Journal of Labelled Compounds — April-June — Vol. V, No. 2

Syntheses of L-β-Cyanoalanine-4-^{14}C, L-Asparagine-4-^{14}C, and L-Aspartic Acid-4-^{14}C *

Y.-H. GIZA and Charlotte RESSLER
Division of Protein Chemistry, Institute for Muscle Disease, Inc.,
New York, New York 10021, U. S. A.
Received on the 28th October 1968

SUMMARY

Syntheses are described from a common labeled intermediate of β-cyanoalanine, asparagine, and aspartic acid, each of the L *configuration and labeled with carbon-14 in carbon-four. Radiochemical purities were 96, 99.7, and 99 %. The common source of carbon-14 was ethyl acetyl-*DL*-β-cyanoalaninate-4-^{14}C, which was prepared from commercially available potassium cyanide-^{14}C by an extension of the studies of Atkinson and Hellmann and Folz.*

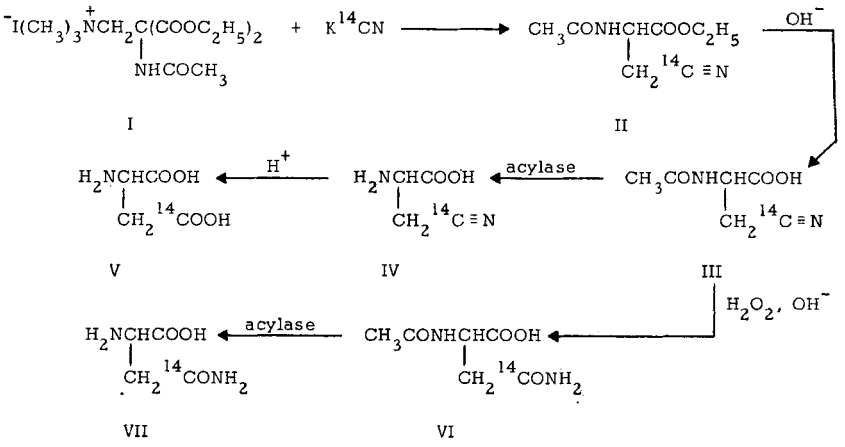
INTRODUCTION

Sources of L-β-cyanoalanine-4-^{14}C, L-asparagine-4-^{14}C, and L-aspartic acid-4-^{14}C were required for a metabolic study in certain *Lathyrus* and vetch plants that led to the elucidation of the role of β-cyanoalanine in the fixation of inorganic cyanide and in asparagine biosynthesis [1]. Possible routes to L-β-cyanoalanine-4-^{14}C could have involved condensation of bromoacetonitrile-1-^{14}C with ethyl acetamidomalonate [2] or dehydration of carbobenzoxy-L-asparagine-4-^{14}C [3]. The latter, however, would have required as starting material suitably labeled asparagine, which itself was not available; only DL-asparagine-2,3-^{14}C was being prepared at the time. Racemic aspartic acid-4-^{14}C was available *via* condensation of ethyl chloroacetate-1-^{14}C with ethyl acetamidomalonate [4] or of methyl bromoacetate-1-^{14}C with ethyl formamidomalonate [5].

Syntheses of β-cyanoalanine, asparagine, and aspartic acid, each with the L configuration and the isotope only in carbon-four, therefore were undertaken. The route developed was based on Atkinson's reaction of potassium cyanide with diethyl α-formamido α-dimethylaminomethylmalonate methio-

* This work was aided by Grant NB 04316 from the U. S. Public Health Service and by Muscular Dystrophy Associations of America.

dide to yield DL-aspartic acid [6]. Hellmann and Folz, studying the mechanism of the condensation of KCN with the analogous acetamido derivative I, isolated and characterized ethyl acetyl-DL-β-cyanoalaninate as the chief product [2]. This reaction now has been extended so that K^{14}CN serves as a readily available, labeled starting material for the condensation with I, and the coupling product, ethyl acetyl-DL-β-cyanoalaninate-4-^{14}C (II), serves as a common intermediate for the synthesis of L-β-cyanoalanine-4-^{14}C and L-asparagine-4-^{14}C, as well as L-aspartic acid-4-^{14}C *. The route to the three amino acids is outlined in the accompanying diagram.



