6-Phenylaminopurine was prepared by the method used for the benzyl derivative. Approximately 2 g. of highly colored product was obtained that melted at about 245°. After several crystallizations from alcohol it melted at 278° (reported[9] 278–281°).

(9) G. H. Hitchings and G. B. Elion, U. S. Patent 2,691,654; Oct. 12, 1954 (*C. A.*, **50**, 1933 (1956)).

[CONTRIBUTION FROM THE DEPARTMENT OF PHARMACOLOGY, MEDICAL COLLEGE OF VIRGINIA, RICHMOND, VIRGINIA]

The Isolation and Structure of a Ketoamide Formed in the Metabolism of Nicotine[1]

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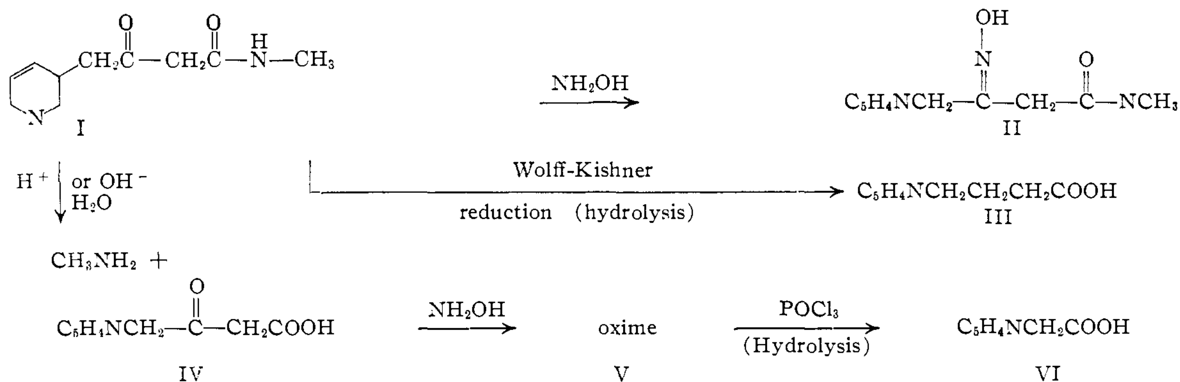
Administration of (–)-nicotine or (–)-cotinine to the dog leads to the excretion of a chloroform soluble amphoteric metabolite which has been separated in crystalline form. Hydrolysis of the metabolite in acidic or basic solution resulted in the formation of methylamine and an oxo acid. The latter yielded γ-(3-pyridyl)-butyric acid upon reduction by the Wolff–Kishner procedure. A Beckmann rearrangement of the oxime from the oxo acid afforded 3-pyridylacetic acid. In consequence the metabolite is assigned the structure γ-(3-pyridyl)-β-oxo-N-methylbutyramide.

The metabolism of (–)-nicotine in the dog leads[3] to the excretion of (–)-cotinine and a variety of Koenig positive components. The rôle of cotinine in the metabolism of nicotine in both the dog and the human is evinced in many ways including metabolism to (–)-desmethylcotinine in the former[4] and hydroxycotinine in both species.[4,5]

During studies on the metabolism of (–)-cotinine and (–)-nicotine it was observed[4] that the urine of dogs following administration of (–)-cotinine contained a chloroform-soluble metabolite which, in contrast to cotinine, hydroxycotinine and desmethylcotinine, was removed from aqueous solution by Dowex 1 (OH^-). Chromatographic evidence has indicated the possible presence of this same substance in the urine of both humans and dogs following administration of (–)-nicotine.

In the current studies (–)-cotinine has been administered to dogs in quantities sufficient to permit isolation of this new metabolite and subsequent structural determination by the reactions



The pooled urine from mongrel dogs following administration of (–)-cotinine was made alkaline with ammonia and then exhaustively extracted with chloroform. An acidic aqueous solution of the residue obtained by evaporation of the chloroform was placed upon a column of Dowex 50 (H^+) which removed all of the Koenig positive components. An ammoniacal eluate of the resin was passed through a column of Dowex 21K (OH^-) or Dowex 1 (OH^-). The effluent contained the Koenig positive compounds cotinine, desmethylcotinine and hydroxycotinine which have been subjected to previous study. By elution with dilute acid or copious quantities of water an additional Koenig positive compound was obtained in crude form. The aqueous solution of this metabolite was concentrated to a yellow oil. A solution of the latter in chloroform was chromatographed on alumina to obtain a crystalline product (I) which melted at 114–116° after recrystallization from benzene. The compound in solution showed no optical rotation. The analyses for C, H and N were in good agreement with the empirical formula $C_{10}H_{12}N_2O_2$.

