[CONTRIBUTION FROM THE LABORATORY OF BIOLOGICAL CHEMISTRY, WASHINGTON UNIVERSITY SCHOOL OF MEDICINE

The Two-Step Oxidation–Reduction of Lapachol, Lomatiol and Related Compounds

BY EDGAR S. HILL

A number of quinones, both naturally occurring and synthetic, have been found to oxidize and reduce in alkaline solution in two steps, with the formation of an intermediate compound, or semiquinone. Among these are the anthraquinonesulfonates,[1] β-naphthoquinone,[2] phenanthrenequinonesulfonate,[3] and phthiocol.[4] Phthiocol is a substituted hydroxynaphthoquinone and related to it by structure are a large number of hydroxynaphthoquinone derivatives, which, depending on the experimental conditions, form reversible oxidation-reduction systems. Some of these hydroxynaphthoquinones occur in a wide variety of plant materials, making their study of more than theoretical interest.

The author has been able to study successfully a number of these compounds by potentiometric methods and has found that their behavior in alkaline solution is similar to that of the quinones mentioned above. The compounds studied were lapachol, lomatiol, hydrolomatiol, hydroxyhydrolapachol, iso-β-lapachol, α-lapachone, β-lapachone, chlorohydrolapachol, bromo-β-lapachone, juglone and lawsone. The first five of these were found to be sufficiently stable in strongly alkaline solution to permit a thorough potentiometric study, and the remainder, although too unstable in alkaline solutions for similar study, were believed to oxidize and reduce in a similar way, on the strength of their structural relationship and on colorimetric experiments.

Fieser[5] has reported the normal potentials of lapachol, iso-β-lapachol, chlorohydrolapachol, hydroxyhydrolapachol, lomatiol, α-lapachone and β-lapachone, working only in 50% alcohol, 0.1 *N* in hydrochloric acid and 0.2 *N* in lithium chloride, using titanous chloride as reducing agent. No titrations were made in alkaline solution. Ball[6] has reported the normal potentials of several of these compounds for *p*H values ranging from 1.1 to 12.6, but obtained his values by the method of mixtures. Ball, working independently, recently has found[7] by titration methods that lapachol, in alkaline solutions, exhibits two-step oxidation and reduction, similar to phthiocol. The normal potentials of juglone have been reported by Conant and Fieser,[8] working again in alcoholic acid solution, and those of both juglone and lawsone by Friedheim,[9] working over a considerably wider *p*H range, who found that rapid decomposition in alkaline solution prevented the obtaining of titration curves reliable enough for analysis. Fieser and Fieser[10] were able to titrate lawsone (2-hydroxy-1,4-naphthoquinone) satisfactorily over the range *p*H 0.33–12.92.

Solutions of the quinones were titrated reductively over a *p*H range from 7.1 to 14.3. The technique of potentiometric titration was the usual one and the concentration of the oxidants used was 0.0005 *M* throughout. All potentials were measured at 30° and have been corrected to the hydrogen standard, and those values obtained in sodium hydroxide solutions have been corrected for liquid junction potentials. The buffers used are tabulated with the potential values.

The compounds successfully titrated were lomatiol, hydrolomatiol, lapachol, hydroxyhydrolapachol and iso-β-lapachol. Since there is little difference in the normal potentials of the various systems (the maximum difference between any two being only 4 to 6 mv.), only one compound, lomatiol, will be discussed in detail and the others but briefly.

Lomatiol is 2-(δ-hydroxy-β-isopentenyl)-3-hydroxy-1,4-naphthoquinone and occurs in the seeds of the Australian plants, *Lomatia ilicifolia* and *Lomatia longifolia*. The coloring matter is the same in both species and was first isolated by Rennie[11] and its constitution, synthesis, relation to and conversion into the other hydroxynaphthoquinone derivatives studied by Hooker.[12] Solutions of lomatiol were titrated reductively over a

(1) E. S. Hill and P. A. Shaffer, *Proc. Am. Soc. Biol. Chem.*, **8**, li (1936); [*J. Biol. Chem.*, **114** (1936)].

