of the cinnamoyl enzymes between water and a number of amines at pH 9.3 and 25 °C. The aminolysis-to-hydrolysis ratios obtained are summarized in Table I. For each amine studied, the ratio of second-order rate constants increases substantially as one proceeds from subtilisin to selenolsubtilisin. The transfer of the cinnamoyl group to butylamine rather than water, for instance, is 14000 times more efficient for selenolsubtilisin than for native subtilisin and more than 20 times more efficient than for thiol-subtilisin. Thus, by converting the active site serine into a selenocysteine it is possible to influence the *selectivity* for aminolysis over hydrolysis of the acyl-enzyme intermediate in a dramatic fashion. Kaiser and co-workers[13] have exploited the increased deacylation selectivity of thiolsubtilisin to catalyze peptide bond formation. Selenolsubtilisin, with its substantially higher aminolysis to hydrolysis ratios, might also find application as a specific peptide ligase[14] useful for convergent chemical syntheses of large proteins.

In summary, we have reengineered the active site of subtilisin by chemical conversion of the catalytically essential serine into a selenocysteine. Not only is the selenol group a valuable mechanistic probe but also through this transformation we have turned a proteolytic enzyme into an acyl transferase with selectivity properties suitable for a peptide ligase. In light of current interest[15] in naturally occurring selenoenzymes, we are now extending these studies to examine the redox and metal binding properties of selenolsubtilisin and are also applying our methodology to other protein active sites. We anticipate that the ability to tailor existing enzymes to perform specific functions through chemical modification will make entirely new enzymatic activities generally accessible for use in research, medicine, and industry.

Acknowledgment. Partial support of this work by the National Science Foundation (Grant CHE-8615992 to D.H.) is gratefully acknowledged.

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Investigations of the Biosynthesis of *trans*-(+)-*S*-1-Propenyl-L-cysteine Sulfoxide in Onions (*Allium cepa*)

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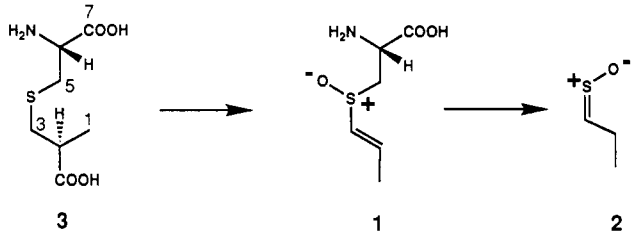
Received February 3, 1989

Both onions (*Allium cepa*) and garlic (*A. sativum*) contain a variety of novel sulfur compounds, some of which possess significant pharmacological activity.[1] The unusual amino acid *trans*-(+)-*S*-1-propenyl-L-cysteine sulfoxide (**1**) has been isolated from onions,[2] and it has been shown[2] to be the precursor of the lachrymatory principle characteristic of this plant, (*Z*)-propanethial *S*-oxide (**2**) (eq 1).[3] Investigations by Granroth[4]



3 **1** **2**

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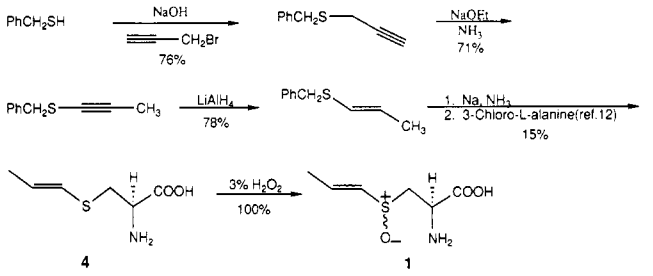
Scheme I



