The Synthesis of Possible Degradation Products of Nicotine*

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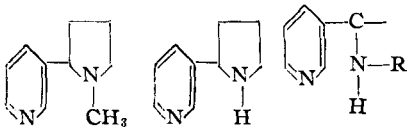
The following compounds have been synthesized as possible degradation products of nicotine: γ-keto-γ-(3-pyridyl)butyric acid, β-amino-β-(3-pyridyl)-propionic acid, and β-methylamino-β-(3-pyridyl)-propionic acid. Certain derivatives of these compounds have been prepared.

LARSON, HAAG, and others (1–4) have been interested in the biological fate of nicotine (I). It has been found (4) that the urine of dogs that had received nicotine contained a substance which gave a red color with cyanogen bromide, while the color reaction was not observed if the dogs had not received nicotine. The urine of dogs that had received nornicotine (II) also gave a red color with cyanogen bromide. This color was due to unchanged nornicotine. It has been established (5) that nornicotine is not a metabolite of nicotine. In order to determine the structural requirements for the red color with cyanogen bromide, a series of compounds was examined and it was found that a red color was obtained when a primary or secondary amino group was attached to the carbon atom adjacent to the pyridine ring (III), (where R may be H or alkyl). This would suggest enzymic cleav-



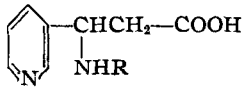
(I) (II) (III)

age of the pyrrolidine ring of nicotine between carbon atom five and the nitrogen atom as shown in Fig. 1. This is in accord with the mechanism postulated by Taggart and Krakaur (6) for the enzymic cleavage of the pyrrolidine ring of proline and hydroxyproline.

(IV) a, R = CH_3
(IV) b, R = H

Figure 1

The corresponding propionic acid analogs of IV are also possible degradation products of nicotine. We have synthesized β-amino-β-(3-pyridyl) propionic acid V_a by the addition of hydroxylamine to β-(3-pyridyl)acrylic acid. V_a was also prepared by the condensation of nicotinaldehyde, malonic acid, and ammonium acetate in absolute ethanol. It was accompanied by β-(3-pyridyl)



(V) a, R = H
(V) b, R = CH_3

acrylic acid and by a large amount of an unidentified substance which could be converted to the above acid by heat. β-Methylamino-β-(3-pyridyl)propionic acid V_b was prepared by the condensation of nicotinaldehyde, malonic acid, and methylammonium acetate in absolute ethanol. Both V_a and V_b gave a red color with cyanogen bromide (5). Compounds V_a and V_b have been characterized by their infrared absorption spectra[1] obtained in a mineral oil mull. Both amino acids show absorption bands in the 6.0–6.7 μ region which are characteristic of amino acids.

For the attempted synthesis of γ-amino-γ-(3-pyridyl)butyric acid IV_b, ethyl nicotinate VI was condensed with diethyl succinate VII by a modification of the procedure of Shivers, Dillon, and Hauser (7). The diethyl α-nicotinoylsuccinate VIII thus obtained was converted to γ-keto-γ-(3-pyridyl)butyric acid IX by acid hydrolysis and decarboxylation (Fig. 2).

After the synthesis of IX was completed Wada and Yamasaki (8) announced the isolation of the keto acid IX from microbial culture media in which nicotine was the only nitrogen-containing substrate. Our synthesis constitutes a proof of structure of their keto acid. It is entirely possible that this compound may be important in other biological degradations of nicotine, although it would not be expected to give a red color with cyanogen bromide.

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[1] These spectra were determined by E. S. Harlow and W. B. Wartman of the Research Laboratory of the American Tobacco Company, Richmond, Va.

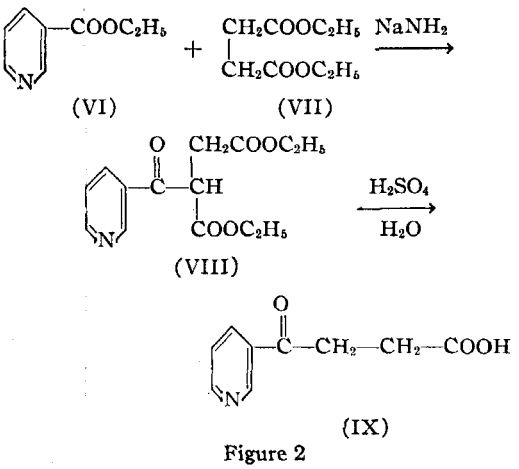
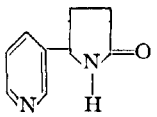


Figure 2

Compound IX was readily converted to the oxime. All attempts to reduce the oxime to the amino acid IV_b or to the lactam X have been unsuccessful. We were also unable to convert the keto acid IX into IV_a or X by catalytic reduction in the presence of ammonia. It was expected



(X)

that the reduction of the acetylhydrazone of IX would furnish IV_a or X since the acetylhydrazone of diethyl β-ketopimelate was a good source of β-aminopimelic acid (9). However, the reduction of the acetylhydrazone of IX with aluminum amalgam gave unidentifiable gums. Other synthetic routes to IV_a, IV_b, and X are being investigated.

