2,6-Dibromobenzoequinone-4-Chloroimide as a Reagent for Determination of Dimethoate, Monoalkyl-Aryl-Phosphorothionates and Some Organic Sulfides

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In studies of enzymatic formation and degradation of the monoalkyl derivatives of the thiophosphorus insecticides, we had a great need for a simple and rapid spectrophotometric method for monoalkyl-aryl-phosphorothionates. The possible use of 2,6-dibromobenzoequinone-4-chloroimide (DQC) as a reagent for these compounds was therefore investigated.

DQC has been very successfully used as a chromatographic spray for detection of phosphorothionates (1-3). Rowlands (4) has used the reagent both for qualitative and quantitative determinations of organophosphorus insecticides and their degradation products. His

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quantitative method involves extraction, extensive purifications and heating to obtain the colour reaction. The heating step is very critical and difficult to reproduce. Kamiya (5) has found that a great number of aromatic and aliphatic thioketones which can enolize, give coloured products with DQC in ethanol. Cuzoni and Lissi (6) have used DQC for qualitative and quantitative determination of aromatic and aliphatic disulfides. The coloured products were found to be quinone-sulfeneimines (7).

In this paper the use of DQC for the determination of monoalkyl-aryl-phosphorothionates and some biological interesting sulfhydryl compounds is reported.

EXPERIMENTAL

Reagents: 0,4% w/v of DQC (Fluka) in:

A) acetic acid

B) acetic acid - ethyl ether (1:1) and

C) absolute ethanol adjusted to pH 1,5 with 1 N HCl

The reagents are stable for some days if kept in dark bottles in refrigerator.

Standard solutions: 0,1% w/v of the compounds in acetone or water according to their solubilities.

The compounds tried were: Parathion-methyl, parathion, demeton-methyl, dimethoate, bromophos, diazinon, trichloronat, fenitrothion, monomethyl-parathion, monomethyl-bromophos (sodium salt), monoethyl-bromophos, monoethyl-

trichloronat (2, 4, 5-trichlorophenyl-ethyl-phosphonothionate), monomethyl-fenitrothion, glutathione reduced, glutathione oxidized, cysteinhydrochloridemonohydrate, 2-mercapto-ethanol.

Procedure: 0, 10, 30 and 50 µl of the standard solutions were transferred to test tubes with a 0,1 ml graduated pipette. 3 ml of the respective reagent solutions were added. When the influence of water should be tried, 1 ml of water plus 2 ml of the reagent solution were used. If no colour appeared at room temperature, heating on a boiling water bath for different periodes of time were tried, eventually one drop of concentrated sulfuric acid was added.

The development and stability of the colour were studied in a spectrophotometer.

RESULTS AND DISCUSSION

With all three reagents the monoalkyl-aryl-phosphorothionates and the sulfhydryl compounds (red. glutathione cysteine and 2-mercapto-ethanol) gave fairly stable colours obeying Beer's law (Fig. 1). To improve the reproducibility the colourometry should be undertaken after a standardized time, e.g. 3 minutes. The colours obtained had their absorbtion maxima between 425 nm and 435 nm. For the same compound the maxima could vary from experiment to experiment, probably depending on the exact pH of the solution.

Water caused rapid fading of the colour after a first-order reaction - using reagent A or C (Fig. 2), and therefore it was impossible to measure the absorbance in these reagents when water was present. Ethylether has a stabilizing effect and with reagent B stable colours were therefore obtained also when great amounts of water was present. Dissolved in 1 ml water, buffer solution, or incubation mixture, e.g. liver or grain homogenate, the compounds could easily be determined by adding 2 ml of reagent B. When much protein was present, the samples had to be centrifuged before their absorbances could be read. Homogenate solutions interfered by giving weak yellow colours, possibly due to the presence of sulfhydryl compounds. By careful preparation of blanks it is, however, possible to determine the compounds directly in incubation mixtures by using reagent B.

With 2 ml 0,4% DQC w/v in propionic acid solution stable colours developed slowly in presence of 1 ml water. The blanks had, however, appreciable colour. Propionic acid and the combination of ether and acetic acid was the only solvents which gave stable colours in the presence of water. The other solvents tried as stabilizing agents (butanol, ethanol, dimethylformamide, acetone, ethylacetate, formic acid, pyridine, strong salt solutions, buffer substances of different pH) did

