The determination of 3-methoxy 4-hydroxy mandelic acid in urine

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SYNOPSIS A simple and accurate method for determining 3-methoxy 4-hydroxy mandelic acid in urine is described. The amounts excreted in urine in 24 hours by nine normal subjects and three patients with phaeochromocytoma have been determined.

The determination of 3-methoxy 4-hydroxy mandelic acid (vanillyl-mandelic acid) in urine is of importance in detecting the increased production of this major metabolite of adrenaline and noradrenaline in patients with phaeochromocytoma (Gitlow, Khassis, Cohen, and Mendlowitz, 1959) and also in investigations on hypertension in which there may be disturbance of catecholamine metabolism leading to increased excretion of vanillyl-mandelic acid (Studnitz, 1960). Various methods for estimating it in urine have been described (Armstrong, McMillan and Shaw, 1957; Studnitz and Hanson, 1959; Sandler and Ruthven, 1959; Gitlow et al., 1959; Robinson, Ratcliffe, and Smith, 1959), but none lends itself to rapid routine investigations with large numbers of urine samples. The method to be described is suitable for this purpose and can be carried out without recourse to special techniques such as paper chromatography, column chromatography, or high voltage electrophoresis. It is based on the fact that the azo dyestuff formed when vanillyl-mandelic acid is coupled with diazotized p-nitroaniline can be separated from interfering coloured substances by extraction from potassium carbonate solution into chloroform from which it can be re-extracted with sodium hydroxide to give a red solution suitable for colorimetry. In developing the method satisfactory conditions for selectively and quantitatively extracting vanillyl-mandelic acid from urine have been determined and the reaction between it and diazotized p-nitroaniline has also been studied in order to obtain maximum reproducibility.

PROCEDURE

REAGENTS In addition to ether A.R., chloroform B.P., and sodium chloride A.R. the following are made up:— p-Nitroaniline reagent To 0.5 g. p-nitroaniline add 3 ml. concentrated HCl and make up to 500 ml. with distilled water, any undissolved solid being filtered off. This solution stores indefinitely. To prepare the reagent add 1 volume of 0.05% (w/v) sodium nitrite A.R. to 1 volume of p-nitroaniline solution, mix and leave two min. then add 1 volume of 0.025% (w/v) ammonium sulphamate to destroy traces of nitrous acid. Prepare the nitrite and sulphamate just before use. The reagent can be used for up to two hours after preparation.

Potassium carbonate solution A 10% (w/v) solution of potassium carbonate A.R. in distilled water.

Standard vanillyl-mandelic acid solution Dissolve 10 mg. DL-3 methoxy-4 hydroxy mandelic acid (Californian Corporation for Biochemical Research) in 100 ml. of distilled water. The solution can be stored at —20° for at least six months and can be kept safely for several weeks at 4°. Standards are prepared as required by appropriate dilution with distilled water.

Magnesium oxide (for chromatography) Mix 1 part by weight of light magnesium oxide (May and Baker L 354) with 3 parts of cellulose powder (Whatman standard grade), any small lumps of the oxide being broken up in a pestle and mortar or small mill before mixing with the cellulose.

Methanolic KOH Dissolve 0.1 g. KOH A.R. in 100 ml. methyl alcohol.

Sodium hydroxide N/10

METHOD

To 2 ml. of urine add 3 ml. of distilled water, 0.5 ml. of concentrated HCl and sufficient solid sodium chloride to saturate the solution and give a slight excess. Extract with five successive 40 ml. volumes of ether, shaking well at each stage. Remove the extracted vanillyl-mandelic acid from ether into aqueous solution by shaking the combined ether extracts with 25 ml. of distilled water containing sufficient N/10 NaOH (about 1 ml.) to give a neutral extract followed by 15 and 10 ml. portions of distilled water. Develop colour by adding to the combined aqueous extracts (which should be neutral to

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Table I shows the excretion of vanillyl-mandelic acid by normal subjects and Table II shows pre-operative vanillyl-mandelic acid excretion figures for three proved cases of phaeochromocytoma, the urines being made available by the courtesy of Dr. M. Sandler. The two values given for subjects J and K were on urine samples obtained on different occasions. In the second column are the values supplied by Dr. Sandler for three of the urines using the method of Sandler and Ruthven (1959) as modified by Sandler and Ruthven (1960). Comparison of the two sets of figures shows reasonably good agreement between the two methods despite the very different techniques employed.

We thank Dr. M. Sandler for making available the phaeochromocytoma urine samples and for giving permission to quote his vanillyl-mandelic acid values for these urines. We also thank Dr. A. D. Munro-Faure for valuable discussion and for providing the urine samples from subjects A and B, and Dr. A. J. Everett for determining the infra-red spectra of the vanillyl-mandelic acid-diazo derivatives.

REFERENCES


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