OXIDATION AND REDUCTION OF ORGANIC COMPOUNDS AT THE DROPPING MERCURY ELECTRODE AND THE APPLICATION OF HEYROVSKÝ'S POLAROGRAPHIC METHOD IN ORGANIC CHEMISTRY[1]

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I. INTRODUCTION

Considerable effort has been made for many years to apply the usually simple and accurate methods of electrochemistry to the study and analysis of organic compounds. Valuable results have been obtained wherever these methods have been applicable. When J. Heyrovský demonstrated the usefulness of the dropping mercury cathode for the study of inorganic reductions, interest in the application of this technic to the field of organic chemistry was aroused. The first organic substance to be reduced at the

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dropping mercury cathode was nitrobenzene. This reduction was studied in 1925 by M. Shikata, who was working at that time in the Prague laboratory (90). Plotting current against applied voltage, he obtained a curve which resembled that due to a reduction of metallic ions. After the development of the polarograph (39), many reducible organic compounds were studied; the results were reproducible, the reduction potentials of the various substances were different, and the current was observed to be a function of concentration. Therefore it seemed reasonable to expect that in organic chemistry qualitative and quantitative analyses should be possible by means of the polarographic method. However, the difficulties encountered were far greater than in inorganic reduction; consequently analyses of organic materials were achieved only in isolated cases under well-controlled conditions. At present the qualitative and quantitative analysis of a mixture of organic compounds in unknown solutions is of doubtful value.

In addition to qualitative and quantitative analyses, the polarographic method may be used in studies of the influence of substituted groups and of conjugated double bonds on the reduction potential, in examinations of organic reactions such as complex formation (including catalysis), tautomerism, and polymerization, and in investigations of the nature of the processes at the electrode surface.

The pioneer studies of organic compounds with the polarograph, done in the laboratories of Heyrovský, Shikata, and Semerano, were devoted to the accumulation of sufficient empirical data to serve as the basis for the development of more refined investigations. While interpretations of these experiments had to remain highly speculative until the value of the "half-wave potential" had been demonstrated by Heyrovský and Ilkovič (38), they often gave enough information to permit predictions of the reducibility of compounds. Refinement of the technic and a better understanding of the principles involved have made necessary a number of corrections of these pioneer exploratory investigations. Today almost one half of over four hundred papers on polarography deal with organic reactions, and it appears that the interest in this field is going to exceed that given to inorganic reductions.

A description of the polarographic method has been omitted in this review because it is given in the preceding paper by Kolthoff and Lingane (49), who also discuss the papers dealing with the suppression of maxima and with the effect of the solvent. Different solvents have been used, but only aqueous solutions are considered in this paper.

In the absence of a theoretical discussion of the organic reactions in general treatments of polarography (36, 43, 75), this paper deals with some of the underlying theories and their relation to established principles of electrochemistry. An attempt is made to show the difference

between reversible and irreversible reactions at the dropping mercury electrode and to demonstrate the importance of buffering and the significance of the half-wave potentials.

II. EVALUATION OF CURVES

In the paper (49) preceding this one it has been shown that the reduction of inorganic ions at the dropping mercury electrode is well enough understood to lend itself to accurate and quantitative description. A similar treatment for organic reactions is not yet possible, since they are much more complex because of the greater number of variable factors. Many of these factors, which also apply to inorganic reactions, have been discussed by Kolthoff and Lingane (49). However, the fundamental difference between inorganic and organic reactions is that hydrogen in some form is always involved in the latter, so that the pH and buffer capacity of the solution and the dissociation constants of reductant and oxidant have to be considered. For studies of tautomerism, polymerization, and reaction kinetics it is necessary to have precise knowledge of the temperature, the age of the solution, and the concentration. So far these factors have not received sufficient attention and often have been entirely neglected.

The greatest difficulty in correlating the results of different authors arises from the varying methods of potential measurement. Considering that a single substance may have any number of reduction potential values depending on the pH (see figure 5), a uniform definition and accurate determination of these values becomes of much greater importance than in the inorganic field. It is by no means sufficient simply to give the polarographically *applied voltage* at a certain point of the curve; the anode potential and the drop in potential (*iR* drop) in the solution have to be known accurately.[3] From these three values the real *potential* at the dropping mercury electrode can be calculated and referred to some common standard.

1. Potential of the non-polarizable (quiet) electrode

The voltage applied to the dropping mercury electrode is measured against the potential of the non-polarizable (quiet) electrode or reference

[3] This cannot be emphasized strongly enough because even in a recent paper (1) a complete failure to realize its importance has led to some obviously wrong conclusions. The potential of the large mercury layer anode was assumed to be of about the same order in an ammonium chloride and in an alkaline solution and only the "potential across the cell" was considered. On this basis it was concluded that variation in the electrolyte in the solution of an organic compound exerted almost no influence on the polarographic reduction potential of the compound. Such a statement seems to me almost equal to saying that the potential of a quinhydrone electrode is the same at pH 1 and at pH 7, when the reference electrode is a hydrogen electrode.

electrode (58). It is essential that the potential of the reference electrode be accurately known or measurable and that it remain constant even when fairly large currents are drawn, if these polarographic measurements are to have any meaning. Normally this electrode serves as anode, because most polarographic investigations deal with reductions at the dropping mercury cathode. While the anode may consist of any system meeting the above requirements, it has been the custom to use a large mercury layer at the bottom of the electrolytic vessel covered with a solution of a known chloride-ion concentration. Such an electrode has been found very satisfactory in inorganic reductions, as it immediately brings back into solution the metal which was deposited at the cathode, thus insuring an unchanged solution. This particular advantage does not exist for organic reactions, most of which are irreversible. The disadvantages encountered are as follows: (*1*) The anode potential cannot be assumed to be that of a calomel electrode at the corresponding concentration of chloride ions; for accurate work this potential has to be measured in each solution against a standard half-cell. (*2*) The potential of the large mercury layer, when used as the cathode, is very poorly poised unless a layer of calomel has previously been deposited on its surface.

A standard calomel half-cell with a large surface, the potential of which is accurately known and which can be checked from time to time, has also been found very satisfactory (59). Its potential remains constant within a few millivolts up to fairly high currents whether it serves as anode or cathode. It is connected to the solution by means of agar bridges. A comparison of the polarograms is simplified because all curves are automatically referred to the same electrode. If potassium chloride–agar bridges are used, the potential measurements are limited to a range between the deposition of chloride and potassium ions. This can be increased, when more positive potentials have to be measured, by using potassium nitrate bridges (58). Perhaps lithium nitrate or tetramethylammonium nitrate bridges would be still better. The disadvantages of this sort of a reference electrode are that the use of the agar bridges introduces some errors due to liquid junction potentials and increases the resistance in the circuit. Repeated resistance measurements become necessary, therefore, when bridges are changed and relatively large currents are drawn, to correct the observed potentials for the iR drop.

