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Registry No. (±)-2, 86064-46-2; 4, 120-57-0; (±)-5, 582-84-3; 6, 86046-45-9; 8, 86046-46-0; 9, 86046-47-1; 11, 86046-48-2; 12, 86046-49-3; 13, 86046-50-6; 14, 86046-51-7; (±)-15, 86064-47-3; (±)-16, 86101-22-6; (±)-17, 86101-23-7; (±)-18, 86116-85-0; [bis-(trifluoroacetoxy)iodo]benzene, 2712-78-9; 2,2,2-trichloroethyl chloroformate, 17341-93-4.

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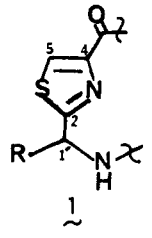
Absolute Configuration of Thiazole Amino Acids in Peptides

Summary: A general method is presented for determining the absolute configuration of 2-(1-aminoalkyl)thiazole-4-carboxylic acids, based on the reaction of thiazoles with $^1O_2$. This newly developed method is used to assign the absolute configuration of thiazole amino acids in the cytotoxic peptides isolated from the marine tunicate *Lissoclinum patella*.

Sir: Previously, we reported the structures and pharmacological profiles of a series of cytotoxic peptides from the tunicate *Lissoclinum patella*,[1,2] all of which contain 2-(1-aminoalkyl)thiazole-4-carboxylic acid moieties (1). These



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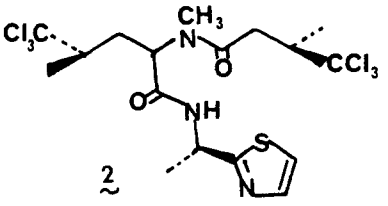

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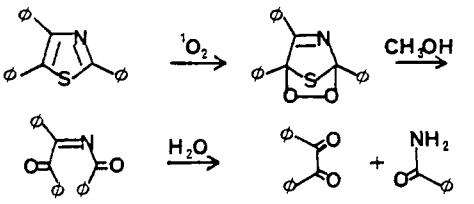
unusual sulfur-containing amino acids are believed to arise biosynthetically from the dehydrative cyclization of a cysteinyl peptide such that C-2 of the thiazole originates from the carboxyl of an amino acid on the N-terminal side of cysteine.[3] Inspite of the probability that thiazoles are derived from chiral precursors, uncertainty existed in the literature, for several years, regarding the chirality of the C-2 substituent. For example, the first thiazole amino acids isolated from the acid hydrolysis of thiostrepton[4] and bottromycin[5] were racemic. We have corroborated this result with the lissoclinum peptides, finding that the thiazoles isolated from 6 N HCl hydrolysis are racemic. Subsequent to degradation studies, the structure of thiostrepton was determined by X-ray studies, and the thiazoles were shown to have the *S* absolute configuration,[6] corresponding to a L-amino acid as the C-2 substitutent. In another X-ray investigation, Tursch's group assigned the *R* configuration to the thiazole in isodysidenin (2), a highly modified peptide from a marine sponge,[7] indicating the presence of a D-alanine in the precursor peptide chain. It is quite clear and not surprising that acid hydrolysis of a peptide results in racemization at C-1′ of a thiazole amino acid of the general formula 1. To our knowledge, the only previous method for determining absolute configuration of these amino acids in peptides was X-ray analysis. We now report a general and mild method for determining the

Scheme I. Reaction of Triphenylthiazole with $^1O_2$[a]



