ON UROCANIC ACID.

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In 1908 the writer isolated from a long-continued pancreatic digest of casein a crystalline substance, which he was able shortly afterwards to identify as urocanic acid ("Urocaninsäure"). Up to that time the substance had been known only as an occasional constituent of the urine of dogs. Two cases of its occurrence had been reported; the first in 1874 by its discoverer Jaffé, and the second in 1898 by Siegfried. It would seem that the two animals concerned presented a rare anomaly of metabolism, not attributable to any definite cause. Jaffé examined the urine of other dogs, and also of men, without again encountering the condition. Siegfried could find no urocanic acid in 110 liters of human urine.

The origin and constitution of urocanic acid have till now remained uncertain. Siegfried conjectured a relation to the purines. The appearance of the substance among the products of casein digestion pointed at once in another direction. In preliminary communications I indicated the probability that the mother substance of urocanic acid is histidine. Observations made soon afterwards suggested a more definite conclusion regarding its structure; but the hypothesis formed could not at the time be decisively tested without a fresh supply of material. Efforts to procure this have consumed a great deal of time. During the last three
years large quantities of casein have been subjected for six or seven month periods to trypic digestion, and in the product urocanic acid has been sought by the method which originally led to its isolation. Disappointment has been the uniform result; the original experience has not once been duplicated. Attempts to discover a dog which excreted urocanic acid have met with no greater success. The statement of Swain that a possibly related substance may occur in coyote urine led to an examination of that source also; but the animal investigated produced neither Swain's substance nor urocanic acid.

It is therefore fortunate that recent work by others has afforded the means of deciding immediately the question at issue, and of settling the problem of constitution. The circumstance removes any reason that may have hitherto existed for withholding the details now communicated.

The digest from which urocanic acid was obtained had been made with the object of preparing a supply of the "polypeptide of pancreatic digestion" described by Fischer and Abderhalden.

In 5 liters of water, containing 10 cc. of concentrated Liquor ammoniae, there were dissolved 500 grams of "Plasmon" and 10 grams of "pankreatin absolut., Rhenania." The mixture was maintained at 40° in the presence of abundant toluene and chloroform. Two days later 10 cc. of ammonia, and sixteen days later 10 grams of pancreatin were added. Digestion was continued for seven months, at the end of which time the biuret reaction was but feebly positive. A loose jelly-like clot (plastein?), impregnated with tyrosine crystals, was filtered off, and the filtrate was concentrated in vacuo at 40° to 50°. The second crop of tyrosine crystals having been removed, the liquid was diluted to about 6 liters, and treated with phosphotungstic acid. The washed precipitate was decomposed in the cold with baryta, and excess of the latter removed by sulphuric acid. The product was concentrated in vacuo and dried over sulphuric acid. The yield of crude "polypeptide" was 56 grams. This was dissolved in 1 liter of 5 per cent

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5 In the somewhat laborious operations involved I had during the Summer Session of 1911 the assistance of Miss Ruth Wheeler, to whom I here gratefully acknowledge my indebtedness.


7 Hunter and Givens: This Journal, viii, p. 449, 1910.

sulphuric acid, and the precipitation with phosphotungstic acid was repeated. From the precipitate were finally obtained 42 grams of brownish-yellow, extremely hygroscopic material, which dissolved in water with strongly alkaline reaction, and gave no biuret reaction whatever.

From this product it was decided to separate, if possible, arginine and histidine. One would naturally have expected the material to contain these bases in considerable amounts. It did give the intense "diazo reaction" shown by histidine. But it was found that towards silver nitrate and fixed alkali it did not react in the way expected of an arginine solution. As a matter of fact subsequent application of the silver-baryta method showed that neither arginine, nor yet histidine, was present in quantities that could be isolated. On the other hand there was produced by silver nitrate alone a quite considerable precipitate, which dissolved at once in the slightest excess of either acid or alkali. Attention was thereupon directed to the separation of the substance so reacting.

To this end the whole material was brought into aqueous solution (600 to 700 cc.), very nearly neutralized with nitric acid, and treated with 10 per cent silver nitrate as long as a precipitate resulted. Six or seven grams of the nitrate were required. The light brown gelatinous precipitate was collected on a filter, and thoroughly washed. It was then suspended in water, and dissolved by the aid of a little dilute sulphuric acid. The solution was freed from silver by hydrogen sulphide, from the latter by a stream of air, and from sulphuric acid by baryta. It reacted now acid, and on concentration deposited 1.45 grams of crude crystalline material. This was purified by boiling with charcoal and by several crystallizations. The final yield was 0.92 gram. The amount originally present in the digest must have been considerably greater.