Intermediates N-acetyl-DL-β-cyanoalanine-4-^{14}C (III) and N-acetyl-DL-asparagine-4-^{14}C (VI) obtained from II proved to be suitable substrates for stereospecific enzymatic deacylation by a slight modification of the general procedure of Greenstein and coworkers for resolving amino acids [8]. Asparagine and β-cyanoalanine thus were obtained in the desired L form; L-aspartic acid was obtained from the latter by acid hydrolysis. In view of their common derivation from II, the three amino acids are considered to have the isotope localized in carbon-four.

RESULTS AND DISCUSSION

The yield of II from the condensation of I with cyanide was lower than the 60 % reported in the literature [2]. However, additional product was isolated from the reaction mixture in the form of the de-esterified product, acetyl-DL-β-cyanoalanine-4-^{14}C (III), which made the condensation yield, after purification of these two products, at least 44 %. H^{14}CN escaping the condens-

* Since this work was undertaken, DL-aspartic acid-4-^{14}C has been synthesized in similar ways by condensing I and K^{14}CN. The condensation product was hydrolyzed directly [7a, b].

ation mixture was recovered in traps of potassium ethoxide in ethanol. Determined gravimetrically as silver cyanide by the procedure described by Prelog and coworkers [9], recovery amounted to 20-30 %. $H^{14}CN$ from several runs was combined and reused for another condensation with I. *

II was hydrolyzed in aqueous dioxane in the presence of an equiv of sodium hydroxide to give acetyl- DL-β-cyanoalanine-4-^{14}C (III) in nearly 80 % yield. This product, combined with III obtained directly from the condensation mixture, was treated with hog kidney acylase I. The enzymatic reaction was accelerated by the use of a pH (8.5) somewhat higher than customary and was complete within 4 hr. L-β-Cyanoalanine-4-^{14}C (IV) was isolated with the aid of a small column of Dowex-50 (H^+) and then crystallized from aqueous dioxane in 80 % yield.

Another portion of III was converted with hydrogen peroxide in alkaline solution to acetyl-DL-asparagine-4-^{14}C (VI), as with previous conversions into the corresponding asparagine compounds of carbobenzoxy-L- and DL-β-cyanoalanine-*carboxyl*-^{18}O [10] and certain fully protected β-cyanoalanine derivatives [11]. Conditions were somewhat more alkaline than in the ^{18}O work and the yield, which was 83 %, was higher. VI was treated similarly with hog kidney acylase I;** the liberated L-asparagine-4-^{14}C (VII) was isolated by crystallization and recrystallized in 87 % yield. Since an acidic radioactive contaminant was still present, the product was purified further by passing it through a small column of anion exchange resin.

L-Aspartic acid-4-^{14}C (V), obtained by hydrolysis of a portion of IV in 1 N HCl, was isolated by crystallization and then recrystallized in 67% yield.***

The L-asparagine and L-aspartic acid had the expected optical rotations. L-β-Cyanoalanine, which has a low rotation [3], was converted into its carbobenzoxy derivative with a correct rotation. Examined by electrophoresis on a paper strip that was then scanned for radioactivity, L-β-cyanoalanine-4-^{14}C, L-asparagine-4-^{14}C, and L-aspartic acid-4-^{14}C had radiochemical purities of 96 to 99 %.

Scope

This may be expected to include syntheses of *i* acetyl-D-β-cyanoalanine-4-^{14}C and acetyl-D-asparagine-4-^{14}C, by isolation from the enzymatic digest left after removing L-β-cyanoalanine-4-^{14}C and L-asparagine-4-^{14}C, respectively [8]; *ii* D-aspartic acid-4-^{14}C, by hydrolyzing the enzymatic digest left after

* Pichat and coworkers recently showed it to be advantageous to carry out the condensation of I and $K^{14}CN$ in a sealed tube [7b].

