[CONTRIBUTION FROM THE STERLING-WINTHROP RESEARCH INSTITUTE]

Reactions of Ethyl (2-Cyanoethyl)-acetoacetate

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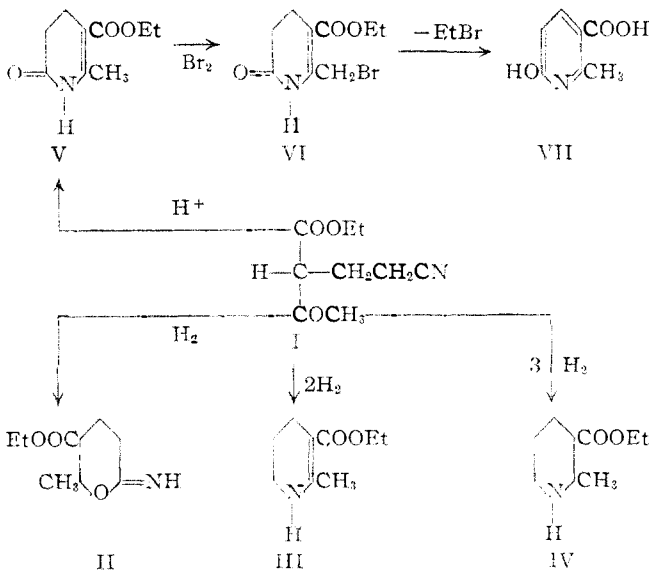
The title compound, I, may be reduced to ethyl 2-methyl-1,4,5,6-tetrahydronicotinate from which either 6-amino-2-hexanone or 1,4,5,6-tetrahydro-α-picoline may be obtained on hydrolysis. Cyclization of I with acid gives ethyl 1,4,5,6-tetrahydro-2-methyl-6-oxonicotinate (V). The bromo derivative of V loses ethyl bromide on heating to give 2-methyl-6-oxynicotinic acid.

Although ethyl (2-cyanoethyl)-acetoacetate (I) has been known for more than twenty years,[1] not much has been published concerning the reactions of this now readily available compound. In two recent publications it was shown that I (a) was hydrolyzed by aqueous sodium carbonate to 5-oxocapronitrile,[2] (b) was reduced in the presence of a nickel catalyst with the uptake of one mole of hydrogen to give a compound formulated as either ethyl 2-(2-cyanoethyl)-3-oxybutyrate[3] or the corresponding pyroneimine, II,[2] and (c) was reduced at a higher temperature with Raney nickel catalyst with the uptake of three moles of hydrogen to give the piperidine, IV.[2]



By using an intermediate temperature for the reduction, two moles of hydrogen are taken up with the production of ethyl 2-methyl-1,4,5,6-tetrahydronicotinate (III). At 84° in 1.5 hours, for example, there was obtained a 10% yield of IV and a 76% yield of III, whereas after eight hours at 108°, the yield of IV was 50% and of III was 39%. Thus, by proper choice of temperature any of three reduction products may be obtained.

The presence of a double bond in III was demonstrated by hydrogenation with a platinum catalyst with the uptake of one mole of hydrogen to give IV, identified as the phenyl isocyanate derivative. The double bond was shown to be in the conjugated 2-position by an absorption curve.[4] When III was refluxed with benzoyl chloride in benzene and the product isolated (after treatment with water) there was obtained ethyl (3-benzamidopropyl)-acetoacetate. Hydrolysis of III with hydrochloric acid gave 6-amino-2-hexanone hydrochloride which reacted with sodium cyanide to give 2-methylpipecolonitrile in nearly quantitative yield. The amino ketone readily cyclized in the presence of base to give the known 1,4,5,6-tetrahydro-α-picoline.[5]

Henecka has reported that I is not reduced to a piperidine unless it is first converted to the amino crotonic ester with ammonia.[2] Accordingly, I was refluxed with butylamine in toluene until the theoretical amount of water was removed and the resulting Schiff base was reduced with Raney nickel in ethanol at 78°. The product was III rather than the N-butyl nipecotate.

