(9) Several anionic bases are known to catalyze H–D exchange of hydrogen: E. A. Symons and E. Buncel, *Can. J. Chem.*, **51**, 1673 (1973), and references therein. Surface phenomena may play a role in generating a microenvironment suitable for catalytic exchange which might not be duplicable in bulk solution. Also, the possibility of impurities should not be overlooked.

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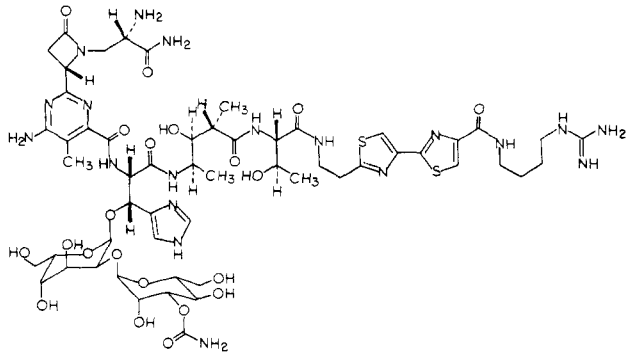
A Biomimetic Synthesis of the Bithiazole Moiety of Bleomycin

Sir:

The antibiotic bleomycin is of current interest because of its clinically useful anticancer activity.[1] As part of a total synthesis of bleomycin B_2 (**1**), we have been investigating the



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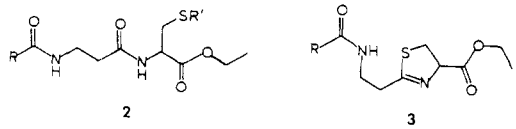
chemistry of the bithiazole moiety, the biosynthetic elaboration of which probably involves dehydrative cyclization of β-alanylcysteinylcysteine and dehydrogenation of the intermediate Δ^2-thiazolines.[2] Although the preparation of Δ^2-thiazolines from certain cysteinyl peptides has been reported not to be possible,[3] and no efficient methods have been recorded for the oxidation of complex Δ^2-thiazolines, we report herein a biomimetic synthesis of the bithiazole moiety of bleomycin. Since several other natural products contain single thiazoles or Δ^2-thiazoline groups,[4] this synthetic approach should also be of more general utility.

Although several agents previously employed for the preparation of simple thiazolines[5] failed to effect the conversion of dipeptide **2a**[6] to the corresponding thiazoline, treatment of



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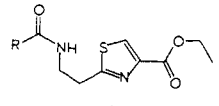

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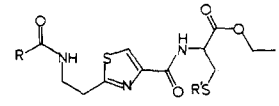
a, R = CH_3
b, R = C_6H_5

chloroform solutions of **2a** (R′ = H or $(C_6H_5)_3C$) with hydrogen chloride at 0 °C afforded ethyl 2-(2-acetamidoethyl)-Δ^2-thiazoline-4-carboxylate (**3a**), mp 156–158 °C, in yields up to 77% (purification by crystallization from benzene–chloroform–petroleum ether or distillation at 160 °C/(0.1 mm)), λ_{max} (1:1 HCl-C_2H_5OH) 267 nm. Of the reagents previously used for the oxidation of thiazolines,[7] only activated MnO_2 ($CHCl_3$, room temperature, 4 days) gave significant conversion of **3a** to **4a;** the latter was obtained as colorless crystals in 65% yield. A much better yield of **4a** (93%) was obtained by the use of NiO_2. In a typical experiment 293 mg (1.20 mmol) of **3a** and 762 mg of NiO_2[8] in 25 mL of $CHCl_3$ was shaken for 42 h. After filtration, concentration of the filtrate and crystallization of the residue (ether) gave **4a** in a good state of purity[9] as colorless needles: mp 83–84 °C; λ_{max} (C_2H_5OH) 236 nm; NMR ($CDCl_3$, $(CH_3)_4Si$) δ 1.45 (t, 3), 2.00 (s, 3), 3.28 (t, 2), 3.74 (m, 2), 4.42 (q, 2) 6.70 (br, 1), 8.09 (s, 1). Analogous conversion of **2b** to **4b** was also effected, although the transformation **2b** (R′ = H) → **3b** generally proceeded in somewhat lower yield than **2a** → **3a.**

