40. *Polarographic Studies. Part II.* Mould Metabolites and Related Quinones.*

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The behaviour at the dropping-mercury electrode of twelve mould metabolites and related quinones has been examined. They produce well-defined current–voltage curves in buffered and in unbuffered 75% alcoholic solutions and can be determined polarographically. The half-wave potentials (versus the saturated calomel electrode at 25°) for these quinones are recorded.

We shall show elsewhere that there is no direct relationship between the normal reduction potentials of these quinones and their bacteriostatic activity against strains of *Staph. aureus* and *Bact. coli*.

In view of the present interest in antibacterial substances produced by moulds, we have examined the behaviour of several such substances at the dropping-mercury electrode.

The polarogram of citrinin, a metabolic product of *Penicillium citrinum*, has already been described by Hirschy and Ruoff (*J. Amer. Chem. Soc.*, 1942, **64**, 1490), who found that in concentrations of 0·001—0·003M it gives well-defined current–voltage curves in buffered and in unbuffered 75% alcoholic solutions. The limiting current was proportional to the concentration of the citrinin, and the half-wave potential in buffered alcoholic solution (pH 2·05) was about 0·80—0·82 v. versus the saturated calomel electrode (S.C.E.). Citrinin was reduced in 0·1N-potassium chloride–75% alcoholic solution, but no reduction occurred in alcoholic acetate buffer (pH 6·0) or in alcoholic phosphate buffer (pH 7·4).

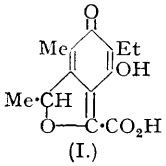
We have confirmed these observations and extended the work to include penicillic acid, 4-methoxy-2 : 5-toluquinone, 4 : 6-dimethoxy-2 : 5-toluquinone, fumigatin, spinulosin, and 2-methyl-1 : 4-naphthaquinone.

The reduction of benzoquinone and the oxidation of quinol at the dropping-mercury electrode were investigated by Müller and Baumberger (*Trans. Amer. Electrochem. Soc.*, 1937, **71**, 169) and by Müller (*Chem. Rev.*, 1939, **24**, 95; *J. Amer. Chem. Soc.*, 1940, **62**, 2434). Smith, Kolthoff, Wawzonek, and Ruoff (*ibid.*, 1941, **63**, 1018) examined the behaviour of many quinones related to vitamin-E, and found that in buffered methanol solutions (pH 5·40), benzoquinone, toluquinone, ψ-cumoquinone, and duroquinone yield anodic waves, whereas 2 : 3-dimethyl-1 : 4-naphthaquinone yields a cathodic wave. As a preliminary to the examination of the mould products, we examined the simpler quinones and obtained results in fair agreement with those of Smith *et al.* (*loc. cit.*) (see Table II).

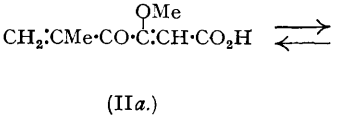
Experimental.

The general procedure adopted was similar to that described in Part I (*loc. cit.*), a Cambridge recording instrument being employed. This was calibrated to read directly in microamps., by Kolthoff and Lingane's procedure (" Polarography," New York, 1941). Dr. Jessop's cell was modified so that a saturated calomel electrode could be used as a permanent external anode.

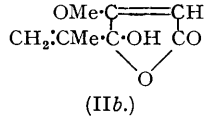
The capillary used had the following characteristics. At a pressure of 41·2 cm. of mercury, the drop time (t) on open circuit in 0·1N-potassium chloride solution was 3·00 secs., weight of mercury dropping per second (m) = 1·60 mg., and $m^{2/3}t^{1/6} = 1{\cdot}638$. The symbols are those adopted by Kolthoff and Lingane (*op. cit.*). Where necessary, 0·01% of gelatin was added to the solutions to depress maxima; methyl-red and similar dyes were not so satisfactory for this purpose. Oxygen was removed from all solutions by bubbling nitrogen for 15 mins. Experiments were carried out at 25°.



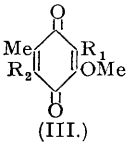
(I.)



(II*a*.)



(II*b*.)



(III.)

The mould products examined had the following properties: (i) Citrinin is a yellow crystalline solid, m. p. 168° (Hetherington and Raistrick, *Phil. Trans.*, 1941, *B*, **220**, 269), and has been assigned the constitution (I) by Coyne, Raistrick, and Robinson (*ibid.*, p. 297). (ii) Penicillic acid forms colourless crystals, m. p. 86° (Black and Alsberg *U.S. Dept. Agric. Bureau Plant Ind. Bull.* No. 199, 1910), and is a tautomeric mixture of (II*a*) and (II*b*) (Birkinshaw,

* Part I was published in *J. Soc. Chem. Ind.*, 1942, **61**, 93.

