Table II

solvent	diene	irrad time, min	% diene (EE)-1	(EZ)-2	(ZZ)-3
acetone-d_6	1	35	100.0	0.0	0.0
	2	35	2.9	95.4	1.7
	3	35	0.0	4.6	95.4
THF-d_8	1	60	100.0	0.0	0.0
	2	60	0.0	100.0	0.0
	3	60	0.0	4.5	95.5

hindered dioxetane products (the "cis alkoxy" effect[15]) in the more polar media[16] are indicative of 1,4-zwitterion formation. As the medium becomes less polar the character of the intermediate shifts its position on the continuum toward the 1,4-diradical extreme. Diagnostic of this mobility on the continuum is the decreasing predominance of the cis dioxetanes and finally reversion of the intermediate to form the stereochemically scrambled diene.

The rapid loss of oxygen from the diradical intermediate in comparison to the zwitterionic intermediate is most likely related to the ease of intersystem crossing[17] in the former species. Decomposition of a triplet intermediate to form triplet oxygen and singlet ground state diene is 22.5 kcal/mol more exothermic than decomposition of a singlet to form two singlet products.

Studies to examine these diradical intermediates and their formation in other singlet oxygen reactions is currently under way.

Experimental Section

Preparative gas chromatographic separations were carried out on a Varian Aerograph 90-P utilizing a 0.25 in. by 20 ft column packed with 20% Carbowax 20M on Chromosorb W. Proton NMR spectra and integrations were obtained for dienes 1-3 and their reaction mixtures with a Jeol FX270 at 270 MHz, and the chemical shifts were referenced to Me_4Si. Acetone-d_6 (Aldrich) was distilled from $CaSO_4$ under a N_2 atmosphere and stored over 4A molecular sieves. Methylene chloride-d_2 (Aldrich) was filtered through activity 1 basic alumina prior to use. Acetonitrile-d_3 was obtained from Aldrich and used without further purification.

(EE)-, (EZ)-, and (ZZ)-1,4-Di-*tert*-butoxy-1,3-butadiene (1-3). All three dienes were synthesized by the method of Hiranuma and Miller[2] and purified by preparative gas chromatography. ^1H NMR (acetone-d_6): 1, δ 6.39 (dd, J = 8.1, 2.9 Hz, 2 H), 5.45 (dd, J = 8.1, 2.9 Hz, 2 H), 1.18 (s, 18 H); 2, δ 6.54 (d, J = 12.4 Hz, 1 H), 6.02 (d, J = 6.6 Hz, 1 H), 5.79 (dd, J = 12.4, 11.0 Hz, 1 H), 4.94 (dd, J = 11.0, 6.6 Hz, 1 H), 1.21 (s, 9 H), 1.20 (s, 9 H); 3, δ 6.09 (dd, J = 3.2, 1.5 Hz, 2 H), 5.33 (dd, J = 3.2, 1.5 Hz, 2 H), 1.22 (s, 18 H). ^{13}C NMR (acetone-d_6): 1, δ 141.4, 107.6, 76.4, 28.4; 2, δ 142.4, 137.0, 104.9, 104.6, 76.3, 76.1, 28.1; 3, δ 138.0, 101.9, 76.2, 28.1.

Isomerization Studies. The extent of isomerization was determined in acetone-d_6 and THF-d_8 solutions. A solution that was 0.014-0.03 M in diene and 10^{-5} M in rose bengal was saturated with nitrogen (acetone-d_6) or argon (THF-d_8) for 30-60 min while being protected from room lights. The sample was then irradiated for 35-60 min with continuous bubbling of the inert gas. The extent of isomerization was determined by integration of the NMR spectra (Table II).

Photooxidation Conditions. To a 1-mL volumetric flask were added 2-4 mg (0.01-0.02 mol) of the diene and 10 μL of a 10^{-3} M sensitizer solution, and the resultant mixture was diluted to volume with the NMR solvent. A portion of this solution (0.5-0.7 mL) was pipeted into a 5-mm NMR tube and saturated with oxygen for 25 min at -76 °C while being protected from the room lights. The acetone-d_6 solutions were irradiated through a 0.5% $K_2Cr_2O_7$ filter solution and the THF-d_8 and CD_2Cl_2 through a $NaNO_2$ (75 g in 100 mL of water) filter. Rose bengal was used as a sensitizer in all reactions containing acetone-d_6 and THF-d_8 and TPP in all reactions with pure CD_2Cl_2.

