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Supplementary Material Available: ^{1}H NMR and other data for **1b,d**, **2b,d**, **1-9**, and unnumbered compounds (8 pages). Ordering information is given on any current masthead page.

Concerning the Role of Nidurufin in Aflatoxin Biosynthesis

Craig A. Townsend*† and Siegfried B. Christensen

Department of Chemistry
The Johns Hopkins University
Baltimore, Maryland 21218

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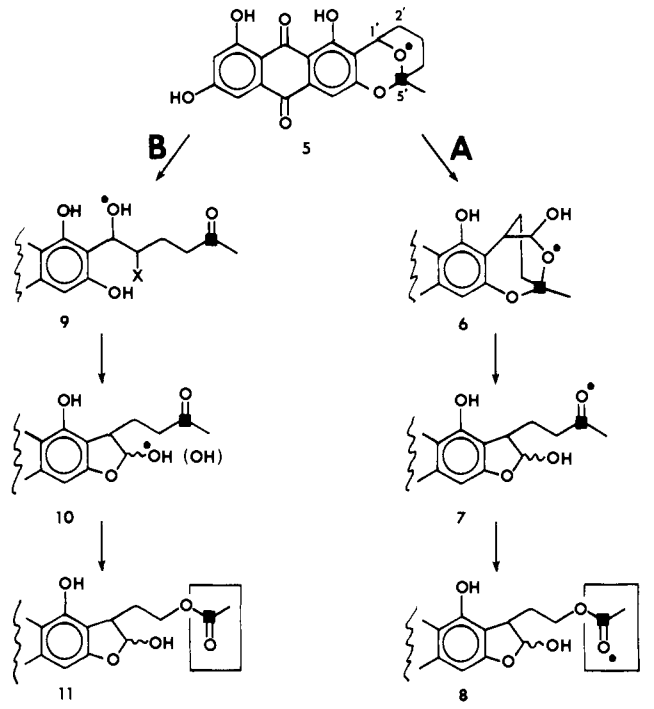
Experiments with specifically labeled specimens of averufin (**1**) have shown its intact incorporation into aflatoxin B_1 (**2**).[1-3] In particular racemic [1′-^{2}H,^{13}C]averufin (**1**) was found to label C-13 in **2** without detectable loss of deuterium relative to ^{13}C internal standard, despite a net change in oxidation state at this carbon. This observation was interpreted[4] as suggesting a pinacol-like rearrangement involving migration of the anthraquinone nucleus to C-2′ in nidurufin (**3**) with departure of the 2′-hydroxyl whose exo orientation[3,5] would particularly favor rearrangement on stereoelectronic grounds (Scheme I, path A). In this paper we examine the intermediacy of nidurufin (**3**) and its 2′-epimer pseudonidurufin (**4**) and, while both fail to give detectable levels of incorporation into aflatoxin B_1 (**2**), further evidence is presented to support important limiting features of biogenetic Scheme IA.

Scheme I



Racemic [1′-^{2}H]nidurufin (**3**) and [1′-^{2}H]pseudonidurufin (**4**) were prepared[3-5] by extension of previously developed methods[2,6] (both >98% d_1). When administered to mycelial suspensions of *Aspergillus parasiticus* (ATCC 15517) under conditions where averufin (**1**) gave >20% specific incorporation,[2] **3** and **4** gave no detectable incorporation into **2** (mass spectrum). Impermeability may be excluded as the mycelial pellets turn perceptibly from white to yellow with orange centers within 3 h of exposure to the labeled anthraquinones. However, unlike averufin (**1**), after an additional 3 h the medium gradually became deeper yellow in color as the administered **3** and **4** were excreted as polar, highly water-soluble conjugates.[7] This disappointing outcome was further tested using FLUFF, a variant of *A. parasiticus* isolated by Bennett,[8] which produces at most only a trace of aflatoxin and appears to be blocked before the anthraquinone stage of the pathway. Parallel experiments revealed that while added averufin (**1**) supported markedly enhanced aflatoxin production, the two hydroxylated derivatives **3** and **4** did not.[9]

Having now to exclude nidurufin (**3**) and pseudonidurufin (**4**) as efficient precursors of aflatoxin B_1 (**2**), consideration of how the side chain of averufin itself might be transformed into the bisfuran leads to a mechanistic distinction that is accessible to experimental test (Scheme I). Path A, invoking oxidation at C-2′ in averufin but not hydroxylation to nidurufin, would generate a reactive intermediate which itself may rearrange to **6**, collapse to **7**, and finally, upon Baeyer-Villiger-like reaction, yield versiconal acetate (**8**).[10] If in averufin (**5**) the 1′-oxygen were labeled

† Research Fellow of the Alfred P. Sloan Foundation 1982-1986; Camille and Henry Dreyfus Teacher-Scholar 1983-1988.

(1) Simpson, T. J.; deJesus, A. E.; Steyn, P. S.; Vleggaar, *J. Chem. Soc., Chem. Commun.* **1982**, 631-632.

(2) Townsend, C. A.; Christensen, S. B.; Davis, S. G. *J. Am. Chem. Soc.* **1982**, *104*, 6252-6153. Townsend, C. A.; Davis, S. G. *J. Chem. Soc., Chem. Commun.* **1982**, 1420-1422.

