Additional Routes in the Metabolism of Nicotine to 3-Pyridylacetate

THE METABOLISM OF DIHYDROMETANICOTINE*

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SUMMARY

The metabolism of dihydrometanicotine was investigated in the rat and dog. In preliminary studies, dihydrometanicotine difumarate was administered to rats. The RF values and retention times of methyl esters prepared from acidic Koenig-positive metabolites in the urine suggested the presence of 3-pyridylacetate and 4-(3-pyridyl)butyrate. Following administration of dihydrometanicotine difumarate to dogs, the pattern of excretion of acidic metabolites in the urine was similar to that found in the rat. Chemical conversion of the acidic metabolites to their methyl esters for separation by preparative gas chromatography afforded methyl 3-pyridylacetate and methyl 4-(3-pyridyl)butyrate. Methyl 3-pyridylacetate was identified by melting point, elemental analysis, and the infrared spectrum of its picric acid salt. Methyl 4-(3-pyridyl)butyrate was identified by mass spectroscopy and by the melting point, elemental analysis, and the infrared spectrum of the methyl ester picrate. Previous studies have provided evidence for the formation of 3-pyridylacetate from (-)nicotine via either (-)-cotinine or (-)-demethylcotinine. In view of the report (DE CLERCQ, M., AND TRUHAUT, R. (1962) Bull. Soc. Chim. Biol., 44, 227) that dihydrometanicotine is a metabolite of nicotine in the rat, the present findings in the rat and dog suggest a third alternate route (via dihydrometanicotine) to 3-pyridylacetate.

Truhaut and de Clercq (1) administered nicotine to rats and obtained evidence for the formation of dihydrometanicotine (3-[4-(methylamino)butyl]pyridine) in the metabolism of nicotine. Other investigators (2, 3) had previously considered dihydrometanicotine as a possible metabolite of nicotine, but their studies provide little or no data which would indicate formation of dihydrometanicotine. In addition to providing the first direct evidence for the natural occurrence of dihydrometanicotine, and a possible conjugate with phenylalanine, de Clercq and Truhaut (4) noted that dihydrometanicotine is more active than the parent nicotine in stimulating the synthesis of diphosphopyridine nucleotide from the precursors tryptophan, nicotinamide, and nicotinic acid, which were supplied to the experimental animals. Evidence for the presence of dihydrometanicotine in tobacco smoke has been supplied by other investigators (5).

One distinguishing feature in the general mammalian metabolism of nicotine is access to two distinct routes, one via cotinine and the other via demethylcotinine, which provide alternate pathways to the production of 3-pyridylacetic acid. The reported production of dihydrometanicotine as a nicotine metabolite would provide on theoretical grounds a third alternate route to 3-pyridylacetic acid: via 4-(3-pyridyl)butyric acid and subsequent oxidation.

These and other considerations led to our exploration of the metabolism of dihydrometanicotine in the rat and the dog. As part of the initial study, primary attention was directed to characterization and identification of pyridylcarboxylic acids that might arise from the oxidative metabolism of dihydrometanicotine.

EXPERIMENTAL PROCEDURE

Chromatography—Paper chromatography was conducted on Whatman No. 1 paper with solvents B and C (7). Thin layer chromatography was carried out on silica gel (Eastman Chromatogram sheets, type K 301 R and 6061) with solvent H (8), which consisted of absolute ethanol-acetone-benzene-concentrated NH₄OH (5:40:50:5 by volume), and solvent I, which contained absolute ethanol-chloroform-isobutylamine (5:94:1 by volume). Koenig-positive zones (generally indicative of pyridine compounds) were disclosed as previously described (9).

Analytical and preparative gas chromatography were performed with an Aerograph Autoprep 700, fitted with an aluminum column (10 feet x ½ inch OD and packed with 30% SE-30 on 80/80 mesh firebrick) at 221°C; helium carrier, 100 ml per min; injection port at 243°C; and thermal conductivity detector at 225°C.
Melting Points—All melting points are micromelting points unless otherwise noted.

