[CONTRIBUTION FROM THE SCHOOL OF MEDICINE AND DENTISTRY AND THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF ROCHESTER]

A New Synthesis of DL-Lysine-6-C^{14} and DL-α-Aminoadipic Acid-6-C^{14} [1]

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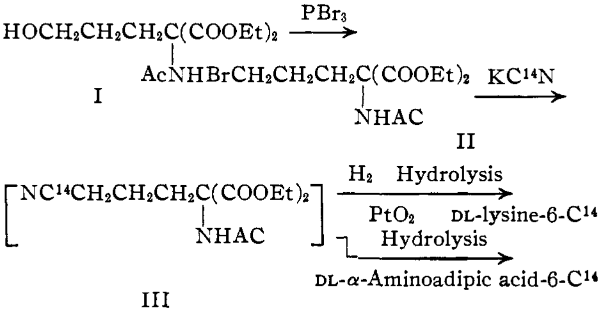
A new method for the preparation of DL-lysine-6-C^{14} and DL-α-aminoadipic acid-6-C^{14} has been described. The synthesis is based on the reaction of $KC^{14}N$ with ethyl α-acetamido-α-carbethoxy-δ-bromovalerate to form a nitrile which by reduction followed by hydrolysis yields DL-lysine-6-C^{14}. Direct hydrolysis of the same nitrile yields DL-α-aminoadipic acid-6-C^{14}.

A simple method for the preparation of DL-lysine-6-C^{14} was desirable since considerable amounts of the amino acid were required in this Laboratory for metabolic studies. A suitable precursor would be ethyl α-acetamido-α-carbethoxy-δ-bromovalerate (II), which after nitrilation with $KC^{14}N$ could easily be converted to DL-lysine-6-C^{14} by reduction and subsequent hydrolysis. Furthermore, DL-α-aminoadipic acid-6-C^{14} could be obtained by direct hydrolysis of the nitrile. Conversion of the bromo ester II to the corresponding monocarboxylic acid would offer the attractive possibility of optical resolution prior to the addition of radiocyanide, thus making possible a direct synthesis of the biologically desirable L-isomers of the amino acids.

Olynyk, *et al.*,[2] were unable to obtain II by condensing 1,3-dihalopropanes with acetamidomalonic ester. A more indirect route was chosen in our work, based on the bromination of ethyl α-acetamido-α-carbethoxy-δ-hydroxyvalerate (I).[3] This yielded the desired bromo analog II.



$$\underset{\mathrm{AcNH}}{\mathrm{HOCH_2CH_2CH_2C(COOEt)_2}}\ (\mathrm{I}) \xrightarrow{\mathrm{PBr_3}} \underset{\mathrm{NHAC}}{\mathrm{BrCH_2CH_2CH_2C(COOEt)_2}}\ (\mathrm{II}) \xrightarrow{\mathrm{KC^{14}N}}$$

$$\left[\underset{\mathrm{NHAC}}{\mathrm{NC^{14}CH_2CH_2CH_2C(COOEt)_2}}\right] (\mathrm{III}) \begin{cases} \xrightarrow[\mathrm{PtO_2}]{\mathrm{H_2}\quad \mathrm{Hydrolysis}} \text{DL-lysine-6-C}^{14} \\ \xrightarrow{\mathrm{Hydrolysis}} \text{DL-}\alpha\text{-Aminoadipic acid-6-C}^{14} \end{cases}$$

Treatment of II with potassium cyanide led to an oily product III which could not be crystallized. In view of this, optimum conditions for cyanation were determined by hydrolysis to the easily crystallized DL-α-aminoadipic acid without prior isolation of the nitrile. The best results were obtained by refluxing the cyanide with the bromo ester for two to three hours in 70% ethanol, or by allowing the mixture to stand for 5 days at room temperature. Both procedures led to a 44% yield of α-aminoadipic acid. Dilution of the ethanol to 50% had no noticeable effect, but shorter or longer periods of refluxing led to decreased yields. The use of methanol or ethylene glycol as solvent led to a darkening of the reaction mixture and lowered yields. Similarly, dropwise addition of either reactant to the other led to decreased yields of α-aminoadipic acid.

In spite of the relatively low yield, no starting material could be recovered and the theoretical amount of potassium bromide was always obtained from the nitrilation. Use of a 25% excess of bromo ester did not improve the yield. It thus appeared that in some manner, hydrobromic acid was being split off preventing the full utilization of potassium cyanide. In the radioactive preparation, the gases from the refluxing cyanation mixture were trapped in alkali resulting in a 45% recovery of radioactive cyanide as $KC^{14}N$, which could be used in further preparations.

