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Preliminary note

ELECTROCHEMICAL STUDY OF SOME PENICILLIN ANTIBIOTICS BY RAPID AC POLAROGRAPHY

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INTRODUCTION

Penicillin antibiotics do not contain a polarographically reducible or oxidizable functional group, making the direct determination impossible. However, we have reported polarographic methods for several penicillins and cephalosporins [1—8] containing an alpha-aminogroup in their side-chains.

These methods were based on the electroactivity of degradation products formed by acidic hydrolysis, an approach which has proved applicable to all penicillins and cephalosporins containing an alpha-aminogroup in their structures.

The electroreducible species was the azomethine group containing in the pyrazine deviate formed by acidic hydrolysis [7]. On the other hand, the other group of well-known penicillins, which lack the alpha-aminogroup in their structures, do not yield the electroactive pyrazine derivate.

Some structures of these penicillin antibiotics are shown in Fig. 1. From these structures, it is clear that an acidic hydrolysis can be expected to break the C—S bond and to lead to an electrooxidizable species R—SH. The present work was undertaken to explore this possibility.

EXPERIMENTAL

Penicillin G, Penicillin V and Cloxacillin were obtained from Laboratorios Chile, Santiago, Chile. Carbenicillin was obtained from Pfizer Laboratories, Santiago, Chile and Flucloxacillin from Beecham Research Laboratories, England. All the other products were AR grade.

We used a Tacussel EPL-2 recorder equipped with a Tipol plug-in GCMS pulse generator for hammer control, an Adapal unit for polarography with superimposed ac signal and a CPRA measuring cell fitted with a PMO-3 hammer.

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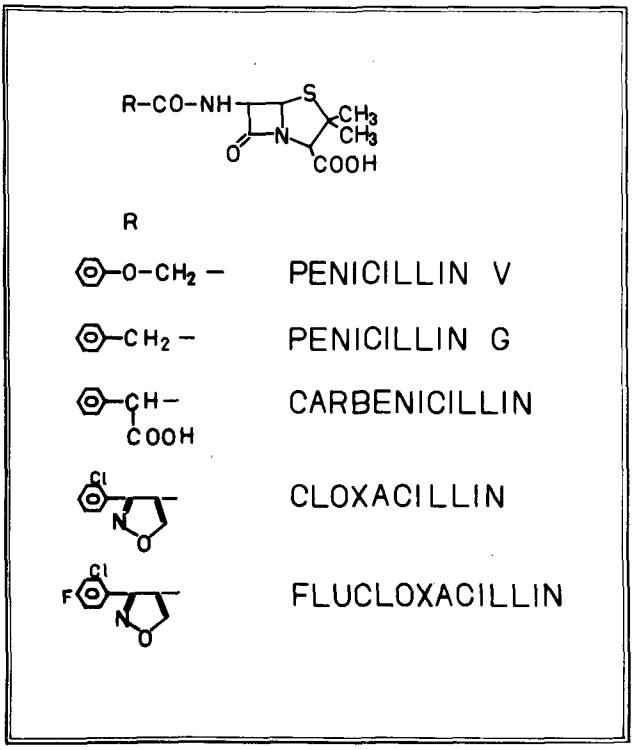


Fig. 1. Structures of the penicillines.

All the following results have been obtained with the following values for the polarographic parameters: drop time 1 s, potential sweep rate 10 mV s^{-1}, frequency of the superimposed signal 20 Hz, amplitude of the superimposed signal 10 mV, and phase angle 0°. The hydrolyses were performed in different buffer solutions at constant ionic strength (μ = 1.0 *M*).

