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The effect of pH and sodium metabisulphite on the stability of physostigmine sulphate solutions to heat and ionizing radiation

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A method which assays the alkaloid in the presence of its breakdown products has been used to investigate the effects of heating and exposure to ionizing radiations on the degradation kinetics of aqueous physostigmine solutions. Maximum stability at 90° is between pH 2·2 and 3·0. Sodium metabisulphite retards the degradation of physostigmine when exposed to gamma irradiation, but has no effect on the degradation by heat.

A MAJOR difficulty in investigating the stability of physostigmine in aqueous solution has been the lack of a specific assay technique whereby physostigmine may be assayed in the presence of its degradation products. Haugas (personal communication) has devised a method based on the reaction of physostigmine with sodium nitrite in acid solution to form a yellow nitroso-compound which, after stabilizing with ammonium sulphamate, is extracted with chloroform, and the intensity of the colour measured at 417 mμ. A modification of Haugas' method (Fletcher, 1968) has enabled physostigmine to be successfully assayed in the presence of its breakdown products and has afforded a fresh opportunity to assess the chemical stability of physostigmine solutions. In this paper a comparison of the influence of pH and sodium metabisulphite on the effects of heat or exposure to ionizing radiation on the degradation kinetics of aqueous physostigmine systems is reported.

Experimental

Materials. Ammonium sulphamate (Laboratory reagent, B.D.H.); citric acid, chloroform, disodium phosphate, lactic acid, sodium chloride and sodium nitrite were AR grade, glycine (Biochemical grade, B.D.H.) and physostigmine sulphate B.P.C. (suppl. 1966).

Reagent solutions. Lactic acid 20% w/v in distilled water. Sodium nitrite 1% w/v in distilled water.

Buffer solutions. (a) For aqueous solutions of physostigmine sulphate in the *absence* of sodium metabisulphite, Sorensen's glycine–sodium chloride–0·1N hydrochloric acid buffer (pH range 1·2 to 3·6) was used. For the range 2·2 to 8·0 McIlvaine's citric acid–disodium phosphate buffer was used.

(b) For aqueous solutions of physostigmine sulphate at pH 7·0 to contain sodium metabisulphate the formulae used were: (i) sodium metabisulphite 0·2 g, citric acid (0·1M) 12·0 ml, disodium phosphate (0·2M) to 100·0 ml; (ii) sodium metabisulphite 0·5 g, citric acid (0·1M)

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5·0 ml. disodium phosphate (0·2M) to 100·0 ml. Unlike the buffers described in Documenta Geigy (p. 315) these proved satisfactory in controlling the pH.

ASSAY METHOD

Physostigmine sulphate solution (0·3 ml), lactic acid solution (10 ml) and sodium nitrite solution (1 ml) were shaken for 30 sec. After 30 min to allow development of the yellow nitroso-compound, ammonium sulphamate (1 g) was added and the yellow compound extracted with 8, 4, 4 and 4 ml aliquots of chloroform. The combined chloroform layers were made up to 25 ml with chloroform. The absorbance of the chloroform solution was determined at 417 mμ in a Unicam SP 600 spectrophotometer using 1 cm glass cells. Each determination was in duplicate, the results averaged and used to calculate the percentage of physostigmine in the sample by reference to a Beer-Lambert plot. The ratios of the standard errors of the slopes to the slopes were better than 0·02. Because of batch to batch variation Beer-Lambert plots were constructed for each fresh batch of physostigmine sulphate used.

HEATING EXPERIMENTS

The effect of pH on the degradation rate of aqueous physostigmine sulphate solutions at 90°. To 2 ml, of an approximately 10% w/v solution of physostigmine sulphate were added 38·0 ml of the required buffer solution, previously heated to 90°, to provide a final concentration of 0·5% w/v. This solution was shaken continuously in an oil bath at 90° $\pm$ 0·5°, 1·0 ml samples being withdrawn at suitable intervals of time and 0·3 ml aliquots assayed. Solutions of physostigmine sulphate at pH 8·0, 7·0, 6·0, 5·1, 4·05, 3·0, 2·2 and 1·5 were used and the degradation rate constants at each of these pH values were calculated. The results are shown in Fig. 2.

The effect of sodium metabisulphite on the degradation rate of aqueous physostigmine sulphate solutions. Solutions of physostigmine sulphate 0·5% w/v containing 0·2 or 0·5% w/v sodium metabisulphite were buffered to pH 7·0 as previously described and 2 ml of each solution was placed in 5 ml neutral glass ampoules and heated at 90° $\pm$ 0·5°. Samples were removed at the requisite time intervals, 0·3 ml aliquots assayed and the degradation rate constants for the two solutions calculated.