Acknowledgment. We thank the National Science Foundation (Grant CHE-8418603) and the Petroleum Research Foundation, administered by the American Chemical Society, for their generous support of this research.

(15) Houk, K. N.; Moses, S. R.; Wu, Y.-D.; Rondan, N. G.; Jager, J.; Schohe, R.; Fronczek, F. R. *J. Am. Chem. Soc.* **1984**, *106*, 3880.
(16) E_T(acetone) = 42.2; $E_T(CH_2Cl_2)$ = 41.1; E_T(THF) = 37.4.
(17) (a) Scaiano, J. C.; Lissi, E. A.; Enciana, M. V. *Rev. Chem. Int.* **1978**, *2*, 139. (b) Caldwell, R. A.; Majima, T.; Pac, C. *J. Am. Chem. Soc.* **1982**, *104*, 629.

Communications

Synthesis and Absolute Configuration of (+)-Averufin

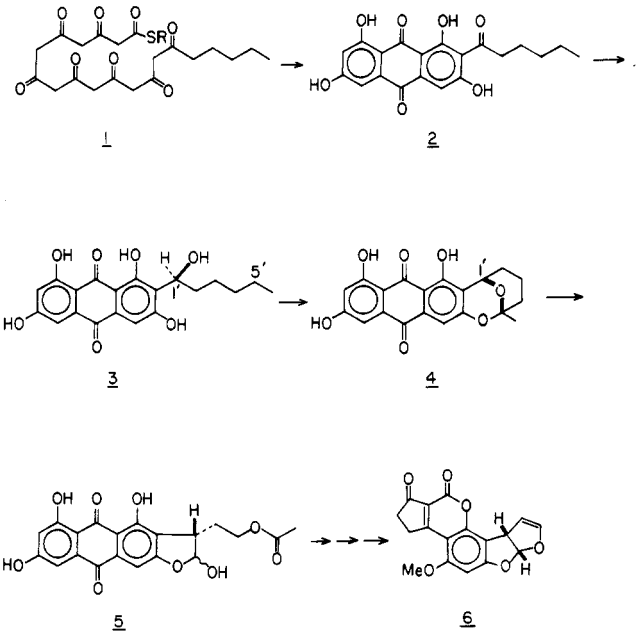
Summary: The absolute configuration of (+)-averufin, a key intermediate in aflatoxin biosynthesis, has been determined to be 1′-*S* by total synthesis from an intermediate whose absolute stereochemistry was established by application of the exciton chirality circular dichroism method.

Sir: Extensive radiochemical experiments with *Aspergillus parasiticus* mutants[1] and the demonstration of a shared polyketide folding pattern[2] have established a sequence of C_{20}-anthraquinone intermediates in the aflatoxin B_1 (**6**) biosynthetic pathway (Scheme I). Notably, hexanoic acid has been found to initiate[3] assembly of the hypothetical intermediate **1** which, on self-condensation and oxidation, affords norsolorinic acid (**2**). Reduction of **2** gives averantin (**3**), whose single asymmetric center[4] has been proposed to prefigure the stereochemical outcome of all subsequent transformations involving the C_6-side chain.[5] Oxidation at C-5′ of **3** and internal ketalization generates averufin (**4**), the key advanced intermediate of the aflatoxin path-

