peptides which contain L-amino acid residues.[11] As reported earlier the *S* form of **3** competitively inhibits aminopeptidase M with a K_I of 1.2 mM.[1] More recent studies have shown that the *R* form of **3** also inhibits competitively, but with a K_I of 9.2 mM, reflecting an eightfold preference for the *S* form. The corresponding constants for Gly-L-Leu and Gly-D-Leu were found to be 4.8 and 24 mM, respectively.[12]

When considering the preparation of other Gly–X analogues, it appears that if the displacement of bromide from the 2-bromo acid precursor is facile, then the preferred nucleophile is 2-mercaptoethylamine. However, in some instances the more powerful nucleophile, trithiocarbonate, may be required. The combination of the two synthetic approaches provides the basis for preparation of Gly–X analogues of substantial biochemical interest.

References and Notes

(1) (a) K.-F. Fok and J. A. Yankeelov, Jr., *Biochem. Biophys. Res. Commun.*, **74**, 273 (1977); (b) K.-F. Fok and J. A. Yankeelov, Jr., *Fed. Proc.*, **36**, 721 (1977).

(2) The term "peptide-gap inhibitor" (alternatively "pseudopeptide") is used to describe compounds whose structures are identical with dipeptides with the exception that the atoms of the peptide linkage (–CONH–) have been replaced by a methylene thioether linkage (–CH_2S–). Thus, any peptide containing this alteration exhibits a "gap" in its peptide backbone at the position of replacement. The support of this research by Grant EY 00969 from the National Eye Institute is gratefully acknowledged. Thanks are due to Dr. Arno F. Spatola of the Department of Chemistry for determination of the NMR spectra reported in this communication.

(3) K. Pfister III, E. E. Howe, C. A. Robinson, A. C. Shabica, E. W. Pietrusza, and M. Tishler, *J. Am. Chem. Soc.*, **71**, 1096 (1949).

(4) (a) W. Gaffield and W. G. Galetto, *Tetrahedron*, **27**, 915 (1971); (b) P. A. Levene, T. Mori, and L. A. Mikeska, *J. Biol. Chem.*, **75**, 337 (1927); (c) J. P. Greenstein and M. Winitz, "Chemistry of the Amino Acids", Vol. I, Wiley, New York, N.Y., 1961, p 165.

(5) A. Dréze, S. Moore, and E. J. Bigwood, *Anal. Chim. Acta*, **11**, 554 (1954).

(6) J. R. Benson in "Applications of the Newer Techniques of Analysis", I. L. Simons and G. W. Ewing, Ed., Plenum Press, New York, N.Y., 1973, p 223.

(7) D. J. Martin and C. C. Greco, *J. Org. Chem.*, **33**, 1275 (1968).

(8) R. D. Cole, *Methods Enzymol.*, **11**, 315 (1967).

(9) A. Neuberger, *Adv. Protein Chem.*, **4**, 297 (1948).

(10) J. H. Brewster, *J. Am. Chem. Soc.*, **81**, 5475 (1959).

(11) A. Light, *Methods Enzymol.*, **25**, 253 (1972).

(12) The methods employed for these determinations are described in ref 1.

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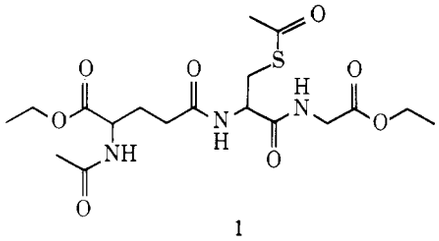
Thiazoles from Cysteinyl Peptides

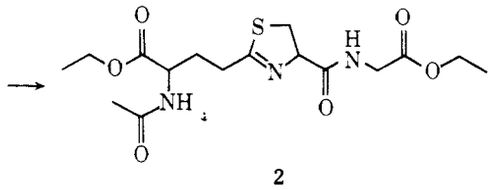
Summary: Certain thiazoles are obtained via dehydrative cyclization of the corresponding cysteinyl peptides and oxidation of the resulting thiazolines with NiO_2; the biomimetic syntheses of two natural products are reported, as is the potential of NiO_2 as an oxidant for other partially reduced heterocycles.

