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DETERMINATION OF THE STABILITY OF MITOMYCIN C BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

The stability of mitomycin C in normal saline solution at elevated temperatures has been investigated by an high-performance liquid chromatography method. The reaction is found to follow first order kinetics and the rate constant for the decomposition at 25°C is estimated by extrapolation. The decomposition of mitomycin C is shown to produce different products in acid and basic solutions by utilising the technique of soap chromatography. First order rate constants are reported as a function of pH and the advantages of the high-performance liquid chromatography method when applied to such rate studies are discussed.

INTRODUCTION

Mitomycin C (Fig. 1), an important antitumour antibiotic, is stated (Korsybski et al., 1967) to be stable in the solid state but to lose activity in solution. Since mitomycin C already mixed with sodium chloride is administered after dissolving the solid in distilled water it was intended, in this investigation, to determine its stability under these solution conditions. Physical chemical investigations of porfiromycin, which differs from mitomycin C only in the methyl substituent on the aziridine nitrogen, have indicated that the decomposition of that compound is complex and dependent on the pH of the solution in

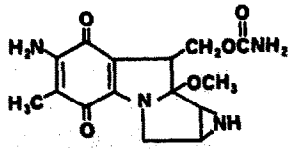

Fig. 1. Mitomycin C.

which the decomposition occurs (Garrett, 1963). Kinetic measurements on porfiromycin (Garrett and Schroeder, 1964) utilizing visible and ultraviolet spectrophotometry have enabled the stability of this drug to be estimated.

While spectrophotometry has been the traditional method of following the kinetics of drug decomposition it is accepted that the method is relatively non-specific especially if absorbing products are formed during the reaction. The rapidly developing high-performance liquid chromatography (h.p.l.c.) technique offers the advantage of much higher specificity as well as providing qualitative and quantitative information on the products formed during decomposition (Taylor and Sood, 1978). Chromatographic methods have been used to study the stability of ampicillin where specificity with respect to degradation products is required (Tsuji and Robertson, 1975) using ion exchange. The ion-exchange method has the disadvantage of relatively low efficiency and the more modern technique of soap chromatography on reverse phase columns can provide a more highly resolving chromatographic system (Gilbert and Pryde, 1979). No chromatographic investigation of mitomycin C and its degradation products has been previously reported and it is intended in this work to show that the h.p.l.c. method has adequate precision for the determination of rate constants as well as providing information on the type of reaction products produced during the decomposition.

MATERIALS AND METHODS

Mitomycin C (Kyowa), obtained from Dales Pharmaceuticals Ltd. in the form of stoppered vials containing 2 mg mitomycin C mixed with 48 mg sodium chloride, was dissolved in distilled water. Chromatographic solvents were obtained from Rathburn Chemicals and other reagents were of BDH Analar grade.

The chromatographic system used was a modular Applied Chromatographic Systems liquid chromatograph using a pneumatic syringe pump and fixed wavelength (254 μm) detector. For confirmatory work a Pye Unicam LC3 variable wavelength detector was also used together with a Pye Unicam SP1800 spectrophotometer with 10 μl flow cell.

Stainless steel (100 X 4.6 mm) columns were used. These were packed at 6000 p.s.i. with Hypersil ODS 5 μm (Shandon Southern Products Ltd.) using methanol/acetate buffer solutions. Syringe injection was used (5–25 μl) and the internal standard, when used, was n-propyl-p-hydroxybenzoate.

Rate measurements at high temperatures: individual samples of the mitomycin C sodium chloride mixture (1.04 mg/ml) were sealed in 0.5 cm^3 ampoules, placed in a drilled aluminium block and heated in an air oven at the required temperature for appropriate times. Decomposed samples were then chromatographed directly or after addition of the internal standard.

Rate measurements in buffer solutions: a standard solution of mitomycin C in either acetate or borate buffer was prepared in a volumetric flask and immersed in a darkened constant temperature bath at 25°C for the required time intervals. Samples were removed, internal standard added, and injected as above. The peak height of the mitomycin was found to be linearly related to the concentration (R^2 = 0.998) and a relative standard deviation of 4.3% was obtained on replicate injections of a partially decomposed solution.

