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DETECTION OF β,β'-DICHLOROETHYL SULPHIDE ON THIN-LAYER CHROMATOGRAMS

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SUMMARY

Thin-layer chromatography (TLC) has been employed to detect and quantify very low concentrations of β,β'-dichloroethyl sulphide (sulphur mustard, HD), on the basis of visualization of spots and R_F values. Various reagents and reactions were examined with respect to their sensitivity and selectivity. In particular, chemically similar compounds like thiodiglycol or β,β'-dichloroethyl oxide as well as a variety of organophosphorus esters (pesticides) and halogen-containing organic compounds were subjected to the same analytical procedures so as to check the selectivity of the TLC methods developed for HD. The best separations were usually obtained by developing silica chromatoplates in dichloromethane–n-hexane (1:1) solvent. The most satisfactory evidence for the presence of HD is based on a reaction with iodoplatinate $[PtI_6]^{2-}$ and the subsequent application of starch solution (ultimate sensitivity '0.1 $\mu g/\mu l$). Somewhat less sensitive (0.5 and 1 μg, respectively), but quite selective, is a reaction utilizing Michler's ketone in the presence of mercury bromide, which gives rise to intense purple spots on a yellow background, and a photochemical reaction using silver nitrate. Other sensitive and selective reactions are those with triphenylmethane dyes in conjunction with $AgNO_3$ and with selenic acid.

INTRODUCTION

In contrast to sophisticated analytical techniques, surprisingly little effort has so far been made to determine trace quantities of sulphur mustard (HD) by means of thin-layer chromatography (TLC), despite the sensitivity and simplicity of this technique[1–3]. Well established analytical procedures using colorimetric methods are based on the so-called "DB 3" reaction in solution in which HD reacts with 4-(p-nitrobenzyl)pyridine to form an intense blue dye[4]. In order to detect rapidly contamination of water, soil and vegetation with mustard outside the laboratory there is need for a much simpler but nevertheless reliable and sensitive method of detecting HD. TLC seems to fulfil most of these requirements if the sensitivity, selectivity and possible interferences of similar compounds are known and certain precautions are taken. This led us to conduct a systematic study of the versatility and utility of TLC for identification and determination of HD.

EXPERIMENTAL

Thin-layer chromatography

Pure freshly distilled HD was dissolved in reagent-grade dichloromethane and diluted to give standard solutions of 100, 10, 1 and 0.1 $\mu g/\mu l$. For the TLC experiments, commercial glass plates coated with 0.25 mm silica gel (Fertigplatte Type 60 F_{254}, E. Merck) were used. A 1-μl volume of standard HD solution was carefully applied to a chromatoplate with a micropipette to form a very small sample starting spot. Thereafter the plates were immersed vertically in glass development chambers maintained at 22°C and containing ʻ100 ml of reagent-grade dichloromethane and *n*-hexane (1:1). This solvent is known to yield rather high R_F values for mustards but only very low R_F values for organophosphorus compounds (pesticides, etc.). Uniform saturation of the chamber atmosphere was achieved in the usual manner by lining the chamber with filter-paper. After development, the chromatoplates were first dried in air to remove most of the solvent. Thereafter they were uniformly sprayed with appropriate reagents (see below) using a spray gun applicator (Desaga, Heidelberg, G.F.R.). If necessary, the plates were heated in an oven to a defined temperature for a certain period of time, or exposed to UV light (mercury line at 253.7 nm) in order to accelerate photochemical reactions.

Chemicals and procedures

Five different reactions were studied in detail (see below), requiring the following reagents.

Detection via $[PtI_6]^{2-}$–starch solution. (a) One millilitre of an aqueous solution of 5% hexaiodoplatinate (potassium salt) was added to 3.5 ml sodium iodide solution (ʻ1 *M*) (prepared immediately before use) and diluted in distilled water to a total of 60 ml. (b) One gram of soluble starch was moistened and stirred and then dissolved in 100 ml boiling water (prepared shortly before use). (c) Reagent grade glacial acetic acid.

The spray reagent comprised 1 ml of reagent a plus 3 ml of b plus 2 ml of c plus 1 ml distilled water and was prepared immediately before use. The developed chromatoplate was uniformly sprayed with this reagent. The presence of HD is indicated by blue spots on a white or pink background. The intensity of the spots fades after a while. Detection limit: < 0.1 μg HD.

