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VESICANT STUDIES I SPECTROPHOTOMETRIC DETERMINATION OF
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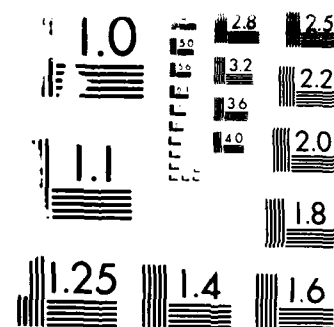


Figure 1. Resolution Test Chart.

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Laboratory Note No. 87-65

AD-A175 334

VESICANT STUDIES
I. SPECTROPHOTOMETRIC DETERMINATION OF N-BUTYL
2-CHLOROETHYL SULFIDE, AN ANALOG OF MUSTARD GAS

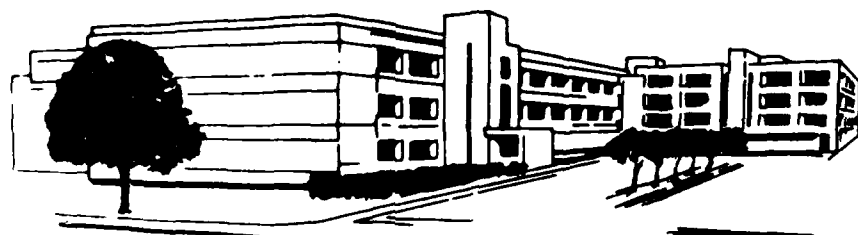
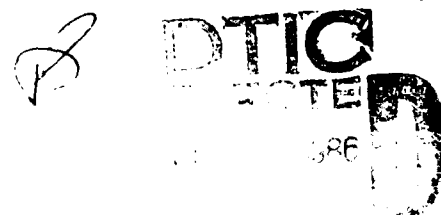
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and
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DIVISION OF CUTANEOUS HAZARDS

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| 20. ABSTRACT (Continue on reverse side if necessary and identify by block number) A spectrophotometric method for the quantitation of n-butyl 2-chloroethyl sulfide (BCS) has been developed using thymolphthalein as the chromogen. The chromophore produced by the reactants has a maximum absorbance at 444 mμ and absorbance is linear up to 1×10^{-6} M BCS. This assay can provide quantitative measurements of the monofunctional mustard down to 3×10^{-6} M. | | |

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Vesicant Studies. I. Spectrophotometric Determination of n-Butyl 2-Chloroethyl Sulfide, an Analog of Mustard Gas--Schmid

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INTRODUCTION

n-Butyl 2-chloroethyl sulfide (BCS) is a monofunctional mustard, which, like mustard gas, is a potent vesicant (1). We failed to find a published quantitative assay for monofunctional mustard. On the assumption that BCS might react similarly to mustard gas, we reviewed methods for mustard gas for their practicality, simplicity and speed. Methods based on titration of hydrogen-ion liberation, bromine titration, ion selective electrodes, thin-layer chromatography and gas-liquid chromatography were excluded for various reasons. The spectrophotometric method based on the Epstein reagent, γ -p-nitrobenzylpyridine, produces a specific chromophore when the reaction product containing the 2-chloroethyl moiety is deprotonated with base (2,3). However, it was reported (4) that the color is unstable and appears to fade after 2 minutes at alkaline pH.

The use of thymolphthalein (Fig 1, structure I) to determine bis-2-chloroethyl sulfide (sulfur mustard) was published in the Russian literature by Telicinem, as reported by Matoušek and Tomeček (5). In 1951, Adamovic and Stromskij (6) showed that nitrogen mustard also reacted with thymolphthalein. This observation was confirmed for nitrogen and sulfur mustard by Issa et al (7), who suggested that condensation of mustard occurred both at the carboxyl group of ring 1 and the phenolic hydroxyl group of ring 2 (Fig 1, structure II). If this were the case, BCS would yield a chromophore (Fig 1, structure III) which would most likely be different from that given by Issa et al (7). Matoušek et al (8), however, showed that a number of chloroethyl compounds, including methyl 2-chloroethyl sulfide, react with thymolphthalein to give a common chromophore with a maximum absorbance at around 450 nm. They also showed that infrared spectra for the reaction product of thymolphthalein with either mustard or methyl 2-chloroethyl sulfide were similar, with the exception of the methyl group for the monofunctional sulfur mustard. On the basis of the work in references (7) and (8), the reaction was studied in our laboratory and a method developed for the rapid determination of n-butyl 2-chloroethyl sulfide.

