hydrogenated in the Parr apparatus at 3-atm. pressure, using Raney nickel as catalyst. After 18 hr., the reaction mixture was filtered and extracted with benzene in the manner described above. The aqueous solution containing the 1,2-diamino-1,2-dideoxy-D-glucitol was treated with 2 g. of sodium bicarbonate and 4.68 g. of salicylaldehyde and the mixture was heated over the steam bath, with stirring, for 3 hr. The product, 1,2-diamino-1,2-dideoxy-D-glucitol bis(salicylaldehyde Schiff base), was recrystallized from 95% ethanol; yield 2.34 g., m.p. 208–208.5°, $[\alpha]_D^{20}$ −83° (*c* 4.04, *N*,*N*-dimethylformamide), x-ray powder diffraction data:[32] 15.17w, 11.87m, 8.04w, 5.93m, 5.55vw, 5.15s(3), 4.90vs(1,1), 4.62s, 4.47s, 4.14vs(1,1), 3.85vw, 3.71vw, 3.53vw, 2.72 vw.

Anal. Calcd. for $C_{20}H_{24}N_2O_6$: C, 61.84; H, 6.23; N, 7.21. Found: C, 61.65; H, 6.01; N, 7.00.

2-Amino-2-deoxy-D-glucose oxime hydrochloride[23] (11.5 g.) was dissolved in 200 ml. of 75% ethanol and the solution was hydrogenated as in the above experiment, but with palladium-charcoal catalyst. The reaction mixture was then evaporated under reduced pressure and a portion of the product (10%) was converted to 1,2-dideoxy-1,2-bis(salicylideneamino)-D-glucitol. This compound had the same x-ray powder diffraction pattern and physical properties as the product synthesized above from 2-amino-2-deoxy-D-glucose phenylhydrazone.

1,2-Dideoxy-1,2-bis(salicylideneamino)-D-mannitol. D-*arabino*-Hexose phenylosazone (3.6 g., 10 millimoles) was dissolved in 100 ml. of 95% ethanol containing 40 millimoles of hydrogen chloride and the solution was hydrogenated in the Parr apparatus at 3-atm. pressure, using palladium-charcoal as catalyst. The reaction mixture was filtered and the filtrate was concentrated to 25 ml., diluted with 100 ml. of water, neutralized with sodium bicarbonate, and extracted with four 100-ml. portions of benzene. The aqueous solution of the reduction product was treated with 4 g. of sodium bicarbonate and 1 ml. of salicylaldehyde and heated over the steam bath with stirring for 2 hr. The resulting yellow precipitate of 1,2-diamino-1,2-dideoxy-D-mannitol bis(salicylaldehyde Schiff base) was purified by three recrystallizations from 95% ethanol; yield 0.15 g., m.p. 223–224°, $[\alpha]_D^{23}$ +54.2° (*c* 2.15, *N*,*N*-dimethylformamide), x-ray powder diffraction data:[32] 14.98w, 11.33m, 8.67m, 6.71w, 5.40m, 5.10vs(1), 4.80m, 4.60vs(2), 4.31vw, 4.00s(3), 3.73vw, 3.48vw, 3.29vw, 3.14w.

Anal. Calcd. for $C_{20}H_{24}N_2O_6$: C, 61.84; H, 6.23; N, 7.21. Found: C, 62.02; H, 6.46; N, 7.17.

COLUMBUS 10, OHIO

(32) Interplanar spacing, Å, CuK_α radiation. Relative intensity, estimated visually; s, strong; m, medium; w, weak; v, very. First three strongest lines are numbered (1, strongest); double numbers indicate approximately equal intensities.

[CONTRIBUTION NO. 2255 FROM THE GATES AND CRELLIN LABORATORIES OF CHEMISTRY, CALIFORNIA INSTITUTE OF TECHNOLOGY]

Synthesis of β-(4-Pyridyl)-DL-alanine and of β-(4-Pyridyl-1-oxide)-DL-, D-, and L-alanine[1]

ROBERT L. BIXLER AND CARL NIEMANN[2]

Received September 30, 1957

A practical synthesis of β-(4-pyridyl)-DL-alanine, suitable for application on a gram scale, has been developed. β-(4-Pyridyl-1-oxide)-DL-alanine has been prepared in good yield, by a procedure capable of being employed on a much larger scale, and a satisfactory resolution of *N*-benzoyl-β-(4-pyridyl-1-oxide)-DL-alanine has been achieved.

Two methods have been reported for the synthesis of β-(4-pyridyl)-DL-alanine. The first[3] was based upon the sequence, 4-pyridylcarbinol→4-pyridylmethyl bromide→diethyl benzamido-(4-pyridylmethyl)malonate→β-(4-pyridyl)-DL-alanine and the second[4] upon the sequence, 4-picoline→ethyl α-oximino-β-(4-pyridyl)propionate→α-oximino-β-(4-pyridyl)propionic acid→β-(4-pyridyl)-DL-alanine. Both syntheses involved a step in which poor yields were obtained. The malonic ester condensation gave but a 4% yield, and the Claisen condensation a 12% yield. It was decided to study the malonic ester condensation with the aim of improving the yield.

The starting material for the malonic ester condensation, 4-pyridylmethyl bromide hydrobromide, was obtained in 85% yield from 4-pyridylcarbinol. This hydrobromide, and its parent amine, are severe vesicants.

The principal competing side reaction in the malonic ester condensation is the polymeric quaternization of 4-pyridylmethyl bromide. This quaternization has been studied by Sorm and Sedivy,[5] who also observed that 2-pyridylmethyl bromide quaternized at a slower rate. The quaternization of 4-bromopyridine is much faster than that of 2-bromopyridine,[6] the difference in rate being attributed to steric effects.[7] Presumably the same effects are operative in the case of 2- and 4-pyridylmethyl bromide. Examination of various modifi-

(1) Supported in part by a grant from the National Institutes of Health, Public Health Service.

(2) To whom inquiries regarding this article should be sent.

(3) C. Niemann, R. N. Lewis, and J. T. Hays, *J. Am. Chem. Soc.*, **64**, 1678 (1942).

(4) D. F. Elliott, A. T. Fuller, and C. R. Harington, *J. Chem. Soc.*, 85 (1948).

(5) F. Sorm and L. Sedivy, *Coll. Czech. Chem. Comm.*, **13**, 289 (1948).

(6) H. S. Mosher in R. C. Elderfield, *Heterocyclic Compounds*, John Wiley and Sons, Inc., New York, 1950, vol. 1, p. 516.

(7) F. W. Bergstrom, *Chem. Revs.*, **35**, 138 (1944).

cations of the malonic ester condensation,[8] such as using 4-pyridylmethyl tosylate, in lieu of the bromide, did not lead to a significant increase in the yield of desired product.

Considering the nature of the competing reactions, it would be predicted that use of a less polar solvent, *e.g.*, benzene instead of ethanol, would favor the rate of condensation and retard that of quaternization.[9] It was determined that approximately 50% benzene in ethanol was the most satisfactory solvent for effecting condensation.

It was necessary to neutralize the hydrobromide of 4-pyridylmethyl bromide. The rapid quaternization of the free base led to the use of an excess of the sodium salt of the acylamidomalonic ester in the neutralization reaction, which was conducted by adding the hydrobromide to a solution of the sodium salt. This procedure had the added effect of providing a higher concentration of the attacking group during the early part of the reaction and thus minimized the possibility of ether formation due to attack of the halide by alkoxide ion.

One would expect the rate of condensation of 4-pyridylmethyl bromide with the malonic ester to be reasonably fast, if the usual comparison of nitrophenyl and pyridyl groups can be made.[10] Dornow and Winter[11] obtained at least a 60% yield of the desired product from the condensation of *p*-nitrobenzyl chloride with diethyl formamidomalonate and it has been shown that *p*-nitrobenzyl bromide is about 400 times more reactive than *n*-butyl bromide in S_N2 reactions.[12] On the other hand, *p*-nitrobenzyl bromide exhibited the slowest rate of several *p*-substituted benzyl bromides in a quaternization reaction with triethylamine.[13] There are no data that afford comparison of the rate of an S_N2 reaction with that of quaternization.

The addition of the dry hydrobromide to the sodio-acylamidomalonic ester was abandoned for two reasons. One, the salt was hygroscopic, which complicated its addition. Two, high local concentration of 4-pyridylmethyl bromide formed when the addition was conducted with the dry salt favored quaternization. Since the hydrobromide was soluble only in water, it was necessary to add it as a slurry in benzene-ethanol.

As a result of studying the effect of time, temperature, solvent, mode of addition of the hydrobromide, and concentration of reactants, on the yields obtained in sixteen preparations, a procedure was devised which gave consistent 60–70% yields of diethyl acetamido(4-pyridylmethyl)malonate on a 0.02-mole scale. However, when scaled up to 0.08-mole quantities, the yield dropped to 30%, largely because of slurrying problems.

