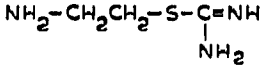
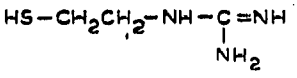
CHROM. 5894

Thin-layer chromatography of salts of S-(2-aminoethyl)-2-thiopseudourea and its transformation products

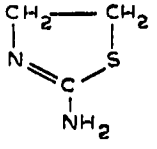
In connection with the preparation of some salts of S-(2-aminoethyl)-2-thiopseudourea (AET) and its transformation products, *i.e.*, 1-(2-mercaptoethyl)guanidine (MEG), bis (2-guanidinoethyl)disulphide (GED) and 2-aminothiazoline (2-AT), we have attempted to find a simple and rapid method for the verification of the purity of these compounds. This is especially important because of the known[1,2] instability of their aqueous solutions, in which, for example, AET is transformed to MEG or 2-AT, depending on the pH value, and MEG is readily oxidized to GED by the oxygen dissolved in natural water. The described[3,4] methods of paper chromatography seemed to be too slow to follow these transformations.



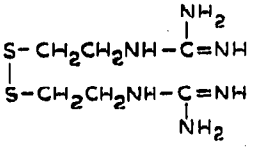
AET



MEG



2-AT



GED

In our experiments, thin-layer chromatography on two types of cellulose plates was used. Development in three different solvent systems was tried for the resolution of the compounds, and potassium ferricyanide–sodium nitroprusside reagent [5,6] was used for their detection.

Experimental

Materials. All reagents used were of analytical or practical grade and were obtained from Lachema (Czechoslovakia). The solvents used for the development of chromatoplates were redistilled before use.

Except for GED dihydrochloride, the compounds studied were prepared by known methods, but their melting points (measured on a Kofler block) were not in all cases in good agreement with those reported in the literature: AET sulphate, m.p. *ca.* 213° (decomp.) (refs. 7,8: m.p. 257° and 236–238°); MEG hemisulphate, m.p. 181–184° (ref. 9: m.p. 177–178°); GED sulphate, m.p. 268–269° (ref. 9: m.p. 257°; GED dihydrochloride (prepared from GED sulphate by reaction with barium chloride), m.p. 160–165°; 2-AT hydrochloride, m.p. 202–203° (ref. 10: m.p. 198–199°).

The elemental analyses of all these compounds were in good agreement with the theoretical values.

Apparatus. Two types of chromatoplates were used. For the first type a homogenized suspension of cellulose powder MN 300 (Macherey, Nagel and Co., G.F.R.)

was used for coating the glass plates (26 × 76 mm) in the usual way. These plates were allowed to dry at room temperature for three days before use. The average thickness of the layer obtained was *ca.* 200 μm. The second type was the commercially available Lucefol-Quick® chromatoplate (Kavalier, Votice, Czechoslovakia), which is an aluminium foil (200 × 200 mm, 0.1 mm thick) coated with a layer of average thickness 200 μm of cellulose without a binder. This chromatoplate may be cut into smaller sizes as required.

Procedure. The chromatograms were obtained partly on the above two types of microplates (26 × 76 mm) and partly on larger chromatoplates (70 × 190 mm) of the Lucefol-Quick® type. The samples were dissolved in 0.2 *N* hydrochloric acid (all compounds are sufficiently stable in this solution apart from the very slow oxidation of MEG to GED). A 0.5-μl aliquot of the 2.5 % solution of each compound was spotted along the starting line of the microplates, and 1 μl of the same solution was applied in the case of the larger chromatoplates. The plates were run by using a one-dimensional ascending technique in glass tanks of corresponding sizes saturated by lining three of the walls with a layer of filter-paper soaked with the solvent system being used.

Development. The chromatoplates were developed to a height of 60 mm or 155 mm in the solvent systems S_1, *n*-butanol–acetic acid–water (4:1:5); S_2, *n*-butanol–2-propanol–0.2 *N* hydrochloric acid (100:10:50); and S_3, *n*-butanol–acetone–0.2 *N* hydrochloric acid (100:25:30). The two-phase systems S_1 and S_2 were saturated by shaking them at room temperature for 30 min and the separated organic phases were then used for development.