METHOD

Reagents: All reagents were analytical grade. n-Butyl 2-chloroethyl sulfide was purchased from Waleree Chemical Company, Camden, SC and Columbia Organic Company, Columbia, SC. The laboratory procedure



for handling this chemical is described in the Appendix. Thymolphthalein, reagent grade, was purchased from Eastman Kodak, Rochester, NY. BCS was diluted to a working concentration with dry acetone, or as specified. A 0.02M solution of thymolphthalein was prepared by dissolving 430 mg of the solid in 30 ml of acetone, adding 13.5 ml of 0.1M NaOH and diluting the solution to 50 ml with distilled water.

Spectrophotometric Assay: A working standard solution of BCS (up to 1.8 ml) was added to 3 ml thymolphthalein reagent and 5 ml ethanol. Screw cap test tubes were used and the tubes heated at 70 to 80°C for 25 minutes, or as indicated for a particular experiment. Initially, a water bath was used and later, a Thermolyne Dry Bath Heater, Model 828125, was used. Following cooling of the hot samples to room temperature, two drops of glacial acetic acid were added and the volume made to exactly 10 ml. Spectra were measured with an ACTA III (Beckman Instruments, Palo Alto, CA). Appropriate solvent and reagent blanks were used. Absorbance was measured at the wavelength where absorbance was maximal, or as indicated.

RESULTS AND DISCUSSION

Absorption Spectrum: n-Butyl 2-chloroethyl sulfide reacts with thymolphthalein to give a yellow color with an absorbance maximum at 444 nm (Fig 2). At this wavelength, the concentration is linearly related to the absorbance value in the range up to a concentration of 1×10^{-4} M. The regression equation takes the form of absorbance = $k \times c = 10,100 \times c$, where the constant is the extinction coefficient and c is the molar concentration of the BCS. The correlation coefficient for the regression curve (concentration vs absorbance) was 0.991. The lower detection limit is approximately 3×10^{-6} M for this assay.

Stability of n-Butyl 2-Chloroethyl Sulfide in Acetone and Ethanol: Initially, variations were noticed in the absorbance produced by BCS, which was diluted in 100% ethanol. It was suspected that the BCS might react with the hydroxyl group of ethanol. To test this hypothesis, we prepared samples at two concentrations of 6.42×10^{-5} and 9.63×10^{-5} M in dry acetone and ethanol. The solutions were kept for several days at room temperature. The colors were developed and the spectra determined. The extinction coefficients for the acetone diluted samples were 9550 and 9960 in duplicates, and for ethanol solutions, 6330 and 6380. The alcohol samples used in these stability experiments were yielding about 35% less color than samples prepared and stored in acetone for the same period of time. It would appear that even when 100% water-free ethanol is used to prepare the standard BCS solution, stability of BCS is affected by hydroxylic solvents. Although we have not made a detailed study of the stability of BCS in acetone, we did not find any serious deterioration in samples kept for about 10 days. On theoretical grounds, it would not be expected that BCS would react with acetone; however, care must be taken to prevent introducing water

into the acetone.

Stability of Thymolphthalein Reagent: From references (7) and (8), it is not clear if the amount of sodium hydroxide added to thymolphthalein affects the extinction coefficient. Issa et al (7) state that thymolphthalein is prepared by dissolving the calculated amount in the appropriate volume of specially pure ethanol to which is added sodium hydroxide until the solution acquires a blue color. The colorless reagent, thymolphthalein, has the structure of a lactone, which upon addition of sodium hydroxide, is converted to the sodium salt of the carboxylic acid. Since the reaction of BCS is with the carboxylate anion of thymolphthalein, it is essential to add at least one mole of sodium hydroxide per mole of thymolphthalein. When the reagent was prepared with this in mind, the daily variations in absorbance values of the standard solution disappeared.