Saponification of **4a** and **4b** (KOH, aqueous dioxane) gave the respective carboxylates in yields of 96 and 95%. While the carboxylate derived from **4a** had appreciable solubility only in water, and could not be condensed conveniently with *S*-tritylcysteine ethyl ester, condensation of the acid derived from **4b** with *S*-tritylcysteine ethyl ester (*N,N*′-dicyclohexylcarbodiimide, tetrahydrofuran) afforded tripeptide analogue **5b**



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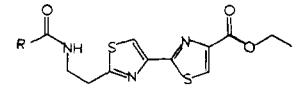

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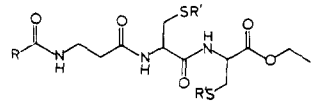

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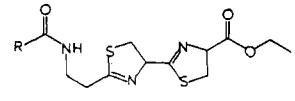

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(R′ = $(C_6H_5)_3C$; 96%) as a white foam. Treatment with $AgNO_3$ (1.3 equiv, pyridine–methanol, 12 h) at room temperature gave the corresponding silver mercaptide (100%, R′ = Ag) as pale yellow crystals. The mercaptide was converted to mercaptan **5b** (100%, R′ = H) by treatment of a methanolic suspension of the silver salt with H_2S: NMR ($CDCl_3$, $(CH_3)_4Si$) δ 1.33 (t, 3), 1.47 (t, 1), 3.12 (dd, 2), 3.35 (t, 2), 3.88 (m, 2), 4.27 (q, 2), 4.98 (m, 1), 7.3–7.5 (m, 3), 7.7–8.2 (m, 3), 8.50 (t, 1), 8.96 (d, 1). Compound **5b** (R′ = H) was dissolved in $CHCl_3$ and treated with a slow stream of hydrogen chloride (36 h, room temperature). After concentration of the reaction mixture, the residue was partitioned between ethyl acetate and aqueous Na_2CO_3. Workup of the organic phase afforded a clear oil (90% recovery; λ_{max} (1:1 C_2H_5OH–HCl) 233 and 300 nm; presumably the thiazolylthiazoline) which was redissolved in $CHCl_3$ and shaken in the presence of MnO_2 or NiO_2[10] (5 days, room temperature). Workup gave a yellow oil which deposited colorless needles of the known[11] ethyl 2′-(2-benzamidoethyl)-2,4′-bithiazole-4-carboxylate (**6b**) from ethyl acetate–petroleum ether: yield 24%; mp 143–144 °C; λ_{max} (EtOH) 290 nm (log 4.17; NMR $CDCl_3$, $(CH_3)_4Si$) δ 1.46 (t, 3), 3.36 (t, 2), 3.93 (t, 2), 4.47 (q, 2), 7.35–7.9 (m, 6), 8.06 (s, 1), 8.19 (s, 1).

Having obtained the desired bithiazole (**6**) via stepwise dehydrative cyclization and oxidation, it was of interest to attempt the direct conversion of β-alanylcysteinylcysteine derivative **7** to **6** via bithiazoline **8.** Treatment of an ethanol-free $CHCl_3$ solution of **7a** (R′ = H)[12] with a slow stream of HCl (24 h, room temperature, followed by concentration under diminished pressure) afforded a water-sensitive residue having the UV spectrum (λ_{max} (1:1 C_2H_5OH–HCl) 266 nm (ε 9200)) expected of bithiazoline **8a.**[13] Attempted oxidation of the putative bithiazoline to **6a** (NiO_2, $CHCl_3$) gave instead the disulfide derived from **5a** (R′ = H), whose formation may pro-

ceed via hydrolysis of **8a** by water associated with the oxidant or formed during the oxidation.

Although NiO_2 could not be employed for the conversion **8a** → **6a**, this reagent has also been used for the attempted oxidation of other partially reduced N-, O-, and S-containing heterocycles, many of which were dehydrogenated in good yield. Compounds oxidized successfully with NiO_2 included 2-methylthio-Δ^2-thiazoline (60%), methyl 2-methyl-Δ^2-imidazoline-4-carboxylate (81%), 1,5-diphenyl-3-(*p*-bromophenyl)pyrazoline (95%),[14] 2,3-dihydrobenzofuran (52%),[15] and several 2,4-disubstituted Δ^2-thiazolines, including phleomycin A_2 (83%).[16]

Acknowledgments. We thank Professor George Büchi and Dr. John Vederas for helpful discussions during the course of this work, Dr. Robert Engle (National Cancer Institute) for providing an authentic sample of 2′-(2-aminoethyl)-2,4′-bithiazole-4-carboxylic acid, and Dr. H. Umezawa for samples of phleomycin A_2 and bleomycin A_2. This investigation was supported by contract N01-CM-43712 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare.

