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Note

Thin-layer chromatographic detection of glycolaldehyde using a fluorescence reaction with *o*-aminodiphenyl

II. An improved procedure

TADASHI NAKAI, TAKAFUMI OHTA, NORIKO WANAKA and DAIJI BEPPU

Faculty of Pharmaceutical Sciences, Science University of Tokyo, Ichigayafunagawara-machi, Shinjuku-ku, Tokyo (Japan)

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Glycolaldehyde exists in the solid state as a symmetrical dimer (2,5-dihydroxy-1,4-dioxan) and in solution as an equilibrium mixture of the monomer, the above dimer and an unsymmetrical dimer (4-hydroxy-2-hydroxymethyl-1,3-dioxolane)[1,2]. Probably as a result of these facts, glycolaldehyde is usually detected as double to triple spots on its thin-layer chromatograms which have been rendered visible with some of the colour reagents for carbohydrates[3], for example, as shown in Fig. 1. Such behaviour makes it difficult to detect and identify glycolaldehyde by means of thin-layer chromatography (TLC), especially when the aldehyde is contained in mixtures with many other sugars, and also lowers its response to the reactions on chromatograms.

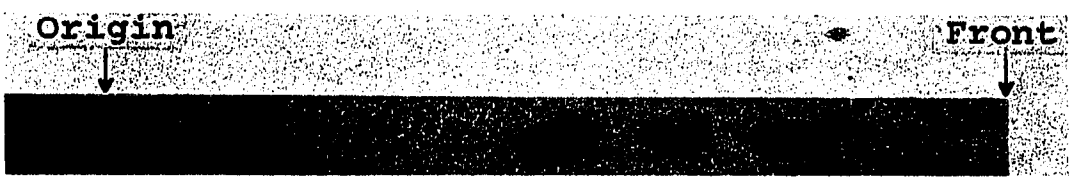

Fig. 1. Example of the formation of multiple spots of glycolaldehyde. Sample solution: 0.5% aqueous solution of the aldehyde. Adsorbent: Kieselgel G nach Stahl (E. Merck). Solvent system: *n*-butanol–ethyl acetate–isopropanol–acetic acid–water (15:40:30:5:10). Detection reagent: naphtho-resorcinol–sulphuric acid solution.

An interesting fact was observed that, when a solution of glycolaldehyde in glacial acetic acid was submitted to silica gel TLC, the aldehyde appeared as a single spot on the finished chromatogram. This paper describes an improved procedure for the detection of glycolaldehyde in complex mixtures of sugars, which is based on this newly found fact.

MATERIALS AND METHODS

The silica gel chromatoplates (0.25-mm layer) were prepared in the usual manner, using Kieselgel G nach Stahl (E. Merck, Darmstadt, G.F.R.), and activated at 110° for 1 h.

The sample to be tested was dissolved in a 10% aqueous solution of acetic acid. A 1-μl portion of the sample solution was applied to the chromatoplate with a microsyringe (application of heat must be avoided). The plate was allowed to stand at room temperature for about 20 min and development was then carried out by the ascending technique with a solvent system consisting of chloroform–ethanol–water (5:1:1; lower layer). Pre-equilibration of the plate in the developing tank was continued for 30 min. At the end of a 10-cm solvent run, the plate was air-dried at room temperature for a short time and sprayed with a slight excess of one of the following two reagents: (1) a freshly prepared 1:1 (v/v) mixture of 1%* *o*-aminodiphenyl in ethanol and 20% sulphuric acid; (2) a freshly prepared solution of 20 mg of naphthoresorcinol, 10 ml of ethanol and 0.4 ml of concentrated sulphuric acid[4]. The former is a fluorigenic reagent, which was used mainly for detecting glycolaldehyde only, and the latter was used for the colour development of the spots of all of the sugars chromatographed, including glycolaldehyde. The chromatoplate sprayed with the fluorigenic reagent was heated at 105–110° for 15 min and submitted to UV irradiation in the dark as described in the previous paper[3]. The chromatoplate sprayed with the naphthoresorcinol reagent was heated at 105–110° for 3–5 min.

