Urinary Excretion of Conjugate Forms of 1-(3-Pyridyl)ethanol after Administration of 3-Acetylpyridine*

(Received for publication, December 20, 1965)

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SUMMARY

Oral administration of 3-acetylpyridine to the dog led to the excretion of 1-(3-pyridyl)ethanol in two acidic conjugate forms which were fractionated with the aid of ion exchange resins.

The more acidic of the conjugate forms had the properties of a sulfate ester in comparison with synthetic material. Acidic hydrolysis of the sulfate ester fraction led to the release of (±)-1-(3-pyridyl)ethanol. Acidic hydrolysis of the other conjugate form, which had properties of a glucuronide, led to the release of 1-(3-pyridyl)ethanol which after chromatographic purification was approximately 95% pure on the basis of extinction coefficient and which was approximately 60% in the levorotatory form on the basis of optical rotation. The pyridylethanol separated after the hydrolysis of the glucuronide contained, in addition, two other Koenig-positive materials. By the chromatographic behavior of some of the latter and by analogy to the metabolism of phenyl-1,2-ethanediol, the formation of a glucuronide of (3-pyridyl)-1,2-ethanediol is suggested.

In a preceding paper (2) from this laboratory, an abridged tentative scheme representing the metabolism of 3-acetylpyridine in the dog was presented. Preliminary evidence suggesting the urinary excretion of 1-(3-pyridyl)ethanol in conjugate form was obtained. The isolation of (3-pyridyl)-1,2-ethanediol (2) from basic fractions of urine of the treated animals, in addition to providing evidence on which to construct the scheme for the metabolism of 3-acetylpyridine, enlarged the number of precursors which possibly contribute to increased excretion of glucuronide (3) after administration of 3-acetylpyridine to the dog.

Early work of Beher, Marley, Anthony, and Gaebler in which increased urinary excretion of glucuronide was noted after administration of 3-acetylpyridine to dogs led to their suggestion (3) that 1-(3-pyridyl)ethanol (3-pyridylmethylcarbinol) was formed during the metabolism of 3-acetylpyridine. More recently, Känig, Koransky, Münch, and Schulz (4) have followed the metabolism and distribution of tritiated 3-acetylpyridine in the rat. In their studies, it was noted that 5 hours after injection of the compound, 0.7% of the administered radioactivity resided in the brain, and approximately one-half of this radioactivity could be attributed to the presence of 1-(3-pyridyl)-ethanol. Intraperitoneal administration of this alcohol (100 mg per kg) led to the production of a toxicity considered to be equal in severity to that produced by 3-acetylpyridine. It was considered also that 1-(3-pyridyl)ethanol had a significant role in bringing about the toxic effects attributable to 3-acetylpyridine, since administration of the alcohol did not lead to the occurrence of demonstrable amounts of 3-acetylpyridine in the central nervous system. Treatment with nicotinamide after administration of the alcohol produced an inhibition of the toxicity of 1-(3-pyridyl)ethanol paralleling previous experiments of others (5-7) in which the toxicity of 3-acetylpyridine was similarly inhibited.

Natural means for protection against the potential toxicity of smaller doses of 3-acetylpyridine and 1-(3-pyridyl)ethanol would appear to include pre-existing nicotinic acid and nicotinamide (8, 9) of the body and at least some of the established metabolic degradations of 3-acetylpyridine which led to the formation of nicotinic acid and other metabolites that are excreted in the urine (2, 3, 10, 11).

Our current report provides chemical evidence for the urinary excretion of 1-(3-pyridyl)ethanol as a sulfuric acid ester and as a glucuronide after oral administration of 3-acetylpyridine to the dog.

EXPERIMENTAL PROCEDURE

Chromatographic Procedures—All paper chromatograms were run on Whatman No. 1 paper with Solvents A, B, and C as previously described (2). Thin layer chromatography was carried out on silica gel (Eastman chromatogram sheet, Type K 301 R) which was used as received and without additional activation. The solvent systems were: I, methanol-chloroform (15:85 by volume) (12); II, absolute ethanol-chloroform (20:100 by volume) (13); and III, acetone-water (100:20 by volume). The Koenig-positive areas were disclosed as previously described (14). All Dowex resins were 50 to 100 mesh.