(2) L. Michaelis, THIS JOURNAL, **58**, 873 (1936).

(3) L. Michaelis and M. P. Schubert, *J. Biol. Chem.*, **119**, 133 (1937).

(4) E. S. Hill, *Proc. Soc. Expll. Biol. Med.*, **35**, 363 (1936).

(5) L. F. Fieser, THIS JOURNAL, **50**, 349 (1928).

(6) E. G. Ball, *J. Biol. Chem.*, **114**, 649 (1936).

(7) E. G. Ball, THIS JOURNAL, **59**, 2071 (1937).

(8) J. B. Conant and L. F. Fieser, *ibid.*, **46**, 1858 (1924).

(9) E. A. H. Friedheim, *Biochem. J.*, **28**, 180 (1934).

(10) L. F. Fieser and M. Fieser, THIS JOURNAL, **56**, 1565 (1934).

(11) E. H. Rennie, *J. Chem. Soc.*, **67**, 784 (1895).

(12) S. C. Hooker, THIS JOURNAL, **58**, 1181 (1936).

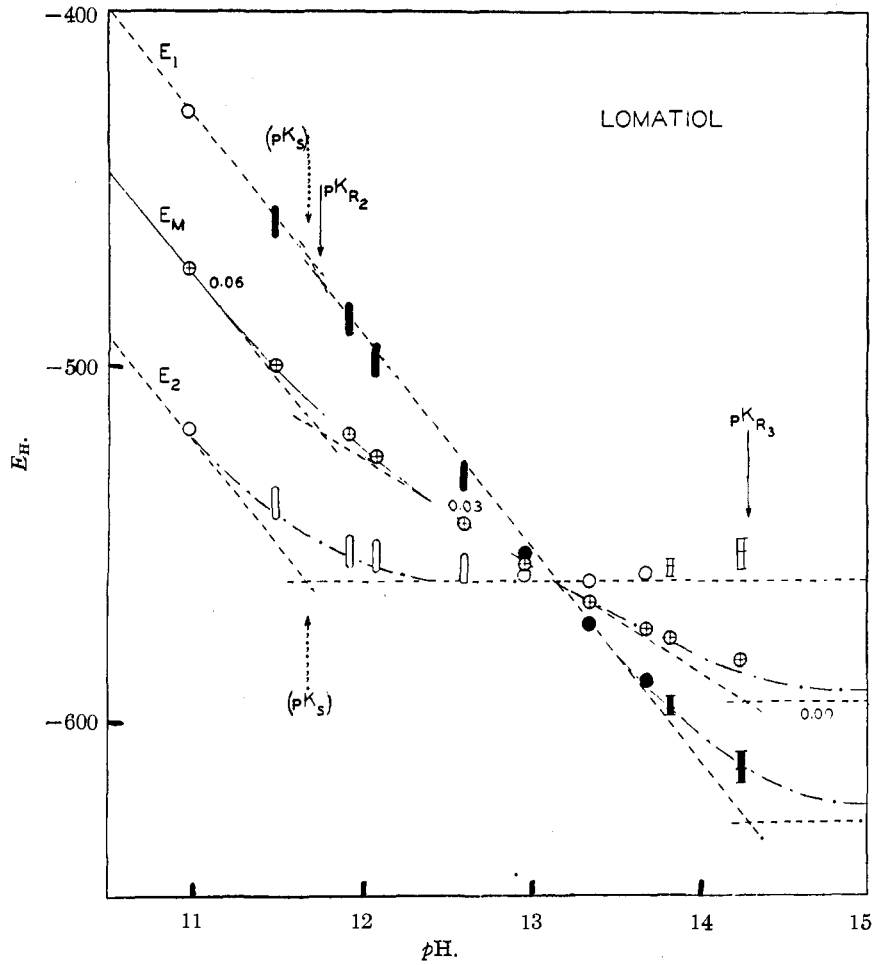

Fig. 1.—The three normal potentials, referred to the normal hydrogen electrode at 30°, of lomatiol, plotted against *p*H: E_m, normal potential of the system fully oxidized–fully reduced form; E_1, that of the system semiquinone form–fully reduced form; E_2, that of the system totally oxidized form–semiquinone form.