Scheme II

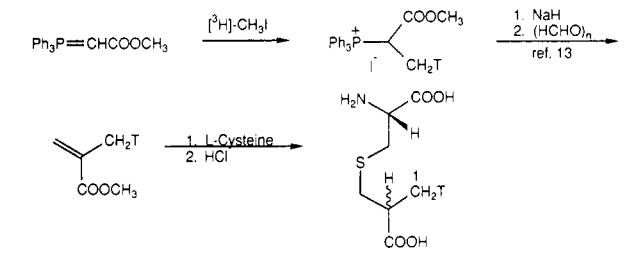


Table I. Administration of Labeled 2(*RS*)-*S*-(2-Carboxy-*n*-propyl)-L-cysteine to *A. cepa*

expt no.	labeling pattern in precursor **3**	isotope ratio in precursor **3**	isotope ratio in product **1**[a]	% ^3H retention
1	[^{35}S,1-^3H]	$^3H/^{35}S$=5.03	$^3H/^{35}S$=4.68	93
2	[1-^3H,5,6,7-^{14}C]	$^3H/^{14}C$ = 4.21	$^3H/^{14}C$ = 4.09	97
3	[^{35}S,2-^3H]	$^3H/^{35}S$ = 7.85	$^3H/^{35}S$ = 6.50	83
4	3(*RS*)-[^{35}S,3-^3H]	$^3H/^{35}S$ = 7.35	$^3H/^{35}S$ = 3.43	47

[a] In experiments with ^{35}S, the ratio is corrected for decay.

demonstrated that radioactivity from labeled (−)-*S*-(2-carboxy-*n*-propyl)-L-cysteine (CPC) (**3**) is incorporated into **1** by *A. cepa*, but rigorous evidence for the specific incorporation of **3** into **1** was not obtained. We now summarize the results of investigations that (a) provide unequivocal evidence for the specific incorporation of CPC into **1** and (b) place limitations on the number of mechanisms that can be envisioned for this interesting transformation.

In order to investigate the biosynthesis of the amino acid **1**, it was necessary to have access to adequate quantities of the compound for isotope dilution purposes. Attempts to isolate significant quantities of **1** from dehydrated onion powder[5] were unsuccessful, and a total synthesis of **1** reported[6] in 1975 proved to be unsatisfactory. We therefore devised the new synthesis of *trans*-*S*-1-propenyl-L-cysteine sulfoxide that is shown in Scheme I. The synthesis yields an approximately 1:1 mixture of diastereomeric sulfoxides. This mixture is adequate for dilution purposes, since it can be cleanly reduced[7] (70%) back to *trans*-*S*-1-propenyl-L-cysteine (**4**) using $K_3W_2Cl_9$. The sulfide **4** is considerably more stable than the corresponding sulfoxides, and it is therefore more suitable for isolation and purification after isotopic dilution with the sulfoxides.

Biosynthetic investigations began with experiments to prove that CPC is a specific precursor of **1**. 2(*RS*),6(*R*)-[1-^3H]CPC was synthesized in the manner outlined in Scheme II and mixed with 2(*RS*),6(*R*)-[^{35}S]CPC prepared by addition of [^{35}S]-L-cysteine to methacrylic acid.[8] The resulting doubly labeled precursor was administered to 3-week-old onion plants by the cotton wick method, and amino acid **1** was isolated by isotope dilution after 3 days. The crude amino acid was reduced to the

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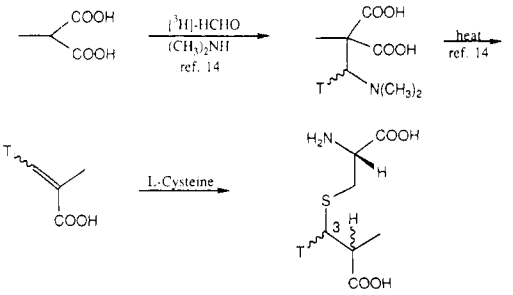
Scheme III



sulfide **4** with $K_3W_2Cl_9$. The reduced amino acid was purified by ion exchange (IR 120-plus) and by chromatography on cellulose, and it was then converted to the *N*-acetyl *p*-bromophenacyl ester. This derivative was recrystallized to constant radioactivity (0.25% incorporation) and constant isotopic ratio. The results, which are summarized in Table I (expt 1), demonstrate that C-1 to C-3 and the sulfur atom of CPC are incorporated specifically into **1**. Evidence for the intact incorporation of CPC into **1** was obtained by means of a double label experiment utilizing a mixture of 2(*RS*),6(*R*)-[5,6,7-^{14}C]CPC, prepared from methacrylic acid and [U-^{14}C]-L-cysteine, and the previously prepared [1-^{3}H]CPC. This doubly labeled precursor was converted into **1** without significant alteration of the isotopic ratio (Table I, expt 2).

