(1.032 × 10^6 c.p.m./µmole). The gas phase was 95% O_2–5% CO_2, and the time of incubation was 3 hours at 34°. The conditions used were those found to be optimum for cholesterol biosynthesis.[2] To each preparation 5 mg. of senecioic acid was added prior to incubation. Following the incubation period, 15 mg. of senecioic acid and 0.5 mg. of cholesterol were added to each flask. Each specimen was saturated with KCl acidified to *p*H 2 with phosphoric acid, and extracted continuously with ether for 20–30 hours. The ether solution was evaporated to dryness and kept in a desiccator over KOH for 24 hours. The dark oily residue was extracted with a few ml. of 10% acetic acid and again extracted continuously with ether. The ether extract was afterwards evaporated to dryness. The second oily residue was transferred to a vacuum microsublimation apparatus and heated at 60° at 3 mm. for 48 hours. A colorless crystalline sublimate was obtained. The product (8–12 mg.) melted at 68–70°. A mixed melting point with authentic senecioic acid showed no depression. The sublimed acid was plated and assayed for radioactivity; the counts were corrected to infinite thinness. The acid was subsequently converted to α,β-dibromoisovaleric acid[7] and recrystallized (m.p. 105–107°). A mixed melting point with authentic dibromoisovaleric acid showed no change. Both the radioactive senecioic acid and α,β-dibromoisovaleric acid were diluted with carrier and recrystallized. Subsequent radioassay indicated the expected reduction in activity. Cholesterol was isolated as the digitonide and plated.[2] The slight discrepancy between the activities of the original senecioic acid and the dibromo derivative is within the range of counting errors. The average specific incorporation of acetate into senecioic acid was 3.35 × 10^{-4} µmole senecioic acid/µmole acetate/flask.

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[7] W. Massot, *Ber.*, **27**, (1894).

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The Synthesis of δ-Aminovaleric Acid-δ-C^{14},[1]

BY MORTON ROTHSTEIN

RECEIVED DECEMBER 28, 1953

The preparation of δ-aminovaleric acid-δ-C^{14} (or any C^{14}-radioisomer of δ-aminovaleric acid) has not been reported previously. Since the δ-labeled compound was required in this Laboratory for metabolic studies, its preparation was carried out by a new synthesis based on the following sequence of reactions

$$\begin{aligned}
&KC^{14}N + Br(CH_2)_3COOC_2H_5 \longrightarrow \\
&\qquad N^{14}C(CH_2)_3COOC_2H_5 \xrightarrow[Ac_2O]{PtO_2} \\
&\qquad\quad [AcNHC^{14}H(CH_2)_3COOC_2H_5] \xrightarrow{HCl} \\
&\qquad\qquad HCl\cdot H_2NC^{14}H_2(CH_2)_3COOH \xrightarrow{\text{Amberlite IR-4}} \\
&\qquad\qquad\qquad H_2NC^{14}H_2(CH_2)_3COOH
\end{aligned}$$

Because ethyl γ-cyanobutyrate is an oil, optimal conditions for the cyanation reaction were determined from the yield of glutaric acid obtained by hydrolysis of the ester. With this procedure it was found that by refluxing a slight excess of ethyl γ-bromobutyrate with potassium cyanide in 70% ethanol for 12 to 16 hours a 61% yield of glutaric acid was obtained based on the total amount of potassium cyanide added. Use of a 30% excess of bromo ester did not affect the yield.

During the radioactive preparation, gases produced by the cyanation reaction were bubbled through a solution of sodium hydroxide. It was found that by this procedure 36% of the cyanide originally present in the reaction mixture could be recovered. This is an amount similar to that recovered in the somewhat analogous preparation of lysine-6-C^{14},[2] and represents a considerable saving of radioisotope. The utilization of this technique should therefore be worthwhile in other cyanations involving isotopic cyanide.

In non-radioactive runs the isolated δ-aminovaleric acid hydrochloride contained small amounts of ammonium chloride, probably from the hydrolysis of unreduced nitrile. This impurity was successfully eliminated by treatment of the amino acid hydrochloride solution with the anion exchanger Amberlite IR-4, yielding a halogen free product.

Experimental

Ethyl γ-Cyanobutyrate-cyano-C^{14}.—A solution of 13.2 mg. (0.4 mc.) of $KC^{14}N$[3] in 2 ml. of water containing 8 mg. of potassium hydroxide was added to 2 ml. of water containing 790 mg. of inert potassium cyanide (95% minimum purity), making a total of 803 mg. (0.0124 mole) of potassium cyanide in 4 ml. of water. This solution was then added to a solution of 2.5 g. (0.0128 mole) of ethyl γ-bromobutyrate in 22 ml. of 95% ethanol. An additional 3 ml. of water in two portions was used to complete the transfer. The resulting solution was refluxed for 14 hours under a slow stream of nitrogen which finally was passed through 10 ml. of 10% sodium hydroxide. Argentimetric titration of this trapping solution indicated a recovery of 36% of the potassium cyanide from the reaction mixture.

