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Note

Thin-layer chromatography of some sulfur and nitrogen mustards

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The need existed for an analytical procedure, with minimum clean-up, that could be used to detect and differentiate trace quantities of sulfur and nitrogen mustards when in soil, vegetation, water, mammalian tissue or fluids; or when impinged on other surfaces. Thin-layer chromatographic (TLC) studies had been reported elsewhere on radioactive mustard (β,β'-dichloroethyl)sulfide[1] and on most of the compounds discussed in this present report[2]. The TLC procedure developed here employs a silica gel G chromatoplate with dichloromethane as the developing solvent. Visualization of the spots is accomplished using the NBP reagent [4-(*p*-nitrobenzyl)-pyridine[3] which allows sensitivities to slightly less than a microgram. The mustards included in this study, their respective R_F values and their detection sensitivities are shown in Table I.

TABLE I

R_F VALUES OF SELECTED MUSTARDS

Mustard	*Code*	R_F	*Sensitivity threshold (μg)*	
			NBP reagent (blue)	*Copper–dianisidine (violet)*
Bis(2-chloroethyl)sulfide	HD	0.77	1	≈45
1,2-Bis[(2-chloroethyl)thio]ethane	Q	0.68	1	1
2,2',2''-Trichlorotriethylamine	HN-3	0.66	1	≈100
2,2',2''-Trichlorotriethylamine–HCl	HN-3–HCl	0.60	1	≈100
Unknown and 2-chloro, 2'-hydroxy dithioethane	Hemi-Q	0.48	10	1
Bis[2-chloroethyl)-thio]ethyl ether	T	0.38	2	4
Unknown and 2-chloro,2'-hydroxyethyl sulfide	Hemi-H	0.33	10	–
N,N-Bis(2-chloroethyl)ethylamine	HN-1	0.16	1	≈100
N,N-Bis(2-chloroethyl)methylamine	HN-2	0.13	1	≈100
N,N-Bis(2-chloroethyl)ethylamine–HCl	HN-1–HCl	0.11	1	≈100
N,N-Bis(2-chloroethyl)methylamine–HCl	HN-2–HCl	0.88	1	≈100
Unknown and (1,2-bis[(2-hydroxyethyl)thio]ethane	Q-glycol	0.00	>1000	1
Bis(2-hydroxyethyl)sulfide	H-glycol	0.00	>1000	≈50

EXPERIMENTAL

Apparatus and reagents

Glass plates (50 × 200 mm or 200 × 200 mm) were coated with 0.5-mm layer

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of silica gel G (Merck, Darmstadt, G.F.R.) using a Desaga thin-layer applicator (Brinkmann, Westbury, NY, U.S.A.). The plates were air dried and stored over silica gel until used. Thin-layer chromatograms were developed employing reagent grade dichloromethane. The solvents and other compounds were of reagent grade and required no further purification. The primary reagent for visualization of developed chromatograms was NBP procurable from Aldrich (Milwaukee, WI, U.S.A.) or K&K Labs. (Plainview, New York, NY, U.S.A.). The NBP is used as a 5% solution in acetone. Other reagents such as potassium acid phthalate, sodium perchlorate and sodium hydroxide are as described in the procedures.

Thin-layer chromatography

The 50 × 200 mm silica gel G chromatoplates were scribed 150 mm above the point of sample application as a horizontal break. Vertical scribing allowed up to five separate chromatography channels formed by scribing parallel breaks in the absorbent. This vertical discontinuity prevented overlapping of developing samples and allowed selective masking of channels for use of different detector systems on the same plate. To determine the amount of unknown sample to apply to the chromatoplate 5 to 50 μl of the compounds (in chloroform or carbon tetrachloride) were applied to a test plate. Addition via capillary or micropipets was made slowly such that the sample spot was kept reasonably small. Known samples could be applied in similar fashion from the same solvents or as smaller aliquots.

The 50 × 200 mm plate developing chamber was maintained at 23 to 25°C and contained 20 ml of dichloromethane. The chamber was lined with filter paper or filter paper wick (50 × 150 mm) to aid in saturating the chamber atmosphere. Where particular mustards or their residues were suspected, known samples of these were pipetted as possible references on a parallel channel.

The mustards were visualized as follows. The chromatoplate was air-dried to a minimum of residual dichloromethane and then sprayed with the NBP solution. This was followed with a spray of an aqueous solution of 5% sodium perchlorate (in 92.1 mg of potassium acid phthalate/100 ml of water adjusted to pH 5.0 with 1 *N* sodium hydroxide solution). The plate was then heated in an oven to 105°C for 10 min. After cooling, the plate was sprayed with piperidine. The mustards (and other alkylating agents) show up as blue spots, the intensity of which is proportional to their concentration.

