by these earlier workers. It is our opinion that much of this work must be re-investigated on a quantitative basis as outlined in this and in the preceding article of this series. We are now engaged in work of this nature in these laboratories.

Experimental

Hydrolysis of the Diacyls.—The hydrolyses of the mixed diacyls were carried out in aqueous sodium hydroxide solution using an excess of sodium hydroxide. The pure mixed diacyls both went into solution slowly, but there was a noticeably greater rate of solution on the part of the N-benzoyl-O-acetyl isomer. After solution was made complete by stirring and warming to 50°, the solutions were allowed to cool and the monoacylated products precipitated by the addition of 6 *N* hydrochloric acid dropwise until the solution was slightly acid to Hydrion paper. The precipitates were filtered off and dried, and in most cases a second crop of crystals was obtained by evaporation of the filtrates to dryness under vacuum. The first crop material was a light tan solid melting at about 150° over a range of 4–5°. There was no observable physical difference between the products obtained in this way from the two isomeric mixed diacyls.

Analysis of the Hydrolysis Products.—The analysis method used was essentially the same as that described in ref. (1) for the mixed diacyls. Using the same system of adsorbents and developing agents it was found that the *o*-acetylaminophenol was adsorbed near the top of the column while the *o*-benzoylaminophenol moved fairly rapidly down the chromatographic column. The same streak reagent was also effective in locating these adsorbate zones. The ultraviolet absorption peak at 284 mμ was used for quantitative estimation of the monoacetyl compound, and the absorption peak at 296 mμ was used for the monobenzoyl material. By using these wave lengths rather than the shorter wave length maxima, interference due to benzoic acid, which was found to contaminate the *o*-benzoylaminophenol adsorbate zone, was practically eliminated.

Summary

1. Contrary to previously published reports, the hydrolysis of the mixed acetyl–benzoyl diacyl derivatives of *o*-aminophenol in aqueous sodium hydroxide solution has been shown to yield both *o*-acetylaminophenol and *o*-benzoylaminophenol. Mixtures of these two products containing about 63% of the N-benzoylate were obtained irrespective of the relative amounts of the two isomeric mixed diacyls in the sample hydrolyzed.

2. The basic outlines of a new theory of the mechanism of acyl migrations has been given. This theory is based on modern organic theory involving a consideration of the relative inductive and resonance effects in the molecules concerned and good agreement has been found between the results reported in the literature and those predicted by theory.

3. A new principle has been developed for predicting which of several isomers will be the most stable. This principle has been called "the principle of minimum charge concentration" and states that the most stable isomer will be that one having the best distribution of charge over its reactive centers so that there is no one point of strong positive or negative charge.

4. In the light of our findings in this, and in the preceding article of this series, it seems desirable to re-examine much of the past work in this field.

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[CONTRIBUTION FROM THE STERLING-WINTHROP RESEARCH INSTITUTE]

A Synthesis of DL-Proline

BY NOEL F. ALBERTSON AND JEANNE L. FILLMAN[1]

Perhaps no amino acid is easier to synthesize—on paper—than proline. However, the reported solubility of proline in alcohol makes its isolation and purification somewhat more difficult than is the case with other amino acids. All of the recorded syntheses of proline make use of silver salts to remove halide ions, and most of the methods also make use of the copper salt of proline in the purification. This makes the preparation of proline both tedious and somewhat expensive. In addition, some of the methods previously reported did not give crystalline intermediates so that the final product in some instances also contained glycine or glutamic acid. In fact, in a chapter on amino acids published recently[2] Dunn and Rockland point out that there is at present no satisfactory laboratory procedure for the preparation of proline and that, "even today, the physical properties of analytically pure DL or L-proline have not been determined."

The method reported here affords a simple and practical synthesis of pure DL-proline, through the reactions diagrammed.

The over-all yield is approximately 20% when a single batch of starting material is carried all the way through to proline.[3]

The use of bromine in place of sulfuryl chloride to give the 3-bromo-3-carbethoxy-2-piperidone led to unsatisfactory results since the sodium bromide subsequently formed was difficult to remove (*cf.* ref. 13).

