Table I. [13]C Chemical Shifts of Cefoxitin (**1a**) and Its Ammonolysis Products

carbon atom	1a	2a[a]	3a[a]
C_2	26.05	28.39	30.04
C_3	114.30	101.74	131.33
C_4	134.05	139.60	172.09
C_6	63.42	58.36	67.21
C_7	95.43	89.38	87.73
C_8	160.72	171.32	171.50
3-CH_2	64.59	66.63	123.25
carbamate	159.16	159.93	168.39
4-CO_2^-	166.26	169.67	165.66
OCH_3	53.30	51.84	52.33
7-NHCOR	172.88	171.32	171.13
thienyl CH_2	37.24	37.24	37.05
thienyl C_2	138.04	138.62	138.62
thienyl C_3 } thienyl C_4	127.74, 127.91	not observed	127.55, 127.83
thienyl C_5	126.27	126.09	126.27

[a] Numbering for **2a** and **3a** follows that of cefoxitin.

Table II. [13]C Chemical Shifts of Cephamycin C (**1b**) and Its Ammonolysis Products

carbon atom	1b	2b[a]	3b[a]
C_2	~26.0		~30.0
C_3	113.90	101.43	131.31
C_4	134.04	139.64	172.37
C_6	63.31	58.34	67.29
C_7	95.30	88.88	87.34
C_8	160.90	171.31	171.49
3-CH_2	64.66	66.52	123.23
carbamate	159.13	159.82	168.37
4-CO_2^-	166.32	169.75	165.76
OCH_3	53.19	51.63	52.30
7-NHCOR	176.64	174.81	174.81
C_δ	36.2	36.2	36.2
C_γ	23.22	23.22	23.22
C_β	36.2	36.2	36.2
C_α	56.88	56.88	56.88
CO_2^-	181.51	181.51	181.51

[a] Numbering for **2b** and **3b** follows that of cephamycin C.

cephamycin C (**1b**) is treated similarly (Table II).

Analysis of the carbon-13 intensities of the methoxyl groups of species **1–3** affords approximate rates of β-lactam opening and side-chain expulsion. Although proton-decoupled carbon-13 FTNMR spectra may exhibit systematic departures from quantitative accuracy due to differences in relaxation rates and Overhauser enhancements,[3] use of the methoxyl signals as measures of relative concentrations minimizes such factors.

Intensity data for each cephamycin were analyzed by standard kinetics techniques[6] and were found consistent with a reaction having two consecutive, first-order steps. The following rate constant values were calculated: $k_{1a} = 0.00036 \pm 0.00004$ s^{-1}, $k_{1b} = 0.00018 \pm 0.00002$ s^{-1}, and $k_{2a} = k_{2b} = k_{1a}$. A weak effect of the 7-acyl group on the rate of β-lactam cleavage was noted in that k_{1a} was twice k_{1b}.

In summary, we have characterized the intermediates of cephamycin ammonolysis and confirmed that such can occur via a two-step process. Although anhydrous ammonia is unlike physiological media, it does represent solvolysis in a dipolar, protic medium in which all species can be observed. This provides a unique means for gaining insight into β-lactam chemistry not possible or recognized previously.[7,8]

(6) Frost, A. A.; Pearson, R. G. "Kinetics and Mechanism"; Wiley: New York, 1958; Chapter 8.

(7) Hamilton-Miller, J. M. T.; Richards, E.; Abraham, E. P. *Biochem. J.* **1970**, *116*, 385.

(8) Schanck, A.; Coene, B.; Dereppe, J. M.; Van Meerssche, M. *Bull. Soc. Chim. Belg.* **1983**, *92*, 81.

# On the Antarafacial Stereochemistry of the Thermal [1,7]-Sigmatropic Hydrogen Shift

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Received August 17, 1984

The classical thermal [1,7]-sigmatropic hydrogen migration[1] is considered to be a pivotal event in the metabolic production of vitamin D.[2] Although the intramolecular nature of this thermal process has been established,[3,4] and the stereochemistry of the corresponding [1,5] migration has been demonstrated to be suprafacial,[1,5] no direct evidence has yet been obtained for the antarafacial nature of the [1,7] process.[1] Our interest in the chemistry of vitamin D prompted us to prepare the labeled *cis*-isotachysterols **1** and **2** and to study their thermal behavior.[6] We herein wish to report the first example of the antarafacial nature of this rearrangement (Scheme I).