The analytical data and positive Koenig reaction of the compound immediately suggested a 3-pyridyl compound derived from the metabolic oxidation of cotinine. The metabolite yielded a crystalline oxime (II) and upon reduction under Wolff-Kishner conditions yielded γ-(3-pyridyl)-butyric acid (III). The latter was identified by

(1) For preliminary reports see: E. R. Bowman, L. B. Turnbull and H. McKennis, Jr., *Fed. Proc.*, **18**, 371 (1959); *Bull. Va. Section Am. Chem. Soc.*, **36**, 184 (1959). Aided by grants from the Tobacco Industry Research Committee and the American Tobacco Company.

(2) Public Health Research Fellow of the National Heart Institute.

(3) (a) H. McKennis, Jr., L. B. Turnbull and E. R. Bowman, THIS JOURNAL, **79**, 6342 (1957); (b) *ibid.*, **80**, 6597 (1958).

(4) H. McKennis, Jr., L. B. Turnbull, E. R. Bowman and E. Wada, *ibid.*, **81**, 3951 (1959).

(5) E. R. Bowman, L. B. Turnbull and H. McKennis, Jr., *J. Pharmacol. Exp. Therap.*, **127**, 92 (1959).

melting point, mixed melting point and infrared spectra in comparison with an authentic sample obtained by reduction of γ-(3-pyridyl)-γ-oxobutyric acid.

Upon hydrolysis in the presence of acid or base the metabolite yielded methylamine and an oxo acid (IV), m.p. 159-161°, which gave an elementary analysis corresponding to a γ-(3-pyridyl)-oxobutyric acid. The latter differed from γ-(3-pyridyl)-γ-oxobutyric acid,[6,7] the only previously described γ-(3-pyridyl)-oxobutyric acid,[8] and yielded a crystalline oxime (V) which differed from the known γ-oximino compound.[6,7,9]

The oxime of the new γ-(3-pyridyl)-oxobutyric acid then was subjected to a Beckmann rearrangement[9] with phosphorus oxychloride. The mixture from this reaction yielded 3-pyridylacetic acid (VI) which was identified by melting point, mixed melting point and conversion to the picric acid salt which corresponded in properties to an authentic sample. The identification of 3-pyridylacetic acid led to the conclusion that the metabolite contained an oxo group in the β position of the side chain and led to the structure γ-(3-pyridyl)-β-oxo-N-methylbutyramide (I).

The isolation of γ-(3-pyridyl)-β-oxo-N-methylbutyramide from the urine as a result of administration of (−)-cotinine further enlarges the known rôle of continine as an intermediate in nicotine metabolism.[10] Werle, *et al.*,[13] reported that incubation of nicotine in the presence of liver slices led to the production of methylamine. It becomes desirable to determine the extent of hydrolysis of γ-(3-pyridyl)-β-oxo-N-methylbutyramide *in vivo* since this would lead to methylamine. The experiments of Hucker, Gillette and Brodie[11] already indicate that liver preparations are capable of converting nicotine to cotinine. γ-(3-Pyridyl)-β-oxobutyric acid which arises from hydrolysis of the ketoamide under the vigorous conditions used in the present study has an exceptional stability to both acid or base. This indicates that if hydrolysis of keto acid takes place *in vivo* to give 3-pyridylacetic acid or 3-pyridylacetone the process would be efficient only as an enzymatic reaction.

Experimental[14]

Isolation of γ-(3-Pyridyl)-β-oxo-N-methylbutyramide.—(−)-Cotinine, 13.89 g., in physiological saline, was administered (100 mg./kg.) intravenously to 13 mongrel dogs (both sexes) under pentobarbital anesthesia during an 8-hr. period. During administration and a subsequent 10-hr. period bladder urine was collected *via* an indwelling catheter. The combined urine (4 l.) was adjusted to *p*H 8–9 with concentrated ammonia water in a Rinco extractor and then exhaustively extracted with chloroform. The residue (10.3 g.) from evaporation of the chloroform gave on paper chromatography Koenig positive zones. These zones, R_f 0.61 and 0.74 (ammonia–ethanol–butanol)[7] and R_f 0.48 and 0.30 (formic acid–*sec*-butyl alcohol–water),[15] corresponded in value to those obtained from the urine of dogs after intravenous administration of nicotine. Similar values were also obtained after oral or intraperitoneal administration of (−)-cotinine to dogs. The residue was dissolved in water and adjusted to a volume of 50 ml. and *p*H 2 with dilute hydrochloric acid. The solution was placed on a column of Dowex 50 (H^+) (200 g. of wet weight). After a copious water wash the column was eluted with *N* ammonia water (*ca.* 1 l.). The Koenig positive eluate was placed on Dowex 21K (OH^-) (3 × 13 cm.). The column was washed with water until the Koenig reaction of the effluent was positive and then eluted with *N* acetic acid until the Koenig reaction of the eluate was negative. The combined solutions of Koenig positive material were treated with decolorizing carbon. The filtrate was concentrated to a light-colored viscous oil (963 mg.).