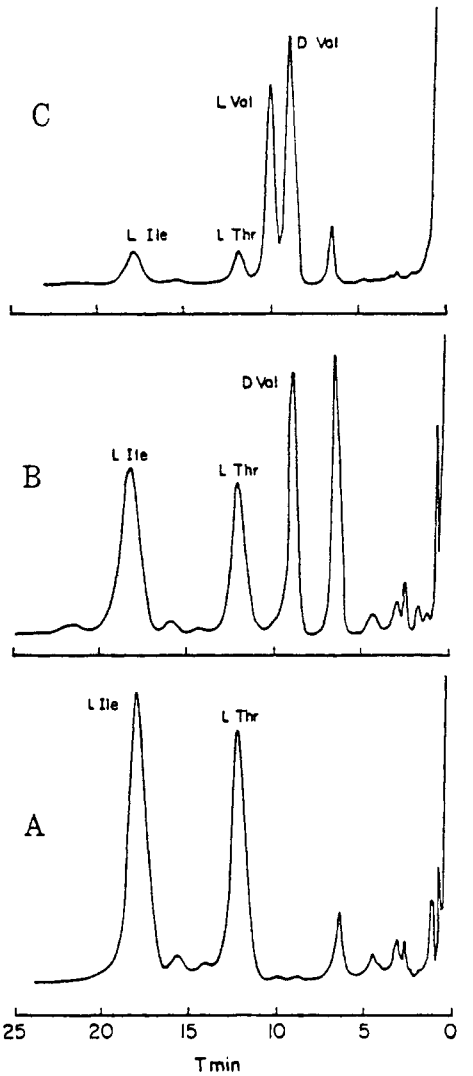


Figure 1. GC analysis of patellamide A (5) hydrolysis products as ME-TFA derivatives (column, SP-300 12 ft × 1/8 in.; program, 110–140 °C at 2°/min, 30 min at T_1. (A) 6 N HCl hydrolysis of 5 without $^1O_2$ pretreatment. L-Serine elutes at 30 min. Valine thiazole does not elute off this column. (B) 6 N HCl hydrolysis of 5 treated with $^1O_2$. (C) Coinjection of the previous sample with D,L-valine.

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(5) Waisvisc, J. M.; van der Hoeven, M. G.; te Nijenhuis, B. *J. Am. Chem. Soc.* **1957**, *79*, 4524–27.
(6) Anderson, B.; Hodgkin, D. C.; Viswamitra, M. A. *Nature (London)* **1970**, *225*, 233–35.
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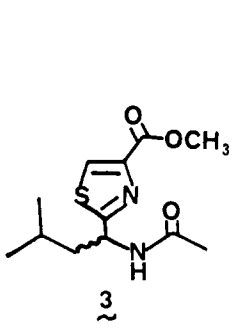
chirality of thiazole amino acids (1) in peptides and report that the thiazoles present in the lissoclinum peptides possess predominantly the *R* configuration.

Our initial inclination was that protonation of the ring nitrogen of the thiazole facilitates racemization at C-1′ via a relay-type mechanism involving the 2,3-double bond and that if the aromaticity of the system were destroyed prior to hydrolysis, the C-2 side chain could be carved out with retention of chirality. The desired product of such a scheme is the α-amino acid. It has already been demonstrated that the chirality of amino acids can be determined by GC using a chiral liquid phase.[2,8]

Wasserman reported several years ago that thiazoles undergo 4 + 2 cycloaddition with $^1O_2$ to give a thioozonide, which decomposes in MeOH to an amide such that C-2 of the thiazole becomes the amide carbonyl (Scheme I).[9] This reaction was attractive for several reasons, in addition to disrupting the aromaticity of the system: the desired oxidation state is retained at C-2 and acid workup should give the desired amino acid directly. To test the utility of this reaction, thiazole 3 obtained from the acid hydrolysis of ulithiacyclamide (4) was allowed to react with singlet oxygen,[10] followed by acid hydrolysis and conversion of the product to the methyl ester trifluoroacetate (ME-TFA). The product was a nearly 50:50 mixture of D- and L-leucine as determined by GC.[8] Interestingly, thiazole 3 exhibited an optical rotation $[\alpha]^D$ +5.3° but was also racemic.[11] In contrast, reaction of ulithiacyclamide (4) with $^1O_2$ followed by an identical workup gave 2 molar equiv of D-leucine, indicating that both thiazoles in 4 are chiral, possessing an *R* absolute configuration.[12] As expected, L-threonine and L-cystine were also obtained. Figure 1 illustrates the general applicability of this method. Direct comparison of total acid hydrolysates of a peptide with and without singlet oxygen treatment allows immediate identification of amino acids generated by $^1O_2$ degradation of a thiazole.[13] The absolute configuration of the new amino acid is determined by coinjection with standards. Ulithiacyclamide (4) and patellamides A–C (5–7) have been analyzed by using this method. All four peptides contain exclusively thiazoles with *R* absolute configuration.