In the presence of cold concentrated hydrochloric acid I is rapidly cyclized to ethyl 1,4,5,6-tetrahydro-2-methyl-6-oxonicotinate (V)—a reaction typical of many 5-oxonitriles.[6] The cyclization may also be brought about by passing a stream of hydrogen chloride through a chloroform solution of I. Upon bromination, V readily gives a monobromo compound formulated as VI, although the position of the bromine atom has not been proved. The same bromo compound may be obtained directly, though in poorer yield, by brominating I. The hydrogen bromide which is liberated causes cyclization. Several methods of aromatizing VI were tried with only fair results until the behavior of VI on melting was recalled. The originally turbid melt cleared up with gas evolution when held above the melting point. Upon warming a sample of VI, ethyl bromide (identified by boiling point) was evolved and 2-methyl-6-oxynicotinic acid (VII) was obtained in nearly quantitative yield. Rearrangement of VI may take place before loss of ethyl bromide, but this point was not investigated.

Saponification of V led to 1,4,5,6-tetrahydro-2-methyl-6-oxonicotinic acid.

The 5-oxocapronitrile obtained from I by carbon-

(1) S. Keimatsu and S. Sugasawa, *J. Pharm. Soc. (Japan)*, **48**, 755 (1928); *C. A.*, **23**, 834 (1929).

(2) N. Albertson, THIS JOURNAL, **72**, 2594 (1950).

(3) H. Henecka, *Ber.*, **82**, 104 (1949).

(4) The absorption curve showed a maximum epsilon value of about 20,000 at 290 mμ indicating a β-amino crotonic ester type. *Cf.* S. Glickman and A. Cope, THIS JOURNAL, **67**, 1017 (1945). We are indebted to Dr. F. C. Nachod and Miss C. Martini for the absorption data.

(5) A. Lipp, *Ann.*, **289**, 173 (1896).

(6) For examples of this type of reaction, see (a) E. Kohler, A. Graustein and D. Merrill, THIS JOURNAL, **44**, 2536 (1922); (b) E. Kohler and B. Souther, *ibid.*, **44**, 2903 (1922); (c) C. Allen and W. Ball *ibid.*, **59**, 686 (1937); (d) Brit. Patent 549,673 (1942). The example cited by E. Farmer and J. Ross, *J. Chem. Soc.*, **129**, 3233 (1926), violates Bredt's Rule.

ate hydrolysis[2] was also cyclized with hydrogen chloride to give 3,4-dihydro-6-methyl-2-pyridone.

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Experimental[7]

Ethyl 2-Methyl-1,4,5,6-tetrahydronicotinate (III).—When 183 g. of ethyl (2-cyanoethyl)-acetoacetate in 420 ml. of ethanol was shaken at 84° in the presence of hydrogen and Raney nickel there was an uptake of 2.2 moles of hydrogen in 1.5 hr. Distillation of the product gave 17.3 g. (10%) of ethyl 2-methyl nipecotate (IV) boiling at 51–80° at 0.07 mm. and 120 g. (76%) of ethyl 2-methyl-1,4,5,6-tetrahydronicotinate (III) boiling at 110–121° at 0.08 mm. and solidifying in the receiver; m.p. 45–47.5°. A sample was recrystallized from Skellysolve B for analysis.

Anal. Calcd. for $C_9H_{15}NO_2$: C, 63.88; H, 8.94; N, 8.28. Found: C, 63.35; H, 9.21; N(AP), 7.85.

Ethyl 2-Methylnipecotate by Reduction of III.—A solution of 6 g. of ethyl 2-methyl-1,4,5,6-tetrahydronicotinate in 100 ml. of ethanol was reduced at room temperature in the presence of 0.15 g. of Adams platinum oxide in about 45 minutes. Distillation gave 5.4 g. of product; $n^{25}D$ 1.4572. When treated with phenyl isocyanate the 1-carbanilino derivative which was obtained did not depress the melting point of the derivative previously prepared from ethyl 2-methylnipecotate.[2]

Ethyl (3-Benzamidopropyl)-acetoacetate.—A solution of 12.7 g. of ethyl 2-methyl-1,4,5,6-tetrahydronicotinate in dry benzene was refluxed with 11.5 g. of benzoyl chloride for 18 hours. Then 5.5 ml. of methanol was added and refluxing continued until one phase was obtained. The benzene layer was washed with dilute hydrochloric acid, water and sodium bicarbonate solution, dried and distilled. The product distilled at a bath temperature of 173–176° at 0.6 μ. It gave an intense purple color with alcoholic ferric chloride.

