# IMIDAZOLEPROPIONIC ACID AS A URINARY METABOLITE OF L-HISTIDINE

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Received September 10, 1962

Imidazolepropionic acid has often been proposed as a metabolite of L-histidine, but convincing data on its formation by mammalian tissue has been lacking. Hanke and Koessler (1922) originally described imidazole-propionic, -acetic, -lactic, and -acrylic acids as metabolites of L-histidine in bacteria, but their methods were inadequate to distinguish these products. One route of L-histidine metabolism was subsequently established as proceeding through urocanic acid to formiminoglutamic acid and other aliphatic derivatives (Fruton and Simmonds, 1958). Recently, Baldridge and Tourtellotte (1958) showed that imidazolepropionic acid was found in rat urine following the administration of a large load of L-histidine by stomach tube. Auerbach et al. (1962) identified imidazolepropionic acid in the urine following administration of a load of urocanic acid to an infant with a presumed defect in histidine metabolism.

This paper is to present evidence showing that imidazolepropionic acid is a normal constituent of human urine, and that it is derived from urocanic acid.

## EXPERIMENTAL

Spot 140b, previously characterized by us (Smith et al., 1959) as a normal constituent of extracts of human urine, was shown to be identical with authentic imidazolepropionic acid when chromatographed in six solvent systems and developed with four spray reagents (Table I). Furthermore, spot 140b was separated from human urine by precipitation with alkaline silver nitrate

TABLE I.

Chromatographic Characteristics of Imidazolepropionic and Urocanic Acids

| Imidazolepropionic acid | Urocanic acid | |
|---|---|---|
| $R_f$ data | | Solvent* |
| 0.45 | 0.49 | A |
| 0.24 | 0.26 | B |
| 0.56 | 0.62 | C |
| 0.54 | 0.51 | D |
| 0.80 | 0.77 | E |
| 0.36 | 0.58 | F |
| Color reactions | | Spray reagents** |
| - | - | Nin |
| Red | Yellow → Orange-red | DSA |
| Purple | Purple | DNA |
| Purple | Blue | Hg-DPC |

* A = Butanol-acetic acid-water (4:1:1)
B = Isopropanol-ammonium hydroxide-water (8:1:1)
C = Methanol-butanol-benzene-water (2:1:1:1)
D = Butanol-pyridine-water (1:1:1)
E = Isobutyric acid-0.5N ammonium hydroxide (10:6)
F = t-Butanol-acetone-formic acid-water (160:160:1:39)

** For spray reagents see McGeer et al. (1961). Whatman No. 1 filter paper by descending chromatography was used.

at pH 8.5 (Kerr and Seraidarian, 1945). It was isolated from other components of the precipitate by repeated paper chromatography and strip elution; solvents E, A, D, and C (Table I) were used in that order. The single product so obtained was converted to the methyl ester by the 'Fisher esterification method'. The methyl ester behaved the same as authentic methyl

imidazolepropionate when a chromatogram was prepared in solvent B and developed with DSA. The density of color produced by spot 140b on chromatograms would suggest that approximately 1 mg per day is excreted in human urine.

In an effort to determine the origin of urinary imidazolepropionic acid, two rats were each given approximately 12 microcuries of L-histidine-ring-2-$C^{14}$ (specific activity 2 mc/mM) by intraperitoneal injection. Aliquots of the subsequent 24-hour urine specimen were chromatographed with carrier urocanic and imidazolepropionic acids unidimensionally in solvent F and bidimensionally in solvents A plus F and B plus F. Solvent F was chosen because known metabolites of histidine other than imidazolepropionic and urocanic acids have low $R_f$'s in this solvent system. Urocanic acid was located on the chromatograms by Ultraviolet (2570 AU) fluorescence, and imidazolepropionic acid by its $R_f$ relative to urocanic. The appropriate areas were cut from the chromatograms, and counted in a liquid scintillation counter (Wang and Jones, 1959). Accurate location of urocanic and imidazolepropionic acids was confirmed by later spraying of the papers with DSA. Duplicate chromatograms were also run which were first sprayed with DSA and then counted by strip scanning using a Tracerlab Omniguard counter. The latter gave less efficient counting but established that there were no other radioactive spots in the regions of urocanic and imidazolepropionic acids. Further evidence of purity was that the spots corresponding to urocanic and imidazolepropionic acids on the unidimensional chromatogram gave the same counts as corresponding spots on either of the bidimensional chromatograms. An average of 4.7% of the injected radioactivity was recovered in the first 24-hour urine specimen. Of this urinary radioactivity, 1.4% was associated with urocanic acid and 11.7% associated with imidazolepropionic acid. No attempt was made to break down possible conjugates of these acids.

Approximately 17,000 dpm's of urocanic acid were obtained from the remainder of the rat urines by repeated strip chromatography in solvent F, using a small amount of cold urocanic acid as a carrier. This radioactive

urocanic acid was injected intraperitoneally into another rat, and a 24-hour urine specimen collected. This contained 38% of the injected radioactivity. The total urine was streaked on several chromatograms, and, after running in solvent F, the strips corresponding to urocanic and imidazolepropionic acids were cut, eluted, concentrated and counted in a liquid scintillation spectrometer. About 10% of the urinary radioactivity was in urocanic acid and 10% in imidazolepropionic acid.

Finally, a load of 50 mg/kilo of cold urocanic acid was administered intraperitoneally to another rat. Chromatograms of the subsequent 24-hour urine specimen showed a prominent DSA-positive spot not visible in similar chromatograms of the control urine; this spot behaved identically with imidazolepropionic acid in solvents C, D, and F. A spot corresponding to urocanic acid was also seen.

Although these experiments leave little doubt that one pathway of L-histidine metabolism is to imidazolepropionic acid via urocanic acid, the exact mechanism of the formation, and the proportion of urocanic acid going through this route, are not known. Presumably there is a direct reduction of urocanic acid, which does not involve gut bacteria.

Urocanic acid itself, although a normal constituent of human urine (Acheson et al., 1958), is not readily detected except in certain pathological conditions such as megaloblastic anemia and kwashiorkor (Whitehead, 1962). In these conditions there is evidently a block in the route through formimino-L-glutamic acid. It would be interesting to know whether such cases would also show an elevation in imidazolepropionic acid.

ACKNOWLEDGEMENT

We wish to thank Dr. Edith McGeer for many valuable suggestions. This work was supported by grants from the National Institute of Health (Grant No. H-5664), and Federal-Provincial Mental Health Grants Nos. 609-5-130 and 609-5-141.

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