Detection with Michler's ketone and mercury salts. (a) A 0.1% solution of Michler's ketone [4,4′-bis(dimethylamino)benzophenone] in toluene (p.a.). (b) A 5% solution of mercury dichloride or bromide ($HgCl_2$, $HgBr_2$) in absolute ethanol.

The spray reagent comprised equal parts of reagents a and b, thoroughly mixed. The developed chromatoplate was uniformly sprayed with the reagent and then heated to 80°C. HD is indicated by intense purple spots on a yellowish background. The detection limit depends strongly on the concentration of the mercury salt solution, and is lowest for the conditions given above. With the standard chromatoplates described above the detection limit is approximately 0.5 μg HD. If high-performance TLC chromatoplates are used the detection limit can be lowered to 0.01 μg.

Detection with silver nitrate using a photochemical reaction. The spray reagent comprised a 0.1 *M* aqueous solution of silver nitrate, prepared immediately before

use, stored in the dark. The developed chromatoplate was uniformly sprayed with the reagent, then placed in an oven for *ca.* 5 min at 80°C before exposure to UV light (mercury line at 253.7 nm) for 3 min. HD is indicated by gray or purple spots. Detection limit: 1 μg HD.

Detection with triphenylmethane dyes and silver nitrate. (a) Thymolphthalein (0.17 g) was dissolved in 20 ml absolute ethanol to which 2 ml of 5 M sodium hydroxide solution were added. Instead of thymolphthalein, 0.17 g α-naphtholphthalein, 0.13 g phenolphthalein or 0.15 g alizarine-S (sodium sulphonate salt of dihydroxyanthraquinone), respectively, can be used. (b) A 0.1 M aqueous solution of silver nitrate, prepared shortly before use. (c) 25% Sulphuric acid or acetic acid.

The developed chromatoplate was uniformly sprayed with reagent a. A dark blue colour is obtained with thymolphthalein and α-naphtholphthalein, a purple colour with phenolphthalein or alizarine-S. Thereafter reagent b was carefully sprayed; a thin homogeneous coating is crucial. A heat treatment in an oven at 120 or 140°C then follows, for about 10 min until dark brown spots appear. Excessive heat will result in a brown background, thus preventing visualization of the spots. After this treatment solution c was sprayed which helps to lighten the background to a yellowish finish. The detection limit is around 1 μg HD.

Detection with selenic acid. One gram of selenic acid (H_2SeO_3) was dissolved in 25 ml sulphuric acid (25%). The developed chromatoplate was homogeneously sprayed with this solution. Subsequent heating in an oven at 120°C for about 10 min (too long a heat treatment is deleterious) produced orange or light-brown spots on a white background if HD was present. The detection limit is approximately 1 μg HD. A photometric evaluation (by shining light through the transparent plate) is recommended since it increases the detection limit by a factor of 2. The spots are very stable and do not change in intensity even weeks after the reaction.

RESULTS

The experiments were first performed in order to test the applicability of various analytical reactions (so far mostly carried out in solution) to TLC. Secondly, the sensitivity was determined and the physicochemical parameters (concentration, composition of mixtures, temperature, exposure, etc.) were varied systematically so as to improve the suitability and detection limit for HD. Thirdly, the selectivity of a given reaction was checked by directly comparing HD with various chemically related compounds and some other harmful substances such as organophosphorus esters or irritants.

Some of the results of our investigations concerning the first two of these categories have already been touched upon in that only those reactions listed gave satisfactory chromatograms. Also, the best analytical procedures were also briefly reported. Here, we concentrate on the third category, namely, the selectivity of a given reaction and possible interferences in mixtures. Experiments were performed in which HD was allowed to migrate together with a variety of other chemical compounds in similar concentrations, under otherwise identical conditions (same developing solvent, heat treatment, etc.). Basically three different groups of compounds were investigated:

(i) Chemically related species: "oxygen mustard" β,β'-dichloroethyl ether,

TABLE I

SUMMARY OF THE TLC RESULTS OF THE DETERMINATION OF HD

| Reaction | Temperature (°C) | Detection limit (μg) | Interferences | | Reproducibility |
			Irritants related components	Pesticides	
DB 3 (NPB reagent)	110	1	+	?	Very good
Iodoplatinate	25	0.1	−	Few	Very good
Michler's ketone + mercury salts	80	0.5	+	+	Very good
Silver nitrate + UV light	80	1	−	−	Very good
Triphenylmethane dyes + AgNO$_3$	120–140	1	+	+	Poor
Selenium dioxide	120	1	−	Few	Very good