** Recently L-asparagine-2,3,3-d_3 was prepared by hydrolysis of acetyl-DL-asparagine-2,3,3-d_3 with hog kidney acylase. A longer reaction time at lower pH was used [12].

*** This product recently was subjected to β-decarboxylation with *Clostridium welchii* and was found to liberate 80 % of its radioactivity as CO_2. The apparent partial racemization during acid hydrolysis might be avoided, as indicated in preliminary work, by the use of enzymatic hydrolysis (see "Alternate routes").

removing L-β-cyanoalanine-4-^{14}C [8]; *iii* L-β-cyanoalanine labeled with ^{15}N in the cyano group and *iv* L-asparagine labeled with ^{15}N in the amide group, by substituting K^{15}N in the reaction with I; and *v* L-2,4-diaminobutyric acid-4-^{14}C, by reducing L-β-cyanoalanine-4-^{14}C with hydrogen over platinum under acidic conditions [13] or possibly in the presence of Rancy Nickel (7[b]), or with sodium-ammonia-methanol [14].

Alternate, biosynthetic routes

Economical syntheses of L-asparagine-4-^{14}C, L-β-cyanoalanine-4-^{14}C, and L-aspartic acid-4-^{14}C may now be based on the recently established biosynthetic fixation of inorganic cyanide in plants. Feeding K^{14}CN to seedlings of certain legumes and other plants can give 14-21 % yields of L-asparagine-4-^{14}C, and from it, L-aspartic acid-4-^{14}C [1, 15, 16]. Feeding K^{14}CN to *V. sativa* seedlings affords 27-63 % yields of γ-glutamyl-β-cyanoalanine-4-^{14}C, which quantitatively liberates L-β-cyanoalanine-4-^{14}C when treated with rat kidney γ-glutamyl peptidase or which can be hydrolyzed in acid to L-aspartic acid-4-^{14}C [1, 17]. Moreover, L-β-cyanoalanine-^{14}C and L-aspartic acid-^{14}C specifically labeled in carbons 1, 2, or 3 can likely be obtained through the same route if *V. sativa* seedlings are fed KCN *plus* L-serine-^{14}C labeled in carbons 1, 2, or 3, respectively [18, 19]. In preparing L-aspartic acid-4-^{14}C the possible occurrence of partial racemization during acid hydrolysis might be circumvented by hydrolyzing L-β-cyanoalanine-4-^{14}C or L-asparagine-4-^{14}C enzymatically with asparaginase. L-Aspartic acid-4-^{14}C has been prepared commercially *via* enzymatic condensation of phosphoenolpyruvate and $^{14}CO_2$ with carboxylase (Calbiochem, Los Angeles, Calif.).

EXPERIMENTAL

Potassium cyanide-^{14}C was purchased from Nuclear-Chicago Corp., Des Plaines, Ill. Hog kidney acylases I and II were obtained from Worthington Biochemical Corp., Freehold, N. J. Acylase II had been prepared as described [20] but had not been assayed.

Analysis of Products

Melting points were taken in open capillaries and are corrected. Optical rotations were taken in a 2-dm cell in a Rudolph polarimeter, Model 80. Elemental analyses were performed by Schwarzkopf Microanalytical Laboratory, Woodside, N. Y., and Micro-Tech Laboratories, Skokie, Ill. Elemental and electrophoretic-ninhydrin analyses, rotations, and melting points were on nonisotopic products of trial runs. Evaporations were carried out on a rotary evaporator at reduced pressure with a water bath below 30°. Extractions were as described for preparation of II.

Electrophoresis was carried out in sodium barbital buffer, pH 8.5, or pyridinium acetate buffer, pH 5.7, on strips of Whatman No.1 paper at 9-10 V/cm for 3 hr. The strips were developed with 0.15 % ninhydrin in acetone.