Sir: Thiazolines and thiazoles are structural components of a number of peptide-derived natural products; among these are the antibiotics siomycin,[1] thiostrepton[2] and micrococcin P,[3] the antitumor antibiotics phleomycin[4] and bleomycin[4] (elaborated by *Streptomyces verticillus*), and Jadot's novel dicarboxylic amino acid (**4**),[5] isolated from the mushroom *Xerocomus subtomentosus*. Several lines of evidence suggest that the biosyntheses of these natural products proceed via the dehydrative cyclization of the corresponding cysteinyl peptides and subsequent oxidation to thiazoles.[6] The facility with which polypeptides may now be assembled makes the biomimetic preparation of peptide-derived thiazoles an attractive synthetic approach; the cysteinyl peptide → thiazoline → thiazole transformation has also been of interest as a possible peptide sequencing tool.[7] However, in spite of the potential utility of such transformations, and the likelihood that biosynthesis proceeds in this fashion, attempted chemical syntheses of all but the simplest thiazoles have failed during dehydrative cyclization[8] or subsequent dehydrogenation.[9] Our interest in the total synthesis of the thiazole-containing antibiotic bleomycin prompted us to reinvestigate the conversion of cysteinyl peptides to their corresponding thiazoles. We report herein the realization of this transformation in a synthetically useful fashion.

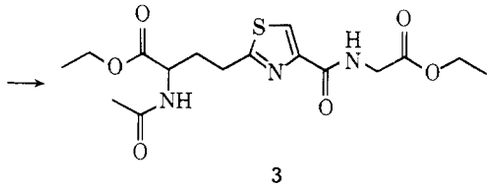
Cyclization of glutathione to the corresponding thiazoline was first reported by Calvin,[10] who observed its formation in strong mineral acid by monitoring changes in the ultraviolet spectrum of the reaction mixture. This observation has been verified by others, but it has not been possible to isolate the product.[11] Indeed, Hirotsu et al.[12] reported that their "attempt to secure a pure thiazoline compound by dehydration of *N*-acylglutathione dibenzyl ester in nonaqueous acidic medium ... failed". In spite of the reported experimental difficulties, we observed that the slow addition of anhydrous hydrogen chloride to *N*,*S*-diacetylglutathione diethyl ester (**1**)[13] in a 5% ethanolic chloroform solution over a period of 24 h effected its cyclization to thiazoline **2**. Treatment of the reaction mixture with solid sodium bicarbonate, followed by filtration, concentration of the filtrate, and trituration of the residue with benzene afforded the thiazoline as a white solid in 70% yield. The proton NMR spectrum of thiazoline **2** included signals characteristic[14] of Δ^2-thiazolines at δ 3.61 (d, 2, J = 9.5 Hz) and 5.07 (t, 1, J = 9.5 Hz) and the UV spectrum had the expected[12] λ_{max} (1:1 C_2H_5OH–HCl) 267 nm (ϵ 5400); $[\alpha]^{25}_D$ +40° (*c* 2.0, $CHCl_3$). Dehydrative cyclization of several cysteinyl peptides not requiring prior ethanolysis of S-protecting groups has been accomplished conveniently in chloroform solution;[6,15] the choice of protecting groups was important in such cases, since better yields were generally obtained when the desired thiazolinium chlorides were insoluble in the reaction medium.