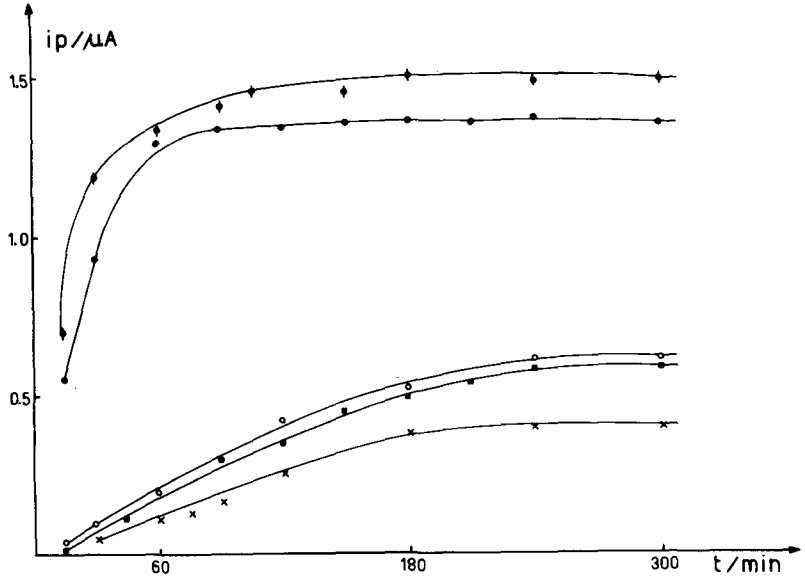
Clark—Lubs buffer was used for pH 1—2, McIlvaine buffer for pH 3—8 and Sorensen buffer for pH 9—12. These buffered solutions were used for the polarographic studies after bubbling with a stream of pre-purified nitrogen for 5 min.

Potentials were measured with respect to a saturated calomel electrode (SCE).

RESULTS AND DISCUSSION

The acidic hydrolysis of the penicillins (Fig. 1) produce electroactive products(s) oxidizable at the dropping mercury electrode. The hydrolyses were performed in the whole range of pH; however, the optimal range was pH 4—5. It should be noted that, for the five antibiotics tested, the maximum peak currents values are not obtained by the same hydrolysis conditions. The optimum pH for the hydrolysis of Carbenicillin and Penicillin G was 5.0 and of Penicillin V, Cloxacillin, Flucloxacillin 4.0.

The maximum peak current values at different hydrolysis time are shown in



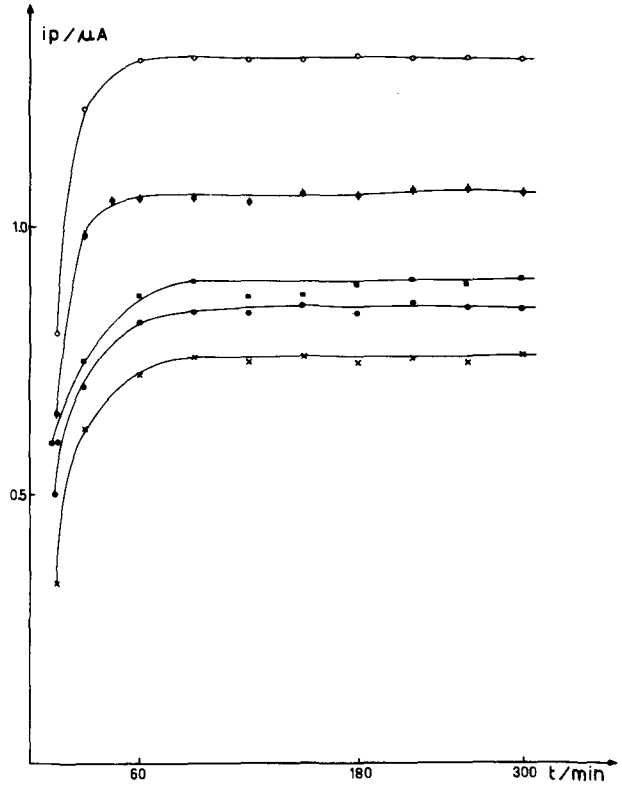


Fig. 2. Maximum peak current for hydrolysed penicillins at pH 5.0, 90°C, 60 min. (♦) Carbenicillin, (•) Penicillin G, (○) Cloxacillin, (▪) Flucloxacillin, (×) Penicillin V. All concentrations were 1.0 mg ml^{-1}.

