PHYSICO-CHEMICAL STUDIES RELATED TO CHEMOTHERAPY

by

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INTRODUCTION

Since very early times the naturally occurring derivatives of salicylic acid, notably methyl salicylate and salicin, found in the bark, leaves and fruit of a number of trees, such as the willow, have been used as remedies against many diseases. Various authors, e.g. Pliny, during the Classical Period recommended extracts now known to contain salicylic acid as common factor in the treatment of a number of ills, though not all of these recommendations are in line with current medical thought.

However, it was with the discovery in 1874 by Kolbe[1] of a process by which salicylic acid could be synthesised on a technical scale that it became the first organic compound occurring in nature to be manufactured as a commercial article. This also resulted in the much wider availability of the pure compound for medical use.

During the second half of the nineteenth century the effect of salicylic acid, either as the acid or in the form of one of its salts, on a wide variety of diseases was investigated. Probably the most important result of this was the simultaneous

finding by Stricker[2] and MacLagan[3] that salicylate was not only an antipyretic but was a specific remedy for rheumatic fever.

Acetylsalicylic acid was first introduced into medical practice by Wohlgemut[4] and by Dreser[5] in 1899 as a form of salicylic acid more readily tolerated by the patient. Although this derivative was intended for the treatment of Rheumatic fever, the marked analgesic properties of this particular salicylate were soon noted and its greatest use arose in that connection.

Modern research and clinical experience have confirmed the value of several of the therapeutic uses discovered earlier for salicylate, such as its analgesic, antipyretic and anti-rheumatic properties[6]. Many other biological actions of salicylate have been reported, including its action as a "metabolic stimulant" by increasing the rates of oxygen consumption in animals and its ability to control mild hyperglycaemia and glycosuria in diabetes mellitus. (Hyperglycaemia and glycosuria are the respective states in which the blood glucose concentration is abnormally elevated and glucose is present in the urine).

It is generally agreed that the analgesic[7] and antipyretic[8,9] actions of salicylate are produced by a central action of the drug on the brain. However, in the case of rheumatic fever, its mode of action is unknown. In the earlier part of the century it was believed to act through the Central Nervous System, but in view of the number of pharmacological properties of salicylate occurring at cellular level, this is perhaps now less certain.

As mentioned before, at a relatively early stage in its therapeutic use salicylate was found to be of value in the treatment of rheumatic fever. Although early reports on its use cast some doubt on its real benefits in this disease, this was probably because the dosage in common use up to the second decade of this century was considerably less than the 10 to 12 g. salicylate per day considered necessary at the present time. Today, salicylic acid in the form of its sodium salt or as the acetyl derivative is recognised as one of the best remedies for rheumatic diseases[10]. Stockman[11] observed that the m- and p- isomers of salicylic acid did not share the beneficial action of the o-isomer, and it has been found that the sodium salts of the o-cresotic acid (3-methylsalicylic acid)[12], γ-resorcylic

acid (2,6-dihydroxybenzoic acid)[13], and gentisic acid (2,5-dihydroxybenzoic acid)[14] are as effective as sodium salicylate in this disease. It would appear that the therapeutic action of these compounds is a property of the salicylic acid grouping.

During the latter part of the last century there were numerous clinical reports that the drug inhibited diabetic glycosuria[6]. That salicylate is therapeutically active in diabetes mellitus has been established clearly in animals by Ingle[15], and confirmed by Smith, Meade and Bornstein[16]. In the diabetic patient it has been shown[17] to reduce blood sugar and glycosuria, and to control moderately severe ketosis (i.e. the presence of ketone bodies in the urine). Reid and Lightbody[18] showed that it was possible to replace a proportion of the insulin required by diabetic patients by salicylate.

It is of interest to note that o-cresotic acid has a similar hypoglycaemic action[19].

The effect of sodium salicylate in increasing the rate of oxygen consumption of experimental animals[20,21] and man[22,23] is now well established. It would appear that this phenomenon occurs only in those benzoic acids possessing the

salicylate grouping. Thus Meade[24] found that only salicylic acid among all the mono- and di-hydroxybenzoic acids increased the rate of oxygen consumption of rats. Hall, Tomich and Woollett[25] investigated a number of compounds chemically related to salicylic acid. Only salicylic and acetylsalicylic acid were found to increase the rate of oxygen consumption of rats or mice; 2,5- and 2,6-dihydroxybenzoic acid, m- and p-hydroxybenzoic acid, and salicylamide were found to be inactive. The effect of eighteen substituted benzoic acids on rats was examined by Andrews[26]. Of the compounds studied at the doses used, only salicylic acid and o-, m-, and p-cresotic acid were active, the molar potency ratios of each of the three cresotic acids as metabolic stimulants relative to salicylic acid being 2.61, 1.78, and 1.89 respectively. All the mono-hydroxysalicylic acids, salicyluric acid, and 5-aminosalicylic acid were either inactive or were metabolic depressants. 6-Methylsalicylic acid, at the low dose used, owing to its toxicity, was inactive. These results indicated that the introduction of a methyl group into the benzene ring at positions other than the 6-position increased the potency of salicylic acid as a metabolic

stimulant, the greatest increase being with the methyl group in the ortho position to the phenolic OH. With a methyl group in the 6-position the toxicity of the compound was greatly increased, possibly through steric inhibition of detoxication reactions such as the conjugation of the carboxyl group with glycine. Measurements of the metabolic stimulant action of 6-methylsalicylic acid could not therefore be made with doses as high as with the other acids. It is also of interest that some preliminary measurements with 3-phenylsalicylic acid made by Andrews[26] suggested that it was even more potent than o-cresotic acid. The results in this paper also established that the differences in potencies of the different cresotic acids relative to salicylate did not depend on the rates of degradation or excretion of the drug.

