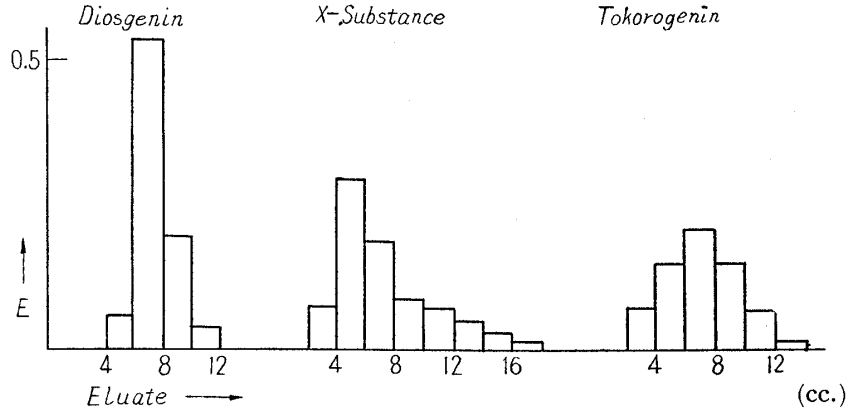

$CHCl_3$ (1% EtOH)-benzene (8:2) CH_2Cl_2 (1.5% EtOH) CH_2Cl_2 (7% EtOH)

Fig. 5. Histogram of Elution

Table II. Comparison of the Values

Sample	By Inoue's method (%)	By this method (%)
1	2.00	2.04
2	1.13	1.21
3	0.34	0.36
4	2.22	2.43

From the result in Table II and from those mentioned in 1) and 2), the present method for determing diosgenin, X-substance, and tokorogenin in the sample seems to be fully reliable.

The authors wish to express their grateful thanks to Dr. Satoru Kuwada and Dr. Atsushi Watanabe for their encouragement throughout the present work and to Dr. Masamoto Nishikawa for his kindness in supplying the sapogenin crystals.

Summary

The alcoholic extract of roots of Dioscorea growing in Japan was hydrolyzed with hydrochloric acid and the resulting mixture of sapogenins was separated into pure diosgenin, tokorogenin, and yonogenin by adsorption chromatography on Florisil. Each of the products was determined colorimetrically with reagents containing antimony trichloride as the chief component.

(Received March 28, 1958)

UDC 545.33 : 547.655.6

82. Morizo Ishidate, (the Late) Takashi Isshiki, and Keizo Tada : Nonaqueous Polarography of Quinones. V.* Half-wave Potentials of *o*-Quinones in Relation to Carcinogenesis of Polyaromatic Hydrocarbons.

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Various hypotheses or theoretical treatment have recently been developed for evaluation of the mechanism of carcinogenesis of polyaromatic hydrocarbons, but keys to solving this troublesome problem have not yet been provided by any of these speculations. It seems rather strange that none of them have referred to the probable rôle of quinones, some of which have recently been proved to result from hydrocarbons in a living body, in spite of the fact that *p*-benzoquinone and 1,4-naphthoquinone have some

* Part IV : This Bulletin, **3**, 312(1955).

** Hongo, Tokyo (石館守三, (故)一色 孝, 多田敬三).

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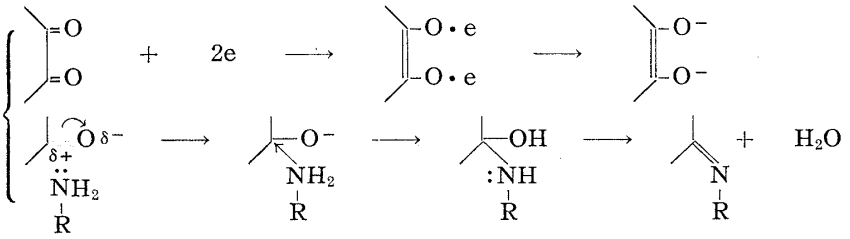
carcinogenic activity.[1] Moreover, A. and B. Pullman[2] postulated that, through *o*-quinonoid form in the "K region," benz[*a*]anthracene should be bound to the receptor in cells according to their "localization energy theory," and Heidelberger, *et al.*[3] demonstrated the intermediate formation of *o*-quinone or its diimide in their studies on the protein-bound compounds of dibenz[*a,h*]anthracene after its topical application to the skin of mice.