The mechanism of the oxidative decarboxylation of CPC to *trans*-*S*-1-propenyl-L-cysteine sulfoxide was examined with the aid of two doubly labeled forms of CPC. The addition of L-cysteine to methacrylic acid in tritiated water yielded 2(*RS*),6-(*R*)-[2-^{3}H]CPC. The position of the isotopic label was confirmed by examination of the NMR spectrum of the corresponding deuterated amino acid prepared in D_2O. Administration of this form of specifically tritiated CPC to *A. cepa* in conjunction with 2(*RS*),6(*R*)-[^{35}S]CPC showed that there is no significant tritium loss from C-2 of CPC as the result of the oxidative decarboxylation (Table I, expt 3). This observation rules out the possibility that the reaction proceeds by dehydrogenation of CPC to (*Z*)-*S*-(2-carboxy-1-propenyl)-L-cysteine followed by decarboxylation of the α,β-unsaturated acid with retention of configuration.[9] The second experiment examined the fate of the hydrogen atoms present at C-3 of CPC. 2(*RS*),3(*RS*),6(*R*)-[3-^{3}H]CPC was synthesized by the route outlined in Scheme III. Administration of a mixture of this precursor and 2(*RS*),6(*R*)-[^{35}S]CPC to young onion plants yielded the amino acid **1** that exhibited ca. 53% loss of tritium (Table I, expt 4). This result suggests that the decarboxylation reaction proceeds with the stereospecific loss of one hydrogen atom from C-3 of CPC, and it rules out the intermediacy of a 3-keto CPC derivative.

The results of the preceding experiments suggest that the oxidative decarboxylation process associated with the biosynthesis of *trans*-(*S*)-1-propenyl-L-cysteine sulfoxide from CPC may be mechanistically related to the formation of the vinyl groups in heme and chlorophyll from propionic acid side chains.[10] The transformation also appears to resemble the formation of uneven numbered 1-alkenes from fatty acids in higher plants.[11] Additional studies of both a stereochemical and enzymatic nature will be required before a clear picture of the mechanism of formation of the amino acid **1** emerges.

Acknowledgment. We are pleased to acknowledge the support of this work by the National Science Foundation (CHE8604611) and the Robert A. Welch Foundation (C-729).

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Enantiomerically Pure Vinylketene Acetals as Dienes in the Diels-Alder Reaction†

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Received January 17, 1989

The asymmetric variant of the Diels-Alder reaction[1] has emerged in recent years as an important method for the construction of complex molecules in enantiomerically pure form. In most instances, diastereomeric transition states arise through the intervention of a chiral auxiliary attached to the *dienophile*. Conversely, there are few examples of the use of chiral *dienes* in the Diels-Alder reaction. While the first definitive experiments appeared in 1976,[2] it has only been in the last decade, through the work of Trost,[3] Kozikowski,[4] Frank,[5] McDougel,[6] and others,[7] that the synthetic versatility of this approach has become evident.

Our quest for an enantiomerically pure diene of general utility in cycloaddition reactions led us to consider vinylketene acetals such as **1**,[8,9] and in this communication we present the results of our preliminary experiments.

The rationale for the use of these reagents is presented in Scheme I, which depicts the normal endo transition state of the intermolecular Diels-Alder reaction. Due to the chirality of the system, approach of the dienophile from the top face of **1** (eq 1) is diastereomeric with approach from the bottom (eq 2). Within this context, steric hindrance between the electron-withdrawing group of the dienophile (Z) and the large -R group of **1** in the transition state of eq 2 will raise the energy of that reaction pathway relative to eq 1, where the complimentary interaction is between the relatively small hydrogen and the -Z group. This results in the net production of **2** at the expense of **3** and, consequently, net enantiomeric excess following ketal removal. Use of the opposite enantiomer of the chiral auxiliary results in production of the enantiomeric product.

The analysis above implies that the primary factor determining diastereomeric excess is steric,[10] which affords this approach simple predictive capabilities as to the major isomer produced. In addition, the product of the Diels-Alder reaction is a ketal,[11] fully

† Dedicated to Professor Carl Djerassi on the occasion of his 65th birthday.
‡ American Cancer Society Junior Faculty Research Awardee, 1987-1990.
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