(±)-1-(3-Pyridyl)ethanol—This compound was prepared by the reduction of 3-acetylpyridine with sodium borohydride (2), comparable to that described by others (15). The homogenous
product showed a retention time of 1.15 min upon gas-liquid chromatography (evaporator, 200°C; column, 6 feet x ½ inch (outside diameter) stainless steel, 5% DC-11 on Gas-Chrom P (60 to 80 mesh) at 154°C; helium flow, 50 ml per min). 3-Acetylpyridine under the same conditions showed a retention time of 0.65 min. At a column temperature of 110°C, 1-(3-pyridyl)ethanol showed a retention time of 1.8 min and 3-acetylpyridine emerged at 1.0 min.

Resolution of (±)-1-(3-Pyridyl)ethanol—(±)-1-(3-Pyridyl)ethanol was resolved with (+)-tartaric acid as previously described (2). The acid tartrate salt from methanol, \([\alpha]_D^{22} = +0.4°\), was recrystallized further, with considerable loss, from ethanol-water to obtain a product, m.p. 148-149°C, of apparently greater optical purity, \([\alpha]_D^{22} = +2.2°\) (20% in water). For confirmation of identity, the product was dissolved in methanol and placed on a column of Dowex 21 K (OH⁻). The effluent and methanolic washings, which contained (−)-1-(3-pyridyl)ethanol, were treated with a calculated 2 eq of methyl iodide and then allowed to stand for 14 hours. The residue from evaporation of the solvent was recrystallized from 2-propanol to obtain (−)-1-(3-pyridyl)ethanol methiodide, m.p. 99-99.5°C, \([\alpha]_D^{22} = -24.4°\) (5% in methanol), in good agreement with the reported value (2).

The foregoing tartrate was also converted to (−)-1-(3-pyridyl)ethanol, \([\alpha]_D^{22} = -56.3°\) (5% in absolute ethanol), by the previously described procedure (2) and then treated with an equivalent of picric acid. After two recrystallizations from absolute ethanol, the picrate (759 mg) from 349 mg of (−)-1-(3-pyridyl)ethanol, \([\alpha]_D^{22} = -24.4°\) (5% in methanol), in good agreement with the reported value (2).

The potassium salt, prepared by a similar procedure, softened between 182°C and 235°C.

(−)-1-(3-Pyridyl)ethanol Glucuronate from Metabolism of 3-Acetylpyridine—A male mongrel dog (25 kg) was allowed food and water ad libitum. An oral administration of an aqueous solution of 3-acetylpyridine (1.25 g) was made daily on each of 4 successive days. The urine was collected daily as runoff from the metabolism during administration of the compound and for 1 subsequent day. The combined urine was filtered and then placed on a column of wet Dowex 21 K (OH⁻). The effluent was evaporated to dryness, and the residue was dissolved in 15 ml of water. The solution was adjusted to pH 8 to 9 by addition of 2.5 N sodium hydroxide. The potassium salt, prepared by a similar procedure, softened between 182°C and 235°C.

C₇H₆N₂O₂K (241.3)
Calculated: C 37.33, H 3.38, N 5.81
Found: C 37.29, H 3.36, N 5.70

(−)-1-(3-Pyridyl)ethyl-1-sulfate Salts—A mixture of 500 mg of (−)-1-(3-pyridyl)ethanol, 65 ml of dry benzene, and 1200 mg of pyridine-sulfur trioxide reagent (16) was heated under reflux for 2 hours. The solvent was removed with a stream of dry nitrogen and the residue was dissolved in 15 ml of water. The solution was adjusted to pH 8 to 9 by addition of 2.5 N sodium hydroxide. The residue obtained on evaporation of the acetate solution was extracted with 25 ml of cold acetone for removal of some colored impurities. The resulting colorless solid was extracted with 250 ml of hot acetone and residual sodium sulfate was removed by filtration. The acetone solution was concentrated to 30 ml and cooled to obtain 318 mg of sodium (+)-1-(3-pyridyl)ethyl-1-sulfate as colorless crystals. These softened between 157°C and 159°C upon heating. An aqueous solution of the salt remained clear upon addition of 5% barium chloride solution.

C₇H₆N₂O₂Na (225.2)
Calculated: C 37.33, H 3.38, N 6.22
Found: C 37.53, H 3.68, N 6.24

The potassium salt, prepared by a simple procedure, softened between 182°C and 235°C.