The diethyl acetamido(4-pyridylmethyl)malonate was hydrolyzed and decarboxylated with 48% hydrobromic acid to give 76% of the dihydrobromide of β-(4-pyridyl)-DL-alanine. Treatment of this salt with "Amberlite IR-4B" resin gave the desired α-amino acid in 90% yield. β-(4-Pyridyl)-DL-alanine reacted rapidly in the cold with ninhydrin to give a red color,[3,4] and was soluble in water to the extent of 3.4 g. per 100 ml. at 25°.

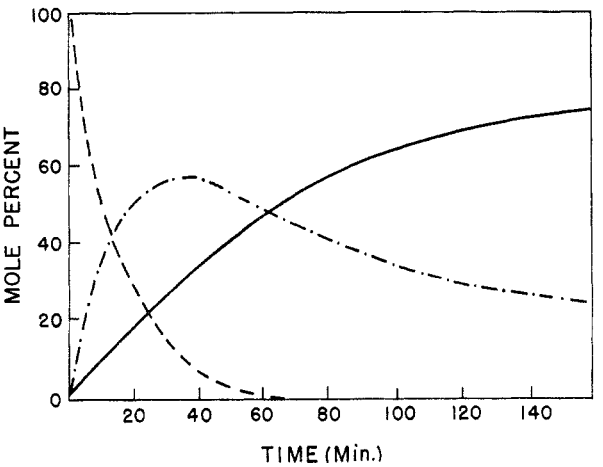

Fig. 1. The catalytic hydrogenation of β-(4-pyridyl-1-oxide)-DL-alanine: --- β-(4-pyridyl-1-oxide)-DL-alanine; -·- β-(4-pyridyl)-DL-alanine; — β-(4-piperidyl)-DL-alanine

Another route to β-(4-pyridyl)-DL-alanine that was considered was from isonicotinaldehyde *via* the azlactone. However, the aldehyde is stable only as a hydrate, the anhydrous compound being very sensitive to air oxidation.[14] It also readily undergoes a Cannizzaro reaction in the presence of air.[15] No product could be isolated from a standard Erlenmeyer synthesis[16] using the anhydrous aldehyde, or its diacetate, prepared from the hydrate by reaction with acetic anhydride. A modified Erlenmeyer synthesis involving rhodanine[17] gave only non-characterizable products.

One possible route that was not investigated was the Knoevenagel condensation of isonicotinaldehyde with nitroacetonitrile. The yield of 1-γ-pyridyl-2-cyano-2-nitroethylene has been reported as 35%.[18] The reaction of ethyl nitroacetate with picolinaldehyde gave an 81% yield of diethyl

(8) A. C. Cope, H. L. Homes, and H. O. House, *Org. Reactions*, 9, 107 (1957).

(9) E. R. Alexander, *Principles of Ionic Organic Reactions*, John Wiley and Sons, Inc., New York, 1950, pp. 85–87.

(10) R. H. Wiley in H. Gilman, *Organic Chemistry, An Advanced Treatise*, John Wiley and Sons, Inc., New York, 1953, p. 837.

(11) A. Dornow and G. Winter, *Ber.*, **84**, 307 (1951).

(12) S. Sugden and J. B. Willis, *J. Chem. Soc.*, 1360 (1951).

(13) J. W. Baker and W. S. Nathan, *J. Chem. Soc.*, 519 (1935).

(14) V. M. Mitchovitch and M. Lj. Mihailovitch, *Rec. trav. chim.*, **71**, 970 (1952).

(15) J. P. Wibaut, C. E. Kooyman and H. Boer, *Rec. trav. chim.*, **64**, 30 (1954).

(16) H. E. Carter, *Org. Reactions*, **3**, 198 (1946).

(17) P. L. Julian and B. M. Sturgis, *J. Am. Chem. Soc.*, **57**, 1126 (1935).

(18) W. Ried and E. Kohler, *Ann.*, **598**, 145 (1956).

α,α-dinitro-γ-(4-pyridyl)glutarate[19] and probably could be controlled to give a 1:1 condensation product. It appears that the double bond and nitro group of 1-γ-pyridyl-2-cyano-2-nitroethylene could be reduced without reducing the pyridine ring on the basis of work done by Walter *et al.* on the reduction of the pyridine acrylic acids.[20]

Although the goal of raising the yield in a series of reactions leading to β-(4-pyridyl)-DL-alanine was achieved, the improved synthesis was not useful for the preparation of large quantities of this amino acid. While there was no reason to expect that the yield obtained by Elliott, Fuller, and Harington[4] could be improved, more recent studies indicated that the electron deficiency at the methyl group of 4-picoline was greatly enhanced in 4-picoline-1-oxide[21] thus implying that a Claisen condensation based upon 4-picoline-1-oxide would be considerably more successful than one based on 4-picoline. This was shown to be the case by Adams and Miyano,[22] who obtained a 48% yield of ethyl β-(4-pyridyl-1-oxide)pyruvate from the condensation of ethyl oxalate and 4-picoline-1-oxide, in the presence of potassium ethoxide. The α-keto ester was isolated *via* aqueous hydrolysis of its potassium salt and extraction with chloroform. A modification of this reaction, conducted in these laboratories, involved aqueous hydrolysis of the sodium salt of the keto ester and, after cooling, isolation of the crystalline solid that had formed. This product was impure, and was found to contain a component that was insoluble in chloroform. Recrystallization of the impure material from water gave a 48% yield of the α-keto ester. Concentration of the filtrate gave a yellow crystalline light sensitive solid, m.p. 186–187°, of unknown constitution. This latter compound was insoluble in chloroform, and would not form a water insoluble oxime, as would the α-keto ester or α-keto acid.

The oxime of the α-keto ester was prepared by Adams and Miyano[22] in 57% yield, and this result was confirmed in these laboratories. A modification of the method for the preparation of the oxime, not involving isolation of the α-keto ester, gave consistent 55–60% yields of recrystallized ethyl α-oximino-β-(4-pyridyl-1-oxide)propionate, directly from 4-picoline-1-oxide.

Oximes are readily reduced to amines, but α-oximino esters require rather strenuous conditions. Hartung and co-workers[23] have examined the catalytic reduction of a number of α-oximino esters. Generally, five grams of palladium on charcoal plus one to five grams of palladium chloride with 150 p.s.i. of hydrogen at room temperature was used for 0.15 mole of oximino ester. In addition, a threefold excess of concentrated hydrochloric acid was necessary to prevent formation of secondary amines. Hartung and Waters[24] have shown that α-amino acids poison the catalytic reduction of α-oximino acids. In the case of oximinomalonic ester, 1500 p.s.i. of hydrogen were necessary to obtain reduction with the palladium catalyst.[25] With Adam's catalyst the reduction of 3-oximino-1-dimethylaminobutane gave a 25% yield of the diamine.[26] Shivers and Hauser[27] found that reduction of α-oximino esters with Raney nickel gave 85% yields of the amino esters and Ried and Schiller[28] employed Raney nickel in glacial acetic acid for the reduction of ethyl α-oximino-β-(2-quinolyl)-propionate. In the latter instance, considerable amounts of β-(2-tetrahydroquinolyl)-DL-alanine were obtained and in other cases only the α-hydroxylamino ester was isolated.[29]

In all of the above procedures it would be expected that concurrent reduction of a pyridine nucleus would occur. This has been shown to be the case for α-oximino-β-(4-pyridyl)propionic acid by Elliott, *et al.*,[4] and for its *N*-oxide in this investigation.

Other methods used for the reduction of oximes, such as lithium aluminum hydride, or sodium amalgam, are not applicable to the case at hand. The use of sodium borohydride was investigated, but no reduction was obtained.

Elliott, Fuller, and Harington[4] found that their oximino acid could be reduced with stannous chloride in concentrated hydrochloric acid. The tin salts were removed by precipitation with hydrogen sulfide after dilution of the reaction mixture with water. A more satisfactory procedure was developed in this investigation and was based upon removal of most of the hydrochloric acid by distillation *in vacuo* and neutralization of the residue to *p*H 6.7 with ammonium hydroxide. The precipitation of stannous and stannic hydroxides was so complete that no further precipitate could be obtained with hydrogen sulfide. The aqueous solution was evaporated to dryness to give a mixture of ammonium chloride and the amino acid, in a ratio of about four to one. The ammonium chloride was removed by extraction of the powdered mixture with methanol, the salt being soluble in methanol to the extent of 3.35 g. per 100 g. at

(19) A. Dornow and G. Wiehler, *Ann.*, **578**, 113 (1952).
(20) L. A. Walter, W. H. Hunt, and R. J. Fosbinder, *J. Am. Chem. Soc.*, **63**, 2771 (1941).
(21) E. Ochiai, *J. Org. Chem.*, **18**, 534 (1953).
(22) R. Adams and S. Miyano, *J. Am. Chem. Soc.*, **76**, 3168 (1954).
(23) R. H. Barry and W. H. Hartung, *J. Org. Chem.*, **12**, 460 (1947).
(24) K. L. Waters and W. H. Hartung, *J. Org. Chem.*, **10**, 524 (1945).
(25) H. R. Snyder and C. W. Smith, *J. Am. Chem. Soc.*, **66**, 350 (1944).
(26) D. E. Pearson, W. H. Jones, and A. C. Cope, *J. Am. Chem. Soc.*, **68**, 1225 (1946).
(27) J. C. Shivers and C. R. Hauser, *J. Am. Chem. Soc.*, **69**, 1264 (1947).
(28) W. Ried and H. Schiller, *Ber.*, **86**, 730 (1953).
(29) W. Ried and K. Lotterhos, *Ber.*, **88**, 38 (1955).