2. Potential of the dropping mercury electrode

The potential of the dropping mercury electrode is determined from the polarogram. The applied voltage is read at a well-defined point on the current–voltage curve, then iR is subtracted, and this corrected voltage is added to the known potential of the large reference electrode. The latter

may be referred to any standard. The finding of a suitable point on the current-voltage curve for characterization of the reducible substance has been one of the major problems in polarography. It was solved by Heyrovský and Ilkovič (38), who proposed the so-called "half-wave potential". This is the point of inflection of the smooth "S" curve which polarographers have called "wave", and it is very easily measured by drawing two tangents to the ends of the "wave". The tangents must be parallel. A third line midway between the tangents will cut the wave at its point of inflection and give the uncorrected half-wave potential. If the two tangents should not be parallel, one tangent to the most easily determinable end of the wave will suffice. The second line is then drawn parallel to the first at a point which seems to indicate the end of the wave. The half-wave potential is obtained as above; it will be changed but little by slight variations of the second line.

This half-wave potential has been found very convenient in inorganic work (for a more detailed discussion see reference 49), as it is a constant and is independent of the drop time, the rate of flow of mercury, the concentration of the reduced material, and the sensitivity of the galvanometer. Its significance in organic reductions and oxidations has been demonstrated by Müller and Baumberger (59) for reversible oxidation-reduction systems. Irreversible reductions can also be well characterized by it, as will be shown later in this paper. It seems, therefore, justifiable to recommend strongly the use of the half-wave potential for future determinations wherever possible. Its use is still somewhat doubtful for curves with maxima (see, however, references 18 and 124), and its application becomes impossible when the galvanometer deflections are so great that the whole wave cannot be brought onto the polarogram. There is also some indication (1) that the potentials are not constant when more than one organic substance is reduced.

For cases in which the half-wave potential method fails and for an understanding of the older literature the following alternative methods are listed: (1) A 45° tangent is drawn to the curve, and the applied E.M.F. corresponding to the point of contact is used (Heyrovský's old method). (2) The applied E.M.F. at the point of maximum curvature (tangent at 35°16′) of the current-voltage curve is used (Semerano). (3) That value of the applied E.M.F. is taken at which an increase of 10 millivolts has caused a rise in current of 1.9×10^{-8} amperes (Shikata).

The most outstanding shortcomings of these methods are that the potentials change with the concentration of the reducible substance, with the sensitivity of the galvanometer, and with the drop time and the rate of flow of the mercury. The exact conditions for standardization of the values always had to be stated in order to make a reproduction of the

results possible. Furthermore, the third method necessitates a high sensitivity of the galvanometer, which makes the determination of a second reduction potential on the same polarogram very difficult. In addition to the variations and combinations of these methods, proposed by different authors, LeBlanc's well-known method of extrapolation to zero current has been used (126, 127); the measurement of R is thus eliminated (see the interrupted lines in figure 3), but the fact that only a very small portion of the wave resembles a straight line makes the extrapolation uncertain, and difficulties arise when more than one wave is present on the polarogram.

If sufficient care is taken the half-wave potentials can easily be obtained with an accuracy of $\pm$ 10 millivolts. It should be possible to increase this accuracy to about $\pm$ 1 millivolt with the apparatus which is available at present.

3. Corrections for the "iR" drop of potential in the solution

These corrections become necessary when the product of current and resistance exceeds 1 millivolt. This means that in the case of a resistance of 5000 ohms and a galvanometer sensitivity of 2×10^{-9} amperes per millimeter per meter, corrections will have to be made only when the deflection exceeds 1 cm. at a galvanometer sensitivity of 1/10. At a sensitivity of 1/100, the correction will be 10 millivolts if the deflection is 1 cm. and 20 millivolts if the deflection is 2 cm. R is measured by means of a Wheatstone bridge or by determination of the slope of a maximum (44). A simple graphic correction of the half-wave potential for iR is demonstrated by Müller and Baumberger (59) for reversible oxidation–reduction systems. Another method for the correction of the half-wave potential for iR in irreversible reductions is demonstrated in figure 4. Without change of the bridge, indifferent electrolyte, or galvanometer sensitivity, polarograms are taken of the reducible substance at slightly different concentrations. A line drawn through the different half-wave potentials thus obtained will cross the galvanometer zero line at a point corresponding to the potential value which is already corrected for iR.

4. Current considerations

Since the various currents observed have been discussed in detail by Kolthoff and Lingane (49), little need be said about them here. In general the same considerations hold for organic reactions as for inorganic reductions.

The currents are usually plotted on the vertical axis of the polarograms, while the applied voltage is plotted horizontally. If *no* current is flowing while the potential across the cell is increased, the galvanometer will

remain at rest and a horizontal line on the polarogram will be obtained, which may be called the "galvanometer zero" line. If a substance is reduced and the galvanometer deflection is above the line, the dropping mercury electrode is the cathode; conversely, oxidation of a substance at the dropping mercury electrode, now anode, results in deflection of the galvanometer below this line (59). The currents are cathodic and anodic, respectively.

In inorganic reactions only cathodic currents have been observed, with the exception of anodic currents due to the oxidation of mercury. However, in reversible organic reactions curves are often obtained which go continuously from anodic to cathodic current. The later models of polarographs are equipped with an attachment (55) which makes such continuous anodic and cathodic polarizations possible.

III. REVERSIBLE OXIDATION–REDUCTION SYSTEMS

1. E_0' and half-wave potentials in well-buffered solutions

Certain compounds, when in solution with their reduction products, form systems which are strictly reversible under ordinary conditions. When equilibrium is established, an indicator electrode (58) immersed in such a solution has a definite and reproducible potential, which is a logarithmic function of pH and of the ratio of the concentrations of the oxidized to the reduced substances. These systems are the ones in which electrochemists have been interested, because the potentials obtained can be used for a very rigorous and accurate treatment of oxidation and reduction processes. The classical example is the quinhydrone electrode; oxidant (quinone) and reductant (hydroquinone) are present in equal concentrations, and the potential is entirely a function of pH. The excellent work of Clark and his collaborators on a series of dyes (20) has greatly increased our knowledge of such processes.