The substance thus obtained was sparingly soluble in cold, readily soluble in hot, water. Its solubility in alcohol was very slight, while in ether, acetone, ethyl acetate and carbon disulphide it was almost, if not quite, insoluble. It was dissolved with ease by glacial acetic acid, and by aqueous ammonia or sodium hydroxide. Its aqueous solution reacted acid to litmus. When rapidly crystallized from hot water, it formed branching groups of slender, beautifully iridescent, doubly refracting needles, sometimes nearly a centimeter long; on more gradual separation it appeared as well formed tetragonal prisms of the first and second orders.
**Urocanic Acid**

The water-free substance melted with decomposition at $224^\circ$ (corrected).

**Analysis and Molecular Weight Determination.** 0.1369 gram air-dried substance lost 0.0281 gram at $110^\circ$.

0.2208 gram, dried at $110^\circ$, yielded 0.4182 gram CO$_2$ and 0.0879 gram H$_2$O.

0.1193 gram gave 21.1 cc. N at $17^\circ$ and 746 mm.

0.1031 gram, dissolved in 10.48 grams glacial acetic acid, depressed the freezing point of the solvent 0.324$^\circ$.

\[
\begin{array}{c|c|c}
\text{H}_2\text{O} & 20.7 & 20.5 \\
\text{C} & 52.1 & 51.7 \\
\text{H} & 4.4 & 4.5 \\
\text{N} & 20.3 & 20.5 \\
\text{Molecular weight} & 138.0 & 124.0 \\
\end{array}
\]

Calculated for $\text{C}_6\text{H}_4\text{O}_2\text{N}_2\cdot2\text{H}_2\text{O}$:  Found:

In crystalline form, solubility, melting point, and elementary composition the substance agreed exactly with the descriptions of urocanic acid. The two following reactions removed any uncertainty as to its identity therewith. (1) If a small quantity of the hydrated substance is treated with a drop of glacial acetic acid the crystals at first dissolve; but almost immediately thereafter, especially if the solution is shaken or rubbed, they separate again as a thick mass of small, opaque, white needles. This behavior is described by Siegfried as characteristic of urocanic acid. The opaque needles dissolve readily on addition of water or alcohol, or an excess of acetic acid. (2) When an aqueous solution of the substance is treated with an equal volume of 50 per cent nitric acid, a heavy microcrystalline precipitate of the nitrate is very rapidly deposited. The crystals of urocanic acid nitrate, produced in a similar manner, have a highly characteristic appearance. They are described by Jaffé as "small plates, bent in the form of a sickle, with the ends apparently frayed or eaten away; frequently several such plates are united to cross- or rosette-shaped aggregates." A reference to the photographs reproduced will demonstrate the quotation to be an exact description of the crystals yielded by the substance from casein.

Concerning the identity of the latter there was therefore no reasonable room for doubt. But the molecular weight determination above reported led to the formula $\text{C}_6\text{H}_4\text{O}_2\text{N}_2$. This was in

*The negatives were very kindly made for me by Dr. R. Cattley, University of Leeds, whom I take this occasion of thanking for the service.*
disagreement with the view of Jaffé (concurred in by Siegfried), which assigned to the acid the double formula $\text{C}_{12}\text{H}_{22}\text{O}_{4}\text{N}_4$. Jaffé's choice was determined by the single circumstance, that urocanic acid, when heated, yields by loss of carbon dioxide and water a base—"urocanine"—to which apparently must be ascribed the formula $\text{C}_{12}\text{H}_{10}\text{O}\text{N}_4$. The evidence for the chemical individuality of this substance is not altogether convincing. Neither the base itself nor its salts with mineral acids could be obtained in crystals.

Various Forms of Urocanic Acid Nitrate.

talline form. Its formula rests entirely upon analyses of an exceedingly hygroscopic chloroplatinate. The nature of urocanine

10 It was at first suspected that the determination itself might be in error. It was made with an apparatus, the only one then at my disposal, which did not exclude moisture. Experiment proved that it was nevertheless easy to obtain with the same instrument and the same sample of acetic acid satisfactory approximations to the calculated molecular weight of other organic substances.
Urocanic Acid
decidedly calls for further investigation. Whatever may be the
mechanism of its formation, the sequel will show with sufficient
clearness that urocanic acid at any rate does possess the simpler
formula indicated by its cryoscopic effect.