The labeled *cis*-isotachysterols **1** and **2** were synthesized as outlined in Scheme II (steroid numbering). Treatment of Grundmann's ketone **3**[6b] with trimethylsilyl iodide (generated in

Scheme I

1a–d   **2a–d**
R_1 = OH; R_2 = H R_1 = H; R_2 = OH
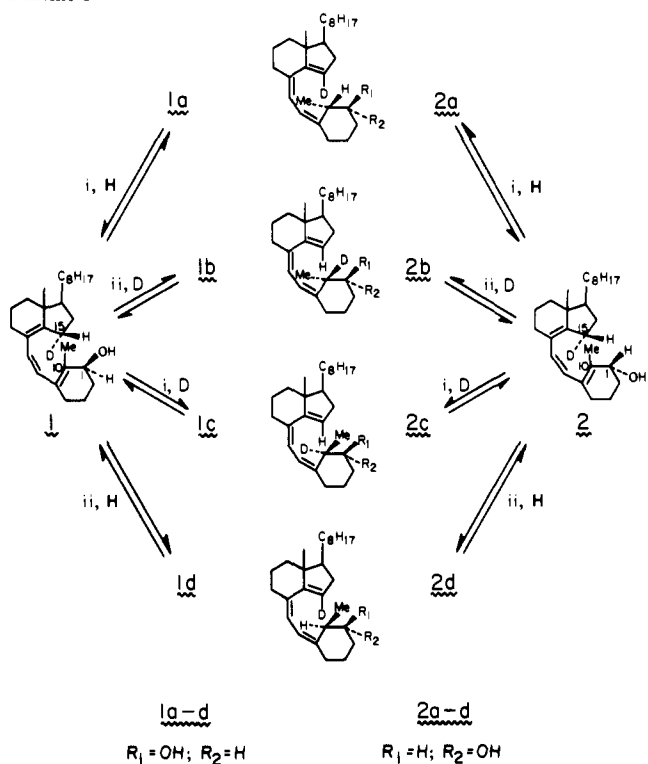
(i = Suprafacial; ii = Antarafacial;
H = Hydrogen Migration; D = Deuterium Migration)

(1) (a) Woodward, R. B.; Hoffmann, R. *J. Am. Chem. Soc.* **1965**, *87*, 2511. (b) Spangler, C. W. *Chem. Rev.* **1976**, *76*, 187.

(2) (a) Schlatmann, J. L. M. A.; Pot, J.; Havinga, E. *Recl. Trav. Chim. Pays-Bas* **1964**, *83*, 1173. (b) Hollick, M. F.; Frommer, J.; McNeill, S.; Richt, N.; Henley, J.; Potts, J. T., Jr. *Biochem. Biophys. Res. Commun.* **1977**, *76*, 107.

(3) Akhtar, M.; Gibbons, G. J. *Tetrahedron Lett.* **1965**, 509.

(4) (a) Sheves, M.; Berman, E.; Mazur, Y.; Zaretskii, Z. *J. Am. Chem. Soc.* **1979**, *101*, 1882. (b) Moriarty, R. M.; Paaren, H. E. *Tetrahedron Lett.* **1980**, 2389.

(5) Roth, W. R.; König, J.; Stein, K. *Chem. Ber.* **1970**, *103*, 426.

(6) (a) Hammond, M. L.; Mouriño, A.; Okamura, W. H. *J. Am. Chem. Soc.* **1978**, *100*, 4907. (b) Condran, P. C., Jr.; Hammond, M. L.; Mouriño, A.; Okamura, W. H. *Ibid.* **1980**, *102*, 6259. (c) For references to related derivatives, see Onisko, B. L.; Schnoes, H. K.; DeLuca, H. F. *J. Org. Chem.* **1978**, *43*, 3441. Okamura, W. H. *Acc. Chem. Res.* **1983**, *16*, 81.