*p*H range from 7.1 to 14.3, and it was found from an analysis of the titration curves that the separation of the two steps begins a little above *p*H 9.0 and is more distinct in more alkaline regions.

A summation of the data for lomatiol is shown graphically in Fig. 1, giving the normal potentials plotted against *p*H. The separation never becomes so great as to produce a jump of the potential at 50% of the whole titration. The slopes of the titration curves varied all the way from 14.5 mv. at the lower limit to 36 mv. at about *p*H 14.24(3 *M* sodium hydroxide). Table I shows the variations in the slopes (E_i) and the attending variations in the formation constant, *K*, of the semiquinone and in the maximum ratio, *M*, of semiquinone to total material. The two steps always overlap so much that, at the most, about 61% of the total material in the form of the semiquinone is in equilibrium with the other forms. The curve (Fig. 1) shows a second dissociation constant of the reductant at about *p*H 11.74, which is in agreement with the findings of Ball,[6] and possibly a third in the neighborhood of *p*H 14.3, although titration data at this *p*H (3 *M* sodium hydroxide) are not too reliable on account of badly drifting potentials and the high liquid junction potentials involved. The separation of the two component systems is small and the precise location of the dissociation constant of the semiquinone is a bit uncertain. It is very close to the second dissociation constant of the reductant, possibly in the neighborhood of *p*H 11.68–11.7. The points on the E_1 and E_2 curves in this region are drawn elliptically to show possible variations; slight variations in the *S* value in this region will cause rather large varia-

TABLE I

LOMATIOL

Temperature: 30°. $E_m(E_0')$ represents the mean normal potential; E_1, E_2, normal potential of the lower and of the higher step; E_i, index potential; S, ½(100% axis − 0% axis) for a tangent drawn to all curves at 50% reduction, according to Elema[13]; K, semiquinone formation constant; M, maximum ratio of semiquinone to total material. Concentration of quinone: 0.0005 M.

pH	Buffer	$E_m(E_0')$, mv.	E_i, mv.	S	$E_2 - E_1$, mv.	E_1, mv.	E_2, mv.	K	M
7.1	Phosphate	−191.2	14.5	28	−104	−139.2	−243.2	0	0
9.87	Carbonate	−409	14.5	28.5	− 90	−364	−454	0.031	0.08
10.97	Phosphate	−473	14.5	28.5	− 90	−428	−518	.031	.08
11.49	Phosphate	−500.2	15.5	29.25	− 76	−462.2	−538.2	.055	.105
11.92	Phosphate	−520	16	29.75	− 66	−487	−553	.08	.124
12.08	Phosphate	−525.2	16.5	31	− 52	−499.2	−551.2	.17	.17
12.6	0.1 M NaOH	−545	18.5	35	− 20	−535	−555	.59	.277
12.96	0.2 M NaOH	−555.5	19.5	37.5	− 5	−553	−558	.83	.31
13.34	0.5 M NaOH	−566.9	22.5	42	+ 10	−571.9	−561.9	1.45	.39
13.68	1.0 M NaOH	−574	26.5	50	+ 32	−590	−558	3.4	.46
13.82	2.0 M NaOH	−577	32	55	+ 42	−598	−556	5.02	.54
14.24	3.0 M NaOH	−583	36	68.5	+62–66	−614–616	−552–550	10.0	.61

$K = (76.63S - 2)^2$, (13) $E_2 - E_1 = 0.06 \log K$, (14) $M = \sqrt{K}/(\sqrt{K} + 2)$. (3)

tions in the value of $E_2 - E_1$. The largest value for K was found to be 10. The fact that the titration curves were smooth, with no jump in the middle, is in keeping with the mathematical interpretations of Michaelis and Schubert,[3] which allow for no lateral points of inflection unless K is greater than 16.

