

THE IDENTIFICATION OF 3-METHOXYANTHRANILIC ACID,  
ADDITIONAL TRYPTOPHAN METABOLITE, IN HUMAN URINE

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The 8-methylether of xanthurenic acid has been found as a normal metabolite of tryptophan in the urine of humans and swine. (Price et al. 1956) In an attempt to determine the pathway of biosynthesis of this quinoline compound, it was proposed that 3-methoxykynurenine is one of its possible precursors. (Price et al. 1960) The present authors have a hypothesis that if the 3-methoxykynurenine occurs in the body, the formation of 3-methoxyanthranilic acid is also possible by the action of kynureninase or a kynureninase-like enzyme and then it might be excreted in urine of humans and pigs. As far as the authors are aware the occurrence of 3-methoxyanthranilic acid in urine has not been previously reported. This communication will serve to describe the isolation and identification of the new tryptophan metabolite from urine. A complete evaluation of chemical data will be published later.

Experimental

The method for the determination of anthranilic acid in human urine that has been commonly employed, was scrutinized and revealed some limitation and liability to get contamination of 3-methoxyanthranilic acid. Results of paperchromatography of synthesized anthranilic acid and related compounds are shown in Table. It indicates that the properties of anthranilic acid are very like that of 3-methoxyanthranilic acid. Besides, the ultraviolet absorption spectrum of anthranilic acid in 0.1 M phosphate buffer at pH 7.4 and 12.0 exhibited a maximum 310 mμ and that of 3-methoxyanthranilic acid was 315 mμ.

Table 1

Results of paperchromatography of synthesized AA and related compounds

	3HAA	5HAA	AA	3MAA
in BuOH, AcOH, H <sub>2</sub> O 4:1:2	0.90	0.61	0.92	0.92
in MeOH, BuOH, Bz, H <sub>2</sub> O 2:1:1:1				
containing 1% AcOH	0.79	0.58	0.87	0.88
1% 15N NH <sub>4</sub> OH	0.21	0.51	0.65	0.70
% KCl	0.62	0.66	0.73	0.67
ultraviolet Fluorescence	YG	B	P	BP
A	UC	RBr		
Man's reagent	UC	SDB	M	SDB

Abbreviations: AA, anthranilic acid; 3HAA, 3-hydroxyanthranilic acid; 5-HAA, 5-hydroxyanthranilic acid; 3MAA, 3-methoxyanthranilic acid; BuOH, n-butanol; AcOH, acetic acid; MeOH, methanol; Bz, benzene; DSA, diazotized sulfanilic acid; Y, yellow; G, green; B, blue; P, purple; UC, unclear; R, red; Br, brown; B, slightly dark blue; M, magenta. The reagents employed were prepared and used according to C. E. Dagliesh, Biochem. J., 52, 3(1952).

Although the fluorescence and ultraviolet spectrum of 3-methoxyanthranilic acid was very similar to that of anthranilic acid, it was clearly separated from the latter by the use of 20% KCl and MeOH/BuOH/Bz/H<sub>2</sub>O, 2:1:1:1 as paperchromatographic solvents and by application of fluorometric assay. Twenty four hour urine collections from normal human subjects, before and after ingestion of 2.0 g. of L-tryptophan, were obtained. An aliquot was hydrolysed by heating it with 6N HCl for 2 hours, cooled down, adjusted to pH 4.0 with NaOH and saturated with NaCl. It was then extracted with ether. The ether extract was concentrated and paperchromatography in the solvent systems described above on Toyoroshi No.5 showed two principal spots corresponding to authentic anthranilic and 3-methoxyanthranilic acids. The spots were cut out, macerated and eluted with 1 N NH<sub>4</sub>OH. The fluorescence characteristics of synthesized anthranilic and 3-methoxyanthranilic acids and the unknown compound are shown in Table 2. The fluorescence spectra of the isolated and authentic 3-methoxyanthranilic acid in 0.1 N NH<sub>4</sub>OH were determined and found to be 3-methoxyanthranilic acid.

Table 2

Fluorescence characteristics of authentic 3MAA, AA and unknown compound

	AA	3MAA	Unknown compound
excitation maximum mμ	320	330	330
fluorescence maximum mμ	395	410	410
medium	0.1 N NH <sub>4</sub> OH	0.1 N NH <sub>4</sub> OH	0.1 N NH <sub>4</sub> OH

### Discussion

It was apparent that the unknown urinary compound was indistinguishable from synthesized 3-methoxyanthranilic acid. The fluorometric spectrum of the new urinary product was almost identical with that of the authentic 3-methoxyanthranilic acid in 0.1 N NH<sub>4</sub>OH. Ingestion of 2.0 gm. of L-tryptophan was always followed by an increase in the intensity of the fluorescence of the spots corresponding to each of anthranilic and 3-methoxyanthranilic acids. When the procedure was applied to normal rat urine, it was possible to detect anthranilic acid but 3-methoxyanthranilic acid could not be detected in rat urine.

### Summary

A compound identified as the 3-methoxyanthranilic acid was found in normal human urine. The excretion of this compound invariably increased after the ingestion of L-tryptophan. A procedure was described for the purification and separation of 3-methoxyanthranilic acid from urine.

### References

- Price, J. M., and Dodge, L. W., J. Biol. Chem., 223, 699, 1956  
Roy, J. K., Price, J. M., and Brown, R. R., J. Biol. Chem., 236, 146, 1960