19°.[30] The residue analyzed correctly for β-(4-pyridyl-1-oxide)-DL-alanine.

The amino acid reacted very slowly at room temperature with an aqueous solution of ninhydrin to give an orange color. It was soluble in water to the extent of 47 g. per 100 ml. at 25° and was essentially insoluble in the usual organic solvents.

In the preparation of acylated β-(4-pyridyl-1-oxide)-DL-alanines, one potential complicating factor has to be considered, *i.e.*, that amine oxides occasionally react with anhydrides and acid chlorides. In the case of heterocyclic aromatic amine oxides, the literature, while consistent, is difficult to explain. Thus, 4-nitroquinoline-1-oxide reacted with acetyl chloride at room temperature to give 4-chloroquinoline-1-oxide while reaction with benzoyl chloride gave 4-chlorocarbostyril.[31] The reaction of 4-aminopyridine-1-oxide with benzoyl chloride under Schotten-Baumann conditions gave a dibenzoyl derivative, which was converted to 4-benzamidopyridine-1-oxide upon recrystallization.[32] Acylation of 4-aminoquinoline-1-oxide with benzoyl chloride under Schotten-Baumann conditions gave a dibenzoate, a monobenzoate, and a carbostyril.[33] Treatment of the same amine with acetic anhydride or benzoic anhydride at room temperature gave only the 4-acylamidoquinoline-1-oxides.[33] Both anhydrides reacted with pyridine-1-oxide at temperatures near 140° to give α-pyridone.[34] With the 2-and 4-picoline-1-oxides, reaction with acetic anhydride at room temperature gave the pyridylcarbinol acetates.[35] However, reaction of acetyl chloride with 4-nitro-2-picoline-1-oxide gave 4-chloro-2-picoline 1-oxide, with no evidence of rearrangement.[36]

The attempted acylation of β-(4-pyridyl-1-oxide)-DL-alanine with benzoyl chloride under Schotten-Baumann conditions gave only red oils, from which no single product could be isolated.[37] However, benzoic anhydride in the presence of triethylamine gave *N*-benzoyl-β-(4-pyridyl-1-oxide)-DL-alanine in 74% yield. The product was separated from benzoic acid by acidifying the reaction mixture until it was 2*N* in hydrochloric acid. The precipitated benzoic acid was removed and the solution then adjusted to *p*H 2.3, to precipitate the product. *N*-Benzoyl-β-(4-pyridyl-1-oxide)-DL-alanine was also obtained in 70% yield from the oximino acid by reduction with stannous chloride, precipitation of stannous and stannic ions with sodium hydroxide, and benzoylation of the filtrate after removal of the stannous and stannic hydroxides.

The pK_A' of the 1-hydroxypyridinium ion derived from β-(4-pyridyl-1-oxide)-DL-alanine was determined to be 1.15 by the spectrophotometric method of Flexser, Hammett, and Dingwall.[38] This value is in good agreement with the values for this type of ion obtained by Jaffe and Doak.[39] With a value of 3.5 for the pK_A' of the carboxyl function,[40] the above value of 1.15 was used to calculate the *p*H for the minimum solubility of *N*-benzoyl-β-(4-pyridyl-1-oxide)-DL-alanine noted above, *i.e.*, 2.3.

It was found that β-(4-pyridyl-1-oxide)-DL-alanine could be acylated with acetic anhydride under Schotten-Baumann conditions in 87% yield. A unique feature of this acetylation was that no excess of the anhydride was required. In fact, an excess led to the immediate formation of colored products, presumably arising from the reaction of the anhydride with the *N*-oxide function. This observation suggests that the amino group is more nucleophilic than the *N*-oxide oxygen with its formal negative charge. *N*-Acetyl-β-(4-pyridyl-1-oxide)-DL-alanine was so soluble in water, about 50 g. per 100 ml. at 25°, and so insoluble in organic solvents that it could not be separated from sodium chloride. This property led to the use of triethylamine as the base in the Schotten-Baumann acetylation, for after acidification of the solution to *p*H 2 and evaporation to dryness, the triethylamine hydrochloride that was formed could be removed by extraction of the solid residue with chloroform since its solubility in this solvent was 17.4 g. per 100 g. at 25°.[41] As no free amino acid could be detected in the extracted residue, it may be inferred that the acylation was quantitative.

In order to determine whether the apparent low order of reactivity of anhydrides toward the *N*-oxide function could be observed under more drastic conditions equimolar amounts of β-(4-pyridyl-1-oxide)-DL-alanine and phthalic anhydride were heated in the dry state at 140°. *N*-Phthaloyl-β-(4-pyridyl-1-oxide)-DL-alanine was isolated in 64% yield. This compound was characterized by its

(30) C. A. L. de Bruyn, *Z. physik. Chem.*, **10**, 782 (1892).

(31) T. Itai, *J. Pharm. Soc. Japan*, **65**, 70 (1945); *Chem. Abstr.*, **45**, 8525h (1951).

(32) E. Ochiai, T. Teshigaqara, K. Oda, and T. Naito, *J. Pharm. Soc. Japan*, **65**, 1 (1945); *Chem. Abstr.*, **45**, 8527i (1951).

(33) K. Oda, *J. Pharm. Soc. Japan*, **64**, 6 (1944); *Chem. Abstr.*, **45**, 8523h (1951).

(34) M. Katada, *J. Pharm. Soc. Japan*, **67**, 51 (1947); *Chem. Abstr.*, **45**, 9536d (1951).

(35) O. H. Bullitt and J. T. Maynard, *J. Am. Chem. Soc.*, **76**, 1370 (1954).

(36) I. Suzuki, *J. Pharm. Soc. Japan*, **68**, 126 (1948); *Chem. Abstr.*, **47**, 8074b (1953).

(37) Attempts to benzoylate β-(4-pyridyl)-DL-alanine with benzoyl chloride under Schotten-Baumann conditions led to the same result. However, benzoic anhydride in the presence of triethylamine gave *N*-benzoyl-β-(4-pyridyl)-DL-alanine.

(38) L. A. Flexser, L. P. Hammett, and A. Dingwall, *J. Am. Chem. Soc.*, **57**, 2103 (1935).

(39) H. H. Jaffe and G. O. Doak, *J. Am. Chem. Soc.*, **77**, 4441 (1955).

(40) E. J. Cohn and J. T. Edsall, *Proteins, Amino Acids and Peptides*, Reinhold Publishing Corp., New York (1943), p. 135.

(41) C. J. Peddle and W. E. S. Turner, *J. Chem. Soc.*, 1205 (1913).

strong ultraviolet absorption at 260 mμ,[42] its failure to give a positive ninhydrin reaction, and by its ability to give a positive ferric hydroxamate test.

The methyl esters of *N*-benzoyl- and *N*-acetyl-β-(4-pyridyl-1-oxide)-DL-alanine, and the ethyl ester of the former compound were prepared by the thionyl chloride procedure of Brenner and Huber.[43] The product first isolated was the hydroxypyridinium chloride which was converted to the free ester by reaction with ammonia in chloroform at ice temperatures.[44] The acetyl ester was soluble in water at room temperature to the extent of 50 g. per 100 ml. at 25° and the benzoyl ester to the extent of 8 g. per 100 ml. at 25°.

The resolution of *N*-benzoyl-β-(4-pyridyl-1-oxide)-DL-alanine methyl ester with α-chymotrypsin was conducted at *p*H 7.9, the solution acidified to *p*H 2.2, and after standing for one hour at 4°, the *N*-benzoyl-β-(4-pyridyl-1-oxide)-L-alanine was recovered by filtration. The filtrate was neutralized to *p*H 7 and saturated with salt, whereupon the *N*-benzoyl-β-(4-pyridyl-1-oxide)-D-alanine methyl ester precipitated. The L-acid was purified by solution in aqueous sodium bicarbonate, at *p*H 7, filtration, reprecipitation at *p*H 2.2, and recrystallization from water. The compound appeared to be a monohydrate. Esterification of the L-acid with methanol and thionyl chloride[43] gave *N*-benzoyl-β-(4-pyridyl-1-oxide)-L-alanine methyl ester, with the same melting point and the same rotation, but of opposite sign, as the D-ester.