RESULTS AND DISCUSSION

With the fluorigenic reagent in the present method, glycolaldehyde was detected as an spot with an R_F value of *ca.* 0.25 showing an intense greenish or bluish white fluorescence against a dark violet background under UV irradiation, and with the naphthoresorcinol reagent as a cobalt blue-coloured spot with the same R_F value on an orange-coloured or light brown background in daylight.

The effect of the presence of free acetic acid in sample solutions of glycolaldehyde on its TLC pattern was investigated. The aldehyde was dissolved in 0.5–10% aqueous solutions of acetic acid and the solutions obtained were chromatographed as described above. For comparison, a water solution of the aldehyde was also chromatographed on the same plate. The results obtained are shown in Fig. 2. These results show that the presence of acetic acid in sample solutions greatly hindered the formation of the double spots of the aldehyde. The sample solutions that were prepared with diluted acetic acid of not less than 5% concentration gave only one spot, and when treated with the fluorigenic reagent, this single spot exhibited an intense fluorescence. Of the two spots that appeared on the chromatogram of the aqueous solution of glycolaldehyde, the spot with the higher R_F value (0.25) showed a positive reaction with the fluorigenic reagent, whereas the spot with the lower R_F value (0.04) showed no positive reaction. The previous paper[3] reported that the monomer of glycolaldehyde showed the fluorescence reaction with *o*-aminodiphenyl but that the dimer gave no positive reaction. It therefore seems likely that the compound with $R_F = 0.25$ was monomeric glycolaldehyde and the compound with $R_F = 0.04$ was dimeric.

Of the three spots that appeared on the chromatogram shown in Fig. 1, only the spot with the highest R_F value gave a positive reaction with the fluorigenic reagent, which indicates that the spot was probably that of the monomeric aldehyde. By means

* In the previous paper in this series[3], the concentration of *o*-aminodiphenyl was incorrectly given as 0.1% instead of 1%.

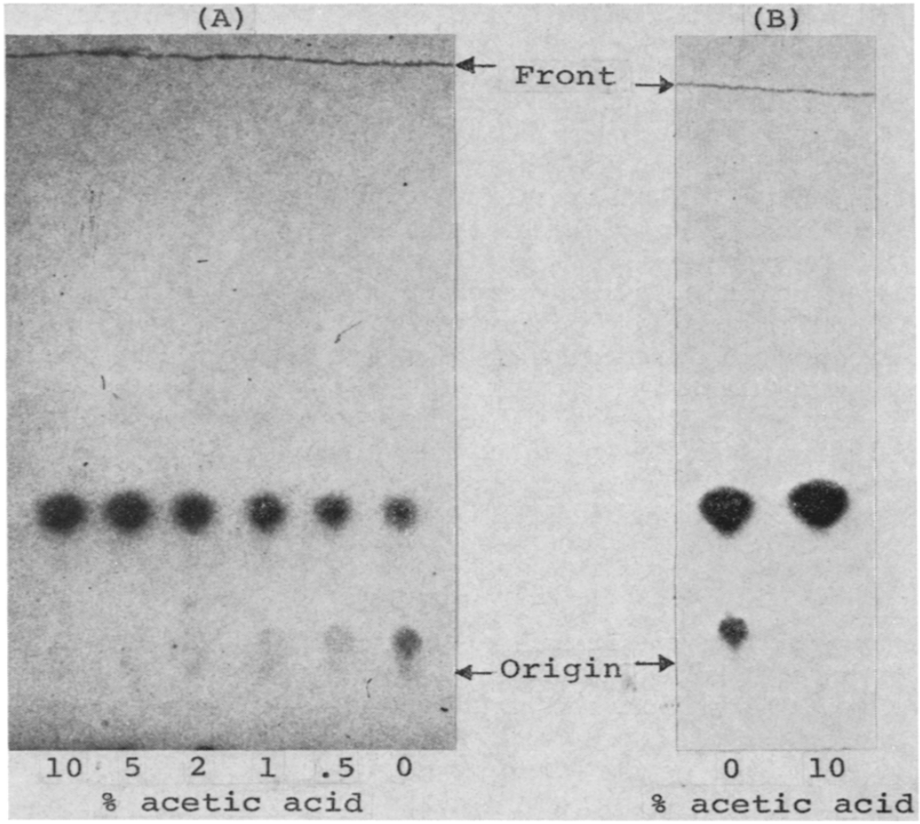

Fig. 2. Effect of the presence of free acetic acid in sample solutions. (A) 0.3% and (B) 0.5% solutions of glycolaldehyde. Adsorbent: Kieselgel G nach Stahl (E. Merck). Solvent system: chloroform–ethanol–water (5:1:1). Detection reagent: naphthoresorcinol–sulphuric acid solution.