References and Notes

(1) (a) H. Umezawa, *Prog. Biochem. Pharmacol.*, **11**, 18 (1976); (b) T. Ichikawa, *ibid.*, **11**, 143 (1976); (c) S. K. Carter and R. H. Blum, *ibid.*, **11**, 158 (1976); (d) G. Bonadonna, G. Tancini, and E. Bajetta, *ibid.*, **11**, 172 (1976); (e) A. Depierre, *ibid.*, **11**, 195 (1976); (f) J. Rygard and H. S. Hansen, *ibid.*, **11**, 205 (1976); (g) P. Rathert and W. Lutzeyer, *ibid.*, **11**, 223 (1976).

(2) See G. E. Hall, N. Sheppard, and J. Walker, *J. Chem. Soc. C*, 1371 (1966), and references therein. The peptide antibiotic bacitracin A, e.g., has a cysteinyl residue that exists preferentially as the corresponding thiazoline (E. P. Abraham and G. G. F. Newton, *Biochem. J.*, **53**, 604 (1953); L. C. Craig, W. Hausmann, and J. R. Weisiger, *J. Am. Chem. Soc.*, **76**, 2839 (1954)) and the production of micrococcin P ceased when cysteine was omitted from the fermentation medium (P. Brookes, R. J. Clark, A. T. Fuller, M. P. V. Mijovic, and J. Walker, *J. Chem. Soc.*, 916 (1960)). Further support for this suggestion may be inferred from bleomycin and phleomycin, which are both produced by *Streptomyces verticillus* and differ structurally only in a single double bond, such that phleomycin (which contains a thiazolinylthiazole) may be regarded as the biosynthetic precursor of bleomycin. Also, the substitutents and substitution patterns associated with all of the cited compounds are consistent with their derivation from peptides.

(3) E.g., (a) I. Goodman and L. Salce, *Biochim. Biophys. Acta*, **100**, 283 (1955); (b) Y. Hirotsu, T. Shiba, and T. Kaneko, *ibid.*, **222**, 540 (1970). See, however, Y. Hirotsu, T. Shiba, and T. Kaneko, *Bull. Chem. Soc. Jpn.*, **40**, 2950 (1967).

(4) E.g., (a) bottromycin, J. M. Waisvisz, M. G. Van der Hoeven, and B. te Nijenhuis, *J. Am. Chem. Soc.*, **79**, 4524 (1957); (b) althiomycin, B. W. Bycroft and R. Pinchin, *J. Chem. Soc., Chem. Commun.*, 121 (1975), and references therein; (c) siomycin, M. Ebato, K. Miyazaki, and H. Otsuka, *J. Antibiot. (Tokyo)*, **22**, 423 (1969), and Y. Wikasaka, T. Nagasaki, and H. Minato, *ibid.*, **26**, 104 (1973); (d) thiostrepton, B. Anderson, D. C. Hodgkin, and A. Viswamitra, *Nature*, **225**, 233 (1970); (e) zorbamycin, Y. Ohashi, H. Abe, S. Kawabe, and Y. Ito, *Agr. Biol. Chem.*, **37**, 2387 (1973); (f) saramycetin, A. Aszalos, A. I. Cohen, J. Alicino, and B. T. Keeler, *Antimicrob. Agents Chemother.*, 456 (1967); (g) nosiheptide, H. Depaire, J.-P. Thomas, and A. Brun, *Tetrahedron Lett.*, 1403 (1977). See also J. Jadot, J. Casimir, and R. Warin, *Bull. Soc. Chim. Belg.*, **78**, 299 (1969).

(5) E.g., (a) phosphorus pentachloride, S. Gabriel, *Chem. Ber.*, **24**, 1110 (1891), and S. Gabriel, *ibid.*, **49**, 1110 (1916); (b) phosphorous oxychloride, J. C. Vederas, Ph.D. Thesis, Massachusetts Institute of Technology, 1973.