Anal. Calcd. for $C_{16}H_{21}NO_4$: C, 65.96; H, 7.26; N, 4.92. Found: C, 66.61; H, 7.07; N(K), 4.87; N(D), 5.04.

6-Amino-2-hexanone Hydrochloride.—Thirty grams of ethyl 2-methyl-1,4,5,6-tetrahydronicotinate were refluxed two hours with concentrated hydrochloric acid and then concentrated to dryness *in vacuo* to give 25 g. of small, nearly colorless needles. These were recrystallized from isopropyl alcohol with a fair loss to give crystals melting at 153–155°. Samples of this compound liquefied within a few days to a few weeks on storage in stoppered bottles. Gabriel[8] prepared this compound but did not isolate it. Apparently Lipp also made it, but found that it was too hygroscopic to characterize.[9].

Anal. Calcd. for $C_6H_{13}NO{\cdot}HCl$: C, 47.53; H, 9.31. Found: C, 47.65; H, 8.85.

A 2,4-dinitrophenylhydrazone, on recrystallization from acetic acid, melted at 215.0–215.7° cor.

Anal. Calcd. for $C_{12}H_{17}N_5O_4{\cdot}C_2H_4O_2$: N, 19.71; Ti equiv., 14.00. Found: N(D), 20.25; Ti equiv., 14.14 (for mol. wt. of 355.4).

1,4,5,6-Tetrahydro-α-picoline.—Treatment of 6-amino-2-hexanone hydrochloride in an equal weight of water with solid sodium hydroxide gave an oil which was extracted with ether, dried with sodium hydroxide and distilled; b.p. 131–134° at 761 mm. (reported:[5] 131–132° at 716 mm.). It gave a picrate melting at 120.4–121.4° cor. (reported:[5] m.p. 119–120°).

Anal. Calcd. for $C_6H_{11}N{\cdot}(NO_2)_3C_6H_2OH$: N(AP), 4.29. Found: N(AP), 4.32.

2-Methylpipecolonitrile.—To a solution of 15.1 g. of crude 6-amino-2-hexanone hydrochloride in 50 ml. of water was added a solution of 5.5 g. of sodium cyanide in 25 ml. of water. Potassium carbonate was added to the resulting solution to salt out the product. It was extracted with ether and distilled to give 9.5 g. boiling at 77–78° at about 6 mm.; $n^{25}D$ 1.4621. About 2 g. of product was not distilled because of column hold-up.

Anal. Calcd. for $C_7H_{12}N_2$: N(AP), 11.28. Found: N(AP), 11.29.

A portion was converted to the phosphate, m.p. 126–128°. This was unstable and could not be recrystallized without loss of HCN. The crude material was analyzed directly.

Anal. Calcd. for $C_7H_{12}N_2{\cdot}H_3PO_4$: N, 12.60; H_3PO_4, 44.1. Found: N(D), 11.47; H_3PO_4, 44.40.

A second portion was treated with phenyl isocyanate to give 1-carbanilino-2-methylpipecolonitrile, m.p. 180–182°.

Anal. Calcd. for $C_{14}H_{17}N_3O$: N, 17.27. Found: N, 17.46.

Ethyl 1,4,5,6-Tetrahydro-2-methyl-6-oxonicotinate (V).—To 50 ml. of concentrated hydrochloric acid maintained at 0 to 5° there was added 50 g. of ethyl (2-cyanoethyl)-acetoacetate at such a rate that the temperature did not exceed 5°. After stirring 20 minutes, the mixture (from which some solid had separated) was poured onto ice and water to give 43 g. (86%) of white crystals. The crude product usually melted at about 148°. Recrystallization from methanol gave material melting at 154–156°.

With 90% sulfuric acid, the crude yield was 84%. When the starting material was dissolved in commercial chloroform and HCl bubbled through for one hour, the crude yield was 74%. With less pure starting material there was an induction period, so that considerably longer reaction times were necessary.

This compound has been prepared from ammonia and 2-acetoglutaric ester.[10]

Anal. Calcd. for $C_9H_{13}NO_3$: C, 59.00; H, 7.15; N, 7.64. Found: C, 58.89; H, 7.10; N(D), 7.60.