C₇H₆N₂O₂K (241.3)
Calculated: C 37.34, H 3.34, N 5.81
Found: C 37.29, H 3.29, N 5.70

After removal of the solvents from Solution I under diminished pressure, the solids were extracted with boiling methanol. The filtered methanolic solution was concentrated to dryness. The residue was heated under reflux with 100 ml of 2 N hydrochloric acid for 17 hours. The mixture was treated with decolorizing carbon and then filtered. After removal of hydrochloric acid under diminished pressure and 40°C, the residue was treated with 25 ml of 1 N sodium hydroxide. The alkaline solution was extracted continuously with chloroform. The chloroform solution was evaporated to obtain crude 1-(3-pyridyl)ethanol as a brown oil (187 mg). An aqueous solution of the oil was processed on Dowex 50 (H⁺) and Dowex 21 K (OH⁻) by the pro-

1 After completion of this work, a preliminary report by Neuhoff and Köhler (17) described the formation of the glucuronide of 1-(3-pyridyl)ethanol as the result of the metabolism of 3-acetylpyridine in the rat.
To analyze the preceding paragraph. The effluent and water washings from the Dowex 21 K (OH-) column were extracted with chloroform. The residue from evaporation of the chloroform was dissolved in acetone and placed on a column of Florisil (6 g). Elution with benzene and benzene containing 10% acetone provided 140 mg of colorless oil, \( [\alpha]_{D}^{25} +73.6^\circ \) (2% in absolute ethanol). This product accounted for approximately 28% of the administered 3-acylpyridine. The ultraviolet absorption spectrum of the oil in comparison with a sample of redistilled 1-(3-acylpyridyl)ethanol indicated a purity of approximately 95%.

A sample of the foregoing oil was treated with a calculated equivalent of picric acid (10% water). The product readily dissolved in absolute ethanol to give a clear solution which clouded upon cooling. After clarification by centrifugation the solution deposed a crystalline picrate, m.p. 122-122.5° (micro melting point). Upon thin film chromatography with Solvent I, the product produced Koenig-positive zones at \( R_F 0.06 \) (very faint), \( R_F 0.36 \) (faint and corresponding in \( R_F \) value to 1-(3-acylpyridyl)-1,2-ethanediol), and \( R_F 0.64 \) (strong and corresponding in \( R_F \) value to 1-(3-acylpyridyl)ethanol). In Solvent II, Koenig-positive areas were formed at \( R_F 0.10 \) (very faint), \( R_F 0.31 \) (faint), \( R_F 0.72 \) (strong). Authentic (3-acylpyridyl)-1,2-ethanediol migrated with a value of 0.31 and 1-(3-acylpyridyl)ethanol migrated with a value of 0.72 in Solvent II. Repeated recrystallizations from methanol and ethanol failed to alter the melting point substantially although the minor Koenig-positive contaminants were eliminated.

A larger sample (84.6 mg) of the liquid, crude 1-(3-acylpyridyl)ethanol, was treated with picric acid as above. The product was recrystallized from methanol to obtain an initial crop which yielded upon recrystallization from ethanol a product similar to that described above, \( [\alpha]_{D}^{25} -7.6^\circ \) (2.1% in methanol). Repeated recrystallizations again failed to alter the melting point or optical rotation substantially. The methanolic mother liquor was cooled in an ice bath to obtain a second crop which yielded upon recrystallization from methanol to obtain an initial crop which yielded upon recrystallization from ethanol a product similar to that described above, \( [\alpha]_{D}^{25} -18.5^\circ \) (1.5% in methanol). Repeated recrystallizations from methanol and ethanol failed to alter the melting point substantially although the minor Koenig-positive contaminants were eliminated.

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In another experiment, a male mongrel dog (12.5 kg) received 3-acylpyridine (108 mg per kg) orally in aqueous solution for 4 days. The combined urine, which was collected as daily runoff into sodium fluoride, was treated with Dowex 50 (H+) and subsequently with Dowex 21 K (Cl-) essentially as in the foregoing. The sulfate ester fraction that was obtained by elution with 2 N sodium chloride and subsequent evaporation of the ethanolic solution was dissolved in water. After treatment with 5% barium chloride until no more precipitate formed for removal of possible extraneous anions, the solution was filtered and was then made approximately 2 N with respect to HCl. During a period of 1 hour at 100°, 1-(3-acylpyridyl)ethanol and sulfate ion were produced. Sulfate was qualitatively determined with benzidine. A sample of the resultant benzidine sulfate was recrystallized from water and dried under diminished pressure at 60°.
**RESULTS AND DISCUSSION**

The results of the present study serve to extend and confirm the previous investigation on the metabolism of 3-acetylpyridine (2), in which the involvement of the 1-(3-pyridyl)ethanol, as itself or in derivative form, and (3-pyridyl)-1,2-ethanediol was established. Although earlier workers (3), on the basis of increased glucuronide excretion after administration of both 3-acetylpyridine and (±)-1-(3-pyridyl)ethanol, had suggested that the glucuronide of 1-(3-pyridyl)ethanol arose as the result of administration of 3-acetylpyridine, direct chemical evidence for conjugates of 1-(3-pyridyl)ethanol was lacking. This study supplies part of the required chemical evidence.