For determination of specific activities, compounds were combusted by the wet oxidation technique to carbon dioxide [21] which was precipitated, plated, and counted as barium carbonate. A Nuclear Chicago C-115 automatic low background counting system was used with a "Micromil" window and gas flow detector. Corrections for self-absorption were made. For determination of radiochemical purity, 100 μg of the labeled amino acids were electrophoresed, the strips were cut into segments, and scanned for activity in planchets as solids.

Diethyl α-Acetamido-α-dimethylaminomethylmalonate Methiodide (I)

This compound was prepared as described by Atkinson [6]. As noted elsewhere [22], it melted at 167.5-168.5° C (lit [6] 384° C).

*Ethyl Acetyl-*DL*-β-cyanoalanine-4-^{14}C (II)*

A 25 ml, rb, $T/_S$ 19/22, 3-necked flask was equipped with thermometer, reflux condenser, and gas capillary inlet-tube and was set up for magnetic

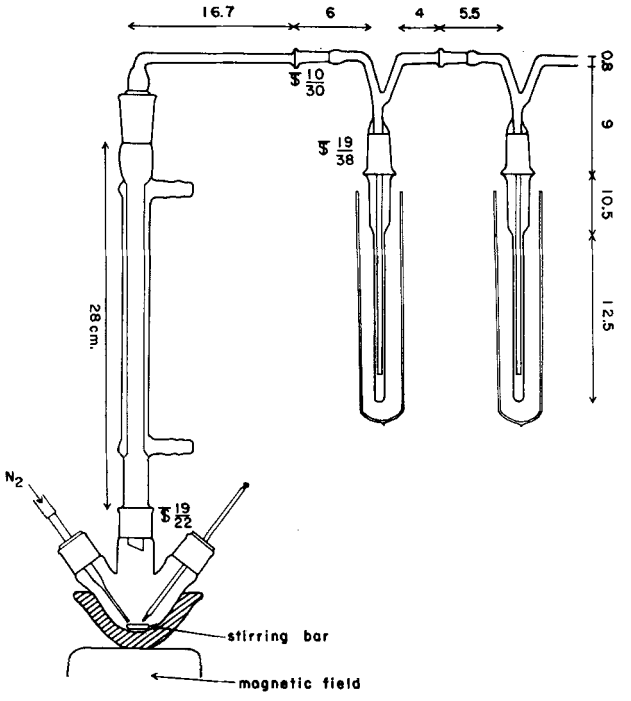


FIG. 1. Apparatus for preparing ethyl acetyl-DL-β-cyanoalaninate-4-^{14}C (II) from K^{14}CN and diethyl α-acetamido-α-dimethylaminomethylmalonate methiodide (I).

stirring. In it were placed 2 g (4.8 mm) of I, 5.73 mg (0.09 mm, 1.5 mCi) of $K^{14}CN$ dissolved in 0.25 ml of water, 469 mg (7.2 mm) of nonisotopic KCN, and 2.5 ml of 95 % alcohol. Connected at the top of the condenser (Figure 1) was a trap containing a solution of 74 mg (1.9 mm) of potassium in 2 ml of 95 % ethanol, and, in series, another trap with 39 mg (1 mm) of potassium dissolved in 2 ml of ethanol. The mixture was stirred in a moderate stream of nitrogen and heated with a mantle at 80° C for 2 hr.

The mixture was transferred to a $T/_S$ 24/40 20-ml test tube and concentrated to 5 ml of a mixture of solid and brown viscous liquid and extracted five times with 2-ml portions of benzene by forcing it through the mixture with a capillary pipette. The extracts were combined, dried, ($MgSO_4$), and then concentrated to a pale yellow, flaky solid or a yellow viscous liquid which was solidified by trituration with hexane and cooling; wt 325 mg (1.76 mm) (37 %) of II, mp 86.5-88.5° C. The product was crystallized from 5.5 ml of absolute ethanol; wt 292 mg (1.59 mm) (33 %), mp 88-89° C, lit [2] mp 89° C. Two such condensations were carried out, each with 1.5 mCi of $K^{14}CN$, and the products were combined.

