mixtures in EtOH were spotted on silica gel TLC plates, and the plates were developed in 10% EtOH in $CHCl_3$ (a, b, c) or 5% EtOH in $CHCl_3$ (d). R_f values for standards in 10% EtOH in $CHCl_3$ were 0.63 (**13**), 0.61 (**12**), 0.57 (**11**), 0.51 (**9**), and 0.55 (**10**).

(a) 19 and 16. Spots were observed at R_f 0.62, 0.61, 0.56, and 0.51, corresponding to all four possible products (**13**, **12**, **11**, and **9**). Addition of each pure component showed a selective increase in fluorescence intensity of the corresponding spot in the mixture.

(b) 18 and 17. Spots were observed at R_f 0.63, 0.61, 0.57, and 0.51, corresponding to all four possible products as in a above.

(c) 9 and 13. Spots corresponding only to the two starting materials were observed at R_f 0.51 and 0.63, respectively.

(d) 16 and 14. Spots were observed at R_f 0.36 and 0.41, corresponding to a physical mixture of **10** (R_f 0.36) and **9** (R_f 0.42), respectively. Heating of **14** alone gave unidentifiable products which remained at the origin on TLC plates; **14** itself had R_f 0.26.

Note Added in Proof. It was recently reported that several ethyl benzylcyanoacetates were prepared from benzyl chlorides and ethyl cyanoacetate (in large excess) in the presence of sodium methoxide in methanol (Calas, M.; Pages, C.; Pastor, G.; Giral, L.; Despaux, E. *Eur. J. Med. Chem.* **1979**, *14*, 529).

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Isourea-Mediated Preparation of Dehydro Amino Acids

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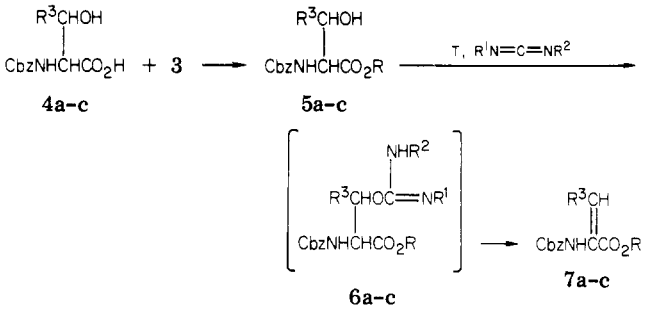
α,β-Dehydro amino acids are constituents of many recently discovered peptides including several fungal metabolites which possess antibiotic activity.[1] Dehydroalanine residues have also been implicated at the active sites of some enzymes.[2] In addition, the chemical reactivity of dehydroalanine generated from serine has been utilized for the site-specific cleavage of proteins.[3]

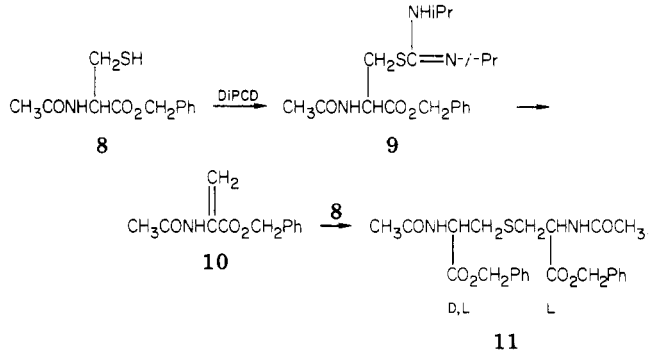
Of the many methods available for the synthesis of dehydro amino acids, β-elimination, the process which mimics the apparent biosynthetic process,[4] is most attractive since the elimination reaction can be performed as the final step on previously incorporated serine, cysteine, or threonine derivatives. Several methods for the preparation of dehydro amino acids by β-elimination processes have been described.[1,5,6] However, these methods often

Scheme I



a, R^3 = H, R = CH_3; b, R^3 = H, R = CH_2Ph; c, R^3 = CH_3, R = CH_3

Scheme II



are low yielding, multistep, require tedious purification steps to remove reagent side products, or incorporate unusual, difficultly obtained amino acid intermediates. Described here is a mild, efficient, and experimentally simple, isourea-mediated β-elimination process for the synthesis of dehydro amino acid derivatives from serine, cysteine, and threonine.