RESULTS AND DISCUSSION

Chromatography

It was observed during the investigation that the undecomposed mitomycin C could be measured as a unique peak when the degraded mixture was eluted with acetonitrile/water solvents. The retention time could be adjusted, and thus the analysis time, by varying the acetonitrile/water ratio. The purity of the mitomycin C peak was verified by its absence on completely decomposing the sample, by failure to increase resolution on decreasing the solvent strength and by measuring its ultraviolet spectrum by the stopped flow technique. Using these chromatographic conditions the products of the reaction were unretained by the column and appeared with the solvent front.

Since the most likely reason for the lack of retention of the products by the reverse phase system was that the products were appreciably ionised in the eluting solvent, the technique of soap chromatography was utilised in order to demonstrate the formation of ionised reaction products. Specimen chromatograms are shown in Figs. 2 and 3. Chroma-

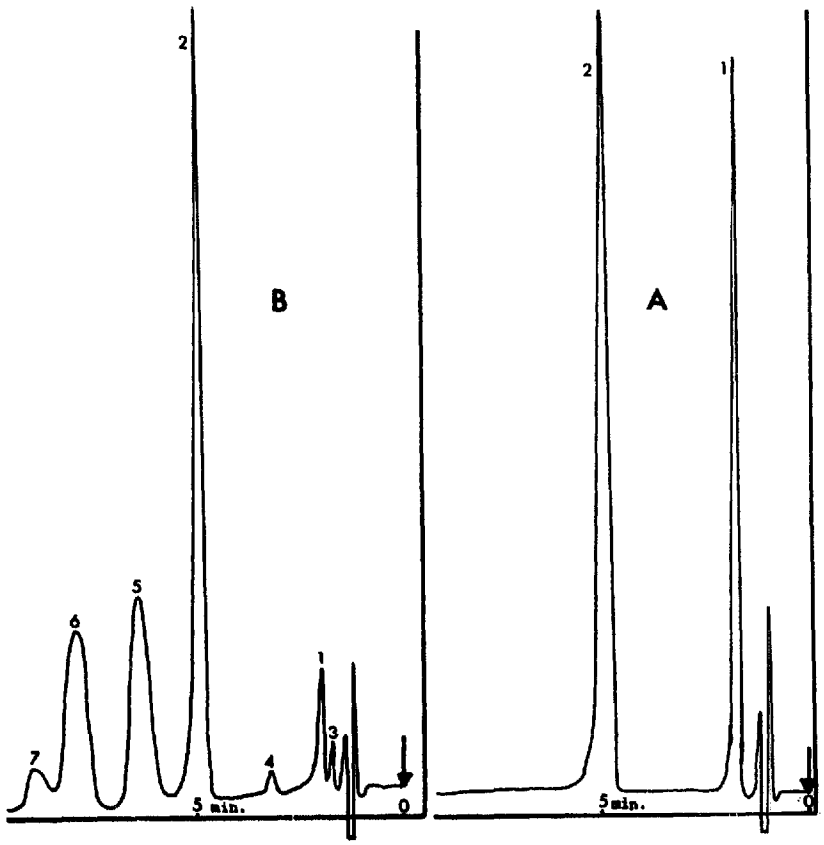

Fig. 2. Specimen chromatograms of mitomycin C decomposition in pH 3.45 buffer. A: initially; B: after 211 min. Eluant, acetonitrile : water 25 : 75 + 0.2% w/v sodium lauryl sulphate + 0.025 M sodium acetate. The full scale absorbance value is shown after each peak: 1, mitomycin C (0.16); 2, internal standard (0.16); 3–6, decomposition products (0.04).