$(ClCH_2CH_2)_2O$; thiodiglycol; β,β'-dihydroxyethyl sulphide, $(OHCH_2CH_2)_2S$; "nitrogen mustard", $(ClCH_2CH_2)_3N$; 1,4-dithiane;

(ii) Irritants and organic compounds containing active halogen atoms: "CN", chloroacetophenone, $CH_2Cl\text{-}CO\text{-}C_6H_5$; "CS", o-chlorobenzalmalondinitrile, $ClC_6H_4CHC(CN)_2$; benzoyl chloride, C_6H_5COCl; benzyl bromide, $C_6H_5CH_2Br$; bromoacetic acid methyl ester, $CH_2BrCOOCH_3$.

(iii) Pesticides (organophosphorus esters, thioesters, etc.) (trivial names given only): dimethoat, $(CH_3O)_2PSSCH_2CONHCH_3$; parathion, $(C_2H_5O)_2PSOC_6H_4NO_2$; malathion, $(CH_3O)_2PS\text{-}SCH(COOC_2H_5)\text{-}(CH_2COOC_2H_5)$; ethion, $(C_2H_5O)_2PS\text{-}SCH_2S\text{-}SP(OC_2H_5)_2$; dimefox, $[(CH_3)_2N]_2POF$; DDVP (Dichlorvos), $((CH_3O)_2POOCH = CCl_2)$; metasystox, (demeton-o-methyl), $(CH_3O)_2PO\text{-}S\text{-}CH_2\text{-}CH_2SCH_2CH_3$; chlorfenvinfos, $(C_2H_5O)_2POOC = CHClC_6H_3Cl_2$.

In the chromatographic experiments, 10 μg HD were directly compared to 100 μg each of the compounds listed above. The developing solvent was again dichloromethane–n-hexane (1:1). The chromatoplates were then subjected to the various analytical procedures described. The results will be reported in the same order.

Selectivity of the iodoplatinate–starch reaction

Chemically related species, organic halides, irritants. Only HD forms intense blue spots; CN and CS are visible as white spots, but their R_F values are much lower than that of HD. Thiodiglycol does not perturb the confirmation of HD. Nitrogen mustard gives no response.

Pesticides. Of all the compounds studied only metasystox slightly perturbs the HD detection in that it gives rise to weak blue spots (a group of two spots with different R_F values) the first of which has a similar R_F to that of HD. In order unequivocally to determine HD a second reaction has to be performed, *e.g.*, the photochemical reaction with silver nitrate which is negative with metasystox.

Selectivity of the reaction using Michler's ketone and mercury salts

Chemically related compounds. Only HD forms an intense purple spot, whereas oxygen mustard gives no response. Thiodiglycol produces a white spot with very low R_F value and 1,4-dithiane gives a white spot with a lower R_F value than that of HD.

Nitrogen mustard likewise gives a positive reaction with Michler's ketone, its R_F value and detection limit being similar to those of HD. As will be shown later, HD can be distinguished from nitrogen mustard by means of the reaction with iodoplatinate–starch or with SeO_2 which gives a negative result in the presence of nitrogen mustard.

Active organic halides, irritants. CN produces a very weak dark spot, whereas CS shows an orange spot whose intensity increases if $HgBr_2$ is used instead of $HgCl_2$. Compounds like "Clark 1", diphenylarsine chloride, $(C_6H_5)_2AsCl$, or "Pfiffikus", phenylarsine dichloride, all show negative reactions. Benzoylchloride reacts immediately with Michler's ketone (without any mercury salt) to give a sea-green precipitate; 1 μg gives a clearly visible spot. It is worth mentioning that aromatic halides substituted in the side chain like xylyl bromide, $CH_3C_6H_4CH_2Br$, or benzyl bromide, $C_6H_5CH_2Br$, give the same reaction as does HD, *viz.,* a purple spot. Here, only the different R_F values would render a distinction from HD.