In the attempted resolution of *N*-acetyl-β-(4-pyridyl-1-oxide)-DL-alanine methyl ester, solubility problems were encountered. Because of the high water solubility of both the ester and parent acid, the attempted separations were based on differing solubilities in organic solvents. Both compounds had rather low solubilities in methanol or ethanol. However, the ester was soluble in chloroform, while the acid was only slightly soluble in this solvent. Since the resolution was conducted in aqueous solution at *p*H 7.9, it was necessary to add base to maintain a constant *p*H. Two choices were available, sodium hydroxide or triethylamine. When sodium hydroxide was used, the sodium salt of the L-acid was formed, and this compound was only slightly in methanol or ethanol. When the solution containing the D-ester and L-acid salt was acidified, sodium chloride was formed. It was not possible to separate the salt from the L-acid. When triethylamine was used the solution again had to be acidified because the triethylammonium salt of the L-acid was soluble in chloroform. Acidification of the solution produced triethylammonium chloride which is soluble in chloroform. Evaporation of the acidified solution to dryness gave a dry powder only a small portion of which would dissolve in chloroform. The low solubility of this product in chloroform was due to the fact that enzyme was present in the dry powder. The dry enzyme is very hygroscopic. Also, triethylammonium chloride is very hygroscopic in the presence of an excess of hydrochloric acid. These two features led to the wetting of the methyl ester, which then was insoluble in chloroform.

An alternative method considered for the resolution of *N*-acetyl-β-(4-pyridyl-1-oxide)-DL-alanine was *via* a papain catalyzed synthesis of the phenylhydrazide. This method is based on the formation of a water-insoluble phenylhydrazide, which for the case at hand would not be likely, since *N*-acetyl-β-(4-pyridyl-1-oxide)-DL-alaninamide was prepared and found to be very water soluble.

Since the original aim of this study was an improved route to β-(4-pyridyl)-DL-, D-, and L-alanine, it was necessary to consider the problem of removal of the *N*-oxide group. Aliphatic amine *N*-oxides or those represented by dimethylaniline-*N*-oxide are readily reduced to the corresponding tertiary amine. On the other hand, aromatic heterocyclic amine *N*-oxides are characterized by a marked resistance to reduction. The oxide oxygen of pyridine- and quinoline-*N*-oxide is not readily removed by catalytic hydrogenation at ordinary temperatures and pressures.[45] For example 4-benzyloxypyridine-1-oxide can be hydrogenolyzed to 4-hydroxypyridine-1-oxide.[32] However, the use of acetic acid–acetic anhydride as a solvent apparently led to reduction of the *N*-oxide group.[46] Raney nickel at elevated temperatures gave a fair yield of the tertiary amine but also a large amount of by-products.[47] Of the many chemical reducing agents that have been investigated, few have proved successful. One of the most useful, *i.e.*, phosphorus trichloride,[48] has obvious limitations. Hertog, *et al.*, has reported the use of iron and glacial acetic acid.[49]

A catalytic reduction would be the most desirable. However, one is handicapped by the ease of reduction of the pyridine nucleus. Exploratory studies were based on the reduction of 4-picoline-1-oxide with platinum and palladium on charcoal. The extent of reduction at room temperature was followed by means of pressure-drop in a low pressure hydrogenation apparatus. The presence of secondary amine was determined by means of the

(42) H. H. Jaffe, *J. Am. Chem. Soc.*, **77**, 4451 (1955).

(43) M. Brenner and W. Huber, *Helv. Chim. Acta*, **36**, 1109 (1953).

(44) R. P. Bell, *J. Chem. Soc.*, 1371 (1931).

(45) E. Ochiai and M. Katada, *J. Pharm. Soc. Japan*, **63**, 186 (1943); *Chem. Abstr.*, **45**, 5151i (1951).

(46) E. Ochiai and I. Suzuki, *J. Pharm. Soc. Japan*, **67**, 185 (1947); *Chem. Abstr.*, **45**, 9541d (1951).

(47) T. Ishii, *J. Pharm. Soc. Japan*, **71**, 1092 (1951); *Chem. Abstr.*, **46**, 5046b (1952).

(48) M. Hamana, *J. Pharm. Soc. Japan*, **71**, 263 (1951); *Chem. Abstr.*, **46**, 4542c (1952).

(49) M. J. den Hertog, C. R. Kolder, and W. P. Combs, *Rec. trav. chim.*, **70**, 591 (1951).

nickel chloride–carbon bisulfide reagent described by Shriner and Fuson.[50] With 5% palladium on charcoal, no reduction was obtained using absolute ethanol or 1*N* hydrochloric acid in absolute ethanol. With glacial acetic acid and one mole equivalent of acetic anhydride, reduction of the pyridine nucleus was extensive. With Adams' catalyst, and the above three solvents, reduction of the nucleus proceeded concomitantly with that of the *N*-oxide.

It was decided to study the catalytic reduction of β-(4-pyridyl)-DL-alanine and of the corresponding *N*-oxide spectrophotometrically, using the method outlined by Friedel and Orchin.[51] The conditions employed involved the use of platinum dioxide at 40 p.s.i. of hydrogen at room temperature in water. The results are summarized in Figure 1. Although the data are not precise, there is good evidence that concomitant reduction of β-(4-pyridyl-1-oxide)-DL-alanine and β-(4-pyridyl)-DL-alanine was encountered under the above conditions.

In conjunction with the previous study, a sample of β-(4-pyridyl-1-oxide)-DL-alanine was hydrogenated at 40 p.s.i. and room temperature over a platinum dioxide catalyst, allowing 2.5 equivalents of hydrogen to be absorbed instead of the 4 equivalents required for complete reduction. These conditions should have led to a 1:1 mixture of β-(4-pyridyl)-DL-alanine and β-(4-piperidyl)-DL-alanine. A sample of the latter compound was prepared and was found to be very soluble in absolute methanol. The reduction product was isolated, dried, and extracted with methanol. The ultraviolet absorption spectra of the dried product exhibited about one-half of the theoretical molar absorption for β-(4-pyridyl)-DL-alanine and gave a blue-purple ninhydrin reaction, in contrast to the red color given by β-(4-pyridyl)-DL-alanine. The same product was isolated after a second methanol extraction. This product appears to be the β-(4-piperidyl)-DL-alanine salt of β-(4-pyridyl)-DL-alanine.

Other methods for the reduction of the amine oxide group that were investigated involved the use of hypophosphorous acid and triphenyl phosphine. In both cases no reduction was obtained. Horner and Hoffmann[52] recently reported the failure of attempted reductions of pyridine- and quinoline-1-oxides with triethyl- and triphenylphosphine. Because of the similarity of the semi-polar N→O bond in heterocyclic aromatic amine *N*-oxides to that of the N→O bond in aromatic nitro compounds, it was hoped that the use of hydrazine and Raney nickel in alcohol, a method which works well for aromatic nitro compounds,[53] would be applicable to the N-oxides. No reduction was obtained with 4-picoline-1-oxide. A reduction of the same compound was attempted, using sulfur in morpholine under the conditions of the Willgerodt reaction. The product obtained was thioisonicotinyl morpholine. These conditions appear to be too strenuous for application to more complex compounds.

This investigation has resulted in the development of a synthesis of β-(4-pyridyl)-DL-alanine which is satisfactory if quantities of 1 to 2 grams of this α-amino acid are all that are required. An alternative synthesis has been developed to the point where substantial quantities of *N*-benzoyl-β-(4-pyridyl-1-oxide)-DL-, D-, and L-alanine may be prepared with relative ease. The problem of transforming these latter compounds to β-(4-pyridyl)-DL-, D-, and L-alanine in good yield has not been solved.

EXPERIMENTAL[54,55]

4-Pyridylmethyl bromide hydrobromide. A solution of 20 g. of 4-pyridylcarbinol in 180 ml. of 48% hydrobromic acid was held at reflux for 4 hr. The reaction mixture was concentrated *in vacuo* to a thick paste, diluted with 100 ml. of absolute ethanol, filtered at ice temperature, and the residue washed with 20 ml. of absolute ethanol. The product, colorless needles, was dried *in vacuo* to give 38.7 g. of the hydrobromide (84%), m.p. 185–187° with dec., lit., 145–150°[3], 187°[5].

Diethyl acetamido-(4-pyridylmethyl)malonate. As a result of sixteen preparations of this compound under varying conditions, the procedure given below was found to be optimal for a 0.02-mole scale.

