Remarkable, Enantiodivergent Biogenesis of Brevianamide A and B

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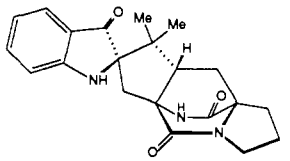
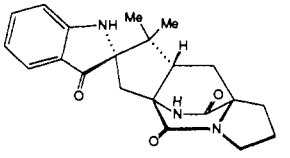
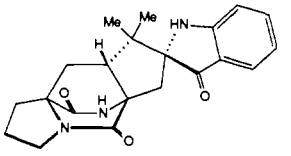
The bright yellow mycotoxins, brevianamide A (**1**) and B (**2**), were isolated from culture extracts of *Penicillium brevicompactum* almost two decades ago by Birch and Wright.[1] The structure



1, (+)-BREVIANAMIDE A



2, (-)-BREVIANAMIDE B from brevianamide A or total synthesis

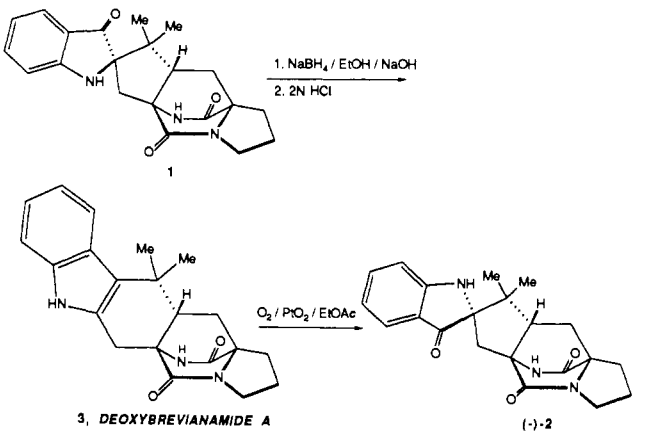


2, (+)-BREVIANAMIDE B from Penicillium brevicompactum

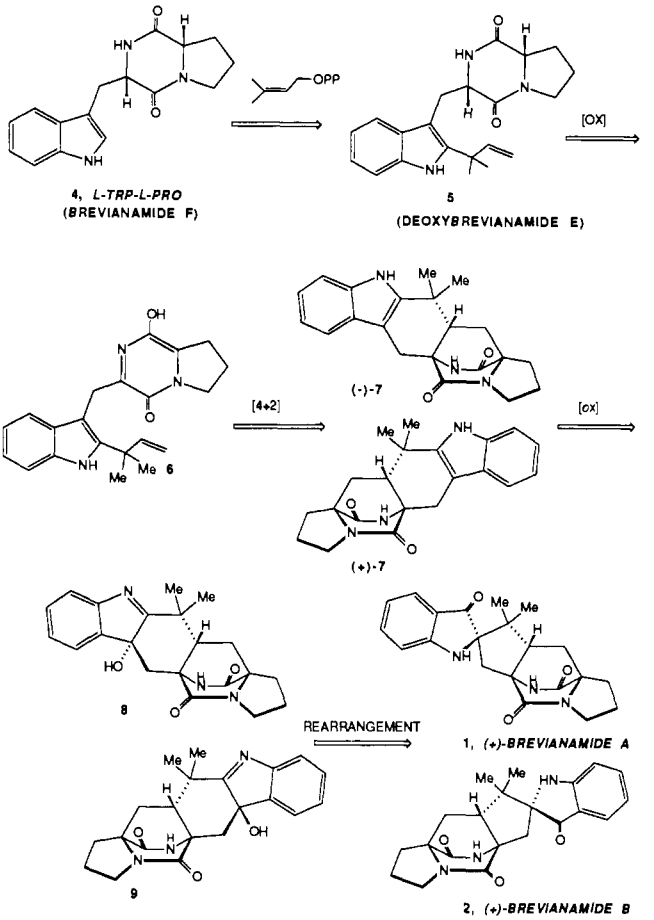
originally proposed by Birch for brevianamide A, the major metabolite, was later confirmed by single-crystal X-ray analysis[2] of 5-bromo-brevianamide A; this study elucidated both the relative and absolute configuration of **1** as that depicted. The structure of brevianamide B (the minor metabolite) was ascertained[1c] by the reduction of **1** to deoxybrevianamide A (**3**) and (the completely stereoselective) oxidation of **3** to brevianamide B (Scheme I). This structure has recently been rigorously confirmed by total synthesis.[3] In the course of securing the identity[3] of the synthetic and natural materials, it was found that synthetic **2** and natural **2**, derived directly from the culture extracts (and not via semisynthetic conversion from **1**), were of the *opposite absolute configuration.* Through a careful study of the chiroptical properties of naturally derived **1** and **2** as well as synthetic[3] and semisynthetic[1c] **2**, we now report that *Penicillium brevicompactum* constructs brevianamide A and brevianamide B in optically pure form but as enantiomorphs with respect to the bicyclo[2.2.2] ring system. These findings have significant biogenetic as well as possible genetic implications for the producing organisms.