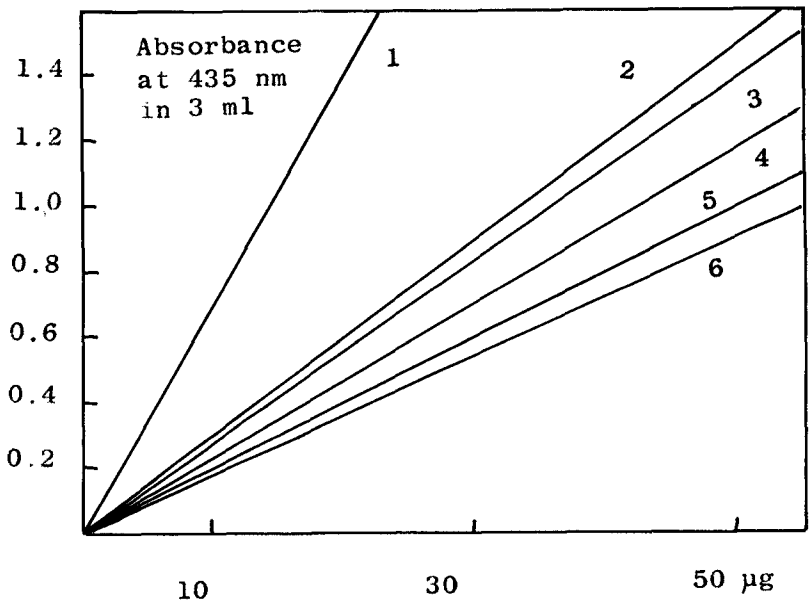

Fig. 1. Standard curves for some of the sulfhydryl compounds, disulfides and mono alkyl-aryl-phosphorothionates. The absorbance is read at the time of maximum colour which was about 3 min. for all but glutathione (35 min.) and dimethoate (15 min.).

1) 2-mercapto-ethanol, 2) Dimethoate (425 nm), 3) Cysteinhydrochloride monohydrate, 4) Monomethylparathion, 5) Monoethyl-bromophos, 6) Oxidized glutathione.

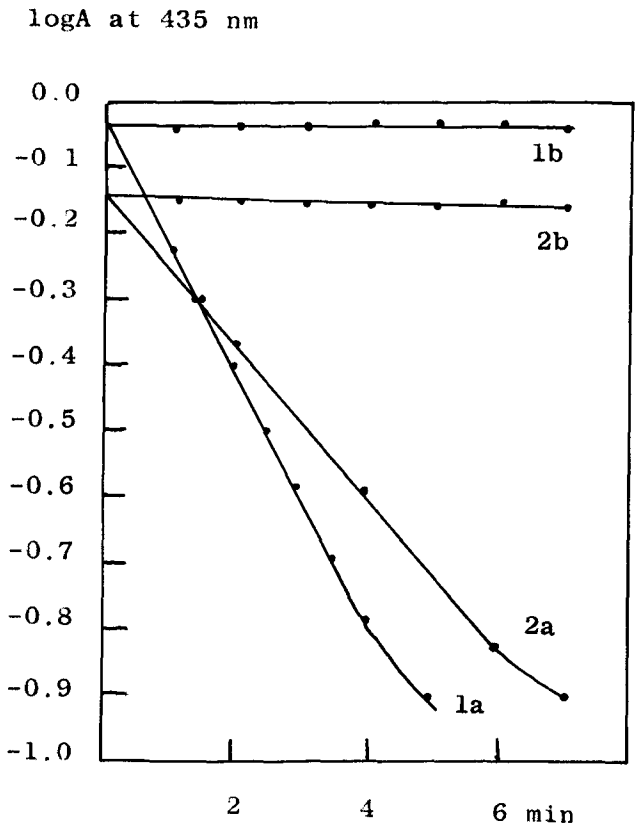

Fig. 2. The fading of the colour produced by 50 μg monomethyl parathion (1) and 10 μg 2-mercaptoethanol (2) in a) 2 ml reagent A plus 1 ml water, b) 2 ml reagent B plus 1 ml water.

not however by themselves inhibit colour development or stability when reagent B was used. Presence of acid buffers (e.g. succinate) may prevent the colour development also in case B by decreasing the acidity of the solution. This may be overcome by adding mineral acid to pH 2 or by using larger amounts of reagent B.

Oxidized glutathione gave a weak yellow colour with reagent B and water (2 ml B plus 1 ml water). With reagent A the colour was completely developed after 35 minutes, and then faded slowly. Beer's law was obeyed. Monoethyl-trichloronat gave a yellow colour with the reagents after addition of one drop of concentrated sulfuric acid. Beer's law was obeyed (max = 412 nm). Water inhibited the colour development in all cases.

The only commercial thiophosphorus insecticide found to give a stable and reproducible colour was dimethoate. With reagent A or C a yellow colour developed to a maximum after 15 min. Beer's law was obeyed (λmax = 425). Reagent B plus water gave no colour.

The molar absorbances of all the compounds obeying Beer's law were between 1.8×10^4 and 2.2×10^4, measured at their respective absorbance maximum.

The methods as described are not suitable for parathion-methyl, parathion, demeton-methyl, bromophos, diazinon, trichloronat or fenitrothion. However, some of these insecticides gave a colour with reagent A or B

after sulfuric acid addition or by heating. As these colours did not follow Beer's law and gave bad reproducibility a hydrolytic step, giving the monoalkyl compounds could probably be included if a general analytical procedure for thiophosphorus insecticides should be developed.

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