(3) The absolute configuration of averufin (**1**) is as shown and by inference (ORD/CD)[5] that of nidurufin (**3**) (unpublished results, these laboratories in collaboration with Professor M. Koreeda, University of Michigan). The relative configurations of nidurufin (**3**) and pseudonidurufin (**4**) have been unambiguously established by total synthesis (Townsend, C. A.; Christensen, S. B., ref 4).[5]

(4) Townsend, C. A.; Christensen, S. B. *Tetrahedron* **1983**, *39*, 3575-3582. For a somewhat different proposal, see: Sankawa, Y.; Shimada, H.; Kobayashi, T.; Ebizuka, Y.; Yamamoto, Y.; Noguchi, H.; Seto, H. *Heterocycles* **1982**, *19*, 1053-1058.

(5) The relative configuration of nidurufin was incorrectly assigned endo in the original isolation from *A. nidulans*: Aucamp, P. J.; Holzapfel, C. W. *J. S. Afr. Chem. Inst.* **1970**, *23*, 40-56. See also the isolation of 6,8-di-*O*-methylnidurufin: Kingston, D. G. I.; Chen, P. N., Vercellotti, J. R. *Phytochemistry* **1976**, *15*, 1037-1039.

(6) Townsend, C. A.; Davis, S. G.; Christensen, S. B.; Link, J. C.; Lewis, C. P. *J. Am. Chem. Soc.* **1981**, *103*, 6885-6888. Townsend, C. A.; Bloom, L. M. *Tetrahedron Lett.* **1981**, 3923-3924.

(7) 48 h after administration of the anthraquinones, the medium had become bright yellow in the case of nidurufin and somewhat less intensely yellow for pseudonidurufin. Neither pigment could be extracted into organic solvent, but on standing in aqueous solution over 3 weeks, the conjugates had largely decomposed to cleanly return the respective labeled anthraquinones.

(8) Bennett, J. W.; Silverstein, R. B.; Kruger, S. J. *J. Am. Oil Chem. Soc.* **1981**, *58*, 952A-955A.

(9) The failure of the "diol" oxidation state to function in the masked pinacol rearrangement is reminiscent of the nonconversion of *ent*-6α,7α-dihydroxykaurenoic acid to GA_{12}-aldehyde in gibberellin biosynthesis: Hanson, J. R.; Hawker, J.; White, A. F. *J. Chem. Soc., Perkin Trans. 1* **1972**, 1892-1895. Graebe, J. E.; Hedden, P.; MacMillan, J. *J. Chem. Soc., Chem. Commun.* **1975**, 161-162.

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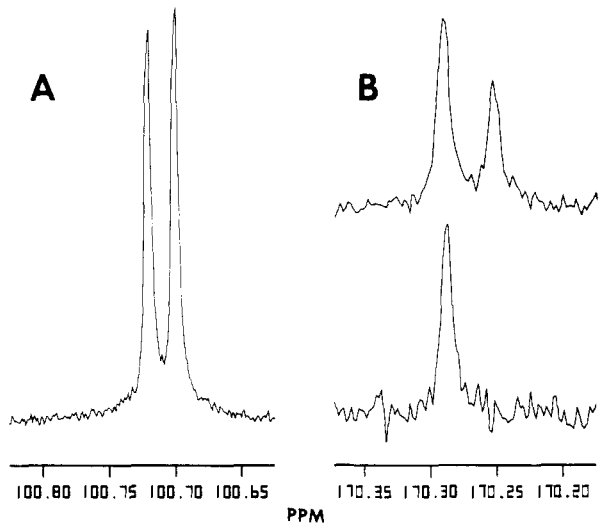


Figure 1. 50.3-MHz $^{13}C\{^1H\}$ NMR spectra recorded on a Varian XL-200 as noted (A) (±)-[1′-^{18}O,5′-^{13}C]averufin (**5**, 17 mg in 2.5 mL of 1:1 Me_2SO-d_6/$CDCl_3$) sweep width 200 Hz, 4000 points, 32 transients, zero filling. (B) Top: [5′-^{13}C,^{18}O]versiconal acetate (**8**, 8.5 mg in 2.5 mL of Me_2SO-d_6), sweep width 1600 Hz, 16 000 points, 12 896 transients, line broadening 0.25 Hz, zero filling. Bottom: versiconal acetate (natural abundance, 34 mg in 2.5 mL of Me_2SO-d_6), acquisition parameters as above, 3136 transients.

with ^{18}O (●) and C-5′ with ^{13}C (■) and, further, if oxygen exchange of the proposed methyl ketone intermediate **7** were not too rapid, a heavy isotope at the carbonyl oxygen of **8** would be expected to be revealed in the upfield shift[11] of the 5′-carbon in its $^{13}C\{^1H\}$ NMR spectrum.[12] In contrast to this highly restrictive criterion, a large number of other mechanistic possibilities may be represented by path B which, as a group, are distinguished by hydrolytic opening of the averufin ketal and hence cleavage of the 1′-oxygen/5′-carbon bond with ^{18}O label being lost rapidly in **11**.

