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Note

2-Thiobarbituric acid as a reagent for the detection of meconic acid by thin-layer chromatography

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Poisoning by morphine is not uncommon in India and, in forensic toxicological work, it often becomes necessary to establish the source of morphine in such cases. A number of morphine formulations are in use in medicine and opium may also be acquired.

Certain 9-N-phenanthrene alkaloids are closely related to the morphine group in structure, e.g., sinomenine¹ from *Sinomenium diversifolius* and *Sinomenine actum*, the Japanese plant belonging to the menispermaceae. Another interesting variant with a structure similar to morphine is hasubanonine from *Stephania japonica*². On the other hand, thebaine, an alkaloid of the morphine group, has also been found in *Rauwolfia serpentina* Benth and in oriental papaver³. Opium is described in the Indian Opium Act, 1878, as capsules or coagulated juice of such capsules of *Papaver somniferum*. A study of different species of papaver in India showed that only *Papaver somniferum* contains all five of the alkaloids morphine, codeine, thebaine, papaverine and narcotine⁴. Most of the alkaloids from opium are present in the form of meconates and lactates, and meconic acid is strictly peculiar to opium. Consequently, in a case of poisoning, the detection of meconic acid together with morphine and the above opium alkaloids in the test samples is necessary in order to establish the source of morphine as opium.

Meconic acid is usually detected by its colour reaction with iron(III) chloride. However, in toxicological work, tissue extracts, particularly from a decomposed body, may show trace amounts of phenolic groups by the iron(III) chloride test. There are certain morphine preparations, such as elixir of dimorphine and terpene, camphorated linctus of dimorphine and camphorated linctus of opium, that contain pine and/or borneol (terpene)⁵, which also react with iron(III) chloride. In a search for an alternative reagent, we found that 2-thiobarbituric acid (2-TBA) in dimethyl sulphoxide gives a cherry red colour with meconic acid.

EXPERIMENTAL AND RESULTS

Preparation of plates

Thin-layer chromatographic (TLC) plates were prepared with silica gel G (E. Merck, Darmstadt, G.F.R.) as adsorbent and were activated by heating for half an hour at 110°. The thickness of the layer was 250 μm .

Solvent system

Methanol–benzene–acetone–perchloric acid (7:2:1:0.2) was found to be the best solvent system.

Spray reagent

2-TBA (7.2 g) was dissolved in about 75 ml of dimethyl sulphoxide and the volume made up to 100 ml with the same solvent, giving a concentration of 0.5 *M*.

Procedure

Meconic acid extracted from an aqueous acidic solution of opium with chloroform–ethanol (4:6) and an authentic sample of pure meconic acid were spotted on a TLC plate and a chromatogram was developed by the ascending technique using the above solvent system. After a 10-cm run, the plate was taken out of the chromatographic chamber and allowed to dry, then sprayed with 0.5 *M* 2-TBA. The plate was then heated in a vacuum oven at 140° for about 30 min, and cherry red spots (R_F 0.64) were observed on very faint yellow background.

Several workers have used 2-TBA as a reagent for TLC with some prior treatment. Copius-Peereboom and Beekes⁶ and Pinella *et al.*⁷ used this reagent for the detection of sorbic acid after spraying with potassium dichromate. Nisbet⁸ described a TLC method for the detection of polyhydric alcohols after treatment with potassium dichromate. Feigl and Libergott⁹ converted sugars into aldehydes before treatment with 2-TBA. Pyrimidine derivatives, after hydrolysis, form coloured compounds with 2-TBA¹⁰. Feigl *et al.*¹¹ also reported a spot test for sulphadiazine using 2-TBA. A quantitative method has been described for the determination of chloral hydrate¹².

In our work, 2-TBA in dimethyl sulphoxide is directly sprayed on to the thin-layer plate. Under the above conditions, phenol and phenolic compounds such as salicylic acid derivatives and 2,4-dichlorophenol did not show any coloured spots. Lactic acid gave a yellow spot slightly more intense than the background yellow colour (R_F 0.45). The sensitivity of 2-TBA in dimethyl sulphoxide for meconic acid was found to be *ca.* 10 μ g, compared with *ca.* 20 μ g for iron(III) chloride.

When the test was carried out in a test-tube, adding the 2-TBA reagent to an authentic sample of meconic acid, a clear cherry red solution with an absorption maximum at 529 nm was obtained. This reaction can be used for the quantitative determination of meconic acid.

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