On the basis of the results of feeding experiments with ^{14}C-labeled precursors[1] Birch postulated that cyclo-L-Trp-L-Pro (brevianamide F, **4**, Scheme II) is prenylated with dimethallyl pyrophosphate to furnish deoxybrevianamide E (**5**). Two-electron oxidation of the Trp moiety and enolization (Pro) would then give the achiral diene **6** that was postulated[1] to suffer intramolecular [4 + 2] cycloaddition to furnish the hexacyclic compound **7**. Oxidation and pinacol-type rearrangement of the diastereomeric hydroxyindolenines **8** and **9** would provide the spiroindoxyls, brevianamide A and B. It is significant that if the intermediacy of **7** is correct, the major metabolite brevianamide A would result from oxidation on the *more hindered face* of **7** (providing **8**), and

Scheme I



Scheme II



the minor metabolite brevianamide B would result from oxidation on the least hindered face of **7** (via **9**).

As shown in Figure 1, the CD spectra of natural **1**, natural **2**, synthetic **2**, and semisynthetic **2** (derived from natural **1**) clearly show that semisynthetic brevianamide B and synthetic brevianamide B have the same absolute configuration (with respect to the bicyclo[2.2.2]piperazinedione nucleus) as brevianamide A. Natural brevianamide B on the other hand, is the *enantiomorph* of these substances. The cotton effect at 200–250 nm is due to an n,π^* transition of the amide bonds and is a reliably diagnostic method to discern the absolute stereochemistry of bicyclic piperazinediones.[4] It is further quite interesting, that the absorption between 250 and 450 nm gives an indication of the *absolute*

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(2) Coetzer, J. *Acta Crystallogr.* **1974**, *B30*, 2254.

(3) Williams, R. M.; Glinka, T.; Kwast, E. *J. Am. Chem. Soc.* **1988**, *110*, 5927.

(4) (a) Herscheid, J. D. M.; Tijhuis, M. W.; Noorkik, J. H.; Ottenheijm, H. C. J. *J. Am. Chem. Soc.* **1979**, *101*, 1159 and references cited therein. (b) Nagarajan, R.; Woody, R. W. *J. Am. Chem. Soc.* **1973**, *95*, 7212.