A 500-ml. three-necked round-bottomed flask, equipped with a Teflon-bladed Trubore stirrer, reflux condenser, drying tube, and a slurrying device containing a separate Teflon-bladed stirrer, was purged with dry nitrogen. After 0.92 g. (0.04 g.-atom) of sodium had been dissolved in 70 ml. of a 1:1 mixture of benzene and anhydrous ethanol, 8.46 g. (0.04 mole) of diethyl acetamidomalonate was added and the resultant solution heated to reflux. With the stopcock on the slurrying device closed, 100 ml. of the benzene-ethanol solvent was added to the bulb of the slurrying device with its stirrer on, followed by 5.06 g. (0.02 mole) of 4-pyridylmethyl bromide hydrobromide. With the stirrer in motion in the reaction flask, the stopcock of the slurrying device was opened. The slurry was added to the reaction flask over 40 min., the rate of addition being controlled by the stirring rate in the slurrying device. The resultant red reaction mixture was held at reflux for 2 hr., then allowed to stand overnight at room temperature. The solvent was removed by evaporation under a stream of air, leaving a brick red solid. The solid was extracted with 100 ml. of dry chloroform, and the chloroform solution extracted with 40 ml. of 4*N* hydrochloric acid. The acid phase was neutralized at ice temperatures with 30% aqueous sodium hydroxide to *p*H 6. The resultant precipitate was recovered by filtration, and combined with a small amount of material obtained by chloroform extraction of the filtrate to give *ca.* 4 g. of product (70%). The ester was recrystallized from water; m.p. 121.7–122.0°.

(50) R. L. Shriner and R. C. Fuson, *The Systematic Identification of Organic Compounds*, 3rd Ed., John Wiley and Sons, Inc., New York, 1948, p. 111.

(51) R. A. Friedel and M. Orchin, *Ultraviolet Spectra of Aromatic Compounds*, John Wiley and Sons, Inc., New York, 1951, pp. 29–32.

(52) L. Horner and H. Hoffmann, *Angew. Chem.*, **68**, 473 (1956).

(53) D. Balcom and A. Furst, *J. Am. Chem. Soc.*, **75**, 4334 (1953).

(54) All melting points are corrected.

(55) Microanalyses by Dr. A. Elek.

Anal. Calcd. for $C_{15}H_{20}O_5N_2$: C, 58.4; H, 6.5; N, 9.1. Found: C, 58.5; H, 6.5; N, 9.1.

This procedure gave only 30% yields when 0.08-mole quantities of the bromide-hydrobromide were used.

β-(4-Pyridyl)-DL-alanine. A solution of 15 g. of recrystallized diethyl acetamido(4-pyridylmethyl)malonate in 75 ml. of 48% hydrobromic acid was held at reflux for 6 hr. The solvent was removed *in vacuo* until a dense white solid separated. The crystalline material was recovered by filtration and washed with 20% hydrobromic acid. The filtrates were concentrated to 10 ml., and another batch of crystals obtained. The combined solids were dried *in vacuo* to give 12.2 g. (76%) of β-(4-pyridyl)-DL-alanine dihydrobromide, m.p. 250–252° (dec.).

Anal. Calcd. for $C_8H_{12}O_2N_2Br_2$: C, 29.0; H, 3.7; H, 8.5; Br, 48.4. Found: C, 29.1; H, 3.6; N, 8.7; Br, 48.5.

An aqueous solution of 11.2 g. of dihydrobromide was shaken with water washed Amberlite IR-4B until the solution was neutral. The resin was removed by filtration, and the yellow solution treated with Norit to give a colorless filtrate. The water was removed *in vacuo* to yield after recrystallization from water, 5.11 g. (90%) of β-(4-pyridyl)-DL-alanine, m.p. 234–235° with dec., lit.,[3] 235–236°.

Anal. Calcd. for $C_8H_{10}O_2N_2$: C, 57.8; H, 6.1; N, 16.9. Found: C, 57.5; H, 6.1; N, 16.2; Br, 0.3.

The addition of ninhydrin to an aqueous solution of the amino acid resulted in the formation of a red color.[3,4] The amino acid is soluble in water to the extent of 3.4 g. per 100 ml. at 25°, and is slightly soluble in pyridine.

N-Benzoyl-β-(4-pyridyl)-DL-alanine. To 0.5 g. (3.02 mmole) of β-(4-pyridyl)-DL-alanine dissolved in 20 ml. of water at 10° was added 0.835 ml. of triethylamine (6.04 mmole) and 0.75 g. (3.32 mmole) of benzoic anhydride. The suspension was stirred for 18 hr., filtered to remove residual anhydride, the filtrate acidified to *p*H 1 with concd. hydrochloric acid, the precipitated benzoic acid removed, and the filtrate adjusted to *p*H 4.3 with aqueous sodium bicarbonate. A ter standing overnight at 4°, the crystalline product was recovered and recrystallized from 50% aqueous methanol to give 0.67 g. of the acylated amino acid, m.p. 246° with dec.

Anal. Calcd. for $C_{15}H_{14}O_3N_2$: C, 66.7; H, 5.2; N, 10.4. Found: C, 66.4; H, 5.3; N, 10.3.

Ethyl β-(4-pyridyl-1-oxide)pyruvate. To a solution of 28 g. (1.22 g.-atom) of sodium in 300 ml. of absolute ethanol was added 109.2 g. (1.00 mole) of 4-picoline-1-oxide dissolved in 300 ml. of absolute ethanol. The solution was stirred at reflux for 15 min. to give a clear red solution. To this solution was added 146.2 g. (1.00 mole) of ethyl oxalate over a period of 5 min. After about 2 min., the yellow sodium salt of the keto ester began to precipitate. Heating was discontinued and the resultant yellow paste stirred for 2 hr. The solvent was removed *in vacuo* to leave a yellow paste, which was dissolved in water to give a dark red solution. The solution was neutralized with 12*N* aqueous hydrochloric acid, the precipitate recovered and recrystallized from water to give 100 g. of product A, yellow needles, m.p. 140.2–141.5°. Concentration of the aqueous filtrate gave 20 g. of product B, yellow prisms, m.p. 182.8–183.0°. Analyses of both samples showed neither to be pure keto ester. B would not dissolve in chloroform, while A dissolved nearly completely. Recrystallization of A from water after prior solution in chloroform and removal of the insoluble material, *i.e.*, B, gave 95 g. of keto ester, m.p. 129° with dec., lit.,[22] 122–123°. Another 5.0 g. was obtained from a chloroform extract of B to give a total yield of 48%.

Recrystallization of B from water, after prior extraction with chloroform, gave a pale yellow solid, m.p. 187–188° with dec. This compound was an acid of molecular weight *ca.* 300 and would not form an oxime. When heated, the compound suddenly decomposed and evolved a purple vapor. The compound turned bright yellow after prolonged exposure to light. No consistent analyses could be obtained but it was shown that the compound contained nitrogen.

Ethyl-α-oximino-β-(4-pyridyl-1-oxide)propionate. The condensation of 4-picoline-1-oxide and ethyl oxalate was conducted as described above. However, the solvent was not removed *in vacuo*. Instead, the yellow paste was washed from the reaction flask into a 4-l. beaker with 2 l. of 50% aqueous ethanol. The resultant red solution heated to *ca.* 60°, and 85 g. (1.22 mole) of hydroxylamine hydrochloride and 100 g. (1.22 mole) of sodium acetate were added. The reaction mixture was allowed to cool overnight, the crystals which had formed collected, the filtrate concentrated to one-half its original volume, and a second crop of crystals recovered. The two crops were combined and recrystallized from water to give 136 g. (60%) of oximino ester, m.p. 210° with dec., lit.,[22] 221–222°. Although the oximino ester was dried over phosphorus pentoxide at 65° and 100 mm., the low melting point and the analysis indicated that a hydrate had been obtained.

Anal. Calcd. for $C_{10}H_{12}O_4N_2 \cdot H_2O$: C, 49.6; H, 5.8; N, 11.6. Found: C, 49.7; H, 5.6; N, 11.7.

The same product was obtained from ethyl β-(4-pyridyl-1-oxide)pyruvate. To a hot solution of 15 g. (0.0702 mole) of keto ester in 100 ml. of water was added 4.9 g. (0.0702 mole) of hydroxylamine hydrochloride, 2.8 g. (0.0702 mole) of sodium hydroxide, and 5.75 g. (0.0702 mole) of sodium acetate. A white flocculent precipitate formed as the solution cooled. The precipitate was recrystallized from water to give 8.2 g. (52%) of fibrous needles, m.p. 210° with dec.