The rate constant (k sec^{-1}) for physostigmine sulphate solutions containing sodium metabisulphite 0·2, 0·5 and 0·5 (but irradiated with 2·5 megarads γ-radiation) (% w/v) are respectively $3{\cdot}9650 \times 10^{-4}$, $3{\cdot}6580 \times 10^{-4}$ and $3{\cdot}2973 \times 10^{-4}$.

IRRADIATION EXPERIMENTS

Physostigmine sulphate solution (40 ml) at a known pH was placed in a "Graviner Gravatom $1\frac{1}{2}$ in Fixed Cobalt 60 Source" whose characteristics have been described elsewhere (Fletcher, 1968). At the requisite time intervals for the required dose, 1 ml samples were withdrawn and 0·3 ml aliquots assayed. The following systems were investigated and

degradation rate constants obtained. Physostigmine sulphate solutions at pH 1·5, 5·1 and 7·0 and at pH 7·0 containing 0·2 or 0·5% w/v sodium metabisulphite.

The results for physostigmine alone at pH 1·5, 5·1 and 7·0 are in Table 1. The degradation rate constants of the physostigmine solutions containing sodium metabisulphate at 0·2 and 0·5% w/v are 0·0580 and 0·0475. The value without the metabisulphite is 0·1029.

PRE-IRRADIATION AND SUBSEQUENT HEATING EXPERIMENTS

Quantities of 40 ml of two 0·5% w/v physostigmine sulphate solutions, one containing 0·5% w/v sodium metabisulphite, were irradiated with a sterilizing dose of 2·5 megarads. Aliquots of 2 ml of the solutions were then placed in 5 ml neutral glass ampoules and heated at 90° ± 0·5°. Samples were removed at suitable time intervals and 0·3 ml aliquots assayed as before and the degradation rate constants obtained.

Results and discussion

On all occasions when solutions of physostigmine were heated or irradiated, plots of log percentage residual concentration against time or dose of radiation were rectilinear. Thus the initial rate of disappearance of physostigmine follows first-order kinetics.

The curves obtained when solutions of different pH were heated at a constant temperature are shown in Fig. 1 and a plot of log k (specific rate constant) against pH is in Fig. 2. These show that physostigmine is more stable at acid pH than at alkaline pH and that the pH of maximum stability exists between pH 2·2 and 3·0.

The curves obtained by irradiation of solutions of different pH were superimposable indicating that the specific rate constant does not change over the pH range of 1·5 to 7·0. The observed rate constant for irradiation will include a temperature effect and the true radiation constant is

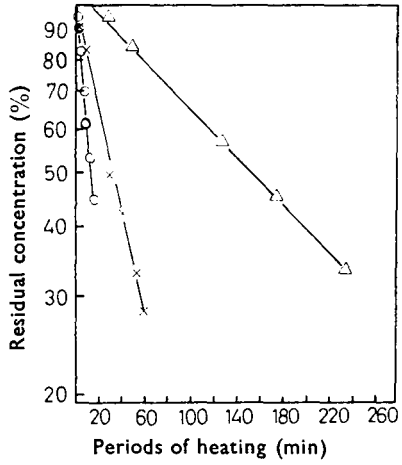


FIG. 1. Plot of "percentage residual concentration" against "period of heating" for solutions of physostigmine sulphate at pH 8·0 ○ —— ○; pH 7·0 × —— ×; and pH 6·0 △ —— △, all at 90° C.

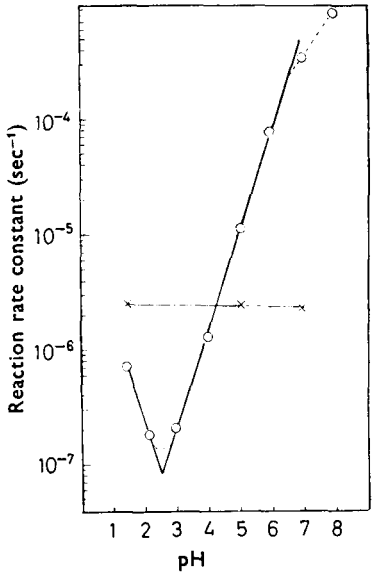


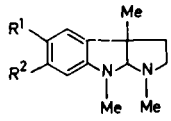
FIG. 2. Plot of "reaction rate constant" against pH for solutions of physostigmine sulphate heated at 90° C ○ —— ○; and for solutions irradiated × —— ×.

given by $k_{irr} = k_{exp} - k_{heat}$. The temperature of the Cobalt 60 source used was about 23° and Table 1 shows the calculated values for k_{irr} in units of time and units of radiation dose. Fig. 2 clearly shows that in the plot of log k_{irr} against pH, the rate of degradation caused by radiation is almost independent of pH.

