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Sodium Ethanemonothiophosphonate. A Weak Antidote to Mustard Gas

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The following work was done in 1940 as part of an antigas programme directed by Prof. R. A. Peters on behalf of the Ministry of Supply. The objective was to discover an antidote to mustard gas (*H*) which could act at a late stage when the vesicant had soaked into living tissue. A review by Peters (1947) gives the references to the original reports to the Ministry and the contribution made by the Oxford group to this problem.

It was assumed as a working hypothesis that the toxicity of *H* was due to the chlorine replacement reaction, the *in vitro* kinetics of which were studied as part of the same programme by Ogston (1948). The interesting feature of this reaction is that the slow disappearance of *H* in aqueous media cannot be accelerated by reagents which replace the chlorine, but that such reagents compete with each other to form *H* substitution products, and that an active reagent may almost completely suppress an inactive one. The power to compete is quantitatively expressible by the 'competition factor' which was measured by Ogston for about eighty substances.

Only the most hopeful of these substances were tested biologically because of shortage of animals at that time. The few rats available sufficed to show that none of the selected substances was a sufficiently good antidote to be of practical importance; but by using forty rats on one substance (sodium ethanemonothiophosphonate) a weak protective action was

demonstrated statistically. This seems to be the only substance which has been proved to give any internal protection against *H*. It was prepared by a new method which is described below together with such biological tests as could be done in the circumstances.

EXPERIMENTAL

Preparation of mono- and di-thiophosphonates

In 1940 three methods were available for the preparation of thiophosphonates. Hofmann & Mahla (1892) treated substituted phosphines with sulphur; Guichard (1899) obtained RPSCl_2 by the action of S on RPCl_2 and Strecker & Grossmann (1916) prepared R_2PSOH from PSCl_3 and Grignard reagents. Since none of these methods was satisfactory, the reaction between Grignard reagents and P_2S_5 was investigated and found to be convenient for the preparation of alkyl or aryl dithiophosphonates (RPOS_2Na_2). All the thiophosphonates were made by the same process, the ethanethiophosphonates being typical examples.

A more complete investigation of the reaction between P_2S_5 and Grignard reagents was subsequently carried out by Malatesta & Pizzotti (1946). These authors claim that quantitative yields of dithiophosphates can be obtained by a suitable choice of experimental conditions.

Disodium ethanedithiophosphonate. Ethyl bromide (327 g.) was converted to the Grignard reagent by dropping on to Mg (72 g.) in dry ether (600 ml.). The solution was then slowly added to a suspension of finely powdered (60 mesh sieve) P_2S_5 (426 g.) in dry ether (400 ml.) at such a rate that the ether was kept gently boiling. This took about 2 hr.

During the initial reaction and for a further 1-2 hr. the mixture was kept vigorously stirred. The product was then decomposed with a small amount of ice and 10N-H₂SO₄ (800 ml.). A sample of the ether was then titrated to pH 7.6 (blue to bromothymol blue) with 0.1N-NaOH and two-thirds of the equivalent amount of 10N-NaOH added to the bulk of the ether. This gave a pH of about 5. The aqueous solution was then heated for 10 min. at 100°, cooled and the remaining one-third of the 10N-NaOH added. (This is probably unnecessary for most purposes, but it served to remove a trace of trithio compounds which were toxic towards the pyruvate oxidase system (Peters, unpublished).

Inorganic compounds were precipitated by the addition of 5 vol. of ethanol and the filtrate evaporated *in vacuo* at 50°. The residual sticky solid was crystallized from a mixture of ethanol (250 ml.) and *isopropanol* (750 ml.); the first crop weighed 245 g. (Found: S, 24.4; P, 12.0; loss at 110° for 18 hr. 13.3. C₂H₅POS₂Na₂·4H₂O, requires S, 24.2; P, 12.0; H₂O, 13.95%). By evaporation of the mother liquor a second crop of material (39 g.) was obtained. This was shown by titration of the free acid to pH 4 and 8 and by acid and alkaline iodine titrations to be probably (C₂H₅)₂PS₂Na.

Ethane monothiophosphonate. Disodium ethanedithiophosphonate (92 g.) was shaken for 10 min. with 10N-H₂SO₄ (330 ml.) and ether (400 ml.). The ether was separated, dried over Na₂SO₄, filtered and the Na₂SO₄ washed thoroughly with ether. A sample of the ether was titrated to pH 7.5 with 0.1N-NaOH to determine the dithiophosphonic acid content, and after evaporating the bulk of the ether *in vacuo* at room temperature, the residue was taken in sufficient water to give a 2-3M-solution. This solution was then heated in a water bath at 100° for 20 min. The time of heating was decided by taking portions from a small sample and, after evacuation to remove H₂S, estimating the I₂ uptake at various intervals. The bulk solution was heated for that time which decreased the I₂ uptake to half its original value. The solution was cooled, evacuated, treated with the calculated amount of 10N-NaOH (as determined by the preliminary titration) and evaporated *in vacuo* at low temperature. Traces of water were removed by re-evaporation with the addition of ethanol. The residue was dissolved in hot ethanol, and *isopropanol* added until a turbidity was produced. On cooling, 68 g. of sodium ethanemonothiophosphonate (C₂H₅PO₂SN₂) crystallized out, and a second crop of 9 g. was obtained by concentration of the mother liquor.