(1) Hsieh, D. P. H.; Lin, M. T.; Yao, R. C.; Singh, R. *J. Agric. Food Chem.* **1976**, *24*, 1170-1174. Singh, R.; Hsieh, D. P. H. *Arch. Biochem. Biophys.* **1977**, *178*, 285-292. Hsieh, D. P. H.; Singh, R.; Yao, R. O.; Bennett, J. W. *App. Environ. Microbiol.* **1978**, *35*, 980-985. Bennett, J. W.; Lee, L. S.; Shoss, S. M.; Boudreaux, G. H. *Ibid.* **1980**, *39*, 835-839. For a review, see: Heathcote, J. G.; Hibbert, J. R. "Aflatoxins: Chemical and Biological Aspects"; Elsevier: Oxford, 1978; pp. 151-172.
(2) Steyn, P. S.; Vleggaar, R.; Wessels, P. L. In "The Biosynthesis of Mycotoxins: A Study in Secondary Metabolism"; Steyn, P. S., Ed.; Academic Press: London, 1980; pp. 105-155 and references cited therein.
(3) Townsend, C. A.; Christensen, S. B.; Trautwein, K. *J. Am. Chem. Soc.* **1984**, *106*, 3868-3869.
(4) Townsend, C. A.; Christensen, S. B., unpublished results.
(5) Townsend, C. A.; Christensen, S. B. *Tetrahedron* **1983**, *39*, 3575-3582. For a similar proposal, see: Sankawa, Y.; Shimada, H.; Kobayashi, T.; Ebizuka, Y.; Yamamoto, Y.; Noguchi, H.; Seto, H. *Heterocycles* **1982**, *19*, 1053-1058.

0022-3263/85/1950-5426$01.50/0

Scheme I

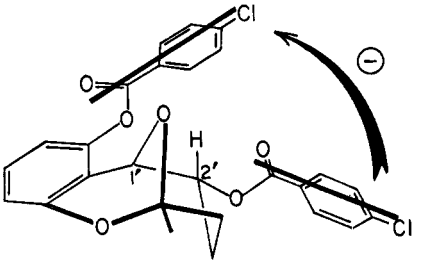


way,[5,6] which undergoes oxidative chain-branching and Baeyer–Villiger-like reaction[7] to give versiconal acetate (**5**) and ultimately aflatoxin B_1 (**6**). The configuration of the bisfuran characteristic of this family of mycotoxins was secured by chemical degradation to (*S*)-(+)-2-methylbutanoic acid.[8] In this paper, we record experiments which establish the absolute configuration of averufin (**4**) as identical with that required by recent biogenetic considerations.[5]

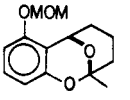
A series of attempts to isolate the averufin side chain by chemical degradation for the purpose of a stereochemical correlation by classical means failed owing to ready epimerization at the benzylic carbon. Therefore, the known[9] aldehyde **7** was converted through Wittig–Schlosser[10] and lithium-free Wittig conditions[11] with the requisite C_5-phosphorane[12] to the (*E*)- and (*Z*)-olefins **8** and **10**, respectively (Scheme II). The modified Wittig–Schlosser condensation was stereoselective (*E*/*Z*, 20:1) while the lithium-free reaction gave a 1:5 *E*/*Z* mixture of olefins that could be conveniently separated by flash column chromatography.[13] Cis diol formation[14] followed by treatment with dilute mineral acid resulted in selective removal of one of the methoxymethyl (MOM) protecting groups[9] and spontaneous ketalization, providing the corresponding racemic endo or exo alcohol, **9** or **11**, in the complete absence of possible crossover products from epimerization at the benzylic carbon.

Optical resolution of the endo alcohol **9** was conveniently achieved through conversion to its (−)-α-methoxy-α-(trifluoromethyl)phenylacetic (MTPA) esters and separation of the resulting diastereoisomers initially by preparative HPLC (Waters Prep-500) and subsequently by fractional recrystallization from hexanes/ether. Saponification of the higher (mp 109.5–110 °C, less polar) and lower (mp 83–84 °C, more polar) melting esters afforded the antipodes of **9**, enantiomer A, $[\alpha]^{23}_D$ +2.0° ($CHCl_3$, *c* 1.64), and enantiomer B, $[\alpha]^{23}_D$ −2.1° ($CHCl_3$, *c* 1.51), respectively. The optical purities of A and B were estimated to be over 98% based on the diastereoisomeric purity of their corresponding MTPA esters as judged by 360-MHz proton NMR. Removal of the MOM protecting group of enantiomer A gave the diol which was in turn converted to its bis-*p*-chlorobenzoate **12**, mp 122–124 °C, $[\alpha]^{25}_D$ −137.6° ($CHCl_3$, *c* 0.10). The circular dichroism spectrum of **12** showed a pair of strong Cotton effects with opposite signs ($\Delta\epsilon_{252.5}$ −49.5, $\Delta\epsilon_{234.5}$ +23.8 in MeOH–dioxane, 9/1). The negative, longer wavelength Cotton effect defines a negative chirality between the two (*p*-chlorobenzoyl)oxy chromophores, thus providing the absolute configuration of the dibenzoate as shown in **12**.[15]

