

Note

Improved amine spray reagent for the detection of sugars

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In the course of our investigations on naturally occurring flavonoid glycosides¹, the sugars obtained after acid hydrolysis were mainly analysed by gas-liquid chromatography and/or paper chromatography, using *p*-anisidine phosphate² as the spray reagent. Often only very small amounts of the isolated compounds are available, in which case the flavonoid aglycone, possible degradation products³ and sugars resulting from the acid hydrolysis are analysed by paper chromatography on the same sheet. Flavonoids are detected by, *e.g.*, measuring their UV fluorescence, which can be intensified by spraying with diphenylboric acid-ethanolamine ester (flavone reagent)⁴, and the sugars can subsequently be detected by overspraying with *p*-anisidine phosphate followed by heating at 100–120°.

During this procedure, a change in colour of the pentose sugars arabinose and xylose was observed, going from brown-yellow when using *p*-anisidine phosphate to purple-red when both spray reagents were used. This effect has proved a useful aid in the separation of pentoses and hexoses, because at the low concentrations used the differences between brown and yellow commonly observed² tend to disappear. In further studies a second effect of the use of the diphenylboric acid complex became apparent. Not only did the colour obtained become much brighter, but also the sensitivity of the sugar detection increased about 10-fold. The latter effect is only partly due to the increased colour brightness; with glucose, for which no colour change occurs, an increase in sensitivity was also obtained. Delayed background coloration may be partly responsible for this effect.

The use of diphenylboric acid ethanolamine ester as a 1% solution in ethanol as a pre-spray for *p*-anisidine phosphate turns the colour of aldopentoses from brown to purple red, without changing the brown-yellow of the aldohexoses or the specific lemon yellow colour of fructose. In addition a 5–10-fold increase in sensitivity is obtained.

REFERENCES

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