Probably because these equilibrium studies seemed quite different from those with a moving electrode surface and continuous reaction, polarographers paid little attention to such systems until 1937, when Müller and Baumberger (59) made a detailed study of the behavior of quinhydrone at the dropping mercury electrode. They used well-buffered solutions containing either quinone, or hydroquinone, or both (quinhydrone). The observed current–voltage curves showed that the oxidation of hydroquinone gave rise to a wave of *anodic* current which was identical in appearance with the wave of *cathodic* current due to the reduction of quinone. Quinhydrone in solution dissociates into equivalent amounts of quinone and hydroquinone, and the polarogram shows a wave, half of which is made of anodic current and half of cathodic current (figure 1). At the midpoint of this curve no current flows. This point is the half-wave

potential and corresponds to the well-known E_0' value that has been obtained with indicator electrodes when the galvanometer serves only as null-point instrument. Interestingly enough, when *only* reductant or *only* oxidant is present, the half-wave potentials obtained (corrected for iR) are identical with the E_0' value (figure 1). This means that at the half-wave potential the conditions at the electrode surface must have been the same in the three instances, i.e., the concentrations of oxidant and reductant were equal, and the pH was constant. Thus one-half of all the molecules of quinone which diffuse to the cathode in unit time must have been instantly reduced at the half-wave potential, and the hydroquinone formed must have remained at the electrode surface long enough to pro-

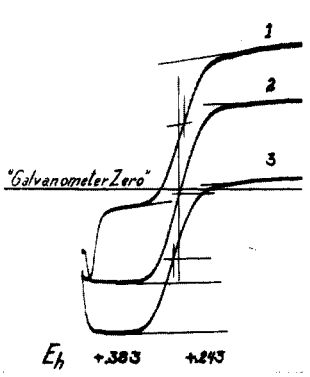

FIG. 1

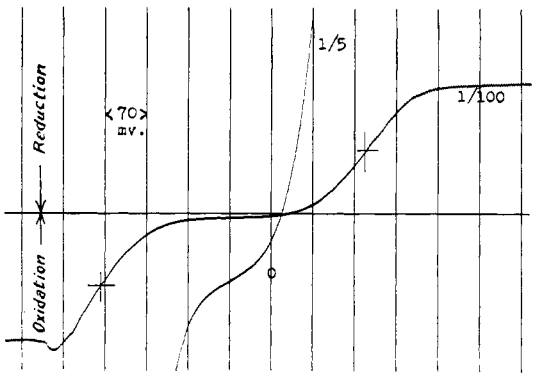

FIG. 2

FIG. 1. Polarogram of (1) quinone reduction, cathodic current, sensitivity 1/70, (2) quinhydrone, anodic and cathodic current, sensitivity 1/70, and (3) hydroquinone oxidation, anodic current, sensitivity 1/40. Buffer solution at pH 6.67. Half-wave potential corrections are indicated.

FIG. 2. Polarogram of quinhydrone (10^{-3} M) in 0.1 N potassium nitrate solution, unbuffered. 0 indicates zero applied voltage (anode potential = +0.250 volt).

duce the potential. Similarly, one-half of all the hydroquinone ions which diffuse to the anode in unit time are oxidized to quinone and remain at the electrode surface long enough to establish the condition of E_0'.

For further discussion it must be clear that an electrode can be polarized to any desired potential without any appreciable flow of current as long as no depolarizer is present. Any material which can furnish or accept electrons can act as a depolarizer, but only when the electrode has been brought to a suitable potential. When that potential is reached by applying voltage from some other source, it is kept constant by the transfer of electrons between depolarizer and electrode, that is, by the flow of current. An increase in applied voltage will produce an increase in

current, while the electrode potential will still be practically the same (59), provided no concentration polarization occurs. This is another way of stating the formula which has been emphasized:

$$\text{Electrode potential} = \text{applied voltage} - (\text{current} \times \text{resistance})$$

The ability to depolarize is, of course, a function of concentration (among other factors), so that an applied voltage can be reached at which polarization of the electrode will be again possible (diffusion current). In inorganic reductions usually a metallic ion is the electron acceptor, and the reduction product is a metal (amalgam). In organic reductions, either the organic molecule or its ion is the acceptor, and the reduction product is a negatively charged organic ion which may or may not undergo secondary reactions. If the reduction product is stable, its dissociation constant is, of course, of prime importance. For instance, if the formed ion is in a medium which is on the acid side of its pK_a, it will at once combine with hydrogen ions and thus alter the pH at the electrode surface unless it is well buffered.

2. E_0' and half-wave potentials in unbuffered solutions

As is well known, the quinhydrone electrode is used for the determination of pH because its potential is a linear function of pH at constant temperature in the range of pH 1 to pH 8. This dependence is due to the weak acidity of hydroquinone and to the fact that in the electrode reaction only the ionic form of hydroquinone can be considered:[4]

$$Q + 2\ominus + 2H^+ \rightleftharpoons Q^{--} + 2H^+ \rightleftharpoons H_2Q$$

The identity of the half-wave potentials of quinone reduction and hydroquinone oxidation demonstrates that the equilibria shown in this formula must be established with extreme rapidity. The constancy of the pH at the electrode surface implies, furthermore, a similarly rapid dissociation or association of the buffer molecules and ions present.

This last consideration led the author to a polarographic investigation of quinhydrone in unbuffered solutions (57). It was found that the smooth single wave obtained with quinhydrone in buffered solutions (figure 1) broke up into two entirely separate waves when a potassium nitrate solution was used (figure 2). It could be ascertained that these were caused by the reduction of quinone and the oxidation of hydroquinone, respectively. The half-wave potentials, of course, were no longer equal and did not indicate the pH of the potassium nitrate solution, which was practically neutral. The pH calculated from the quinone reduction half-wave potential was about 10, and that from the hydroquinone oxidation half-wave

[4] For an excellent treatise on this subject see Clark (20).

potential was about 3. These phenomena could be satisfactorily explained by a consideration of the above formula, where hydrogen ion can come only from water or hydroquinone.

In the oxidation of hydroquinone to quinone, two hydrogen ions will be liberated for each hydroquinone molecule oxidized. In an unbuffered solution those hydrogen ions will remain at the electrode surface, changing the reduction potential by a change of pH. Therefore, when one-half of all the hydroquinone molecules are oxidized, the hydrogen-ion concentration at the electrode surface will be equimolar to the original concentration of hydroquinone or quinhydrone. In the above case the quinhydrone concentration was $10^{-3}\,M$; the pH at the half-wave potential should then be 3, which is the observed value. When the concentration of quinhydrone was changed to $10^{-4}\,M$, the half-wave potential of the corresponding wave indicated a pH of 4 at the electrode surface.