Low pressure reduction of four runs of the crude non-radioactive nitrile with Adams catalyst in acetic anhydride yielded 27–29% of DL-lysine based on the total potassium cyanide added. A fifth run gave a 39% yield. For no apparent reason, the yield of DL-lysine-6-C^{14} (0.95 mc./g.) was only 21% based on total cyanide added. Since 45% of the radiocyanide was recovered, the actual yield of radioactive lysine, based on cyanide utilized, was 38%. No α-aminoadipic acid, which might arise from incomplete reduction of the nitrile, could be detected by two-dimensional paper chromatography (collidine–lutidine–water and phenol–water).

A low activity preparation of DL-α-aminoadipic acid yielded 41% of the isotopic amino acid. No attempt was made to recover the unutilized cyanide. Assuming a similar recovery of radiocyanide to that above, the yield would become 75%.

Experimental

γ-Acetamido-γ,γ-dicarbethoxybutyraldehyde.—This compound was prepared by the method of Moe and Warner[3] using benzene as the solvent. After neutralization with glacial acetic acid, the mixture was filtered and the benzene distilled *in vacuo* without heating to more than 60°. A nearly colorless viscous product resulted.

Ethyl α-Acetamido-α-carbethoxy-δ-hydroxyvalerate (I).—The aldehyde was reduced and isolated according to Moe and Warner[3] except that 6 times the reported amount of catalyst was used. The yield of alcohol was 82% based on crude aldehyde.

Ethyl α-Acetamido-α-carbethoxy-δ-bromovalerate (II).—A vigorously stirred solution of 20 g. of the crude hydroxy ester in 90 ml. of dry benzene was cooled to 16° in a water-bath, and a solution of 2.8 ml. of phosphorus tribromide in 20 ml. of dry benzene added dropwise over a period of 30 minutes. The mixture was then refluxed for 20 minutes, and the benzene evaporated on a steam-bath. The yellow residue was repeatedly extracted with portions of low boiling petroleum ether (b.p. 35–60°). The extracts, after being cooled in a refrigerator, yielded 16.8 g. (68%) of fine white needles, m.p. 58–59°. This material was used without further purification for the preparation of lysine and α-aminoadipic acid.

A sample recrystallized four times from low boiling petroleum ether melted at 60.5°.

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[1] This paper is based on work supported jointly by the United States Atomic Energy Commission under contract with the University of Rochester, W-7401-eng-49, and the Office of Naval Research, United States Navy.

[2] P. Olynyk, D. B. Camp, A. M. Griffith, S. Woislowski and R. W. Helmkamp, *J. Org. Chem.*, **13**, 465 (1948).

[3] O. A. Moe and D. T. Warner, THIS JOURNAL, **70**, 2763 (1948).

Anal. Calcd. for $C_{12}H_{20}O_5NBr$: C, 42.64; H, 5.96; N, 4.14. Found: C, 43.01; H, 6.14; N, 4.15.

Potassium Cyanide-C^{14}.—Radioactive barium carbonate-C^{14} (0.1686 g., 11.11 mc.), obtained from Oak Ridge National Laboratories, was converted to potassium cyanide-C^{14} in 86.8% yield by the sodium azide method.[4] By the addition of non-radioactive potassium cyanide, 0.0541 g. of this radioactive potassium cyanide, representing 9.64 mc., was diluted to 3.25 g. (50 mmoles, 0.19 mc./mmole).

Ethyl α-Acetamido-α-carbethoxy-δ-cyano-C^{14}-valerate (III).—To a solution of 3.25 g. of potassium cyanide-C^{14} (50 mmoles, 0.19 mc./mmole) in 49.5 ml. of water and 165 ml. of absolute alcohol, was added 16.20 g. (50 mmoles) of ethyl α-acetamido-α-carbethoxy-δ-bromovalerate, and the mixture refluxed for 2.25 hours. The hydrogen cyanide evolved during refluxing and subsequent solvent distillation was absorbed in a trap containing 35 ml. of 1.5 *N* sodium hydroxide (carbonate free). The reaction flask was placed in a refrigerator overnight. The solvent was then completely removed by distillation under diminished pressure, and the residue extracted with several small portions of ether. The residual potassium bromide corresponded to the theoretical yield.

Evaporation of the dried extracts (Na_2SO_4) yielded a dark oil, presumably ethyl α-acetamido-α-carbethoxy-δ-cyano-C^{14}-valerate.

DL-Lysine-6-C^{14} Monohydrochloride.—The crude cyano compound was dissolved in 60 ml. of acetic anhydride, 0.3 g. of platinum oxide (Adams catalyst) was added and hydrogenation was carried out at 60 p.s.i. and 50° for 12 hours, then 0.2 g. of PtO_2 was added and the hydrogenation continued for an additional 24 hours. At the end of this time, 50 ml. of ice-water was added to hydrolyze the acetic anhydride, and the mixture allowed to stand for 1 hour. The catalyst was filtered off, 1$^1/_2$ volumes of concentrated hydrochloric acid were added, and the mixture was then refluxed for 20 hours. The lysine dihydrochloride was isolated and converted to the monohydrochloride by the usual methods.[5] The yield of DL-lysine-6-C^{14} monohydrochloride was 1.90 g., which, after taking into account the recovery of 45% of the original radiocyanide, corresponds to 38% of theoretical.