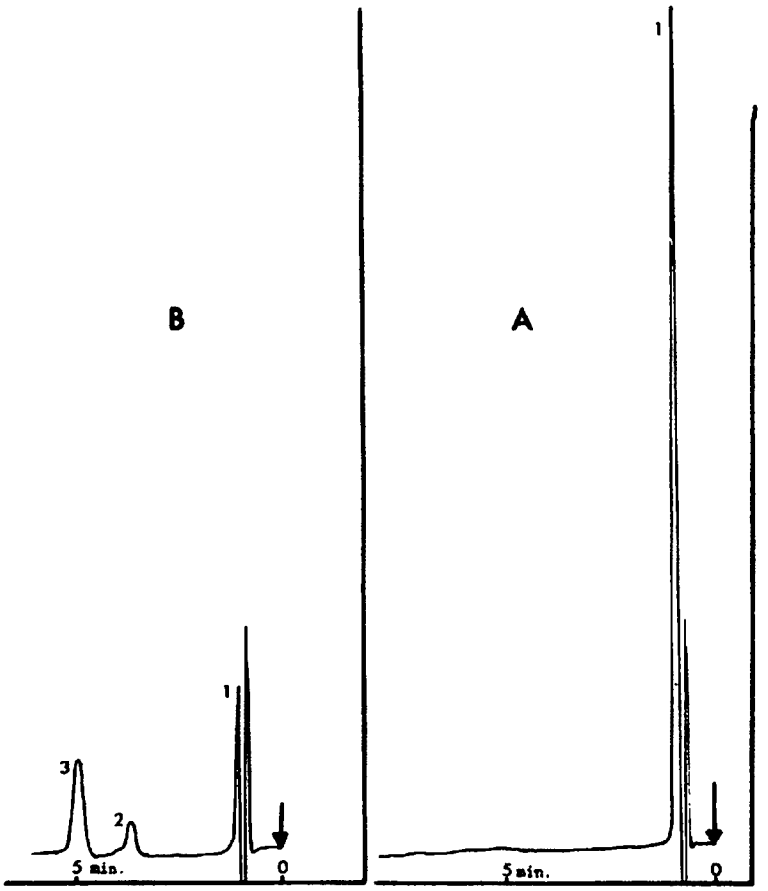

Fig. 3. Specimen chromatograms of mitomycin C decomposition in pH 13.1 buffer. A: initially; B: after 106 min. Eluant, acetonitrile : water 20 : 80 + 0.2% w/v cetrimide + 0.0125 M sodium acetate. Full scale absorbance value is shown after each peak: 1, mitomycin C (0.16); 2 and 3, decomposition products (0.04).

topographic results are summarized in Table 1. Using each type of detergent it was found that retention of the products could best be modified to produce optimum resolution by adjusting the ionic strength by means of sodium acetate. Table 1 shows that the acid degradation produced five detectable products. These were retained only by the ion-exchange or ion-pairing mechanism, with lauryl sulphate as the pairing ion which demonstrated their basic nature. They were almost completely unretained by cetrimide. In the case of the decompositions carried out in alkaline solution the reverse is true and two acidic products appear to be retained by the cetrimide pairing ion only. These results show the complexity of the reaction and indicate that mitomycin C behaves similarly to porfiromycin (Garrett and Shroeder, 1964) in its decomposition pattern. No attempt is made in this report to assign structures to these products nor to indicate if they are final products or intermediates. The ability to resolve the products from the parent mitomycin C indicates further the specificity of the method for the unchanged drug and indicates that different mechanisms operate in acid and basic decompositions.