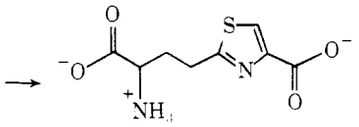
1



2



3



4

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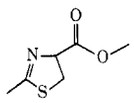
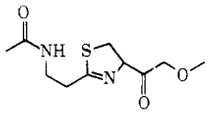
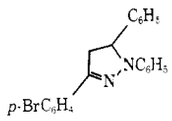
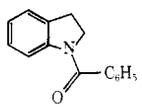
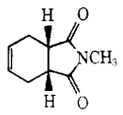
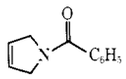
Table I. Nickel Peroxide Oxidations of Partially Reduced Heterocycles

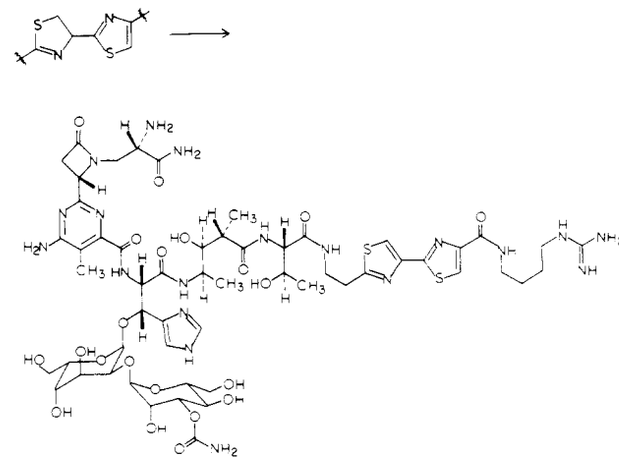
Heterocycle	NiO_2 (equiv O_2/equiv substrate)	Solvent	Conditions	% yield[a]
	2.0	$CHCl_3$	3 days, room temperature	81[b]
	3.7	C_6H_6	4 h, reflux	60
	2.4	$CHCl_3$	3 days, room temperature	93[c]
		C_6H_6	3 h, reflux	95[d]
	2.2	C_6H_6	11 h, reflux	52
	4.4[e]	C_6H_6	24 h, reflux	54
	2.3	C_6H_6	7 h, reflux	62[f]
	3.4	C_6H_6	3.5 h, reflux	59

[a] Isolated yields. The products were obtained by filtration of the catalyst through Celite, concentration of the filtrate, and purification where necessary by chromatography or crystallization. [b] Previously oxidized with phenanthrenequinone in 45% yield. [c] Oxidized in 65% yield with MnO_2. [d] Reference 16. [e] Oxidant was added in three equivalent portions. [f] Product was *N*-methylphthalimide.

The oxidation of several peptide-derived thiazolines was attempted using each of the reagents reported to have utility for this type of transformation,[9] and others not previously used for this purpose. Of the reagents tested, only manganese dioxide effected the desired transformation in a synthetically useful fashion, giving moderate yields of the corresponding thiazoles. In an effort to improve the yields, we considered the use of nickel peroxide[16] as oxidant, since this reagent is believed to function mechanistically in similar fashion to MnO_2.[17] Although preparations of nickel peroxide contain fewer oxidizing equivalents per gram of catalyst than does tion, we reasoned that the greater oxidizing power (or possibly instability) of Ni(IV) as compared with Mn(IV) should make NiO_2 the more effective oxidant.[18] In fact, treatment of thiazoline **2** with NiO_2 afforded the corresponding thiazole (**3**) as a clear oil in 75% yield; λ_{max} 232 nm; NMR ($CDCl_3$, $(CH_3)_4Si$) δ 1.28 (2t, 6), 2.05 (s, 3), 2.27 (bm, 2), 3.07 (t, 2, J = 7.5 Hz), 4.0–4.5 (m, 6), 4.77 (dd, 1, J = 7.5 Hz), 6.55 (bd, 1, J = 7.5 Hz), 7.90 (bs, 1), 8.00 (s, 1). As shown in Table I, the efficient oxidation of other thiazolines has also been achieved with NiO_2. Acid hydrolysis of thiazole **3** afforded a new compound (**4**) in 95% yield, identical with Jadot's mushroom acid.[19]

The mild, selective nature of the dehydrogenations achieved with NiO_2 can be judged by the successful conversion of

