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THIN-LAYER CHROMATOGRAPHIC DETECTION OF GLYCOLALDEHYDE USING A FLUORESCENCE REACTION WITH *o*-AMINODIPHENYL

TADASHI NAKAI, HITOSHI DEMURA AND MIDORI KOYAMA

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

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SUMMARY

It was found that glycolaldehyde reacted with *o*-aminodiphenyl in the presence of sulphuric acid on silica gel thin-layer chromatograms to form a compound which, on UV irradiation, gave an intense bluish white fluorescence. This reaction was used to detect glycolaldehyde in complex mixtures of sugars.

The reversible interconversion of the monomer and dimer of glycolaldehyde is discussed.

INTRODUCTION

The aldol-type condensation of formaldehyde[1-6] gives complex mixtures of monosaccharides as products[7-9]. Glycolaldehyde is apparently the primary condensation product[8-10]. For the further study of this reaction, a method was needed for detecting micro-quantities of glycolaldehyde in the monosaccharide mixtures. This paper describes a thin-layer chromatographic (TLC) technique which is applicable. The method is based on a fluorescence reaction of the aldehyde with *o*-aminodiphenyl. The latter is one of the chromogenic agents generally used in the paper chromatography of sugars[11,12], but no report has appeared describing the use of this compound to detect glycolaldehyde in the manner described here. This paper also describes some of the information obtained about the reversible interconversion of the monomer and dimer of glycolaldehyde in aqueous solution.

MATERIALS

Chemicals

Glycolaldehyde, DL-glyceraldehyde and dihydroxyacetone were purchased from Tokyo Kasei Kogyo Co. Ltd., Japan. They were in the dimeric form[13]. Other sugars were also purchased from commercial sources. *o*-Aminodiphenyl, organic solvents and formalin were obtained from Wako Pure Chemical Industries Ltd., Japan. All these chemicals were of the highest grade commercially available and were used without further purification.

Chromatoplates

A slurry of 30 g of Kieselgel G according to Stahl (E. Merck, A G) in 60 ml of 0.03 *M* boric acid[14] was applied to glass plates in the usual manner (0.25-mm layer). These plates were left to stand for 24 h at room temperature and then heated for 1 h at 110°.

PROCEDURE

A volume of 1 μl of the sample solution to be tested was applied to the chromatoplate. Development was then carried out by the one-dimensional ascending technique with a solvent system of ethyl acetate–isopropanol–acetic acid–water (4:2:1:1) 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 thoroughly air-dried with the aid of a fan at room temperature and sprayed with a slight excess of a freshly prepared 1:1 (v/v) mixture of 0.1 % *o*-aminodiphenyl in ethanol* and 20 % sulphuric acid. The plate was then heated in an oven at 105–110° for 15 min; heating to above 110° should be avoided. The finished chromatogram was submitted to UV irradiation at about 365 nm in the dark to detect glycolaldehyde. In the present work, a Super-Light, Model LS-DI (Osawa Shigaisen Kogyo Kenkyusho Ltd., Tokyo, Japan) was used as a UV light source.

RESULTS

With the present method, glycolaldehyde was detected as a spot with R_F = 0.70 showing an intense bluish white fluorescence against a blue-violet background under long-wavelength UV light. On the other hand, when the solvent-developed chromatoplate was treated with naphthoresorcinol–sulphuric acid spray reagent[14], which is one of the chromogenic agents widely used in the TLC of sugars, glycolaldehyde was detected as double spots, blue-coloured in daylight and with R_F values of 0.70 and 0.45, respectively. With the *o*-aminodiphenyl–sulphuric acid reagent of the present fluorogenic method, the spot with R_F = 0.45 was very faintly brown in daylight and, unlike the spot with R_F = 0.70, gave no fluorescence under UV irradiation.

With the object of clarifying the relation between the two compounds which had R_F values of 0.70 and 0.45, the compound with R_F = 0.70, separated on the chromatoplate, was eluted and re-chromatographed as follows. A 0.5 % aqueous solution of glycolaldehyde was allowed to stand at room temperature for more than 24 h after preparation. A 0.5-ml volume of the solution was placed on the chromatoplate as a streak along the starting line. Development was then carried out with the solvent system described above. After the solvent front had run 10 cm from the line, the plate was air-dried, and the part of the adsorbent on which the compound with R_F = 0.70 had been adsorbed was scraped off the plate. The adsorbent was stirred with water to dissolve the compound with R_F = 0.70, and then centrifuged. The supernatant solution was evaporated to dryness *in vacuo*. The residue obtained was

* The 0.1 % *o*-aminodiphenyl solution does not keep for more than a week. A freshly prepared solution is preferable.

mixed with 1 ml of water to dissolve the compound. A large proportion of the residue remained insoluble, and was probably boric acid. The mixture was centrifuged. The supernatant solution obtained was spotted on the chromatoplate, and then the development was carried out in the above manner. The chromatogram obtained was visualized with naphthoresorcinol–sulphuric acid spray reagent. Two blue spots were found on the chromatogram; one of these had $R_F = 0.70$ and the other had $R_F = 0.45$. This result showed that an appreciable proportion of the compound with $R_F = 0.70$ changed to the compound with $R_F = 0.45$ during the above operation.

A 1-μl portion of each of the 0.1 % and 0.2 % glycolaldehyde solutions (freshly prepared and also those solutions that had been allowed to stand at room temperature for 24 h after preparation) was applied to the chromatoplate with a microsyringe and solvent-developed as described above. The chromatogram was then revealed with the naphthoresorcinol reagent. For both the 0.1 % and 0.2 % solutions, the spot with $R_F = 0.70$ of the solution that had stood for 24 h was larger in size and deeper in colour than the spot of the freshly prepared solution, while the spot with $R_F = 0.45$ of the solution that had stood for 24 h was smaller and fainter than that of the freshly prepared solution. These results showed that the compound with $R_F = 0.45$ partially changed into the compound with the $R_F = 0.70$ with the passage of time after dissolution.