Recovery of cyanide in traps was estimated from a "cold" run of 902 mg (2.17 mm) of I and 237 mg (3.64 mm) of KCN with a trap containing 84 mg (2.15 mm) of potassium in 2.5 ml of ethanol. The contents of the trap were diluted to 10 ml; to 5 ml were added 30 ml of 0.1 N KOH, and the solution was titrated with 0.1 N silver nitrate. A white solid precipitated after 2.55 ml had been added. Titration was continued cautiously until 3.35 ml had been added when some brown solid, presumably silver oxide, began to form. The white silver cyanide was collected by centrifugation, washed with water, then with acetone, and dried in a vacuum; wt 52.8 mg (0.39 mm) (22 %).

The contents of the first trap from both radioactive runs were combined and concentrated to dryness. The residue was diluted with 261 mg (4.0 mm) of nonisotopic KCN dissolved in 0.3 ml of water and 3 ml of 95 % ethanol, and 2 g of I (4.8 mm) were added. The mixture was refluxed under nitrogen for 2 hr as in the preparation of II already noted; it yielded 207 mg of crude and 147.5 mg (0.8 mm) (16.7 %) of recrystallized II of lower specific activity.

*Acetyl-*DL-*β-cyanoalanine-4-^{14}C (III)*

(*a*) *By hydrolysis of II.* A suspension of 597 mg (3.24 mm) of recrystallized II in 1.5 ml of freshly distilled dioxane in a 50-ml rb $T/_S$ 19/38 flask was stirred magnetically and 3.3 ml of 1 N NaOH were added dropwise at room temperature over a period of 1 hr. The mixture, which remained slightly alkaline, was stirred for an additional 2 1/2 hr, then adjusted to pH 7 with 6 N HCl, and concentrated to 1 ml. It was adjusted to pH 1 to 2 and extracted several times with 3-ml portions of ethyl acetate. The extracts were combined, dried ($MgSO_4$), and then concentrated to a white solid; wt 435 mg (86 %), mp 118-121° C. Recrystallization from 12 ml of ethyl acetate left III as short rectangles; wt 388 mg (77 %), mp 122-124° C.

Anal. Calcd for $C_6H_8N_2O_3$: C, 46.2; H, 5.17; N, 17.9. Found : C, 46.4; H, 5.27; N, 17.5.

(*b*) *Isolation of III from reaction mixture of II.* The brown two-phase concentrate of the original reaction mixture, after extraction with benzene to remove II, was concentrated to dryness; combined weight from both runs was 2 g. The test tube was connected, *via* glass tubing equipped with a stopcock and addition funnel, to a 15-ml test tube containing 272 mg of potassium in 6 ml of ethanol which served to trap residual $H^{14}CN$ and other volatiles. The system was evacuated, and 4 ml of 2 N H_2SO_4 were added to the residue. Both tubes were cooled in Dry-Ice baths, and the system was re-evacuated. The cooling bath surrounding the acidified mixture was removed, and it was allowed to concentrate in a vacuum in the closed system with magnetic stirring and occasional warming for 45 min.

The concentrate (1 ml) was extracted several times with 4-ml portions of ethyl acetate. The combined extracts were dried ($MgSO_4$) and then concentrated to dryness. The brown residue was taken up in 3 ml of water; the solution was heated and treated briefly with activated C, then concentrated to 0.5 ml, and extracted with ethyl acetate. The extract was dried ($MgSO_4$) and taken to dryness. The residue was cooled and triturated with hexane to a light brown solid; wt 253 mg, mp 113-116° C. Recrystallization from 3.5 ml of ethyl acetate yielded 169 mg (1.08 mm) of white solid; mp 121-122° C. Combined yield of purified III from *a* and *b*, based on I, is 37 %; based on $K^{14}CN$, 25 %.