All the above papers have described experiments on the whole animal. However, a number of in vitro studies have shown that salicylate stimulates oxygen uptake at cellular level. Fishgold, Field and Hall[27] observed an increase in the rate of oxygen consumption of rat brain slices at concentrations of 0.06 to 0.56 mM, followed by a

progressive fall at higher concentrations. No stimulation of respiration was noted with thick liver slices at any concentration of sodium salicylate. Sproull[28] investigated the effect of sodium salicylate on the rate of oxygen consumption of mouse liver slices. He established reproducible dose-response curves in the presence of concentrations of salicylate in the range 3.5×10^{-4}M to 7.5×10^{-3}M: from concentrations of 3.5×10^{-4}M to 2×10^{-3}M there was a progressive increase in the rate of oxygen consumption, followed by a fall at higher concentrations.

From this brief survey of previous work it would appear that only those benzoic acids having hydroxyl groups in the ortho position to the carboxyl group possess anti-rheumatic, anti-diabetic, or metabolic stimulant properties. However, it should be noted that the presence of this salicylate grouping in a molecule does not necessarily cause the molecule to possess all three or even any one of the above properties, since other groups in the benzene nucleus may counter its activity in any of these respects. Thus γ-resorcylic acid which, is reported to possess anti-rheumatic characteristics[13], has no action as a metabolic stimulant.

At this point one might mention 2,4-dinitrophenol, a compound with a number of structural similarities to the salicylate ion, such as the phenolic hydrogen hydrogen-bonded to an oxygen atom of an electron withdrawing group. This compound has a surprising number of pharmacological properties specific to that structure and similar to those of salicylic acid. It is a metabolic stimulant[29,30] this property being found in the case of nitrophenols only in compounds possessing the 2,4-dinitrophenol grouping[31], and is also found to have a qualitatively similar anti-diabetic action to salicylate[32]. However, it was found inferior to aspirin in this last respect, though a more potent metabolic stimulant.

To summarise the preceding paragraphs, it can be said that the biological activity of salicylate respecting its anti-rheumatic, anti-diabetic, and metabolic stimulant properties would appear to be a function of the 2-hydroxybenzoic acid grouping. The position is, of course, complicated by the presence of further substituents, loss or enhancement of activity occurring in some respects but not in others. It was also mentioned that 2,4-dinitrophenol shows similar activity to salicylate in its anti-diabetic

and metabolic stimulant properties at least. In this case the biological activity is inherent in the 2,4-dinitrophenol grouping.

Physico-chemical aspects

To explain these biological properties, perhaps the first step is to compare salicylic acid with its isomers m- and p-hydroxybenzoic acid, the first being biologically active in the respects previously considered, and the latter two inactive. One notable difference is the much stronger acidity of salicylic acid (see Table 1). This has been attributed[33,34] to hydrogen bonding between the phenolic hydrogen and the carboxylate anion, resulting in stabilisation of the molecule as the salicylate ion. As a concomitant of this, the phenolic pK_a is higher in the salicylate ion than with the other two isomers, and this for the same reason. Intramolecular hydrogen bonding is not, of course, possible between the phenolic hydrogen and the carboxylate group in the m- and p- isomers for steric reasons.

The structural and resulting physico-chemical properties of salicylic acid are sufficiently unusual to warrant further investigation of the physical properties of its chelate bond in an attempt to correlate physical with the biological properties of the molecule.

TABLE 1.

The carboxylic dissociation constants of the monohydroxy benzoic acids[35].

	10^5. K_a
o-hydroxybenzoic acid	101
m-hydroxybenzoic acid	8.27
p-hydroxybenzoic acid	2.95

It has been suggested that compounds possessing both chelating groups and biological activity may possess this latter function through their ability to complex with metal ions. A few examples may be instanced. The action of compounds analogous to 8-hydroxyquinoline in inducing diabetes mellitus in rabbits parallels their chelating tendencies, and the idea was put forward that their diabetogenic action might be associated with their ability to react with trace metal ions[36]. Some evidence has recently been obtained that the antibiotic terramycin inhibits the alanine dehydrogenase of Mycobacterium Tuberculosis through the formation of a chelate compound with the magnesium ion, this being the actual inhibitor[37]. The antibiotic tetracycline, of which terramycin is an alcoholic derivative, has been shown to be bound to deoxyribonucleic acid and to serum albumin in the form of a macromolecule-metal ion-tetracycline complex.[38] This result would strengthen the view that the tetracyclines may exert some of their biological effects by complexing with macromolecules through metal ions.

It is well established[39,40] that the total concentration of salicylate in human blood plasma is much greater than the concentration of free salicylate. Using dialysis and ultrafiltration

techniques it was found[39] that at a concentration of 4 mg per 100 ml 90% of the total salicylate was bound to protein constituents; at a concentration of 70 mg per 100 ml, the figure was 54%. This phenomenon of the binding of organic molecules by proteins may arise through physical forces such as electrostatic interactions or hydrogen bonding[41], but it has been shown that it can occur through the mediation of a metal ion between the protein and the small molecule[42]. In the case of a molecule such as salicylic acid with its known metal complexing properties it is possible that its binding to proteins is by such a chemical mechanism.