Scheme II
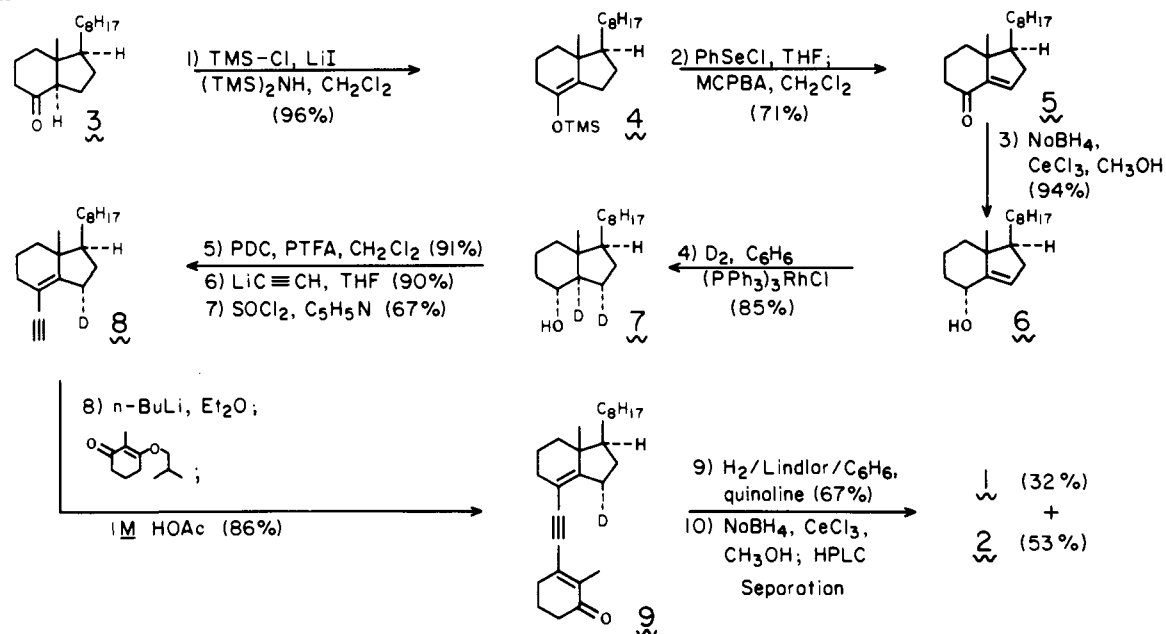
Table I. ^{1}H NMR Chemical Shifts (δ) and Relative Integrations^a

compd	$H_{6,7}$^{b}	H_{15}	H_1	H_{10}
1b (1S,10S)	6.22 (2.08 ± 0.01)[2.03 ± 0.02]	5.52 (0.95 ± 0.05)[0.98 ± 0.03]	3.84 (0.92 ± 0.01)[0.97 ± 0.02]	3.06 (0.99 ± 0.06)^c
1d (1S,10R)	6.17, (2.05 ± 0.04)[2.00 ± 0.06]	5.50 (0.88 ± 0.03)[0.033 ± 0.007]	3.71 (0.95 ± 0.04)[0.99 ± 0.06]	3.24 (1.02 ± 0.05)[0.88 ± 0.07]
2b (1R,10S)	6.14 (2.08 ± 0.02)[2.06 ± .01]	5.49 (0.91 ± 0.02)[0.89 ± 0.03]	3.70 (0.92 ± 0.02)[0.94 ± 0.01]	3.20 (0.89 ± 0.05)[0.053 ± 0.011]
2d (1R,10R)	6.25 (2.06 ± 0.01)[2.08 ± 0.03]	5.49 (0.97 ± 0.04)[0.022 ± 0.002]	3.82 (0.94 ± 0.01)[0.92 ± 0.03]	3.10 (0.99 ± 0.04)[1.02 ± 0.07]