The color changes are worthy of comment. In all lomatiol solutions above *p*H 8.0, the fully oxidized form is cherry-red. Between *p*H 10.0 and 12.6, the intermediate color is brownish-orange with a bluish tinge appearing as the *p*H increases. Above *p*H 13.0 the semiquinone color is brownish-violet and almost pure purple at *p*H 14.24. The completely reduced form is pale yellow below *p*H 13.0 and greenish-yellow above this point.

Parallel investigations on hydrolomatiol, lapachol, hydroxyhydrolapachol, and iso-β-lapachol resulted in potential values almost identical with those of lomatiol, and with only minor variations in the amount of semiquinone formation (Table II). All of these compounds have been described by Paternò, Hooker, Fieser, *et al.* Hydrolomatiol[12] is prepared by the hydrogenation of lomatiol. Lapachol[15-17] is 2-(γ,γ-dimethylallyl)-3-hydroxy-1,4-naphthoquinone and is found in the grain of a number of South American woods. Hydroxyhydrolapachol[15,16] can be prepared by the action of potassium hydroxide on β-lapachone.

(13) B. Elema, *Rec. trav. chim.*, **54**, 76 (1935).
(14) L. Michaelis, *J. Biol. Chem.*, **96**, 703 (1932).
(15) E. Paternò, *Gazz. chim. ital.*, **12**, 337 (1882).
(16) S. C. Hooker, *J. Chem. Soc.*, **61**, 611 (1892).
(17) L. F. Fieser, THIS JOURNAL, **49**, 857 (1927).

Iso-β-lapachol[18,5] is isomeric with lapachol, and differs only in color from the other compounds of this series. Solid iso-β-lapachol is brick-red, while the other compounds mentioned are yellow. In alkaline solution, it is intensely purple, the intermediate reduction stage is brownish-red and the completely reduced form is greenish-yellow.

TABLE II

COMPARISON OF THE VALUES OF K, THE SEMIQUINONE FORMATION CONSTANT

pH	Lomatiol	Hydro-lomatiol	Lapachol	Hydroxy-hydro-lapachol	Iso-β-lapachol
7.1	0	..	..	..	..
9.87	0.08	..	..	..	..
10.2	..	..	0.034	..	..
10.4	..	..	..	..	0.11
10.97	.08	..	..	..	..
11.18	..	0.09	.05	0.05	.15
11.49	.10	..	..	..	..
11.75	..	..	.11	..	.19
11.92	.12	..	..	..	..
12.08	.17	.16	.14	.12	.25
12.6	.27	.24	.26	.21	.26
12.96	.31	.28	.29	.28	.29
13.34	.39	.38	.35	.34	.37
13.68	.46	.47	.46	.46	.46
13.82	.54	.55	.55	.55	.56
14.24	.61	.61	.61	.61	..

All of these compounds gave stable potentials during titration in all of the alkaline buffers except the strong sodium hydroxide solutions. Here, the quinones undergo decomposition and irreversible changes, causing drifting potentials. It is due to this instability that the values for E_2 in the range of *p*H 13.6–14.24 deviate from the

(18) S. C. Hooker, *J. Chem. Soc.*, **69**, 1355 (1896).

base line (Fig. 1). Table II shows a comparison of the maximum ratios of semiquinone to total material for all of the quinones. The E_1 and E_2 curves all intersect the E_0' curves in the neighborhood of *p*H 13.1–13.2. The values of pKr_2 agree closely with those of Ball,[6] and the values of E_0' vary but slightly. The E_0':*p*H curve for these compounds is about 10–12 mv. more positive than that of phthiocol. These quinones are extremely insoluble in acid solution, but titrations in alcoholic acid buffers failed to show any step formation in this region.