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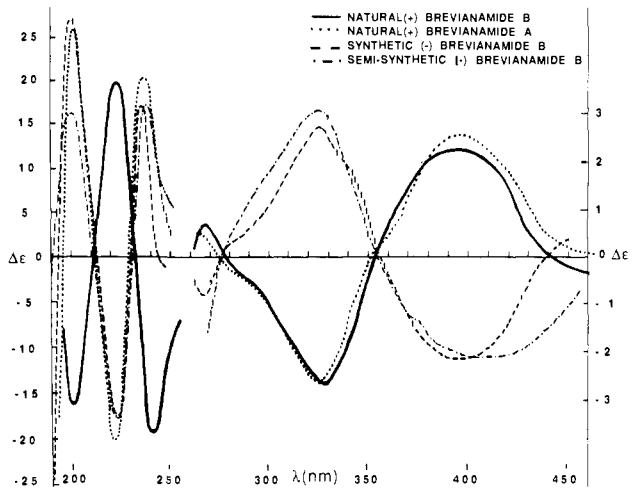

Figure 1. CD spectra of **1** and **2** recorded in trifluoroethanol between 200 and 250 nm and in 2.5% formic acid/dichloromethane between 250 and 450 nm.

stereochemistry at the spiroindoxyl stereogenic center which is (*R*)- for brevianamide A *and* (*R*)- for *natural* brevianamide B. The CD spectra between 250 and 450 nm for these two substances are virtually identical even though they are diastereomers belonging to unique enantiomorphic groups. The UV spectra of these substances show an absorbtion at ~400 nm which is attributable to the indoxyl chromophore.[1]

If the Birch biosynthetic pathway[1] is correct with respect to the structures of intermediates **5**–**7**, our results require that (1) both enantiomorphs of **7** are synthesized by *Penicillium brevicompactum* in *unequal* amounts and either (2) two distinct oxidases having unique *enantioselectivity* for (+)-**7** and (−)-**7** must also have *opposite diastereoselectivity* delivering oxygen from the more and less hindered faces of **7** (furnishing **8** and **9**, respectively) resulting in optically pure **1** and **2** or (2) that a single oxidase recognizes only the binding orientation of the indole moiety, delivering oxygen from the (*R*)-face of each enantiomer of **7**. The contention that the oxidation of **7** to **1** or **2** is enzyme-mediated is supported by the following observation. An authentic, synthetic sample of the proposed shunt metabolite (−)-**7** was prepared by removal[6] of the *p*-methoxybenzyl group from N-9 of the corresponding synthetic[3,7] derivative. Allowing this compound to stand in ethyl acetate solution exposed to air for several days resulted in a myriad of decomposition products of which no identifiable trace of either **1** or **2** could be detected. However, m-CPBA oxidation of (−)-**7** followed by exposure of the incipient hydroxyindolenine to NaOMe in methanol gave in high yield (exclusively) (−)-brevianamide B. Thus, unlike deoxybrevianamide A (**3**) which autoxidizes[1] to (−)-**2**, compound **7** does *not* autoxidize to either **1** or **2** implicating a specific, enzyme-mediated process. Attempts to identify **7** in culture extracts of *Penicillium brevicompactum* were completely unsuccessful. While this does not rule out the possibility that **7** is a short-lived, tightly enzyme-bound intermediate that is not excreted into the culture medium, further experiments are required to prove the validity of this reasonable biosynthetic scheme. The relative proportions of brevianamide A and B produced suggests that **7** is produced in partially racemic form. The failure to detect **7**, particularly (+)-**7** which precedes **2**, supports the notion that both enantiomorphic precursors to **1** and **2** are produced in unequal amounts and are completely consumed by the oxidase(s). An interesting mystery that remains is to elucidate the mechanism for the formation of the two enantiomorphic series, regardless of the validity of structure **7**.

The above facts lead to the conclusion that *Penicillium brevicompactum* has evolved genes encoding for enantio- and diastereodivergent pathways specifically for the biosynthetic production of **1** and **2** regardless of the structural uncertainties of the intermediates following **4**. Planar, achiral intermediate **6** would nicely accommodate the occurrence of the two enantiomeric series; a single oxidase displaying complete (*R*)-facial selectivity toward the indole, or two distinct enantio- and diastereoselective oxidases, would then effect a resolution producing the two optically pure diasteromers **1** and **2**. Experiments aimed at validating the intermediacy of **5**, **6**, and **7** as shunt metabolites and elucidating the nature of the oxidase(s) are in progress in these laboratories.