In another experiment, acetone was used to dilute BCS and the solution reacted with two different solutions of thymolphthalein. One solution was prepared with the minimal amount of sodium hydroxide so as to obtain a light blue color of the assay solution; whereas, for the "alkaline" thymolphthalein solution 1.1 mole of sodium hydroxide per mole of thymolphthalein was used. The results showed a mean extinction coefficient for the "alkaline" thymolphthalein of 10,100; the mean value for the "minimal" amount of NaOH was only 3500. From this experiment, it can be concluded that the amount of sodium hydroxide definitely and pronouncedly affects the amount of color produced by a standard amount of BCS. Our limited number of tests suggests that the routine of preparing the thymolphthalein reagent on a daily basis is not necessary and that the reagent can be kept for one or two days without significant variation in the results of the standards. A one-week-old and a fresh solution of thymolphthalein were prepared and tested with an ethanolic fresh solution of BCS. The "old" thymolphthalein solution gave an extinction coefficient of 4880 and 5340 for duplicate samples; whereas, the "new" thymolphthalein solution gave values of 5760 and 5500. Spectra of the "old" and "new" thymolphthalein solutions did not differ significantly. However, ethanol as a solvent for the standard BCS solution is not advisable since it results in approximately one-half the absorbance, under standard conditions, as that of a solution of BCS in acetone.

Effect of Heating Time on the Development of Color: According to the procedure of Issa et al. (7), a reaction temperature of 70 to 80°C is recommended. We did not investigate the effect of temperature on color development for BCS with thymolphthalein, but 70 to 80°C appears to be a reasonable temperature which does not create a serious fire hazard. For sulfur mustard gas, the same authors recommended a heating period of 20 to 25 minutes. However, they showed a considerably shorter period of heating was necessary for nitrogen mustard. In view

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of the different reaction rates for the sulfur and nitrogen mustards, and in view of the structural differences of the monofunctional BCS and the bifunctional sulfur mustard, samples were incubated for variable periods of time up to 100 minutes. The results in Fig 3 indicate that a heating period of about 1 hour is required to develop the optimal amount of color.

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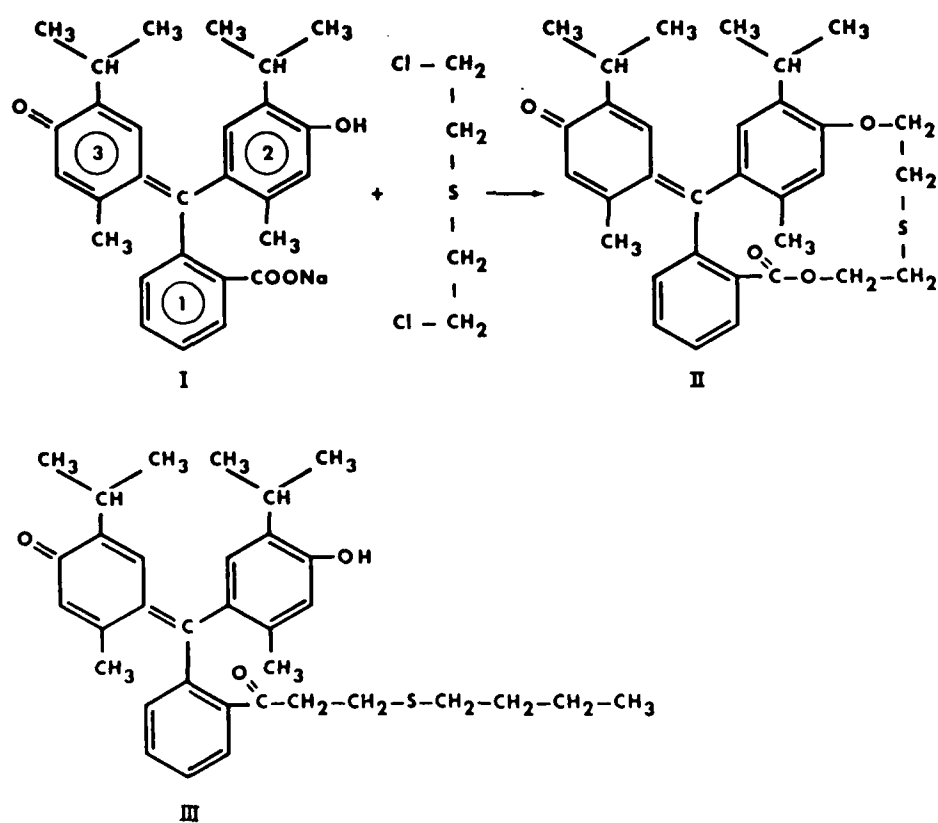
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LEGEND

