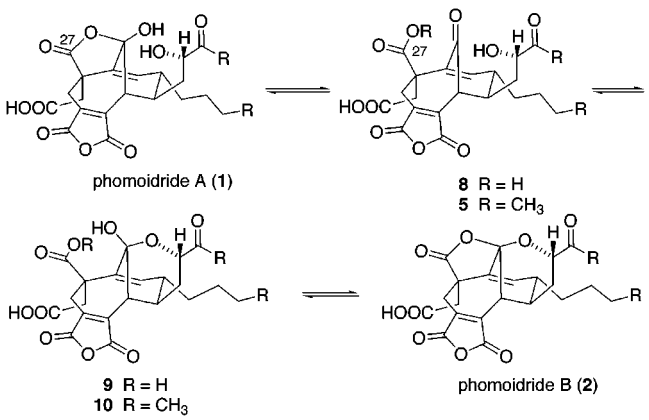
(5) Spencer, P.; Agnelli, F.; Williams, H. J.; Keller, N. P.; Sulikowski, G. A. *J. Am. Chem. Soc.* **2000**, *122*, 420−421.

(6) We thank Professor Samuel Danishefsky and Dr. Dongfang Meng for providing spectral data for **3**.

1). Parenthetically, during the course of recording spectral data on methyl ester **5**, an interesting observation was made. A sample of **5** stored over a 3 week period in d_6-acetone underwent exchange of C(13) methylene protons for deuterium (**5** → **7**). The exchange showed selectivity for the C(13) *exo* proton as determined by ^{1}H NOESY experiments following spontaneous cyclization to phomoidride B (**2**) in chloroform. Since **2** shows no tendency to exchange protons, this may prove to be a useful route to tritium labeling of phomoidride B for biological studies.

The above observations support the reaction pathway outlined in Scheme 2 for the interconversion of phomoidride

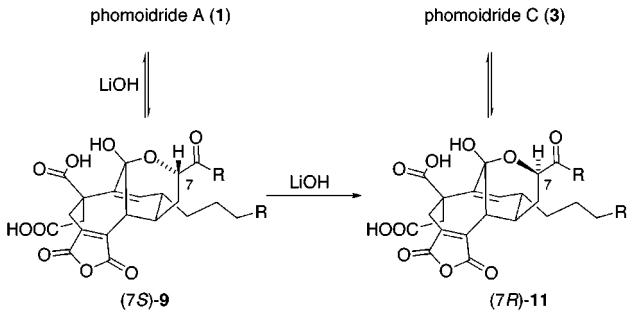
Scheme 2



A (**1**) and phomoidride B (**2**). In this scheme, cyclodehydration of **1** to **2** and hydrolysis of **2** to **1** proceed via open-chain ketone **8**. Under anhydrous acidic conditions, **1** presumably undergoes ring−chain tautomerization to ketone **8** which on closure to **9** undergoes rapid lactonization to **2**. In the reverse direction, phomoidride B (**2**) on treatment with aqueous base undergoes hydrolytic opening to **9** which unravels to ketone **8** due (in part) to the sterically demanding endo orientation of the C(7) alkyl side chain. Keto acid **8** undergoes bond reorganization to phomoidride A (**1**), the thermodynamically favored ring tautomer. In the case where methoxide is the nucleophile, intermediate **10** opens to ketone **5**.

Danishefsky has attributed the favored epimerization of phomoidride B (**2**) to phomoidride D (**4**) to the preferred exo orientation of the C(7) hexanoyl side chain with respect to the bicyclo[3.3.1]nonane substructure. In contrast, the apparent thermodynamic preference for the 7*R*-isomer, phomoidride C (**3**), in the open series (**1** and **3**) remains largely unexplained. Only the possibility of a unique hydrogen-bonding array has been offered as a potential explanation.[2n] Here we offer a second proposal as outlined in Scheme 3 involving the interconversion of ring−chain

Scheme 3



tautomers **1** to **9**. For reasons identical to those of the C(7) epimerization of **2**, pyran (7*S*)-**9** is expected to isomerize to (7*R*)-**11** and upon tautomerization afford phomoidride C (**3**). Currently, we are developing experiments to support this mechanistic hypothesis.

In summary, we have isolated, for the first time, the 7*R*-isomer of phomoidride A as well as the previously identified fermentation product **4** from ATCC 74256. We propose that these secondary metabolites adopt the names phomoidrides C (**3**) and D (**4**). The dependency of product distribution on pH and fermentation time, as well as the stability of **1** and **2** to the culture conditions, suggests that phomoidride B (**2**) is the primary biosynthetic product and the remaining three phomoidrides are derived from **2**.

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Supporting Information Available: ^{1}H and ^{13}C NMR data for compounds **1**−**5**. This material is available free of charge via the Internet at http://pubs.acs.org.

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