In the reduction of quinone hydrogen ions are necessary to combine with the hydroquinone ions which are formed. In the absence of a buffer they must come from water, the pH of which is of course changed simultaneously. Around pH 10, however, the dissociation constants of hydroquinone are reached, hydroquinone is now capable of existing in the ionic form in the solution, and, as a rough approximation, no more hydrogen ions are necessary for further reduction. It can be argued from this that the half-wave potential reaches a limit in the neighborhood of the dissociation constant of the reductant in unbuffered solutions.[5]

When to such unbuffered solutions a suitable buffer is added in a concentration below that of the quinhydrone, a third wave appears between the two waves mentioned; this indicates the buffer action at the electrode surface (57). In other words, the trace of buffer added has a certain capacity, limited by its concentration, to keep the pH constant; when the buffer at the electrode surface is exhausted, the pH can change. The sum of these three waves is, of course, a constant and depends on the concentration of quinhydrone. When the buffer concentration is made greater than that of quinhydrone, it is fully able to keep the pH at the electrode surface constant even though all of the hydroquinone be oxidized, or all of the quinone be reduced. In this case the continuous anodic–cathodic curve characteristic of reversible oxidation–reduction systems is obtained.

3. Applications

The polarographic method can be applied to the study of reversible oxidation–reduction systems, the potentials of which fall within the range

[5] For convenience in this treatment, the two ionization constants of hydroquinone, which are 1.75×10^{-10} and 4×10^{-12}, have been taken as equal. The conclusions reached are not thereby invalidated.

of $E_h = +0.65$ to -1.60 volts (58, 59, 117). The reversibility of a system can be ascertained when curves of anodic current due to oxidation of the reductant show the same half-wave potential as curves of cathodic current due to the reduction of the oxidant. It has also been demonstrated that semiquinones can be studied polarographically (59). However, because the polarographic method is considerably less accurate than the standard equilibrium procedures with a platinum electrode, its use will probably be limited to exploratory investigations.

IV. IRREVERSIBLE REDUCTION

1. Polarographic apparent reduction potentials

The large majority of organic reductions which have been investigated polarographically are not of the type mentioned in the preceding section but are more or less *irreversible*. In many of these reductions a smooth "S" curve is obtained on the polarogram, and a regular shift of the reduction potential with pH is observed; this suggests a reversible process. For this reason they have often erroneously been called reversible, although the corresponding oxidations of the end products have not been possible at the dropping mercury electrode.[6] The simplest explanation of this phenomenon is the assumption of a reversible step in the reduction, which is on the whole irreversible. Fortunately, analogies in the electrochemical literature were available to test this supposition, which was first made by Müller and Baumberger (60). Conant (21) had occasion to study a number of irreversible reductions which proceeded at different rates depending on the oxidation–reduction potential of the system which was used for the reduction. He concluded that the first step in the reduction must have been reversible and instantaneous, while the next, irreversible step was slow enough to permit measurement. The irreversible system could be characterized by a potential, the "apparent reduction potential" (A.R.P.). This was defined by Conant as the potential of a "critical reagent" which would just cause "appreciable reduction" (20 to 30 per cent in 30 min.).

Only a few of the systems described by Conant have been studied polarographically. Müller and Baumberger (60) evaluated some data in the polarographic literature for dinitrobenzene, nitrobenzene, and maleic acid, and found fair agreement with the A.R.P. of Conant (21). As a further check, the two polarograms in figures 3 and 4 were prepared in this laboratory.[7] The half-wave potentials are independent of concentration in

[6] The nature of the end product is of course never known with certainty, because not enough is formed at the mercury droplets to permit an analysis. A reasonable guess can, however, be made on the basis of long-time experiments with larger electrodes.

[7] Unpublished results.

these highly acid solutions and can be measured easily. The corresponding A.R.P.'s of Conant are indicated by arrows. It may be seen that they differ from the half-wave potentials, but that they coincide with the

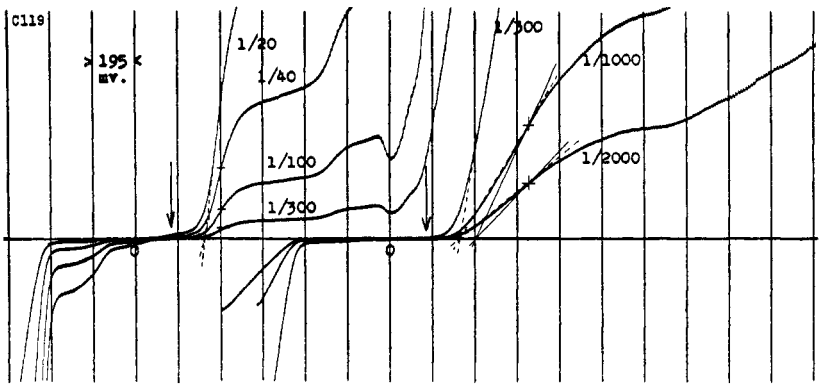

FIG. 3. Polarogram of nitrobenzene in acetone and nitric acid. Comparison of half-wave potentials and Conant's (21) A.R.P. (↓). At sensitivities 1/1000 and 1/2000 the correction for iR is drawn in. Notice also LeBlanc's method of extrapolation to zero current (interrupted lines); iR is graphically eliminated.

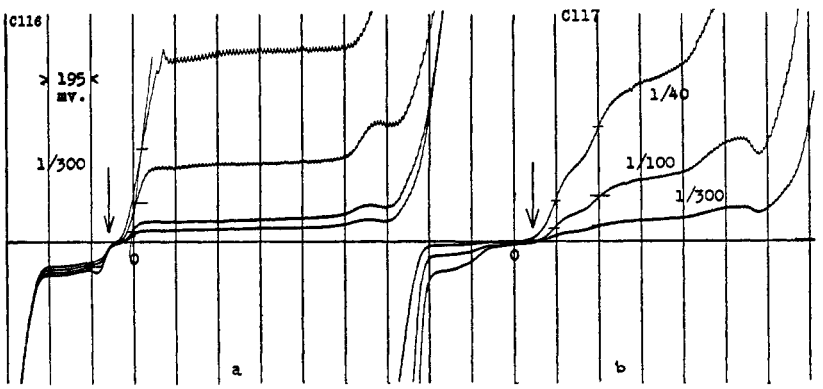

FIG. 4. Polarograms of (a) azobenzene in acetone and nitric acid and (b) dinitrobenzene in acetone and nitric acid. Comparison of half-wave potentials and Conant's (21) A.R.P. (↓). Note graphic correction of the half-wave potentials of azobenzene (see text). 0 indicates zero applied voltage (anode potential = +0.247 volt, referred to the hydrogen electrode). The dropping mercury electrode is polarized negatively to the right of 0 and positively to the left of 0; each abscissa represents 195 millivolts.

beginning of a rise in current due to the onset of reduction. This can be expected, considering the methods of measurement; while Conant measured the A.R.P. indirectly by observing the slow, irreversible process, the

polarograph appears to record selectively the reversible portion of the reaction. We may designate those potentials which are not truly reversible, which show dependence on pH, and which demonstrate partial reversibility by means of smooth "S" curves as "polarographic apparent reduction potentials" (P.A.R.P.).