With regard to the rôle of quinones in the carcinogenesis of their parent hydrocarbons, we had already made the following postulations: Quinones may react (or contact) with a receptor of some specific enzymes that govern or control the reduction-oxidation enzyme system, and then through chemical changes of the quinones, the enzyme system may occasionally but steadily be disturbed. The accumulation of irregular or irreversible changes in the reduction-oxidation system finally leads to exhibition of carcinogenesis. In this assumption, at least the next three factors seem to be of importance. First, a quinone must possess such physical and chemical properties that it can penetrate the cell membrane, reach the receptor, and stay there as long as possible without any detoxications or, if detoxicated, to a minimum degree. Second, the redox potential of a quinone should be high enough to be reduced in the living body so that it disturbs the enzyme system. Third, the *o*-quinone should be active enough to condense with active amines of the receptor through a Schiff base.

Now, if we can assume that the reduction of a quinone to a hydroquinone anion is preceded by each uptake of an electron by each oxygen radical following the restoration of aromatic conjugated system, and moreover, that the condensation of *o*-quinones with active amines be activated with the excitation of π-electrons in the carbonyl bond, the ease with which the two reactions proceed should be the reverse of each other.



As the order of the redox potentials of quinones directly indicates the ease with which they are reduced, the sequence may suggest indirectly the order of the inactivity of imine-formation, and consequently, the measurement of redox potentials of quinones, especially of *o*-quinones, becomes of significance.

On the other hand, we already have reported a series of studies[4] on the nonaqueous polarography of several *p*-quinones in glacial acetic acid containing 0.25*N* ammonium acetate. It was found that their half-wave potentials could satisfactorily be measured under identical conditions although it was necessary in each run to correct the I.R. drop caused by inner resistance through the circuit.

Similar to the case of *p*-quinones, we selected the following four *o*-quinones and phenanthrenequinone, used instead of *o*-benzoquinone, because of the extreme unstability of the latter: 1,2-Naphthoquinone (*o*-NQ), 1,2-anthraquinone (*o*-AQ), 9,10-phenanthrenequinone (Ph.Q), benz[*a*]anthra-5,6-quinone (*o*-BAQ), and dibenz[*a,h*]anthra-5,6-quinone (*o*-DBAQ).

For these five quinones, all measurements were carried out under identical conditions as in the case of *p*-quinones, and as described in the previous papers[4] except

1) e.g., N. Takigawa: Proc. Imp. Acad. (Tokyo), **16**, 309(1940).
2) "Advances in Cancer Research," Academic Press, New York, III, 166(1955).
3) P.M. Bhargava, C. Heidelberger: J. Am. Chem. Soc., **78**, 3671(1956).
4) T. Isshiki, K. Tada: This Bulletin., **2**, 266; K. Tada: *Ibid.*, **2**, 270, 272(1954).

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for those given in the experimental part of this paper.

Result and Discussion

The half-wave potentials of *o*-quinones were satisfactorily measured and their values are summarized in Table I.

Table I. Half-wave Potentials of *o*-Quinones

	o-NQ	*o*-AQ	Ph. Q	*o*-BAQ	*o*-DBAQ
$E_{1/2}$ volt vs. S.C.E.	$+0.20_7$	$+0.12_7$	$+0.08_1$	$+0.01_6$	$+0.04_1$

From this table, the order of the ease with which they are reduced at dropping mercury electrode is shown by the sequence (1).

$$o\text{-NQ} > o\text{-AQ} > \text{Ph.Q} > o\text{-DBAQ} > o\text{-BAQ} \quad \cdots\cdots\cdots\cdots\cdots\cdots (1)$$

In the previous paper,[5] it was shown that a similar order of *p*-quinones as in the sequence (2) well coincided with the resonance theory.

p-Benzoquinone (*p*-BQ) > 1,4-naphthoquinone (*p*-NQ) > dibenz-[*a*,*h*]anthra-7,14-quinone (*p*-DBAQ) > benz[*a*]anthra-7,12-quinone (*p*-BAQ) > 9,10-anthraquinone (*p*-AQ) ·····(2)

Now, the reduction of *o*-quinones must be much affected by the so-called *ortho*-effect, but if the degree of the effect be identical for all of these *o*-quinones, the order shown in the sequence (1) should also agree with the order in which the ratios of *o*-hydroquinone anion to *o*-quinone decrease in the number of principal limiting structures as discussed in detail in a previous paper.[5] This accordance is illustrated in Table II.

