Determination of the Absolute Configuration of the Thiazolinylthiazole Moiety of Phleomycin

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The phleomycins (PLM) were isolated from *Streptomyces verticillus* in 1956.[1] Their promising antitumor properties[2] prompted a search for related species, which led to the discovery of the bleomycins (BLM).[3] These families of compounds are closely related structurally (Figure 1);[4] they are thought to be biosynthesized via a common pathway.[5]

As shown for BLM, PLM also forms complexes with redox-active metal ions such as Fe, Co, and Cu.[1b,6,7] Following reductive activation in the presence of O_2, the Fe and Co complexes have been shown to mediate the oxidative destruction of DNA.[6] The DNA cleavage selectivities of PLM and BLM were remarkably similar,[6] in spite of the evidence that their C-terminal domains may interact with DNA by different mechanisms. BLM, which has a planar bithiazole moiety, has been shown to unwind DNA[7-9] and cause DNA helix elongation[7,8] and has been postulated to be a (partial) intercalator.[7,8,10] In contrast, PLM contains a thiazolinylthiazole moiety whose sp^3 center must preclude intercalation.[11] It seems likely that PLM binds to the minor groove of DNA, which is the site of DNA damage.[12] Thus the presence of the chiral center in the DNA binding domain would seem to alter the strategy by which the antibiotic binds to DNA, but a detailed analysis of the nature of this interaction has been hindered by the absence of an assignment for the stereochemistry of the chiral sp^3 carbon atom in the thiazolinylthiazole moiety of PLM. Presently, we establish the absolute configuration of this center.

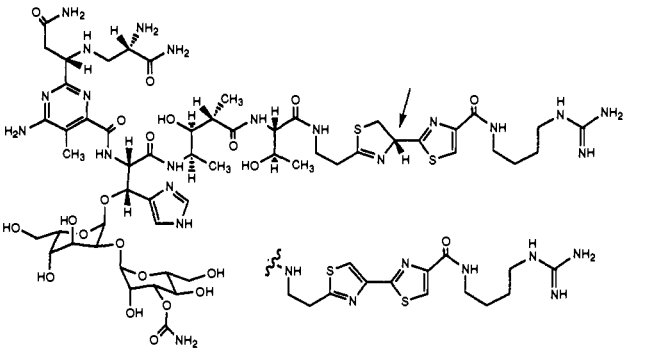

Figure 1. Structure of phleomycin D_1, illustrating the *R* configuration of the newly established stereocenter (arrow). Also shown is a partial structure for bleomycin B_2.

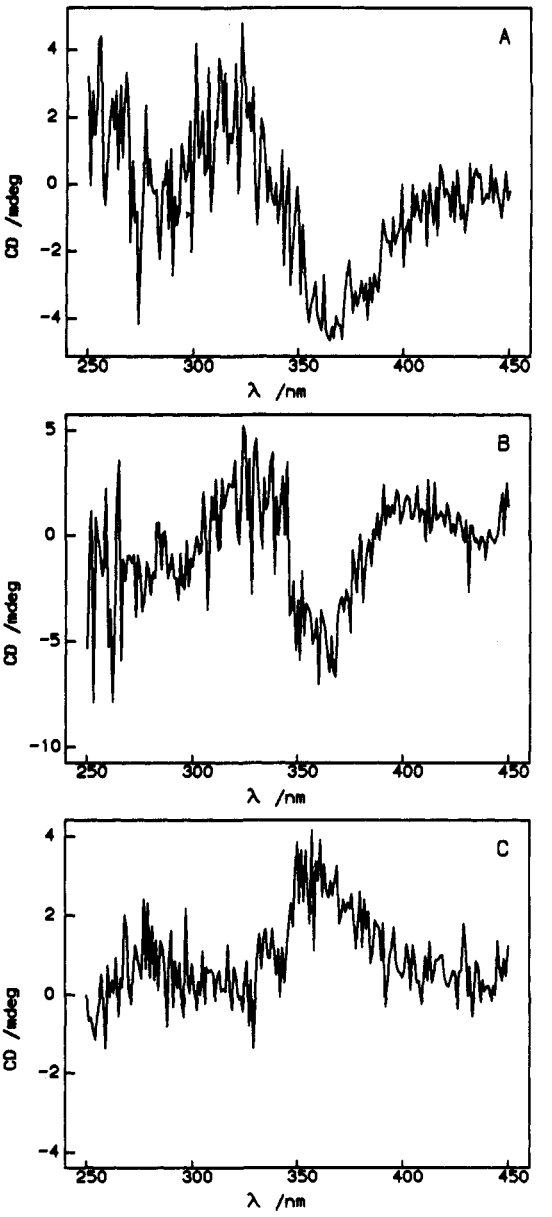

Figure 2. CD spectra of thiazole **2** derived from phleomycin (A), (*R*)-**1** (B), and (*S*)-**1** (C).

