

CHROM. 5648

Specific spray reagent for antioxidants

The non-specific spray reagent molybdophosphoric acid¹ has been generally used for the detection of antioxidants on paper or thin-layer chromatograms. The reagent now described is specific and 100 times more sensitive.

Experimental

Reagents. Solution A consisted of a 2 % solution of crude β -ocimene* in hexane purified immediately before use by passing it through a column packed with magnesium silicate (Florisol from Floridin Co., W. Va., U.S.A.), 10 mm \times 100 mm for 100 ml of solution. Solution B consisted of a 2 % solution of 2,4-dinitrophenylhydrazine in 30 % perchloric acid.

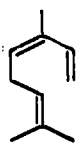
Procedure. The antioxidants are separated by sheet chromatography on paper or thin layers with a suitable solvent. The chromatographic sheet is air-dried, sprayed with solution A (1 l/m²), and then either allowed to stand at room temperature for about 1 h, or heated at 50° for 10–15 min, both treatments being equally effective. The antioxidants are revealed as white spots on a yellow-brown background by spraying the sheet with solution B. On inert supports the spots remain visible for days. A permanent record is obtained by photographing the sheet through a dark blue filter (Kodak, Wratten filter No. 47) on Polaroid black and white film.

Results

The reagent detects antioxidants in general, the sensitivity depending on the efficiency of the antioxidant in preventing the autoxidation of ocimene. 1 ng of butylated hydroxyanisole (BHA) can be detected on thin-layer plates. Under the same conditions 100 ng of BHA was the limit of detection with the molybdophosphoric acid spray. The detection of some antioxidants is shown in Fig. 1. The reagent may also be used as a spot test for antioxidants.

Discussion

α -Farnesene, from stored apples, was originally used in solution A instead of β -ocimene since the method was first employed in the study of compounds capable of inhibiting farnesene autoxidation. Both compounds contain the C=C-CH₂-C=C-C=C system and autoxidise with extreme ease, one of the hydrogen atoms being readily removed from the methylene group². Ocimene has the advantage of being commercially available since it is used in perfumery.



(Z)-3,7-dimethyl-1,3,6-octatriene (*cis*- β -ocimene)

As soon as the chromatogram is sprayed with solution A, the ocimene begins to autoxidize except where antioxidants are present. If the ocimene solution contains

* "Ocimene 80" (Bush Boake Allen Australia Ltd.) was found by gas chromatography-mass spectrometry to be a mixture containing *cis*- α -, *trans*- α -, *cis*- β -, and *trans*- β -ocimenes in the ratio of 4:4:40:1. That the predominant isomer was *cis*- β -ocimene was confirmed by NMR spectroscopy. Presumably the active components are the β -ocimenes.

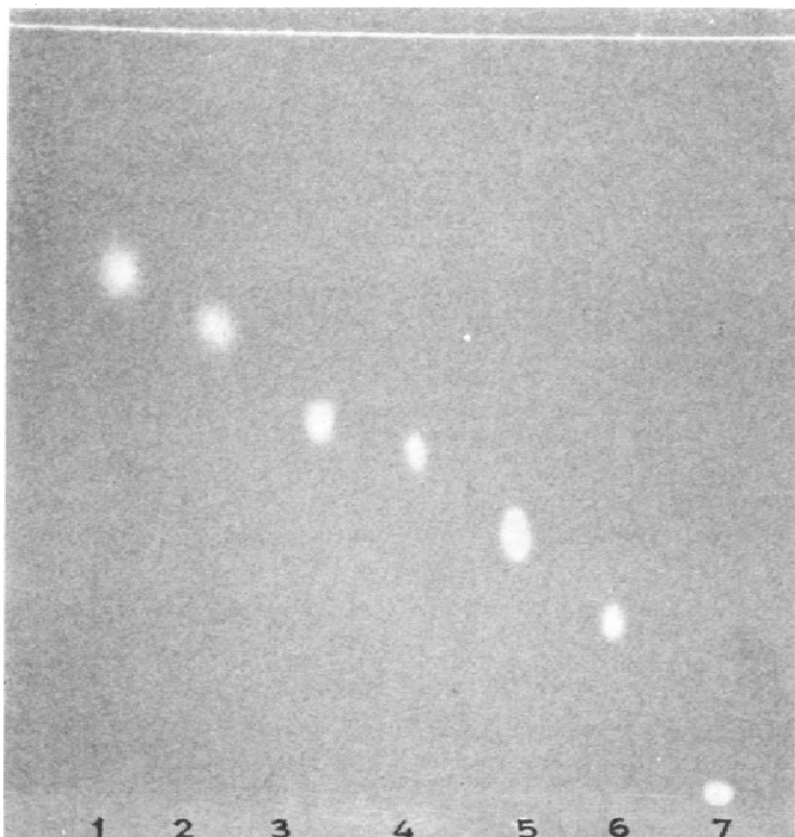


Fig. 1. Thin-layer chromatogram on Eastman silica gel sheet 6060 developed in benzene and sprayed with solutions A and B. 1 = Butylated hydroxytoluene; 2 = Phenyl- β -naphthylamine; 3 = N,N-diphenyl-1,2-phenylenediamine; 4 = α -tocopherol; 5 = butylated hydroxyanisole; 6 = ethoxyquin; 7 = lauryl gallate.

antioxidants, the autoxidation is delayed, and if it contains peroxides the applied antioxidant spots may not be able to stop the autoxidation; in both instances the sensitivity of the reagent is reduced. Both types of impurity are removed with Florisil.

The initial products of the autoxidation of ocimene, hydroperoxides and peroxides, decompose to yield carbonyl compounds. Two reagents were found useful for detecting oxidation products of ocimene. Ferrous thiocyanate revealed hydroperoxides and 2,4-dinitrophenylhydrazine carbonyl compounds. The ferrous thiocyanate solution, however, is not stable in air and is marginally less sensitive. The dinitrophenylhydrazine in perchloric acid, solution B, gives much greater contrast than the conventional solution¹ in hydrochloric acid.

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¹ E. STAHL (Editor), *Thin-Layer Chromatography*, 2nd Ed., Springer, New York, 1969.

² E. F. L. J. ANET, *Aust. J. Chem.*, 22 (1969) 2403.

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