A solution of the foregoing oil in chloroform gave Koenig positive zones at R_f 0.72 (ammonia–ethanol–butanol) and 0.43 (formic acid–*sec*-butyl alcohol–water[16]). The chloroformic solution was placed on acid-washed alumina (10 g.). The column was eluted with ether containing successively increasing concentrations of methanol to a final concentration of 20%. The latter concentration removed most of the Koenig positive material. The Koenig positive fractions were combined and concentrated to an oily crystalline mass (415 mg.). The residue was dissolved in a minimal amount of hot benzene. Upon cooling the solution deposited 325 mg. of oxoamide, m.p. 114–116°. For analysis the sample was dried at 60° and 1 mm. over KOH.

Anal. Calcd. for $C_{10}H_{12}N_2O_2$: C, 62.48; H, 6.29; N, 14.59. Found: C, 62.46; H, 6.17; N, 14.45.

The compound was optically inactive and gave a positive enol test with picric acid.[18]

γ-(3-Pyridyl)-β-oximino-N-methylbutyramide.—To a solution of 70 mg. of the metabolic oxoamide in 1.5 ml. of 10% sodium hydroxide was added 100 mg. of hydroxylamine hydrochloride. The stoppered mixture was allowed to stand at room temperature overnight and then was neutralized with dilute hydrochloric acid. The solution deposited colorless crystals which, after several recrystallizations from water, melted at 160–165° on the hot stage.

Anal. Calcd. for $C_{10}H_{13}N_3O_2$: C, 57.96; H, 6.32. Found: C, 57.60; H, 6.10.

Acidic Hydrolysis of γ-(3-Pyridyl)-β-oxo-N-methylbutyramide.—A solution of 200 mg. of γ-(3-pyridyl)-β-oxo-N-methylbutyramide in 15 ml. of 5 *N* hydrochloric acid was heated under reflux for 8 hr. The cooled solution was placed on Dowex 50 (H^+) (1.5 × 7 cm.). The column was washed with water until the effluent was neutral and then eluted with 40 ml. of *N* ammonia water. The ammoniacal solution was placed on Dowex 21K (OH^-). The column was washed with water and then eluted with *N* acetic acid. The acid eluate was concentrated to a crystalline mass (145.5 mg.). The residue was dissolved in methanol and treated with decolorizing carbon. The filtrate yielded col-

(6) R. N. Castle and A. Burger, *J. Am. Pharm. Assoc. Sci. Ed.*, **43**, 163 (1954).

(7) H. McKennis, Jr., L. B. Turnbull, H. N. Wingfield, Jr., and L. J. Dewey, THIS JOURNAL, **80**, 1634 (1958).

(8) Synthesis of the ethyl ester of γ-(3-pyridyl)-β-oxobutyric acid has been noted, *C. A.*, **50**, 10796 (1956). The original reference, U. S. Patent No. 2,727,899, reveals, however, that only the γ-oxo compound was investigated.

(9) E. Wada and Y. Yamasaki, THIS JOURNAL, **76**, 155 (1954).

(10) The possible utilization of cotinine as an intermediate in the metabolism of nicotine in mammalian species other than man and the dog has been indicated by the reported appearance of cotinine in the urine of rabbits following administration of nicotine.[11,12]

(11) H. B. Hucker, J. R. Gillette and B. B. Brodie, *Nature*, **183**, 47 (1959); *Fed. Proc.*, **18**, 404 (1959).

(12) T. Kitamura, *Folia Pharmacol. Japon.*, **54**, 825 (1958).

(13) E. Werle and A. Meyer, *Biochem. Z.*, **321**, 221 (1950).

(14) Microanalyses by Spang Microanalytical Laboratory and Weiler and Strauss.