It is very probable that a number of the biological actions of salicylate may arise as a result of its tendency to associate with plasma protein. In 1959 Christensen[43] noted that using thyroxine labelled with ^{131}I and bound to protein within a dialysis apparatus, there was a substantial increase in the amount of ^{131}I passing through the membrane on adding salicylate or 2,4-dinitrophenol to the protein compartment. This he interpreted as being due to an increase in free thyroxine. It would appear that the salicylate or 2,4-dinitrophenol displaced

the thyroxine bound to the protein. A later paper[44] noted that with plasma salicylate concentrations of 30 mg per 100 ml or higher there was an increased activity of plasma enzymes. This phenomenon could readily be explained if it were assumed that salicylate was more strongly bound to protein at the enzyme binding sites than the enzyme itself. The release of thyroxine by a mechanism such as this might explain the increase in metabolic rate resulting in mammals treated with salicylate, this being one of the hormonal actions of thyroxine.

A mechanism of this nature would explain the wide range of the biological actions of salicylate on whole animals. Furthermore, if the binding sites of these biologically active materials are metal ions already complexed to the plasma proteins, a ready explanation of their displacement by salicylate would be at hand.

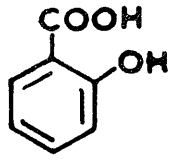
However, this is all surmise, and the true picture may be very different. But, whatever the mechanism of action of salicylate may be, it would be expected that it ~~should~~ might be possible in a group of substituted derivatives to correlate the complexing tendency of the molecule with metal ions with its

biological action, the stability of the complex being an indication of the total effect of the various factors of molecular structure and environment which would affect its biological action. For that reason the subject of this work was an investigation into the complex formation of some derivatives of salicylic acid with ferric ion. In view of the similarity in biological action between salicylic acid and 2,4-dinitrophenol in many respects, this compound was also included for comparison. The compounds studied either had been or were about to be investigated with regard to their biological activity.

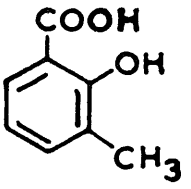
General Methods

The substances studied can conveniently be divided into three groups: firstly, salicylic acids substituted in the 3-position with alkyl or aryl groups; secondly, the hydroxy-salicylic acids; and thirdly, the remaining acids. The first group contained o-cresotic acid, 3-iso-propylsalicylic acid, 3-tert-butylsalicylic acid, and 3-phenylsalicylic acid. The second comprised γ- and β-resorcylic acid (2,6- and 2,4-dihydroxybenzoic acid respectively), and would have included gentisic acid (2,5-dihydroxybenzoic acid), a minor detoxication product of salicylic acid, but for its ready oxidation by ferric ions. The remaining acids, namely m- and p-cresotic acid (4- and 5-methylsalicylic acid respectively), 6-methylsalicylic acid, salicyluric acid (the principal detoxication product of salicylic acid), and 2,4-dinitrophenol made up the third group. The structural formulae of the compounds whose complex formation with iron(III) was studied are shown in Figure 1.

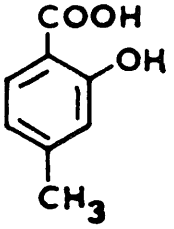
The choice of metal ion in this investigation was somewhat arbitrary, and partly arose from the known ability of the ferric ion to form stable complexes with phenols in general and



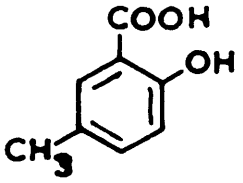
salicylic acid



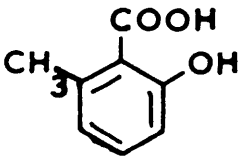
o-cresotic acid



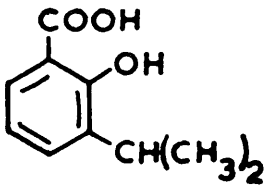
m-cresotic acid



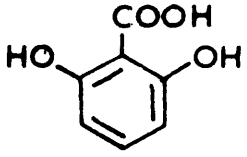
p-cresotic acid



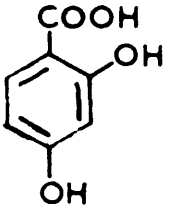
6-methylsalicylic acid



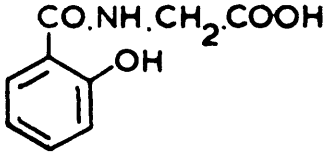
3-iso-propyl-salicylic acid



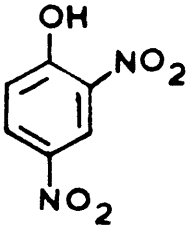
γ-resorcylic acid



β-resorcylic acid



salicyluric acid

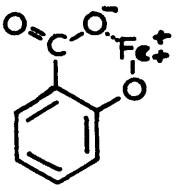


2,4-dinitrophenol

Fig. 1. The structural formulae of the acids studied.

salicylates in particular, and also from the known importance of iron in metabolism.