Oxford, and Raistrick, *Biochem. J.*, 1936, **30**, 394). (iii) Fumigatin, 3-hydroxy-4-methoxy-2 : 5-toluquinone (III; R_1 = OH, R_2 = H), consists of maroon-coloured crystals, m. p. 116° (Anslow and Raistrick, *ibid.*, 1938, **32**, 687). (iv) Spinulosin, 3 : 6-dihydroxy-4-methoxy-2 : 5-toluquinone (III; $R_1 = R_2$ = OH), forms purplish-bronze plates, m. p. 201° (Birkinshaw and Raistrick, *Phil. Trans.*, 1931, *B*, **220**, 245).

In addition, the following synthetic substances were examined: (1) *p*-Toluquinone, m. p. 69°. (2) ψ-Cumoquinone, yellow needles, m. p. 29—30° (Smith, *J. Amer. Chem. Soc.*, 1934, **56**, 472). (3) Duroquinone, yellow needles, m. p. 111° (Smith and Dubrovolny, *ibid.*, 1926, **48**, 1420). (4) 6-Bromo-2 : 3 : 5-trimethylquinone, yellow needles, m. p. 79—80° (Smith and Johnson, *ibid.*, 1937, **59**, 673). (5) 4-Methoxytoluquinone (III; $R_1 = R_2$ = H), yellow plates, m. p. 172—173° (Ashley, J., 1937, 1471). (6) 4 : 6-Dimethoxy-2 : 5-toluquinone (III; R_1 = H, R_2 = OMe), yellow needles, m. p. 125° (Anslow, Ashley, and Raistrick, J., 1938, 439). (7) 2 : 5-Dihydroxy-4 : 6-dimethoxytoluene, obtained by reduction of (6), colourless needles, m. p. 148°; this is a new compound, prepared by Dr. J. N. Ashley. (8) 2-Methyl-1 : 4-naphthaquinone, a lemon-yellow powder, m. p. 106° (Anderson and Newman, *J. Biol. Chem.*, 1933, **103**, 406); this can be regarded as a benzotoluquinone: it has a greater vitamin-K activity than any other known substance (Ansbacher and Fernholz, *J. Amer. Chem. Soc.*, 1939, **61**, 1924), and is used clinically to treat prothrombin deficiency.

The buffer solutions were prepared from " AnalaR " reagents and each was examined on the polarograph to ensure freedom from impurities. They had the following compositions: (I) A mixture which was 0·1M in acetic acid and 0·1M in sodium acetate in water (pH 4·63). (II) A mixture 0·05M in potassium chloride and 0·01M in hydrochloric acid in 75% (vol.) ethyl alcohol (pH 2·03). (III) A mixture 0·05M in sodium acetate and 0·05M in acetic acid in 75% (vol.) ethyl alcohol (pH 6·24). (IV) A mixture 0·0134M in sodium dihydrogen phosphate and 0·0536M in disodium hydrogen phosphate in 75% (vol.) ethyl alcohol (pH 7·4).

The unbuffered 0·1N-potassium chloride solutions had pH values in the range 6·5—8·5. Müller and Baumberger (*loc. cit.*) have pointed out that small quantities of chloride seriously interfere with the steps due to the more easily reducible quinones in acid solution. At the relatively high positive potential of quinhydrone at low pH, the mercury is oxidised, with the formation of mercurous chloride.

The pH of all solutions were checked by means of a Cambridge pH meter, which was also employed for the electrometric estimation of the dissociation constant (K_a) of penicillic acid.

TABLE I.

Cathodic and anodic waves of various quinones in different buffer solutions.

	Compound.	Molar concn. (C) × 10^5.	Buffer.	pH.	Diffusion current (i_d) × 10^6	$10^3 i_d/C$.	Half-wave potential (S.C.E.).
(1)	.....	83	III	6·24	4·3	5·2	+0·05
		,,	IV	7·4			−0·01
		,,	0·1N-KCl	—			+0·06
(2)	.....	100	III	6·24	4·7	4·7	−0·07
(3)	.....	100	III	6·24	4·3	4·3	−0·14
(4)	.....	100	III	6·24	3·9	3·9	−0·03
(5)	.....	66	III	6·24	2·8	4·2	−0·06
		,,	IV	7·4			−0·10
		,,	0·1N-KCl	—			−0·10
(6)	.....	55	III	6·24	2·6	4·6	−0·16
		,,	IV	7·4			−0·22
		,,	0·1N-KCl	—			−0·31
(7)	.....	54	III	6·24	2·8	5·2	−0·16
		,,	IV	7·4			−0·23
		,,	0·1N-KCl	—			−0·32
(iii)	.....	59	III	6·24	1·9	3·2	−0·16
		,,	IV	7·4			−0·24
		,,	0·1N-KCl	—			−0·25
(iv)	.....	54	III	6·24	1·9	3·5	−0·30
		,,	IV	7·4			−0·34
		,,	0·1N-KCl	—			−0·28
(8)	.....	63	III	6·24	2·5	3·9	−0·17
		,,	IV	7·4			−0·24
		,,	0·1N-KCl	—			−0·33
(i)	.....	40	I	4·63	4·3	2·5	−1·14
		170	II	2·03			−0·82
		170	0·1N-KCl	—			−0·90
(ii)	.....	58	I	4·63	2·9	5·0	−0·69
		,,	0·1N-KCl	—			−0·85

Results.—(Values of all half-wave potentials and steps recorded are against the S.C.E.) The experimental results are summarised in Table I. The values of the diffusion currents (i_d) have been corrected for the residual current, and the half-wave potentials corrected where necessary for the resistance of the cell (iR).