Fig. 3. Maximum peak current for hydrolysed penicillins at pH 4.0, 90°C, 60 min. (♦) Carbenicillin, (•) Penicillin G, (○) Cloxacillin, (▪) Flucloxacillin, (×) Penicillin V. All concentrations were 1.0 mg ml^{-1}.

Figs. 2 and 3 at pH 5.0 and pH 4.0 respectively, for all antibiotics studied.

Increase of temperature produces an increment of the electroactive product(s). This effect is summarized for Carbenicillin in Figure 4.

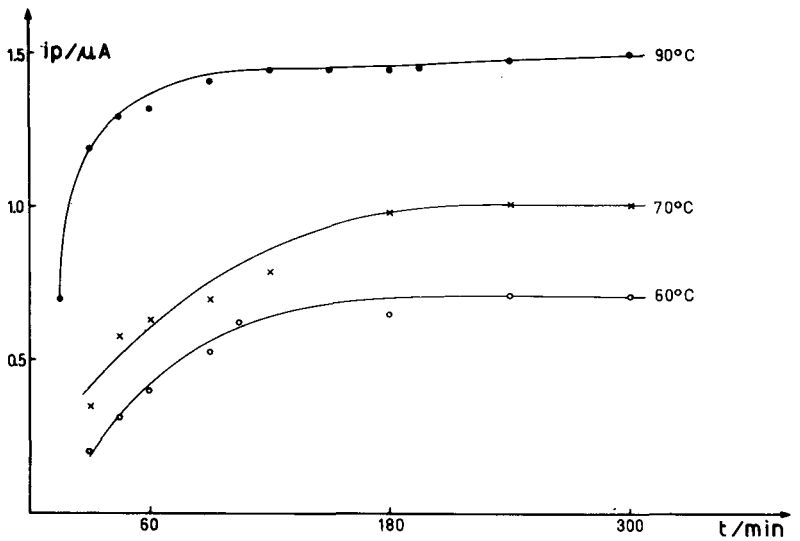


Fig. 4. Maximum peak current for hydrolysed Carbenicillin at pH 5.0 and different temperatures. Initial concentration 1.0 mg ml^{-1}.

TABLE 1

Peak potentials at pH 5.0 for the different hydrolysed antibiotics. Runs at 25°C

Antibiotic	E_p/V vs. SCE
Penicillin G	−0.245
Penicillin V	−0.248
Flucloxacillin	−0.245
Cloxacillin	−0.238
Carbenicillin	−0.240

The potential values of the ac peaks (Table 1) cannot be utilized for the qualitative determination of these penicillins in mixture because they are very similar. This is explained by the similarities of the structures of these antibiotics (Fig. 1) which can lead to similar electroactive product(s) after hydrolysis. The polarographic peaks of the hydrolysed penicillin solutions are shifted towards more negative potential when the pH of the solutions increases. The E_p vs. pH plot (Figs. 5 and 6) gives a straight line in the pH 1—8 region and then a curve of decreasing slope as the pH becomes higher. Penicillin G (Fig. 6) and Carbenicillin show a similar second peak between pH 1—4. However, the other antibiotics studied exhibit a second peak between pH 9 and 12 (Fig. 5).

The variations of the peak potentials versus pH are summarized in Table 2. The slope of the straight lines suggested a one-electron process. This type of behaviour of peak potential vs. pH is characteristic of the anodic behaviour of the sulphydryl group [9,10].