O-Alkylisoureas (**3**) have received increased attention as alkylating agents.[7] These reagents are conveniently prepared by the CuCl-catalyzed reaction of the corresponding alcohol **1** with a carbodiimide (**2**) (eq 1). How-

$$\underset{\mathbf{1}}{ROH} + \underset{\mathbf{2}}{R^1N{=}C{=}NR^2} \xrightarrow{CuCl} \underset{\mathbf{3}}{ROC(NHR^2){=}NR^1} \quad (1)$$

ever, attempts to isolate the isoureas (**6**) from the reactions of various carbodiimides and the hydroxyl group of serine and threonine derivatives **5** gave only the elimination products **7** (Scheme I).[8] Thus treatment of Cbz serine methyl ester **5a** with diisopropylcarbodiimide in the presence of 30 mol % (0.3 equiv) CuCl gave an 82% yield of the corresponding dehydroalanine **7a** after filtration, evaporation, and chromatography to remove the diisopropylurea. The results of other trials are given in Table I.

Several additional points are noteworthy. The use of a water-soluble carbodiimide [WSC, 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate] eliminated the need for chromatographic separation of the resulting urea. Peroxide-free solvents were required for both the reaction and workup since the unsaturated amino acid derivatives were found to polymerize rapidly in the

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Table I. Preparation of Dehydro Amino Acids

compd	CuCl, mol %	carbodiimide[a]	(mol %)	solvent	temp, °C	time, h	product	(% yield)[b]
5a	30	DiPCD	(110)	CH_3CN	40	6	7a	(82)
5b	4	DiPCD	(130)	CH_3CN	20	23	7b	(72)
5b	30	WSC	(110)	CH_2Cl_2	20	12	7b	(96.5)
5c	30	DiPCD	(110)	CH_3CN	40	24	7c (E + Z)	(65)[c]
8	30	DCCD	(110)	THF	50	3	10	(68)
8	90	WSC	(110)	CH_2Cl_2	20	12	10 + 11	(76-88) (12-20)
8	0	DiPCD	(110)	CH_3CN	20	5	11	(79)

[a] DiPCD = diisopropylcarbodiimide, WCS = the water-soluble carbodiimide 1-cyclohexyl-3-(2-morpholinoethyl)-carbodiimide metho-*p*-toluenesulfonate, DCCD = dicyclohexylcarbodiimide. [b] Isolated purified yields. [c] NMR spectrum of crude 7c showed a 1:1 mixture of E + Z; chromatographic separation gave the E and Z isomers in a 3:2 ratio. The geometry of these isomers was assigned by comparison of NMR and melting point data with that in the literature.[5,9]

presence of ether containing trace amounts of peroxide. The threonine derivative gave a 3:2 mixture of the E and Z isomers which could be chromatographically separated and characterized by comparison with literature data.[5,9] No reaction of the β-hydroxy amino acids **5a–c** occurred in the absence of CuCl. However, without added CuCl the cysteine derivative 8 gave the symmetrical thioether **11** in 79% yield as a mixture of two diastereomers. Since the starting material was an L-cysteine derivative, the formation of the diastereomers presumably occurred by an elimination–addition mechanism (Scheme II). In the presence of CuCl the dehydroalanine derivative **10** was preferentially formed from 8.

Some restrictions of this method of dehydro amino acid synthesis are apparent. The need to avoid peroxide-containing solvents has already been mentioned. Aqueous, alcoholic, and carboxylic acid solvents and similarly reactive substituents must be avoided since they would competitively consume the carbodiimide. A distinct advantage of this one-pot process, however, is the ability to use water-soluble reagents to facilitate the reaction workup.

Experimental Section

Melting points were taken on a Thomas-Hoover melting-point apparatus and are uncorrected. NMR spectra were determined in chloroform-*d* with tetramethylsilane as a reference, using a Varian A-60A spectrometer. Infrared spectra were recorded on a Perkin-Elmer Infracord. Mass spectra were recorded on a Dupont DP102 spectrometer. THF was distilled from $LiAlH_4$ directly before use. High-pressure liquid chromatography was performed with a Beckman/Altex Model 332 system. Elemental analyses were performed by Midwest Microlabs.

