Identification Test for Tolbutamide and Chlorpropamide

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To date no simple and rapid identification test for tolbutamide or chlorpropamide is available, although more elaborate qualitative and quantitative methods of detecting and determining the compounds have been proposed (1–12). The qualitative test presented here is based on a "spot" test discussed by Feigl (13) for the detection of methenamine.

PROCEDURE

To a 100-mg. sample of each of the drugs listed in Table I, 100 mg. of powdered zinc and 100 mg. of granular ammonium chloride were added and intimately mixed in a mortar. The powder mixture of each drug was transferred to an 8-in. Pyrex test tube. Test papers were made by dipping Whatman No. 5 filter paper into a 2% solution of 2,4-dinitrochlorobenzene dissolved in ethyl ether. The paper was dried, passed through the vapors of concentrated ammonium hydroxide, and cut into squares large enough to completely cover the test tube opening.

The identification test for the compounds consisted of placing the square of test paper over the tube opening and putting the tube in a Slaco tube heater (Hallikainen Instruments, Berkeley, Calif.) preset at 150 ± 1° for exactly 10 minutes. The paper was held tightly in place by resting a small Pyrex beaker on top of it during the heating period. A yellow circular stain on the part of the paper over the tube orifice at the end of the heating period indicated a positive result for a particular compound. The lower limits of the method for the detection of tolbutamide and chlorpropamide under these conditions were determined by keeping the weights of zinc powder and ammonium chloride constant and decreasing the quantity of added sulfonylurea until a definite yellow paper color was seen at the end of 35 minutes.

Tolbutamide, chlorpropamide, methenamine, and sulfisoxazole all gave color reactions on the paper within the 10-min. time period. The yellow color of the first three substances on paper was the one expected for the reaction between a volatile amine and 2,4-dinitrochlorobenzene, while the brown color seen with sulfisoxazole was atypical and due to the staining of the paper by colored distillation products.

The method could detect 0.4 mg. of either tolbutamide or chlorpropamide after 35 minutes of heating.

Table I.—Drugs to which Powdered Zinc and Granular Ammonium Chloride Were Added to Make Test

	Official or Common Name[a]	Results
1.	Aprobarbital	−
2.	Barbital	−
3.	5-*n*-Butyl-5-ethylthiobarbituric acid	−
4.	Chlorpropamide	+
5.	5,5-Diethylthiobarbituric acid	−
6.	Methenamine	+
7.	Pentobarbital	−
8.	Phenobarbital	−
9.	Sulfacetamide sodium	−
10.	Sulfaguanidine	−
11.	Sulfamethazine	−
12.	Sulfamethizole	−
13.	Sulfisoxazole	+[b]
14.	Thiopental	−
15.	Tolbutamide	+

[a] 1. 5-Allyl-5-isopropylbarbituric acid; 2. 5,5-diethylbarbituric acid; 4. 1-propyl-3-(*p*-chloro-benzenesulfonyl)-urea; 6. formin or hexamethylenetetramine; 7. 5-ethyl-5-(1-methylbutyl)barbituric acid; 8. 5-ethyl-5-phenylbarbituric acid; 9. N¹-acetylsulfanilamide sodium; 10. N¹-Guanylsulfanilamide; 11. N¹-(4,6-dimethyl-2-pyrimidinyl)sulfanilamide; 12. N¹-(5-methyl-1,3,4-thiadiazol-2-yl)sulfanilamide; 13. N¹-(3,4-dimethyl-5-isoxazolyl)-sulfanilamide; 14. 5-ethyl-5-(1-methylbutyl-)2-thiobarbituric acid; 15. 1-butyl-3-(*p*-tolylsulfonyl)urea. [b] Brown tint to paper due to volatile decomposition products.

DISCUSSION

The pyrolysis reaction here probably involves first the liberation of hydrogen chloride from the heated ammonium chloride-zinc dust mixture, which then causes cleavage of the substituted sulfonylurea. An amine is liberated as a decomposition product. If ammonium chloride is omitted from the procedure, the development of the yellow ring is greatly delayed. The identification test described here is also suitable for qualitative detection of the hypoglycemic agents in commercially available tablets.

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