α-Lapachone, β-lapachone, chlorohydrolapachol and bromo-β-lapachone were not titratable in alkaline solution due to their rapid decomposition. α-Lapachone, first obtained by Paternò[15] by the action of nitric acid on lapachol, and later described by Hooker,[16] decomposes rapidly in alkali. The same is true of β-lapachone,[15,16] its ortho isomer. Both of these compounds are insoluble in alkalies in the cold, but are dissolved gradually by a boiling 1% solution of sodium hydroxide, and in so doing undergo change. Bromo-β-lapachone[15,16] can be obtained by treating lapachol with hydrogen bromide gas in chloroform solution, and is also extremely insoluble. The bromine of bromo-β-lapachone is easily displaced from the molecule by alkalies and the substance reverts to β-lapachone, making it useless for titration. Chlorohydrolapachol[16] is a product of the direct addition of hydrogen chloride to lapachol. In contact with dilute alkalies, chlorohydrolapachol is converted into a mixture of lapachol, hydroxyhydrolapachol, α-lapachone and β-lapachone, the latter two of which crystallize out, all of these complications making it impossible to study the compound potentiometrically. The similarity in structure of these compounds with the ones successfully titrated, coupled with the fact that slow reduction in test-tube experiments gives rise to similar intermediate color changes, seem to indicate that they too possess the ability to form semiquinones.

For the opportunity to study potentiometrically these compounds of the lapachol group, the author is greatly indebted to Dr. Louis F. Fieser, who kindly placed at his disposal pure samples of these materials left by the late Dr. Samuel C. Hooker, whose exhaustive study of hydroxynaphthoquinone chemistry is unique in the literature of organic chemical research.

The potentials of the naturally occurring hydroxynaphthoquinones, juglone and lawsone, were reinvestigated. Lawsone is 2-hydroxy-1,4-naphthoquinone and occurs in the leaves of the henna plant (*Lawsonia inermis*) and juglone, the coloring matter contained in the husks of walnuts, is 5-hydroxy-1,4-naphthoquinone. Friedheim[9] reported an irregularity in the slopes of titration curves obtained with these substances at *p*H 7 and above, and suggested that this irregularity pointed toward semiquinone formation. This possibility was not pursued, due to the instability of the pigments in alkaline media. On reinvestigating these compounds, the author also found it impossible to obtain reliable titration curves with juglone and lawsone at *p*H greater than 7.0 due apparently to the decomposition of these compounds in alkaline buffers. In the case of lawsone, which was extracted from henna leaves by the method of Tommasi,[19] the fault probably lay in the purity of the material used, since Fieser and Fieser,[10] working with a completely pure substance, were able to titrate this compound successfully in buffers as alkaline as *p*H 12.92. It is the intention of the author to reinvestigate the potentials of lawsone, using a thoroughly pure synthetic sample. The juglone used was prepared synthetically by oxidation of 1,5-dihydroxynaphthalene with chromic–sulfuric acid mixture, according to Bernthsen and Semper.[20] Test-tube experiments seemed to show intermediate colors when these quinones were oxidized and reduced, and, since they are so similar in structure to phthiocol, lomatiol, *et al.*, it might be suggested, pending further work, that these pigments be included among anionic semiquinone formers. On comparing juglone with lawsone and the aforementioned hydroxynaphthoquinones, one might draw the conclusion that substitution of the benzene ring containing the quinone oxygens has considerable to do with potentiometric stability.

Conclusion

Semiquinone formation by lomatiol, lapachol, and other hydroxynaphthoquinones has been investigated. The two-step character of the oxidation–reduction becomes evident at about *p*H 9.5 and becomes more distinct as the solution becomes more alkaline. The ratio of semiquinone to total material reaches a maximum of 0.61 at *p*H 14.24. It corresponds to an index potential of 36 mv. and a formation constant of 10. The

(19) G. Tommasi, *Gazz. chim. ital.*, **50**, 263 (1920).
(20) A. Bernthsen and A. Semper, *Ber.*, **20**, 938 (1887).

data presented add both to the knowledge of naturally occurring pigments, and of substances with a quinoid structure as a group.