DL-α-Aminoadipic Acid-6-C^{14}.—To a solution of 2 g. of ethyl α-acetamido-α-carbethoxy-δ-bromovalerate in 20 ml. of ethanol, was added 400 mg. of radioactive potassium cyanide in 5.4 ml. of water. An additional 2 ml. of water was used to rinse the last of the cyanide into the reaction flask. The mixture was refluxed for 2.5 hours, distilled to dryness *in vacuo*, and the residue extracted four times with ether. The residual potassium bromide weighed 0.73 g. (100%).

The ether extracts were evaporated and hydrolyzed for 5 hours with 30 ml. of concentrated hydrochloric acid. The resulting solution was distilled to dryness under diminished pressure. Addition of water and distillation to dryness was repeated twice, yielding a crystalline residue which was dissolved in a small amount of water and filtered. The volume was made up to about 12 ml. with water and 15 ml. of ethanol was added. The solution was treated with a slight excess of pyridine, stirred and placed in the refrigerator overnight. The white, crystalline DL-α-aminoadipic acid-6-C^{14} was filtered, washed with 50% ethanol until halogen free, and dried; yield 0.389 g. (41%). A paper chromatogram (phenol–water) showed one spot only, *Rf* = 0.33.

Benzoylation of material from a similar but non-radioactive preparation gave α-benzamidoadipic acid, m.p. 183.5–184.5° (lit.[6] m.p. 184–185°).

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[4] C. J. Claus, D. C. Camp, J. L. Morgenthau, Jr., P. Olynyk and R. W. Helmkamp, Abstracts of Meeting of Am. Chem. Soc., Buffalo, N. Y., March 1952, p. 51K.

[5] "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1941, p. 375.

[6] R. Gaudry, *Can. J. Research*, **27B**, 21 (1949).

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE UNIVERSITY OF ROCHESTER]

Studies on Model Compounds for Coenzyme A. A Kinetic Study of Aminolysis and Hydrolysis of Ethyl Thioacetate and β-Acetaminoethyl Thioacetate in Aqueous Solution

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A kinetic study of the reaction of *n*-butylamine with ethyl thioacetate (I) and with β-acetaminoethyl thioacetate (II) in aqueous solution showed the rate of hydrolysis of the thioesters in aqueous amine solution to be appreciable. The rate of disappearance of ester by aminolysis and hydrolysis was in agreement with the following rate equation: $-d([\text{Ester}]/dt) = k_4[\text{Ester}][\text{RNH}_2][\text{OH}^-] + k_5[\text{Ester}][\text{OH}^-]$. The rate of reaction of the ethyl thioacetate is similar to that of β-acetaminoethyl thioacetate, indicating little effect on the reaction rate of the amide group in the β-position. Bis-(β-acetaminoethyl) disulfide and N-*n*-butylacetamide have been isolated in high yield from the action of *n*-butylamine on β-acetaminoethyl thioacetate in aqueous solution.

The interest in this Laboratory in the general problem of the cleavage of the carbon–sulfur bond[1] has recently included kinetic studies of the alkaline and acid hydrolysis of thioesters.[2] The properties of this class of compound are at present of both biochemical and chemical significance, due to the discovery that Coenzyme A, which plays a key role in metabolism, is an N-acylated derivative of β-aminoethyl mercaptan.[3] The S-acylated Coenzyme A rapidly transfers its acyl group to other substrates (in the presence of the appropriate enzyme), and it was the purpose of the present work to relate, if possible, the high reactivity of the S-acylated Coenzyme A to structural features and thus explain its reactivity on purely chemical grounds.

The present paper describes kinetic studies of the aminolysis and hydrolysis of ethyl thioacetate (I) and β-acetaminoethyl thioacetate (II), using *n*-butylamine, to determine the effect on the reactivity of the thiolester of the amide group on the β-carbon.

$CH_3COSCH_2CH_3$ I $\qquad$ $CH_3COSCH_2CH_2NHCOCH_3$ II

The reactions were carried out in aqueous solution in order to approach biological conditions more

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[1] D. S. Tarbell and D. P. Harnish, *Chem. Revs.*, **49**, 1 (1951).

[2] (a) P. N. Rylander and D. S. Tarbell, THIS JOURNAL, **72**, 3021 (1950); (b) B. K. Morse and D. S. Tarbell, *ibid.*, **74**, 416 (1952).

[3] (a) J. D. Gregory and F. Lipmann, *ibid.*, **74**, 4017 (1952), and numerous earlier papers; (b) F. Lynen, E. Reichert and L. Rueff, *Ann.*, **574**, 1 (1952); (c) E. E. Snell, *et al.*, THIS JOURNAL, **72**, 5349 (1950); (d) J. Baddiley and E. M. Thain, *J. Chem. Soc.*, 3421 (1951).