Scheme I



phleomycin A_2 to bleomycin A_2 (Scheme I).[20] The phleomycin molecule, which has substantial solubility only in water and stability only at neutral pH, is a complex, densely functionalized molecule.[4] Exacting requirements are thus made of any oxidant utilized for the conversion of phleomycin to bleomycin, since it must have a high degree of selectivity under a narrow range of conditions. Phleomycin A_2 was oxidized in neutral, aqueous solution by stirring with portions of nickel peroxide at room temperature. The course of the dehydrogenation was monitored by the increase in λ_{max} 290 nm and concomitant decrease in λ_{max} 242 nm;[21] analysis of the supernatant revealed 83% conversion to bleomycin A_2. The purified reaction product was shown to be identical with bleomycin A_2, as judged by proton NMR and chromatography on paper and cellulose TLC in five different solvent systems.[22] Parallel oxidation with MnO_2 revealed <30% conversion of phleomycin A_2 to bleomycin A_2 and much more extensive loss of material by irreversible adsorption to the oxidant.

Since NiO_2 has not been utilized as a reagent for heterocyclic dehydrogenations, we have begun to examine its reaction with partially unsaturated heterocycles. Several examples are included in Table I.

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Supplementary Material Available: Details of preparation procedures for compounds **1**, **2**, **3**, and **4** and for oxidation of compounds listed in Table I (4 pages). Ordering information is given on any current masthead page.

References and Notes

(1) (a) M. Ebato, K. Miyazaki, and H. Otsuka, *J. Antibiot.*, **22**, 423 (1969); (b) Y. Wikasaka, T. Nagasaki, and H. Minato, *ibid.*, **26**, 104 (1973).
(2) B. Anderson, D. C. Hodgkin, and A. Viswamitra, *Nature (London)*, **225**, 233 (1970).
(3) (a) A. T. Fuller, *Nature (London)*, **175**, 722 (1955); (b) M. N. G. James and K. J. Watson, *J. Chem. Soc. C*, 1361 (1966).
(4) (a) T. Takita, Y. Muraoka, T. Yoshioka, A. Fujii, K. Maeda, and H. Umezawa, *J. Antibiot.*, **25**, 755 (1972); (b) H. Umezawa, *Pure Appl. Chem.*, **28**, 665 (1971).
(5) J. Jadot, J. Casimir, and R. Warin, *Bull. Soc. Chim. Belg.*, **78**, 299 (1969).
(6) D. A. McGowan, U. Jordis, D. K. Minster, and S. M. Hecht. *J. Am. Chem. Soc.*, **99**, 8078 (1977).
(7) (a) J. Walker, *J. Chem. Soc. C*, 1522 (1968); (b) N. A. Fuller and J. Walker, *ibid.*, 1526 (1968).
(8) Reversible cyclization of simple *N*-acylcysteines in concentrated, aqueous mineral acid has been demonstrated repeatedly [see, e.g., ref 7; W. Stoffel and L. C. Craig, *J. Am. Chem. Soc.*, **83**, 145 (1961); H. A. Smith and G. Gorin, *J. Org. Chem.*, **26**, 820 (1961)]. However, decomposition of the thiazolines upon neturalization or dilution renders the procedure preparatively useless [see also R. E. Barnett, *Acc. Chem. Res.*, **6**, 41 (1973)].

Several Lewis acids have also been utilized for the preparation of relatively simple thiazolines [e.g., phosphorous pentachloride: S. Gabriel, *Ber.*, **24,** 1110 (1891); S. Gabriel and W. Coblentz, *ibid.*, **24,** 1122 (1891); phosphorous pentoxide: S. Gabriel, *ibid.*, **49,** 1110 (1916); phosphorous oxychloride[14]], but were found to be ineffective for the dehydrative cyclization of cysteinyl peptides. See, however, Y. Hirotsu, T. Shiba, and T. Kaneko, *Bull. Chem. Soc. Jpn.*, **40,** 2950 (1967); **43,** 1564)1970).