- Fig. 1 Reaction of thymolphthalein with mustard gas and n-butyl 2-chloroethyl sulfide, a vesicant analog of mustard.
- Fig. 2 Absorption spectrum of the chromophore produced by the condensation of thymolphthalein with BCS.
- Fig. 3 Effect of time on the development of color in the reaction of thymolphthalein with BCS.

Figure 1



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Figure 2

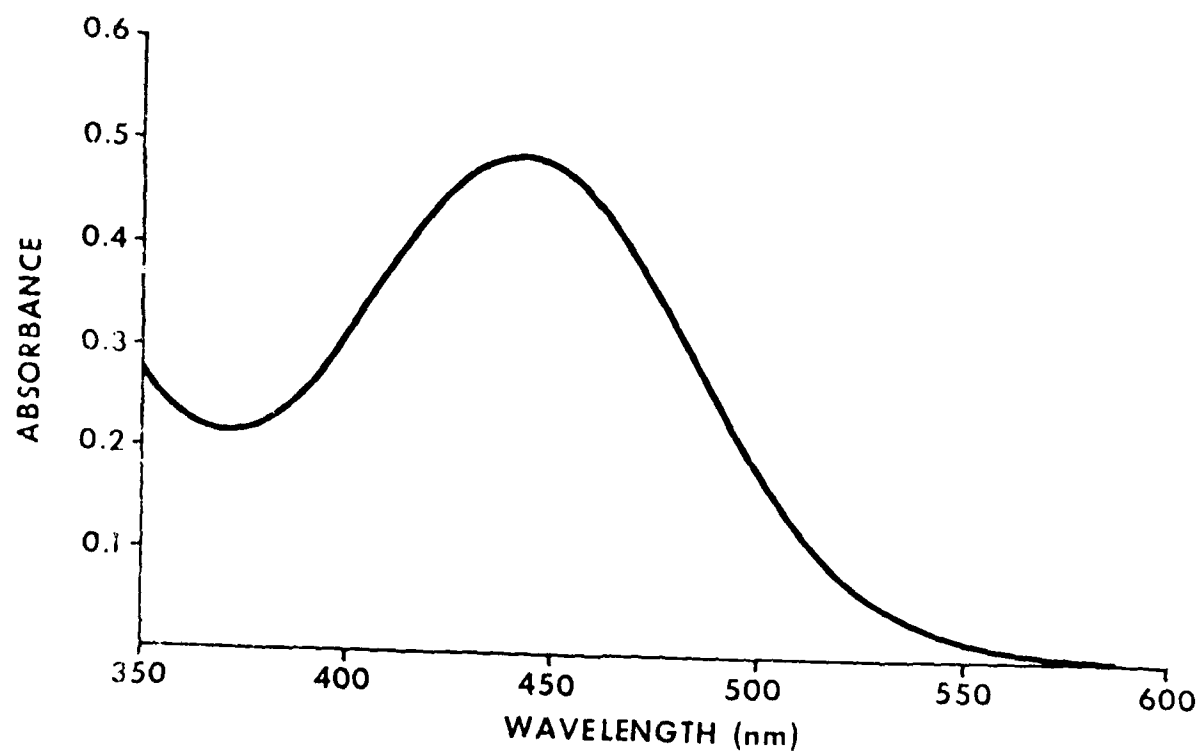
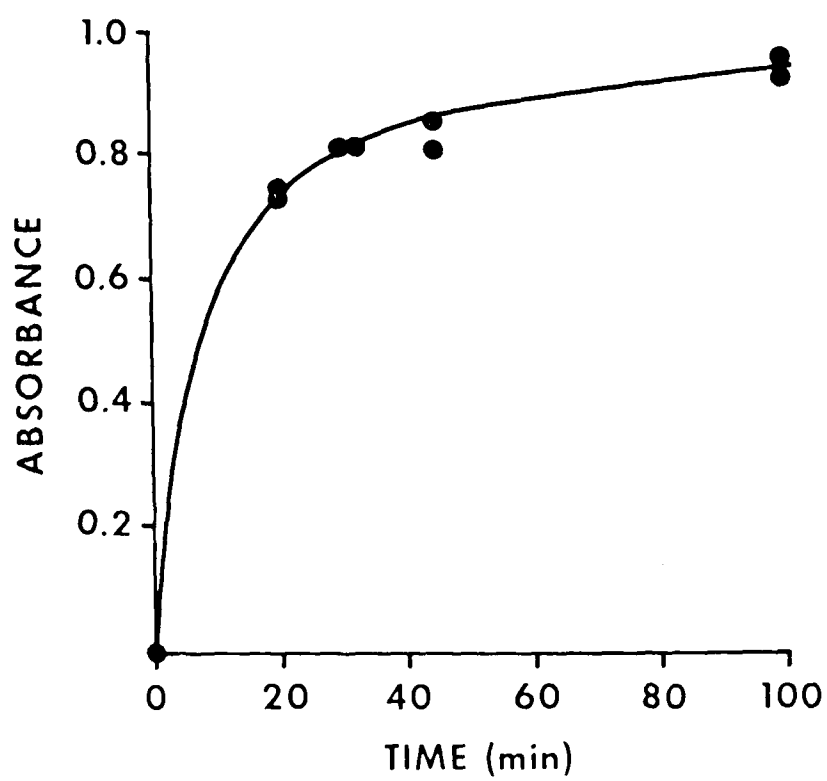