To the older descriptions of urocanic acid I am able to add the
following points. Its solutions are optically inactive. They are
precipitated by silver nitrate; the precipitate increases in bulk upon
exact neutralization with ammonia, but dissolves instantly in the
slightest excess of either ammonia or nitric acid. Urocanic acid
is precipitated also by mercuric chloride, and by phosphotungstic
and picrolonic acids. The phosphotungstate dissolves in hot water,
from which it crystallizes in minute cubes or short rectangular
prisms. The picrolonate, gelatinous when formed by bringing
together aqueous solutions but granular if precipitated in alcohol,
is dissolved with great difficulty in boiling absolute alcohol, with
less difficulty in boiling water, and with comparative ease in boiling
dilute alcohol. It can be recrystallized from water as bright yel-
low sheaves of long filamentous needles, from 75 per cent alcohol
as dense clumps of yellow plates, which singly take the form of
elongated rhombs. It decomposes about 268° (corrected), after
gradual discoloration from about 230°.

On bringing together saturated aqueous solutions of urocanic
acid and picric acid there is no immediate precipitate; but there
gradually separate yellow iridescent macroscopic prisms of the
picrate, which melt at 224° to 225° (corrected).

Urocanic acid in dilute sodium carbonate solution gives a very
intense red reaction with diazobenzenesulphonic acid. It does not
evolve any nitrogen on treatment with nitrous acid. It instantly
reduces a cold alkaline permanganate solution with immediate
liberation of manganese dioxide.

A substance giving the diazo reaction, and obtained from pro-
tein in the way described, could hardly be other than an imidazole
derivative, standing in some relation to histidine. Its precipita-
tion reactions were in harmony with this conclusion. The immedi-
ate reduction of cold alkaline permanganate, which the imidazole
ring itself will not bring about, pointed to the possession of an
unsaturated side chain. These considerations, taken in con-
junction with the empirical formula C₆H₅O₂N₂, suggested the
probability that urocanic acid is an imidazole-acrylic acid, related
therefore to histidine in the same way as cinnamic acid is to phenylalanine.\textsuperscript{11}

\[
\begin{align*}
\text{CH-NH} & \bigg\| \text{CH} \\
\text{C} & \bigg\| \text{N} \\
\text{CH}_2 \\
\text{CH.NH}_2 \\
\text{COOH}
\end{align*}
\]

Histidine

\[
\begin{align*}
\text{CH-NH} & \bigg\| \text{CH} \\
\text{C} & \bigg\| \text{N} \\
\text{CH} \\
\text{CH} \\
\text{COOH}
\end{align*}
\]

Imidazole-acrylic or Urocanic acid

Such a constitution would account for the acid reaction to indicators with simultaneous possession of basic characters, the want of optical activity, and the failure to react with nitrous acid. But before it could be held to be fully established, confirmatory evidence of a stricter nature was essential. This can now be supplied. A substance known independently to have the structure represented above has been recently described by Barger and Ewins.\textsuperscript{12} They obtained it in two ways: (1) from ergothioneine (the betaine of thiohistidine) in the manner illustrated by the scheme below:

\[
\begin{align*}
\text{CH-NH} & \bigg\| \text{C.SH} \\
\text{C} & \bigg\| \text{N} \\
\text{CH}_2 \\
\text{CH}_{-N}(\text{CH}_3)_3 \\
\text{CO} & \bigg\| \text{O}
\end{align*}
\]

KOH

\[
\begin{align*}
\text{CH-NH} & \bigg\| \text{C.SH} \\
\text{C} & \bigg\| \text{N} \\
\text{CH} \\
\text{CH} \\
\text{COOH}
\end{align*}
\]

HNO\textsubscript{3}

\[
\begin{align*}
\text{CH-NH} & \bigg\| \text{CH} \\
\text{C} & \bigg\| \text{N} \\
\text{CH} \\
\text{CH} \\
\text{COOH}
\end{align*}
\]

\textsuperscript{11} This view of the constitution of urocanic acid occurred independently, as I learn from a private communication, to Professor Treat B. Johnson of Yale University, whose studies on thiohydantoins have led him to an interest in the derivatives of histidine (see Johnson and Guest: Amer. Chem. Journ., xlvi, p. 242, 1912). It was Professor Johnson who drew my attention to the paper of Barger and Ewins mentioned below, and he therefore who furnished the stimulus that occasioned the immediate publication of my results. It is a pleasure to record my appreciation of the friendly spirit that has characterized Professor Johnson’s side of the correspondence.