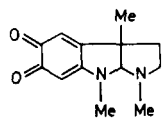
TABLE 1. DEGRADATION RATE CONSTANTS $K_{exp.}$, K_{heat} AND $K_{irr.}$ FOR THE IRRADIATION OF PHYSOSTIGMINE SULPHATE SOLUTIONS AT pH 1·5, 5·1 AND 7·0

	$k_{exp.}$		k_{heat}	k_{irr}	
pH	Mega-rad^{-1}	sec^{-1}	sec^{-}	sec^{-1}	Mega-rad^{-1}
1·5	0·1119	$2{\cdot}551 \times 10^{-6}$	$1{\cdot}068 \times 10^{-11}$	$2{\cdot}551 \times 10^{-6}$	0·1119
5·1	0·1078	$2{\cdot}458 \times 10^{-6}$	$6{\cdot}267 \times 10^{-10}$	$2{\cdot}458 \times 10^{-6}$	0·1078
7·0	0·1029	$2{\cdot}345 \times 10^{-6}$	$1{\cdot}933 \times 10^{-8}$	$2{\cdot}326 \times 10^{-6}$	0·1021

It is therefore apparent that the mechanism of breakdown of physostigmine sulphate in these two processes is different. When the alkaloid is heated in aqueous solution it undergoes hydrolytic cleavage of the methylurethane side chain.



Physostigmine: $R^1 = MeNHCO_2^-$; $R^2 = H$
Eseroline: $R^1 = OH$; $R^2 = H$
Leuco-rubreserine: $R^1 = R^2 = OH$



Rubreserine

The irradiated alkaloid may decompose by two possible mechanisms: (a) a direct ionizing effect on the molecule leading to disruption, and (b) an indirect oxidative chain reaction effect due to attack of such agents as $OH^{\cdot}$, $HO_2^{\cdot}$, $H^{\cdot}$ and H_2O_2 resulting from the irradiation of water.

Sodium metabisulphite has little effect on the degradation rate of physostigmine sulphate, the plot of % residual concentration against period of heating being superimposable in solution with and without metabisulphite. This confirms the reports of Hellberg (1949) and Riegelman & Vaughan (1958) that the presence of an antioxidant does not stabilize physostigmine solutions but simply masks the development of colour resulting from further degradation. This prevention of colour development is probably due to reduction of rubreserine to the colourless leuco-rubreserine (Heacock, 1959).

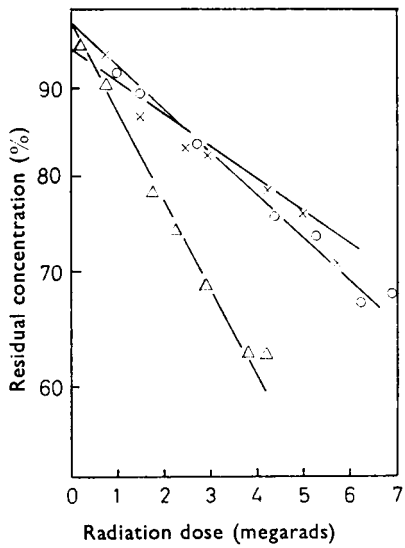


FIG. 3. Plot of "percentage residual concentration" against "dose of radiation" for 0% w/v △ —— △; 0·2% w/v ○ —— ○; 0·5% w/v × —— × sodium metabisulphite in physostigmine sulphate solutions at pH 7·0.

Fig. 3 shows that the presence of sodium metabisulphite in solutions of physostigmine improves the stability of the alkaloid towards irradiation under the conditions described: 0·5% w/v solutions are more effective than 0·2% w/v. The mechanism of degradation is probably oxidative.

Irradiation with 2·5 megarads has no effect on the degradation rate of the solution when subsequently heated at 90°. Since the plots of % residual concentration against dose of irradiation and subsequent period of heating and period of heating with no irradiation are superimposable, it may be concluded that a solution, when stored after irradiating, will degrade at the normal rate for the storage temperature. The presence of 0·5% w/v sodium metabisulphite does not affect the rate

of degradation of irradiated solutions when subsequently heated, but does affect the amount of degradation that occurs during the exposure to 2·5 megarads. Knowing the amount of degradation resulting from 2·5 megarads, it is possible to formulate eye-drops to include the required excess of drug. Thus the solutions could be presented sterile and containing the full amount of the stated amount of drug in the preparation.

Another aspect of the results is that the only effective method of stabilizing physostigmine solutions to heat degradation is to reduce the pH of the solution. For maximum stability, a pH of 3·0 is required and this is generally considered too low for ophthalmic solutions. Schradie & Miller (1959), however, have suggested that provided a solution is buffered with a low capacity system such that, on administration, it quickly adjusts to the normal pH of the eye, a solution at pH 3·0 is quite satisfactory. Using ionizing radiation where the degradation of the alkaloid is independent of the pH of the solution, it is possible, however, to formulate the eye-drops at pH values more acceptable for administration.

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