Consequently, irreversible oxidations of this nature should be called "polarographic apparent oxidation potentials" (P.A.O.P.). Because of the difficulty of measuring very positive potentials at the dropping mercury electrode, those compounds which show an apparent oxidation potential, measured by Conant, cannot be compared. Recently, however, a typical case was found by Kodíček and Wenig (47) in vitamin C, which can be oxidized polarographically but cannot be reduced.

It is premature to speculate on the nature of the reversible step, but it has been suggested that electromotively active ions and molecules may exist which change slowly into an inactive form. Also, it has been suggested that hydrogen ions may be reduced at the electrode to hydrogen atoms which then combine in the nascent state with the organic molecule.

2. Effect of buffers

That buffering is as essential in these reactions as in the reversible ones may be seen from a comparison of some curves of Shikata (91, 100), who was the first to study the influence of pH on the polarographic reduction potentials of organic compounds. In the earlier work (97 to 100) Shikata and Tachi measured the pH of their solutions by means of quinhydrone or hydrogen electrodes, but did not concern themselves with the capacity of the solutions to keep this pH constant. As a result these curves show peculiar inflections around neutrality (see the curves of benzil and diacetyl reproduced in figure 5). When buffer solutions were employed in the later work of Shikata and collaborators (91, 92, 101, 103), relatively smooth curves were obtained (see the curves of nitrobenzene and *o*-dinitrobenzene in figure 5).

It may be worthwhile to restate that pH is an expression of *intensity*; the concentration of the buffer determines the *capacity* of the solutions to keep the pH constant. This has been pointed out repeatedly, but is still occasionally neglected. As a rule one may consider the solution well buffered if the concentration of the buffer is one hundredfold that of the substance which is being reduced or oxidized.

Outside of Shikata's work relatively little has been reported on the change of reduction potentials with a change in pH of the solutions. Examples of potential–pH curves are given in figure 5; they include an aldehyde (benzaldehyde (120)), ketones (benzil, diacetyl (100)), unsaturated acids (fumaric and maleic acids (124)), and aromatic nitro compounds

(nitrobenzene and *o*-dinitrobenzene (91)). Our knowledge of the electrode processes in these reductions is still too meagre to permit satisfactory explanations of the inflections seen in these curves.

Organic reduction potentials measured in neutral potassium chloride or similar unbuffered solutions must be interpreted with caution. As an example of the fallacies encountered in the interpretation of polarograms obtained in unbuffered solutions may be cited the case of acetylacetone. This compound has been reported reducible by several investigators (100,

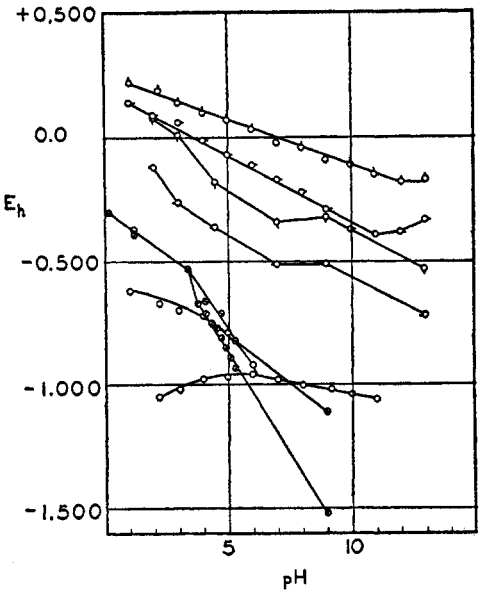

FIG. 5

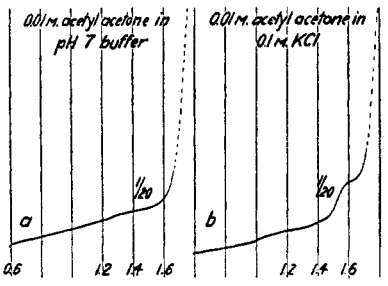

FIG. 6

FIG. 5. Potential–pH curves. ♁, *o*-dinitrobenzene; ○-, nitrobenzene; ♀, benzil; -○, diacetyl; ⊘, maleic acid; ⊘, fumaric acid; ○, benzaldehyde.

FIG. 6. Curves for acetylacetone (a) in a solution buffered at pH 7 and (b) in 0.1 *M* potassium chloride

127), who worked with unbuffered solutions. A wave is indeed observed when the reduction is carried out in potassium chloride solution (see figure 6b), but the wave disappears when the solution is buffered at pH 7 (figure 6a). Titrations showed[7] that the unbuffered solution was acid, no doubt owing to the dissociation of the enol form of acetylacetone; therefore it seems probable that the wave was caused by the reduction of free hydrogen ions (see also reference 1).

It has been customary to report reduction potentials of organic compounds obtained in ammonium chloride solutions. This is an unfortunate

choice of electrolyte, because it constitutes but one component of a buffer. As is well known, a buffer is most effective when its two components, acid and salt, are in equal concentration (123). Therefore it seemed probable that when the half-wave potential is reached during a reduction, the pH at the mercury surface would be different from that of the solution. This conclusion was verified using quinhydrone as a criterion (57). Depending on the concentration of ammonium chloride, the pH at the electrode surface, as indicated by the half-wave potential of quinone reduction, was 8, 9.3, 10.0, and 10.2 when the ammonium chloride concentrations were 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} M, respectively. The oxidation of hydroquinone, however, proceeded as if the solution had been unbuffered. At the same time the pH of the solution was found by indicators to be about 5. The results reported in the literature must, therefore, be taken as representing reduction potentials not in a slightly acid solution but rather in a slightly alkaline solution.

Brdička (7, 8, 9) recommends the addition of ammonia to the ammonium chloride solution as a buffer for the cysteine determination. Since Brdička's results are essentially the same as those obtained in phosphate buffers (11), it would be of interest to study this buffer system using quinhydrone.

3. Applications

It is impossible in this short review to do justice to the many excellent papers that deal with applications in this field of irreversible organic reductions. They are especially numerous because obviously the polarographic method excels, owing to the fact that it picks out the reversible step and often gives an indication of the nature of the reaction and the degree of reversibility. Here may be mentioned only examples of the most important and promising types of investigations. Since the conclusions reached in these papers are based on experiments which were carried out under identical conditions, although not always in perfectly buffered solutions, they may have significance. It should be strongly urged, however, that future workers report half-wave potentials which are accurately measured, corrected for iR, and referred to the most common standard in electrochemistry, which is the hydrogen electrode. Furthermore, all measurements should be carried out in well-buffered solutions of known pH. In the table of organic compounds that is included in this discussion, the nature of the study and the corresponding reference are given; the latter is given in italics if the compound has been studied in some detail. Because of the lack of uniformity in the method of measurement, a comparison of the potentials stated in the literature is useless, and a recalculation of the values is impossible because the conditions of measurement are not well enough defined.

