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The detection of the methylenedioxy group on thin-layer chromatographic plates

A number of natural products contain a methylenedioxy group¹⁻⁴ and thus a rapid method for the specific detection of this moiety on thin-layer chromatographic (TLC) plates would be of value both in structural studies and in the identification of these materials. This group has been detected by colour reactions resulting from the treatment of dilute alcoholic solutions with gallic and sulphuric acids⁵⁻⁷, tannic and sulphuric acids⁶ and phloroglucinol and sulphuric acid⁸. The colours, which are apparently due to the reaction of the polyhydroxyphenol with the formaldehyde released upon hydrolysis of the methylenedioxy group⁷, vary in shade and intensity and can often lead to ambiguous results.

In contrast to the above reagents, chromotropic acid (1,8-dihydroxynaphthalene-3,6-disulphonic acid) in the presence of sulphuric acid is specific for formaldehyde⁹, with which it gives a characteristic purple colour. This reagent has been adapted for the quantitative assay of formaldehyde¹⁰⁻¹² and for the analysis of a variety of methylenedioxy derivatives¹³⁻¹⁷. The chromatography of methylenedioxyphenyl compounds has been the subject of a recent review¹⁸. This paper describes two modifications of the reagent for its use with TLC for the rapid detection and tentative identification of compounds containing the methylenedioxy group.

Experimental

Test solutions contained 1 mg per 1 ml and were spotted with lambda micropipettes. Chromotropic acid (supplied as the disodium salt) and sulphuric acid were reagent grade. Chromatoplates were prepared at a thickness of 250 μ using MN Kieselgel G-HR. Self-indicating plates were prepared by adding silica gel (25 g) to a solution of sodium chromotropate (1 g) in water (49.5 ml) containing conc. sulphuric acid (0.5 ml). This quantity was sufficient for five 20 \times 20 cm plates. All plates were activated for 1 h at 90° after which they were stored in the dark over a silica gel desiccant. The chromotropic acid spray was prepared weekly by slowly adding conc. sulphuric acid (15 ml) to a solution of the sodium salt (1 g) in water (15 ml). Colour development for both sprayed and self-indicating plates was achieved by heating for 15-30 min at 110-120°. The chromatographic solvents used were benzene-petroleum ether (1:1), benzene-1 % methanol and benzene-2 % acetone.

Results and discussion

The detection of the methylenedioxy group with chromotropic acid is based upon the release of formaldehyde and thus a mineral acid must be present in order to effect hydrolysis. Use of the spray reagent (cf. refs. 16 and 17) was found to produce the expected purple colour after heating. Clearly defined spots on a nearly colourless background were obtained when dilute solutions (5-10 γ of applied material) were used; concentrated applications led to colour diffusion together with a purple background. Minimum detectable amounts were in the order of 0.5 γ for safrole, isosafrole, dihydrosafrole, piperonal, piperonol, piperonylic acid, piperonyl acetate, piperonyl isobutyrate and piperine. All compounds were detected as distinct purple spots at this level.

In order to apply direct densitometry to the analysis of several methylenedioxy derivatives of importance in the food industry, the use of self-indicating impregnated plates was investigated. These plates, which contain an even distribution of the chromogenic reagent necessary for densitometric measurements, required only heating for colour development. Although the piperonyl esters were left intact during chromatography in benzene-petroleum ether (1:1), the use of self-indicating plates is necessarily limited to acid-stable compounds. Minimum detection limits (1-2 γ) and general plate appearance were similar to those obtained using the spray mode of detection. However, the latter method is more generally applicable and is to be preferred for routine analysis.

Several other types of compounds are also capable of releasing formaldehyde upon hydrolysis. Hexamethylenetetramine and *sym*-trithiane contain =N-CH₂-N= and -S-CH₂-S- groups, respectively, and thus yield the expected purple spots with the above detection systems. These materials can be distinguished from methylenedioxy derivatives by elemental analysis.

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