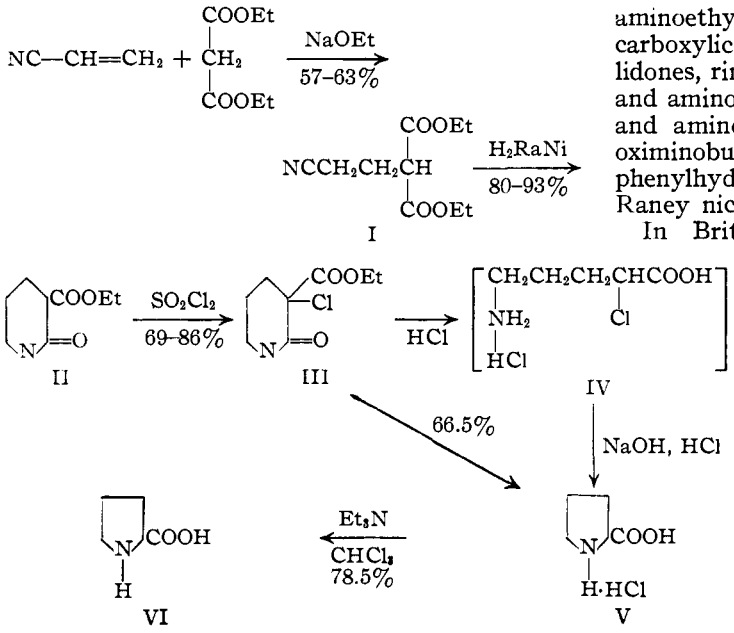
Amberlite resin may be used to liberate proline from its hydrochloride, but the method is not as satisfactory as the use of triethylamine.

It is interesting to note that we were unable to

(1) Present address: Mrs. M. Silvestrone, Scotia High School, Scotia, New York.

(2) Anson and Edsall, "Advances in Protein Chemistry," Vol. III, Academic Press, Inc., New York, N. Y., 1947, p. 322.

(3) Mikeska, in U. S. Patent 2,461,336 which became available to us during the revision of this manuscript, reports the preparation of I in 88% yield using equimolar quantities of malonic ester and acrylonitrile. This would give a 29% over-all yield of proline.



prepare proline by continued refluxing in acid solution of the 2-halo-5-aminovaleric acid hydrohalide. This suggests that Fischer's hydrolysis[4] of 2-bromo-5-benzamidovaleric acid did not give proline hydrochloride, but rather 2-bromo-5-aminovaleric acid hydrochloride which formed proline on treatment with silver sulfate.

Some of the preliminary experiments directed toward the synthesis of proline make it seem very improbable that Willstätter's synthesis[5] of proline from 3-bromopropylbromomalonic ester proceeds through the 3-aminopropylaminomalonic ester as postulated.[6] Reduction of 2-cyanoethylaminomalonic ester, which should proceed through the intermediate 3-aminopropylaminomalonic ester, gave not 2,2-pyrrolidinedicarboxylic ester, but rather 3-amino-3-carbethoxy-2-piperidone. Willstätter's intermediate was probably 3-bromopropylaminomalonic ester, an intermediate used by Putochin[7] in his synthesis of proline.

2-Cyanoethylaminomalonic ester was prepared from aminomalonic ester by forming the Schiff base with benzaldehyde, reacting with acrylonitrile and treating with dilute acid. Raney nickel was used for the reduction.

The 3-amino-3-carbethoxy-2-piperidone was identified by acetylation to give 3-acetamido-3-carbethoxy-2-piperidone. It did not depress the melting point of an authentic sample.[8]

Since 2-cyanoethylaminomalonic ester gave the piperidone on reduction it was thought that this might be due to steric factors, especially in view of the fact that certain substituted benzylaminoethylcyanoacetic esters give pyrrolidine carboxylic esters on reduction[9] rather than pyrrolidones, ring closure occurring between the nitrile and amino group in preference to the carbethoxy and amino group. Therefore, ethyl 4-cyano-2-oximinobutyrate and ethyl 2-oxo-4-cyanobutyrate phenylhydrazone were prepared and reduced with Raney nickel. Both gave 3-amino-2-piperidone.

In British Patent Application No. 35043 (Dec. 31, 1947) it is suggested that proline may be prepared from an acylaminomalonic ester by condensation with acrolein, hydrolysis and reduction. This route to proline was investigated in 1944, but appeared unpromising.

