Separation of isomeric methylated deoxyguanosines on thin cellulose layers prepared with a glass rod applicator*

The separation of nucleic acid derivatives by thin layer chromatography has already been demonstrated by RANDERATH[1-3], who used cellulose powder and Ecteola cellulose[4] for the preparation of thin layers and demonstrated the advantages of this method over the more classical paper chromatography[5]. We report here an application of thin-layer chromatography to the separation of methylated isomeric deoxyguanosines obtained by the action of diazomethane on deoxyguanosine. These products, otherwise separated by paper chromatography and isolated by column chromatography on cellulose powder, have been identified as principally 1-methyl-deoxyguanosine (MGDR-I) and O^6-methyl deoxyguanosine (MGDR-II), R_F values 0.70 and 0.78 (isopropanol–water, 70:30) and 0.72 and 0.80 (isopropanol–water–ammonia, 70:25:5), respectively[6].

In the present study the chromatoplates were prepared by applying cellulose layers with a simple glass rod applicator, as well as by use of a conventional applicator. Plates prepared by both methods gave equally effective separation of MGDR-I and MGDR-II. Glass rod applicator has been used previously by LEES AND DEMURIA[7] and by DUNCAN[8], who successfully applied silica gel G and Kiesel gel G slurries to glass plates.

Experimental

Preparation of chromatoplates. A mixture of 15 g of cellulose powder MN 300 G (Macherey, Nagel and Co., Düren, Germany) having particle size less than 10 mμ and containing plaster of Paris as the binder, and 90 ml of water was vigorously stirred by a mechanical stirrer for about 2 min. The slurry formed was then applied to one side of well-cleaned glass plates (200 mm × 50 mm in size) arranged in a row on a template. In place of a template a slab of glass plate of appropriate size may be used. A thick uniform glass rod, preferably with a ground surface (10 mm in diameter), around which Scotch tape was wound to the desired thickness, 250 mμ, at two positions less than the width of the glass plate apart, was drawn over the cellulose slurry in the manner described by LEES AND DEMURIA[7]. The uniform thin layer of cellulose obtained was dried and heated in the usual manner[9,10]. LEES AND DEMURIA applied Scotch tape to the sides of the glass plates. Their method also gave satisfactory results although it is less simple than the present method.

Glass plates coated with cellulose layers to a thickness of 250 mμ by a conventional adjustable applicator were prepared for purpose of comparison.

Comparison of chromatoplates. A mixture of the methylated products, MGDR-I and MGDR-II[6], in methanol (5 μg in 4 μl) was applied to plates prepared by the two methods, at a distance of 1/2 in. from one end on as small a spot as possible. The plates were developed simultaneously in the same bath, using a mixture of isopropanol and water (70:30) as a solvent, by the ascending technique. After 2 h when the solvent had risen to an appreciable height the plates were taken out, dried and examined under short wave ultraviolet light.

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There was good reproducible resolution of the mixture in both cases. Two distinct spots, one dark and the other brightly fluorescent in ultraviolet light, separated in each case. These spots, scraped from the glass plates and eluted with $N/10$ hydrochloric acid or $N/100$ sodium hydroxide, gave products with ultraviolet spectra dentical to those of the corresponding compounds isolated earlier[6]. R_F values are listed in Table I.

TABLE I

No. of Expt.	*Spots*	*R_F values on cellulose plates prepared with applicator*	*R_F values on cellulose plates prepared with glass rod*
1	Dark spot (MGDR-I)	0.72	0.76
	Bright spot (MGDR-II)	0.86	0.88
2	Dark spot (MGDR-I)	0.74	0.76
	Bright spot (MGDR-II)	0.88	0.87

Although the small differences in R_F values in the two cases are possibly attributable to differences in the thickness of the layers, it is clear that satisfactory thin layers of cellulose can be prepared simply and inexpensively by this glass rod technique. The rapidity and sensitivity of the method made it particularly useful for monitoring the fractions eluted from cellulose columns. This technique has also been extended with success to the preparation of plates with ion-exchange cellulose powder (MN 300 G/Ecteola). These latter plates have been used for separation of isomeric methylated deoxyribonucleotides to be reported later.

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