DETECTION OF TUMOUR-INHIBITING MANNITOL DERIVATIVES BY MEANS OF PAPER CHROMATOGRAPHY

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(Received March 13th, 1962)

Research work concerning the mechanism of the action by which anti-tumour agents produce their inhibitory effect has made it necessary to separate and analyse both the original substance and its decomposition products. Owing to its sensitivity, paper chromatography seems to be suitable for the identification of those agents that give a colour reaction. Degranol (1,6-bis(β-chloroethylamino)-1,6-dideoxy-D-mannitol dihydrochloride)[1] and Mannitol-Myleran (1,6-dimethanesulphonyl-D-mannitol)[2,3] both mannitol derivatives, were justifiably expected to lend themselves for such experiments. Runs were performed on Schleicher & Schüll paper No. 2043/b with ascending development. The test substances were dissolved in distilled water and transferred to the paper as samples containing 50 to 200 μg. Mannitol was run at the same time as model substance. Various solvent mixtures and spraying reagents were compared in the course of our experiments. It was found that both Degranol and Mannitol-Myleran were detectable by means of the procedures employed (Fig. 1). The R_F values obtained with various solvent systems are listed in Table I. With *n*-

TABLE I

R_F VALUES OF MANNITOL, DEGRANOL AND MANNITOL-MYLERAN (MM) IN VARIOUS SOLVENT SYSTEMS

	R_F			
	1	*2*	*3*	
Degranol	0.12	0.15	0.25	0.06
MM	0.45	0.43	0.62	0.57
MM-decomposition product	0.19	0.16	0.28	0.18
Mannitol	0.18	0.17	0.28	0.20

1 = *n*-butanol–acetic acid–water (5:2:1)[4]; 2 = *n*-butanol–ethanol–water (4:1:5)[5]; 3 = *n*-propanol–ethyl acetate–water (7:1:2)[6]; 4 = *tert.*-isoamyl alcohol–*n*-propanol–water (8:2:3)[7].

propanol–ethyl acetate–water (7:1:2) the highest R_F values were obtained. While the R_F values of Degranol and mannitol were of approximately the same order of magnitude, the R_F of Mannitol-Myleran proved to be significantly higher.

Preliminary investigations had proved that spraying reagents containing an indicator were best suited for the demonstration of tumour-inhibiting mannitol derivatives. The results obtained with different reagents are assembled in Table II, in

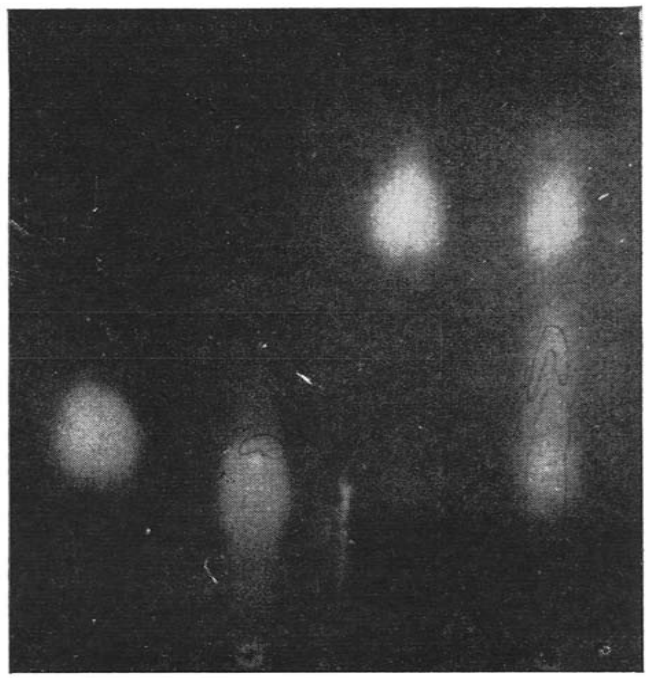

Fig. 1. Chromatogram of anti-tumour mannitol derivatives. Solvent system: *n*-butanol–ethanol–water (4 : 1 : 5). Spraying reagent: phenol red. Ascending development. (1) Mannitol; (2) Degranol; (3) Mannitol-Myleran; (4) Mannitol-Myleran, 6 day old solution. Amount applied 200 μg in each case.