α-Oximino-β-(4-pyridyl-1-oxide)propionic acid. A solution of 20 g. (0.0893 mole) of the ester and 7.15 g. (0.1786 mole) of sodium hydroxide in 180 ml. of water was held at the boiling point for 5 min. The reaction mixture was cooled to about 50°, neutralized with 14.9 ml. of concd. hydrochloric acid, diluted with 25 ml. of water, kept in an ice bath for 4 hr., the solid which had formed recovered and washed with a small amount of cold water. The product was dried *in vacuo* to give 14.3 g. (82%) of acid, m.p. 140–141° with dec. Recrystallization from water gave the acid, clusters of fine needles, m.p. 139.8–140° with dec.

Anal. Calcd. for $C_8H_8O_4N_2$: C, 49.0; H, 4.1; N, 14.3. Found: C, 49.2; H, 4.2; N, 14.4.

β-(4-Pyridyl-1-oxide)-DL-alanine. To 1 l. of concd. hydrochloric acid and 260 g. (1.15 mole) of stannous chloride dihydrate was added, in portions, 100 g. (0.51 mole) of α-oximino-β-(4-pyridyl-1-oxide)propionic acid. The clear solution was allowed to stand at room temperature overnight and then concentrated *in vacuo* to a thick paste. The paste was dissolved in 750 ml. of water and the solution neutralized to *p*H 6.8 with *ca.* 375 ml. of 28% aqueous ammonia. The precipitate was removed and washed with 200 ml. of water. The filtrate was evaporated to dryness *in vacuo*, and the granular residue ground to pass an 80-mesh screen. This powder was extracted with 6 l. of absolute methanol to give 90 g. of crude product. Further extraction with two 500-ml. portions of methanol gave 78 g. (84%) of β-(4-pyridyl-1-oxide)-DL-alanine, m.p. 238.2° with dec. An aqueous solution of the product did not give a positive test with silver nitrate.

Anal. Calcd. for $C_8H_{10}O_3N_2$: C, 52.7; H, 5.5; N, 15.4. Found: C, 52.7; H, 5.6; N, 15.4.

The product could be obtained more rapidly, but in a lower yield, *i.e.*, *ca.* 45%, by removing the solid, formed on partial evaporation of the filtrate obtained by adjustment of the acidic reaction mixture to *p*H 6.8 with aqueous ammonia followed by filtration, and continuing this process until the volume of the filtrate was reduced to *ca.* 150 ml. The filtrate was then evaporated to dryness and the residue, mainly product, extracted with methanol to remove residual ammonium chloride.

The amino acid gave an orange color, preceded by a violet-blue color, with ninhydrin solution. The reaction was very slow. The amino acid was soluble in water to the extent of 45 g. per 100 ml. at 25° and was slightly soluble in pyridine.

N-Benzoyl-β-(4-pyridyl-1-oxide)-DL-alanine. To a solution of 61.0 g. (0.33 mole) of β-(4-pyridyl-1-oxide)-DL-alanine in

600 ml. of ice cold water and 92 ml. (0.66 mole) of triethylamine was added 82.2 g. (0.363 mole) of finely ground benzoic anhydride. The mixture was stirred for 18 hr., filtered, and the filtrate acidified with 246 ml. of 12*N* aqueous hydrochloric acid. The precipitated benzoic acid was removed, washed with a small amount of 2*N* aqueous hydrochloric acid, and the combined filtrate and washings adjusted to *p*H 2.3 with 91.2 g. of sodium hydroxide in 200 ml. of water. The resultant paste was cooled to 4°, the product collected, washed with 250 ml. of water, and dried to give 73 g. (77%) of acid, m.p. 228.5–229.2° with dec. The acid was recrystallized from 60% aqueous ethanol to give 70 g. (74%) of acid, m.p. 229.5° with dec.

Anal. Calcd. for $C_{15}H_{14}O_4N_2$: C, 62.9; H, 4.9; N, 9.8. Found: C, 62.9; H, 5.0; N, 9.8.

The compound was soluble in water to the extent of 0.5 g. per 100 ml. at 25°.

*N-Acetyl-β-(4-pyridyl-1-oxide)-*DL*-alanine.* To 30 g. (0.165 mole) of the amino acid dissolved in 165 ml. of water was added 23.1 ml. (0.165 mole) of triethylamine. The solution was cooled to *ca.* 0° and portions, each of 5.8 ml. (0.041 mole) of triethylamine and 3.85 ml. (0.041 mole) of acetic anhydride, were added in that order with vigorous stirring and at 10-min. intervals. When a few drops of the fifth portion of acetic anhydride was added, the solution became bright orange, and no further addition was made. The solution was acidified with 100 ml. of 4*N* hydrochloric acid and concentrated *in vacuo*. The residual paste was dried *in vacuo* over potassium hydroxide and phosphorus pentoxide for two days. The dried material was powdered in a dry box, shaken with 400 ml. of dry chloroform for 20 min. to remove the triethylamine hydrochloride, and then filtered to give 32.9 g. of crude product. An additional 3.6 g. separated from the filtrate to give a total yield of 36.5 g. The crude product was stirred with 100 ml. of dry chloroform for 10 min., filtered, and dried to give 32.3 g. (87%) of chloride-free product. This product was recrystallized from absolute ethanol to give the acid, m.p. 210.2° with dec.

Anal. Calcd. for $C_{10}H_{12}O_4N_2$: C, 53.6; H, 5.4; N, 12.5. Found: C, 53.5; H, 5.1; N, 12.5.

The acid was soluble in water to the extent of 60 g. per 100 ml. at 25°.

*N-Phthaloyl-β-(4-pyridyl-1-oxide)-*DL*-alanine.* β-(4-Pyridyl-1-oxide)-DL-alanine, 1.0 g., (5.5 mmole) and phthalic anhydride, 0.816 g., (5.5 mmole) were thoroughly mixed and heated at 140° for one hour. The yellow paste was cooled and treated with saturated aqueous sodium bicarbonate. The residual anhydride was removed and the filtrate acidified with hydrochloric acid to give an oil which crystallized upon trituration with water. The crystalline solid was collected to give 1.06 g. (64%) of product, m.p. 253–254° with dec. This product was recrystallized from 200 ml. of water to give the acid, rhombs, m.p. 254.5–255.0° with dec. The ultraviolet spectrum of the acid exhibited a characteristic *N*-oxide absorption at 260 mμ and the acid did not give a positive ninhydrin reaction. It gave a positive ferric hydroxamate test, similar to that observed with *N*-phthaloyl-DL-phenylalanine.

Anal. Calcd. for $C_{16}H_{12}N_2O_5$: C, 61.5; H, 3.9; N, 9.0. Found: C, 61.5; H, 4.0; N, 9.1.

*N-Benzoyl-β-(4-pyridyl-1-oxide)-*DL*-alanine methyl ester.* To 200 ml. (4.88 mole) of absolute methanol, cooled in an ice-salt bath and stirred, was added over a period of 15 min., 26.7 ml. (0.366 mole) of thionyl chloride. To this solution was added, in portions, over a period of 20 min., 70.0 g. (0.244 mole) of *N*-benzoyl-β-(4-pyridyl-1-oxide)-DL-alanine. The clear solution was warmed to 40°, and after about 15 min., a white solid began to precipitate. The slurry was stirred overnight at room temperature, filtered, and the filtrate evaporated to dryness. The residues from the filtration and evaporation were combined and dried *in vacuo* to give 77 g. (94%) of ester hydrochloride. The dry hydrochloride was suspended in 600 ml. of ice cold dry chloroform and treated with 150 ml. of 1.8*N* ammonia in chloroform (0.270 mole). The ammonium chloride that formed was removed and the filtrate evaporated to dryness. The residue was washed with ligroin and dried *in vacuo* to give 63.5 g. (86%) of ester, m.p. 190.0–190.5°. The melting point was not raised by recrystallization of the ester from chloroform. The ester was soluble in water to the extent of 8.0 g. per 100 ml. at 25°.

Anal. Calcd. for $C_{16}H_{16}O_4N_2$: C, 64.0; H, 5.4; N, 9.3. Found: C, 63.9; H, 5.4; N, 9.5.

*N-Benzoyl-β-(4-pyridyl-1-oxide)-*DL*-alanine ethyl ester.* This ester, m.p. 141–143°, was prepared from the acid and anhydrous ethanol as described for the methyl ester.

Anal. Calcd. for $C_{17}H_{18}O_4N_2$: C, 65.0; H, 5.8; N, 8.9. Found: C, 65.2; H, 5.6; N, 9.0.

*N-Acetyl-β-(4-pyridyl-1-oxide)-*DL*-alanine methyl ester.* This ester was prepared in 75% yield from *N*-acetyl-β-(4-pyridyl-1-oxide)-DL-alanine in a manner identical with that used for the *N*-benzoyl-compound. The ester was very soluble in chloroform and water, soluble in methanol, less soluble in ethanol and 2-propanol, and insoluble in ethyl acetate and toluene. The ester was recrystallized from absolute ethanol to give clusters of hygroscopic needles, m.p. 193.5–194.2° with dec.