***N*-Protected amino acid esters 5a–c and 8** were prepared by reaction of the appropriate amino acid derivative **4a–c** and *N*-acetylcysteine, respectively, with the appropriate isourea **3**, according to the reported procedures.[7] These esterifications proceeded in high yield as described. However, the reaction of *N*-acetylcysteine with *O*-benzyl-*N*,*N*′-diisopropylisourea gave a mixture of products. Chromatographic separation (silica gel, ethyl acetate–hexane, 3:7) gave the desired *N*-acetyl-L-cysteine benzyl ester 8 in 44% yield: mp 78.5–79.5 °C (after recrystallization from ethyl acetate–hexanes); NMR δ 1.25 (t, SH), 2.05 (s, 3 H), 2.98 (dd, 2 H), 4.9 (m, 1 H), 5.23 (s, 2 H), 6.5 (br, NH), 7.4 (s, 5 H); mass spectrum (CI with CH_4), m/e 254 (M + 1). A prior chromatographic fraction also contained *S*-benzyl-*N*-acetylcysteine benzyl ester in 6% yield: mp 64–66.5 °C dec; NMR δ 1.96 (s, 3 H), 2.85 (d, 2 H), 3.66 (s, 2 H), 4.85 (m, 1 H), 5.17 (s, 2 H), 6.6 (br d, NH), 7.27 (s, 5 H), 7.34 (s, 5 H); mass spectrum (CI with CH_4), m/e 344 (M + 1). Competitive carboxyl and sulfhydryl alkylation by *O*-alkylisoureas has been previously observed.[1]

General Method of Preparation of Dehydro Amino Acids. The reaction conditions for the β-elimination processes are given in Table I. All solvents were dried before use and the reactions were carried out under a drying tube or a nitrogen atmosphere. When the water-insoluble carbodiimides were used the following workup was employed. One volume of ethyl acetate or methylene chloride was added and the precipitated urea was removed by filtration. The organic solution was then extracted with two separate volumes of water, dried over $MgSO_4$, filtered, and evaporated. The residue was chromatographed on silica gel. When the water-soluble carbodiimide was used the chromotographic purification could be avoided, and the product was purified by recrystallization or vacuum distillation. The products were characterized by comparison of their physical and spectral properties with those reported.[1,5,9] The assignment of the E and Z isomers **7c** obtained from **5c** was made by comparison of the reported melting point and NMR data.[9]

Diasteromeric Thioether 11. *N*-Acetylcysteine benzyl ester 8 (253 mg, 1 mmol) was dissolved in 5 mL of CH_3CN and stirred under nitrogen at room temperature while 126 mg of freshly distilled diisopropylcarbodiimide (DiPCD) was added. The solvent was then evaporated, and the residue was chromatographed on silica gel with ethyl acetate–hexanes (1:1) and then pure ethyl acetate. The symmetrical thioether 11 was thereby obtained in 79% yield as a mixture of diastereomers: mp 128–138 °C (after recrystallization from ethyl acetate–hexanes); NMR δ 2.0 and 2.04 (2 s, 3 H total), 2.95 (d, 2 H), 4.8 (m, 1 H), 6.7 (br d, NH), 7.4 (s, 5 H). The two singlets at δ 2.0 and 2.04 correspond to the acetyl methyl groups of the two diastereomers. High-performance LC (RP-18 column; 70% CH_3OH, 30% H_2O at 1 mL/min) gave retention times of 5.4 and 5.6 min. With 65% CH_3OH the retention times increased to 15.5 and 16.6 min.

Anal. Calcd for $C_{24}H_{28}N_2O_6S$: C, 61.00; H, 5.97; N, 5.93; S, 6.79. Found: C, 60.63; H, 5.92; N, 5.92; S, 6.85.

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Registry No. L-**5a**, 1676-81-9; L-**5b**, 21209-51-8; L-**5c**, 57224-63-2; **7a**, 21149-17-7; **7b**, 59524-07-1; (*E*)-**7c**, 60027-55-6; (*Z*)-**7c**, 60027-61-4; L-**8**, 73908-42-6; **10**, 73908-43-7; D,L-**11**, 73908-44-8; L,L-**11**, 73908-45-9; *S*-benzyl-*N*-acetyl-L-cystein benzyl ester, 73908-46-0.

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Constituents of *Trichilia hispida* (Meliaceae). 2. A New Triterpenoid, Hispidone, and Bourjotinolone A

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In an earlier paper we reported the isolation and characterization of two major constituents, sapelins A (1) and

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