Citrinin.—The polarographic behaviour of citrinin in alcoholic solution has already been reported by Hirschy and Ruoff (*loc. cit.*). 0·004M-Citrinin in an aqueous 0·1M-acetate buffer (pH 4·63) gives a step at −1·14 v.

Penicillic acid. Over the concentration range 0·0001—0·001M, penicillic acid in aqueous 0·1M-acetate buffer (pH 4·63) yields a well-defined step at about −0·69 v., the limiting current being proportional to the concentration. The method could therefore be used for estimating penicillic acid in aqueous solution; the steps in 75% alcoholic buffered and unbuffered solutions are not satisfactory for quantitative purposes. The dissociation constant of penicillic acid was also determined; it is not a strong acid, K_a being $1·26 \times 10^{-6}$ at 25° in aqueous solution.

Toluquinones.—As is shown in Table II, our values for the half-wave potentials of toluquinone, ψ-cumoquinone, and duroquinone at pH 6·24 agree reasonably well with those of Smith *et al.* (*loc. cit.*) at pH 5·4, when due allowance is made for the difference in pH. It can be shown theoretically, and has been confirmed experimentally by Smith *et al.* (*loc. cit.*), that, in the reversible reduction of a substituted quinone to a quinol, the reduction potential increases by 0·0591 v. per unit decrease of pH at 25°. We have assumed this value in calculating the data for Table II.

TABLE II.

Comparison of half-wave potentials calculated for pH 5·4 *with values of Smith* et al.

Compound.	Half-wave potential. Calc.	Smith *et al.*
Toluquinone	+0·10	+0·090
ψ-Cumoquinone	−0·02	−0·027
Duroquinone	−0·09	−0·093

At pH 6·24 (Buffer III), the half-wave potential for spinulosin is −0·30 v. and at pH 7·4 (Buffer IV) it is −0·34 v. The following colour changes were observed for spinulosin at different hydrogen-ion concentrations. Below pH 2·8, the solution is slightly buff-coloured, but at 3·1 it becomes a pinkish-violet and gradually attains a deep-purple at pH 5·5; the intensity of the colour then gradually diminishes, solutions above pH 8·0 being almost colourless.

Fumigatin yields a satisfactory step, suitable for its quantitative estimation, in a 75% alcoholic acetate buffer (pH 6·24) at about −0·16 v. The step becomes less distinct in more acid solution. Below pH 2·5, alcoholic solutions of fumigatin are cherry-red, but on increase of pH, the colour gradually darkens and changes to mauve at pH 4·0, and deep violet above pH 5·4.

4 : 6-Dimethoxy-2 : 5-toluquinone forms the most clearly defined steps in unbuffered 75% alcoholic potassium chloride and in 75% alcoholic acetate buffer (pH 6·24). The half-wave potentials are respectively −0·16 v. and −0·22 v. 2 : 5-Dihydroxy-4 : 6-dimethoxytoluene has approximately the same half-wave potential as the quinone, this result being in keeping with observations on other similar pairs of compounds such as benzoquinone and quinol (Müller, *loc. cit.*, 1940). The height of the step for a solution of the dihydroxytoluene is slightly greater than that for the same molar concentration of the toluquinone (cf. Smith *et al.*, *loc. cit.*).

4-Methoxytoluquinone is readily reduced in a 75% alcoholic phosphate buffer (pH 7·4), the half-wave potential being −0·10 v. The wave becomes anodic in slightly acid solution.

2-*Methyl*-1 : 4-*naphthaquinone*. This quinone gives a step at −0·17 v. in 75% alcoholic acetate buffer (pH 6·24). From this value we can, following the method of Smith *et al.* (*loc. cit.*), calculate the potential for a pH of zero (see above), and using 0·246 v. as the standard potential of the saturated calomel electrode, deduce that the standard normal oxidation potential of 2-methyl-1 : 4-naphthaquinone is about +0·43 v. This is in harmony with the figure of +0·408 v. obtained by Fieser and Fieser (*J. Amer. Chem. Soc.*, 1935, **57**, 491) using the classical procedure. McCawley and Gurchot (*Univ. Calif. Pub. Pharmacol.*, 1940, **1**, 325; cf. *Chem. Abs.*, 1941, **35**, 1466) employed a polarograph procedure and reported a value for E_0 of +0·458 v.

Discussion.—We had hoped to throw light on the alleged correlation between oxidation–reduction potential and antibacterial and related activities, suggested by certain workers, but we shall show elsewhere that for this limited series, the ability of a substituted quinone to inhibit the growth of strains of the Gram-negative *B. coli* is not related to the normal electrode potential of that quinone. For the Gram-positive *Staph. aureus*, on the other hand, the reduction potentials of all the bacteriostatic quinones fall within certain limits.

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