Experimental

2-Cyanoethylmalonic Ester (I).[3,10]—To a solution of 7 g. of sodium in 200 ml. of alcohol and 945 ml. of malonic ester there was added 208 ml. of acrylonitrile at such a rate that the temperature did not rise above 35°. The product distilled at 104–110° at 0.6 mm. Yields ranged from 57 to 63.5%, the last three experiments giving 420, 426 and 417 g., respectively. Approximately 530 g. of malonic ester and about 150 g. of diethyl bis-(2-cyanoethyl)-malonate are also obtained.

3-Carbethoxy-2-piperidone (II).[10]—A solution of 380 g. of 2-cyanoethylmalonic ester in 1300 ml. of ethanol was reduced using 7–10 g. of Raney nickel catalyst and hydrogen at 80° and 1000 lb. pressure. Reduction required one to six hours. The solvent was removed and the residue poured into one liter of Skelly B with stirring. The product was filtered and air-dried. Yields varied from 80 to 93%, the last three experiments giving 283, 272 and 281 g., respectively, of material melting above 75°.

When the reduction was carried out according to the method of Koelsch,[10] the cooled reduction mixture deposited crystals insoluble in benzene or acetone. Recrystallization from ethanol gave a compound melting at 194°. This proved to be the amide of the above piperidone.

Anal. Calcd. for $C_6H_{10}N_2O_2$: C, 50.69; H, 7.09; N, 19.71. Found: C, 50.62; H, 7.06; N, 19.33.

The major portion of the product could not be crystallized until after distillation.

Reduction of diethyl bis-(2-cyanoethyl)-malonate[11] in ethanol with Raney nickel catalyst gave 2,8-diazaspiro-(5,5)-hendecan-1,7-dione, melting at about 314° when recrystallized from water.

Anal. Calcd. for $C_9H_{14}N_2O_2$: C, 59.32; H, 7.74; N, 15.38. Found: C, 59.25, 59.54; H, 8.03, 7.73; N, 15.29, 15.29.

This compound has been prepared previously by Fischer and Bergmann from diethyl bis-(2-chloroethyl)-malonate and ammonia.[12]

Hydrolysis of 3-carbethoxy-2-piperidone (II) with concentrated hydrochloric acid for four hours gave 3-aminopropylmalonic acid, m. p. 181.5°.

Anal. Calcd. for $C_6H_{11}NO_4$: C, 44.71; H, 6.88; N, 8.69. Found: C, 44.90; H, 6.82; N, 8.61.

3-Chloro-3-carbethoxy-2-piperidone (III).—A solution of 145 ml. of sulfuryl chloride in 300 ml. of dry chloroform

(4) Fischer, *Ber.*, **42**, 1023 (1909).
(5) Willstätter, *ibid.*, **33**, 1160 (1900).
(6) "The Chemistry of Amino Acids and Proteins," Charles C. Thomas Co., Springfield, Ill., 1944, p. 84.
(7) Putochin, *Ber.*, **56**, 2213 (1923).
(8) Albertson and Archer, THIS JOURNAL, **67**, 2043 (1945).
(9) Bergell, Hindler, Morrison and Rinderknecht, U. S. Patent 2,405,555.
(10) This is a modification of the method reported by Koelsch, THIS JOURNAL, **65**, 2458 (1943).
(11) Bruson and Reiner, *ibid.*, **65**, 23 (1943).
(12) Fischer and Bergmann, *Ann.*, **398**, 124 (1913).

was added with stirring to a solution of 283 g. of 3-carbethoxy-2-piperidone in 400 ml. of chloroform at such a rate that the solution refluxed slowly. Removal of the chloroform left a residue which soon crystallized. It was dissolved in 175 ml. of hot ethyl acetate, and brought to turbidity with Skelly B (about 75 ml.). After cooling, 50 ml. of additional Skelly B was added. The product was filtered and dried. The yield was 236 g. (69.5%), m. p. 74–78°. Yields varied from 69 to 86%. An analytical sample melted at 79°.

Anal. Calcd. for $C_8H_{12}ClNO_3$: Cl, 17.20; N, 6.66. Found: Cl, 16.98; N, 6.79.

2-Chloro-5-aminovaleric Acid Hydrochloride (IV).—Two hundred and thirty-six grams of 3-chloro-3-carbethoxy-2-piperidone was refluxed five hours with 500 ml. of concentrated hydrochloric acid. The solution was charcoaled, filtered and concentrated *in vacuo*. The residue is used directly in the next step. A sample, recrystallized from alcohol–ether, melted at 124°.