Enantiomer A, having the absolute configuration shown in **9**, was converted to its phenyl thionocarbonate derivative and reduced with tri-*n*-butyltin hydride[16] to afford the (*S*)-(−)-tricyclic ketal **13**, $[\alpha]^{23}_D$ −55.0° ($CHCl_3$, *c* 0.690) in 56% overall yield. By previously established procedures[9] **13** was converted to a sample of (*S*)-(+)-averufin (**4**), whose spectral and chromatographic properties were identical with natural material obtained from fermentation and whose small but positive optical rotation, +1 < $[\alpha]^{23}_D$ < +2° (EtOH, *c* 0.80), corresponded to those reported for the natural product (lit. +1°,[17] +2°[18]).



12



(−)-**13**

In conclusion, from a resolved intermediate whose absolute configuration was unambiguously defined by non-

(6) Townsend, C. A.; Christensen, S. B.; Davis, S. G. *J. Am. Chem. Soc.* **1982**, *104*, 6152–6153. Simpson, T. J.; deJesus, A. E.; Steyn, P. S.; Vleggaar, R. *J. Chem. Soc., Chem. Commun.* **1982**, 631–632. Townsend, C. A.; Davis, S. G. *Ibid.* **1983**, 1420–1422.

(7) Townsend, C. A.; Christensen, S. B.; Davis, S. G. *J. Am. Chem. Soc.* **1982**, *104*, 6154–6155.

(8) Brechbühler, S.; Büchi, G.; Milne, G. *J. Org. Chem.* **1967**, *32*, 2641–2642.

(9) Townsend, C. A.; Davis, S. G.; Christensen, S. B.; Link, J. C.; Lewis, C. P. *J. Am. Chem. Soc.* **1981**, *103*, 6805–6888.

(10) Schlosser, M.; Christmann, K. F. *Angew. Chem., Int. Ed. Engl.* **1966**, *5*, 126. Schlosser, M.; Christmann, K. F.; Piskala, A. *Chem. Ber.* **1970**, *103*, 2814–2820.

(11) Sreekunar, C.; Darst, K. P.; Still, W. C. *J. Org. Chem.* **1980**, *45*, 4260–4262.

(12) Prepared in situ from the corresponding phosphonium bromide which was in turn synthesized from 2-acetylbutyrolactone in three steps in overall ca. 30% yield [i. HBr/H_2O, reflux, ii. $(CH_2OH)_2$, *p*-TsOH, benzene, reflux, iii. Ph_3P, xylenes, 130 °C, 6 days].

(13) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923–2925.

(14) VanRheenan, V.; Kelly, R. C.; Cha, D. Y. *Tetrahedron Lett.* **1976**, 1973–1976.

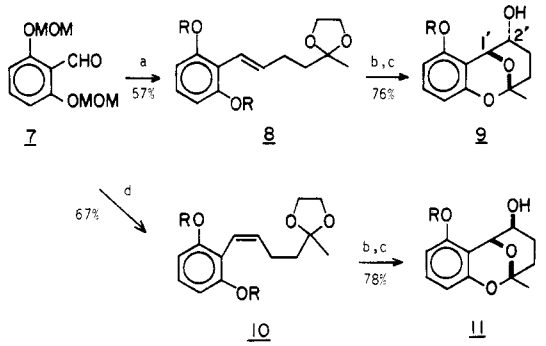
(15) Harada, N.; Nakanishi, K. *J. Am. Chem. Soc.* **1969**, *91*, 3989–3991. Harada, N.; Nakanishi, K. *Acc. Chem. Res.* **1972**, *5*, 257–263. Harada, N.; Nakanishi, K. "Circular Dichroic Spectroscopy–Exciton Coupling in Organic Stereochemistry"; University Science Books: Mill Valley, CA, 1983.

