316. *The Colouring Matters of* Drosera Whittakeri. *Part IV. The Reduction Potentials of Some Naphthaquinones.*

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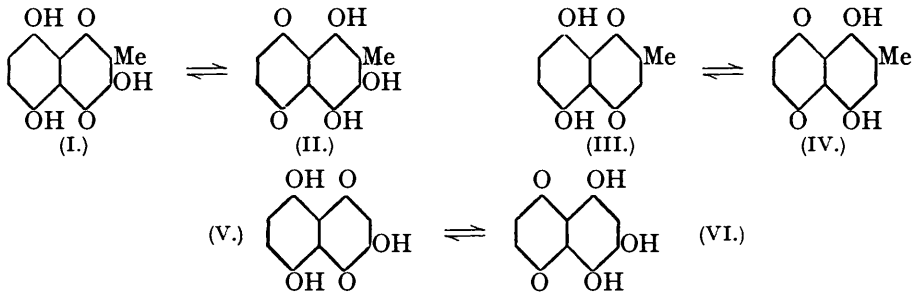
Macbeth, Price, and Winzor (J., 1935, 325) assigned the names droserone * and hydroxydroserone to the two associated colouring matters of *Drosera Whittakeri*, and the reactions of the compounds subsequently indicated that, whereas hydroxydroserone was 3 : 5 : 8-trihydroxy-2-methyl-1 : 4-naphthaquinone, droserone was probably the 3 : 5(or 8)-dihydroxy-compound (Macbeth and Winzor, *ibid.*, p. 334). The constitution of the former was verified by synthesis (Winzor, *ibid.*, p. 336), but the structure of droserone is as yet unconfirmed.

The presence of the two *peri*-hydroxyl groups in hydroxydroserone introduces the possibility of tautomerism, and the substance may exist in the two forms (I) and (II). Similar behaviour is found for methylnaphthazarin (III, IV) and might occur in naphthapurpurin (V, VI). It is, however, excluded in the case of droserone, the boroacetate and pyridine salt of which indicate the presence of one *peri*-hydroxyl and one hydroxyl group in the quinone ring.



Reduction-potential measurements are well adapted for examination of such tautomerism and Fieser (*J. Amer. Chem. Soc.*, 1928, **50**, 439) has shown that the normal reduction potentials of many simple and substituted naphthaquinones are almost independent of the solvent when it is sufficiently acidic, and that the effects of substituents in the naphthaquinone nucleus are largely additive. The object of the present work was to obtain comparable values for a series of naphthaquinones in the *same* solvent, so that the effect of substituents might be more closely examined and the principle of additivity (if valid) be applied to consideration of the equilibrium of the tautomeric forms of methylnaphthazarin and hydroxydroserone. A calculated value for droserone may also be derived which will provide a check on the constitution already proposed when a sufficiently pure sample of the natural product becomes available.

Experimental.

To determine the normal reduction potentials, Fieser's apparatus (*loc. cit.*) was slightly modified. The solvent, which was employed throughout for both the oxidising and the titrated solutions, was made by dissolving hydrogen chloride to 0·1*M* and lithium chloride to 0·2*M* in 50% (by wt.) aqueous alcohol—Fieser and Fieser's solvent B (*J. Amer. Chem. Soc.*, **1935**, **57**, 491). The normal reduction potentials are based on a conventional zero potential for the hydrogen electrode at 760 mm. of mercury total pressure above the solvent. As the vapour pressure of the solvent was assumed to be the same as that found by Dobson (J., 1925, **127**, 2866) for 50% alcohol, *viz.*, 50 mm. at 25°, the standard pressure of hydrogen at the electrode was taken as 710 mm., and the corrections for variation of atmospheric pressure were seldom more than a few tenths of a millivolt.

The quinone solutions were reduced by hydrogen in the presence of a suitable catalyst and

* The name droserone has also been given by Witanowsky (*Wiadomosci Pharm.*, 1934, **16**, 420, 432; *Amer. Chem. Abs.*, 1934, **28**, 7257) to the hydroxynaphthaquinone isolated from the round-leafed sundew, *D. rotundifolia*, L.

then forced over by nitrogen from the reduction flask to the titration vessel. Graduation marks were etched on the latter which enabled the volume of the quinol solution transferred (about 50 c.c.) to be estimated to within 1%. The hydrogen electrode unit was connected with the titration vessel by a tube filled with solvent and plugged with filter-paper at both ends.

