CHROM. 3718

Paper chromatography of glucose–formic acid solutions

During an investigation of the compounds found in the defensive scent fluid of the ground beetles *Dicaelus splendidus* (Say) and *D. dilatatus* (Say)[1], two-dimensional paper chromatography followed by aniline hydrogen phthalate detection revealed the presence of at least three different reducing sugars. All the spots were the same color, brown, which suggested that aldohexoses were involved but not pentoses, as the latter give reddish spots with the detecting agent used[2]. However, only one of these spots matched the R_F values of any of the listed aldohexoses, namely glucose[3].

Since formic acid was the major component in the beetles' defensive fluid, it was suspected that formate esters of glucose were present in addition to the free glucose. Several solutions of α-D-glucose in different concentrations of formic acid were prepared and chromatographed. In most cases two or three separate but sometimes hazy spots appeared, as shown in Table I.

TABLE I

R_F VALUES FOR VARIOUS GLUCOSE SOLUTIONS AT 30°

Solvents: (A) phenol saturated with water; (B) 2-butanol saturated with water; (C) 1-butanol–acetic acid–water (2:1:1, by volume).

Glucose solution	*Solvent A*	*Solvent B*	*Solvent C*
1 % in water	0.47	0.25	0.43
1 % in 55% formic acid	0.46	0.24	0.41
	0.67	0.38	0.53
1 % in 83 % formic acid	0.47	0.25	0.41
	0.66	0.39	0.52
	0.83	0.56	0.66
2 % in 97 % formic acid (14 months old)	0.46	0.27	0.43
	0.67	0.40	0.55
	0.85	0.58	0.67

The existence of formate esters of glucose was demonstrated by TARKOW AND STAMM[4] in a rate study which showed that esterification proceeded fairly rapidly when glucose and formic acid were mixed and that only two of the five hydroxyl groups of glucose reacted, those on positions four and six. Thus the three spots obtained from paper chromatography quite likely correspond to glucose, a glucose formate, and glucose 4,6-diformate.

These esters when spotted on paper will withstand 100° for an hour with no apparent change. However, they can be quickly and completely hydrolyzed at room temperature during paper chromatography when solvents containing NH_4OH, aliphatic amines, or HCl are used; in each instance only one spot, corresponding to free glucose, was obtained. This spot was always clear without streaking or haziness, indicating a rapid and complete conversion of the esters to free glucose. The possibility

that those solvents had been unable to separate glucose from its formate esters was excluded by two-dimensional paper chromatography. A solvent suspected of causing hydrolysis was used for the first dimension. For the second dimension, any solvent from Table I was chosen, a solvent definitely capable of separating glucose from its formate esters. Under these conditions with aniline hydrogen phthalate only one spot was detected, whose R_F values matched those of glucose. The presence of formic acid, acetic acid, or pyridine in the chromatography solvent did not cause any hydrolysis.

Lactic acid, acetic acid, and chloroacetic acid were tried as ester-forming reagents with glucose but these failed to produce any compounds different from glucose, as monitored by paper chromatography. In addition, these three acids were rather poor solvents for glucose as compared to formic acid. D-Mannose and L-rhamnose were also studied and found to react with formic acid in like manner. Mannose in 80 % formic acid yielded three spots while rhamnose, lacking a hydroxyl group on position number six, produced only two spots. Whatman No. 1 paper with ascending flow was used for all the chromatograms.

We wish to thank the National Science Foundation for the financial assistance given under Grant No. GY-2982.

St. Edward's University,
Austin, Texas 78704 (U.S.A.)

BROTHER THOMAS MCCULLOUGH, CSC
EDWARD DEJONG
BROTHER CHESTER CASTER, CSC

1 BROTHER T. MCCULLOUGH, *Ann. Entomol. Soc. Am.*, 60 (1967) 861.
2 S. M. PARTRIDGE, *Nature*, 164 (1949) 443.
3 K. FINK, R. E. CLINE AND R. M. FINK, *Anal. Chem.*, 35 (1963) 389.
4 H. TARKOW AND A. J. STAMM, *J. Phys. Chem.*, 56 (1952) 262.

Received July 30th, 1968

J. Chromatog., 37 (1968) 545–546

CHROM. 3723

The separation of Alizarin Complexan from impurities by paper chromatography

Alizarin Complexan (3-[di-(carboxymethyl)aminomethyl]-1,2-dihydroxyanthraquinone) is the most important reagent for the spectrophotometric determination of fluoride[1–3]. The reagent is synthesized by Mannich condensation[4] from alizarin, iminodiacetic acid and formaldehyde in strong alkaline media[5]. The yield of the synthesis is very satisfactory, but the reagent may be contaminated by small amounts of the starting products. The different solubilities found for various samples of Alizarin Complexan confirms this opinion. Furthermore, other reagents that are synthesized