In the first studies on the effect of substitutions in an organic molecule upon its reduction potential, Shikata and coworkers investigated different ketones and aromatic nitro compounds (91, 92, 98, 100, 104, 106). On the basis of extended research, Shikata and Tachi formulated the "electronegativity rule of reduction potentials" (102), which states that organic compounds are more easily reduced as more electronegative groups are substituted in the same compound. This is of interest because the dissociation constants (102, 18) and the Raman and absorption spectra (127) of compounds are similarly influenced. The following two lists of Shikata may serve as examples; the compounds are listed in the order of decreasing reducibility:

benzil/diphenyltriketone/diacetyl/dibenzoylmethane/benzoylacetone/benzoin/benzophenone/acetophenone/acetoin/acetylacetone/acetone

and

dinitrophenol/dinitrobenzene/nitrophenol/nitrobenzene/nitroaniline

Similar studies were made by Semerano and Chisini (81), who used benzaldehyde as a reference. They arranged the following groups in the order in which they change the reduction potential to more negative values:

o-Cl/*m*-Cl/*p*-Cl/*o*-CH_3/H/*m*-CH_3/*p*-CH_3/*o*-OH/CH_2O_2/*p*-OCH_3/4-OH, 3-OCH_3

Many more aldehydes and ketones were studied by other investigators, notably Winkel and Proske (126, 127) and Adkins and Cox (1). As far as a comparison of the results of different authors is permissible, it shows that the above influence of different groups upon the reduction potential is essentially correct.

While the aromatic series show such easily reproducible differences in reduction potential, the saturated aliphatic ketones and aldehydes that have been studied are reduced at about the same potential, regardless of the length of the chain. Aldehydes, in general, are more easily reduced than ketones, with the notable exception in the sugar series, where fructose is reducible while glucose is not (40). This fact has been used for analytical purposes (40, 41, 46).

Organic acids in general are not reducible unless they contain carbonyl groups or ethylene linkages. Even in the latter case reduction is possible only when the double bonds are conjugated. This was demonstrated by the investigations of Schwaer (71), who also found that under certain conditions the *cis*- and *trans*-isomers of acids could be distinguished by their polarographic potential (see also 78, 79, 88). As might be expected,

unsaturated aldehydes and ketones are relatively easy to reduce (1). Semerano and Chisini (83) studied cinnamic acid, cinnamaldehyde, and hydrocinnamaldehyde for the purpose of comparing the influence of a C=O group on the reduction potential with that of a C=C linkage.

After it had been established that the currents obtained in the reductions of some organic compounds were proportional to concentration, these currents could be used for the study of reaction kinetics, polymerization, and tautomerism. So far relatively few such experiments have been done, but they indicate the possibilities of polarography.

Herasymenko (34) studied the rate of formation of fumaric acid from molten malic acid by polarographing samples at different time intervals, making use of the fact that malic acid does not produce a wave on the polarogram. Semerano and dePonte (86) found that waves of benzaldehyde reduction decreased with time, owing to the Cannizzaro reaction (see, however, Tokuoka (120)). The inversion of sucrose was followed polarographically by Heyrovský and Smolef, who measured the currents due to the fructose formed (40). Polymerization studies were made on formaldehyde (45) and pyruvic acid (60). Some papers on keto–enol tautomerism (1, 60, 77, 82, 100, 113) have also been published, but verification of the results by comparison with established methods is still lacking. A very clear demonstration of the reliability of polarographic results was made by Borcherdt and Adkins (5). They studied the rate of tautomerization of an optically active azomethine by noting the loss in rotatory power of the reaction mixture and by polarographic analysis of the products of hydrolysis of the components.

As has been pointed out in the introduction, the analysis of several organic compounds in the same solution is possible only under well-standardized conditions. An analysis of a mixture of compounds in an unknown solution is, at present, impossible. For instance, the analysis of pyruvic acid cannot be carried out in blood or urine until satisfactory methods for its separation from the rest of the solution are available. Therefore, one of the most important tasks in the field of organic polarography seems to be to find suitable procedures for the fractionation of solutions in order to make the method available for analytical purposes.

V. ORGANOMETALLIC COMPLEXES

The organometallic complexes may be divided into an inorganic and an organic group, depending on the nature of the electrode reaction.

In the inorganic group a metallic ion is reduced at a potential which varies with the nature of the organic component and with the relative concentrations of the metallic and organic components of the complex. Because the latter may be influenced by hydrogen-ion concentration, a

knowledge of the pH of the solutions is essential. A discussion of this group of complexes is found in the preceding review (49).

In the organic group the metallic component acts only as a catalyst for the reduction of hydrogen. Such catalytic reactions were first observed by R. Brdička in 1933 (7). He found well-reproducible maxima on the polarograms when sulfur-containing proteins or amino acids were added to buffered cobalt salt solutions. His further investigations (8 to 16) show that these reactions can be used for analytical purposes. Sladek and Lipschütz (109) observed that amino acids that do not contain sulfur (arginine, histidine, and others) can also produce specific waves in similar cobalt solutions.

Brdička's work may be summarized as follows: In solutions of ammonium chloride to which ammonia has been added (or in otherwise buffered solutions (11)), cysteine or cystine and sulfur-containing proteins cause characteristic reduction curves when cobalt or nickel salts are present. These curves are much higher (three hundred fold) than those due to the normal reduction of cystine in the absence of metals (8). Amino acids can be distinguished polarographically from the proteins, because low molecular compounds (cystine, cysteine) give the reaction only with divalent cobalt salts, while proteins give similar reactions also with trivalent cobalt salts. The catalytic reduction maxima do not increase proportionally with concentration but reach a maximal height; calibration curves are therefore necessary for quantitative analysis. Cystine is first reduced to cysteine; its reduction curve is twice as high as that of cysteine for an equimolar solution, but does not differ otherwise. A differentiation between cystine and cysteine is possible only by indirect means: The —SH group reacts with monoiodoacetate to form hydrogen iodide and $—SCH_2COO^-$, which do not show the polarographic reaction. In monoiodoacetate the —S—S— group remains unaltered.

According to Brdička (8), these specific curves obtained from solutions containing organic and metallic ions are to be attributed to a catalyzed reduction of hydrogen from the organometallic complex. The abnormal height of the wave (maximum) speaks for a catalytic process (see also reference 56). Further evidence that the reaction is catalytic in nature is provided by the negative influence of arginine, tryptophan, histidine, casein, and other substances (109, 68), which suppress the cysteine reaction completely.