The well-known colour reaction of salicylates with ferric ions was reported as early as 1798 by White[45] in connection with studies of extracts from willow bark. It was suggested by Vogel[46] in the 1870's that the reaction might be used in the detection of iron, and since then it has become a standard method for the determination of iron [47,48] and of salicylic acid [49]. This colour reaction results from the association of the ferric ion and the salicylate molecule. In a neutral solution of salicylic acid the carboxylic group is completely ionised. On adding a solution of ferric ions to it there is a very considerable liberation of hydrogen ion which can be explained on the assumption that the phenolic hydrogen is released as an ion. In the event of a 1 : 1 complex being formed this would indicate a structure[51] such as:



At this date there are a very considerable number of methods available for the quantitative investigation of complex formation. Of these methods, potentiometry, polarography, spectroscopy, solubility and ion exchange have all been used in experiments involving iron(III) complexes[50]. Although other methods have their uses, by far the most accurate and widely applicable technique currently available is that of potentiometry. In fact, this method, often in combination with spectrophotometry, has been used by most of those investigating the iron(III)-salicylate system. This is due, perhaps, to the most notable properties associated with the complex, viz., the marked colour of the complex, the drop in pH on its formation, and the reduction in the free iron(III) concentration in solution caused by the complexing.

A number of authors have examined the iron(III)-salicylic acid complexes using a combination of pH measurements and spectrophotometry[51-58]. Only two papers[53,59] appear to have been published using combined pH and oxidation-reduction potential measurement to investigate the system. However, the

consensus of opinion of all these papers is that iron(III) forms three complexes with salicylic acid, of formula FeR^{+}, FeR_2^{-} and FeR_3^{3-}, where R represents $^{-}O.C_6H_4.COO^{-}$. The complex FeR^{+} is of a violet colour and is formed in strongly acid solution. On raising the pH the species FeR_2^{-} and FeR_3^{3-} make their appearance in turn, the first of these being red and the second yellow.

Of the other salicylic acid derivatives, only o-cresotic acid, m-cresotic acid and β-resorcylic acid have been investigated previously as regards their complex formation with iron(III) in aqueous solution. Tripathi and Prakash[60], using a spectrophotometric technique, found that o-cresotic acid formed a 1:1 complex with iron(III) ions. The complexes of ferric ion with m-cresotic acid[58] and with β-resorcylic acid[58,61-63] were found to be similar in type to those of salicylic acid, and it has been suggested[63] that the 1:1 complex of the latter acid be used for the determination of ferric iron.

As regards the experimental technique used in this work, it was decided to adopt the procedure of simultaneous pH and oxidation-reduction

potential measurement in the system, since this was the most versatile method and at least as accurate as any other. The elegant graphical method of analysis of results used by Ågren[64] was followed, as was his use of sodium perchlorate as "swamping" electrolyte. Using this experimental method a number of assumptions are made, and these will be considered in turn.

Initially it was hoped to use 3 M sodium perchlorate as swamping electrolyte to overcome activity factor and liquid junction difficulties[65], and because values for the iron(III) hydrolysis constants were obtained in that medium by Hedström. (It has been reported[67,68] that the perchlorate ion has a weak complex forming tendency, but in the presence of a large and constant excess of perchlorate ion this need not be taken into account in the calculation of the equilibria). However, as will appear later, in most of the experiments the concentration was 0.1 M.

The equilibrium solutions studied contained both ferric and ferrous ions, each kept at a fixed, total concentration in each experiment. Since the concentration of free ferric ions was obtained from the oxidation-reduction potential of the system not

in absolute terms but relative to the ferrous ion concentration, it was important that this should remain constant and not be decreased by complexing with either organic acid or hydroxyl ions. From Hedström's work on its hydrolysis[69], the degree of hydrolysis of ferrous ion is negligible below pH 6.5 and is still less than 1% at pH 7.5. Thus the influence of this on the determination of the stabilities of the 1:1 and 1:2 complexes, predominant in the acid range of pH, will be negligible. As regards complexing of the ferrous ion by the chelating agent, Ågren[53] investigated the possibility in the case of salicylic acid by varying the ratio of the concentrations of ferric to ferrous ion in the system. The values of the constants obtained were not dependent on the ratio of ferrous to ferric iron. From this result it was concluded that the complexing of ferrous ions by salicylate under the conditions of the experiments was negligible. This was repeated to a lesser extent in this series of experiments in that each compound was investigated using solutions containing different ratios of the two forms of iron ion.

Certain practical difficulties arose in using the 3 M perchlorate medium, so that for most of the measurements a medium brought to an ionic strength of 0.10 was used. One difference resulting from this was that while the complexing of ferric ions

by perchlorate would now be negligible, it would no longer be correct to assume that the activity factors, particularly those of polyvalent ions, would be constant. It was, however, assumed that, within experimental error, they were constant over the concentration range studied. Also, it was not possible to utilise a value for the liquid junction potential at the solution phase/salt bridge solution phase boundary.

