

Note

Detection of citrazinic acid by paper and thin-layer chromatography

A. CEE* and B. HORÁKOVÁ

Research Institute for Organic Synthesis, Pardubice-Rybitví (Czechoslovakia)

(Received April 25th, 1985)

The spots of citrazinic acid (2,6-dihydroxypyridine-4-carboxylic acid) obtained in paper or thin-layer chromatography can be detected by the use of several colour reactions, but these are not specific or are of low sensitivity. The ammonium salt of citrazinic acid¹ gives blue or blue-violet and citrazinamide light-blue fluorescence under UV light. Impurities present in citrazinic acid of technical purity also give blue or blue-violet fluorescence and in the colour reaction based on coupling with diazotized sulphanic acid² the impurities give yellow spots similar to that given by citrazinic acid. Citrazinic acid undergoes oxidation³ and the reaction of ammonium salts can be used, after distribution on a silica gel layer under atmospheric oxygen, for the detection of blue dyestuffs of the azoxonolic type. The colouring formation can be accelerated by illumination with a UV lamp and the oxidation reaction is carried out to the formation of brown products, this being the final phase of the reaction⁴. Citrazinic acid on chromatograms can also be detected by other reagents, for example AgNO₃ solution-1% ammonia solution (1:9) gives after heating the chromatogram, a dark brown spot, which after spraying with 1% potassium hexacyanoferrate(III)-15% FeCl₃ solution, appears as a blue spot.

In our laboratory, we have detected citrazinic acid by reaction with *p*-dimethylaminobenzaldehyde⁵, which gives red-violet spots of the free acid and the amide. The impurities present in the technical product do not react. The colour of the spots is stable and the detection limit is 0.2 µg.

EXPERIMENTAL

Silufol plates (Kavalier, Votice, Czechoslovakia) (15 × 15 cm), chromatographic chambers (18 × 8 × 17 cm) and Whatman No. 1 chromatographic paper (27 × 52 cm) were used.

Solutions of citrazinic acid and citrazinamide were prepared by dissolving 50 mg of the sample in 2 ml of ethanol-pyrimidine (9:1).

Preparation of the detection agent

p-Dimethylaminobenzaldehyde (1 g) was dissolved in 100 ml of ethanol-concentrated hydrochloric acid (95:5).

p-Dimethylaminobenzaldehyde (1 g) dissolved in 20 ml of acetic anhydride was mixed with 100 ml of acetone. The uniformly sprayed chromatogram was heated in a dryer for 1-2 min at 125°C.

Thin-layer chromatography

Volumes of 1–2 μl of sample solution were applied on the Silufol plates, 2 cm from the periphery. After the solvent had been evaporated, the plates were eluted with 1-propanol–ammonia solution (2:1).

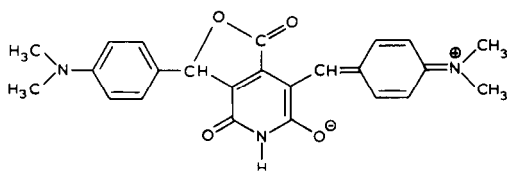
Paper chromatography

Volumes of 5–7 μl of sample solution were applied on the chromatographic paper, 7 cm from the periphery. Elution was effected with 1-propanol–ammonia solution (2:1).

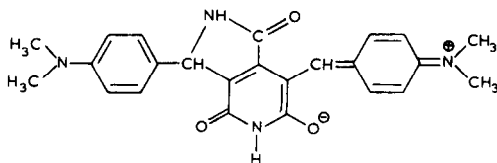
RESULTS AND DISCUSSION

Under the above conditions, the spot of citrazinic acid was separated from the spots of citrazinamide and other compounds. Citrazinic acid gave R_F values of 0.16 and 0.21 and citrazinamide gave R_F values of 0.32 and 0.46 in paper and thin-layer chromatography, respectively. Hence citrazinic acid can be reliably identified.

The red product of the reaction of citrazinic acid with *p*-dimethylaminobenzaldehyde was a methine dyestuff with the following formula, established by mass spectrometry ($M^+ = 417$ a.m.u.):



Citrazinamide gave a red-violet product on reaction with *p*-dimethylaminobenzaldehyde, with the following formula.



ACKNOWLEDGEMENTS

Mass spectrometric measurements on a JSM 01 SG-2 instrument (JEOL) and interpretation of the mass spectra were carried out by Ing. I. Kolb and Ing. J. Čermák of the VÚOS analytical-physical laboratory.

REFERENCES

- 1 F. Feigl, V. Anger and O. Frehden, *Mikrochemie*, 17 (1935) 29.
- 2 A. Cee, J. Dvořák, V. Chmátal, P. Kuthan and J. Vencl, *Czech. Pat.*, 216 606, 1976.
- 3 F. L. Thomas, *U.S. Pat.*, 2 971 957, 1961.
- 4 A. Cee and B. Horáková, unpublished results.
- 5 R. H. Wiley and J. Kraus, *J. Org. Chem.*, 21 (1956) 757.