(6) Compound **2a** (R′ = H) was obtained (100%) by successive treatments of *N*-acetyl-β-alanyl-*S*-tritylcysteine ethyl ester (**2a**, R′ = $(C_6H_5)_3C$) with $HgCl_2$ or $AgNO_3$ and then with methanolic H_2S. The fully blocked dipeptide was prepared by condensation of *N*-acetyl-β-alanine and *S*-tritylcysteine ethyl ester (79%; DCC, *N*-hydroxysuccinimide).

(7) E.g., (a) potassium ferricyanide and mercuric acetate, J. Walker, *J. Chem. Soc. C*, 1522 (1968), and N. A. Fuller and J. Walker, *ibid.*, 1526 (1968); (b) hydrogen peroxide and potassium dichromate, F. Asinger, M. Thiel, and L. Schroeder, *Justus Liebigs Ann. Chem.*, **610**, 49 (1957); (c) cupric sulfate;[5b] (d) MnO_2 and various quinones, M. A. Barton, G. W. Kenner, and R. C. Sheppard, *J. Chem. Soc. C*, 1061 (1966).

(8) Containing 2.83 mequiv of active O_2, as measured by iodide titration: K. Nukagawa, R. Konaka, and T. Nakata, *J. Org. Chem.*, **27**, 1597 (1962).

(9) New compounds gave satisfactory elemental analyses or high resolution mass spectra.

(10) The two oxidants gave comparable yields for this transformation.

(11) Compound **6b**, mp 143–145 °C, λ_{max} (C_2H_5OH) 290 nm (log ϵ 4.18), has been prepared previously: K. Y. Zee-Cheng and C. C. Cheng, *J. Heterocycl. Chem.*, **7**, 1439 (1970).

(12) Obtained from **2a** (R′ = $(C_6H_5)_3C$) in 67% overall yield via saponification (methanolic NaOH, reflux, 10 min), condensation with *S*-tritylcysteine ethyl ester (DCC, *N*-hydroxysuccimmide) to give **7a** (R′ = $(C_6H_5)_3C$) and deblocking via the monomercuric mercaptide.

(13) Alternate workup procedures, which permitted the product to come in contact with an aqueous phase, gave material with much smaller apparent absorptivitiy. Although ϵ is dependent on the nature of the ring substituents and the medium in which the UV spectrum is recorded, molar absorptivity values of 5000 for single thiazolines are typical. See, e.g., (a) W. Stoffel and L. C. Craig, *J. Am. Chem. Soc.*, **83**, 145 (1961); (b) W. Konigsberg and L. C. Craig, *J. Am. Chem. Soc.*, **81**, 3452 (1959), and ref 7a.

(14) K. S. Balachandran, I. Bhatnagar, and M. V. George, *J. Org. Chem.*, **33**, 3891 (1968).

(15) This oxidation was carried out by Mr. David Evans.

(16) Conversions of phleomycin D_1 and E to bleomycins B_2 and B_4, respectively, have been reported previously in unspecified yields. See T. Takita, Y. Muraoka, A. Fujii, H. Itoh, K. Maeda, and H. Umezawa, *J. Antibiot. (Tokyo)*, **25**, 197 (1972); H. Umezawa, *Biomedicine*, **18**, 459 (1973).

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Oxidation of 9-Hydroxy- and 9-Methoxyfluorene Carbanions by Flavin. Proof of Radical Mechanism

Sir:

Flavin mediated dehydrogenation reactions which introduce unsaturation α,β to carbonyl groups are of considerable biochemical interest (lactic acid oxidase, amino acid oxidases, succinic acid dehydrogenase, etc.) and have been the subject of numerous investigations.[1-3] Model studies from this laboratory[2b,3b,d] have firmly established that it is the resonance stabilized carbanion of the substrate which undergoes oxidation by flavin. Kinetic and other evidence supports a radical mechanism (Scheme IA) or, less likely, a mechanism involving a 4a adduct which goes on to product by specific base catalysis (Scheme IB).[2b,3,4]

The mechanism of Scheme IA has been favored[3] on the basis of free-energy calculations,[3c,e] arguments centered around the requirement of specific base catalysis of 4a-adduct decomposition,[3d] and the results of studies with 1,5-dihydro-3,5-dimethyllumiflavin.[5] However, direct evidence for the formation of a flavin–substrate radical pair, as required by Scheme IA, has not been obtained. The present study deals

Scheme I