The reaction mixture was distilled to dryness *in vacuo*. The distilling head was then washed down with a little absolute ethanol, the mixture once more taken to dryness, and the residual solid, presumably potassium bromide, extracted four times with a total of 40 ml. of dry ether. The combined extracts were filtered and evaporated in an air stream with gentle warming. The yellow oily residue was dissolved in 20 ml. of ethanol, boiled with a little Norite for 10 minutes, and then filtered. Evaporation of the solvent yielded a small amount of colorless oil, presumably ethyl γ-cyanobutyrate-cyano-C^{14}. In similar but non-radioactive runs, a 61% yield of glutaric acid (based on potassium cyanide) was obtained after hydrolysis of this material with hydrochloric acid.

δ-Aminovaleric Acid-δ-C^{14}.—The cyano ester was dissolved in 28 ml. of acetic anhydride and 250 mg. of platinum oxide was added. The mixture was hydrogenated in a Parr hydrogenator for 18 hours at 40° under a pressure of 50 lb./sq. in. The resulting mixture was filtered, distilled to dryness *in vacuo* and hydrolyzed for 5.5 hours with 20 ml. of concentrated hydrochloric acid. The solution was then distilled to dryness *in vacuo*, water added, and the distillation repeated. The residue was dissolved in 50 ml. of water and slowly passed through a column containing the anion-exchange resin IR-4 (carbonate form). The column was washed with distilled water until the solution emerging was both ninhydrin negative and free of radioactivity. The final volume was about 250 ml. This was distilled to dryness *in vacuo* and the residue which contained a yellow impurity from the resin was dissolved in ethanol, treated with Norite, filtered, and evaporated to dryness. The residue was dried thoroughly over phosphorus pentoxide and then treated with warm 95% ethanol to which acetone was added until the cloud point was reached. After standing overnight in a refrigerator, a faintly yellow solid was obtained. A small second crop was obtained by evaporation of the mother liquors to near dryness followed by the addition of 5 ml. of absolute ethanol to the residue. The total amount

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[1] This research has been supported in part by the United States Atomic Energy Commission under contract with the University of Rochester, W-7401-eng-49, and in part by funds from the Office of the Surgeon General.

[2] M. Rothstein and C. J. Claus, THIS JOURNAL, **75**, 2981 (1953).

[3] Purchased from Nuclear Instrument and Chemical Corp., Chicago, Illinois.

of δ-aminovaleric acid-δ-C^{14} obtained was 0.55 g. This represents a yield of 59% after taking into account the recovered cyanide. After trituration with a little cold absolute ethanol, 25 ml. of acetone was added and the product was obtained by filtration. Paper chromatography both with collidine–lutidine–water and phenol–water yielded only one ninhydrin reactive spot which corresponded in each case to that obtained with authentic δ-aminovaleric acid (Eastman Kodak Co.). From the radioactive preparation only one radioactive spot was obtained which coincided exactly with the ninhydrin reactive spot of δ-aminovaleric acid. The product was halogen free and melted at 155–156° (reported for δ-aminovaleric acid, 156°).

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Polyphosphoric Acid as a Reagent in Organic Chemistry. V. Conversion of Acids to Substituted Amides; Further Observations on the Lossen Reaction

BY H. R. SNYDER AND CLAYTON T. ELSTON[1]

RECEIVED JANUARY 21, 1954

In an earlier paper[2] it was postulated that the reaction of hydroxylamine with aromatic carboxylic acids in the presence of polyphosphoric acid proceeds to the amine by way of an intermediate hydroxamic acid. In agreement with this postulate it has been found that carboxylic acids react with weakly basic amines under similar conditions to yield the corresponding amides.

The yields of substituted amides formed from benzoic acid and various amines increase markedly as the basic strength of the amine decreases (Table I). Thus aniline was found to be unreactive while 2,4-dinitroaniline reacts almost quantitatively. The reaction is not limited to aromatic acids since acetic acid and 2,4-dinitroaniline give a 92% yield of 2,4-dinitroacetanilide.

The acylation of *p*-nitroaniline with various carboxylic acids was investigated. In these reactions, the ease of amide formation appears to increase as acid strength decreases. However, as shown in Table I, the correlation is not so clearly defined as that pertaining to the basic strength of the amines. Of the nitrobenzoic acids only the *meta* isomer reacts to give the amide and the yield is very low (8%). Neither *o*-nitrobenzoic acid nor the para isomer reacts with 2,4-dinitroaniline.

