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Note

A general spray reagent for the detection of terpene derivatives on thin-layer plates

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Thin-layer chromatographic (TLC) procedures involving the use of silica gel G plates have been developed for the specific detection of terpene derivatives at the 2-μg level with benzene–ethyl acetate (9:1) as the developing solvent.

A number of spray reagents for the detection of terpene derivatives on silica gel G TLC plates are known[1]. Anisaldehyde–sulphuric acid detects terpenes in addition to sugars and steroids. Very sensitive but non-specific detection is possible by using molybdophosphoric acid reagent. After spraying and heating, amounts as low as 0.05–1 μg can be detected as blue spots on silica gel layers. Antimony(III) and antimony(V) chloride reagents are less sensitive and give grey to violet colours. When these reagents are used, the chromatogram should be examined under natural and longwave UV light both before and after the heat treatment. Limited use has been made of antimony(III) chloride as a spray reagent for the identification of isoprenoids[2]. Other spray reagents, such as tin(IV) chloride, chlorosulphonic acid–glacial acetic acid and acetic anhydride–sulphuric acid, have been used for detecting triterpenes. Benzidine detects only terpene aldehydes. Arsenic(III) chloride–acetic acid has been used for the detection of monoterpenoids and sesquiterpenoids[3].

It was therefore considered necessary to find a sensitive spray reagent that was suitable for the detection of various types of terpene derivatives, and a solution of tin(II) chloride (5 g) and monochloroacetic acid (5 g) in chloroform (90 ml) was found to be appropriate. This reagent is capable of detecting different types of terpene derivatives as listed in Table I. The colours reported are produced at the 2-μg level, although the reagent appears to be sensitive to amounts down to 0.5 μg for many terpene derivatives. Grey to light violet spots were observed against a light blue background after heating the developed plates for 3 min at 100°.

The reagent gives the observed colour reaction with terpenoid derivatives due to the complex-forming ability of tin(II), which can be further enhanced as a result of activation by the conveniently located neighbouring group. Interestingly, an activation effect was observed with khusinol, in which a methylenic bond is present in the position nearest to the secondary hydroxyl group, and the colour reaction occurs immediately. Very surprisingly, khusinol benzoate and tosylate are even more sensitive than khusinol to this colour reaction, as tosylate and benzoate act as more powerful neighbouring groups than the hydroxyl group. Menthol did not produce any colour with this spray reagent under similar conditions, possibly due to conformational and

TABLE I

DETECTION OF TERPENE DERIVATIVES (2-μg LEVEL) BY SPRAYING WITH A SOLUTION OF TIN(II) CHLORIDE AND CHLOROACETIC ACID IN CHLOROFORM

Compound	*Colour observed*	*Fluorescence in UV light*
Khusinodiol	Light brown	Yellowish
Carotol	Orange	Yellowish
Daucol tosylate	Violet	Light yellowish
Menthol	—	Light blue
Khusol	Brown	Pinkish
Khusinol	Grey	Yellowish
Khusinol benzoate	Dark brown	Bluish
Khusinol tosylate	Pink	Yellowish
Khusilol	Light blue	Brownish
Khusilal semicarbazone	Light violet	Light yellowis..
17α-Hydroxyprogesterone	—	—
Cholesterol	Violet	Brownish
Estradiol	Light orange	Brownish
Quercetin	Yellow	Light yellowish
Rutin	Yellow	Light yellowish

electronic effects. Kohli *et al.*[4] have already confirmed the neighbouring group participation by secondary hydroxyl groups using antimony(III) chloride as a Lewis acid. However, antimony has a lower tendency than tin(II) to undergo complex formation. A deactivation effect was noticed in the absence of neighbouring group participation. Thus, khusol, khusilol and khusilal semicarbazone did not produce colours immediately. Owing to the lack of authentic samples, it has not been possible to test for reactions with diterpene or triterpene derivatives, but as they contain activated groups they are likely to give characteristic colour reactions.

It may be mentioned that the reagent is also very sensitive towards steroids and flavonoids. It gives violet and light orange colours with cholesterol and estradiol, no colour is produced with 17α-hydroxyprogesterone. Quercetin and rutin produce a yellow colour with this reagent.

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