# A REACTION FOR PHENOBARBITAL.*,[1]

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The tests described in the pharmacopœias of the United States, Great Britain and Germany direct the use of mercuric nitrate or mercuric chloride for the identification of barbital and phenobarbital. Since barbital, phenobarbital, dial (diallylbarbituric acid) and other barbiturates produce a white precipitate with both of these reagents it is the opinion of the authors that a more specific test is desirable in order to differentiate between the official compounds.

Barbituric acid derivatives are usually identified by recrystallization (1) and few chemical tests for their identity are described in the literature. Most of the tests are not specific and the presence of phenobarbital cannot be established with Millon's reagent, Denigès' reagent, phenylhydrazine and sodium nitroprusside, or sulphuric acid and naphthol.

Paget and Desogt (2) used Millon's reagent to differentiate allylbarbituric acids from other barbituric acid derivatives. Cobalt salts were used for the detection of barbiturates by Parri (3), Zwikker (4), Bodendorf (5), and Koppanyi *et al.* (6), but they did not prove them to be entirely specific. The murexide test, described by Handorf (7), is not sufficiently specific.

Ekkert (8) used formaldehyde and sulphuric acid to differentiate phenobarbital from barbital and proponal. He also (9) used selenious acid to distinguish phenobarbital from barbital. Cinchophen and neocinchophen show a similar reaction with selenious acid.

Lyons and Dox (10) employed *p*-nitro benzoyl chloride in the identification of alkylbarbituric acids. van Itallie and Steenhauser (11) reported the behavior of barbituric acid derivatives toward solutions of ammonium phosphate, bromine water, Schweitzer's reagent, silver nitrate and barium hydroxide.

Denigès (12) recrystallized the barbiturate and identified it by means of its crystal formation under a microscope. Zamporo (13) showed that phenobarbital produces a color with sodium nitrite in presence of sulphuric acid while barbital does not. Ramwez (14) nitrated the phenyl group and in this way was able to differentiate between barbital and phenobarbital.

David (15) was able to differentiate between barbital and phenobarbital in admixture by dissolving the sample in ammonium hydroxide and treating with hydrogen peroxide. He was able to show the presence of phenobarbital by the formation of a wine color. He has also described a reaction for barbital with alcohol, nitrous acid and sulphuric acid, afterward neutralizing with sodium hydroxide. Under his conditions barbital produces an orange-yellow color and phenobarbital a lemon-yellow. Lagrace (16) used vanillin in sulphuric acid to identify dial. This reaction is also obtained with allyl alcohol, terpenes, menthol, camphor and other similar compounds.

Mohler's (17) test for benzoic acid seemed to the authors to offer the possibility of differentiating between phenylated and non-phenylated barbiturates by

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nitration of the phenyl group. Benzoic acid is converted to *m*-dinitrobenzoic acid by heating with sulphuric acid and potassium nitrate, made alkaline with ammonia, and reduced with colorless ammonium sulphide to the *m*-diamino compound, producing a red-brown color which changes to greenish yellow.

We have found that the following procedure will yield a definite reaction with phenobarbital that is not duplicated by any of the non-phenylated derivatives of barbituric acid.

In a large, hard glass test-tube mix 0.2 Gm. of the sample with 0.5 Gm. of potassium nitrate, and add 2 cc. of sulphuric acid. Heat the entire mixture in boiling water for twenty minutes, cool, and carefully add 3 cc. of distilled water. Make the solution strongly ammoniacal and boil gently to destroy any ammonium nitrite formed. To the cooled mixture, add without mixing 2 or 3 drops of colorless ammonium sulphide U. S. P. T. S.

Phenobarbital will show the formation of a reddish brown ring which diffuses and forms an orange color throughout the mixture. On heating, the color becomes a little more intense and finally becomes greenish yellow. Barbital does not give this reaction. Benzoic acid produces a reddish brown ring on the addition of the ammonium sulphide, but on heating becomes yellow-green

## SUMMARY.

Mohler's reaction may be used to distinguish phenobarbital from barbital. The progress of the reaction differs slightly from that with benzoic acid. In the latter case the reddish brown ring diffuses to form a solution of the same color, and on heating changes to a yellowish green solution. With phenobarbital, on the other hand, the reddish-brown ring diffuses to form an orange-colored mixture which is turbid. Upon heating the color becomes more intense, shading into orange-red, and eventually becomes greenish yellow, the precipitate persisting.

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