Further attempts were made to extend the amine synthesis[2] to aliphatic acids. With slightly modified conditions it was found that aliphatic acids do react with hydroxylamine to give low yields of the expected amines. *n*-Amylamine and cyclohexylamine were obtained in yields of 25 and 36%, respectively. The reaction, in contrast with that of aromatic acids, is difficult to control and gives considerable amounts of dark ether-insoluble oil.

Experimental

Table I gives the yields of crude amides obtained. The melting points are given as an indication of purity.

A. Amide Formation.—The preparation of *p*-nitroacetanilide is typical of the method used.

TABLE I

Acid	Amine	Amide		
		Yield, %	M.p., °C.	Reported m.p., °C.
Benzoic	Methoxyamine	0		
Benzoic	Aniline	0		
Benzoic	*o*-Chloroaniline	39	98–99	99
Benzoic	*p*-Nitroaniline	54	196–197	199
Benzoic	*o*-Nitroaniline	71	90–92	94
Benzoic	2,4-Dinitroaniline	98	196–200	200–202
Acetic	2,4-Dinitroaniline	92	119–120	121
Acetic	*p*-Nitroaniline	67[a]	213–214	214
o-Chlorobenzoic	*p*-Nitroaniline	64	180–182	180
p-Toluic	*p*-Nitroaniline	60	201–203	206.5
m-Nitrobenzoic	*p*-Nitroaniline	8[b]	248–249	249
o-Nitrobenzoic	*p*-Nitroaniline	0		
p-Nitrobenzoic	*p*-Nitroaniline	0		
Monochloroacetic	*p*-Nitroaniline	7[b]	184–185	185
o-Nitrobenzoic	2,4-Dinitroaniline	0		
p-Nitrobenzoic	2,4-Dinitroaniline	0		

[a] Recrystallized from a mixture of dimethylformamide and water. [b] Recrystallized from ethanol.

To a mixture of 3.5 g. of acetic acid and 4.0 g. of *p*-nitroaniline in a three-necked flask, fitted with a reflux condenser and stirrer, was added 70 g. of polyphosphoric acid. The flask was placed in an oil-bath and the temperature was raised to 160° in the course of 20 minutes. The mixture was then hydrolyzed by pouring it over 200 g. of crushed ice. The precipitated amide was filtered off, washed with water and then recrystallized from a mixture of dimethylformamide and water. The *p*-nitroacetanilide was obtained in the form of fluffy white crystals melting at 213–214°. The yield was 3.5 g. (67%).

B. **Amine Synthesis.**—*n*-Amylamine and cyclohexylamine were prepared by essentially the same procedure.

A mixture of 7.0 g. of hydroxylamine hydrochloride and 85 g. of polyphosphoric acid was placed in a three-necked flask fitted with a reflux condenser and a stirrer. The mixture was heated to 135°, held at that temperature until the evolution of hydrogen chloride had ceased and then 10.0 g. of cyclohexanecarboxylic acid was added. The vigorous evolution of carbon dioxide which occurred was complete in about ten minutes. The mixture was hydrolyzed by pouring it over 200 g. of crushed ice and the resulting brown solution was made strongly basic with potassium hydroxide. This caused the separation of a brownish-black oil. The mixture was then extracted continuously with ether for 15 hours. The extract was dried over potassium hydroxide and then distilled through a Holtzmann column. The fraction boiling at 136° was collected. The yield was 2.8 g. (36%). The identity of the amine was confirmed by its conversion to the corresponding phenylthiourea, melting point 148°.

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[1] Visking Corporation Fellow, 1953–1954.

[2] H. R. Snyder, C. T. Elston and D. B. Kellom, THIS JOURNAL, **75**, 2014 (1953).

Polyphosphoric Acid as a Reagent in Organic Chemistry. VI.[1] The Hydrolysis of Nitriles to Amides

BY H. R. SNYDER AND CLAYTON T. ELSTON[2]

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The use of 100% phosphoric acid in the hydrolysis of nitriles to acids has been known for some time.[3] Recently, however, the effectiveness of polyphosphoric acid in the Beckmann rearrangement[4] and in the acylation of amines[1] indicated the rather exceptional stability of amides in this reagent. The reaction of polyphosphoric acid with various nitriles was therefore investigated.

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[1] For the preceding paper, see H. R. Snyder and C. T. Elston, THIS JOURNAL, **76**, 3039 (1954).

[2] Visking Corporation Fellow.

[3] G. Berger and S. C. J. Olivier, *Rec. trav. chim.*, **46**, 600 (1927).

[4] E. C. Horning, V. L. Stromberg and H. A. Lloyd, THIS JOURNAL, **74**, 5153 (1952).