(*c*) *Recovery as material of lower specific activity.* II, 101 mg, as second crops from recrystallization of II from both runs; II, 139 mg, of lower specific activity obtained from the condensation in which recovered $K^{14}CN$ was used; and nonisotopic II, 142 mg, were combined, suspended in 1.5 ml of dioxane, and hydrolyzed with 2.5 ml of 1 N NaOH as described under III*a*. The pale yellow crude product weighed 279 mg (86 %). This was combined with 114 mg of second crops of III from recrystallization of III derived from the initial hydrolysis of II (Section III*a*), and from recrystallization of III isolated from the original condensation mixtures (Section III*b*). The combined solids were recrystallized from 5.5 ml of ethyl acetate; wt 306 mg of III as a white solid. This served as starting material for acetyl-DL-asparagine-4-^{14}C (VI).

L-β-*Cyanoalanine-4-*^{14}C *(IV)*

Judged from the rate of uptake of base required to maintain the starting pH and checked occasionally by amino acid analysis, the rate of deacylation of III in 0.016 M solution with 7 % by weight of hog kidney acylase I at pH 8.5 was twice that at pH 7.0 at 1 hr. Hydrolysis of III was catalyzed also by a preparation of hog kidney acylase II [20] and was 85 % complete at pH 8.5 and 37° C within 37 hr. The rate was only one-fourth that with acylase I at 1 hr.

A solution of 300 mg (1.92 mm) of III and 110 mg (0.7 mm) of nonisotopic III in 160 ml of water was adjusted at the pH meter to pH 8.5 with 1.3 ml of 2 N LiOH. The mixture was stirred magnetically at 38° C, and acylase I, 28.9 mg, 900 units per mg, was added. The pH was maintained at 8.5 with 1 N LiOH as required (1.3 ml). Uptake of alkali ceased after 4 hr. Acetic acid, 1.0 ml, and C, 36 mg, were added. The mixture was stirred well and then filtered through a thin wet layer of C. The filtrate was concentrated nearly to dryness and taken up in 1.5 ml of water. Some insoluble material was removed by centrifugation. The solution was adjusted to pH 4.7 with 6 N HCl. The white solid (**A**) that formed was collected at the centrifuge and dried. The centrifugate was concentrated to dryness, and the residue was dissolved in a minimum volume of water. The solution, adjusted to pH 2 with 6 N HCl, was applied slowly to a column of Amberlite CG 120 (H^+) resin, 0.9 × 8 cm. The column was washed with water until a neutral and chloride-free effluent resulted and then was eluted with 1 N ammonia. Eluates were examined for β-cyanoalanine by the green spot test on paper with 0.15 % ninhydrin in acetone [3]. The β-cyanoalanine-containing eluates appearing near the breakthrough of base were combined and rapidly concentrated to dryness. The residue was dissolved in 1 ml of water, adjusted to pH 4.8, and again taken to dryness. This material and (**A**) were combined and dissolved in 0.75 ml of warm water. Freshly distilled dioxane was added slowly to incipient crystallization. After several hours in the cold, the white crystals were collected at the centrifuge; wt 120 mg (80 %); mp 215-216° C; lit [3] for synthetic material 218-218.5° C dec; lit [14] for the natural amino acid 214.5° C dec. Nonisotopic product, 81 mg, dissolved in 0.8 ml of water was stirred with 0.18 ml of carbobenzoxy chloride and 85 mg of magnesium oxide for 90 min. After one recrystallization, the carbobenzoxy-L-β-cyanoalanine had $[\alpha]_D^{22}$-46.2° C (*c* 0.9, dimethylformamide); lit [3] $[\alpha]_D^{22}$-45.2° C (*c* 0.93); lit [14] $[\alpha]_D^{23}$-46.0° C (*c* 0.47).

Electrophoresis at pH 8.6 located 96 % of the radioactivity in the position of β-cyanoalanine. The residual activity, 2.6 %, was present at the origin, and 1.4 % trailed from the origin to β-cyanoalanine. Specific activity was 7.82 × 10^5 cpm/mg, or 8.92 × 10^7 cpm/mmole.