The methane-, pentane-, hexane- and toluene-thiophosphonates were prepared in exactly the same way except for the solvent used for crystallization. The solvent selected was designed to give a sharp separation of the mono- and di-alkane compounds. Thus, pentanedithiophosphonate was crystallized from a mixture of ethanol, ethyl acetate and ether or dioxan; hexanedithiophosphonate from ethanol and *isopropanol*. Methanedithiophosphonate had solubility properties very similar to those of inorganic thiophosphate, and was therefore obtained by fractional precipitation from aqueous solution at pH 12.0 with ethanol.

Biological tests

Compared with the inorganic thiophosphates and the thiophosphoric *O*-esters, which readily hydrolyse to hydrogen sulphide, all the thiophosphonates studied were surprisingly stable, both dry and in aqueous solution. The ethane- and butane-thiophosphonates had very low toxicity. This is shown in comparison with other selected thiols in Table 1, in which *D* is a rough estimate of the 50% lethal dose in mmol./g. of mouse, given by the geometric mean of the highest non-lethal and the lowest lethal dose in a series differing by a factor of two, with one mouse per dose. *D* is probably correct to a factor of two since overlapping responses were never observed. *F* is the kinetic competition factor described above. The substances are tabulated in order of the product *FD* which is a semi-theoretical therapeutic index useful in deciding which thiols to test.

Therapeutic test of sodium ethanemonothiophosphonate. Forty white rats, of both sexes, were each injected in the thigh muscles with 0.5% (w/v) *H* in ethanol (0.2 ml./200 g. body weight). Twenty of the injected rats were kept as controls and the other twenty were immediately injected intraperitoneally with 1 ml. of a M-aqueous solution of the thiophosphonate. Table 2 shows the mortality after 10 days. The survivors were kept under observation for some weeks and at the end of that time no further deaths had taken place.

Table 1. Toxicities and competition factors of SH compounds in mice

(*D*, approximate LD₅₀ in mmol./g.; *F*, competition factor against *H* *in vitro*. All compounds administered as Na salts.)

Substance	<i>D</i>	<i>F</i>	<i>FD</i>
Ethanedithiophosphonate, C ₂ H ₅ .POS ₂ .Na ₂	0.016	1.2 × 10 ⁵	1920
Butanedithiophosphonate	0.016	—	—
Hexanedithiophosphonate	<0.007	1.2 × 10 ³	<840
Bishydroxyethylthiocarbamate, (HOC ₂ H ₄) ₂ N.CS.SNa	0.022	3.2 × 10 ⁴	705
Thiosulphate	0.019	2.5 × 10 ⁴	475
Diethylthiocarbamate, (C ₂ H ₅) ₂ N.CS.SNa	0.0083	3.1 × 10 ⁷	257
Diethanedithiophosphonate, (C ₂ H ₅) ₂ PS.SNa	>0.02	6.7 × 10 ³	>107
Monothiophosphate	0.0025	3.5 × 10 ⁴	88
Thioglucose	0.020	2.6 × 10 ³	52
Ethyl xanthate	0.0025	1.6 × 10 ⁴	40
Phenyldithioacetate	0.0013	4.7 × 10 ³	6.1
Trithiocarbonate	0.0004	—	—
Sulphide	0.0004	—	—
Trithiophosphate	0.0002	—	—

The tests on other substances are given in Table 3, from which an upper limit to the possible protective power of each substance is deducible. This may be useful if any of these substances are re-examined.

Table 2. *Protection of rats against injected mustard gas (H) by sodium ethanemonothiophosphonate*

	No. of rats		
	Total	Survived	Died
H alone	20	10	10
H + thiophosphonate	20	18	2

$P=0.0068$ (Fisher, 1946).

SUMMARY

1. A new method for preparing some mono- and di-thiophosphonates is described.

2. Injected sodium ethanemonothiophosphonate protected a significant percentage of rats against death from injected di-(2-chloroethyl) sulphide (mustard gas).

3. A search for more strongly protective sulphydryl compounds was unsuccessful.

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Table 3. *Preliminary tests on antimustard gas substances*

Substance on skin (1)	Substance injected (2)	No. of rats	
		Died	Survived
None	Sodium diethyldithiocarbamate	3	1
Phenyldithioacetic acid $C_6H_5 \cdot CSSH$	$(C_2H_5)_2N \cdot CSSNa$	4	1
Ethylmercaptomalonate $SH \cdot CH_2 \cdot (CO_2Et)_2$	None	4	1
Sodium diethyldithiocarbamate	None	4	0
None	Sodium bishydroxyethylthiocarbamate	1	0
Ethylmercaptomalonate	$(HOC_2H_4)_2N \cdot CSSNa$	2	1
Sodium bishydroxyethylthiocarbamate	„	5	1
Ethylmercaptomalonate	Sodium thiosulphate	1	1
None	Sodium thioglucose	1	0
Tetraethylthiuram disulphide $[(C_2H_5)_2N \cdot CSS]_2$	None	1	0
Ethyl mercaptomalonate	None	1	0
Phenyldithioacetic acid	None	3	0
None (Control; untreated except with H)	None	19	0

(H: 0.001 ml. was placed in a clipped dorsal region of a 150–200 g. rat. 5 min. later 50–100 mg. of the substance in column 1 was rubbed in and 1 ml. of a *m*-aqueous solution of the substance in column 2 was injected intraperitoneally.)

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