(15) W. Hausmann, THIS JOURNAL, **74**, 3181 (1952).

(16) These values are experimentally indistinguishable from those of cotinine in descending chromatograms conducted at ambient room temperatures on Whatman No. 1 paper. The processed urine from some dogs was found at this stage to contain material giving three Koenig positive zones when developed in benzene–methanol–acetate buffer (60:15:25 volume; 0.2 *M* sodium acetate, *p*H 5.6).[17] Zones at R_f 0.11 and 0.17 appear to represent small amounts of pyridine compounds derived from the metabolism of cotinine. A zone at R_f 0.05 corresponded in value to authentic γ-(3-pyridyl)-β-oxo-N-methylbutyramide.

(17) This solvent system corresponds to one employed in studies on the metabolism of nicotine by insects, F. E. Guthrie, R. L. Ringer and T. G. Bowery, *J. Econ. Entomol.*, **50**, 822 (1957), as described in a private communication from Dr. F. E. Guthrie.

(18) J. V. Koštíř and V. Rábek, *Biochem. et Biophys. Acta*, **5**, 210 (1950).

orless crystals which were recrystallized from methanol for analysis, m.p. 159–161°.

Anal. Calcd. for $C_9H_9NO_3$: C, 60.32; H, 5.06; N, 7.82. Found: C, 60.47; H, 5.29; N, 7.77.

Basic Hydrolysis of γ-(3-Pyridyl)-β-oxo-N-methylbutyramide.—A solution of 200 mg. of ketoamide in 50 ml. of 20% potassium hydroxide was heated at 200° for 20 hr. The cooled solution was placed on a column of Dowex 21K (OH^-) (1 × 10 cm.). The column was washed with water until neutral and then eluted with 100 ml. of *N* acetic acid. The acidic solution showed only one Koenig positive zone R_f 0.26 (ammonia–ethanol–butanol), R_f 0.76 (formic acid–*sec*-butyl alcohol–water), corresponding in value to those obtained with an authentic sample of γ-(3-pyridyl)-β-oxobutyric acid.

The oxoamide also was hydrolyzed by heating to reflux a solution of the compound (400 mg.) in 60 ml. of 20% potassium hydroxide for 36 hr. The solution at room temperature then was placed upon a column of Dowex 21K (OH^-) (4 × 17 cm.). The column was washed with water and then eluted with *N* acetic acid. The acidic solution was evaporated to a crystalline residue (260 mg.) of γ-(3-pyridyl)-β-oxobutyric acid. After two recrystallizations from methanol the compound melted at 159–161° and did not depress the melting point of the analytical sample obtained by acid hydrolysis of the oxoamide.

Methylamine from Hydrolysis of γ-(3-Pyridyl)-β-oxo-N-methylbutyramide.—The oxoamide (200 mg.) was hydrolized in 10 ml. of 4 *N* HCl as above. Aliquots of the acidic solution were treated with an excess of 10% sodium hydroxide in the outer chambers of Conway dishes containing saturated aqueous picric acid solution[19] (4 ml. total volume) in the center chamber. The sealed dishes were allowed to stand at 40° for 17 hr. The combined picric acid solutions were cooled to 0°. The precipitated yellow crystalline methylamine picrate was collected and recrystallized four times from methanol for analysis, m.p. 210–212°, 50 mg. or 20% of the calculated amount. The compound did not depress the melting point of an authentic sample.[20]

Anal. Calcd. for $C_7H_8N_4O_7$: C, 32.31; H, 3.10; N, 21.54. Found: C, 32.43; H, 3.00; N, 21.57.

γ-(3-Pyridyl)-β-oximinobutyric Acid.—γ-(3-Pyridyl)-β-oxobutyric acid (30 mg.) in 0.05 ml. of water was added to 17 mg. of hydroxylamine hydrochloride in 0.17 ml. of 10% sodium hydroxide. The mixture was allowed to stand overnight. Upon neutralization with *N* hydrochloric acid, the solution deposited 30 mg. of colorless oxime. For analysis the compound was recrystallized from hot water, m. p. 167.5–169° dec.

Anal. Calcd. for $C_9H_{10}N_2O_3$: C, 55.66; H, 5.19; N, 14.43. Found: C, 55.73; H, 5.02; N, 14.45.

