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SHORT COMMUNICATION

The polarography of cephalosporin C derivatives

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WE report some preliminary measurements on the polarographic reduction of cephalosporin C, cephalothin [7-(2-thienylacetamido)-cephalosporanic acid] and cephaloridine [7-(2-thienylacetamido)-3-(1-pyridylmethyl)-3-cephem-4-carboxylic acid betaine].

Green, Page & Staniforth (1965) have shown that cephalosporin C derivatives give well-defined infrared and nuclear magnetic resonance spectra that can be used for their qualitative identification. Martin & Shaw (1965) have reviewed the application of methods such as ultraviolet spectrophotometry, paper chromatography and paper electrophoresis for cephaloridine, while Chapman, Page & others (1968) have described infrared and X-ray powder measurements for this compound. The application of mass spectroscopy to the structural elucidation of cephalosporin derivatives has been reported by Richter & Biemann (1965).

EXPERIMENTAL

Polarograms were obtained using a Tinsley Mark 19 pen-recording polarograph and an Electrochemical Laboratories manual polarograph. A dropping mercury electrode was used as cathode and a saturated calomel electrode as external anode; the latter was connected to the cell solution by means of a potassium chloride salt-agar bridge. The polarographic cell which held about 10 ml of solution, was maintained in a thermostat at 25° (±0·1°). All solutions were deoxygenated at 25° with solvent-saturated, oxygen-free nitrogen for 10 min before electrolysis. Solutions of Britton–Robinson buffer pH 3–7 and 0·1M hydrochloric acid were used as supporting electrolytes.

The half-wave potentials ($E_{0.5}$) reported (Table 1) are averaged values from replicate polarograms recorded on the manual apparatus. Capillary drop times were recorded at the appropriate half-wave potential. The diffusion currents reported refer to the maximum diffusion currents registered on the recording polarograph without condenser damping.

RESULTS AND DISCUSSION

Derivatives of cephalosporin C differ from those of benzylpenicillin (Brezina & Zuman, 1958) in showing reduction waves, the shapes and half-wave potentials of which are pH dependent. Typical polarograms of cephalosporin C, cephalothin and cephaloridine are shown in Fig. 1 and the change in the wave form of cephaloridine with pH in Fig. 2. We have used the polarograms of cephaloridine for quantitative analysis, those for the other compounds are reported for qualitative comparison.

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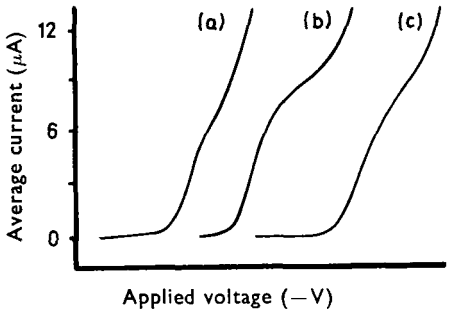

FIG. 1. Polarographic waves for (a) 1·0 mM cephalosporin C, (b) 1·0 mM cephaloridine and (c) cephalothin, in Britton–Robinson buffer pH 3·05. Each wave starts at 0·85 V. Scale 10 mm = 0·25 V.

