(36.76 mmoles) $AcONa \cdot 3H_2O$ were added consecutively and stirred for 24 h at about 20°C. The reaction mixture was diluted with water and treated with excess sodium carbonate. The impurities were extracted with ether. The aqueous alkaline solution was acidified with a solution of one part water and one part concentrated hydrochloric acid and extracted with EA. The extract was dried over $MgSO_4$ and evaporated in vacuum. The residue consisted of alkylation product (IX). This product was stirred with 30 ml acetic acid and 5 ml water and heated at reflux for 3 h. Then, 3 ml concentrated hydrochloric acid was added and the mixture was heated at reflux for an additional 3 h and evaporated in vacuum. The residue was treated with 10-15 ml of water, held at 0°C for 24 h, filtered, washed with water and dried in the air to give 1.4 g (38%) ketodiacid (X), mp 106-107°C (from EA) [5], R_f 0.74 (ether as the eluent, development of the spot with saturated aqueous $KMnO_4$). IR spectrum (ν, cm^{-1}): 1698 (CO_2H), 1703 (CO). PMR spectrum in CD_3OD (δ, ppm): 1.32 m ($(CH_2)_5$), 2.06-2.87 m ($2CH_2CO$, $2CH_2CO_2H$). The yield of ketodiacid (X) was 1.1 g (30%) when 0.8 ml (15.70 mmoles) bromine, 1.5 g (25.0 mmoles) urea, 5 g (36.76 mmoles) $AcONa \cdot 3H_2O$, and 2.2 g (15.26 mmoles) Meldrum's acid were used to treat 3 g (14.97 mmoles) ketoester (VII).

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PREPARATION OF α-DEUTERATED L-AMINO ACIDS USING E. coli CELLS CONTAINING TRYPTOPHANASE

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A preparative method has been developed to obtain a series of α-deuterated L-amino acids with high chemical yields and quantitative optical yields by stereospecific isotope exchange in D_2O by the action of E. coli cells with high tryptophanase action.

The study of the mechanisms of biological transformations of natural amino acids is markedly facilitated by the use of isotope-labelled substrates, in particular, α-deuterated derivatives. The chemical methods for the preparation of α-deuterated amino acids lead to the formation of racemic products [1]. Methods using the diastereomeric properties of complexes of transition metals with Schiff bases of amino acids [2, 3] permit the preparation of α-deuterated L-amino acids with high optical purity.

Enzymatic methods have been reported for the introduction of hydrogen isotopes into the α-positions of natural amino acids by stereospecific isotope exchange in water enriched by the corresponding isotope. This process is catalyzed by pyridoxal-5'-phosphate-dependent enzymes specific for the given amino acid [4, 5]. These methods require only mild conditions without the need for auxiliary steps and have high steric specificity. A significant disad-

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TABLE 1. Stereospecific Exchange of the α-Proton of L-Amino Acids in D_2O by the Action of Lyophilized *E. coli* B/1t7-A Cells Containing Tryptophanase (optical purity > 95%)

Amino acid	C, moles/liter·10^2	Cell mass, mg	Incubation time, h	Chemical yield, %
L-Valine	5	50	24	59
L-Leucine	5	50	24	64
L-Isoleucine	5	50	24	60
L-Norvaline	5	50	24	63
L-Norleucine	5	50	24	58
L-Methionine	10	50	24	76
L-Phenylalanine	10	50	24	60
L-Histidine	5	100	24	55
L-Arginine	5	250	48	45
L-Glutamic acid	10	100	24	0
L-Aspartic acid*	10	100	24	20
L-Cysteine*	5	50	24	0
L-Threonine*	5	50	24	0
3-Nitro-L-tyrosine	10	50	24	65
3-Amino-L-tyrosine	5	50	24	68

*Optical purity not determined.

vantage of enzymatic methods is the narrow substrate specificity of the enzymes such that the preparation of a broad range of products requires the use of a similarly broad set of expensive enzymes. The use of highly purified preparations markedly complicates the process and reduces its practical value.

The reaction of tryptophanase (KF 4.I.99.I) with many amino acids is accompanied by the appearance of a UV band at 500 nm, which indicates the formation of a quinoid complex with the loss of the α-proton [6]. This finding shows the possibility of isotope exchange in the α-position of amino acids upon their incubation with tryptophanase preparations. The capacity of this enzyme to form quinoid complexes with a large number of amino acids may be the basis for a method to introduce hydrogen isotopes into the α-position of natural amino acids with the retention of their configuration.