During electrometric titration of the quinol with a suitable oxidiser dissolved in some of the solvent, nitrogen, forced through a fine jet, was bubbled through the solution in the titration vessel and obviated the need for a mechanical stirrer. Commercial nitrogen was purified by passage over heated copper, followed by scrubbing with alkaline pyrogallol; and traces of oxygen were removed from hydrogen by the same reagent. Before being admitted to the various vessels, the gases were saturated with solvent vapour at the appropriate temperature.

As a preliminary to the electrometric work, the approximate solubilities of the quinones in the experimental solvent were determined, a colorimetric method being applicable in all cases. A sub-saturated standard solution was thus compared with various dilutions of a solution saturated at 25°, the temperature selected for the electrometric work. In all cases Beer's law was obeyed within the error of comparison. With synthetic hydroxydroserone, the standard solution and the unknown did not match perfectly, owing to the presence of a trace of coloured impurity. The interference was, however, to a large extent overcome by saturating with the least practicable excess of the material. The solubilities (S) are recorded in Table I as millimols/l. Methylnaphthazarin (0·00064M) was the least soluble of the quinones examined, and a standard concentration of 0·0005M was adopted for each quinone prior to reduction. The oxidisers, except potassium ferricyanide (0·01M), were of concentration 0·005M.

Platinised asbestos, in amount representing some 3% by weight of the solution, was employed as the main catalyst in the reduction of the quinones, and the reaction was frequently completed in less than 10 minutes as judged by loss of colour. In a few instances, upwards of 30 minutes were required, and in difficult cases a little of Adams and Voorhees's platinum–platinum oxide catalyst (*J. Amer. Chem. Soc.*, 1922, **44**, 1397) was mixed with the platinised asbestos to facilitate reaction.

Procedure.—The original scheme underlying a titration was a measurement of the e.m.f. at several different stages of re-oxidation of the quinol solution, a comparison of the successive increases in e.m.f. serving to check the validity of the titration. The method, however, had to be extended to include exact determination of the end-points, since in a few instances in which platinised asbestos was used as the catalyst in reduction, and in practically all cases in which platinum–platinum oxide was employed, there was partial destruction of the quinol or quinone. The degree of destruction depended on the time the solution was left in contact with the catalyst, and was most marked with hydroxydroserone (38—74%) and naphthapurpurin (24—46%). Whatever the nature of the destruction may be, it did not vitiate the results obtained by this procedure, for the normal reduction potentials were sensibly independent of the degree of destruction in particular cases which were exhaustively examined.

The normal reduction potentials of the oxidisers ordinarily used in the course of the work were so much higher than those of the naphthaquinones that the end-points were accurately obtained by inspection. A check on the determination of the end-point may, however, be obtained by extending the titration into the region dominated by the reduced and the oxidised form of the oxidiser. Both normal reduction potentials can be obtained from one extended titration, but if the difference between them is much less than 100 mv. the computations become involved, even when the degree of destruction is known to be negligible. Although *p*-benzoquinone was frequently used as an oxidiser, it was unsatisfactory in extended titrations because the system comprising its reduced and its oxidised form fails to establish a satisfactory potential at the electrode at the low concentrations used. This behaviour is depicted in Figs. 1 and 2 (Curves I). The potentials represented by the continuous lines were those read after a 5-minute interval and were drifting upward through the shaded areas to the broken boundaries which represent values calculated from 0·715 volt as the normal reduction potential of *p*-benzoquinone. Tetrabromo-*o*-benzoquinone and potassium ferricyanide behaved excellently in such extended titrations, but as the former is somewhat unstable in solution, titrations with it must be conducted rapidly.

The measured e.m.f. prior to addition of any of the oxidising solution was usually well poised, and from it could be calculated later the amount of pre-oxidation of the quinol solution. (2-Methyl-1 : 4-naphthaquinone was an outstanding exception in this respect.) The titration figures were always corrected for such observed pre-oxidation, which was usually only a fraction of 1% but occasionally was higher when the reduction period was curtailed to minimise the destruction referred to above.

After each addition of oxidising solution, the e.m.f. was measured at 2, 5, 8 . . . minutes according to the rapidity with which equilibrium was attained, but usually the readings after 2 and 5 minutes were substantially identical. In an attempt to measure the reduction potential of 2-methyl-1 : 4-naphthaquinone, however, with *p*-benzoquinone as oxidiser, it was found that the system was virtually inert at the electrode (black platinum, and gold, being tried as well as the usual smooth platinum). It is probable that, as with benzoquinone itself, the system would behave satisfactorily at much higher concentrations, but the reduction potential at the usual concentrations was determined by the following procedure.