^{a} The chemical shifts are for the unlabeled compounds, which at 300 MHz (Nicolet 300, CDCl$_3$) were identical with the observable resonances of the labeled series. The values given in parentheses are the relative integrated values for the unlabeled derivative; those given in brackets are for the corresponding labeled derivative obtained by heating **1** or **2**. ^{b} Chemical shift for $H_{6,7}$ is that for the center of the AB pattern. ^{c} No signal was observed for the labeled material.

situ) in the presence of base^7 gave exclusively the thermodynamic trimethylsilyl enol ether **4**. Reaction of the latter with benzeneselenenyl chloride followed by direct oxidation with MCPBA and thermolysis of the resulting selenoxide gave the enone **5**.^{8} Reduction of this enone with NaBH$_4$ and catalytic deuteration of the resulting allylic alcohol gave the 14α,15α-dideuterio alcohol **7**.^{9} Oxidation of this alcohol with pyridinium dichromate^{10} followed by addition of lithium acetylide to the ketone afforded a single propargyl alcohol^{6b} which could be subsequently dehydrated with SOCl$_2$ in pyridine to give the 15α-deuterio enyne **8** (>98% d_1). The labeled enyne **8** could then be carried on through **9** to the *cis*-isotachysterols **1** and **2** via methods previously reported by this laboratory.^{11} It should be noted that the two-step sequence

5 → 6 → 7 provides a simple solution to a classic problem in steroid synthesis, namely, the highly selective production of the *trans*-hydrindane nucleus.

With the synthesis of the labeled *cis*-isotachysterols complete, their thermolytic behavior could be studied in a manner similar to that previously described for the unlabeled series.^{6b} Scheme I shows the [1,7] hydrogen migration pathways possible for compounds **1** and **2**. If the [1,7] migration is indeed an antarafacial process, one expects to obtain from the thermolysis of compound **1** only compounds **1b** and **1d**; conversely, suprafacial migration should give **1a** and **1c**. If a random process were operative, all four products (**1a–d**) might be expected. Since the C$_1$ and C$_{10}$ stereochemical assignements have been established earlier by independent synthesis of the corresponding unlabeled compounds, which in turn had been stereochemically correlated to 1α-hydroxycholesterol of known configuration,^{6b} differentiation between the suprafacial and antarafacial modes is possible. A similar analysis of **2** reveals that **2b** and **2d**, and not **2a** and **2c**, should obtain from the antarafacial mode of rearrangement.

The product mixture from heating a solution of **1** in isooctane (~10^{-3} M) at 98.4 °C (sealed tubes) for 26 h consisted of 22% **1**, 47% **1b**, and 31% **1d**; that from heating **2** under identical conditions consisted of 22% **2**, 15% **2b**, and 63% **2d** (HPLC analyses using calibrated columns). Preparative separation of the individual components was readily achieved by HPLC (Whatman M9 Partisil column, 10% EtOAc–hexanes). As summarized in Table I, the deuterium analyses of the products **1b,d** and **2b,d** were conveniently carried out by ^{1}H NMR integration of the resonances assigned to H$_1$, H$_{10}$, and H$_{15}$ of the products of **1** or **2**. The observation of the products **1b** and **1d** from **1** and **2b** and **2d** from **2** thus demonstrates directly for the first time the antarafacial nature of the thermal [1,7] hydrogen migration.

(7) (a) Miller, R. D.; McKean, D. R. *Synthesis* **1979**, 730. (b) For a review concerning Me$_3$SiI, see: Schmidt, A. H. *Aldrichimica Acta* **1981**, *14*, 31 and references therein. (c) Kruerke, U. *Chem. Ber.* **1962**, *95*, 174. (d) Schmidt, A. H.; Russ, M. *Chem.-Ztg.* **1978**, *102*, 26, 65.

(8) (a) Reich, H. J. *Acc. Chem. Res.* **1979**, *12*, 22. (b) Reich, H. J.; Wollowitz, S. W.; Trend, J. E.; Chow, F.; Wendelborn, D. F. *J. Org. Chem.* **1978**, *43*, 1697. (c) Johnston, A. D. Ph.D. Thesis, University of California, Riverside, 1983. Early work on this conversion was also carried out by Dr. Antonio Mouriño.