Owing to lack of space, it is impossible to discuss here the many applications which these reactions have found in the field of biochemistry.

VI. ORGANIC COMPOUNDS THAT HAVE BEEN STUDIED POLAROGRAPHICALLY

In table 1 are listed the organic compounds that have been studied polarographically. The literature has been covered up to June, 1938.

TABLE 1

*Organic compounds that have been studied polarographically**

COMPOUND		REFERENCES
Acetal	−, D	42
Acetaldehyde	R, D	1, 41, 45, 51, *84*, 94, 108, *110*, 126
Acetamide	−	127
4-Acetaminophenylarsonic acid	R	18
Acetic acid	−	30, 65, 94, 108, 109, 126
Acetoacetic ester	R, D	82
Acetoin	R	*98*, 127
Acetone	R	*73*, *98*
	−	1, 126
Acetonylacetone	R	*100*
	−	127
Acetophenone	R, D	1, 5, *6*, *98*, 117, 126
Acetylacetone	R	82, *100*, 127
	−	1
Acetylbenzoylmethane	R	1
α-Acetylbutyrophenone	R	1
α-Acetylcaprophenone	R	1
Acetylenedicarboxylic acid	R, D	71
Aconitic acid	R, D	71, 78, *80*, *88*, 108
Acridine derivative	R	42
Acridone	R	42
Adenine	MS	69
Adipic acid	−	127
Adrenaline	MS	76
Alanine	−	16, 109
Albumin	C	7, 9, 11, 37, 53, 68, 125
Alizarin red	MS	66
Alkaloids	R, MS	*63*, 27
Allyl alcohol	−	*112*
p-Aminoazobenzene	R, D	*103*
4-Aminophenylarsonic acid	R	18
Anisaldehyde	R	1, 81, 127
Anthraquinone	R	1
l-Arabinose	−	40
Arginine	C	7, 109
Arsphenamine	−, D	*10*
Asparagine	C	7, 37, 62
Atropine sulfate	MS	27
Aurantia	MS	66
Azobenzene	R	*101*, 117
Benzal *tert*-butyl acetone	R	1

* − means that the substance is not reducible; C = catalyst (organometallic); D = determinable; MS = maximum suppressor (see 49); R = reducible.

TABLE 1—*Continued*

COMPOUND		REFERENCES
Benzalacetone	R, D	1
Benzaldehyde	R	1, *81*, *86*, 94, 108, *120*, 126, 127
Benzamide	–	127
Benzil	R, D	1, *100*, 117, 126, 127
Benzoic acid	R	97, 120, 126
Benzoin	R, D	1, *77*, *98*, 117, 127
Benzophenone	R, D	1, *72*, *98*, 126
Benzopyrone	R, D	1, 54
p-Benzoquinone	R, D	1, *59*, 127
Benzoylacetone	R, D	100, 117
Benzoyl-*o*-hydroxybenzoylmethane	R	1
Benzylbenzoylacetoylmethane	R	1
Benzyl phenyl ketone	R	1
Betaine	MS	69
Biebrich scarlet	MS	66
Bilirubin	R, D	*111*
Blood pigments		*17*
Bromoacetone	R	126
m-Bromobenzaldehyde	R	127
p-Bromobenzaldehyde	R	127
2-Bromoethyl phenyl ketone	R	1
Bromoisopropyl phenyl ketone	R	1
p-Bromophenyl methyl ketone	R	1
Brucine	MS	63
tert-Butyl benzoylmethyl ketone	R	1
Butyraldehyde	R	1, 83
n-Butyric acid	MS	65
Caffeine	–, MS	62
Camphor	R	107, *115*, 116
Caproates	MS	69
Carbazole	–	42
Carbon tetrachloride	R	52
Casein	MS	53, *68*
Catalase		17
Chloroacetone	R	126
p-Chloroacetophenone	R, D	6, 126
m-Chlorobenzaldehyde	R	81, 127
o-Chlorobenzaldehyde	R	81, 127
p-Chlorobenzaldehyde	R	81, 127
p-Chlorobenzophenone	R, D	1, 5, 6
Cholestenone	R	1
Cholesterol	–	109
Choline hydrochloride	R	64
Chromone	R, D	1, 54

TABLE 1—*Continued*

COMPOUND		REFERENCES
Cinchonidine	R	63
Cinchonine	R	63
Cinnamal benzophenone	R	1
Cinnamic acid	R	71, 83, 108
Cinnamaldehyde	R	83, 94, 108
Citraconic acid	R	71, 78
Citric acid	–	35, 48, 71
Codeine	R	63
Coproporphyrin		17
Creatine	–	7, 37, 62
Creatinine	–	7
Crotonaldehyde	R	1, 83
Crotonic acid	R	108
	–	71
Cyclohexanone	–	126
Cyclopentanone	–	1, 126
Cysteine	C	*8, 9, 15*
Cysteylglycine	C	8
Cystine	C	*7, 8, 9, 15*, 23, 67, 122
Desoxybenzoin	R	77
Diacetyl	R, D	1, 84, *85*, 94, *100*, 117, *127*
Dibenzalacetone	R	1
Dibenzoylmethane	R	1, *100*
2,6-Dibromophenol indophenol	R	59
sym-Dichloroacetone	R	126
2,6-Dichlorophenol indophenol	R	59
2,4-Dichlorophenylarsonic acid	R	18
Dicyanogen	R	*19*
Diethyldibenzoylmethane	R	1
Dimethylaminoazobenzene	R	*114*
Dimethylquinoxaline	R, D	59
m-Dinitrobenzene	R, D	*91*
o-Dinitrobenzene	R, D	*91*
p-Dinitrobenzene	R, D	*91*
2,4-Dinitrophenol (α)	R, D	*92*
2,6-Dinitrophenol (β)	R, D	*92*
2,5-Dinitrophenol (γ)	R, D	*92*
Diphenylamine	–	42
1,2-Diphenylethylene glycol	–	1
Diphenyltriketone	R	*100*
Dyes	MS	*66*
Dypnone	R	1
Erythrosin	MS	66
2-Ethylchromen-4-ol	D	54