of NMR spectroscopy, it has already been demonstrated that in solutions freshly prepared with dimethyl sulphoxide as solvent, only the symmetrical dimer is present and that its depolymerisation in this solvent takes place very slowly[1,2]. In order to locate the symmetrical dimer on the above chromatogram, a 0.5% solution of glycolaldehyde in dimethyl sulphoxide and a 0.5% aqueous solution were chromatographed on a plate in the same manner as that in Fig. 1. The dimethyl sulphoxide solution was prepared just before spotting. Immediately after the dimethyl sulphoxide solution had been applied to the plate, the plate was placed in the developing tank, and pre-equilibration (30 min) and development (10 cm) were carried out successively. The aqueous solution gave three spots, as expected. The dimethyl sulphoxide solution gave a single large spot showing an R_F value identical with that of the middle spot of the above three spots. From those results, it seems that the middle spot corresponds to the symmetrical dimer and, presumably, the lowest spot corresponds to the unsymmetrical dimer.

As it has been reported that acids catalyze the depolymerization of dimeric glycolaldehyde[2,5], 0.3% solutions of the aldehyde dissolved in 1, 3 and 6% hydrochloric acid were chromatographed by the present method. In every instance, the spot

with $R_F = 0.04$ (dimeric glycolaldehyde) was not detected with the naphthoresorcinol reagent, but two spots with $R_F = 0.25$ and 0.57 were found. When the solvent-developed chromatoplate was treated with the fluorigenic reagent, both spots showed intensely positive reactions. This result implies that both compounds on the chromatogram are monomeric. By means of NMR spectroscopy, Collins and George[1] demonstrated that the monomer and the hydrated *gem*-diol form (hydroxyacetal) of the monomer, together with the dimers, are present in aqueous solutions. One of the two compounds on the above chromatogram might be the hydroxyacetal of the monomer. From the above result, it is evident that the dilute acetic acid used for the preparation of sample solutions in the present method cannot be replaced by dilute hydrochloric acid.

Several monosaccharides other than glycolaldehyde, listed in Table I, were chromatographed by the present procedure. Most of these sugars remained at or near their starting points, and none of the sugars overlapped glycolaldehyde on the chromatogram. Of these sugars, only glyceraldehyde showed a weakly positive reaction with the fluorigenic reagent. The presence of these sugars in sample solutions, therefore, does not disturb the detection of glycolaldehyde by the present method.

TABLE I

SUGARS TESTED AND THEIR R_F VALUES

Sample solution: 0.2% solution of each sugar dissolved in 10% acetic acid. Adsorbent: Kieselgel G nach Stahl (E. Merck). Solvent system: chloroform–ethanol–water (5:1:1). Detection reagent: naphthoresorcinol–sulphuric acid solution.

Compound	R_F*
Glycolaldehyde	0.25
DL-Glyceraldehyde	0.11
Dihydroxyacetone**	0.02; 0.14
D-Erythrose**	0.05; 0.32
L-Arabinose	
D-Xylose	
D-Lyxose	
D-Ribose	
D-Deoxyribose	0.05
L-Rhamnose	
D-Glucose	
D-Mannose	
D-Galactose	
D-Fructose	
L-Sorbose	

* The R_F values of the sugars showing no obvious migration are not listed.
** Detected as double spots.

The limit of visual detection of glycolaldehyde in the present method was measured by using 1 μl each of the sample solutions with various concentrations of the aldehyde. The limit of detection with the fluorigenic reagent was found to be 0.05 μg. The present method, therefore, is much better in sensitivity than the method described in the previous paper[3]. With the use of the naphthoresorcinol reagent, the detection limit was 0.2–0.3 μg.

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