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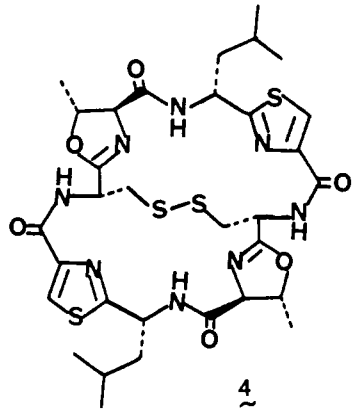

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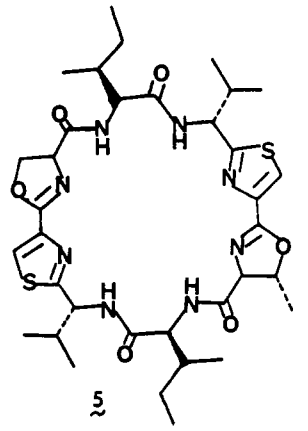

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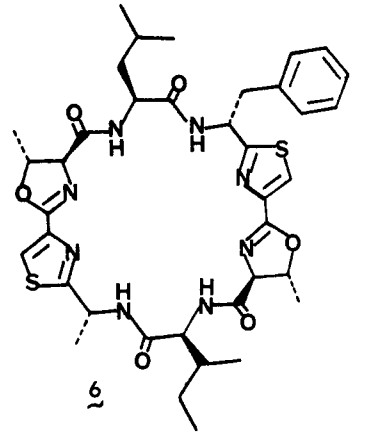

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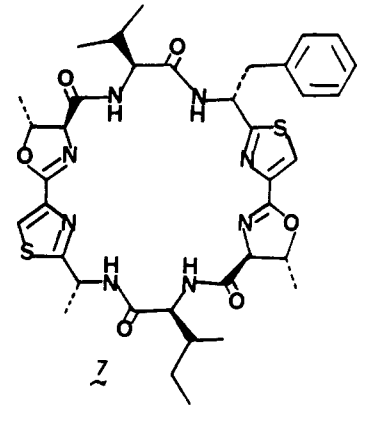

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The only limitation of the procedure as it now exists is that the GC column employed in the analysis (SP-300, Supelco) has a low temperature ceiling (140 °C), precluding the analysis of a small number of amino acids.[14] However,

(8) Charles, R.; Beitler, B.; Gil-Av, E. J. *J. Chromatogr.* **1978**, *112*, 121–33.

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(10) Wasserman, E.; Murray, R. W.; Kaplan, M. L.; Yager, W. A. *J. Am. Chem. Soc.* **1968**, *90*, 4160 and references therein.

(11) The enantiomeric composition of 3 was determined by ^1H NMR spectroscopy using a chiral shift reagent (Eu(hfc)$_3$, Aldrich).

(12) **General Procedure for Reaction of Peptides with $^1O_2$. A.** Triphenyl phosphite (2.0 g, 6.4 mmol) was dissolved in CH_2Cl_2 (100 mL) in a round-bottomed flask and cooled to −70 °C in a dry ice–acetone bath. A stream of ozone was bubbled through the solution until a deep blue ozone color persisted. A stream of N_2 was bubbled through the solution, still at −70 °C, to remove excess ozone. Immediately afterward, the flask was fitted with a connecting-hose adapter outleted to a second round-bottomed flask through Tygon tubing and a pipet. The second flask contained the peptide (5–10 mg) dissolved in CH_2Cl_2 (30 mL) at room temperature. The flask containing triphenyl phosphite ozonide was removed from the −70 °C bath and allowed to warm slowly to room temperature. Vigorous bubbling was observed almost immediately in both flasks. After gas evolution ceased, the progress of the reaction was monitored by TLC. The process was repeated if starting material remained. **B.** After the reaction was completed, the CH_2Cl_2 was removed under a stream of nitrogen and the crude product treated directly with 6 N HCl (6 mL) for 20 h at 100 °C in a Pyrex threaded bomb sealed with a Teflon brand screw cap. Afterwards, the HCl was removed in vacuo to give a mixture of amino acid hydrochlorides. The ME-TFA derivatives were prepared and analyzed by chiral GC.[2,8]

