Fate of 2-Chloro-6-(trichloromethyl)pyridine in the Rat

The white rat excretes two degradation products in its urine following ingestion of 2-chloro-6-(trichloromethyl)pyridine, the active ingredient in N-Serve. The chief product is 6-chloropicolinic acid; the minor product is *N*-(6-chloropicolinoyl)glycine. An explanation for the difference between excretion products observed for the dog and the rat is proposed

A study of the fate of 2-chloro-6-(trichloromethyl)-pyridine in the dog by Redemann *et al.* (1966) demonstrated that this compound, the active ingredient in N-Serve (Dow Chemical Co.) nutrient stabilizer, undergoes hydrolysis and conjugation with glycine to form *N*-(6-chloropicolinoyl)glycine. The fate of this compound in the rat is somewhat similar and forms the subject of this communication.

EXPERIMENTAL

Reference Compounds. 6-Chloropicolinic-carboxy-C^{14} acid, 2-chloro-6-(trichloromethyl)-C^{14}-pyridine, and *N*-(6-chloropicolinoyl)glycine were prepared as described by Redemann *et al.* (1964, 1966).

Feeding. Two male albino rats, housed in a metabolism cage, were fed mixed grains to which had been added 100 p.p.m. of 2-chloro-6-(trichloromethyl)-C^{14}-pyridine of 1 mc. per mmole specific activity. After a single day's feeding, the tagged compound was replaced with untagged compound, and the feeding continued.

Excreta Collection. Urine was collected daily, treated with one drop of toluene as a preservative, and stored at 4° C. for no longer than 10 days.

Paper Chromatography. Immediately after collection, replicate portions of the urine voided during the day following radioactive feeding were placed on strips of Whatman No. 1 filter paper. Each replicate was then developed with a different solvent system. After development, the strips were dried at room temperature, and the radioactive regions located with a Forro gas-flow strip counter.

The results of this chromatography, as well as the results of cochromatographing whole rat urine with 6-chloropicolinic-carboxy-C^{14} acid and with *N*-(6-chloropicolinoyl-C^{14})glycine, are presented in Table I.

Fractionation of Urine. The urine from tagged feeding was added to the urine from untagged feeding and filtered through paper to yield 35.2 grams of filtrate. The filtrate was adjusted to pH 8.5 with saturated sodium bicarbonate and shaken out with two 35-ml. portions of ethyl ether.

Because very little radioactivity was present in the ether extracts, they were discarded.

The aqueous phase was adjusted to pH 2 with 0.5*N* hydrochloric acid and shaken out with three 40-ml. portions of ethyl ether. On evaporation to dryness through a 17-inch Vigreux column, the combined ether extracts left 173 mg. of "ether-soluble acids," containing most of the radioactivity, in the urine.

These "ether-soluble acids" were dissolved in 1 ml. of 1*M* potassium phosphate. The pH was adjusted to 5.6 with 2*N* hydrochloric acid, and the solution was absorbed on 1 gram of Dicalite Speedex (Great Lakes Carbon Co.), and packed at the top of a 1.9 × 18 cm. chromatographic column made from 8 ml. of 1*M* potassium phosphate buffer, pH 5.6, mixed into 20 grams of Dicalite. Development with ethyl acetate saturated with the 1*M* pH 5.6 buffer followed, the effluent being collected in portions of 8 ml. on an automatic fraction collector.

Radiochemical assay detected two zones of radioactivity. The first, I, appeared in the 32- to 56-ml. fractions of effluent. The second, II, appeared in the 96- to 240-ml. fractions.

FRACTION I. When the solutions containing fraction I were evaporated to dryness, the material obtained would not crystallize. Next, it was streaked on strips of Whatman No. 1 filter paper, and replicates were chromatographed with each of the solvent systems listed in Table I. Each chromatogram was dried, sprayed with the 4-dimethylaminobenzaldehyde reagent of Gaffney *et al.* (1954), and heated at 150° C. for 2 minutes. On each strip, a red-orange band appeared exactly coincident with the radioactivity and at the same R_f value observed for the minor metabolite before fractionation of the urine.

These red-orange bands were cut off the paper chromatograms, and suspended in a small volume of methanol. The methanol eluted the pigment, and this solution was found to possess a maximum optical absorption at 486 mμ. The pigment solution prepared in a similar manner from the reaction product of *N*-(6-chloropicolinoyl)glycine with 4-dimethylaminobenzaldehyde reagent also possessed maximum absorption at 486 mμ.

In a separate chromatographic study, starting with a synthetic sample of *N*-(6-chloropicolinoyl)glycine and the identical buffered Dicalite and ethyl acetate system, *N*-(6-chloropicolinoyl)glycine was found to collect in the same portions of column effluent that fraction I did.

FRACTION II. Paper chromatograms of fraction II showed the same R_f values as the major metabolite found before fractionation of the urine.

Table I. R_f Values in Presence of Rat Urine

Compound	Solvent 1[a]	Solvent 2[b]	Solvent 3[c]
Major metabolite of N-Serve	0.38	0.52	0.76
Minor metabolite of N-Serve	0.44	0.52	0.57
6-Chloropicolinic-C^{14} acid	0.38	0.52	0.76
N-(6-Chloropicolinoyl-C^{14})-glycine	0.44	0.52	0.57

[a] Solvent 1. 1-Butanol saturated with 1.5*N* ammonia.
[b] Solvent 2. 1-Butanol–triethylamine–water, 5:1:2, v./v.
[c] Solvent 3. Benzene–propionic acid–water, 2:2:1, v./v.

The ethyl acetate solutions containing fraction II were combined and shaken out with two successive 10-ml. portions of 1.5*N* ammonia. The ultraviolet absorption spectrum of the ammoniacal solution showed the absorption peak at 272 mμ and shoulders at about 265 and 279 mμ, which characterize the absorption spectrum of the ammonium salt of 6-chloropicolinic acid.

In a separate chromatographic study, using identical conditions, a reference sample of 6-chloropicolinic acid was found to collect in the same portions of effluent that fraction II did.

DISCUSSION

Unlike the dog, the rat excretes not one, but two degradation products in its urine after ingestion of 2-chloro-6-(trichloromethyl)pyridine. Paper chromatography, collumn chromatography, and absorption spectroscopy all indicate that the major product is 6-chloropicolinic acid and the minor one is *N*-(6-chloropicolinoyl)glycine.

Both products could be detected by paper chromatography of freshly voided urine. Therefore, it is unlikely that the 6-chloropicolinic acid is an artifact arising from bacterial fermentation of *N*-(6-chloropicolinoyl)glycine. It seems much more probable that the 6-chloropicolinic acid represents an intermediate in the biosynthesis of *N*-(6-chloropicolinoyl)glycine from 2-chloro-6-(trichloromethyl)pyridine. There is considerable evidence that this occurs in the dog. Perhaps the difference between the excretion products in the dog and in the rat lies primarily in a greater ability of the dog to conjugate 6-chloropicolinic acid with glycine.

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