Ultraviolet Absorption Spectra—All ultraviolet absorption spectra were determined in 95% ethanol with a Cary model 11 PM recording spectrophotometer.

Acetyldihydrometanicotine—A modification of the procedure of Liebman, Mundy, and Rapoport (10) and Spith and Bobenberger (11) for the preparation of benzoylmetanicotine was used to prepare acetyldihydrometanicotine from redistilled (---) nicotine (227 g, 1.4 moles) and acetyl chloride (110 g, 1.4 moles; Eastman practical grade) in 200 ml of freshly distilled xylene. The acetyl chloride solution was cooled in the ice bath, and nicotine was slowly added. The mixture was allowed to come to room temperature and then was refluxed for 18 hours under protection of a calcium chloride tube. While still warm and free flowing, the mixture was treated with 100 ml of 10% hydrochloric acid and vigorously shaken in a separatory funnel. The acidic aqueous layer was removed and later combined with three subsequent acidic extractions (50 ml each).

The combined aqueous layers were cooled and adjusted to pH 9 to 10 (pHydrion paper) by addition of 20% potassium hydroxide. The alkaline solution was then continuously extracted for 48 hours with n-hexane, which removed unreacted nicotine ($R_F$ 0.93, solvent II), and a small amount of acetylmethanicotine ($R_F$ 0.82, solvent II). The aqueous phase was then continuously extracted with ether for 48 hours. The ether layer contained a major Koenig-positive component ($R_F$ 0.80, solvent II, corresponding to authentic acetylmethanicotine) and several minor unidentified Koenig-positive components ($R_F$ 0.55 and 0.48, solvent II). The ethereal solution was evaporated under reduced pressure to obtain crude acetylmethanicotine (227 g, 44% as a brown oil residue.

For purification and identification, a sample of the crude acetylmethanicotine (20 g) in 40 ml of chloroform was placed on a column (3 x 34 cm) of acid-washed alumina. The column was developed with methanol-ether (10:90 by volume) until the eluate was no longer Koenig positive. This solution, which contained a single Koenig-positive zone ($R_F$ 0.82, solvent II) was concentrated under diminished pressure to obtain 14 g of acetylmethanicotine as a light brown oil ($R_F$ 0.80, solvent II, corresponding to authentic acetylmethanicotine) and several minor unidentified Koenig-positive components ($R_F$ 0.55 and 0.48, solvent II). The ethereal solution was evaporated under reduced pressure to obtain crude acetylmethanicotine (127 g, 24% as a brown oil residue.

For further identification, dihydrometanicotine (7 g) was heated under reflux with 50 ml of 12 N sulfuric acid for 27 hours. The hydrolysis mixture, which contained Koenig-positive zones (Solvent I) at $R_F$ 0.25 (major) and $R_F$ 0.63 (minor, and corresponding in $R_F$ value to unhydrolyzed acetyldihydromethanicotine), was adjusted to pH 9 to 10 (pHydrion paper) by addition of concentrated ammonium hydroxide. The mixture was then extracted three times with methylene chloride (100 ml portions). The combined methylene chloride extracts were evaporated to a brown residue of crude dihydrometanicotine as a light yellow oil (227 g, 44% as a brown oil residue.

Acetyldihydrometanicotine—A solution of 13 g of acetylmethanicotine in 50 ml of absolute alcohol was stirred at room temperature with 3.3 g of 10% palladium-charcoal and hydrogen at atmospheric pressure and room temperature for 2 hours, or until the calculated amount of hydrogen had been consumed. After removal of the catalyst, the solution which contained two Koenig-positive zones ($R_F$ 0.67 and $R_F$ 0.13, solvent I) was evaporated under diminished pressure to a light brown oil.

