2-HYDROXYIMINOMETHYL-*N*-METHYLPYRIDINIUM METHANESULPHONATE (P2S), AN ANTIDOTE TO ORGANOPHOSPHORUS POISONING. ITS PREPARATION, ESTIMATION AND STABILITY

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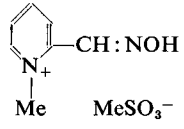
2-Hydroxyiminomethyl-*N*-methylpyridinium methanesulphonate (P2S) is prepared by boiling pyridine-2-aldoxime with methyl methanesulphonate in benzene. It can be estimated both in water and in biological materials by measuring its ultra-violet absorption in alkaline solution. In aqueous solution it is slowly decomposed by heat or prolonged storage with some cyanide ion formation. The stability varies with pH, being optimal at pH 4–5. P2S can be satisfactorily sterilised by filtration through a "millipore" filter.

2-HYDROXYIMINOMETHYL-*N*-METHYLPYRIDINIUM methanesulphonate (P2S) (I) has recently been shown[1] to be very effective when given in conjunction with atropine in the treatment of animals severely poisoned with organophosphate anticholinesterases. It is relatively non-toxic to animals[2] and has been given to man without ill-effects, in doses of a size known to be effective against poisoning in animals[3]. P2S is closely related to the better-known PAM (II) which has been successfully used in parathion poisoning



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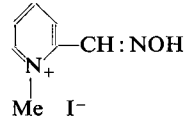

(II)

in man[4], but it has the advantage over PAM in being much more water-soluble so that the relatively large doses necessary may be administered intramuscularly instead of intravenously as with PAM. As P2S may replace PAM we have provided this note on some of its properties of pharmaceutical interest.

EXPERIMENTAL AND RESULTS

Preparation and Physical Properties

100 g. pyridine-2-aldoxime (commercial) was boiled under reflux for about 3 hours with 120 ml. of methyl methanesulphonate in 1 l. of benzene. When cool the crude product was filtered off, sucked fairly dry, and then dissolved in 500 ml. boiling ethanol. Ethyl acetate was added to the filtered hot ethanol solution until crystallisation began. P2S is obtained in a yield of about 100 g. of white, very hygroscopic crystals, m.p. 155°. It is very soluble in water (at least 1 g. in 2 ml.) and is a weak acid (pKa in water, 8·0).

Estimation

P2S ionises in dilute alkaline solution to give a yellow anion with a strong ultra-violet absorption maximum at 335 mμ. (see Fig. 1). This absorption provides a satisfactory method of estimation. 0·2 ml. 20 per cent sodium hydroxide is mixed in a 1 cm. silica cell with 3 ml. of an aqueous solution containing about 30 μg. P2S. The optical density is measured at 335 mμ and the P2S content read from a calibration curve (Fig. 2).

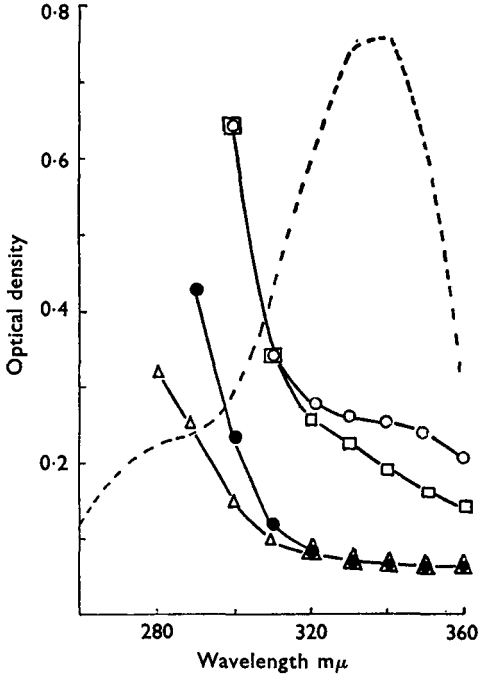

FIG. 1. Ultra-violet spectra of P2S and some tissue extracts in 1·25 per cent NaOH.

- - - 30 μg P2S + 0·2 ml. of 20 per cent NaOH + 3 ml. of water.
—○— Kidney
—□— Liver
—●— Muscle
—△— Whole blood
} 3·0 ml. of protein-free solution (equivalent to 0·6 g. tissue or 0·6 ml. blood) + 0·2 ml. of 20 per cent aqueous NaOH.

None of the likely contaminants of reasonably pure samples, such as pyridine-2-aldehyde or pyridine-2-aldoxime, absorb light appreciably at 335 mμ, and do not interfere. All the spectra were measured with a Unicam SP500 spectrophotometer.