Ethyl 2-Bromomethyl-1,4,5,6-tetrahydro-6-oxonicotinate (VI).—To a stirred solution of 30.5 g. of V in about 250 ml. of dry chloroform there was added dropwise 26.5 g. of bromine in chloroform. The bromine was decolorized instantly. The solvent was removed by distillation *in vacuo* and the crystalline residue recrystallized from methanol to give 39.2 g. (90%); m.p. 118–120° to a turbid liquid.

Bromination of ethyl (2-cyanoethyl)-acetoacetate gave the same cyclic bromo compound in 48% yield of crystalline material melting to a turbid liquid at 117°. The remainder of the reaction product was a sirup not further investigated.

Anal. Calcd. for $C_9H_{12}BrNO_3$: Br, 30.49; N, 5.34. Found: Br, 31.00; N, 5.28.

2-Methyl-6-oxynicotinic Acid (VII).—Twenty grams of bromo compound, VI, was heated for 15 min. at 130°. Ethyl bromide, b.p. 38°, was evolved. The residue was stirred with dilute ammonium hydroxide, filtered and dried to give 11 g. of product (95%) melting at 239–243°. Recrystallization from acetic acid, with practically no loss, gave a product melting at 252°. When prepared on a molar scale from crude bromo compound colored impurities are introduced which are best removed by recrystallization from water with charcoal treatment.

Anal. Calcd. for $C_7H_7NO_3$: C, 54.91; H, 4.61; N, 9.15. Found: C, 55.01; H, 4.57; N(K), 9.05.

The bromo compound was converted to VII in poorer yield by refluxing with 10 volumes of water for six hours or by refluxing with five volumes of acetic acid containing fused sodium acetate (the latter being obviously unnecessary). Use of sodium ethylate in ethanol gave no pure product.

1,4,5,6-Tetrahydro-2-methyl-6-oxo-nicotinic Acid.—Ten grams of the ester, V, was heated with 50 ml. of 10% aqueous sodium hydroxide for one-half hour to effect solution. Upon cooling and acidification with hydrochloric acid (copious evolution of CO_2), the product separated. Reprecipitation from aqueous ammonia gave 1.3 g.; m.p. 212–216°.

Anal. Calcd. for $C_7H_9NO_3$: N, 9.03; neut. equiv., 155.1. Found: N, 8.78; neut. equiv., 154.1.

3,4-Dihydro-6-methyl-2-pyridone.—A stream of hydrogen chloride was bubbled into a solution of 15 g. of 5-oxocapro-

(7) N(AP) refers to nitrogen determined by titration in acetic acid with perchloric acid; N(K) refers to Kjeldahl nitrogen; and N(D) refers to Dumas nitrogen.

(8) S. Gabriel and J. Colman, *Ber.*, **42**, 1243 (1909).

(9) See reference 4, page 201.

(10) W. Emery, *Am. Chem. J.*, **13**, 352 (1891); G. Clemo and K. Welch, *J. Chem. Soc.*, **131**, 2624 (1928).

nitrile in chloroform (dried by distillation; alcohol not removed) for 1.5 hours. There was heat evolution. Concentration gave 17.6 g. of solid which became pink on exposure to air. The product was stirred with water containing enough ammonium hydroxide to make the water phase basic. This was filtered and washed with acetone to remove the now yellow color. The product was dissolved in hydrochloric acid and reprecipitated with ammonium hydroxide, m.p. 234–236°.

Anal. Calcd. for C_6H_9NO: C, 64.86; H, 8.17; N, 12.61. Found: C, 64.75; H, 8.17; N(K), 12.41; N(AP), 6.26 (sharp end-point).

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The Application of *p*-Nitrobenzyl Chloroformate to Peptide Synthesis

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The preparation of crystalline carbo-*p*-nitrobenzyloxy derivatives of several amino acids, through the use of the reagent, *p*-nitrobenzyl chloroformate is described. These crystalline derivatives include those of the amino acids for which crystalline carbobenzoxy derivatives have not yet been reported. The application of this reagent to peptide synthesis through preparation of glycyl-L-leucine and L-leucyl-L-leucine is reported.