In Table I the important features of the ultraviolet absorption spectra of IX and its methyl ester are compared with those of 3-acetylpyridine (10) and with the natural keto acid isolated by Wada and Yamasaki (11).

EXPERIMENTAL

All melting points are uncorrected.

β-(3-Pyridyl)acrylic Acid.—This compound was prepared by the method of Panizzon (12) from nicotinaldehyde[2] and malonic acid in the presence of pyridine and piperidine, m. p. 231–234°.

β-(3-Pyridyl)acrylic Acid Hydrobromide.—It was prepared by refluxing the β-(3-pyridyl)acrylic acid in 40% aqueous hydrobromic acid. The nearly pure hydrobromide separated as white plates upon cooling. A sample purified for analysis melted at 263–265°.

Anal.—Calcd. for $C_8H_7NO_2 \cdot HBr$: C, 41.76; H, 3.51. Found: C, 42.17; H, 3.75.

[2] Supplied by Dr. John Aeschlimann, Hoffmann-La Roche, Nutley, N. J.

TABLE I.—COMPARISON OF ULTRAVIOLET ABSORPTION SPECTRA

Compound	Maxima λ, mμ	Maxima ε × 10⁻³	Minima λ, mμ	Minima ε × 10⁻³
3-Acetylpyridine	228	8.48	250	2.05
	267	2.88		
γ-Keto-γ-(3-pyridyl)-butyric acid	227.5	9.18	250	2.08
	267.5	3.09		
Methyl γ-keto-γ-(3-pyridyl)butyrate	229	9.42	250	2.30
	267.5	3.63	300	0.23
	310	0.38	320	0.13
Natural Keto Acid (11)	228.5	8.63	250	...
	267	2.97	...	...

β-(Amino-β-(3-pyridyl)propionic Acid.—The procedure in *Organic Syntheses* (13) for the preparation of β-phenyl-β-alanine was adapted to the preparation of the 3-pyridyl analog.

To a hot solution of sodium ethoxide, prepared from 2.3 Gm. (0.1 Gm. atom) of sodium and 80 ml. of absolute ethanol, was added with shaking 6.95 Gm. (0.1 mole) of hydroxylamine hydrochloride. The suspended sodium chloride was filtered off and washed with absolute ethanol. To this solution was added 7.45 Gm. (0.05 mole) of β-(3-pyridyl)-acrylic acid. A voluminous precipitate separated which dissolved upon heating. After nine hours of refluxing the reaction mixture deposited 0.6 Gm. of the desired amino acid upon cooling. After repeated purification, accomplished by dissolving the compound in water and precipitating it with absolute ethanol, it gave fine white plates, m. p. 206° (dec.).

Anal.—Calcd. for $C_8H_{10}N_2O_2$: C, 57.82; H, 6.07. Found: C, 58.18; H, 6.11.

The above amino acid was also prepared by refluxing 3.2 Gm. (0.03 mole) of nicotinaldehyde, 3.1 Gm. (0.03 mole) of malonic acid, and 4.6 Gm. (0.06 mole) of ammonium acetate in 25 ml. of absolute ethanol. The evolution of carbon dioxide which began upon mixing the reactants continued for nearly the entire refluxing period of one hour. After allowing the reaction mixture to cool, the white solid (2.8 Gm.) was filtered and dried. This substance decomposes with the evolution of gas (both carbon dioxide and ammonia) at 148–160° and is converted into β-(3-pyridyl)acrylic acid, m. p. 231–234°. After several purifications this extremely water-soluble substance would not give a satisfactory analysis for any possible compound.

The filtrate was refluxed for an additional twenty minutes and allowed to cool. The desired amino acid (0.6 Gm.), which separated, melted at 205–206° (dec.) after purification. Some additional β-(3-pyridyl)acrylic acid was isolated from the mother liquor.

β-Methylamino-β-(3-pyridyl)propionic Acid.—Thirty-three grams (0.36 mole) of methylammonium acetate, 18.6 Gm. (0.18 mole) of malonic acid, and 19.2 Gm. (0.18 mole) of nicotinaldehyde were dissolved in 150 ml. of absolute ethanol and the mixture refluxed for two hours. On cooling the solution, 9.2 Gm. of β-(3-pyridyl)acrylic acid separated. The filtrate solidified to a mush which altogether produced 6.2 Gm. of β-methylamino-β-(3-pyridyl)-propionic acid. A sample purified for analysis by several crystallizations from absolute methanol melted at 171–171.5°.