Estimation of Cyanide in P2S Solutions

One of the decomposition products of P2S on prolonged storage or on heating the aqueous solution is cyanide ion (see below). This cyanide can be satisfactorily estimated by Aldridge's colorimetric method[5], but as the colour produced may be affected by the presence of P2S it was found desirable to incorporate a suitable control (method A below) or separate the cyanide from the P2S before estimation (method B).

Method A. 1 ml. of 20 per cent aqueous P2S was diluted with 3·9 ml. of water and 0·1 ml. 10N sulphuric acid. The cyanide concentration in 3 ml. of this solution was estimated immediately by Aldridge's procedure. To provide a control, the hydrogen cyanide was removed from the remaining 3 ml. of the solution by the passage of a steady stream of air through the solution for 1 hour. A repeat "cyanide" estimation on 2 ml. of this solution gave an appropriate control and the true cyanide concentration was obtained by difference.

Method B. 1 ml. N sodium hydroxide was placed in the inner well of a Conway dish and in the outer compartment were placed (separately, on opposite sides) 1 ml. 20 per cent aqueous P2S and 0·2 ml. 10N sulphuric acid. The lid was rapidly placed in position before the two solutions in the outer compartment had come into contact. The dish was then shaken gently about 120 times a minute for 1 hour. 0·5 ml. of the sodium hydroxide in the inner compartment was then mixed with 0·1 ml. 10N sulphuric acid and 1·4 ml. water and the cyanide was estimated as above.

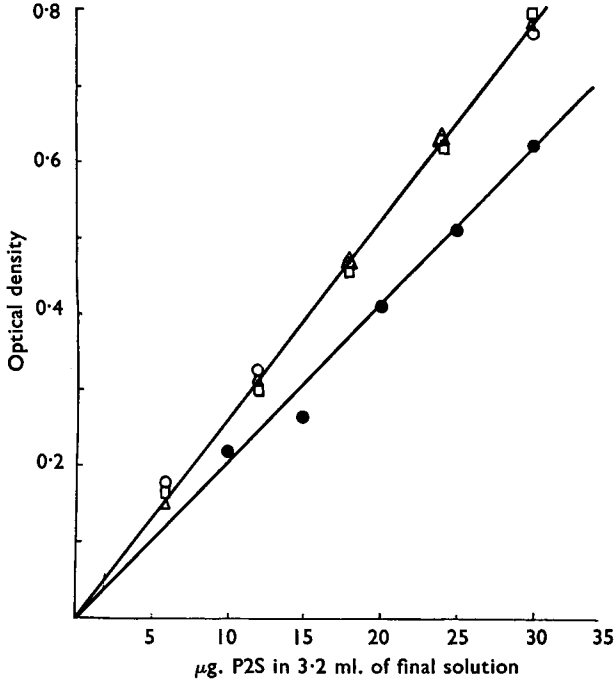

FIG. 2. P2S calibration curve. The tissue curves have been corrected for the absorption due to the tissue itself.
—△— Water. —□— Whole blood. —○—Muscle homogenate. —●— Liver homogenate.

Stability of P2S

When P2S was heated in solution in an attempt to sterilise it, it was found that small quantities of cyanide were formed accompanied by a change in colour of the solution from very pale yellow to orange-brown. These changes are dependent on the temperature and pH of the solution. When 20 per cent aqueous P2S was heated at 100° for 30 minutes the following percentages (by weight based on the initial amount of P2S) of cyanide were found: at pH 5, 0·3; at pH 6, 1·1; and at pH 7, 2·8 per cent. In Table I the amounts of cyanide formed and P2S remaining are shown after storage of 20 per cent aqueous P2S in glass at room temperature for 109 days at pH values in the range 3–7.

TABLE I

pH	3	4	5	7
Cyanide formed, per cent	0·04	0·07	0·30	0·50
Unchanged P2S, per cent	93	97	99	61

When a solution of P2S was adjusted to pH 6·0, freeze dried and stored in a sealed glass container for 6 months some cyanide was detected by its smell on opening the container. But, when P2S was dried from solution at pH 4·5 no cyanide was detected after storage either by smell or by chemical analysis.

Sterilisation of P2S

From the above experiments it is clear that any method involving heat would be unsuitable for the sterilisation of P2S. However, filtration through a "millipore" filter has been found to be adequate. If an asbestos filter is used the P2S solution becomes yellow-coloured with a pH about 6 due to the extraction of alkali from the filter. Our practice is to make a 15 per cent solution of P2S at pH 4·5 in pyrogen-free water which is then filtered. 3 ml. portions of the filtrate are freeze-dried into sterile ampoules, which are then sealed. When required for use the ampoule is opened and the P2S is dissolved in 3 ml. sterile pyrogen-free water. Sg. t. G. D. Wedd tells us this solution can be safely injected into the thigh muscle without causing either pain or tissue damage. Samples from each batch are tested for sterility, cyanide and P2S content.