(9) Reagents employed have included sulfur [F. Asinger, H. Diem, and A. Sin-Gun, *Justus Liebigs Ann. Chem.*, **643,** 186 (1961)], potassium ferricyanide,[7] mercuric acetate,[7] hydrogen peroxide, potassium dichromate [F. Asinger, M. Thiel, and L. Schroeder, *ibid.*, **610,** 49 (1957)], cupric sulfate,[14] ferric chloride [W. D. McElroy, E. H. White, F. McCapra, and G. F. Field, *J. Am. Chem. Soc.*, **83,** 2402 (1961)], MnO_2, and various quinones [M. A. Barton, G. W. Kenner, and R. C. Sheppard, *J. Chem. Soc. C,* 1061 (1966)].

(10) M. Calvin, "Symposium on Glutathione", Academic Press, New York, N.Y., 1954, p 3.

(11) E.g., an attempt to isolate the thiazoline by concentration of the acidic solution over P_2O_5 resulted in a mixture consisting of at least four products: I. Goodman and L. Salce, *Biochim. Biophys. Acta,* **100,** 283 (1955).

(12) Y. Hirotsu, T. Shiba, and T. Kaneko, *Biochim. Biophys. Acta,* **222,** 540 (1970).

(13) Compound **1** was prepared in 70% yield by treatment of glutathione with acetic anhydride and 1 M NaOH, followed by esterification with acetyl chloride in ethanol.

(14) J. C. Vederas, Ph.D. Thesis, M.I.T., 1973.

(15) Workup was simplified in these cases, involving only partition of the reaction mixture with aqueous carbonate and subsequent concentration of the organic layer.

(16) M. V. George and K. S. Balachandran, *Chem. Rev.*, **75,** 491 (1975), and references cited therein.

(17) (a) A. J. Fatiadi, *J. Chem. Soc., B,* 889 (1971); (b) *Synthesis,* 65 (1976); R. Konaka, S. Terabe, and K. Karuma, *J. Org. Chem.*, **34,** 1334 (1969).

(18) F. A. Cotton and G. Wilkinson, "Advanced Inorganic Chemistry", 2nd ed, Wiley-Interscience, New York, N.Y., 1966, pp 798, 837, 838. Consistent with this suggestion was the greater activity of NiO_2 in the conversion of benzaldehyde to benzoic acid and the single report of the use of NiO_2 for the oxidation of nitrogen heterocycles.

(19) The new compound, isolated as the acetate, had the expected NMR spectrum in D_2O [δ 1.93 (s, 3), 2.35 (bd, 2) 3.17 (t, 2), 3.82 (m, 1), 7.97 (s, 1)] and infrared and ultraviolet spectra identical with those reported. (The structures of **2–4** were also verified by low- and high-resolution mass spectrometry). The synthesis of compound **4** in seven steps was reported previously;[5] however, no experimental details or yields were given and both the thiazoline- and thiazole-forming steps involved procedures which we have found to be of marginal utility in related cases.

(20) Conversions of phleomycins D_1 and E to bleomycins B_2 and B_4, respectively, with MnO_2 in unspecified yield has been reported by Umezawa and his co-workers: T. Takita, Y. Muraoka, A. Fujii, H. Itoh, K. Maeda, and H. Umezawa, *J. Antibiot.*, **25,** 197 (1972); H, Umezawa, *Biomedicine,* **18,** 459 (1973).

(21) (a) T. Ikekawa, F. Iwami, H. Hiranaka, and H. Umezawa, *J. Antibiot., Ser. A,* **17,** 194 (1964); (b) H. Umezawa, Y. Suhara, T. Takita, and K. Maeda, *ibid.*, **19,** 210 (1966).

(22) After destruction of the remaining phleomycin A_2 with acid, bleomycin A_2 was treated with copper(II) chloride and purified by chromatography on Sephadex C-25.[21] The product was identical with authentic bleomycin A_2, but not with phleomycin A_2, in five chromatographic systems. See ref 11b; H. Umezawa, K. Maeda, T. Takeuchi, and Y. Okami, *J. Antibiot., Ser. A,* **19,** 200 (1966); T. Takita, Y. Muraoka, A. Fujii, M. Itoh, K. Maeda, and H. Umezawa, *J. Antibiot.*, **25,** 197 (1972). After removal of copper,[21b] the conversion was also verified by proton NMR.

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