Even at the most acid pH's used in this work, a significant proportion of the total ferric iron concentration in a solution of ferric perchlorate is present as hydroxyl complexes. Hedström[66] found that the species $FeOH^{2+}$, $Fe(OH)_2^{+}$, and $Fe_2(OH)_2^{4+}$ are formed, and derived values for their formation constants at 25° and in 3 M perchlorate medium. These values were used in the analysis of the results from the work in 3 M perchlorate. A later paper by Milburn and Vosburgh[70] confirmed Hedström's work, and these authors obtained equations relating the formation constants of the species $FeOH^{2+}$ and $Fe_2(OH)_2^{4+}$ to ionic strength, from which it was possible to calculate their values at an ionic strength of 0.1. An estimate of the value of the formation constant of the species

$Fe(OH)_2^+$ at ionic strength 0.1 was made using the figures from the papers of Hedström and of Milburn and Vosburgh.

Fortunately, the complexing power of all the compounds containing the salicylic acid grouping was sufficiently great for the actual free ferric ion concentration to be very small over most of any one titration. Hence the proportion of the total ferric ion present as hydroxyl complexes was very small, and any possible error in the calculated correction for these hydroxyl complexes would be negligible in the final result.

As stated before, the method of Ågren was used in analysing the experimental results. This[64] is given below.

Consider the theory of the method applied to salicylic acid, represented by RH_2. The method involved the simultaneous measurement of pH and oxidation-reduction potential in a solution containing sodium salicylate, ferric and ferrous perchlorate, sodium hydroxide, perchloric acid and sodium perchlorate. Hence the electromotive force of the following cells was measured:

1. $- \; Ag \,|\, AgCl_{(s)} \,|\, HCl \,|\, \text{glass} \,|\, \text{solution} \,|\, \begin{matrix}\text{reference} \\ \text{electrode}\end{matrix} \; +$

2. $- \; Pt \,|\, \text{solution} \,|\, \text{reference electrode} \; +$

(The reference electrode was a silver/silver chloride electrode in the 3 M perchlorate work, and a calomel electrode for measurements in ionic strength 0.1.)

The equilibrium constants for the formation of the FeR^{+} , FeR_2^{-}, and FeR_3^{3-} complexes are defined by:

$$Fe^{3+} + RH^- \rightleftharpoons H^+ + FeR^+; \qquad K_1 = \frac{h.[FeR]}{[RH].[Fe]}$$

$$Fe^{3+} + 2RH^- \rightleftharpoons 2H^+ + FeR_2^-; \qquad K_2 = \frac{h^2.[FeR_2]}{[RH]^2.[Fe]}$$

$$Fe^{3+} + 3RH^- \rightleftharpoons 3H^+ + FeR_3^{3-}; \qquad K_3 = \frac{h^3.[FeR_3]}{[RH]^3.[Fe]}$$

or in a more general form:

$$Fe^{3+} + nRH^- \rightleftharpoons nH^+ + FeR_n^{(3-2n)}; \qquad K_n = \frac{h^n.[FeR_n]}{[RH]^n.[Fe]} \quad (1)$$

where h is the hydrogen ion concentration and all concentrations are expressed in mM. The signs for electrical charges in the equilibria are omitted for convenience.

The E.M.F. of the first cell above is given by

$$E_{Gl} = E_{o,Gl} - 59.16 \log h - E_j$$

where $E_{o,Gl}$ is a constant (in mV) and E_j the liquid junction potential

The E.M.F. of the second cell is obtained from

$$E_{Pt/Fe^{3+},Fe^{2+}} = E_{o,Pt/Fe^{3+},Fe^{2+}} - 59.16 \log [Fe^{3+}] / [Fe^{2+}] - E_j$$

where $E_{o,Pt/Fe^{3+},Fe^{2+}}$ is a constant.

Whence $E = E_{Pt/Fe^{3+},Fe^{2+}} + E_j = E_{o,Pt/Fe^{3+},Fe^{2+}} - 59.16 \log \frac{[Fe]_t}{[Fe^{2+}]} + 59.16 \log (b + 1)$

where $b = \frac{[Fe]_t - [Fe^{3+}]}{[Fe^{3+}]}$ and $[Fe]_t$ is the total concentration of iron (III).

If the total concentrations of iron (III) and iron (II) are each constant during an experiment, this equation can be written

$$E = E_o + 59.16 \log (b + 1)$$

where

$$E_o = E_{o,Pt/Fe^{3+},Fe^{2+}} - 59.16 \log [Fe]_t / [Fe^{2+}]$$

Since the total concentration of iron (III) is the sum of the concentrations of iron (III) present in the different species containing it, and similarly with the total concentration of salicylate, the following equations are valid:

$$[Fe]_t = [Fe] + [FeOH] + [Fe(OH)_2] + 2[Fe_2(OH)_2] + [FeR] + [FeR_2] + [FeR_3] \quad (2)$$

$$[R]_t = [RH] + [RH_2] + [FeR] + 2[FeR_2] + 3[FeR_3] \quad (3)$$

where $[R]_t$ is the stoichiometric concentration of salicylate.

By a consideration of the reactions producing or consuming hydrogen ion, the following relation is derived:

$$h = [H]_t - [OH]_t + [FeR] + 2[FeR_2] + 3[FeR_3] - [RH_2] + [FeOH] + 2[Fe(OH)_2] + 2[Fe_2(OH)_2] \quad (4)$$

The hydroxyl complexes present are $FeOH^{2+}$, $Fe(OH)_2^{+}$ and $Fe_2(OH)_2^{4+}$.