Pesticides. Chlorfenvinphos, dimefox and metasystox give negative reactions. Dimethoat gives rise to a group of three separate spots, a pink one with very low R_F and two green ones with appreciable R_F values. Parathion exhibits similar behaviour; besides the pink spot only one weak pink-yellow spot was observed. Blue spots are obtained with dichlorvos and malathion, and the latter pesticide produces an additional green starting spot. Ethion gives rise to a very weak greenish spot. So all the pesticides studied essentially do not perturb the HD determination with Michler's ketone.

Selectivity of the photochemical reaction with $AgNO_3$

Chemically related species. Nitrogen mustard gives exactly the same reaction as HD, *i.e.,* a dark purple spot on a white background. Also, the detection limit is similar, namely, 1 μg. Thiodiglycol and oxygen mustard as well as 1,4-dithiane do not show any reaction at all and therefore do not perturb the HD detection.

Irritants and active organic halides. Only CN gives a (washed-out) light spot which shows intense purple fluorescence if irradiated with UV light; its R_F value is about half that of HD. CS gives a negative reaction as does benzyl bromide. Benzoyl chloride, however, gives a brownish spot which can easily be distinguished from the respective HD features.

Pesticides. Immediately after spraying a yellow starting spot appears with dimethoat, a weak yellow spot with parathion, R_F value approximately half that of HD, and an intense yellow spot is obtained with ethion (R_F '2/3 that of HD). After irradiation with UV light for 5 min some alteration of the features occurs in that the parathion and ethion spots become very intense. A weak yellow spot appears with malathion. All the spots, however, do not interfere with the HD spot as far as R_F value and colour are concerned. The other pesticides showed no responses.

In summary, the photochemical reaction with silver nitrate, despite its simplicity, is a quite selective and reasonably sensitive method to detect mustards having activated chlorine atoms in the β-position.

Selectivity of the triphenylmethane dye–silver nitrate reaction (thymolphthalein, in particular)

Chemically related compounds. In contrast to the very intense dark brown spot

obtained with HD, thiodiglycol only shows a brown starting spot and oxygen mustard gives no reaction. 1,4-Dithiane and nitrogen mustard were not studied in this series.

Irritants and active organic halides. Both CN and CS show fairly intense orange and yellow-brown spots, respectively, with slightly different R_F values (about 1/2 to 2/3 that of HD). The CS spot sometimes has a characteristic yellow halo around it. Benzoyl chloride exhibits a quite intense whitish or pink spot with about the same R_F value as that of CN and CS.

Pesticides. Many pesticides give rise to brown or orange spots similar to HD, particularly parathion, malathion, ethion, dimefox, metasystox, dichlorvos and chlorfenvinfos. Dimefox produces an intense brown spot ($R_F \approx 60\%$ that of HD) and a much weaker greyish spot with appreciably higher R_F value ($\approx 80\%$ that of HD) and may therefore render difficult a distinction from HD. All other pesticide spots occur at markedly lower R_F values than that of HD, so a separation should be possible.

However, the thymolphthalein–silver nitrate method, although quite sensitive, cannot be regarded as a very specific reaction for HD. Moreover, it is a rather crucial reaction (also true for the other triphenylmethane dyes investigated) as far as exposure to the spray reagents and the thermal treatment is concerned, and thus cannot be recommended for routine determinations of sulphur mustards, despite the fact that it gives good results in solution[5].

Selectivity of the selenium dioxide reaction

Chemically related compounds. Oxygen mustard does not show up at all, and thiodiglycol only produces an intense orange starting spot. Interestingly, nitrogen mustard does not react with SeO_2. Therefore, this reaction can be used to distinguish between sulphur mustard and nitrogen mustard. 1,4-Dithiane has not been investigated with respect to SeO_2.

Irritants and active organic halides. Here, the excellent selectivity of the HD detection with SeO_2 becomes quite apparent. CN, CS (which produces a brown starting spot), benzoyl chloride end benzyl bromide do not give a positive reaction.

Pesticides. Although somewhat less selective (parathion, malathion and ethion all give pronounced yellowish spots; $R_F = 40\%$ that of HD for parathion, about 10% for malathion and 60% for ethion; dimethoat and metasystox only give brown starting spots), a clear separation from HD is easily achieved by choosing an appropriate developing solvent.

DISCUSSION

The aim of this section is not only to review briefly and discuss analytical reactions that have been used before to detect and identify sulphur mustard (particularly with respect to TLC), but also to correlate the sensitivity and selectivity of the various reactions with the actual chemistry that occurs when HD interacts with the respective reagents.