Urocanic Acid

(2) by the action of trimethylamine on α-chloro-β-imidazole-propionic acid. Of this product sufficient has been placed in my possession\(^{13}\) to enable me to say with certainty that it is identical with urocanic acid. It crystallizes in precisely the same forms; it behaves in the same way with glacial acetic acid; and its nitrate has the peculiar and characteristic shape of urocanic acid nitrate. Specimens of the two products heated side by side melted together at 231\(^\circ\) to 232\(^\circ\) (corrected);\(^{14}\) a mixture of both in equal proportions melted simultaneously with a sample of the compound from casein. Barger and Ewins describe a phosphotungstate crystallizing in small rectangular plates, and a picrate forming golden yellow prisms.

The comparison places it beyond reasonable doubt that urocanic acid is \(\beta\)-imidazole-4(or 5)-acrylic acid.

That such a substance should make its appearance in a pancreatic digest is somewhat astonishing. Its origin certainly cannot be ascribed to the action of trypsin. What the responsible factor was, whether the particular ferment preparation employed contained a deaminizing enzyme of peculiar nature, or whether the responsibility lay with some accidental circumstance in the manipulation of the product, it has not been possible to determine. It is doubtless of significance that from the digestion mixture arginine, as well as histidine, had disappeared. The attempt to duplicate the occurrence has not been abandoned, and an explanation may yet be found. One naturally thinks of bacterial action. The incubated mixture at no time exhibited evidence of organismal growth; yet in the absence of bacteriological control that source of decomposition cannot be by any means excluded. With this in mind I have grown some of the commoner organisms in casein and histidine solutions. The result hoped for has not so far been attained. Experiments in this direction also are being continued, although, so far as I am aware, no analogous case of the conversion by bacteria of an amino-into an unsaturated acid has been reported.

\(^{13}\) To Drs. George Barger and Arthur J. Ewins I take this opportunity of expressing my grateful recognition of the courtesy with which they at once acceded to my request for a specimen.

\(^{14}\) I had previously found for urocanic acid the melting point 224\(^\circ\), while Barger and Ewins report for their substance 235\(^\circ\) to 236\(^\circ\). The fact is, as Siegfried also noticed, that the value found varies widely with the manner of heating. This is probably equally true for the picrate, which according to Barger and Ewins melts at 213\(^\circ\) to 214\(^\circ\), according to my own determination at 224\(^\circ\) to 225\(^\circ\).
The appearance of imidazole-acrylic acid under the circumstances described in this paper is not more remarkable than its occasional occurrence as an excretory product in the dog. In this character it almost certainly represents an intermediate step in the catabolism of histidine. The type of amino-acid transformation which would thus be illustrated apparently occurs in plants—witness the formation of cinnamic and p-cumaric acids—but has not hitherto been met in animals. The production of cinnamoyl-glycocoll observed by Dakin to follow administration of phenylpropionic acid to cats presents perhaps the nearest analogy. Other origins than the one assumed are of course not impossible. But the formation of the unsaturated acid is not the only problem offered.

The phenomenon of its excretion is equally puzzling. It is known that moderate doses of cinnamic acid are easily and completely oxidized within the animal organism. A case where the analogous imidazole derivative cannot be similarly disposed of is almost certainly a metabolic anomaly. The elucidation of the structure of urocanic acid adds therefore a fresh interest to the search, still being prosecuted, for an animal which regularly excretes that substance. It would be of interest to determine whether even normal dogs do not excrete small quantities of urocanic acid in response to enteral or parenteral administration of histidine or its derivatives. Experiments to decide the point are in contemplation. The metabolic fate of histidine has been the subject of studies by Abderhalden and Einbeck, Abderhalden, Einbeck and Schmid, Kowalevsky, and Dakin. But in none of the experiments reported was urocanic acid specifically sought.

15 Dakin: This Journal, v, pp. 173 and 303, 1908; also vi, p. 203, 1909.
20 Dakin: This Journal, x, p. 499, 1912.
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