Anal. Calcd. for $C_{11}H_{14}O_4N_2$: C, 55.5; H, 5.9; N, 11.8. Found: C, 55.4; H, 6.0; N, 11.7.

The ester was also obtained in a 47% yield from α-oximino-β-(4-pyridyl-1-oxide)propionic acid as follows: The oximino acid was reduced with stannous chloride and the tin salts removed as described previously. Following concentration, the filtrate was acetylated with acetic anhydride and sodium hydroxide. The resultant solution was acidified to *p*H 2.0 and evaporated to dryness. Treatment of the residue with thionyl chloride and methanol gave a solution of the ester hydrochloride and a precipitate of sodium chloride. Following filtration, the solution was treated as described above.

*The determination of the pK_A of the N-benzoyl-β-(4-pyridyl-1-hydroxy)-*DL*-alanine ion.* A stock solution, $0.930 \times 10^{-2}M$ in *N*-benzoyl-β-(4-pyridyl-1-oxide)-DL-alanine in *ca.* 0.3*N* hydrochloric acid was prepared. The ultraviolet spectrum of 1.00 ml. of the stock solution, diluted to 50.00 ml. with 4*N* hydrochloric acid, was taken to obtain a value for A_{BH}. The ultraviolet spectrum of 1.00 ml. of the stock solution, made basic with sodium hydroxide and diluted to 100.00 ml. with water (*p*H 10.6), was determined to obtain a value for A_B. From extinction coefficients determined at 260 mμ and proceeding as described by Flexser, Hammett, and Dingwall,[38] the following values were obtained or derived; $A_{BH} = 1.013$, $A = 1.438$, $A_B = 1.648$, $C_{BH} = 1.86 \times 10^{-4}M$, $C = 1.86 \times 10^{-4}M$, $C_B = 0.93 \times 10^{-4}M$, $C_H = 0.309M$, $C_{BH}/C_B = 4.37$, $k'_{BH} = 0.545 \times 10^4$ cm. M^{-1} $k' = 0.773 \times 10^4$ cm. M^{-1}, $k'_B = 1.772 \times 10^4$ cm. M^{-1} $K'_A = 0.0707$ and $pK'_A = 1.15$.

*β-(4-Pyridyl-1-oxide)-*DL*-alanine methyl ester.* The amino acid was suspended in absolute methanol and the mixture saturated with dry hydrogen chloride, allowing the temperature to rise to *ca.* 60°. The clear solution was evaporated *in vacuo*, and the residue neutralized with methanolic sodium methoxide. The sodium chloride was removed, the filtrate concentrated, filtered, and the filtrate evaporated to dryness *in vacuo*. The product, a hard glass, was soluble in chloroform if initially wet with methanol. It was completely insoluble in anhydrous chloroform once exposed to a trace of moisture. The product was deliquescent in air, and it was impossible to obtain a pure sample, even when working in a dry box.

*β-(4-Pyridyl-1-oxide)-*DL*-alaninhydrazide.* Approximately 1.0 g. of the above ester was dissolved in 5 ml. of absolute methanol, 0.5 ml. of anhydrous hydrazine added, the solution held at reflux for one hour and then evaporated in a stream of dry nitrogen to give a viscous oil which crystallized when triturated with chloroform. The solid was collected and dried *in vacuo* to give 0.71 g. (70%) of a hygroscopic product,

TABLE I

SPECTRAL AND RATE DATA FOR HYDROGENATION OF β-(4-PYRIDYL)-DL-ALANINE AND ITS *N*-OXIDE

Optical Density *vs.* Concentration

β-(4-Pyridyl)-DL-alanine			β-(4-Pyridyl-1-oxide)-DL-alanine		
Concn. $M \times 10^{-3}$	O.D.260 mμ	O.D250 mμ	Concn. $M \times 10^{-4}$	O.D.260 mμ	O.D.250 mμ
0.120	0.258	0.246	0.209	0.328	0.213
0.240	0.526	0.498	0.418	0.660	0.434
0.480	1.042	0.981	0.835	1.343	0.878
0.959	2.056	1.947	1.044	1.692	1.106
1.199	2.619	2.416	1.225	2.053	1.402

Optical Density *vs.* Time

β-(4-Pyridyl)-DL-alanine $c_0 = 1.199 \times 10^{-2} M$ Pt = 0.265 g./l.			β-(4-Pyridyl-1-oxide)-DL-alanine $c_0 = 1.074 \times 10^{-2} M$ Pt = 0.265 g./l.		
Time, min.	O.D.260 mμ $\times 10^{-1}$	O.D.250 mμ $\times 10^{-1}$	Time, Min.	O.D.260 mμ $\times 10^{-2}$	O.D.250 mμ $\times 10^{-2}$
0	2.619	2.416	0	1.724	1.185
5	2.323	2.147	10	0.983	0.672
10	2.167	2.010	23	0.504	0.362
21	2.010	1.838	39	0.263	0.213
46	1.767	1.648	64	0.1250	0.118
63	1.672	1.605	109	0.0730	0.0730
91	1.616	1.477	155	0.0581	0.0578
298	1.010	0.990	1130	0.0149	0.0151

Mole Percent *vs.* Time[a]

β-(4-Pyridyl)-DL-alanine		β-(4-Pyridyl-1-oxide)-DL-alanine			
Time, Min.	Mole % PA[b]	Time, Min.	Mole %, POA[c]	Mole %, PA[d]	Mole %, PIA[d]
0	100	0	100	0	0
5	89	10	51	44	5
10	82	23	22	49	29
21	76	39	6.5	63.5	30
46	68	64	1	46	53
63	65	109	0	32.5	67.5
91	61	155	0	25.5	74.5
298	40	1130	0	6.5	93.5

[a] Based upon the relations: at 260 mμ, $O.D._{POA}/c_{POA} = 1.604 \times 10^4 M^{-1}$, $O.D._{PA}/c_{PA} = 2.183 \times 10^3 M^{-1}$; at 250 mμ $O.D._{POA}/c_{POA} = 1.058 \times 10^4 M^{-1}$, $O.D._{PA}/c_{PA} = 2.017 \times 10^3 M^{-1}$. [b] β-(4-pyridyl)-DL-alanine. [c] β-(4-pyridyl-1-oxide)-DL-alanine. [d] β-(4-piperidyl)-DL-alanine.

which was recrystallized from ethanol to give large rhombs, m.p. 147–148°.

Anal. Calcd. for $C_8H_{12}O_2N_4$ (196): C, 49.0; H, 6.2; N, 28.6. Found: C, 48.9; H, 6.0; N, 28.4.

N-Acetyl-β-(4-pyridyl-1-oxide)-DL-alaninamide. A solution of 5.0 g. of *N*-acetyl-β-(4-pyridyl-1-oxide)-DL-alanine methyl ester in 120 ml. of dry methanol saturated with ammonia at room temperature was allowed to stand overnight. The solution was evaporated to dryness *in vacuo*, the residue suspended in absolute ethanol and filtered to give 4.0 g. (86%) of product. This substance was recrystallized from 300 ml. of absolute ethanol to give 3.4 g. of amide, m.p. 235–236° with dec. The amide was soluble in water to the extent of about 50 g. per 100 ml. at 25°.

Anal. Calcd. for $C_{10}H_{13}O_3N_3$: C, 53.8; H, 5.9; N, 18.8. Found: C, 53.8; H, 5.8; N, 18.8.

N-Benzoyl-β-(4-pyridyl-1-oxide)-L-alanine. α-Chymotrypsin, 20 mg., was added to a solution of 10 g. of *N*-benzoyl-β-(4-pyridyl-1-oxide)-DL-alanine methyl ester in 100 ml. of water maintained at *p*H 7.9 by the addition of 1*N* aqueous sodium hydroxide. The asymmetric hydrolysis was complete in one hour. The clear solution was acidified to *p*H 2.3 with 6*N* aqueous hydrochloric acid, the solution held for one hour at 4°, the precipitated L-acid collected, and recrystallized from water to give 2.73 g. (58%) of L-acid, $[\alpha]_D^{25°}$ −45.1° to −43.4° (c, 1.5% in acetic acid), m.p., 216–220°, with oil formation at 145–150°. Analyses for this compound were never satisfactory even after repeated recrystallization. A similar behavior was observed with resolutions conducted with the ethyl ester.

Anal. Calcd. for $C_{15}H_{14}O_4N_2 \cdot H_2O$ (316): C, 60.7; H, 5.1; N, 8.9. Found: C, 59.5; H, 5.2; N, 10.1.