Detection. The dried developed plates were sprayed with fresh potassium ferricyanide–sodium nitroprusside reagent[5,6] prepared as follows[4]. A mixture of 5-ml portions of 10 % potassium ferricyanide, 10 % sodium nitroprusside and 10 % sodium hydroxide solutions, after standing at room temperature for 30 min, was diluted with a mixture comprising 90 ml of water and 120 ml of acetone. The colour of the detected spots was recorded 2–3 min after spraying. Apart from the violet spot of 2-AT, all the other spots were bleached within 15–30 min to provide white spots on a pale yellow background. The limit of the sensitivity of this method of detection in practice was about 1 μg for AET, MEG and GED salts, and 0.1 μg for 2-AT-hydrochloride.

Results and discussion

The R_F and R_S values (with the most mobile MEG as reference compound) are summarized in Table I together with the colours of the detected compounds. In all three solvent systems used, a satisfactory separation of the compounds studied occurred. Although the differences in the R_F values of the same compound developed in all three systems are not very significant, it seems that system S_3 is particularly useful because of the regularity of the distribution of spots between the most rapidly and the most slowly running compounds (see R_S values). A further advantage of this system follows from the comparison of running times of all three systems (measured on the Lucefol-Quick® 70 × 190 mm chromatoplates, the system S_1 running time was 2 h 40 min, system S_2 2 h 7 min and system S_3 1 h 27 min).

Comparing the plates coated with a layer of cellulose powder MN 300 and Lucefol-Quick® chromatoplates, there is no significant difference in the R_F values of

TABLE I

R_F AND R_S VALUES, AND SPOT COLOURS OF COMPOUNDS

R_S values (in parentheses) refer to MEG sulphate. The developing solvent systems were S_1, *n*-butanol–acetic acid–water (4:1:5); S_2, *n*-butanol–2-propanol–0.2*N* hydrochloric acid (100:10:50); S_3, *n*-butanol–acetone–0.2*N* hydrochloric acid (100:25:30). The types of plates used were A, microplates coated with cellulose powder MN 300 (Macherey, Nagel and Co., G.F.R.), size 26 × 76 mm; B and C, Lucefol-Quick® chromatoplates (Kavalier, Votice, Czechoslovakia) cut to the size of 26 × 76 mm (B) and 70 × 190 mm (C). Colours: WP = white spot with a pink demarcation; RP = red–pink spot; P = pink spot; V = violet spot (pale yellow background in all cases).

Compound	*R_F (R_S) values in developing systems*									*Colour*
	S_1			S_2			S_3			
	A	*B*	*C*	*A*	*B*	*C*	*A*	*B*	*C*	
AET sulphate	0.05 (0.11)	0.04 (0.09)	0.08 (0.13)	0.05 (0.12)	0.04 (0.08)	0.07 (0.14)	0.05 (0.10)	0.07 (0.12)	0.12 (0.20)	WP
GED dihydrochloride	0.28 (0.61)	0.24 (0.55)	0.47 (0.78)	0.24 (0.57)	0.24 (0.50)	0.27 (0.54)	0.23 (0.44)	0.24 (0.42)	0.34 (0.59)	RP
2-AT hydrochloride	0.40 (0.87)	0.35 (0.80)	0.55 (0.92)	0.32 (0.76)	0.35 (0.73)	0.39 (0.78)	0.38 (0.73)	0.40 (0.70)	0.44 (0.76)	V
MEG hemisulphate	0.46 (1.00)	0.44 (1.00)	0.60 (1.00)	0.42 (1.00)	0.48 (1.00)	0.50 (1.00)	0.52 (1.00)	0.57 (1.00)	0.58 (1.00)	P

the same compounds (see columns A and B in Table I). We observed a sharper demarcation of spots on the former plates, but their running times were about twice as long in all systems (measured on 26 × 76 mm microplates, the running time of cellulose MN 300 layers was 30–36 min compared with 15–17 min on the Lucefol-Quick® plates). We did not use the cellulose MN 300 layer on the larger chromatoplates. On the other hand, most of our analyses on the microplates were carried out with the use of this kind of cellulose layer because of its better stability and adhesion to the glass plate in comparison with these properties of the cellulose layer on the aluminium foil of the Lucefol-Quick® plates.

The method of thin-layer chromatography described has been found to be useful for its intended purpose. Moreover, with this method we were able to find the optimum conditions for the preparation of AET sulphate and MEG hemisulphate, thus enabling us to obtain these compounds in high yields.

The Lucefol-Quick® chromatoplates were the gift of Kavalier, Votice, Czechoslovakia. We are much indebted to Dr. K. CHMEL for information on the plates prior to their commercial availability.

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