Therefore, 2,6-bis(methoxymethyl)benzaldehyde was exchanged with ^{18}O water, elaborated to (±)-[1′-^{18}O,5′-^{13}C]averufin (**5**) as before[2,6] and administered to cultures of *A. parasiticus* (ATCC 15517) in the presence of dichlorovos.[10] Figure 1A depicts a portion of the 50.3-MHz $^{13}C\{^1H\}$NMR spectrum of averufin (**5**) indicating about a 48/52 $^{16}O/^{18}O$ ratio at C-5′ as revealed in the 0.02 ppm ^{18}O-induced upfield shift.[11] Examination of the analogous spectrum (Figure 1B, top) of the isolated versiconal acetate (**8**) showed an upfield shift of the C-5′ resonance of 0.04 ppm consistent with the location of heavy isotope at the carbonyl oxygen,[11] as hoped. When normalized for natural abundance contributions (Figure 1B, bottom), it could be shown that approximately 80% of the ^{18}O label originally present in averufin (**5**) survived in the derived versiconal acetate (**8**).

In conclusion, neither nidurufin (**3**) nor pseudonidurufin (**4**) under two distinct feeding regimens serves as an effective precursor of aflatoxin B_1. However, the established facile and intact incorporation of averufin into aflatoxin[1,2] and versiconal acetate[10] is further refined herein to require that the side-chain branching and cleavage steps proceed in such a way that the 1′-oxygen/5′-carbon bond remains intact to versiconal acetate. Scheme 1A is one of a very narrow spectrum of possibilities that satisfies this mechanistic stricture.[14]

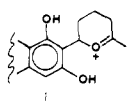
Acknowledgment. We are deeply indebted to Dr. S. L. Pratt (Varian Instrument Group) for running the spectra shown in Figure 1, to Professor J. W. Bennett (Tulane) for providing advice and laboratory space for the microbiological portion of this work, and to C. A. Lisek and the NSF-supported Middle Atlantic Mass Spectrometry Laboratory at Hopkins and J. B. Stanley (Southern Regional Research Center, USDA) for mass spectra. We are pleased to acknowledge the financial support of the NIH (ES01670) and of the A. P. Sloan Foundation.

Note Added in Proof. Very recent relative stereochemical assignments of nidurufin and pseudonidurufin, based on model systems,[13] confirm those made earlier for the natural product and its 2′-epimer from unambiguous total synthesis.[3-5] While we concur that π-participation in the chain-branching reaction is possible,[11,14] the details of the mechanism proposed by these authors must be revised in light of the findings presented herein.

(10) The *O*-acetyl in versiconal acetate is known to be derived by strictly intramolecular oxidative rearrangement of the terminal two carbons of the averufin side chain: Townsend, C. A.; Christensen, S. B.; Davis, S. G. *J. Am. Chem. Soc.* **1982**, *104*, 6154-6155.

(11) Vederas, J. C. *J. Am. Chem. Soc.* **1980**, *102*, 374-376. Moore, R. N.; Diakur, J.; Nakashima, T. T.; McLaren, S. L.; Vederas, J. C. *J. Chem. Soc., Chem. Commun.* **1981**, 501-502.

(12) Net retention of the 1′-oxygen/5′-carbon bond could result from radical or cation formation and migration of the anthraquinone nucleus either directly or through π participation and spiro cyclopropane formation (see: Murphy, W. S.; Wattanasin, S. *Chem. Soc. Rev.* **1983**, *12*, 213-250). Alternative fates of an intermediate as i also may be visualized.



(13) Murphy, R. A., Jr.; Cava, M. P. *J. Am. Chem. Soc.* **1984**, *106*, 7630-7632.

(14) Townsend, C. A.; Davis, S. G.; Koreeda, M.; Hulin, B., unpublished results. A summary of these studies was made at the 188th ACS National Meeting, Philadelphia, PA, August, 1984, ORGN 211.

Solid-State Conformations of Linear Oligopeptides and Aliphatic Amides. A Model of Chiral Perturbation of a Conjugated System

Andrzej Stanislaw Cieplak

Department of Chemistry
Fordham University
Bronx, New York 10458

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The out-of-plane distortions of an amide bond may result from two kinds of torsion, and are correspondingly described by the angles τ, $\theta_{C'}$, and θ_N, where the latter two reflect the degree of pyramidalization of the C′ and N atoms[1] (see Scheme I). Such a pyramidalization does not require large energy expenditure[2] and is in fact often observed in the solid state.[3] Since a nonplanar amide bond is chiral, introduction of chirality in the C_i^α and C_{i+1}^α allylic rotors could in principle affect the magnitude and sign of the $\theta_{C'}$ and θ_N values. Furthermore, it appears that an amide bond interacts differently with the two allylic rotors C_i^α and C_{i+1}^α; as shown by ^{13}C NMR studies, there is an electron density shift into the C_i^α-H bond overlapping the $2\pi_\pi$ system, but this is not seen in the case of the C_{i+1}^α-H bond.[4] Therefore aliphatic amides present an interesting and convenient model for studying the effect

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