Table II. Number of Principal Limiting Structures contributing to the Resonance and Ratio of Hydroquinone Anion to Quinone in the Number of Principal Limiting Structures

	o-NQ	*o*-AQ	Ph. Q	*o*-DBAQ	*o*-BAQ
Hydroquinone anion	3	4	5	12	7
Quinone	2	3	4	10	6
Ratio	1.50	>1.33	>1.25	>1.20	>1.17

The quantitative relationship between the ratios and $E_{1/2}$'s of *p*- and *o*-quinones is given in Fig. 1.

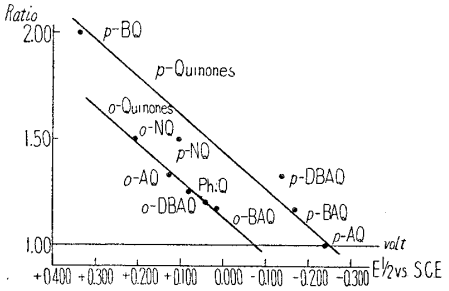

Fig. 1. Ratios of Hydroquinone Anion to Quinone in the Number of Principal Limiting Structures
$E_{1/2}$'s diagram in *p*- and *o*-Quinones

It was found that a fine straight line was obtained for *o*-quinones while not so fine a one for *p*-quinones. The reason for it is yet beyond understanding.

Next, we should like to refer a little to some relations between the above-mentioned rôles of quinones and the $E_{1/2}$'s thus obtained polarographically which are analogous to the redox potentials.

If we can assume that the mechanisms of the reduction of quinones and of condensation of *o*-quinones with amines were both available and the conditions of these reactions were to be almost identical for all quinones, the order of the ease with which *o*-quinones condense with amines of the receptors should be written as in the sequence (3).

5) M. Ishidate, T. Isshiki, K. Tada : *Ibid.*, **3**, 312(1955).

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$$o\text{-NQ} < o\text{-AQ} < \text{Ph.Q} < o\text{-DBAQ} < o\text{-BAQ} \quad \cdots\cdots\cdots\cdots\cdots\cdots (3)$$

Among the six parent hydrocarbons of the quinones in question, only two, benz[*a*]anthracene and dibenz[*a*, *h*]anthracene, are known to be carcinogenic. This distinct difference may probably result from many unknown factors, but, by focussing the problem on the rôle of quinones from the above sequence (2) and (3), one may at least understand the following. *o*-BAQ and *o*-DBAQ are more active to condense with amines than the other *o*-quinones. Though they are more resistant to reduction than the others, they may be still reducible enough in the living cells, and *p*-BAQ and *p*-DBAQ have higher $E_{1/2}$'s than *p*-AQ though lower than *p*-BQ and *p*-NQ. The last two quinones are carcinogenic, but they can scarcely be formed *in vivo* from their parent hydrocarbons probably because they are easily metabolized.

The authors wish to thank Messrs. Kimura and Iwaguchi, and Miss Kyogoku for their technical assistance.

Experimental

(All melting points are uncorrected.)

Apparatus—Instrument : Polarograph used was Yanagimoto PB-4 type (1956).

Reference electrode : As $E_{1/2}$'s of *o*-quinones in question have all positive values vs. S.C.E., a saturated mercurous sulfate electrode was used as the reference electrode for all of *o*-quinones. Its potential vs. S.C.E. was measured immediately before each experiment in the manner described previously.

Reagents—1) *o*-NQ : Orange-red crystals, m.p. 110~117°(decomp.), prepared by oxidation of 1-amino-2-naphthol with $FeCl_3$, were washed several times with benzene-petr. ether (1:4); m.p. 114~119°(decomp.). This substance was very unstable in the solution and could not be purified by recrystallization, reprecipitation, or column chromatography.

2) *o*-AQ : Synthesized from 1-hydroxyanthracene (prepared[6] from 1-amino-9,10-anthraquinone) according to Dienel's method[7] and recrystallized once from boiling water and three times from dehyd. EtOH, m.p. 179°.

3) Ph.Q : Synthesized by CrO_3-oxidation of pure phenanthrene according to Gräbe's method,[8] recrystallized three times from dehyd. EtOH and once from benzene, and washed with petr. ether, m.p. 206.7~207.7°.

4) *o*-BAQ : Synthesized by CrO_3-oxidation of crude 5,6-dihydroxy-5,6-dihydrobenz[*a*]anthracene (prepared[9] by OsO_4-oxidation of benz[*a*]anthracene (Eastman Kodak product), m.p. 165~166°) according to Collin's method[9] and recrystallized from glacial AcOH, m.p. 258~260°. Some unknown orange crystals (m.p. 149~151°) were also obtained, but they have not been identified.