Since the introduction of benzyl chloroformate (carbobenzoxy chloride) by Bergmann and Zervas[1] as a reagent for peptide synthesis, a large number of peptides have been prepared by methods which have made use of this reagent. The wide applicability of the carbobenzoxy method is now well established.[2] One disadvantage of the carbobenzoxy method arises from the benzyl chloroformate. The sirupy reagent, which is generally stored in toluene solution in the cold, gradually decomposes over a period of a few months and as a result there is some uncertainty as to the amount of active reagent present at any one time. A second disadvantage of the method arises from the difficulty experienced in crystallizing certain derivatives. The carbobenzoxy derivatives of DL- or L-hydroxyproline, DL- or L-proline, L-leucine and DL- or L-isoleucine have not been crystallized as yet. In attempts to surmount these difficulties recent modifications of the method have been introduced. These include the use of the carboallyloxy group[3] and of the *p*-bromobenzyloxy group[4] in peptide synthesis.

We have investigated the use of *p*-nitrobenzyl chloroformate in the preparation of carbo-*p*-nitrobenzyloxy derivatives of amino acids and peptides. It contains a nitro group which makes possible the detection of the derivatives in very small amounts by measurement of the ultraviolet absorption at about 265 mμ. The reagent[5] has the further advantage of being a stable, low melting crystalline solid. One preparation of the reagent was stored in a desiccator over phosphorus pentoxide in the cold for over a year, during which time numerous samples were withdrawn. No change in the melting point or other evidence of deterioration of the reagent was detected. The presence in the reagent of a nitro group would be expected to enhance the ease of crystallization of the derivatives prepared from this reagent. This was indeed the case and all derivatives prepared so far, including the carbo-*p*-nitrobenzyloxy derivatives of L-proline, hydroxy-L-proline, L-leucine and DL- and L-isoleucine which yield oils as the carbobenzoxy derivatives, have been obtained in crystalline form with no difficulty. The carbo-*p*-nitrobenzyloxy group was readily removed by hydrogenolysis.

Thiele and Dent[5] reported that phosgene does not react with *p*-nitrobenzyl alcohol in the cold and consequently prepared *p*-nitrobenzyl chloroformate in a sealed tube at 60–65°, using chloroform as a solvent. However, it was found that by using dioxane as a solvent and by permitting the reaction to proceed overnight, the reagent could be prepared in 95% yield at room temperature.

The carbo-*p*-nitrobenzyloxy derivatives of the amino acids were prepared in the usual manner.[1] In a preliminary study using glycine as a model it was found that a ratio of 1.25 moles of *p*-nitrobenzyl chloroformate to one of the amino acid gave the best yield. This ratio was used, therefore, in the preparation of the remainder of the derivatives described below.

It was of interest to discover if the carbo-*p*-nitrobenzyloxy group could be used in peptide synthesis with the same ease as the carbobenzoxy group. With this view in mind, glycyl-L-leucine and L-leucyl-L-leucine were prepared in good yield using the carbo-*p*-nitrobenzyloxy group to mask the amino group. These peptides were prepared through the reaction of the acid chlorides of carbo-*p*-nitrobenzyloxyglycine and carbo-*p*-nitrobenzyloxy-L-leucine, respectively, with L-leucine methyl ester. The resulting esters were saponified and the carbo-*p*-nitrobenzyloxy group was removed by hydrogenolysis. No racemization was observed and all the intermediate carbo-*p*-nitrobenzyloxy derivatives were readily obtained in crystalline form. These results demonstrate the applicability of this reagent to peptide synthesis.

It should be pointed out that although introduction of the nitro group into the reagent gives rise to certain favorable properties for peptide synthesis it also elicits certain disadvantages that are not present in the original carbobenzoxy chloride reagent. The presence of the nitro group precludes the use of the Kjeldahl method for nitrogen determination. In addition the presumed formation of *p*-toluidine

(1) M. Bergmann and L. Zervas, *Ber.*, **65**, 1192 (1932).

(2) J. Fruton, "Advances in Protein Chemistry," Vol. 5, ed. by M. Anson, J. Edsall and K. Bailey, Academic Press, Inc., New York, N. Y., 1949, p. 1.

(3) C. M. Stevens and R. Watanabe, THIS JOURNAL, **72**, 725 (1950).

(4) D. M. Channing, P. B. Turner and G. T. Young, *Nature*, **167**, 487 (1951).

(5) First reported by J. Thiele and F. Dent, *Ann.*, **302**, 258 (1898).