TABLE 1

CHROMATOGRAPHIC COLUMN CAPACITY RATIOS (k') OF MITOMYCIN C AND ITS DECOMPOSITION PRODUCTS FOLLOWING DECOMPOSITION IN ACID, BASIC AND NEUTRAL SOLUTIONS FOR VARIOUS CHROMATOGRAPHIC ELUANTS

Eluant	Solute	k'		
		Decomposition at pH 3.46, 4.65	Decomposition at pH 9.95, 13.1	Decomposition at pH 6.5
CH_3CN/H_2O 20/80 v/v	Mitomycin C	–	–	2.10
	Products	–	–	0
CH_3CN/H_2O 25/75 v/v + 0.2% w/v sodium lauryl sulphate + 0.025 M sodium acetate	Mitomycin C	1.15	1.25	–
	Product 1	0.9	All products	
	Product 2	2.5	<0.2	
	Product 3	6.2		
	Product 4	7.8		
	Product 5	8.9		
CH_3CN/H_2O 20/80 v/v + 0.2% w/v cetrimide + 0.125 M sodium acetate	Mitomycin C	1.20	1.25	–
	Product 1	Both products	7.62	
	Product 2	<1.0	11.0	

Stability

Table 2 summarizes the kinetic results obtained. The reaction was found to be adequately represented as first order with respect to mitomycin C in all situations, R^2 values greater than 0.95 usually being obtained. The decomposition was followed till less than 10% of the mitomycin C peak height remained, except at 60°C when the measurements were discontinued after 60% decomposition. The measured values for the rate constants as a function of temperature at approximately neutral pH in unbuffered solution conformed to the Arrhenius equation over the range of temperature used ($R^2 = 0.998$). This indicated a single mechanism which justified the extrapolation of the results to obtain a rate constant of 7.66×10^{-6} min^{-1} at 25°C. Using the Arrhenius equation the activation energy for the primary decomposition of mitomycin C is estimated to be 81.1 kJ mol^{-1}. Table 2 also shows that the rate of decomposition increases in both acid and basic solutions. Extrapolation of the data obtained yields a minimum rate constant of 2.24×10^{-5} min^{-1} at pH 7.2. Porfiromycin (Garrett and Schroeder, 1964) has been shown to have minimal rate of decomposition in solution at pH 6.9. The rate constant at 25°C in neutral solution extrapolated from the results obtained at elevated temperatures is considerably lower than that estimated from data obtained in buffered systems. This may indicate that the reaction is subject to general acid–base catalysis in which case the more relevant value is that obtained from the results at elevated temperatures.

The results obtained from the correlation coefficients and standard deviations of the

TABLE 2

FIRST ORDER RATE CONSTANTS (k) OBTAINED FROM MITOMYCIN C DECOMPOSITION IN VARIOUS BUFFERS AND AT VARIOUS TEMPERATURES

The standard deviation (S) and correlation coefficient (R^2) quoted are those obtained by regression analysis of the first order plot. Where the decomposition was followed in more than one chromatographic solvent average values are quoted.

pH	Buffer	Temperature (°C)	$k \times 10^3$ (min^{-1})	$S \times 10^4$	R^2
3.45	Acetate	25	8.90	0.85	0.996
4.65	Acetate	25	1.54	1.33	0.954
9.95	Borate	25	0.974	0.830	0.811
13.1	Sodium hydroxide	25	61.0	18.6	0.983
6.5	Unbuffered	60	0.228	0.270	0.990
6.5	Unbuffered	80	1.40	1.15	0.976
6.5	Unbuffered	100	5.53	1.0	0.913
6.5	Unbuffered	120	20.6	14.0	0.994

rate constants indicate that the chromatographic method used here is adequately precise. Rate constants have, in the past, very often been obtained from spectrophotometric data which must be corrected for the presence of absorbing impurities or products. In view of the high specificity of the liquid chromatographic method rapid and reliable estimates of unchanged drug can be obtained by this technique.

CONCLUSION

Since the technique of h.p.l.c. is now well established as an analytical method of drug analysis and the chromatography of most classes of drugs is possible, the method used here for estimating the stability of mitomycin C is very generally applicable. It has been shown that in addition to the high specificity the method has adequate precision for quantitative kinetic measurements. The results obtained for mitomycin C show that it is very stable in neutral solution but that it is subject to more rapid decomposition in acid and base. The decomposition is complex and this technique could be utilised to monitor the kinetics of the reaction as a whole.

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