5) *o*-DBAQ : Synthesized by CrO_3-oxidation of crude 5,6-dihydroxy-5,6-dihydrodibenz[*a*, *h*]anthracene (prepared[10] by OsO_4-oxidation of dibenz[*a*, *h*]anthracene (Eastman Kodak product), m.p. 265~266°) according to Heidelberger's modified method,[11] and recrystallized from AcOEt; m.p. 326~328°.

6) Glacial AcOH : Purified by repeated fractional distillations and freezing method after standing with CrO_3 for 3 days.

Summary

The half-wave potentials of five homologous *o*-quinones shown below were measured under identical conditions by the nonaqueous polarography in glacial acetic acid containing 0.25*N* ammonium acetate : 1,2-Naphthoquinone (*o*-NQ), 1,2-anthraquinone (*o*-AQ), 9,10-phenanthrenequinone (Ph. Q), benz[*a*]anthra-5,6-quinone (*o*-BAQ), dibenz[*a*, *h*]anthra-5,6-quinone (*o*-DBAQ).

6) H.E. Fierz-David, *et al.* : Helv. Chim. Acta, **29**, 1755(1946).
7) H. Dienel : Ber., **39**, 930(1906).
8) C. Gräbe : Ann. **167**, 140(1873).
9) C.G. Collins : J. Am. Chem. Soc., **73**, 5178(1951).
10) J.W. Cook, R. Schoenteil : *Ibid.*, **77**, 2886(1955).
11) C. Heidelberger, *et al.* : *Ibid.*, **77**, 2866(1955).

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The order of the ease with which they are reduced at the dropping mercury electrode are given by the following sequence :

$$o\text{-NQ} > o\text{-AQ} > \text{Ph. Q} > o\text{-DBAQ} > o\text{-BAQ}$$

This order agreed with the resonance theory as in the case of *p*-quinones described in a previous paper.

From the standpoint of the order of $E_{1/2}$'s of the above-mentioned *o*-quinones, it was found that they might play some important rôles in the carcinogenesis of their parent hydrocarbons.

(Received March 29, 1958)

UDC 547.837.07

83. Norio Sugimoto and Hiroshi Kugita : Studies on the Syntheses of Hydrogenated Quinolines and Isoquinolines as Analgesics. XV.[1] Synthesis of 3-Hydroxy-N-methyl-7-aza-des-N-morphinan (9-Hydroxy-3-methyl-5,10b-trimethylene-1,2,3,4,4a,5,6,10b-octahydrobenzo[*f*]isoquinoline).

(*Osaka Research Laboratory, Tanabe Seiyaku & Co., Ltd.**)

Preliminary studies on the synthesis of the subject compound have already been described in one of earlier papers of this series.[2] This compound is one of the most interesting from the point of location of the nitrogen atom among the morphinan isomers in which the position of nitrogen has been transferred, the synthetic studies of which are being undertaken by Sugimoto and his co-workers. The reason for this is that the position (17) of nitrogen in D-ring would correspond to 7-position in C-ring, considering the position (13) of the quaternary carbon atom in morphinan. In this way, this compound is structurally interesting and studies on its synthesis and pharmacological action are of great interest.

The process of synthesis followed the process described earlier[2] and 8-(*p*-methoxybenzyl)-8-hydroxy-5,6,7,8-tetrahydroisoquinoline (II) was prepared by the Grignard reaction of 8-oxo-5,6,7,8-tetrahydroisoquinoline (I) and *p*-methoxybenzylmagnesium chloride. (II) was heated with dilute hydrochloric acid to effect dehydration and the *p*-methoxybenzylidene compound (III) was obtained. Hydrogenation of all the double bonds in (III) was attempted by catalytic reduction over 5% palladium-carbon to obtain methoxybenzyltetrahydroisoquinoline (IV) but the reduction did not proceed at ordinary pressure and the objective compound was not obtained.

As a preliminary experiment, 8-benzylidene-5,6,7,8-tetrahydroisoquinoline methiodide[1] (IIIa) was submitted to catalytic reduction in methanol with Raney nickel, in the presence of potassium hydroxide at ordinary pressure. The base obtained on absorption of three moles of hydrogen was proved to be entirely identical with the base (Va) formed by the same catalytic reduction of 8-benzyl-5,6,7,8-tetrahydroisoquinoline methiodide after absorption of two moles of hydrogen. In accordance with this process, the methiodide (IIIb) of the *p*-methoxybenzylidene compound (III) was submitted to catalytic

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1) Part XIV : This Bulletin, **5**, 378(1957).

2) Part XII : *Ibid.*, **5**, 67(1957).

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