3-Pyridylacetic Acid from Beckmann Rearrangement of γ-(3-Pyridyl)-β-oximinobutyric Acid.—A slurry of the oxime (350 mg.) and 10 ml. of freshly distilled chloroform was treated with 300 mg. of phosphorus oxychloride. After a thorough shaking the mixture was allowed to stand overnight. The oily bottom layer was added to 10 ml. of 5 *N* hydrochloric acid. The mixture was heated to reflux for 8 hr. The solution at room temperature was placed on a column of Dowex 50 (H^+) (1.5 × 7 cm.). After a water wash the column was eluted with 100 ml. of *N* ammonia water. The ammoniacal solution was placed on Dowex 21K (OH^-) (1.5 × 7 cm.). After a water wash the column was eluted with 80 ml. of 1 *N* acetic acid. Paper chromatograms of the acidic eluate showed Koenig positive zones at R_f 0.22 and 0.26 (ammonia–ethanol–butanol) and at R_f 0.61 and 0.34 corresponding in value to γ-(3-pyridyl)-β-oxobutyric acid and 3-pyridylacetic acid, respectively. The acidic solution was concentrated to a yellow solid which was dissolved in a minimal amount of hot methanol. The solution was cooled in an ice-bath. The colorless crystals of 3-pyridylacetic acid, m.p. 144–146° (34.1 mg.), had a melting point in good agreement with literature values and did not depress the melting point of an authentic sample prepared from 3-acetylpyridine by means of the Schwenk modification[21] of the Willgerodt reaction.[22] The 3-pyridylacetic acid (10 mg.) was treated with 18.1 mg. of picric acid as a saturated solution in ethanol. The mixture was heated to boiling and then allowed to cool to room temperature. The yellow crystalline precipitate melted at 167–169°. The melting point was unchanged on further recrystallization.

Anal. Calcd. for $C_{13}H_{10}N_4O_9$: C, 42.63; H, 2.75; N, 15.30. Found: C, 42.65; H, 2.69; N, 15.27.

The compound did not depress the melting point of authentic 3-carboxypyridinium picrate, m.p. 167–169°, which was prepared from authentic 3-pyridylacetic acid. Malon and Dean[22] reported the preparation of 3-carboxypyridinium picrate, m.p. 99–101°, but did not give analytical data.

γ-(3-Pyridyl)-butyric Acid.—A mixture of γ-(3-pyridyl)-γ-oxobutyric acid (2.0 g.), glycerol (10 ml.), 95% hydrazine hydrate (1 ml.) and water (0.25 ml.) was boiled under reflux for one-half hour. Potassium hydroxide pellets (3.0 g.) were added. Heating was continued for 4 hr. at a bath temperature of 195°. The cooled solution was diluted with 50 ml. of water and made slightly acidic with HCl. The solution was placed on a column of Dowex 50 (H^+). After a water wash the column was eluted with *N* ammonia water. A Koenig positive acid (R_f 0.32, ammonia–ethanol–butanol; R_f 0.43, formic acid–*sec*-butyl alcohol–water) was the major component of the ammoniacal solution. By evaporation of the eluate the acid was obtained in crude form (1.5 g.). A sample for analysis was prepared by recrystallization from acetone–alcohol, m.p. 121–122°.

Anal. Calcd. for $C_9H_{11}NO_2$: C, 65.44; H, 6.71; N, 8.48. Found: C, 65.79; H, 6.56; N, 8.62.

A sample of γ-(3-pyridyl)-β-oxo-N-methylbutyramide (500 mg.) was reduced in a similar fashion. The crude product (300 mg.) was recrystallized from ethyl acetate to give colorless crystals, m.p. 121–122°. The mixed melting point with the authentic acid obtained above showed no depression. R_f values identical to those of the authentic acid obtained above showed no depression. R_f values identical to those of the authentic samples (above) were obtained and the infrared spectrograms of the two samples were identical.[23]

(19) E. J. Conway, "Microdiffusion Analysis and Volumetric Error," Crosby Lockwood and Son Ltd., London, 1950.

(20) Literature values for the melting point of this salt range from 207 to 215°. See for example: S. M. McElvain, "The Characterization of Organic Compounds," The Macmillan Co., New York, N. Y., 1945; G. Jerusalem, *J. Chem. Soc.*, **95**, 1275 (1909).

(21) E. Schwenk and D. Papa, *J. Org. Chem.*, **11**, 798 (1946).

(22) R. L. Malon and P. M. Dean, THIS JOURNAL, **69**, 1797 (1947).

(23) Kindly determined by Mr. J. Scott Osborne, American Tobacco Company, Department of Research and Development.