(13) The ME-TFA derivatives of D- and L-alanine chromatograph as shoulders on a ubiquitious peak at t_R 6.5 min (see Figure 1), complicating their identification. However, this problem is easily circumvented by preparing ethyl ester trifluoroacetate derivatives for analysis of peptides containing alanine thiazoles.

(14) Reference 8 reports the successful analysis of 17 amino acids.

in these cases alternate methods of analysis could be employed.[15]

Interestingly, Pettit recently speculated that the glutamine thiazole present in dolastatin, a cytotoxic peptide isolated from a marine mollusc, has the *S* configuration on the grounds that *S* is the natural configuration.[16] Our results certainly indicate that such speculation should be avoided.

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Registry No. 3, 86024-29-5; 4, 74847-09-9; 5, 81120-73-2; 6, 81098-23-9; 7, 81120-74-3.

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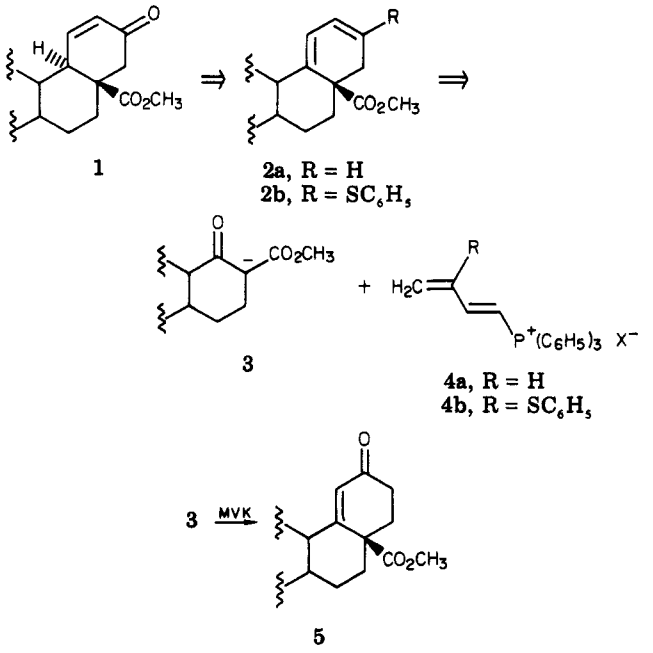
The Robinson Transposition Reaction.[1] Conjugate-Addition/Intramolecular Wittig Reactions of Enolates with [3-(Phenylthio)-1,3-butadienyl]triphenylphosphonium Chloride

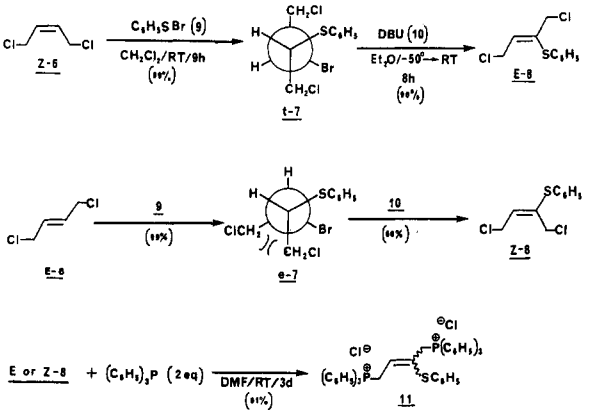
Summary: The phosphonium salt described reacts with certain enolates to produce dienyl sulfides that may be subsequently hydrolyzed to afford enones that are regiotransposed relative to the standard Robinson annulation product.