FIG. 1. FIG. 2.

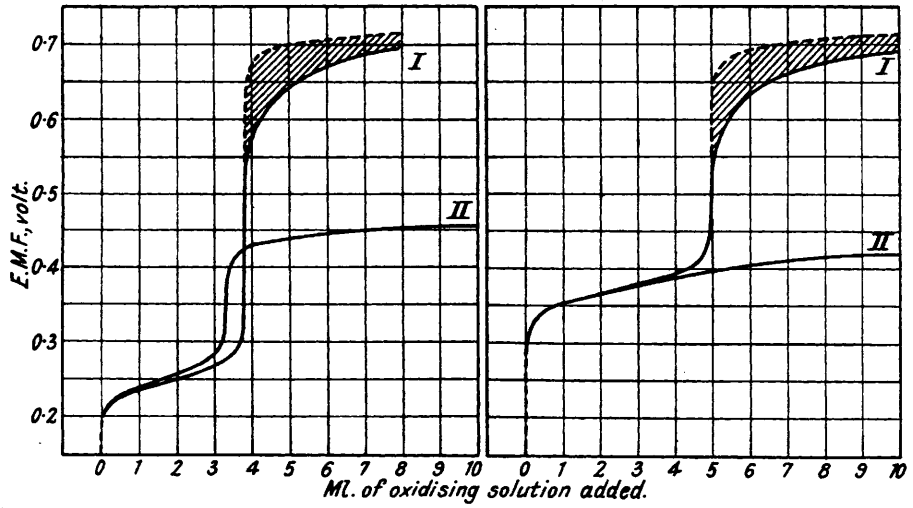


FIG. 1.—*Titration of reduced naphthapurpurin with* p-*benzoquinone* (*Curve I*) *and with juglone* (*Curve II*).

Curve I. 1·0% *Pre-oxidation :* 24% *destruction ; potentials drifting from continuous to broken line through shaded area.*

Curve II. 0·7% *Pre-oxidation :* 36% *destruction.*

FIG. 2.—*Titration of reduced naphthazarin with* p-*benzoquinone* (*Curve I*) *and with* 2-*methyl*-1 : 4-*naphthaquinone* (*Curve II*).

Curve I. 0·3% *Pre-oxidation : negligible destruction ; potentials drifting from continuous to broken line through shaded area.*

Curve II. 0·1% *Pre-oxidation : curve is in harmony with an assumed negligible destruction.*

At any stage of an extended titration of a reduced quinone A with another quinone B, we have at equilibrium

$$E = E_0^{A} - RT/2F \cdot \log_e [A_{red.}]/[A_{ox.}] = E_0^{B} - RT/2F \cdot \log_e [B_{red.}]/[B_{ox.}]$$

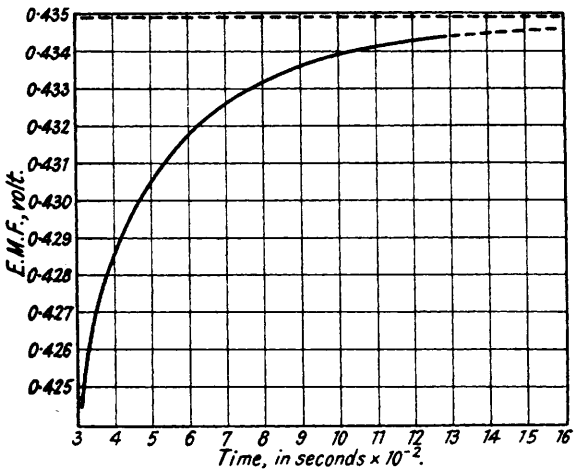
If only the A system readily establishes its potential, it can also act as an indicator for the B system, and the normal reduction potentials of both are obtained. In practice, the reduction potential of A should not be much lower than that of B (some 60 mv.) to ensure that $[A_{red.}]$ shall never be too low to poise the potential effectively. Thus, reduced naphthazarin was satisfactorily titrated with 2-methyl-1 : 4-naphthaquinone (Fig. 2, Curve II). If the reduction potential of A is slightly higher than that of B, a titration in the customary sense cannot be made, but if equimolecular quantities of B and of reduced A are mixed, then the potential at equilibrium must lie midway between the normal reduction potentials of the two systems and should be well poised. The reduction potential of A being known, that of B follows, but errors are magnified. A typical example is afforded by 2-methyl-1 : 4-naphthaquinone and reduced juglone. When equimolecular quantities of these are mixed, well-poised potentials are obtained which increase markedly with time at first, but become almost stationary after some 20 minutes. Since the juglone system is known to establish its potential rapidly, whereas the methylnaphthaquinone system is known to be virtually insensitive at the electrode, the variation of potential with time (Fig. 3) can justifiably be associated with the change in state of oxidation in the former system. On this basis the data were found to accord excellently with the supposition that at