(16) Barton, D. H. R.; McCombie, S. W. *J. Chem. Soc., Perkin Trans. 1* **1975**, 1574–1585. Barton, D. H. R.; Subramanian, R. J. *Ibid.* **1977**, 1718–1723.

(17) Pusey, D. F. G.; Roberts, J. C. *J. Chem. Soc.* **1963**, 3542–3547.

(18) Aucamp, P. J.; Holzapfel, C. W. *J. S. Afr. Chem. Inst.* **1970**, *23*, 40–56.

Scheme II[a,b]



[a] R = MOM. [b] Conditions: (a) $Ph_3P^+(CH_2)_3C(OCH_2CH_2O)(CH_3)$, Br^- (i)/LDA (1 equiv)/THF, 0 °C; addition of **7**, −78 °C, 30 min; LDA (1 equiv), −40 °C → −30 °C, 1 h; MeOH (excess), −30 °C → room temperature, overnight; (b) OsO_4, *N*-methylmorpholine *N*-oxide/THF/H_2O, room temperature, overnight; (c) aqueous 1 N HCl/THF, room temperature, 40 h; (d) phosphonium bromide i, $KN(SiMe_3)_2$ (1 equiv)/THF, room temperature, 20 min; addition of **7** at −78 °C, 10 min; −78 °C → room temperature, overnight.

empirical means,[15] a sample of optically active averufin has been synthesized and thereby the 1′*S* configuration assigned to the natural product. This configuration is in accord with that predicted on the basis of a biogenetic proposal[5] relating the linear side chain of averufin through a chain-branching step to generate ultimately the bisfuran of aflatoxin in the correct absolute stereochemical sense.

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Registry No. **4**, 14016-29-6; **7**, 79834-12-1; **8**, 98634-98-1; (±)-**8** (glycol), 98635-00-8; **9** (R = H), 98635-06-4; **9** (phenylthionocarbonate), 98635-05-3; (±)-**9**, 98635-02-0; (+)-**9**, 98675-11-7; (+)-**9** (MTPA ester), 98635-03-1; (−)-**9**, 98675-12-8; (−)-**9** (MTPA ester), 98717-30-7; **10**, 98634-99-2; (±)-**10** (glycol), 98635-01-9; (±)-**11**, 98675-10-6; **12**, 98635-04-2; **13**, 98675-13-9; i, 5944-33-2; $Br(CH_2)_3COCH_3$, 3884-71-7; (±)-3-acetyldihydro-2(3*H*)-furanone, 98634-97-0; 2-methyl-2-(3-bromopropyl)-1,3-dioxolane, 24400-75-7.

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A Cationic Model of the Chain-Branching Step in Aflatoxin Biosynthesis

Summary: The 2′-mesylates of 6,8-di-*O*-methylnidurufin and 6,8-di-*O*-dimethylpseudonidurufin and simple benzenoid models of these compounds have been treated in 2,2,2-trifluoroethanol to give furanoid products of potential relevance to aflatoxin B_1 biosynthesis.

Sir: Initiation of averufin (**1**, R = H) biosynthesis by hexanoic acid necessitates a sequence of otherwise seemingly redundant redox steps in its formation.[1] In so doing, however, a template is generated whose latent chemistry is revealed in a series of oxidative rearrangement reactions that lead ultimately to the unusual bisfuran of the potent mycotoxin aflatoxin B_1 (**6**). Fermentive incorporation studies of aflatoxin using specifically labeled samples of averufin[2] have led to the proposal that a pinacol-like rearrangement may be initiated by oxidation at C-2′ in **1** (R = H) to achieve the side-chain branching and redox changes required for bisfuran formation[3] (Scheme I). Against expectation, however, nidurufin (**1**, R = OH)[4] and its 2′-epimer, pseudonidurufin, failed to incorporate label into aflatoxin B_1 (**6**) under conditions where averufin (**1**, R = H) was efficiently utilized.[2] In noteworthy juxtaposition, however, [1′-^{18}O,5′-^{13}C]averufin was converted in vivo into versiconal acetate (**5**) with ca. 80% of the ^{18}O-label (●) found at the carbonyl oxygen bound to C-5′ (■). This boundary condition and consideration of stereoelectronic effects,[4] which are supported by the absolute configuration of averufin,[5] in one of a limited number of mechanistic interpretations may argue for direct oxidation at C-2′ and rearrangement through the closed form of the ketal side chain.[4] Of several interesting possibilities that can be advanced for the detailed course of such a transformation, one is fundamentally cationic and may be approximated by the reactions described herein.