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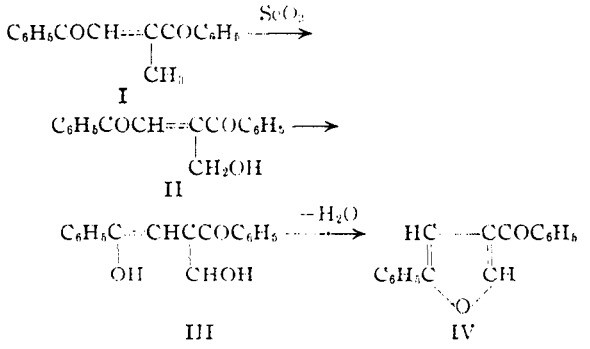
[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF ILLINOIS]

2-Phenyl-4-benzoylfuran

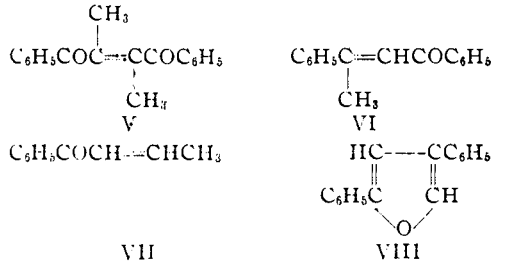
BY REYNOLD C. FUSON, C. L. FLEMING AND ROBERT JOHNSON

Very few β-keto furans are known and of these none has an unsubstituted α-position. 2-Phenyl-4-benzoylfuran (IV) is, therefore, unique. In the study of the application of the principle of vinylogy to ketones we chanced to prepare this furan and noted that its properties reflected its unusual structure. This observation led us to examine these properties in some detail.

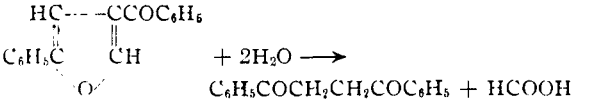
The furan was made by treating 1,2-dibenzoyl-1-propene (I) with selenium dioxide. Since the diketone is a vinylog of acetophenone it was expected that the methyl group would be attacked. Evidently this group is oxidized and the furan is formed by rearrangement and subsequent loss of water. Of especial interest in this connection is the observation that only one-half mole of selenium dioxide is required to convert one mole of the diketone (I) to the furan (IV). This corresponds to the amount of oxidation necessary to form the hydroxy compound (II). The latter could rearrange to the dienol (III) by a 1,5- or a double 1,3-shift. The dienol would be expected to lose water to form the furan.



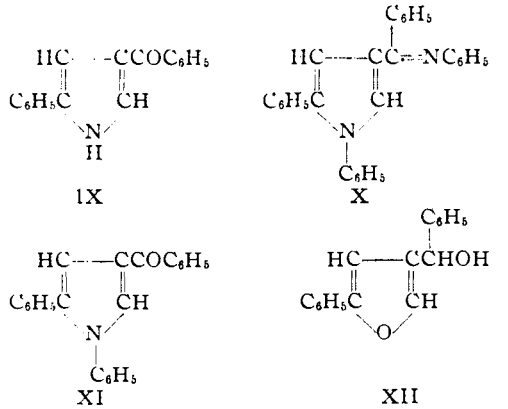
This mechanism suggests that the method might be general for ketones of the type $RCOC(=CH-CH_3)$. To test this idea we subjected 2,3-dibenzoyl-2-butene (V), dypnone (VI) and crotonophenone (VII) to a similar treatment. Only dypnone yielded a furan; the other two ketones were recovered unchanged even after long treatment with the oxidizing agent. This indicates that this method of preparing furans is highly specific. It should be mentioned that 2,4-diphenylfuran (VIII), the product obtained from dypnone, had been prepared in low yields by the oxidation of dypnone with nitrobenzene.[1]



The most striking property of 2-phenyl-4-benzoylfuran is the ease with which it reacts with alkalies, ammonia or aniline. This appears less extraordinary if we consider that the compound is vinylogous with benzoic esters and might, therefore, be expected to be sensitive to hydrolytic and ammonolytic agents. Aqueous alkalies hydrolyze it rapidly to 1,2-dibenzoylethane and formic acid



Ammonia and aniline convert the furan, respectively, into the pyrrole (IX) and the anil (X) of the expected phenylpyrrole.



(1) Engler and Dengler, *Ber.*, **26**, 1446 (1893); *cf.* Delacre, *Bull. soc. roy. Belg.*, [3] **26**, 534 (1893).