The general expression for their equilibrium constants is

$$k_{nm} = \frac{h^m \cdot [Fe_n(OH)_m]}{[Fe]^n} \quad (5)$$

By combining equations (1) and (5) with (2):

$$[Fe]_t = [Fe] + \frac{k_{11}\cdot[Fe]}{h} + \frac{k_{12}\cdot[Fe]}{h^2} + \frac{2k_{22}\cdot[Fe]^2}{h^2} + \frac{K_1\cdot[RH][Fe]}{h} + \frac{K_2\cdot[RH]^2[Fe]}{h^2} + \frac{K_3\cdot[RH]^3[Fe]}{h^3}$$

$$\text{i.e. } \frac{[Fe]_t - [Fe]}{[Fe]} = b = \frac{k_{11}}{h} + \frac{k_{12}}{h^2} + \frac{2k_{22}[Fe]}{h^2} + \frac{K_1[RH]}{h} + \frac{K_2[RH]^2}{h^2} + \frac{K_3[RH]^3}{h^3}$$

$$\text{i.e. } b = k' + \frac{K_1[RH]}{h} + \frac{K_2[RH]^2}{h^2} + \frac{K_3[RH]^3}{h^3} \quad (6)$$

where k', the small correction term for the hydroxyl complexes, is equal to $\left\{ \frac{k11}{h} + \frac{k_{12}}{h^2} + \frac{2k_{22}[Fe]}{h^3} \right\}$

If the last term of equation (6) is assumed to be small compared with the other terms, and this should be so at lower pH's, the following equation is obtained:-

$$(b - k') \frac{h}{[RH]} = K_1 + K_2 \frac{[RH]}{h} \qquad (7)$$

The value of the term on the left hand side of the equation can be calculated from experimental data. If this is plotted against the function $[RH]/h$ a straight line should be obtained, the intercept being K_1 and the slope K_2.

To calculate $[RH]$ the dissociation constant of the salicylic acid must be known:

$$K_a = \frac{[RH]\ h}{[RH_2]} \qquad (8)$$

By subtracting equation (4) from (3):

$$[R]_t - h = [RH] + 2[RH_2] - [H]_t + [OH]_t - [FeOH] - 2[Fe(OH)_2] - 2[Fe_2(OH)_2],$$

and combining this with (8):

$$[RH](1 + \frac{2h}{K_a}) = [R]_t + [H]_t - [OH]_t - h + [FeOH] + 2[Fe(OH)_2] + 2[Fe_2(OH)_2] \qquad (9)$$

from this equation $[RH]$ can be calculated.

At higher pH's where it is no longer correct to assume that the last term in equation (6)

is negligible compared with the others, the value of K_3 can be obtained from the rearranged form of equation (6):

$$\left\{(b - k')\frac{h}{[RH]} - K_1\right\}\frac{h}{[RH]} = K_2 + K_3\frac{[RH]}{h} \qquad (10)$$

Assuming K_1 has been determined at a lower pH range, on plotting the left hand side of equation (10) against $[RH]/h$, a straight line should be obtained with intercept K_2 and gradient K_3.

The experimental details of the method are now given in the following section.

EXPERIMENTAL

Apparatus

The apparatus used in the E.M.F. measurements consisted of a titration cell, a liquid junction assembly, and a reference electrode, all maintained at $25^{\circ} \pm 0.01^{\circ}$ in an oil bath fitted with a toluene-mercury thermo-regulator.

The titration cell was made from a four-necked flask (see Fig. 2). Three of the necks held the two platinum electrodes and the nitrogen inlet. The fourth, and largest neck, was used to hold one limb of the liquid junction assembly, the glass electrode, and the tips of the two burettes.

The design of the liquid junction assembly and reference electrode depended on whether measurements were being made in 3M perchlorate or in a medium of ionic strength 0.1. In the 3M perchlorate measurements the apparatus was similar to that used by Ågren[64] and was of form:

$$+ \quad Ag \;\Big|\; AgCl \;\Big|\; \begin{matrix} 100mM\ NaCl \\ 3000mM\ NaClO_4 \end{matrix} \Big|\; NaClO_4 \quad 3M \;\Big|$$

The liquid junction consisted of a glass tube drawn out to a fine capillary in the form of a J (Fig. 3a). The Ag/AgCl electrode was prepared by the method of Brown[71].

For the series of measurements at an ionic strength of 0.1 various modifications were made to the apparatus. With the liquid junction and reference electrode system shown above, a drift in liquid junction potential with time was noted. This was traced to the coarse nature of the J-shaped liquid junction, which, while being perfectly satisfactory with a solution containing 3M perchlorate, did not function well with a solution containing perchlorate at a strength of 0.1M. It was replaced by a much finer, differently shaped junction (Fig. 3b). To reduce the liquid junction potential, ammonium nitrate was used in the salt bridge on account of the approximately equal ionic mobilities of the ammonium and nitrate ions. However, a solution 5M in ammonium nitrate gave drifting liquid junction potentials, whereas very steady potentials were obtained with a 1M solution, but it possessed too high a resistance. A 2.5M solution in the salt bridge was found to ~~result in~~ give a steady potential and to possess a sufficiently low resistance for practical use.