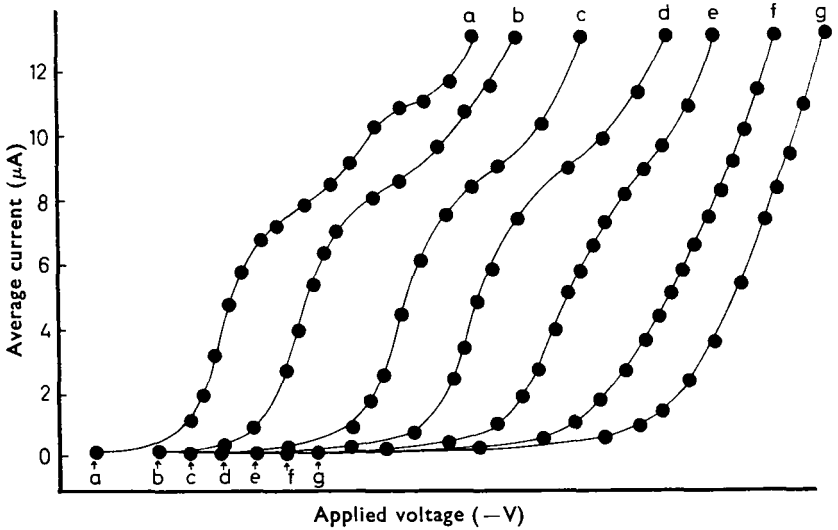

FIG. 2. Effect of pH on wave form of 1·0 mM cephaloridine in hydrochloric acid (a) 1·0 M, (b) 0·1 M. Britton–Robinson buffer (c) pH 3·05. (d) pH 3·98. (e) pH 4·96. (f) pH 6·03. (g) pH 7·02. Each wave starts at 0·70 V. Scale, 10 mm = 0·12 V.

The waves produced in acid solution appear to be diffusion controlled, the diffusion current (i_d) of cephaloridine being a linear function of the height of the mercury reservoir, i.e. $i_d = k\sqrt{h_{corr.}}$, where $h_{corr.}$ is the height of the mercury reservoir corrected for back pressure of mercury. The diffusion current of cephaloridine is linearly related to the capillary characteristics as predicted by the Ilkovic equation, for diffusion controlled waves. The relation between i_d and $m^{2/3}t^{1/6}$ is given by the equation $i_d = 3{\cdot}67m^{2/3}t^{1/6} + 0{\cdot}03$ where m and t have the usual significance (correlation coefficient for four settings of the mercury reservoir height between 43 and 79 cm = 0·99). Preliminary studies on the measurement of the diffusion coefficient suggest that the wave produced by cephaloridine in acidic solution is a two electron step.

The shape of the cathodic waves hindered the accurate measurement of diffusion current. This was overcome by drawing a tangent at the foot of the wave parallel to the limiting current, the diffusion current then being taken as the vertical distance between the two parallel lines.

The measured diffusion current is proportional to the concentration of the depolarizer as predicted by the Ilkovic equation. A linear relation, $i_d = 6{\cdot}59C + 0{\cdot}02$, was obtained for levels of cephaloridine between 2×10^{-3} and 1×10^{-5}M in 0·1M hydrochloric acid (correlation coefficient for 9 values = 0·99). Concentrations as low as 2×10^{-6}M cephaloridine could be detected on the recording polarograph.

TABLE 1

MEAN HALF-WAVE POTENTIALS AND DIFFUSION CURRENTS FOR CEPHALOSPORIN DERIVATIVES AT 25° IN 0·1M HYDROCHLORIC ACID. m=4·41 mg/sec.

Conc. mM	Cephalosporin C			Cephalothin*			Cephaloridine		
	$-E_{0\cdot5}$ (V)	$i_d(\mu A)$	$t_{-E_{0\cdot5}}$ (sec)	$-E_{0\cdot5}$(V)	$i_d(\mu A)$	$t_{-E_{0\cdot5}}$ (sec)	$-E_{0\cdot5}$(V)	$i_d(\mu A)$	$t_{-E_{0\cdot5}}$ (sec)
1·00	1·05	4·31	1·91	1·08	5·62	1·80	0·93	7·28	1·81
0·50	1·01	2·17	1·94	—	—	—	0·90	3·56	1·83
0·10	0·97	0·46	1·96	—	—	—	0·85	0·77	1·86

*Recorded in 0·1M hydrochloric acid containing 50% v/v ethanol.

The half-wave potentials for cephalosporin C, cephalothin and cephaloridine (Table 1) depend on the concentration of the depolarizer, the half-wave potentials becoming more negative with increasing concentration as shown for cephalosporin C and cephaloridine. All $E_{0\cdot5}$ values were adjusted for the potential required to overcome the internal resistance of the cell ("iR drop"). Experiments using a potassium nitrate salt-agar bridge to connect the cell solution to the saturated calomel electrode showed that cephaloridine at potentials up to +0·3V yielded no anodic step at the dropping mercury electrode in the supporting electrolytes examined.

The sensitivity of the polarographic method for cephaloridine is similar to that of the ultraviolet spectrophotometric method (Martin & Shaw, 1965).

References

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