(9) In addition, a small amount of the 14β,15β-dideuterio alcohol was formed (14α,15α:14β,15β = 37:1). For recent related cases of hydroxyl-directed catalytic reductions, see the following: (a) Thompson, H. W.; McPherson, E. *J. Am. Chem. Soc.* **1974**, *96*, 6232. (b) Stork, G.; Kahne, D. E. *J. Am. Chem. Soc.* **1983**, *105*, 1072. (c) Crabtree, R. H.; Davis, M. W. *Organometallics* **1983**, *2*, 681. (d) Corey, E. J.; Engler, T. A. *Tetrahedron Lett.* **1984**, *26*, 149. (e) Evans, D. A.; Morrissey, M. M. *J. Am. Chem. Soc.* **1984**, *106*, 3866.

(10) Corey, E. J.; Schmidt, G. *Tetrahedron Lett.* **1979**, 399.

(11) This is paper 28 in the series Studies on Vitamin D (Calciferol) and Its Analogues. For paper 27, see: Jeganathan, S.; Johnston, A. D.; Kuenzel, E. A.; Norman, A. W.; Okamura, W. H. *J. Org. Chem.* **1984**, *49*, 2152.

Supplementary Material Available: ¹H 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.
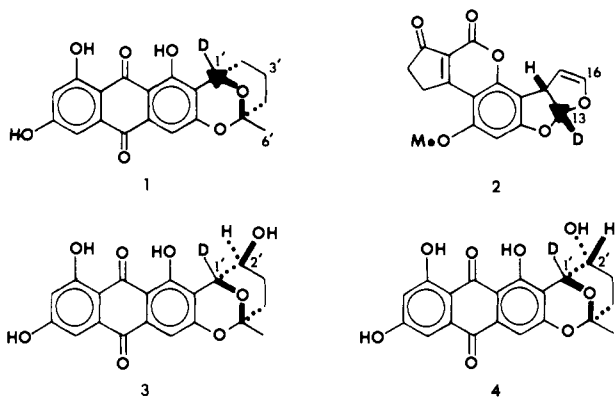
Concerning the Role of Nidurufin in Aflatoxin Biosynthesis

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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

Scheme I

particular racemic [1'-²H,¹³C]averufin (**1**) was found to label C-13 in **2** without detectable loss of deuterium relative to ¹³C 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
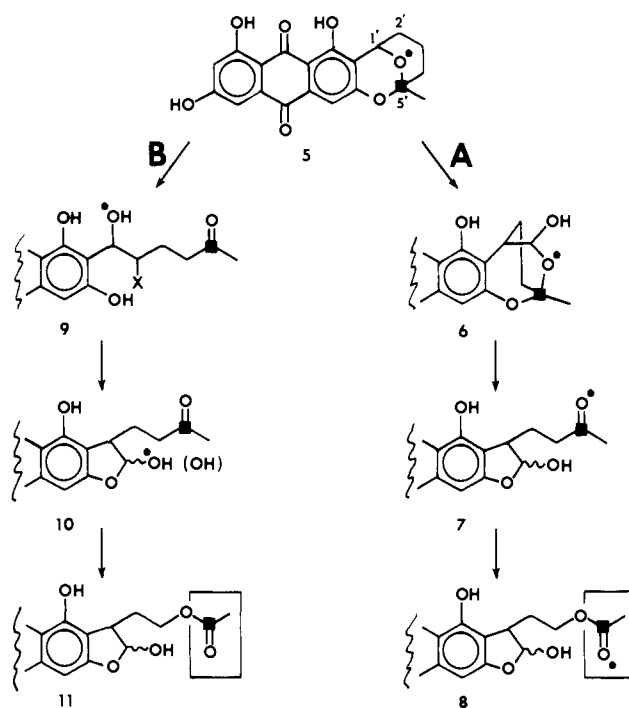
of incorporation into aflatoxin B_1 (**2**), further evidence is presented to support important limiting features of biogenetic Scheme IA.

Racemic [1'-²H]nidurufin (**3**) and [1'-²H]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

(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.