*Acetyl-*DL*-asparagine-4-*^{14}*C (VI)*

N-Acetyl-DL-β-cyanoalanine (III), 300 mg (1.92 mm), derived from various combined mother liquors as described under III*c*, was dissolved with cooling in 1.2 ml of 2 N NaOH in a 50-ml $T/_S$ rb flask. To this 2.25 ml of 30 % hydrogen peroxide were slowly added, and the mixture was stirred magnetically at room temperature for 3 hr. The solution was adjusted to pH 7 with a few drops of 6 N HCl, then concentrated to 0.5 ml, acidified with approximately 15 drops of 6 N HCl, and cooled for 1 1/2 hr. The white solid was collected by filtration and washed with 80 % ethanol. Filtrate and washings were concentrated until crystallization started. Cooling gave a second crop;

wt of combined solids, 316 mg (95 %), mp 176-179° C. Recrystallization from 4.5 ml of 80 % ethanol gave 277 mg (83 %), mp 179-181° C; lit [12] for acetyl-DL-asparagine-2,3,3-d_3 176-177.5° C.

Anal. Calcd for $C_6H_{10}N_2O_4$: C, 41.4; H, 5.79; N, 16.1. Found: C, 41.3; H, 5.80; N, 15.5.

L-*Asparagine-4-*^{14}*C (VII)*

A solution of 277 mg (1.59 mm) of VI in 110 ml of water was adjusted to pH 8.5 with approximately 0.88 ml of 2 N LiOH. Acylase I, 21 mg, was added. The mixture was surrounded by a bath at 37° C and stirred magnetically. The pH was maintained near 8.5 by periodic addition of 1 N LiOH. After 22 hr, the solution was treated with 27 mg of C as described for the preparation of L-β-cyanoalanine. The filtrate was adjusted to pH 7, a trace of Dow Corning Antifoam B was added, and the solution was concentrated to dryness. The residue was taken up in the minimum of water, and the solution was adjusted to pH 5.4 and cooled. A small amount of amorphous solid was filtered off. Absolute ethanol was added at room temperature to incipient crystallization, and the mixture was cooled. The white crystals were collected by filtration and washed with a little ethanol; wt 114 mg (96 %). Recrystallization from water-ethanol followed by drying in a vacuum at room temperature left 104 mg (87 %) of the monohydrate; $[\alpha]_D^{25}$ + 29.8° C (*c* 2.1, 6 N HCl); commercial reference sample $[\alpha]_D^{22}$ + 30° C.

Electrophoresis at pH 8.6 gave a single yellow ninhydrin spot for asparagine. Electrophoresis at pH 5.7 indicated an acidic radioactive impurity. The material, 67 mg, was passed through a column of Amberlite IR-45 (acetate) resin, 0.7 × 27 cm, which was washed with 0.05 N acetic acid. Concentration and recrystallization yielded 37 mg (48 %; no attempt was made to improve yield). Electrophoresis at pH 5.7 located 99.7 % of the radioactivity in the position of asparagine. Specific activity was 2.31 × 10^5 cpm/mg or 3.47 × 10^7 cpm/mmole.

L-*Aspartic acid-4-*^{14}*C (V)*

In a 50-ml T/$_8$ rb flask equipped with reflux condenser were placed 25.3 mg (0.22 mm) of L-β-cyanoalanine-4-^{14}C (IV), 25.5 mg of nonisotopic IV, and 6 ml of 1 N HCl. The mixture was heated in a bath at 111° C for 4 1/2 hr, then cooled, and concentrated to dryness. Several milliliters of water were added and the solution was taken to dryness repeatedly. The white residue was dissolved in 1 ml of water, and the solution was adjusted from pH 1.5 to 2.8 at the pH meter with 10 % pyridine. The white crystalline solid which separated was collected by filtration and washed with a small volume of alcohol; wt 39.5 mg. The filtrate was cooled for several hours, when it yielded a further crop of 10 mg. The combined solids (84 %) were recrystallized from

water-ethanol; wt 39.7 mg (67 %); $[\alpha]_D^{24.5}$ + 25.0° C (*c* 0.35, 5 N HCl); lit [23] $[\alpha]_D$ + 25.4° C.