Because the absolute stereochemistry of the thiazolinylthiazole moiety could not be determined by NMR analysis,[13] we developed a strategy involving chemical degradation of PLM to afford a fragment amenable to structural analysis.[14] The proposed

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(11) Unlike Cu(II)-BLM and Fe(III)-BLM, Cu(II)-PLM did not cause DNA lengthening.[7]

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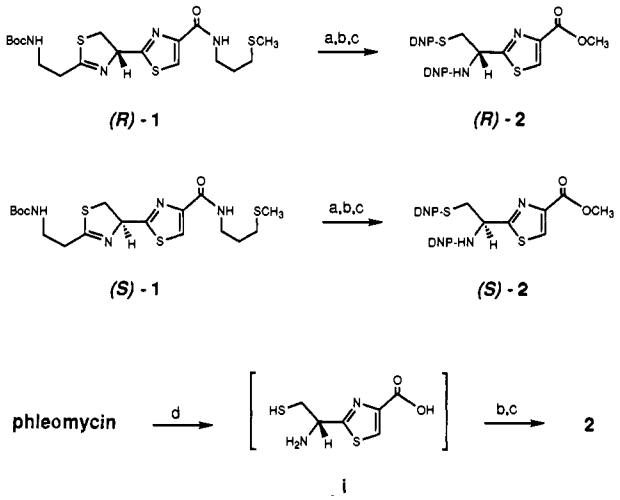
Scheme I. Degradation of Phleomycin To Obtain a Chiral Fragment of the Thiazolinylthiazole Moiety[a]



[a] (a) 4.5 N HCl; (b) 2,4-dinitrofluorobenzene; (c) CH_2N_2; (d) 5.5 N HCl.

transformations are outlined in Scheme I. As shown, treatment of PLM with 5.5 N HCl (105 °C, 15 h) afforded putative i, which was treated successively with 2,4-dinitrofluorobenzene (K_2CO_3 in 4:1 acetone–H_2O, 25 °C, 15 h) and CH_2N_2 to afford thiazole derivative **2**[14b,c] as a yellow solid, $[\alpha]^{25}_D$ –40° (*c* 0.02, $CHCl_3$).[15,16] As shown in Figure 2, the CD spectrum of this sample of **2** exhibited a negative Cotton effect at 360 nm.

Analogous hydrolysis and derivatization of authentic (*R*)-thiazolinylthiazole **1** (4.5 N HCl, 105 °C, 16 h) (Scheme I) also afforded a sample of thiazole methyl ester **2**. This material was indistinguishable chemically from the sample derived from PLM and from authentic **2**,[17] and had a similar optical rotation ($[\alpha]^{25}_D$ –45° (*c* 0.1, $CHCl_3$))[16] and CD spectrum to the sample of **2** derived from PLM (Figure 2). In comparison, the sample of **2** derived from (*S*)-thiazolinylthiazole **1** (Scheme I) had $[\alpha]^{25}_D$ + 50° (*c* 0.1, $CHCl_3$)[16] and had a CD spectrum with a positive Cotton effect at 360 nm. On this basis the chiral center in the thiazolinylthiazole moiety of PLM can be assigned an *R* configuration. Establishment of the absolute stereochemistry of this center in PLM, as well as the elaboration of both isomers of the thiazolinylthiazole moiety as described herein, will allow experimental definition of the role that this structural element plays in PLM–DNA interactions.

On the likely assumption that the thiazol(in)e moieties in PLM and BLM are biosynthesized from cysteinyl peptides,[5] the cysteine that forms the asymmetric center in the thiazoline moiety of PLM must have the *R* configuration.[19]

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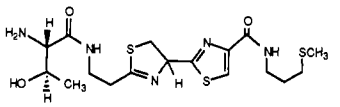
Supplementary Material Available: Experimental procedures and spectral data for the syntheses of compounds **1** and **2** and for the degradation of phleomycin and derivatization of intermediate **i** to afford **2** (15 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(13) (*R*)- and (*S*)-**3** were prepared from the respective isomers of **1** in analogy with published methods: see Hamamichi, N.; Natrajan, A.; Hecht, S. M. *J. Am. Chem. Soc.* **1992**, *114*, 6278. The 500-MHz ^{1}H-NMR spectra of (*R*)-**3**, (*S*)-**3**, and phleomycin had virtually identical chemical shifts for the Hs of the thiazoline moiety (e.g., the thiazoline C-4 Hs in (*R*)-**3**, (*S*)-**3**, and phleomycin resonated at δ 5.89, 5.88, and 5.85, respectively). Likewise, the ^{13}C-NMR resonances had essentially the same chemical shift values.



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(15) ^{1}H NMR ($CDCl_3$): δ 3.78 (dd, 1 H, *J* = 13, 8 Hz), 3.96 (dd, 1 H, *J* = 13, 4 Hz), 3.98 (s, 3 H), 5.50 (m, 1 H), 6.87 (d,1 H, *J* = 9 Hz), 7.66 (d, 1 H, *J* = 9 Hz), 8.17 (s, 1 H), 8.26 (dd, 1 H, *J* = 9, 2 Hz), 8.40 (dd, 1 H, *J* = 9, 2 Hz), 9.05 (d, 1 H, *J* = 2 Hz), 9.17 (d, 1 H, *J* = 2 Hz), 9.20 (d, 1 H, *J* = 6 Hz). λ_{max} (CH_3OH): 233 and 334 nm. λ_{min}: 287 nm. Mass spectrum (chemical ionization): *m/z* 551 $(M + H)^+$ and 351. High-resolution mass spectrum (chemical ionization): *m/z* 551.026 ($C_{19}H_{15}N_6O_{10}S_2$ requires 551.029).

(16) Authentic (*R*)-**2**[17] had $[\alpha]^{25}_D$ –110° (*c* 0.5, $CHCl_3$); authentic (*S*)-**2**[17] had $[\alpha]^{25}_D$ +110° (*c* 0.5, $CHCl_3$). The low optical rotations observed for the samples of **2** derived from phleomycin and **1** were shown to be due to partial racemization during acid hydrolysis.

(17) Prepared starting from (*R*)- and (*S*)-methyl *N*-(*tert*-butoxycarbonyl)-(2-tetrahydropyranyl)cysteinate.[18]

(18) Holland, G. F.; Cohen, L. A. *J. Am. Chem. Soc.* **1958**, *80*, 3765.

(19) There are numerous examples of naturally occurring thiazolines having *R* or *S* configurations. See supplementary material for leading references.