Anal. Calcd. for $C_5H_{10}ClNO_2 \cdot HCl$: Cl, 18.85; N, 7.45. Found: Cl, 18.63; N, 7.28.

The analogous 2-bromo-5-aminovaleric acid hydrochloride (prepared by hydrolysis of the 3-bromo-3-carbethoxy-2-piperidone) melted at 115°.

Anal. Calcd. for $C_5H_{10}BrNO_2 \cdot HCl$: N, 6.02. Found: N, 6.29.

Proline Hydrochloride (V).—The crude residue (V) was dissolved in 200 ml. of water and made basic with a solution of 143 g. of sodium hydroxide[13] in 300 ml. of water. After two days at room temperature the solution was made acid to congo red paper with 315 ml. of concentrated hydrochloric acid. The solution was concentrated to dryness and the residue extracted with 400 and 300 ml. of hot absolute alcohol. The alcohol solution was concentrated *in vacuo* and the residue refluxed with 250 ml. of water and 50 ml. of concentrated hydrochloric acid for one hour. The solution was charcoaled, filtered and taken to dryness. The crude, dry proline hydrochloride weighed 161 g. It was recrystallized from 400 ml. of isopropyl alcohol and washed with 50 ml. of isopropyl alcohol. The yield of product melting at 148–151° amounted to 114 g. (66.5%). An analytical sample melted at 156°.

Anal. Calcd. for $C_5H_9NO_2 \cdot HCl$: Cl, 23.39. Found: Cl, 23.20.

In one experiment in which insufficient hydrochloric acid was added, the alcoholic extract of proline hydrochloride gave, on cooling, crystals melting at 189°. Treatment with hydrochloric acid converted these crystals to proline hydrochloride, identified by mixed melting point with an authentic sample. Treatment of the by-product (m. p. 189°) with phenyl isocyanate gave the same hydantoic acid as was obtained from proline. Treatment of proline with half an equivalent of hydrochloric acid gave on evaporation and recrystallization from ethanol, crystals melting at 189–190°. The melting point of this sample was not depressed when mixed with the by-product melting at 189°. Since the by-product was formed in the presence of large amounts of proline hydrochloride it is evident that proline and proline hydrochloride tend to crystallize from alcohol in a 1:1 ratio. The by-product gave the following analytical results.

Anal. Calcd. for $C_{10}H_{18}N_2O_4 \cdot HCl$: C, 45.03; N, 7.18; Cl, 13.29; N, 5.26. Found: C, 45.38; H, 7.26; Cl, 13.03; N, 5.41.

DL-**Proline**.—One hundred and fourteen grams of proline hydrochloride was suspended in 500 ml. of dry chloroform and 150 ml. of triethylamine dripped in slowly with good stirring. Stirring was continued for one to two hours, and the proline was filtered off. Since proline is soluble in triethylamine, a small additional amount of proline was obtained by concentrating the chloroform solution to dryness and treating the residue with 500 ml. of chloroform. The total yield was 68.4 g., m. p. 203–204° (78.5%). The product was entirely free of halogen.

Anal. Calcd. for $C_5H_9NO_2$: N, 12.17. Found: N, 12.10.

Recrystallization from alcohol–ether, with but little loss, gave a product melting at 210.2–211.4° cor.

Anal. Calcd. for $C_5H_9NO_2$: C, 52.21; H, 7.87; N, 12.17. Found: C, 52.49; H, 7.81; N, 12.19.

Recrystallization of 100 g. of crude proline from 1200 ml. of ethanol gave 86.4 g. of pure proline on chilling overnight, thus indicating that DL-proline is not especially soluble in cold ethanol.

The proline was characterized by making the picrate, phenylhydantoic acid and phenylhydantoin. All melting points agreed with literature values. The phenylhydantoic acid did not depress the melting point of a sample made from proline prepared by the method of Fischer.[14]

Summary

Proline has been synthesized in 20% over-all yield by condensing acrylonitrile with malonic ester, reducing to the piperidone, chlorinating, hydrolyzing to proline hydrochloride and converting to proline with triethylamine and chloroform. All intermediates are readily purified solids. The use of silver salts in the conversion of proline hydrochloride to proline is avoided.

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(13) More recent experiments in connection with another problem suggest that treatment of IV with two equivalents of triethylamine would give VI directly. This has not been tried, however.

(14) Fischer, *Ber.*, **34**, 454 (1909).