A sample of this crude acetyldihydrometanicotine (5 g) in absolute ether was applied to a column (1 x 13 cm) of acid-washed alumina. The column was then developed with 45 ml of absolute ether. The ethereal eluate was dried with anhydrous magnesium sulfate and then evaporated to obtain acetyldihydrometanicotine as a light yellow oil (4.6 g, or a calculated total yield of 11 g, 92%); $R_F$ 0.70 (solvent I); $\lambda_{\text{max}}$ 269 nm ($\epsilon = 2270$), $\lambda_{\text{max}}$ 262 nm ($\epsilon = 3100$), $\lambda_{\text{max}}$ 257 nm ($\epsilon = 2760$), $\lambda_{\text{min}}$ 267 nm ($\epsilon = 2060$), and $\lambda_{\text{min}}$ 259 nm ($\epsilon = 2650$) in 95% ethanol. A sample of this acetyldihydrometanicotine base (96.3 mg, 0.466 mmole) was treated with 84.4 mg (0.486 mmole) of 5-nitrobarbituric acid in a small volume of 95% ethanol. The solvent was removed under nitrogen, and the crystalline product was recrystallized from acetone; m.p. 132-134°, 130 mg. For analysis, the salt was recrystallized from acetone without change of melting point, and was dried at 80° and 1 mm of Hg over KOH.

$C_4H_8N_2O_4$ (379.39)
Calculated: C 50.65, H 5.58, N 18.46
Found: C 50.36, H 5.45, N 18.23

Acetyldihydrometanicotine was previously prepared by Hromatka (12), who acetylated dihydrometanicotine with acetic anhydride and then distilled the product (b.p. 219° at 15 mm of Hg).

Dihydrometanicotine—Acetyldihydrometanicotine (7 g) was heated under reflux with 50 ml of 12 N sulfuric acid for 27 hours. The hydrolysis mixture, which contained Koenig-positive zones (Solvent I) at $R_F$ 0.25 (major) and $R_F$ 0.63 (minor, and corresponding in $R_F$ value to unhydrolyzed acetyldihydrometanicotine), was adjusted to pH 9 to 10 (pHydrion paper) by addition of concentrated ammonium hydroxide. The mixture was then extracted three times with methylene chloride (100 ml portions). The combined methylene chloride extracts were evaporated to a brown residue of crude dihydrometanicotine as a light yellow oil (4.6 g, 44% as a brown oil residue.

A solution of the crude dihydrometanicotine (2.02 g) was treated with 3.01 g of fumaric acid in 40 ml of hot absolute ethanol and then concentrated to approximately 20 ml. The solution was cooled in an ice bath, and ether was added dropwise until no further increase in turbidity resulted. The product (4.5 g, representing a 92% yield based upon the acetyldihydrometanicotine employed in the hydrolysis) melted at 112-115°. For analysis, the distillate was dissolved in a minimal amount of warm 9-propanol and reprecipitated by dropwise addition of ethyl acetate. The sample was dried at 44° and 1 mm of Hg over KOH.

$C_4H_8N_2O_4$ (396.39)
Calculated: C 54.54, H 6.10, N 7.07
Found: C 54.35, H 6.22, N 7.07

For further identification, dihydrometanicotine base (91 mg) was treated with 0.4 ml of 40% HBr. After removal of the solvent by evaporation at 140° in an atmosphere of nitrogen, the dihydrobromide salt was dissolved in 1 ml of ethanol-acetone (1:1 by volume). Upon cooling and scratching the wall of the container, the salt was precipitated. After recrystallization from absolute ethanol, the product was dried at 40° and 1 mm of Hg over KOH for 3 hours, mp. 127-130° (capillary).
**Metabolism of Dihydrometanicotine Difumarate in Rats**—To a group of 25 male rats (165 to 240 g), a total of 962 mg of dihydrometanicotine difumarate was administered by single intraperitoneal injection of 181 mg per kg, 72.4 mg per ml in aqueous solution. The animals were allowed food (Purina Rat Chow) and water ad libitum. Urine was collected for 48 hours as runoff from metabolism cages into flasks containing sodium fluoride. Daily collections of urine were stored frozen until processing. Paper chromatographic examination of the urine revealed six Koenig-positive zones (Table I).