The limit of visual detection of glycolaldehyde in the present method, which was based on the fluorescence reaction of the aldehyde on the spot with $R_F = 0.70$, was measured by using 1 μl each of the sample solutions which had stood at room temperature for 24 h or more after preparation. The detection limit obtained was 0.2 μg* in every case with the above solutions. With the use of freshly prepared solutions of the aldehyde, the detection limit was 0.5 μg*.

Fourteen sugars other than glycolaldehyde, listed in Table I, were also tested

TABLE I

THE SUGARS TESTED AND THEIR R_F VALUES

Adsorbent and solvent system as described in the text.

	$R_F \times 100$
Glycolaldehyde	70; 45
Glyceraldehyde	59
Dihydroxyacetone	60; 50
Erythrose	45
Arabinose	39
Xylose	47
Lyxose	45
Rhamnose	51
Glucose	34
Mannose	36
Galactose	30
Fructose	32
Sorbose	32
Lactose	14
Saccharose	24

* Based on the quantity of the aldehyde-dimer initially spotted.

by the fluorogenic method. Only glyceraldehyde and dihydroxyacetone showed positive reactions. In the intensity of their fluorescence, however, they were considerably inferior to glycolaldehyde. The other sugars gave dark spots showing no fluorescence under UV irradiation.

The aldol-type condensation of formaldehyde was carried out according to LOEW[3], with the use of a mixture of magnesium oxide, magnesium sulphate and metallic lead as catalyst. As shown in Fig. 1, the presence of glycolaldehyde in the products was demonstrated by using the present technique. A detailed report on the condensation will be published elsewhere in the near future.

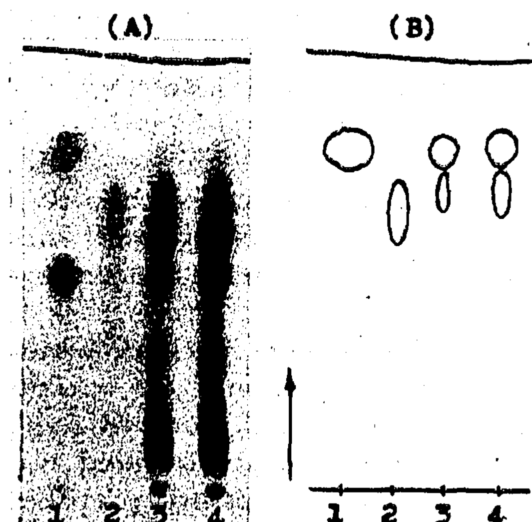

Fig. 1. Thin-layer chromatograms of the products of formaldehyde condensation carried out according to LOEW[3]. Adsorbent: 0.03 *M* boric acid-impregnated Kieselgel G. Solvent system: *n*-butanol–ethyl acetate–isopropanol–acetic acid–water (35:100:60:35:30). Detection: (A) with naphthoresorcinol–sulphuric acid solution, (B) by the present fluorogenic method; 1 = glycolaldehyde; 2 = glyceraldehyde; 3 = product of the condensation continued for 9 h; 4 = product of the condensation continued for 12 h.

DISCUSSION

The results obtained in the experiments which were carried out with the solution of the compound with $R_F = 0.70$ from the chromatogram and with the freshly prepared solutions of glycolaldehyde and solutions that had stood for 24 h clearly show that in aqueous solution glycolaldehyde exists in two forms; one ($R_F = 0.70$) gives the fluorescence reaction with *o*-aminodiphenyl on the chromatograms and the other ($R_F = 0.45$) does not, and these two forms of the aldehyde are interchangeable in aqueous solution.

As described above, the sample solutions which had been allowed to stand for more than 24 h after preparation all showed the same value of the detection limit of the aldehyde (0.2 μg) with the fluorogenic method and always gave the two spots on chromatograms with naphthoresorcinol–sulphuric acid reagent. These results show that in these solutions the two forms are probably in equilibrium.

It is already known that in the solid state glycolaldehyde exists as the dimer and that the dimer reverts gradually to the monomer in aqueous solution[13]. From

this fact and above experimental results, it can be presumed that the compound with $R_F = 0.70$ which shows the fluorescence reaction with *o*-aminodiphenyl on chromatograms is the monomer and the compound with $R_F = 0.45$ is the dimer. The reason why the dimer gives no positive reaction with the fluorogenic reagent has not yet been clarified. It can only be said that under the experimental conditions of the fluorescence reaction on the chromatogram, the dimer, which is apparently a mixture of isomers of 2,5-dihydroxy-1,4-dioxan and 4-hydroxy-2-hydroxymethyl-1,3-dioxolan[13], cannot revert to the monomer.

Glyceraldehyde and dihydroxyacetone, which give weakly positive reactions with the fluorogenic reagent, are readily converted to pyruvaldehyde when they are heated with mineral acids[15]. It can therefore be postulated that during the course of the fluorescence reaction these trioses are first converted to pyruvaldehyde owing to the presence of sulphuric acid in the reagent solution and then the pyruvaldehyde reacts with *o*-aminodiphenyl.

The fluorogenic method, as described above and especially as shown in Fig. 1, is fairly specific for glycolaldehyde. It therefore seems likely that the method can be used effectively for detecting glycolaldehyde in complex mixtures of sugars.

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