SYNTHESIS AND EXCRETION OF TRIGONELLINE*

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(Received for publication, July 10, 1940)

Since Ackermann (1) has demonstrated that ingested nicotinic acid is partly excreted by the dog as trigonelline, and since this substance has been isolated from normal human urine by Linneweh and Reinwein (2), it became obviously important to estimate its excretion under a variety of conditions. In our studies on the estimation of nicotinic acid derivatives in human urine (3) we were greatly handicapped by the general unavailability of pure trigonelline and were obliged to attempt to prepare it synthetically. Two types of method for such a synthesis are available, that of Hantzsch (4) and that of Winterstein and Weinhagen (5). In testing these methods we were able to modify and simplify them as follows:

Trigonelline Hydrochloride Method, Modified from Winterstein and Weinhagen (5)—10 gm. of nicotinic acid (1 mole) and 12 gm. of methyl iodide (1 mole) in the presence of 15 ml. of dry methyl alcohol were heated at 150° for 3 hours in a 300 ml. pressure bottle in an autoclave. The mass was then dissolved in water and treated with an excess of moist silver oxide.\(^1\) The filtrate was evaporated with 7 ml. of concentrated hydrochloric acid (1 mole) to a moist crystalline mass. The trigonelline hydrochloride was crystallized from 300 ml. of hot 90 per cent alcohol after standing in the refrigerator overnight. The precipitate was washed with cold alcohol and ether. 9 gm. of trigonelline hydrochloride melting at 258°–259° were obtained. Concentration

* Grateful acknowledgment is made for aid received from the John and Mary R. Markle Foundation.

\(^1\) At this point the filtrate can be evaporated and the free trigonelline (m.p. 140°) obtained by crystallization from alcohol.
of the mother liquor yielded another 2 gm. of the pure product. The 11 gm. represent a 76 per cent yield.

\[ \text{C}_7\text{H}_8\text{N}_2\text{O}_2\text{Cl} \]

Calculated. Cl 20.46, equivalent weight 173.5

Found. " 20.35, " " 174.5 (titration)

Trigonelline Acid Sulfate Method—There is no record in the literature of the previous isolation and characterization of this salt. Heiduschka and Brüchner (6) on evaporating trigonelline with dilute sulfuric acid apparently obtained a ditrigonelline sulfate, judging from the percentage of sulfur given as 8.61. They give no other data on their salt. The trigonelline acid sulfate

\[
\begin{array}{c}
\text{N} \\
\text{CH}_3 \\
\text{COOH}
\end{array} + \text{HSO}_4^-
\]

may be readily obtained by the following modification of the procedure used by Winterstein and Weinhagen (5). These authors did not isolate or describe the sulfate, but merely used it as an intermediate stage in the preparation of the hydrochloride. In a 1 liter, 3-neck flask, equipped with a mechanical stirrer, 24.6 gm. of nicotinic acid (0.2 mole) and 38.0 gm. of dimethyl sulfate (0.3 mole) were heated at 130° for 4 hours. This viscous mass was dissolved after cooling in 200 ml. of water and 1 ml. of 10 N sulfuric acid to decompose the methyl ester of trigonelline acid sulfate. This was evaporated to 60 ml. of a thick syrup, poured into 200 ml. of hot alcohol, and decolorized by boiling with Darco charcoal and filtering. After crystallization in the cold, 25.6 gm. of the pure trigonelline acid sulfate were filtered off and washed with cold alcohol and ether. Concentration of the mother liquor and addition of alcohol to 90 per cent gave a further crop of 4.7 gm. of pure crystals. The melting point of each sample was 199-200°. The yield was 30.3 gm. or 65 per cent of the calculated amount.

Trigonelline acid sulfate is easily soluble in water, 1 gm. dissolving readily in 1 ml. of water. At 25° about 2.1 gm. are soluble in 100 ml. of 90 per cent alcohol and only 1.2 gm. at 5°. Boiling
90 per cent alcohol dissolves about 8.5 gm. per 100 ml. In ether trigonelline acid sulfate is insoluble.

\[ \text{C}_7\text{H}_7\text{NO}_4\text{S} \]

Calculated. S 13.6, equivalent weight 117.5
Found. " 13.56, " 117.1 (titration)
" 13.51, " 117.2

Hantzsch (2) found that on being heated with alkali trigonelline yielded methylamine. By distillation from strong alkali and titration of the distillate we have accounted for 96 to 98 per cent of the methylamine, identified in the form of N-methylbenzamide, m.p. 78–80°.

We have found that small amounts of trigonelline, when heated to 75° with 6 N KOH in the presence of a source of ammonia (ammonium salts or urea), yield a substance which gives a color identical with that of nicotinic acid in the Bandier and Hald (7) modification of the König reaction (cyanogen bromide and an amine). We believe that the ammonia closes the ring when methylamine is split out and gives nicotinic acid. On the basis of the color reaction, the conversion is about 70 per cent when one starts with less than 300 γ of trigonelline. Larger amounts of trigonelline decrease the yield progressively. We are attempting to adapt this procedure to a quantitative estimation of trigonelline in connection with the metabolism of nicotinic acid and its derivatives.

Our data thus far indicate, on the basis of the above reactions, that normal human subjects excrete daily only 1 to 3 mg. of nicotinic acid and derivatives (amide and glycine conjugate) compared with 30 to 50 mg. of trigonelline, and that nicotinic acid ingested in small doses (100 mg.) is excreted largely as trigonelline.

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