any instant of time a forward reaction between reduced juglone and 2-methyl-1 : 4-naphthaquinone (velocity constant $k_1 = 1{\cdot}3$) and a back reaction ($k_2 = 7{\cdot}1\ k_1$) were occurring. This permitted the e.m.f. at infinite time (equilibrium) to be deduced as 0·4349 volt, a value which is only some 0·5 mv. higher than the e.m.f. at 21 minutes. Hence the normal reduction potential of 2-methyl-1 : 4-naphthaquinone is [0·4349 — (0·4475 — 0·4349)] = 0·4223 volt, in good agreement with the value (0·4216 volt) obtained from its extended titration with reduced naphthazarin.

The mean experimental values of the normal reduction potentials determined for a series of naphthaquinones are shown in col. 7 of Table I, and in col. 6 the individual values obtained by different methods are set out. In the latter case a letter *a* or *b* is placed beside each value, followed by a number which represents in the case of *a* the particular oxidiser used (as numbered in the table), and in the case of *b* the reduced quinone from an extended titration of which the normal reduction potential of the oxidiser was derived.

FIG. 3.

Variation of potential with time when reduced juglone (0·00045M) *reacts with 2-methyl-1 : 4-naphthaquinone* (0·00045M); *asymptote at* 0·4349 *volt.*



The values of E_0 recorded in the table were obtained by the application of the usual basic equation to points that lay in the immediate vicinity of the mid-point (50% oxidation) of the curves drawn for each substance. In the construction of the curves, fewer than 13 experimental values were never employed. The departure of the curves from the theoretical is recorded in the table in a similar way to that adopted by Fieser, who, using the mid-point as a basis of reference, reported the variation in values at 20% and at 80% oxidation. The average differences between E_0 and the potentials at these stages of oxidation are set out in the columns under ΔE_1, and ΔE_2, and as the theoretical difference is 17·8 mv., it will be seen that no serious discrepancies have been encountered in our work.

The quinones used had all been subjected to rigorous purification for use in the absorption spectroscopy already reported or to be described later. The colorimetric determination of the solubilities of the substances also provides a check on their purity.

DISCUSSION.

The Principle of Additivity.—The effects of substituents on the normal reduction potential of 1 : 4-naphthaquinone are summarised in Table II, which has been compiled from the experimental values recorded in Table I. The reduction potential of hydroxydroserone for this purpose was taken as the average of all determinations made with the natural and the synthetic substance. The effect of the introduction of a methyl group in the 2-position is seen to be a lowering of the potential by some 63 mv. compared with the value of 76 mv. deduced by Fieser and Fieser, using different solvents. The effect of substitution of a hydroxyl group in the 3-position is found to be — 126·6 mv., in excellent agreement with Fieser's value.

TABLE I.