N-Benzoyl-β-(4-pyridyl-1-oxide)-D-alanine methyl ester. The acidified filtrate from the above resolution was adjusted to *p*H 7.0 with saturated aqueous sodium bicarbonate and the solution saturated with sodium chloride. The D-methyl ester precipitated. The crude ester was collected, dried, dissolved in chloroform, the solution filtered and the filtrate evaporated to give 2.82 g. (56%) of the D-methyl ester, m.p. 207° with dec., $[\alpha]_D^{25°}$ +95.5° (c, 3.3% in methanol).

Anal. Calcd. for $C_{16}H_{16}O_4N_2$: C, 64.0; H, 5.4; N, 9.3. Found: C, 63.9; H, 5.3; N, 9.2.

N-Benzoyl-β-(4-pyridyl-1-oxide)-D-alanine ethyl ester. This ester was recovered from a resolution of the DL-ethyl ester which was conducted as described for the D-methyl ester. The crude product was recrystallized from 300 ml. of ethyl acetate containing 3 ml. of water to give 3.7 g. (74%) of D-ester, m.p. 181.0–182.0, $[\alpha]_D^{25°}$ +87.6° (c, 1.5% in methanol).

Anal. Calcd. for $C_{17}H_{18}O_4N_2$: C, 65.0; H, 5.8; N, 8.9. Found: C, 65.0; H, 5.9; N, 8.8.

N-Benzoyl-β-(4-pyridyl-1-oxide)-L-alanine methyl ester. This ester was prepared from the L-acid by reaction with methanol and thionyl chloride. The recrystallized product,

m.p. 208°, $[\alpha]_D^{25}$° −95.5° (c, 4% in methanol), was obtained in 88% yield.

Anal. Calcd. for $C_{16}H_{16}O_4N_2$: C, 64.0; H, 5.4; N, 9.3. Found: C, 63.9; H, 5.4; N, 9.3.

*N-Benzoyl-β-(4-pyridyl-1-oxide)-*L*-alanine ethyl ester.* This ester was prepared as described for the DL-ester. Recrystallization of the crude product from wet ethyl acetate gave 86% of the L-ester, m.p. 181.0–182.5°, $[\alpha]_D^{25}$° −87.3° (c, 1.5% in methanol).

Anal. Calcd. for $C_{17}H_{18}O_4N_2$: C, 65.0; H, 5.8; N, 8.9. Found: C, 64.9; H, 5.9; N, 8.9.

4-(Pyridyl-1-oxide)carbinol. To 23 g. of 4-pyridylcarbinol dissolved in 200 ml. of glacial acetic acid was added 30 ml. of 30% aqueous hydrogen peroxide, the solution held at 70° for three hours, another 30 ml. of hydrogen peroxide added and the solution held at 70° overnight. The solvent was removed *in vacuo* and the residue recrystallized from a mixture of ethanol and ethyl acetate to give 19.6 g. (74%) of fine needles, m.p. 111.5–112.0°.

Anal. Calcd. for $C_6H_7NO_2$ (125): C, 57.6; H, 5.6; N, 11.2. Found: C, 57.6; H, 5.6; N, 11.1.

4-(Pyridyl-1-oxide)methyl bromide hydrobromide. A solution of 10.0 g. of 4-(pyridyl-1-oxide)carbinol in 50 ml. of 48% hydrobromic acid was heated twice to the boiling point and allowed to stand overnight. The acid was removed *in vacuo*, 50 ml. of absolute ethanol added, and the solution cooled to 0° to give a paste, which was recrystallized from absolute ethanol to give 8.2 g. of the hydrobromide of 4-(pyridyl-1-oxide)carbinol, m.p. 93–95°. This material, 6.53 g., was dissolved in 25 ml. of hydrobromic acid and held at reflux for 18 hr. The acid was removed *in vacuo*. The addition of absolute ethanol to the residue caused the formation of a white crystalline solid, which was collected, washed with absolute ethanol, and dried *in vacuo* to give 7.2 g. (88%) of a hygroscopic product, m.p. 170.5–171.8°.

Anal. Calcd. for $C_6H_6ONBr \cdot HBr$: C, 26.8; H, 2.6; N, 5.3; Br, 59.4. Found: C, 26.9; H, 2.6; N, 5,2; Br, 59.4.

4-(Pyridyl-1-oxide)methyl bromide. Five g. of the above bromide hydrobromide was dissolved in the minimum amount of water and solid sodium bicarbonate was added until the solution was adjusted to *p*H 7.0. The solution was saturated with salt and extracted with chloroform. The extracts were dried and the solvent removed to give the theoretical amount of solid, m.p. 138–138.5°. No analysis was obtained as the compound becomes colored and decomposes within several hours.

Thioisonicotinyl morpholine. A suspension of 9.6 g. (0.3 g.-atom) of sulfur in a mixture of 10.9 g. (0.1 mole) of 4-picoline-1-oxide and 13.1 g. (0.15 mole) of morpholine was heated at 170° for 12 hr. The reaction mixture was cooled, diluted with 50 ml. of absolute ethanol, the precipitate collected and recrystallized twice from ethanol to give 11.4 g. (55%) of product, m.p. 150–152° lit.,[56] 150–151°.

Anal. Calcd. for $C_{10}H_{12}N_2OS$: C, 57.7; H, 5.8; N, 13.5. Found: C, 57.6; H, 5.8; N, 13.4.

*Catalytic hydrogenation of β-(4-pyridyl)-*DL*-alanine and its N-oxide.* The reductions were conducted in the same manner for both compounds. A weighed sample of each compound was dissolved in 25 ml. of water, the weighed platinum dioxide catalyst added, and the mixture hydrogenated at 40 p.s.i. and 25°. At selected time intervals the hydrogenation was interrupted, a 2.5-ml. aliquot removed, filtered and a 1.0-ml. aliquot of the filtrate diluted 1:10 for β-(4-pyridyl)-DL-alanine and 1:100 to 1:5 for the *N*-oxide. The spectra were taken at 260 and 250 mμ. The data are summarized in Table I. The data for the mole percentage of β-(4-pyridyl)-DL-alanine and β-(4-piperidyl)-DL-alanine in the reduction of β-(4-pyridyl-1-oxide)-DL-alanine have error in them estimated at ± 10 mole percent through the fifth point, and about ± 2 mole percent in the last points. This is due to the fact there is approximately a factor of 10 between the molar extinction coefficients of β-(4-pyridyl-1-oxide)-DL-alanine and β-(4-pyridyl)-DL-alanine and small errors in the concentration of the former component are reflected by *ca.* 10 times that error in the concentration of the latter. β-(4-Piperidyl)-DL-alanine has no absorption in this region.

To a solution of 5.0 g. (.0275 mole) of β-(4-pyridyl-1-oxide)-DL-alanine in 25 ml. of water was added 0.5 g. of platinum dioxide and the mixture hydrogenated at 40 p.s.i. and 25° until 0.069 mole of hydrogen had been absorbed. The catalyst was removed and the solution evaporated in dryness *in vacuo*. The solid was extracted with 25 ml. of dry methanol and the residue, 2.82 g., dried *in vacuo*. An oily solid was isolated from the methanol extract. β-(4-Piperidyl)-DL-alanine is very hygroscopic. A determination of the extinction coefficient at 256 mμ, assuming the absence of β-(4-pyridyl-1-oxide)-DL-alanine, gave a value of 311 ± 16 for the molecular weight of the solid product. After a second extraction with methanol, a value of 380 ± 10 was obtained. The molecular weight of β-(4-piperidinium)-DL-alanine β-(4-pyridyl)-DL-alaninate is 338. The solid decomposed at 250–280° and gave a blue-purple color with ninhydrin. The yield of 2.8 g. compares favorably with the yield of 2.5 g. expected on the basis of hydrogen uptake.

PASADENA, CALIF.

(56) H. D. Porter, *J. Am. Chem. Soc.*, **76**, 127 (1954).

[CONTRIBUTION FROM INDIAN ASSOCIATION FOR THE CULTIVATION OF SCIENCE]

Synthesis of the Dicarboxylic Acid $C_{12}H_{14}O_4$—Degradation Product of Picrotoxin

MONOJIT GHOSAL, BISWAJIT SINHA, AND P. BAGCHI

Received September 9, 1957

γ-(2-Carboxy-6-methylphenyl)butyric acid, a degradation product of picrotoxin, has been synthesized following an unambiguous procedure. The synthetic compound possesses properties similar to those described for the product from natural sources.

Picrotoxin is a molecular compound of picrotin and picrotoxinine. Each of these compounds when boiled with phosphorus and hydriodic acid produces picrotic acid.[1] The latter, on hydrolytic fission produces acetone and a dibasic acid, $C_{12}H_{14}O_4$.[2] Out of the two possible structures for this dibasic acid,

(1) F. Angelico, *Gazz. chim. ital.* **42**, ii, 337 (1911).

(2) F. Angelico and F. Monforte, *Gazz. chim. ital.*, **53**, 800 (1923).