Figure 3



APPENDIX

SOP FOR WORK WITH n-BUTYL 2-CHLOROETHYL SULFIDE (BCS)

1. Storage of BCS:

Neat BCS must be stored in the Chem-Safe located in the refrigerator in LR 1144. During non-duty hours, the Chem-Safe must be locked.

2. Work with BCS:

All work performed with BCS must be done in the hood in LR 1144. Rubber gloves must be worn. A 10% sodium hydroxide solution must be available at all times for detoxification of contaminated areas or equipment. All containers and contaminated materials should be labeled with the yellow CHEMICAL HAZARD label. All lab benches must be covered with absorbent paper.

3. Detoxification of utensils and glassware:

All pipets and glassware that have come in contact with BCS must be placed in the sodium hydroxide solution for at least 18 hours prior to flushing with copious amounts of water and then thoroughly washed. All rubber gloves, paper towels and similar disposable supplies need to be soaked in decontaminant solution and stored in a moist form for at least 18 hours prior to disposal.

4. Detoxification of human skin surfaces:

Use ordinary kitchen flour. Pat flour onto contaminated area to absorb most of the liquid as soon as possible. Contaminated flour must then be disposed of as specified in paragraph 3 after removal with paper towels. Skin will then be thoroughly washed with mild soap solution, followed by copious amounts of water. Accidental contamination of the skin, or inhalation of the vapors, must be brought to the attention of the NCO for initiation of emergency medical treatment.

5. Accidental spillage of BCS:

Following the accidental spillage of BCS on floor surfaces, all personnel should leave the room immediately, except one responsible person, who will put on a gas mask and immediately throw handfuls of flour on the liquid. As soon as it is absorbed, he will proceed to clean up the flour with a hand brush and deposit it into a solution of 10% sodium hydroxide. The contaminated area can be washed with Clorox®, followed by soap and water.

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