The combined urine from the animals was filtered through Whatman No. 1 paper and adjusted with ammonium hydroxide to approximately pH 10. The aqueous solution (A) was then continuously extracted with methylene chloride for 24 hours. The methylene chloride extract (B), which showed a single Koenig-positive zone \( R_F 0.73 \), solvent B) and cochromatographed with authentic dihydrometanicotine, was concentrated to a brown residue (331 mg). Based on the ultraviolet absorption spectrum \( (\lambda_{max} = 262 \text{ nm}) \) of an aliquot of the residue, 24% of the total administered dose of dihydrometanicotine was excreted unchanged in the 48-hour urine. Control urine similarly treated showed little or no ultraviolet absorption under these conditions.

The aqueous phase (A), remaining from extraction with methylene chloride, was passed on a column (6 x 18.5 cm) of Dowex 21-K (OH⁻). After a water wash, material was eluted from the column with 2M acetic acid until the eluate was Koenig negative. The ammoniacal eluate, containing acidic metabolites of dihydrometanicotine, was concentrated on a steam bath and under reduced pressure to a dark brown residue (2.0 g). The residue was heated under reflux with 125 ml of methanol and 3 ml of concentrated sulfuric acid for 16 hours (6). The methyl esters were extracted from the reaction mixture by the procedure previously described (9), with methylene chloride in place of chloroform as the extracting solvent. Paper chromatographic examination (Table I) of the methylene chloride extract of the alkalinized esterification mixture showed the presence of two Koenig-positive zones, \( R_F 0.58 \) and 0.68 (solvent C). The methylene chloride was removed under diminished pressure and the residue was diluted to 1 ml with benzene. Aliquots (50 μl) were subjected to gas chromatographic examination (Fig. 1). Peak A corresponded in retention time (10 min) to authentic methyl 3-pyridylacetate and Peak B corresponded in retention time (21 min) to methyl 4-(3-pyridyl)butyrate (methyl-y-(3-pyridyl)butyrate).

### Table I

| Male albino rat (intraperitoneal, 181 mg/kg) | Male mongrel dog (intravenous, 44.6 mg/kg) |
|---------------------------------------------|---------------------------------------------|
| 48-Hour urine                               | 15-Hour urine                                |
| Solvent C Solvent B                         | Solvent C Solvent B                         |
| \( R_F \)                                   | \( R_F \)                                   |
| 0.05                                        | 0.05                                        |
| 0.14*                                       | 0.16                                        |
| 0.23                                        | 0.21                                        |
| 0.27                                        | 0.31                                        |
| 0.33*                                       | 0.46                                        |
| 0.46                                        | 0.46                                        |
| 0.58                                        | 0.58                                        |
| 0.68                                        | 0.68                                        |

* Obtained by the descending method on Whatman No. 1 paper.
* Acidic fraction after esterification with methanol-sulfuric acid and extraction with methylene chloride (see text).
* Acidic fraction after esterification with methanol-sulfuric acid and extraction with chloroform (see text).
* Corresponded in \( R_F \) value to dihydrometanicotine.
* Corresponded in \( R_F \) value to 3-pyridylacetic acid.
* Corresponded in \( R_F \) value to 4-(3-pyridyl)butyric acid.
* Corresponded in \( R_F \) value to methyl 3-pyridylacetate.
* Corresponded in \( R_F \) value to methyl 4-(3-pyridyl)butyrate.

**FIG. 1.** Gas chromatographic examination of the methyl ester fraction prepared from the 48-hour urine of rats (upper curve) after intraperitoneal administration of dihydrometanicotine difumarate (181 mg per kg). Peak S corresponds to the solvent; Peak A corresponds in retention time to methyl 3-pyridylacetate; attenuation change (X); and Peak B corresponds in retention time to methyl 4-(3-pyridyl)butyrate. Authentic methyl 3-pyridylacetate is shown at C and authentic methyl 4-(3-pyridyl)butyrate at D.
pentobarbital anesthesia (30 mg per kg) were administered dihydrometanicotine difumarate (84.6 mg per kg dissolved in 0.9% aqueous sodium chloride) via a femoral vein at a constant rate of 0.78 ml per min (Sage model 355 syringe pump). During the 41 hours of infusion and a subsequent 10-hour period, bladder urine was collected by an indwelling catheter leading to a bottle immersed in dry ice-acetone and kept frozen until processing.