TABLE 1—*Continued*

COMPOUND		REFERENCES
2-Ethylchromone	R	1, 54
Ethyl isobutyl ketone	–	126
Euglobulin	C	7
Fenchone	R	107
Flavanone	R	1
Flavone	R	1
Flazine	R	42
Formaldehyde	R, D	*45*, 126
Formamide	–	127
Formic acid	–, MS	30, 65, 126
Fructose	R, D	*40*, 41, 46
Fuchsin (acid and base)	MS	66, 76
Fumaric acid	R, D	*29*, *31*, *32*, *34*, *71*, *78*, *79*, 80, *87*, 108, *124*
Furan	–	121
Furfural	R, D	94, 95, 108, *121*
Furfuralacetone	R	1
Furyl acetonyl ketone	R	1
Furyl benzoylmethyl ketone	R	1
Fuscainic acid	MS	69
Galactose	–	40
Gelatin	–	7
Globulin	C	7, 125
Glucose	–	40, 94, 109
Glutaminates	MS	69
Glutaric acid	–	127
Glutathione	C	8
Glycerol		48
Glyceraldehyde	R	127
Glycine	–, R, C	7, 12, 16, 18, 37, 62 ,109
Glyoxal	R	127
Helianthine	MS	66
Hematin	R	17, 99
Hemin		28, 99
Hemoglobin	R	17, 99
Heptaldehyde	R	1
Hexaldehyde	R	1
Histidine	–, C	94, 109
Humic acid	–, D	22, *121*
Hydrastine	R	63
Hydrobenzoin	–	83
Hydrocinnamaldehyde	R, D	*83*
Hydroquinone	R	*59*

TABLE 1—*Continued*

COMPOUND		REFERENCES
Hydroxyacetone	R	127
m-Hydroxybenzaldehyde	R	1, 127
p-Hydroxybenzaldehyde	R	127
o-Hydroxyphenyl methyl ketone	R	1
Hymatomelanic acid	R	*121*
Indanthrene	MS	69
Indole	R	62
Insulin	C, D	7, *61*, *122*
Iodoacetone	R	126
Isoamyl alcohol	–	95
Isobutyraldehyde	R	1, 95
Isocrotonic acid	–	71
Isopropyl phenyl ketone	R	1
Isovaleraldehyde	R	94, 95
Isovaleric acid	MS	65
Itaconic acid	R	108
	–	71, 78
Lactic acid	–	48, 94
Lactones	R	40
Lactose	–	40
Lecithin	–	109
Leucine	–, MS	7, 69
Levulic acid	–	71
Lysine	–	109
Lyxose	–	40
Maleic acid	R, D	*29*, *32*, *71*, 78, *79*, 80, *87*, *124*
Malic acid	D	34, 41, 71
Malonic acid	–	30, 127
Maltose	–	40
Mannitol		48
Mannose	–	40
Melanoidin	R	22
Mesaconic acid	R	71, 108
Mesityl oxide	R	1
Methemoglobin	R	99
p-Methylacetophenone	R	126
Methyl benzyl ketone	–	126
Methylene blue	R	58
	MS	25, 66
4-Methyl ether of phenylarsonic acid	R	18
Methyl ethyl ketone	R	98
	–	126

TABLE 1—*Continued*

COMPOUND		REFERENCES
Methyl green	MS	66
Methyl hexyl ketone	–	126
Methyl α-naphthyl ketone	R	126
Methyl nonyl ketone	–	126
Methyl orange	MS	66
Methyl 1,3-pentadienyl ketone	R	1
4-Methylphenylarsonic acid	R	18
Michler's ketone	R	126
Morphine hydrochloride	R	63
	MS	27
Mustard gas	C	*13*
Myosalvarsan	–	10
Narcotine	R	63
Neosalvarsan	–	10
Neutral red	R	117
Nicotine	D	74
Nicotinic acid	R	*97*
m-Nitroaniline	R	*104*
o-Nitroaniline	R	*104*
p-Nitroaniline	R	*104*
Nitrobenzene	R	*90*, 91, 116, *119*
m-Nitrophenol	R	91, *106*
o-Nitrophenol	R	91, *106*
p-Nitrophenol	R	91, *106*
Nitrophenylarsonic acid	R	18
Oleic acid	–	108
Orange II	MS	66
Oxalic acid	R	29, *30*, 127
	–	19
Oxamic acid	R	*19*
Oxamide	R	127
Palmitic acid	MS	65
Papaverine hydrochloride	MS	27
Paraformaldehyde	R	45
Paraldehyde	–	126
Peptone	C	11, 68
Petroleum		*26*
Phenanthraquinone	R	1
Phenethylamine		6
Phenolarsonic acid	R	18
Phenol indo-2,6-dibromophenol	R	59
Phenolphthalein	MS	66
β-Phenyl-α-alanine	C	109

TABLE 1—*Continued*

COMPOUND		REFERENCES
β-Phenyl-β-alanine	C	109
Phenylarsonic acid	R	18
Phenyl *o*-carbethoxyphenyl ketone	R	1
4-Phenyl-3-ethylbutanedione-2,4	R	1
Phenylethyl phenyl ketone	R	1
Piperonal	R	1, 81, 127
Propionaldehyde	R	1, 94, 126
Propionic acid	–, MS	30, 65
n-Propyl phenyl ketone	R	1
Proteins	C	2, 3, *7*, *8*, *9*, *11*, 14, 15, 24, 41, 50, 89, 122, 125
Purine group	–	62
Pyridine	R	95, *96*
	–	1
Pyromucic acid	–	121
γ-Pyrone	R	1, *54*
Pyronin	MS	66
Pyrrolealdehyde	R	*4*
Pyruvic acid	R, D	1, *60*, 71, 93, 127
Quinhydrone	R, D	*58*, *59*, 127
Quinidine	R	*63*
Quinine	R	33, *63*, 64, *105*
	MS	27
Quinoline	R	1, 62, 118
p-Quinone	R, D	1, *59*, 127
Rhamnose	–	40
Rosinduline GG	R	*59*
Saccharin	R, D	*62*
Safranine	R	42
Salicylic acid	MS	26
Salicylic aldehyde	R	1, 81, 127
Sorbic acid	R	71
Sorbose	R, D	40, 41
Stearates	MS	69
Stearic acid	MS	65
Strychnine nitrate	R	63
	MS	27
Suberic acid	–	127
Succinic acid	–	94, 108, 127
Sucrose	–, MS	*40*, 48, 69, 70
Tartaric acid	–, D	71
Theobromine	–	62

TABLE 1—*Concluded*

COMPOUND		REFERENCES
Thiazane	–	13
Thioglycolic acid	C	8
o-Tolualdehyde	R	81
p-Tolualdehyde	R	81
Triacetoneamine	–	1
Trimethylamine	R	64
Tropaeolin	MS	66, 76, 79, 80
Tryptophan	–, C	109
Tyrosine	MS, –	7, 69, 109
Ultramarine	MS	69
Urea	–	37, 62, 109
Uric acid	–, D	*62*
Valeraldehyde	R	1
n-Valeric acid	MS	65
Vanillin	R	1, 81, 127
Vernin	MS	69
Vitamin C	D	47

A reference number given in italics indicates that the compound has been studied in some detail.

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