For the experiments described in the last paragraph, the silver/silver chloride electrode

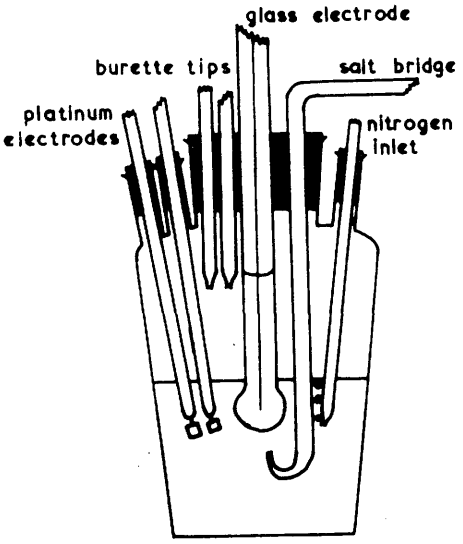


Fig. 2. The titration cell.

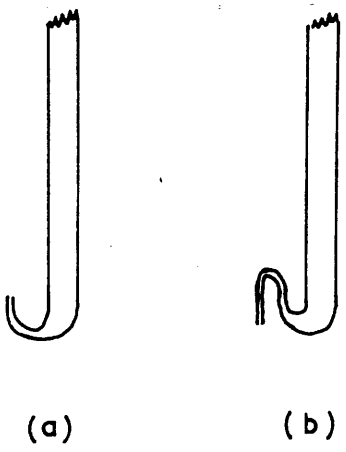


Fig. 3. Liquid junction shapes used (a) in 3M and (b) in 0.1M experiments.

in 0.1 M sodium chloride solution was replaced by a saturated calomel electrode, and the final electrode assembly used is shown in Fig. 4.

During titrations nitrogen was passed through the reaction mixture. The nitrogen used was the "White Spot" variety supplied by the British Oxygen Company. It was freed from any oxygen and/or carbon dioxide present by passing it first through Fieser's solution[72] and then through a glass tube packed with copper turnings at a temperature of 450° [73]. The activity of the copper in the tube was regenerated by passing hydrogen over it on the completion of an experiment. Before entering the titration cell, the nitrogen was bubbled through a solution of sodium perchlorate, 3 M in the case of the experiments in 3 M perchlorate and 0.1 M in the experiments at ionic strength 0.1

In the measurement of the oxidation-reduction potentials, two bright platinum electrodes were used simultaneously. Before use these were cleaned with boiling nitric acid and rinsed with distilled water. Electrodes treated in this way were found to agree in potential to better than 0.1 mV.

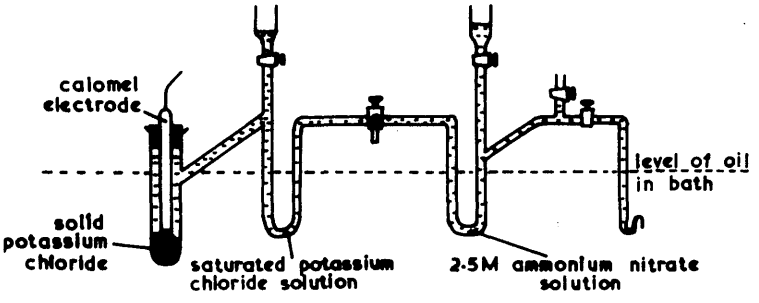


Fig. 4. The saturated calomel half cell and salt bridge used in the 0.1M experiments.

The oxidation-reduction potentials were measured with a Croydon Precision Instruments Co. potentiometer coupled with a Pye "Scalamp" galvanometer, this system having a sensitivity of 0.1 mV.

The hydrogen ion concentration was determined with a Cambridge pH meter, accurate to ±0.01 pH unit. Various commercial glass electrodes were used, in particular the Cambridge glass electrode. The pH meter was standardised with 10^{-2} and 10^{-3} M solutions of perchloric acid made up to the appropriate ionic strength with sodium perchlorate.

The method of standardisation of the pH meter differed slightly between the 3 M perchlorate experiments and the experiments at ionic strength 0.1. In the former experiments the E.M.F.'s of the cells containing 10^{-2} and 10^{-3} M perchloric acid ($[ClO_4^-]$ = 3 M) were measured and the difference between them taken to be the gradient $dE/d(\log h)$ ~~$d(\log h)/dE$~~. The value of this gradient and the value of E at h = 1 mM were substituted in the equation

$$E_{Gl} = E_{o,Gl} - m \log h - E_j,$$

where $E_{o,Gl}$ is a constant (in mV) for Cell 1, m is the gradient dE/d(log h), and E_j the liquid junction potential. Under the conditions of the 3 M experiments the value of E_j was approximately - 0.018h mV[64,65]. Hence the values of h corresponding to different values of E_{Gl} were calculated from this.

In the experiments at ionic strength 0.1, the gradient dE/d(log h) was the theoretical one, viz. 59.16 with E measured in mV, and the pH meter was therefore used directly for measuring pH, being standardised with a 1 mM solution of perchloric acid (ionic strength 0.1) and the performance of the glass electrode checked with a 0.1 mM solution of perchloric acid (ionic strength 0.1), which solutions were assumed to have hydrogen ion concentrations of 10^{-3} and 10^{-4} M respectively.

The glass electrode was, of course, then calibrated in terms of hydrogen ion concentration, not activity.

Chemicals

Constant boiling hydrochloric acid (A.R.) was used as the primary standard for acidimetric and alkalimetric titrations. Standard sodium hydroxide solution was prepared by diluting a 16.5 M sodium Hydroxide (A.R.) solution with carbon dioxide-free

water and standardised against diluted constant boiling hydrochloric acid at the boiling point using phenol red indicator. This method was used in all mineral acid - sodium hydroxide solution titrations.