A fairly detailed study on the TLC determination of HD was recently reported by Sass and Stutz[3] who used the NBP reagent [4-(p-nitrobenzyl)pyridine], a method also known as the "DB 3" reaction[6]. Although reasonably sensitive ($\approx$ 1 μg HD), this method exhibits only poor selectivity since all alkylating agents will give positive

results, and an unequivocal identification of HD is only possible if the R_F values are carefully considered. However, as these authors results showed, this reaction is about a factor of 5 more sensitive than that using o-dianisidine and cupric acetate.

To our knowledge the iodoplatinate reaction has not been employed for TLC before, although its suitability for HD determination has long been known (Tschugajeff reaction)[7]. Although the details of the reaction pattern are quite complex, an essential step is certainly the formation of elemenal iodine which is most sensitively indicated by the well known reaction with starch. Iodine is at least partially released from the hexaiodoplatinate anion as the HD molecule becomes a ligand. Of course, all reagents that lead to the formation of elemental iodine will perturb this method. However, as our results demonstrate, there are very few such compounds amongst the chemically similar species formed in the course of HD hydrolysis. Surprisingly, most of the other materials studied (irritants, pesticides) are inactive, which makes this method rather selective. A serious disadvantage, however, is that the reagents are expensive and, more importantly, not stable and have to be prepared immediately before use. Furthermore, the persistence of the TLC spots is poor.

The other quite promising method to identify HD is based on a reaction with Michler's ketone. This has only been reported before as a drop reaction[8]; its applicability to TLC is one of the key results of this work. It is superior to the DB 3 method, and it competes very well with the iodoplatinate or the thymolphthalein method as far as sensitivity and selectivity are concerned. Excellent results are obtained if high-performance TLC chromatoplates are used, about 10 ng HD being detectable. Again, the underlying chemistry must be very complex. Supposedly, the cyclic dialkylsulphonium ion (into which HD is easily transformed) attacks the base N atom(s) of the dimethylamino groups of Michler's ketone, thereby forming a sort of diphenylmethane dye molecule. Addition of mercury may be essential in order to achieve an auxochromic effect.

Much clearer is the chemistry of the photochemical reaction using silver ions. This method has also not been applied to TLC before, although it is sensitive and extremely simple, and a similar reagent has been employed for the detection of some pesticides[9]. Since it essentially takes place in aqueous solution, the β-activated chlorine atom(s) can react with Ag^+ to form a precipitate of silver chloride, which then suffers photochemical decomposition into chlorine and metallic silver. Accordingly, this reaction should also be given by all those organic halides (except fluorides) that have a high dissociation constant, yielding the respective halogen atom(s). Surprisingly, however, few perturbations arise from the other compounds investigated, the most important being the reaction with nitrogen mustard which proceeds in exactly the same fashion as with HD.

As far as the triphenylmethane dye–silver nitrate reaction is concerned, again quite complex chemical processes are involved. In previous work concerning the detection of HD in solution, the formation of at least five different coloured species was established[10]. The yellow or red colour of the product species (which is believed to consist of an alkylated triphenylmethane dye molecule) very likely arises from an extended system of conjugated double bonds in which, for some (as yet unknown) reasons, the HD molecule has a strong auxochromic effect. However, despite its utility in solution analytical chemistry, its complexity and poor reproducibility make this dye reaction unsuitable for routine TLC determination of HD.

On the other hand, this is not true for the detection of HD via selenium dioxide, wich is the other important result of this work. In the monograph of Stahl[11] the reaction is listed among others for the detection of active organic halides. It had not been employed for the determination of HD. Chemically, the nucleophilic central S atom of the HD molecule can be oxidized to sulphoxide compounds. Apparently, selenium dioxide is a very selective oxidizing agent which is in turn reduced to elementary selenium with its characteristic red or orange colour.

CONCLUSIONS

We have demonstrated that several analytical methods, previously confined to solution or drop reaction methods, can be favourably employed in TLC. Specifically, reactions involving hexaiodoplatinate, Michler's ketone, silver nitrate, triphenyl-methane dyes and selenium dioxide have been investigated as regards their sensitivity and selectivity. Except for the triphenylmethane dye, all the reactions listed gave good analytical results and point to the suitability and versatility of thin-layer chromatography. A summary of our results is given in Table I.

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