Acknowledgment. We thank the National Institutes of Health (CA 43969) and the Colorado State University Agricultural Experiment Station (part of USDA SAES Western Project W-122) for financial support of this work. Prof. Robert W. Woody (CSU) is acknowledged for assistance in obtaining the CD spectra. R.M.W. acknowledges The Alfred P. Sloan Foundation, Eli Lilly Co., and the National Institutes of Health (Research Career Deveopment Award) for additional Fellowship support.

(5) Specific optical rotations for these substances further support the CD data: synthetic **2** $[\alpha]_D^{25} = -124°$ (*c* 0.77, CH_2Cl_2/2.5% HCO_2H); natural **2** (from *Penicillium brevicompactum* directly) $[\alpha]_D^{25} = +124°$ (*C* 0.77, CH_2Cl_2/2.5% HCO_2H); semisynthetic **2** (derived from **1** via oxidation of **3**) $[\alpha]_D^{25} = -124°$ (*c* 0.77, CH_2Cl_2/2.5% HCO_2H). The synthetic material (**2**) was shown to be >99% ee.

(6) Williams, R. M.; Kwast, E. *Tetrahedron Lett.* **1989**, *30*, 451.

(7) Reference 3, compound no. 16.

Studies of the Inactivation of General Acyl-CoA Dehydrogenase by Racemic (Methylenecyclopropyl)acetyl-CoA: New Evidence Suggesting a Radical Mechanism of This Enzyme-Catalyzed Reaction

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General acyl-CoA dehydrogenase (GACD) is a flavin (FAD) dependent enzyme which catalyzes the first step of β-oxidation converting a straight chain fatty acyl thioester substrate **1** to the corresponding α,β-enolyl-CoA product **2**.[1] Studies of this desaturation step are of particular mechanistic interest, since it involves the rupture of two kinetically stable C–H bonds. Evidence has accumulated supporting a C_α deprotonation as the initial step of this dehydrogenation.[1] However, the mechanism of the subsequent transfer of reducing equivalents from the carbanion **3** to the oxidized flavin is still disputable.[1,2] The commonly accepted route consists of C_β–H expulsion from **3** and then hydride addition to FAD yielding, in a net trans elimination, the α,β-enolyl-CoA **2** and the fully reduced flavin (eq 1).[1] While this mechanism appears to be quite feasible, it should be kept in mind that oxidized flavin is a poor hydride acceptor.[3] Oxidation of the carbanion **3** via a one-electron route forming a transient radical species **4** and a semiquinone flavin is a compelling alternative (eq 2).[1d-3] In fact, formation of flavin radical upon addition of substrate to acyl-CoA dehydrogenase has indeed been noted.[4,5] Several recent

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(2) (a) Massey, V.; Ghisla, S. In *Biological Oxidations, 34, Mosbach Colloquim*; Sund, H., Ullrich, V., Eds.; Springer: Berlin, 1983; p 114. (b) Bruice, T. C. In *Advances in Chemistry Series 191: Biomimetic Chemistry*; American Chemical Society: Washington, DC, 1980; p 89.

(3) (a) Farng, O. L.; Bruice, T. C. *J. Chem. Soc., Chem. Commun.* **1984**, 185. (b) Hemmerich, P.; Massey, V.; Ferner, H. *FEBS Lett.* **1977**, *84*, 5.

(4) McKean, M. C.; Sealy, R. C.; Frerman, F. E. In *Flavins and Flavoproteins*; Massey, V., Williams, C. H., Eds.; Elsevier: Amsterdam, 1982; p 614. When the natural acceptor, electron-transfer flavoprotein (ETF), is present with excess substrate, the yield of this radical is increased by 2-fold.

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