We studied the isotope exchange of a series of amino acids in D_2O by the action of lyophilically dried *E. coli* B/1t7-A cells. The cells of this strain contain a considerable amount of tryptophanase [7]. The results of the isotope exchange are given in Table 1, which shows that amino acids with an aliphatic side chain such as valine, leucine, isoleucine, norvaline, and norleucine as well as methionine, phenylalanine, and histidine undergo enantioselective isotope exchange, and the PMR spectra indicate that these amino acids do not undergo side reactions related to the presence of other enzymes in the cells. Large amounts of biocatalyst are not required for these amino acids, and the exhaustive exchange of the α-hydrogen by deuterium requires incubation for 24 h. The isotope exchange for L-arginine proceeds somewhat more slowly, while L-lysine does not undergo isotope exchange at all, which is in accord with the lack of the band at 500 nm in the spectrum of pure tryptophanase in the presence of L-lysine [6]. Side reactions occur extensively with some other amino acids such as aspartic acid, glutamic acid, cysteine, and threonine with the formation of unidentified products. This limits the use of this method for these amino acids.

The use of lyophilically dried *E. coli* cells as a source of tryptophanase permits us to avoid the laborious procedure of isolating and purifying the enzyme and provides for the preparation of a rather broad range of α-deuterated amino acids. The cells used were stored for three months without significant loss of activity.

EXPERIMENTAL

E. coli B/1t7-A cells possessing tryptophanase activity were grown as described in our previous work [8], washed with water, and subjected to lyophilic drying. The dried cells were stored at -4°C.

In order to determine the tryptophanase activity, a sample of dried cells was suspended in 0.1 M potassium phosphate buffer (pH 8.0) and then ultrasonically decomposed with cooling. The activity in the cell extract was determined as in our previous work [8]. The tryptophanase content was 280 units per 1 g dry cells.

The amino acid and lyophilically dried cells were added to 5 ml buffer in D_2O* containing 2.15% $K_2HPO_4 \cdot 3H_2O$, 0.034% KH_2PO_4, and 0.4 μmole pyridoxal-5′-phosphate in the amounts indicated in Table 1. The mixture was maintained for a given period at 30°C and stirred. The cells were removed by centrifugation, and the solution was analyzed by PMR spectroscopy. The extent of deuteration was determined relative to the intensity of the signals of the α-protons and the protons not undergoing exchange. The amino acid was separated on a column packed with Dowex-50 in the H^+ form and subjected to enantiomeric analysis by gas-liquid chromatography [10].

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SYNTHESIS AND X-RAY STRUCTURAL ANALYSIS OF 2-AMINO-4-CYANOMETHYLENE-5,5-DIMETHYLTHIAZOLINE

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The reaction of thiourea with 4-hydroxy-4-methyl-2-pentynenitrile in triethylamine is accompanied by heterocyclization and the formation of 2-amino-4-cyanomethylene-5,5-dimethylthiazoline, whose structure was established by x-ray structural analysis (XSA).

Activated acetylenes (acids and their esters, ketones, and nitriles), in contrast to acetylene [1], react with thiourea under mild conditions with the formation of both S- and N-derivatives [2-5]. The structure of the starting acetylene has a significant effect on the direction of the reaction. Cyanoacetylenes and thiourea gave thiodiacrylonitriles [4], while tertiary acetylenic alcohols in KOH/DMSO gave tetrahydropyrimidine-2-thiones [5].

In the present work, we studied the reaction of thiourea (I) with a tertiary cyanoacetylenic alcohol, namely, 4-hydroxy-4-methyl-2-pentynenitrile (II). This reaction would be expected to yield various derivatives of thiazoline, imidazolidine-2-thione, and dihydrofuran. We found that the nitrogen atom of thiourea (I) in triethylamine attacks the triple bond of alcohol (II) to form a heterocyclization product, namely, 2-amino-4-cyanomethylene-5,5-dimethylthiazoline (III). 5-Amino-2,3-dihydro-2,2-dimethyl-3-furanone (IV) was isolated as a side product

*The pH value for this solution determined potentiometrically with a glass electrode was 8.17, which corresponds to pD 8.57 [9].

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