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A sensitive method for detecting cyanoglycosides on paper and cellulose thin layers

The lack of a suitable detection method for microgram quantities of cyanoglycosides after thin-layer or paper chromatography has been a hindrance to the location of cyanoglycosides.

In the present communication, a procedure is described whereby trace amounts of cyanoglycosides can be accurately located by a modification of the colorimetric method for cyanide of GUILBAULT AND KRAMER[1], after enzymic hydrolysis of the cyanoglycosides.

Experimental

Emulsin. Hopkins and Williams, grade unspecified, 0.1 % solution in 0.02 *M* phosphate buffer, pH 7.4.

Linamarase. Prepared according to the method of COOP[2]. The 20-fold purified enzyme was diluted three times with 0.02 *M* phosphate buffer, pH 7.0.

Solution A. *p*-Nitrobenzaldehyde and *o*-dinitrobenzene solution, 0.05 *M*, in 2-methoxyethanol. This solution remained stable for at least one week in a deep-freeze.

Solution B. Isonitrosobenzoylactone, 0.02 *M*, prepared by the method of WOLF[3], dissolved in solution A. This solution was unstable and was prepared freshly each day.

Method

The TLC plate or paper was given a very light spraying with the appropriate enzyme, taking care to achieve an even and speedy coverage. The plate or paper was then covered with a thin sheet of polythene followed by a 1/4 in. sheet of foam plastic and sandwiched between rigid supporting plates.

The glycosides were allowed to hydrolyse for 30 min. The optimum combination of temperature and time of incubation was found to vary with the different enzyme preparations. In the case of amygdalin-type glycosides, hydrolysis at room temperature was found to give best results, while lotaustralin and linamarin required an incubation temperature of 30° for maximum sensitivity.

Longer incubation times than this were tried (45, 60 and 75 min) but the gains in sensitivity were outweighed by the concomitant disadvantage of diffusion of the spot. For mixtures of glycosides of close R_F value, *e.g.* lotaustralin and linamarin, the diffusion was sufficient to mask the separation.

After incubation the sandwich was unclamped and immediately given a light spraying (*ca.* 1 ml/100 cm^2) with a 10 per cent solution of sodium carbonate. This fixed the HCN and raised the pH of the medium to 8.5, the optimum pH for formation of the purple dye. A final spraying was then given with solution A. Areas corresponding with the glycosides appeared almost immediately as mauve spots reaching maximum intensity after 15 min. Thereafter fading took place, which was accelerated by strong light.

The addition of isonitrosobenzoylacetone to the reagent increased the sensitivity of detection two-fold, but the procedure of spraying was altered. Immediately upon

unclamping, the plate was sprayed with solution B, followed by an exceedingly light spraying (*ca.* 1 ml/300 cm^2) of 10% sodium carbonate solution. Glycoside areas appeared immediately almost at their maximum intensity. Spots appearing by this altered procedure were more compact in size but a high background colouration which soon developed masked areas of low concentration.

Both procedures have been used to detect cyanoglycosides separated on paper chromatograms and cellulose thin layers where as little as 0.005 μmoles was detected. The cyanoglycosides could not be detected on silica gel thin layers and only weakly on plates prepared from a mixture of silica gel and cellulose. No interfering substances were observed in several alcoholic extracts of plant tissue containing cyanoglycosides.

The glycosides dhurrin and taxiphyllin gave purple spots which on standing developed brown centres. This is attributed to a separate reaction of *p*-hydroxybenzaldehyde or its derivatives which are produced in the enzymic hydrolysis and may be useful in giving a partial identification of unknown glycosides.

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A method for the differentiation of histone fractions F2(a)1 and F2(a)2 by starch gel electrophoresis

The histones of calf thymus have been separated into four main fractions designated F1, F2(a), F2(b), and F3.[1] These fractions have all been characterized by total and N-terminal amino acid analyses and by starch gel electrophoresis at pH 2.3[2], which effectively separates the four groups. More recently fraction F2(a), the largest of the four groups has been subdivided into F2(a)1 and F2(a)2 by a variety of methods[3–5]. Starch gel electrophoresis at pH 2.3 does not however differentiate between these subfractions, and although it may be possible to separate them at higher pH values this is not desirable since it has been shown that histones, particularly F3, aggregate with increase in pH[6–8]. Also the proteolytic activity associated with the histones rapidly increases as the pH is raised[9].

A simple modification has therefore been made to the method described previously[2], which enables F2(a)1 and F2(a)2 to be separated in starch gel at about pH 2 and which also enables F2(a)1 to be specifically detected in a mixture of histones.