Throughout our present and previous studies, it was observed that the administration of 3-acetylpyridine to dogs, anesthetized or unanesthetized, was followed by the appearance in the urine of a Koenig-positive substance which was not retained by Dowex 50 (H+). Attention was immediately focused on the possibilities of a sulfate ester formed from the metabolism of 3-acetylpyridine. Sulfate esters are widely known (18) as conjugates of simple alkyl alcohols, various other alcohols, and phenols.

The partially purified urinary fraction (effluent from the Dowex 50 (H+)), which contained Koenig-positive material with RF value corresponding to synthetic (±)-1-(3-pyridyl)ethanol sulfate, was placed on Dowex 21K (OH-) which retains both natural and synthetic sulfate esters. Acidic hydrolysis of the ester, eluted from the column by hydrochloric acid, led to liberation of sulfate ion and (−)-1-(3-pyridyl)ethanol (accounting for approximately 13% of the administered 3-acetylpyridine). The liberated alcohol was chromatographed to remove colored and extraneous material to obtain 129 mg of (−)-1-(3-pyridyl)ethanol, [α]D −53.2°, identified as a picrate and in another experiment, a glucuronide of 1-(3-pyridyl)ethanol was lacking. This study extended to 2 hours.

Liberation of alcohol was followed by the appearance in the urine that the administration of 3-acetylpyridine to dogs, anesthetized or unanesthetized, was followed by the appearance in the urine that the administration of 3-acetylpyridine, direct chemical evidence for conjugates of 1-(3-pyridyl)ethanol was lacking. This study extended to 2 hours.

The fact that levorotatory, rather than racemic, 1-(3-pyridyl)ethanol was obtained from the acidic hydrolysis of the sulfate ester was of interest in view of the previous isolation of the metabolic N-methyl derivative of the racemic alcohol under conditions that presumably had not led to racemization. Our own data (2) in the dog and that of others (4) in the rat had not previously permitted a conclusion on the possible participation of optically active forms of the alcohol in the metabolism of 3-acetylpyridine.

The samples of 1-(3-pyridyl)ethanol obtained by chromatography after acidic hydrolysis of the glucuronide fractions in our studies showed ultraviolet absorption characteristics which suggested a purity of approximately 95%. The optical rotation of this material, however, corresponded to a (−) isomer content of approximately 60%. The low observed rotation may be attributed to a combination of factors: traces of impurities and a mixture of racemic and optically active 1-(3-pyridyl)ethanol arising metabolically or as an artifact in processing. Attempts to racemize (−)-1-(3-pyridyl)ethanol under conditions comparable to those employed in the processing of the urine did not produce significant evidence of racemization. The involvement of racemization arising as an artifact, however, cannot be entirely eliminated due to possible contributions of many urinary components; trace quantities of unidentified Koenig-positive material, which may be optically active, were observed in the picrates initially obtained from the 1-(3-pyridyl)ethanol of the glucuronide fraction.

From both formal and experimental considerations, the involvement of both optical isomers of 1-(3-pyridyl)ethanol in the metabolism of 3-acetylpyridine requires much additional study. The present investigation, in addition to providing evidence for the formation of the glucuronide and sulfate esters of 1-(3-pyridyl)ethanol, points to the possibility of (3-pyridyl)-1,2-ethanediol as a constituent of the glucuronide fraction. The formation of a glucuronide of phenyl-1,2-ethanediol has already been noted (19), and the reports of formation (20, 21) of 3-acetyl-6-pyridone and N-methyl-3-acetyl-6-pyridone from the metabolism of 3-acetylpyridine give additional possibilities for glucuronide formation.

Acknowledgments—We are very grateful to Mr. Quentin S. McKennis, Mrs. Marcela Siao, and Mr. Sidney Vance for technical assistance.

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