Processing of Dog Urine—Celite (Johns-Manville) was added to the urine (3.7 liters) from 13 dogs which had received a total of 8.9 g of dihydrometanicotine, and the urine was filtered through Whatman No. 1 paper. Data obtained by paper chromatographic examination of the urine are shown in Table I. The filtered urine was adjusted to pH 9 to 10 (pHidron paper) by addition of concentrated ammonium hydroxide. This aqueous solution (A) was then continuously extracted with methylene chloride for 48 hours. The methylene chloride solution (B) contained four Koenig-positive zones (solvent I), Rf 0.66, 0.46, 0.32, 0.28. The major zone at Rf 0.28 corresponded in Rp value to dihydrometanicotine. The methylene chloride solution (B) was evaporated to a dark brown residue (1.2 g). The residue was dissolved in distilled water and placed on a column (2 X 7.5 cm) of Dowex 21-K (OH−). The effluent and a water wash were collected until Koenig negative. The combined effluent and wash were made acidic (pH 5 to 6 to pHidron paper) with 10% hydrochloric acid and then placed on a column (2.5 X 4.5 cm) of Dowex 50-W (H+). The effluent and wash were discarded. The column was then treated with 1 liter of 2 N ammonium hydroxide and then with 1 liter of 5 N ammonium hydroxide. The combined ammonium hydroxide eluates were continuously extracted with methylene chloride for 24 hours. The methylene chloride was evaporated to a brown residue (399 mg). The residue was dissolved in absolute ethanol and treated with decolorizing carbon (Norit A). After filtration through Celite, the mixture was concentrated on the steam bath with a stream of nitrogen to a brown residue (371 mg). An aliquot (70.1 mg) was treated with 113.9 mg of fumaric acid dissolved in hot ethanol. After removal of the solvent on the steam bath under nitrogen, the residue was dissolved in 1 ml of 2-propanol. The mixture was treated dropwise with ethyl acetate until no more turbidity developed. After standing overnight at room temperature, the crystals (94.2 mg) were collected and dried with a stream of nitrogen. A solution of the product in hot ethanol was treated with Norit A and then filtered through Whatman No. 1 filter paper with the aid of Celite. Upon cooling and scratching, white crystals were deposited, m.p. 112-113°. An aliquot (48 mg) of Fraction II material (465 mg; Rf 0.56, solvent C) was dissolved in 0.5 ml of methanol and treated dropwise with approximately 90 mg of hydroic picric acid (19% water) as a saturated methanolic solution. The solution was cooled to obtain 127 mg of methyl 3-pyridylacetate picrate, m.p. 127-129°. The sample, which showed a single Koenig-positive zone (Rf 0.69; silicon gel, solvent I), was recrystallized from methanol for analysis and was dried at 93° and 2 mm of Hg over KOH, m.p. 127-129°. Admixture of the methyl ester picrate with an authentic sample (6), m.p. 127-129°, caused no depression of the melting point.

Calculation

An aliquot (29 mg) of the Fraction IV material (80 mg; Rf 0.66, solvent C) was treated with 50 mg of hydroic picric acid.
FIG. 2. Infrared spectra (KBr pellet) of methyl 4-(3-pyridyl)butyrate picrate prepared from the acidic fraction of dog urine after administration of dihydrometanicotine difumarate in comparison with authentic synthetic material.

(15% water) as a saturated solution in absolute ethanol. This provided a yellow crystalline precipitate of methyl 4-(3-pyridyl)butyrate picrate (41.9 mg; m.p. 79-82°C, Koenig-positive at Rf 0.69; silica gel, solvent I). For analysis the ester salt was recrystallized from absolute ethanol and dried over KOH at room temperature and 2.5 mm of Hg for 2 hours, m.p. 83-85°C. There was no depression of the melting point upon admixture with an authentic sample (13).