Anal. Calcd. for $C_9H_{12}N_2O_2$: C, 59.98; H, 6.72. Found: C, 59.43; H, 6.78.

This procedure has been carried out several times. The yield of the amino acid varies with no apparent changes in procedure. In two experiments only β-(3-pyridyl)acrylic acid could be isolated.

γ-Keto-γ-(3-pyridyl)butyric Acid.—To 16.4 Gm. (0.42 mole) of sodium amide, covered with dry ether, were added all at once 69.7 Gm. (0.4 mole) of freshly distilled diethyl succinate and then 33.3 Gm. (0.22 mole) of ethyl nicotinate. Sufficient dry ether was added to bring the total volume of ether to 80 ml. If more ether was used the yields were negligible. The mixture was refluxed and stirred for two and one-half hours when the evolution of ammonia was completed.

The thick brown reaction mixture was poured into a mixture of ice and concentrated hydrochloric acid. The acid mixture was then saturated with solid sodium bicarbonate and repeatedly extracted with ether. This ether extract (*A*) was extracted several times with 5% hydrochloric acid; the acid extract was again saturated with sodium bicarbonate and extracted with ether. After drying the extract (anhydrous sodium sulfate), the ether was evaporated. From the residual oil 8.6 Gm. of ethyl nicotinate was recovered. On fractionation 22.6 Gm. (50%) of diethyl α-nicotinoyl-succinate boiling at 160–162° at 1 mm. was obtained. The maximum yield obtained in other experiments amounted to 56%. From ether extract *A* some diethyl succinate was always recovered together with some diethyl 2,5-diketo-1,4-cyclohexanedicarboxylate, m. p. 124°.

The diethyl α-nicotinoylsuccinate was hydrolyzed and decarboxylated by refluxing a mixture of 15 Gm. of the ester with 26 ml. of water and 2 ml. of concentrated sulfuric acid for forty-eight hours. After cooling and adjustment of the solution to *p*H 4–4.5 with ammonium hydroxide, the γ-keto-γ-(3-pyridyl)butyric acid separated. There was obtained 7.3 Gm. (76%) of compound which after four crystallizations from ethanol melted at 161.5–163°.

Anal. Calcd. for $C_9H_9NO_3$: C, 60.33; H, 5.06; N, 7.82. Found: C, 60.34; H, 5.20; N, 7.63.

γ-Oximino-γ-(3-pyridyl)butyric Acid.—To 9.0 Gm. (0.05 mole) of γ-keto-γ-(3-pyridyl)butyric acid in 50 ml. of 10% sodium hydroxide solution was added 5.25 Gm. (0.075 mole) of hydroxylamine hydrochloride dissolved in 15 ml. of water. The solution, after standing forty-eight hours at room temperature, deposited 8.5 Gm. (88%) of the oxime upon neutralization with dilute sulfuric acid. The white needles melted at 165–166° after three crystallizations from hot water.

Anal. Calcd. for $C_9H_{10}N_2O_3$: C, 55.66; H, 5.19. Found: C, 55.45; H, 5.22.

Acetylhydrazone of γ-Keto-γ-(3-pyridyl)butyric Acid.—A solution of 3.58 Gm. (0.02 mole) of γ-keto-γ-(3-pyridyl)butyric acid and 1.24 Gm. (0.02 mole) of acetylhydrazine in 60 ml. of absolute ethanol was refluxed for one and one-fourth hours. About two-thirds of the ethanol was removed by distillation. The white crystalline solid (3.7 Gm.) separated on cooling. After three crystallizations from absolute ethanol the compound melted at 195–198°.

Anal. Calcd. for $C_{11}H_{13}N_3O_3$: C, 56.16; H, 5.57. Found: C, 55.97; H, 5.51.

Methyl γ-Keto-γ-(3-pyridyl)butyrate.—Five Gm. (0.028 mole) of γ-keto-γ-(3-pyridyl)butyric acid was refluxed in 100 ml. of absolute methanol containing 10 ml. of concentrated sulfuric acid for seven hours. One-half of the methanol was removed by distillation, the residue neutralized with ammonium hydroxide, and the ammonium sulfate removed by filtration. The mother liquor was concentrated to a thick syrup which solidified to a purple solid on refrigeration, amounting to 2.45 Gm. (46%). After two crystallizations (Norite) from hot water, the ester melted at 65.5–67.5°.

Anal. Calcd. for $C_{10}H_{11}NO_3$: C, 62.16; H, 5.74. Found: C, 61.91; H, 5.91.

Ultraviolet Absorption Spectra.—These data (Table I) were determined in 95% ethanol with the Beckman D. U. spectrophotometer.

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