Electrophoresis at pH 8.6 showed a single blue ninhydrin spot with the mobility of L-aspartic acid. Electrophoresis at pH 5.7 located over 99 % of the radioactivity in the position of aspartic acid, with the residual activity at the origin. Specific activity was 3.63×10^5 cpm/mg or 4.83×10^7 cpm/mmole.

BIBLIOGRAPHY

1. (*a*) RESSLER, C., GIZA, Y.-H. and NIGAM, S. N. — *J. Am. Chem. Soc.*, **85** : 2874 (1963); (*b*) *ibid.*, in press.
2. HELLMANN, H. and FOLZ, E. — *Chem. Ber.*, **88** : 1944 (1955).
3. RESSLER, C. and RATZKIN, H. — *J. Org. Chem.*, **26** : 3356 (1961).
4. NOLLER, R. M. and TOLBERT, B. M. — In : *Organic Synthesis with Isotopes*, p. 252, A. Murray, III, and D. Williams, Eds., Interscience Publishers, Inc., New York, Part I, 1958.
5. WANG, S. C., WINNICK, T. and HUMMEL, J. P. — *J. Am. Chem. Soc.*, **73** : 2390 (1951).
6. ATKINSON, R. O. — *J. Chem. Soc.* (1952) 3317.
7. (*a*) LERMAN, M. I. and MAIMIND, V. I. — *Chem. Abstr.*, **56** : 3557 (1962). (*b*) PICHAT, L., HERBERT, M. and FABIGNON, C. — *Proc. 2nd Intern. Conf. on Methods of preparing and storing labelled Compounds, J. Lab. Compds*, Vol. II, n° 4, p. 427 (1966).
8. GREENSTEIN, J. P. — In : *Methods in Enzymology, Vol. III*, p. 554, S. P. Colowick and N. O. Kaplan, Eds., Academic Press, New York, 1957.
9. PRELOG, V., URECH, H. J., BOTHNER-BY, A. A. and WURSCH, J. — *Helv. Chim. Acta*, **38** : 1095 (1955).
10. KASHELIKAR, D. V. and RESSLER. C. — *J. Am. Chem. Soc.*, **86** : 2467 (1964).
11. LIBEREK, B. — *Chem. Ind. (London)*, (1961) 987.
12. BLOMQUIST, A. T., HISCOCK, B. F. and HARPP, D. N. — *J. Org. Chem.*, **31** : 4121 (1966).
13. ZAORAL, M. and RUDINGER, J. — *Coll. Czech. Chem. Commun.*, **24** : 1993 (1959).
14. RESSLER, C. — *J. Biol. Chem.*, **237** : 733 (1962).
15. BLUMENTHAL-GOLDSCHMIDT, S., BUTLER, G. W. and CONN, E. E. — *Nature*, **197** : 718 (1963).
16. TSCHIERSCH, B. — *Flora*, **153** : 115 (1963).
17. SASAOKA, K., LAUINGER, C., NIGAM, S. N. and RESSLER, C. — *Biochim. Biophys. Acta*, **156** : 128 (1968).
18. NIGAM, S. N. and RESSLER, C. — *ibid.*, **93** : 339 (1964).
19. RESSLER, C., NELSON, J. and PFEFFER, M. — *Nature*, **203** : 1286 (1964).
20. BIRNBAUM, S. M., LEVINTOW, L., KINGSLEY, R. B. and GREENSTEIN, J. P. — *J. Biol. Chem.*, **194** : 455 (1952).
21. VAN SLYKE, D. D. and FOLCH, J. — *J. Biol. Chem.*, **136** : 509 (1940).
22. SNOW, M. L., DOMBRO, R. S. and RESSLER, C. — *J. Org. Chem.*, **32** : 246 (1967).
23. MEISTER, A. — In : *Biochemistry of the Amino Acids, Vol.* 1, 2nd Ed., p. 141, Academic Press, New York, 1965.