C_{12}H_{19}N_2O_7 (408.34)

Calculated: C 47.06, H 3.94
Found: C 47.04, H 3.93

The infrared spectrum of the isolated compound showed no essential difference from an authentic sample (Fig. 2).

The mass spectrum of the free methyl ester (Fraction IV), obtained in an Hitachi RMU 6 II instrument, showed ions of high intensity present at m/e 179 (M = C_{12}H_{19}N_2CH_3CH_2COOCH_3), m/e 148 (M-31, loss of -OCH_3), m/e 120 (M-59, loss of -COOCH_3), m/e 106 (M-73, loss of CH_3COOCH_3), and m/e 92 (M-87, loss of -CH_2CH_2COOCH_3).

RESULTS AND DISCUSSION

A number of synthetic sources (14, 15), in addition to natural sources (4), have been described for dihydrometanicotine. In the present study, attention was directed to the preparation of dihydrometanicotine via acetylmethanicotine, since there is some possibility (16) that the latter may be formed in vivo by a reaction analogous to the Pinner-Bard reaction. Acetylmethanicotine, which has been previously prepared (17) from acetic anhydride and nicotine, was conveniently obtained in our studies from acetyl chloride and nicotine. Hydrogenation of acetylmetanicotine in the presence of palladium-charcoal afforded a 92% yield of acetyldihydrometanicotine. Dihydrometanicotine, from the acidic hydrolysis of acetyldihydrometanicotine, was conveniently stored as the stable fumaric acid salt.

Preliminary studies were conducted on the metabolism of dihydrometanicotine in the rat, since it is in this species alone that dihydrometanicotide and the possible phenylalanine conjugate (3-[N-phenylalanyl-4-(methy lamino)butyl]pyridine) have been implicated (1) in the metabolism of nicotine. After the intraperitoneal administration of dihydrometanicotine, the urine of the animals provided evidence by paper chromatography for the presence of 3-pyridylacetate and 4-(3-pyridyl)butyrate. After removal of unchanged dihydrometanicotine by extraction with methylene chloride, the urine was processed and the acidic fraction was subjected to esterification with methanol-sulfuric acid. The resultant methyl ester fraction was examined by both paper and gas chromatography. The Rf values of Koenig-positive materials and the retention times of Koenig-positive materials corresponded to authentic methyl 3-pyridylacetate and methyl 4-(3-pyridyl)butyrate. Experimental work was then extended to the dog to facilitate the use of larger quantities of dihydrometanicotine.

The experimental procedures in the dog experiments closely resembled those chosen for the rat except for the fact that dihydrometanicotine was administered intravenously to the
dogs under pentobarbital anesthesia and bladder urine was collected by means of an indwelling catheter. The methylene chloride-soluble basic fraction of the urine contained unchanged dihydrometanicotine and three other Koenig-positive substances which were not identified. A preliminary chromatographic examination of the acidic components of the urine pointed to the possible presence of 3-pyridylacetate and 4-(3-pyridyl)butyrate. Confirmation of the presence of 3-pyridylacetate was achieved by gas chromatographic separation of sufficient quantities of the methyl ester of 3-pyridylacetate for preparation of the picric acid salt. The analytical sample was identified by melting point, mixed melting point, and comparison of infrared spectra with authentic material.

The gas chromatographic fraction which corresponded in retention time to authentic methyl 4-(3-pyridyl)butyrate showed a principal mass spectral peak m/e 179, corresponding to authentic methyl 4-(3-pyridyl)butyrate (13). Additional confirmation of identify was achieved through favorable comparison of the infrared spectra, melting point, and analysis of the picric acid salt. The isolation of 4-(3-pyridyl)butyrate in this instance affords the first direct evidence for the participation of 4-(3-pyridyl)butyrate in the mammalian metabolism of nicotine, if one assumes that dihydrometanicotine participates in the metabolism of nicotine in the dog along the lines indicated by Truhaut and de Clercq (1) for metabolism of nicotine in the rat. From Knoop's early studies (18) on the metabolism of 4-phenylbutyric acid to phenylacetic acid in the dog, it can be anticipated that 4-(3-pyridyl)butyric acid is a precursor of 3-pyridylacetic acid, which has been shown to arise from the metabolism of nicotine in a variety of mammalian species.