The racemic *exo*- and *endo*-mesylates **7** and **8**, respectively (Table I), were easily prepared from the corresponding 2′-alcohols, which were in turn available as described in the accompanying paper.[5] While the attempted rearrangement of **7** and **8** in dipolar aprotic solvents under a variety of conditions led to complex mixtures of products, the use of 2,2,2-trifluoroethanol (TFE)[6] met with encouraging results. The *exo*-mesylate **7**, analogous in its relative configuration to nidurufin (**1**, R = OH) as correctly constituted,[7] when heated to reflux in TFE, rearranged in 10 min to benzofuran **7a** in 50% yield (Table I, entry 1). Buffering the reaction mixture with sodium bicarbonate or triethylamine resulted in the formation of **7b** and **7c**, mixed acetals/ketals of TFE (entries 2 and 3). In marked contrast, the endo isomer **8** failed to react under the same conditions. The deoxy analogue of **7**, compound **9**, was prepared by a similar route from salicylaldehyde and found to rearrange in like fashion (entries 6 and 7).

To more directly mimic the biogenetic hypothesis illustrated in Scheme I, close models of nidurufin (**1**, R = OH) and pseudonidurufin, its 2′-epimer, were synthesized by modification of the route used earlier.[4,7] 5,7-Dimeth-

(1) Townsend, C. A.; Christensen, S. B.; Trautwein, K. *J. Am. Chem. Soc.* **1984**, *106*, 3868–3869.
(2) Townsend, C. A.; Christensen, S. B.; Davis, S. G. *J. Am. Chem. Soc.* **1982**, *104*, 6152–6153. Simpson, T. J.; deJesus, A. E.; Vleggaar, R.; Steyn, P. S. *J. Chem. Soc., Chem. Commun.* **1982**, 631–632. Townsend, C. A.; Davis, S. G. *Ibid.* **1983**, 1420–1421.
(3) Townsend, C. A.; Christensen, S. B. *Tetrahedron* **1983**, *39*, 3575–3582. For an independent related proposal, see: Sankawa, Y.; Shimada, H.; Kobayashi, T.; Ebizuka, Y.; Yamamoto, Y.; Noguchi, H.; Seto, H. *Heterocycles* **1982**, *19*, 1053–1058.
(4) Townsend, C. A.; Christensen, S. B. *J. Am. Chem. Soc.* **1985**, *107*, 270–271.
(5) Koreeda, M.; Hulin, B.; Yoshihara, M.; Townsend, C. A.; Christensen, S. B. *J. Org. Chem.*, previous communication in this issue.
(6) Shiner, V. J., Jr.; Dowd, W.; Fisher, R. D.; Hartshorn, S. R.; Kessick, M. A.; Milakofsky, L.; Rapp, M. W. *J. Am. Chem. Soc.* **1969**, *91*, 4838–4843. Schadt, F. L.; Bentley, T. W.; Schleyer, P. v. R. *Ibid.* **1976**, *98*, 7667–7674. Johnson, W. S.; DuBois, G. E. *Ibid.* **1976**, *98*, 1038–1039. Johnson, W. S.; Escher, S.; Metcalf, B. W. *Ibid.* **1976**, *98*, 1039–1041. Creary, X.; Geiger, C. C. *Ibid.* **1983**, *105*, 7123–7129.
(7) For a discussion of the correct structure of nidurufin, see ref 4 and the first citation in ref 3.

0022-3263/85/1950-5428$01.50/0