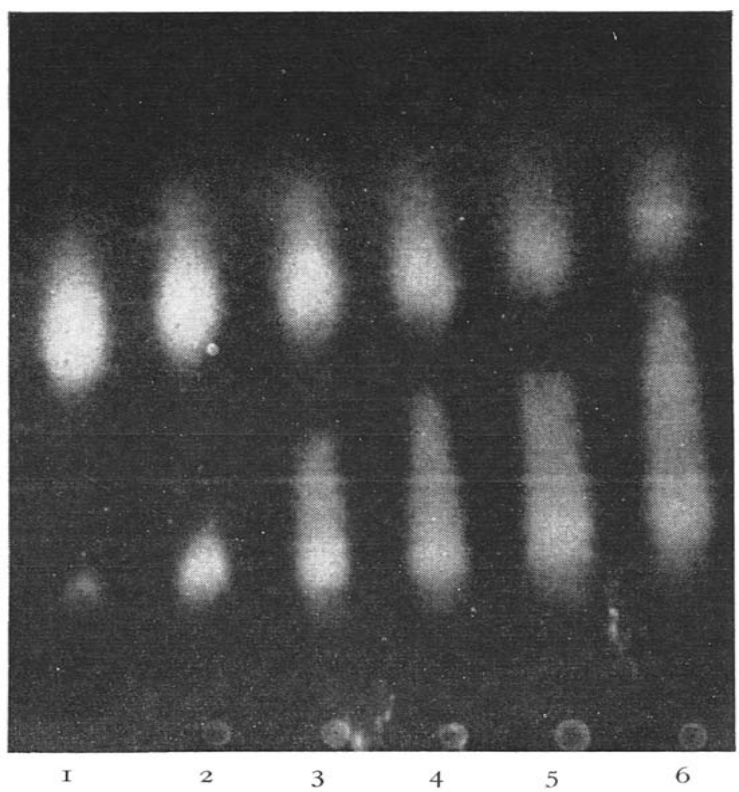

Fig. 2. Chromatograms of solutions of Mannitol-Myleran which were allowed to age. Solvent system: *n*-butanol–ethanol–water (4 : 1 : 5). Spraying reagent: phenol red. Ascending development. A = Mannitol-Myleran; B = decomposition product of Mannitol-Myleran. 1 = fresh solution; 2–6 = 1, 2, 4, 7 and 9 day old solutions, respectively. Amount applied 200 μg in each case.

which the colour reactions as well as the comparative colour intensity of the compounds examined are shown. Of the four indicator-containing reagents, methyl red gave a particularly weak reaction. Degranol gave a moderate reaction, mannitol a less marked one, while with Mannitol-Myleran, no colour reaction at all was obtained.

TABLE II

COLOUR RESPONSE OF MANNITOL, DEGRANOL AND MANNITOL-MYLERAN (MM) TO VARIOUS SPRAYING REAGENTS

	Mannitol	*Degranol*	*MM*
Bromophenol blue[8]	+++ yellow	++ brownish red	+++ yellow
Bromocresol green[8]	+++ yellow	+++ yellow	+++ yellow
Phenol red[9]	+++ yellow	+++ yellow	+++ yellow
Methyl red[10]	+ violet	++ violet	— —
Permanganate[11]	+++ yellow	++ brown	— —

The number of crosses indicates the intensity of the reaction; — = no reaction.

Reagents containing phenol red appeared to be the most sensitive of all the reagents examined. The lower limit of sensitivity was about 2 μg for all the reagents examined.

The reagent $KMnO_4$ yielded a yellow spot with mannitol, a brown one with Degranol, and failed to react with Mannitol-Myleran.

Experiments with tissue cultures showed that if solutions of Mannitol-Myleran were left to stand they gradually lost their cytotoxicity[12,13]. This process ran parallel with a decrease in the value of the pH, a phenomenon that was significant only during the first 24 hours (Table III).

TABLE III

CHANGES IN THE pH VALUE OF MANNITOL-MYLERAN SOLUTION AT DIFFERENT INTERVALS (DISSOLUTION IN DISTILLED WATER)

	Fresh	*24 h*	*48 h*	*72 h*
pH values	3.0	1.8	1.7	1.65

Paper chromatographic analyses of samples collected at different intervals revealed a gradual decrease in the intensity of the Mannitol-Myleran spot, and the simultaneous emergence of a new spot of growing intensity in the zone of mannitol (Fig. 2). In view of the fact that the diminution of the pH value practically terminates after the first 24 hours, while the disappearance of the Mannitol-Myleran spot is a slow process which lasts some 3 to 4 weeks, it seems safe to assume that the two phenomena are not directly connected. If the solution of Mannitol-Myleran is left standing for a

few hours, methanesulphonic acid is split off and this renders the solution strongly acid[3]. The new spot that arises is, therefore, presumably that of mannitol, a supposition which is confirmed by the observed R_F value (Table I, Fig. 1). The comet formation seen in connection with the new spot was probably due to the decomposition products of acid character (Fig. 2). since the spraying reagent was extremely sensitive in respect to pH. No similar phenomenon was observed in connection with Degranol: time produced neither a weakening of its spot nor the appearance of a new spot.

SUMMARY

Paper chromatography has proved to be suitable for the separation of both Degranol and Mannitol-Myleran. Experiments made in this connection showed that Degranol was more stable than the other agent.

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