Sir: During the course of one of our synthetic projects we required a functionalized enone of the general structure **1** (Scheme I). As can readily be seen, **1** is a transposed version of the enone **5** routinely prepared via the Robinson annulation process. Although it seems reasonably likely that transformation of **5** to **1** would be possible,[2] we wished to effect a more direct conversion of **3** to enone **1**. Previous experience by Büchi[3a] and ourselves[3b] concerning the conjugate addition of ketone enolates **3** to butadienyl phosphonium salt **4a** affording cyclohexadienes (**2a**) after an intramolecular Wittig reaction, led us to conclude that a [3-(phenylthio)-1-butadienylphosphonium salt (**4b**) should similarly afford a dienyl sulfide (**2b**) capable of hydrolysis to the requisite transposed enone **1**.[4,5]

Scheme I



Scheme II



Synthesis of an appropriate reagent (albeit not **4b** as a discrete and isolable substance) was accomplished as follows (Scheme II): Reaction of (*Z*)-1,4-dichlorobut-2-ene ((*Z*)-**6**) with phenylsulfenyl bromide (**9**) (generated by the reaction of diphenyl disulfide with bromine in methylene chloride[6]) affords *threo*-trihalo sulfide **t-7**[7,8] in essentially quantitative yield as a colorless oil. Similarly the isomeric dichloride (**E**)-**6** reacts with **9** to produce crystalline *erythro*-trihalo sulfide **e-7** (99% as oil, 77% as crystals, mp

(1) Bruceantin Support Studies. 3. For paper 2, see S. N. Suryawanshi, P. L. Fuchs, *Tetrahedron Lett.*, 4201 (1981).
(2) In point of fact, Watt et al. have attempted a Robinson annulation reaction on a substrate bearing functionality appropriate for Bruceantin synthesis. Although the Michael reaction proceeds smoothly, in this instance the intramolecular aldol/dehydration sequence fails completely. D. L. Snitman, M. Y. Tsai, D. S. Watt, *Synth. Commun.*, **8**, 195 (1978). See also: Pariza, R. J.; Fuchs, P. L. *J. Org. Chem.*, following paper in this issue.
(3) (a) G. Büchi, M. Pawlak, *J. Org. Chem.*, **40**, 100 (1975); (b) P. L. Fuchs, *Tetrahedron Lett.*, 4055 (1974).
(4) Other 4C + 2C approaches to heteroatom-substituted dienes have been reported by Martin. (a) The reaction of a (2-alkoxy)dienylphosphonium salt with ketone enolates to afford, after hydrolysis, an enone bearing the Robinson substitution pattern: S. F. Martin, S. R Desai, *J. Org. Chem.*, **43**, 4673 (1978). (b) The reaction of a [1-(methylthio)dienyl]phosphonate to afford noncyclized dienyl sulfides after *inter*molecular Wadsworth-Emmons reaction with a second carbonyl component: S. F. Martin, P. J. Garrison, *Synthesis*, 394 (1982).
(5) A 3C + 3C annulation approach to enones via conjugate addition of (2-alkoxyallylidine)triphenylphosphorane to enones followed by intramolecular Wittig reaction to yield a dienyl ether has been reported by Martin and Desai (S. F. Martin and S. R. Desai, *J. Org. Chem.*, **42**, 1664 (1977)).
(6) B. M. Trost and S. D. Ziman, *J. Org. Chem.*, **38**, 933 (1973).
(7) All new compounds have been characterized by a combination of ^{1}H NMR, ^{13}C NMR, ^{31}P NMR, IR, mass spectrometry, and/or combustion analysis. Yields refer to material of greater than 95% purity.
(8) Experimental details, including 360- and 470-MHz ^{1}H NMR and ^{13}C NMR spectral evidence for compounds **t-7**, (*E*)-**8**, **11**, **18**, and **19** are in the supplementary material.

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