Evidence for the metabolism of nicotine to 3-pyridylacetate was first achieved (19) following administration of [14C]nicotine to the dog, and later studies from this laboratory indicated a similar type of oxidation of nicotine to 3-pyridylacetate by

![Diagram of nicotine metabolism](image-url)

Fig. 3. Abridged metabolic scheme showing relationships between mammalian metabolites of nicotine (other than quaternary compounds and amine oxides) found in urine.
rabbit liver preparations in vitro (20). Evidence for a metabolic degradation of [14C]nicotine to 3-pyridylacetate in the cat has been recently presented by Turner (21). Routes which prevail in the metabolism of nicotine to 3-pyridylacetate in the cat can only be constructed on the basis of analogy to the metabolic routes in other mammals.

From the data currently available, it is possible to construct a composite picture of routes leading from nicotine to 3-pyridylacetate in a number of mammals (6) with γ-3-pyridyl-γ-oxobutyric acid and γ-3-pyridyl-γ-hydroxybutyric acid, as itself or in the form of its lactone, serving as common intermediates (Fig. 3). Experimental studies (22) on administration of γ-3-pyridyl-γ-oxobutyric acid to the rabbit, dog, and rat provided evidence for the urinary excretion of 3-pyridylacetate. 3-Pyridylacetate is also formed following administration of the lactone of γ-3-pyridyl-γ-hydroxybutyric acid to the rat (23). Similarly, administration of demethylocotinine to the rat and dog gives rise (6) to urinary 3-pyridylacetate, and administration of cotinine to the rat (9), the mouse (24), and man (6) results in urinary excretion of 3-pyridylacetate. Evidence suggests that aldehydes (1-methyl-5-hydroxy-5-(3-pyridyl)-2-pyrrolidinone), (25), an additional urinary metabolite which may be in equilibrium (20) with γ-(3-pyridyl)-γ-oxo-N-methylbutyramide, serves (9) as a key intermediate in transformation of cotinine to γ-(3-pyridyl)-γ-hydroxybutyric acid. Evidence for an analogous reaction leading to the natural occurrence of the hypothetical aldehydehydrometanicotine and the formation of γ-(3-pyridyl)-γ-oxobutyramide has never been obtained.

In early studies on the metabolism of nicotine in vitro, Werle, Koebke, and Meyer (3) considered the possibility that dihydrometanicotine was formed as a result of nicotine metabolism and suggested that this intermediate would undergo oxidation in the presence of monoamine oxidase, thus providing 4-(3-pyridyl)butyraldehyde and subsequently 4-(3-pyridyl)butyric acid. Wada and Yamasaki (27) have shown bacterial metabolism of pseudo-oxynicotine to γ-3-pyridyl-γ-oxobutyric acid.

Although considerable advances have been made in defining the substrate specificities of monoamine oxidase since the early experiments of Werle, the presently available data do not permit conclusion as to whether monoamine oxidase activity is responsible for a direct conversion of dihydrometanicotine to 4-(3-pyridyl)butyraldehyde and methylamine or not. Other alternate routes to 4-(3-pyridyl)butyraldehyde, including possible intermediary participation of 4-(3-pyridyl)butylamine (demethyl dihydrometanicotine) (3), remain to be experimentally considered. These considerations and the isolation of 3-pyridylacetate from the metabolism of dihydrometanicotine in the present study considerably enlarge the number of possible alternate routes to 3-pyridylacetate which are briefly summarized in Fig. 3.
Additional Routes in the Metabolism of Nicotine to 3-Pyridylacetate: THE METABOLISM OF DIHYDROMETANICOTINE
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