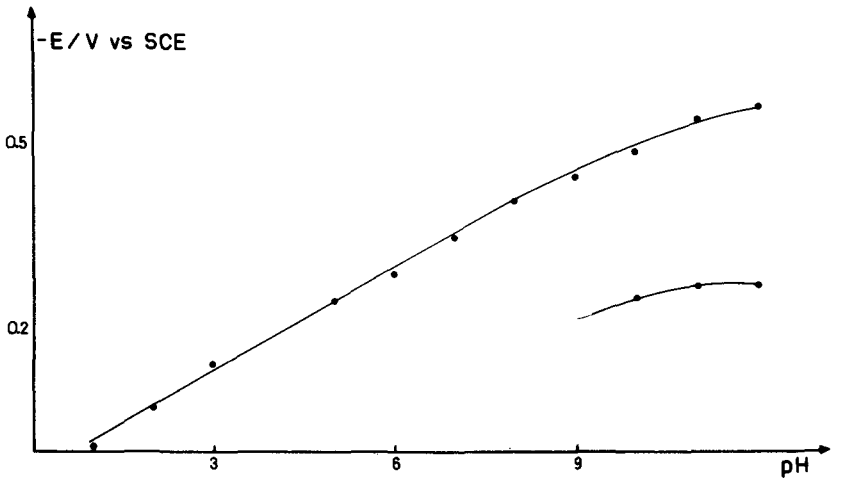


Fig. 5. Plot of E_p vs. pH for hydrolysed Cloxacillin at pH 4.0, 90°C and 60 min.

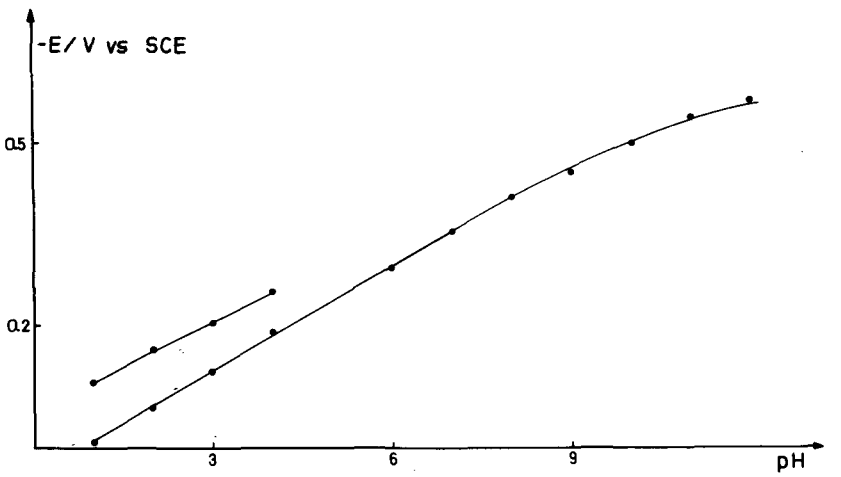


Fig. 6. Plot of E_p vs. pH for hydrolysed Penicillin G at pH 5.0, 90°C and 60 min.

TABLE 2

Slopes of the linear variations of the peak potentials versus pH

Penicillin	$(\Delta E_p/\Delta pH)$/mV	Regression Coeff.
Penicillin V	50.4	0.997
Penicillin G	56.2	0.999
Carbenicillin	55.8	0.999
Flucloxacillin	53.7	0.997
Cloxacillin	55.2	0.998

This statement was confirmed by blocking the ac signal with *p*-hydroxy mercury benzoate.

In aqueous solution, using for each antibiotic the optimal hydrolysis condi-

tions, concentrations between 0.01 and 1.0 mg ml^{-1} can be detected. The variations of the peak currents versus concentration are linear for concentrations below 1.0 mg ml^{-1} provided that the electrode process is diffusion controlled.

The reproducibility of the determination was calculated on the basis of measuring six waves for each of five samples of penicillin solutions hydrolysed in optimal conditions. For 1.0 mg ml^{-1} of Penicillin V the mean current was 0.63 μA, the range 0.03 and the standard deviation 0.02.

CONCLUSION AND FURTHER WORK

The penicillin antibiotics can be determined by polarography with very good results after acidic hydrolysis in buffered aqueous solution. The optimal hydrolysis conditions were pH 4.0, 90°C and 60 min for Flucloxacillin, Cloxacillin and Penicillin V and pH 5.0, 90°C and 60 minutes for Carbenicillin and Penicillin G.

Our work is now devoted to the identification of the hydrolysis products and the study of the electrochemical mechanisms corresponding to the polarographic peaks. In addition, we are studying the extension of this method to other penicillin and cephalosporin antibiotics. The possibility of determining these antibiotics in plasma and urine samples and the analytical application to dosage forms (capsules, tablets, syrups) were also studied. Preliminary results indicate that solvent extraction techniques and protein separation were not necessary.

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