Preparation of sample

The mustards being alkylating agents react readily with hydroxyl groups as with water or alcohols, more slowly with the latter. Solution or extraction of the sulfur compounds is best accomplished with chloroform, carbon tetrachloride or hexane. The nitrogen mustards as the free bases are extractable by all three solvents. The hydrochloride salts of the nitrogen mustards are solubilised by chloroform but not by carbon tetrachloride or hexane. Extraction of the salts from a 0.1% saline water medium (including homogenized tissue) is best accomplished by adding chloroform and then sodium bicarbonate powder with thorough mixing. The transfer efficiency into chloroform is better than 95%. Sodium chloride serves to inhibit dialkylammonium formation which could lead to hydrolysis of the mustard. Chloride ion similarly stabilizes sulfur mustards by slowing down dialkylsulfonium ion formation in the presence of water.

RESULTS AND DISCUSSION

The initial experimentation on the chromatographic analysis of mustards was made in an attempt to separate HD from Q. Since the nature of these compounds precluded their use with polar solvents, emphasis was placed on their chromatographic development utilizing non-polar organic solvents. With silica gel G as adsorbent, and chloroform or chloroform–acetone as developing solvent[1], no separation was observed. Only partial separation was discernible with benzene, while dichloromethane gave complete separation of HD from Q. Due to the large number of components found in some crude samples, it was considered advantageous to allow the solvent front to travel a distance of 150 mm rather than the normal 100 mm. In this manner it was not only possible to separate the HD from the Q, but the impurities associated with each were also separated.

After the achievement of a successful separation of HD from Q, it was decided to test the separation of four additional mustards in the same system. These mustards (T, HN-1, HN-2, and HN-3) were chromatographed on silica gel G with dichloromethane as developing solvent. It was found that they were separable from HD and Q and also from each other. However, judicious care was found necessary when attempts were made to separate HN-1 from HN-2 as the R_F values of these two compounds were very similar. If the spot application was too large there was a tendency for the compounds to merge, resulting in only partial separation.

Further attempts were made to improve the separation of HN-1 from HN-2. Silica gel G with chloroform–acetone as eluent[1] did not improve the separation. Using acid alumina as adsorbent, dichloromethane, cyclohexane and trichloroethylene were tested as developing solvents. None of these gave separation. The same can be said for the following systems: basic alumina–carbon tetrachloride; neutral alumina with either cyclohexane, chloroform, benzene, or dichloromethane; or utilization of the wedged-tip technique with any of the above. The best separation of HN-1 and HN-2 (as well as the other mustards) was achieved with the silica gel G–dichloromethane system. Positive identification was found to be possible when a control sample was chromatographed simultaneously with the suspected HN-1 or HN-2, or both.

The separation of any of the mustards from one another, or from the impurities in a given mustard sample was such that quantitation of the mustard in question could be performed directly using a densitometer or, preferably after excision, by solution colorimetry (NBP method). The TLC procedure was found to be applicable over a wide range of concentrations from a micro scale with development on microscope slides, to a macro scale with development on preparative plates having adsorbent layers 1 mm thick or greater.

An alternate to visualization by NBP consisted of spraying the developed plate with a 0.5% solution of *o*-dianisidine in methanol followed by a spray of a 0.5% aqueous cupric acetate solution containing one drop of acetic acid per 50 ml. This was followed by a spray of concentrated sulphuric acid. A violet coloration formed at once with most of the components, although heating at 110°C for 10 min was required to fully develop the color. This reagent is most effective for Q and T and significantly less so for the other mustards.

The NBP reagent was selected as the primary reagent because of its overall sensitivity in visualizing the mustards by producing deep blue colors. The dianisidine–

cupric acetate reagent was especially effective with Q, hemi-Q and the hydrolysis product, 1,2-bis[(2-hydroxyethyl)thio]ethane or Q glycol and significantly less intensive for H and H glycol.

The hemi-Q referred to above is a product of partial hydrolysis of Q in which one of the chlorines is replaced by a hydroxyl. Similarly a hemi-H can form, also by partial hydrolysis. These have been detected proximal to the R_F values shown as unknowns in Table I. The unknown(s) at the origin can be attributable to the respective H-glycol and Q-glycol (NBP > 1000 μg; copper-dianisidine 50 and 1 μg, respectively) as well as to some other more polar species.

When solubilized in water, the nitrogen mustards cyclize to form their corresponding dialkylammonium salts; the sulfur mustards form their dialkylsulfonium salts. In both situations this is the step that precedes hydrolysis. To preclude or lessen the cyclization, chloride ion via hydrogen chloride or saline (sodium chloride) is kept present in aqueous systems containing the mustards.

The nitrogen mustards discussed here are relatively unstable *in situ* as their free bases. All can self alkylate via aziridine and thence piperizine formation. For practical purposes, the compounds were stored as their hydrochloride salts and converted to their free bases for experimental use. The separate TLC of aged samples of the three nitrogen mustards as their free bases, produced plates that were in their own right too complex for the present discussion.

As can be seen in Table I reasonable separation is obtainable even on composite mixes containing all of the mustards. The salts of the nitrogen mustards are sufficiently separable to allow detection among themselves and their free bases, when compared with known samples. The sulfur mustards HD, Q and T are readily separable from one another and detectable. Even if HN-3 were present with Q, differentiation could be obtained by employment of the copper–dianisidine color system to allow visualization of Q.

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