No.	Substances.	*S.*	ΔE_1, mv.	ΔE_2, mv.	E_0, volts.	Mean.	Previous values, volts.
1	1 : 4-Naphthaquinone	31	19·2	18·2	0·4839 [*a* (i)], 0·4859 [*a* (i)], 0·4852 [*a* (i)], 0·4841 [*b* (dros)]*	0·4848	0·4839,[2] 0·483,[4] 0·492[5]
2	Methylnaphthaquinone	33·7	†	†	0·4216 [*b* (8)], 0·4223 [*b* (4)]	0·4220	0·4080[3]
3	Lawsone	24·4	18·0	18·7	0·3583 [*a* (i)], 0·3580 [*a* (i)]	0·3582	0·356,[4] 0·352,[5] 0·352[7]
4	Juglone	10·0	18·4	18·0	0·4474 [*a* (i)], 0·4482 [*a* (i)], 0·4476 [*a* (i)], 0·4472 [*b* (10)], 0·4471 [*b* (dros)] *	0·4475	0·451,[4] 0·429[7]
5	Phthiocol	12·5	18·0	18·4	0·2988 [*a* (i)], 0·2997 [*a* (i)]	0·2992	0·2987[6]
6	*iso*Naphthazarin	6·8	17·9	18·7	0·2876 [*a* (i)], 0·2883 [*a* (i)]	0·2880	
7	Methylnaphthazarin	0·64	18·8	18·9	0·3213 [*a* (i)], 0·3221 [*a* (ii)]	0·3217	
8	Naphthazarin	1·53	18·4	18·6	0·3724 [*a* (i)], 0·3724 [*a* (i)], 0·3717 [*a* (i)], 0·3722 [*a* (2)]	0·3722	0·361[1]
9	Hydroxyjuglone	9·5	18·4	17·9	0·3151 [*a* (i)], 0·3147 [*a* (i)]	0·3149	
10	Naphthapurpurin	6·07	18·6	19·2	0·2483 [*a* (i)], 0·2491 [*a* (4)], 0·2509 [*a* (i)]	0·2487	0·243[1]
11	Hydroxydroserone (natural)	1·1	19·8	19·7	0·1995 [*a* (ii)], 0·2013 [*a* (iii)]	0·2004	
12	Hydroxydroserone (synthetic)	1·14	20·0	19·4	0·2000 [*a* (i)], 0·1992 [*a* (ii)]	0·1996	
13	Lomatiol	35	18·7	18·8	0·2920 [*a* (i)], 0·2931 [*a* (i)]	0·2926	0·294[1]
i	*p*-Benzoquinone				0·715 (at 0·02*M* of reductant + oxidant)		
ii	Tetrabromo-*o*-benzoquinone	43			0·868		0·872[4]
iii	Potassium ferricyanide				0·688		

* These values obtained from extended titrations of reduced droserone are included here for completeness. † No values obtainable (see text).

[1] Fieser, *J. Amer. Chem. Soc.*, 1928, **50**, 439; *t*, 25°; solvent, same as in our experiments.

[2] Fieser and Fieser, *ibid.*, 1934, **56**, 1565; *t*, 25°; solvent, same as in our experiments.

[3] *Idem, ibid.*; *t*, 25°; solvent, 30% water–ethanol mixture with HCl and LiCl both at 0·2*M*.

[4] Conant and Fieser, *ibid.*, 1924, **46**, 1858; *t*, 25°; solvent, 50% water–ethanol mixture with HCl 0·5—1·0*M*.

[5] *Idem, ibid.*; *t*, 25°; solvent, 5% water–ethanol mixture with HCl 0·5—1·0*M*.

[6] Ball, *J. Biol. Chem.*, 1934, **106**, 515; *t*, 30°; solvent, aqueous buffers with HCl.

[7] Friedham, *Biochem. J.*, 1934, **28**, 180; *t*, 20°; solvent, aqueous buffers with HCl.

The principle of additivity, which postulates that the effect of a number of substituents is the sum of the individual effects, may be tested by comparing the experimental values of the normal reduction potentials of some substituted quinones with those calculated from the data in Table II; *e.g.*, the test may safely be applied to phthiocol, since Fieser's examination of hydroxynaphthaquinone and the ethers of 1 : 2- and 1 : 4-naphthaquinone clearly showed that in the equilibrium of the two forms of a substance such as phthiocol only a negligible amount of the 1 : 2-quinonoid tautomeride is present. Since the calculated normal reduction potential of phthiocol, 0·2954 volt (1 : 4-naphthaquinone, 0·4848, + 2-methyl effect, — 0·0628, + 3-hydroxyl effect, — 0·1266 volt) differs from the experimental value 0·2992 volt by less than 4 mv., the additive character of the effects is evident. If Fieser and Fieser's values are used, a difference of some 18 mv. is obtained.

TABLE II.

No.	2	3	4	5	6	7
Substituents	2-Me	3-OH	5-OH	2-Me 3-OH	2 : 3-$(OH)_2$	2-Me 5 : 8-$(OH)_2$
Effect (mv.).....	— 62·8	— 126·6	— 37·3	— 185·6	— 196·8	— 163·1
No.	8	9	10	11 and 12	13	
Substituents	5 : 8-$(OH)_2$	2 : 5(or 8)-$(OH)_2$	2 : 5 : 8-$(OH)_3$	2-Me 3 : 5 : 8-$(OH)_3$	2-CH:CH·CMe_2·OH 3-OH	
Effect (mv.).....	— 112·6	— 169·9	— 236·1	— 284·8	—	

The case of naphthapurpurin has been discussed by Fieser, and the reduction potentials of the tautomeric forms (V, VI) may be calculated for comparison with the experimental

value. For (V), the calculated potential is 0·2456 volt (1 : 4-naphthaquinone, 0·4848, + 5 : 8-*peri*-hydroxyl effect, — 0·1126, + 2-hydroxyl effect, — 0·1266 volt), which differs from the experimental value by only some 3 mv. instead of some 9 mv. on the basis of Fieser's values. The conclusion previously deduced, that only a negligible proportion of the second tautomeric form (which has a calculated reduction potential of 0·3182 volt) is present in the equilibrium mixture, is confirmed.