Estimation in Biological Materials

The simple ultra-violet absorption method is applicable to estimating P2S in biological material provided a correction is made for the absorption of the tissues themselves (see Fig. 1), and provided these are first deproteinised by treatment with zinc sulphate and barium hydroxide as in the examples below.

Tissues were ground with an equal weight of sand and twice their weight of 0·9 per cent sodium chloride. The mixture was centrifuged and 3 ml. of the supernatant was mixed with 1 ml. 0·3M barium hydroxide and 1 ml. 0·33M zinc sulphate. The slight excess of zinc sulphate was needed to give a clear supernatant. The precipitated protein was removed by centrifugation and the supernatant used in the P2S estimation. With liver extracts, this supernatant was often opalescent because of the presence of glycogen; this was removed without significantly affecting the P2S by heating the solution at 100° for 3 minutes with 1 per cent by volume of concentrated hydrochloric acid.

Blood. 2·0 ml. of whole blood was mixed with 3·8 ml. water, and 1 ml. 0·3M barium hydroxide; after haemolysis was complete, 1 ml. 0·33M zinc sulphate and 0·2 ml. 20 per cent sodium chloride were added and the mixture centrifuged. The sodium chloride caused the precipitate to shrink, thus giving the maximum volume of supernatant; this was then used in the P2S estimation.

Urine. 0·2 ml. of urine was usually diluted 1 : 10 before treatment with the barium and zinc solutions. Diluted urine 0·2 ml. was mixed with water 2·8 ml., 1 ml., 0·3M barium hydroxide and 1 ml. 0·33M zinc sulphate. The supernatant obtained by centrifuging was used for the estimation of P2S.

Faeces. Dried faeces were ground with 3 times their weight of water and were then centrifuged. 1 ml. of the supernatant was deproteinised by addition of 5 ml. water, 1 ml. 0·3M barium hydroxide and 1 ml. 0·33M zinc sulphate followed by centrifugation. The supernatant was used in the estimation of P2S.

3 ml. of the final protein-free supernatant from any of the above materials was mixed with 0·2 ml. 20 per cent sodium hydroxide and the optical density measured at 335 mμ. The P2S concentration was read from a predetermined calibration curve (Fig. 2) after correction for the absorption of the biological materials themselves at this wavelength (Fig. 1). The minimum sensitivity is about 1 μg. of P2S per ml. of the final solution. Complete recovery of P2S added to various tissue homogenates was obtained except in the case of liver homogenate which slowly decomposed P2S (see Fig. 3).

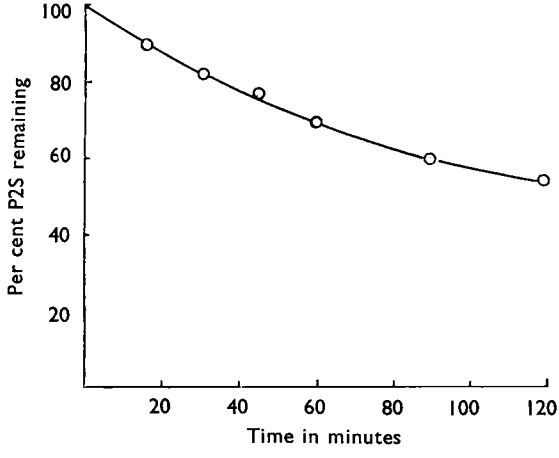

FIG. 3. Breakdown of P2S by liver homogenate at 37°. 300 μg. of P2S added per ml. of 25 per cent. rat liver homogenate in saline.

It was impossible to obtain tissue blanks from the same animals that had been treated with P2S. However, the variation in tissue blanks was not large and a blank value previously determined for a group of animals was used to calculate individual tissue levels within certain limits. The mean value of the blanks for 12 different samples of rat skeletal muscle expressed as μg. of P2S per g. was 5·6 (S.D. 1·1); 5·6 μg./g. was therefore used as a fixed blank to be subtracted from all estimates of muscle P2S levels. The corresponding values used for other biological materials were: whole blood 2·7 μg./ml. (S.D. 0·4), liver 11·4 μg./g. (S.D. 2·4), kidney 21 μg./g. (S.D. 3·7); urine diluted 1 : 10 had a negligible blank.

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