Examination of the cases of hydroxydroserone and methylnaphthazarin indicates that these also exist preponderatingly in the tautomeric forms (I) and (III) respectively: the calculated value for (III), *viz.*, 0·3094 volt (1 : 4-naphthaquinone, 0·4848, + 2-methyl effect, — 0·0628, + 5 : 8-*peri*-hydroxyl effect, — 0·1126 volt) is 12 mv. lower than the observed value; and the mean calculated value of hydroxydroserone (I) is about 14 mv. lower than the mean of the experimental results. In this connection it is noteworthy that the calculated values for hydroxydroserone derived by two distinct methods which depend on entirely different experimental results differ by less than 1 mv.; thus, (*a*) naphthazarin, 0·3722, + 2-methyl-3-hydroxyl effect (from phthiocol), — 0·1856, gives the calculated figure of 0·1866 volt; whereas (*b*) naphthapurpurin, 0·2487, + 2-methyl effect, — 0·0628, gives a value of 0·1859 volt. The calculated values of the other tautomeric forms (II, IV) are of distinctly greater magnitude, but reliable figures cannot be derived on account of the meagre information available concerning the effect of substitution of a methyl group in the benzenoid ring of the naphthaquinone nucleus.

Departures from the principle of additivity may reasonably be associated with disturbance due to tautomerism or interference due to the character of the substituent groups themselves. The case of *iso*naphthazarin seems to provide an example of the mutual effect of the groups, for on a purely additive basis the calculated reduction potential, 0·2316 volt (1 : 4-naphthaquinone, 0·4848, + 2 × 2-hydroxyl effect, 2 × — 0·1266 volt) is less than the observed value by about 56 mv. This is the only serious discrepancy so far observed in this direction, but a disturbance in the opposite sense is noted in the case of the two *peri*-hydroxyl groups of naphthazarin, for the experimental value, 0·3722 volt, is some 38 mv. less than that calculated by subtracting from the normal reduction potential of 1 : 4-naphthaquinone double the effect of substitution of one *peri*-hydroxyl group (obtained from the juglone potential).

In considering the bearing of the principle of additivity on the general question of tautomerism of hydroxynaphthaquinones, six conceivable quinone structures, *viz.*, 1 : 2, 1 : 4, 1 : 5, 2 : 3, 2 : 6, and 2 : 8, may be based on the naphthalene nucleus, but of these only the 1 : 2, 1 : 4, and 2 : 6 are known to exist. From what is known of the effect of substituents on the reduction potentials of 1 : 2- and 1 : 4-naphthaquinones, it may safely be assumed that measurements are not materially affected by 1 : 4 $\rightleftharpoons$ 1 : 2 tautomerism, but in the absence of definite knowledge the same cannot be said of other possible tautomeric shifts. Accordingly, in the strictest sense, some of the reduction potentials now recorded have to be recognised as applying, not necessarily to the pure substituted 1 : 4-naphthaquinones, but to tautomeric mixtures in equilibrium.

The Calculated Normal Reduction Potential of Droserone.—The effects of substitution recorded in Table II do not take into account any difference between the normal reduction potentials of the isomeric 2 : 5- and 2 : 8-dihydroxy-1 : 4-naphthaquinones. It is not definitely known to which constitution hydroxyjuglone conforms, but on the assumption that the hydroxyl groups in droserone occupy the same relative positions, we may deduce a value for its reduction potential by considering the effect of the 2-methyl group (— 0·0628 volt) on the observed reduction potential of hydroxyjuglone (0·3149 volt). This leads to the value 0·2521 volt, which, however, takes no account of the effect (if any) that the substituent methyl group may exert on the equilibrium of any tautomerism in the hydroxyjuglone system. A second value may be derived by considering the effect of introducing a 5-hydroxyl group (— 0·0373 volt) into phthiocol (0·2992 volt), but the resultant figure, 0·2619 volt, does not differentiate between the 2 : 5- and the 2 : 8-isomeride and may be influenced by tautomeric changes.

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