A solution of iron(III) perchlorate was prepared by dissolving ferric chloride (A.R.) in water and precipitating ferric hydroxide from it with aqueous ammonia (A.R.) . The ferric hydroxide was separated from adsorbed salts by dialysing it for several days in running (Glasgow) tap water. Finally it was filtered and washed with distilled water. No precipitate was then obtained from the washings on adding silver nitrate solution acidified with dilute nitric acid to them. To the pure ferric hydroxide so obtained was added an equivalent amount of 60% perchloric acid (A.R.) and the mixture heated on a water bath under reflux for 11 hours. The resulting solution of ferric perchlorate and excess perchloric acid was separated from residual ferric hydroxide by filtration using a filter aid.

Iron(II) perchlorate solution was prepared by the addition to barium perchlorate solution (prepared from barium carbonate (A.R.) and A.R. perchloric acid) of an equivalent amount

of ferrous sulphate (A.R.) solution. The solution obtained by filtration gave negative tests for sulphate with barium chloride and for barium with dilute sulphuric acid.

Stock solutions containing ferric and ferrous perchlorate in different concentration ratios were prepared from the above solutions. The perchlorate ion concentration was determined by an ion exchange method. Total iron concentration was measured by reduction of a sample with stannous chloride and titration against standard potassium dichromate solution. The ferrous ion concentration was found by direct titration with potassium dichromate solution. The ferric ion concentration was determined by difference ($[Fe]_{total} - [Fe^{2+}]$); the hydrogen ion concentration also by difference. ($[ClO_4^-] - 3[Fe^{3+}] - 2[Fe^{2+}]$)

An approximately 8 M solution of sodium perchlorate was prepared from 60% perchloric acid (A.R.) and A.R. sodium carbonate in deficit. After removing dissolved carbon dioxide by bubbling nitrogen through the solution, the pH of the solution was raised to 10 with 16 N sodium hydroxide and the solution left for 24 hours. It was then separated from precipitated impurities by filtration through

acid washed filter aid. The filtrate was acidified with perchloric acid and neutralised with carbon dioxide-free sodium hydroxide solution. The concentration of the final solution was determined using an ion exchange technique.

Initially, the sodium perchlorate solution was prepared from commercially available sodium perchlorate. However, it was found that oxidation of ferrous ion occurred in solutions made up with this sodium perchlorate, probably as a result of the presence of sodium chlorate as an impurity in the sodium perchlorate.

Most of the organic acids were obtained commercially. However, the samples of p-cresotic acid and of 6-methylsalicylic acid were supplied by Dr. C.J.W. Brooks and those of 3-*tert*-butyl- and 3-*iso*-propyl-salicylic acid by Dr. O. Fancher (Miles Laboratories Inc.). The γ-resorcylic and salicyluric acid used were synthesised, the first by the carboxylation of resorcinol under pressure[74], and the second following the method of Fischer[75]. The final purities of all samples were checked by paper chromatography.

All melting points are uncorrected for the emergent stem of the thermometer.

o-cresotic acid

This acid was purified by recrystallizing twice from benzene, then from methanol and water, and finally subliming twice at 80 to 100°C and 0.3 mm. pressure. The final melting point of the sample was 163-164°C (literature value[76], 163-164°C).

salicylic acid

Salicylic acid (A.R.) was recrystallized from benzene and sublimed at 80-100°C and 0.2 mm. pressure. The melting point of the purified sample was 157-159°C (literature value[76], 159°C).

m-cresotic acid

m-cresotic acid was recrystallised twice from benzene, twice from methanol and water, and finally was sublimed twice at 80-100°C and 0.3 mm. pressure. The melting point of the purified compound was 175-176°C (literature value [76], 177°C).

p-cresotic acid

The sample of p-cresotic acid was purified by crystallisation using benzene as solvent, followed by two sublimations at 80-100°C and 0.3 mm. pressure. The final melting point was 149-151°C (literature value[76], 153°C).

6-methylsalicylic acid

A sample of crude acid was readily purified by subliming twice at 130°C and 0.1 mm. pressure. The melting point of the purified sample was 170-171°C (literature value[76], 170-171°C).

3-iso-propylsalicylic acid

A pure sample of 3-iso-propylsalicylic acid was prepared by sublimation from the technical product at 120°C and 0.1 mm. The final melting point was 65-66°C.

γ-resorcylic acid

This acid was prepared, along with the β-isomer, by the carboxylation of resorcinol[74]. It was separated from the β-resorcylic acid by fractional crystallisation from benzene, and was further purified by sublimation at 130°C and 0.1 mm. pressure. The purity of the final product was checked by paper chromatography using an iso-propyl alcohol-ammonia (0.880)-water (10:1:1) solvent. (R_f values; γ acid: 0.70-0.75; β acid: 0.25-0.30). Its melting point was 164.5-165°C (literature value[76] 150-170°C).

β-resorcylic acid

β-resorcylic acid was purified by

recrystallisation from water and by sublimation at 160°C and 0.1 mm. pressure. Its melting point was 222-223°C (literature value[76], 213°C).

3-phenylsalicylic acid

Technical grade acid was recrystallised several times from a benzene-acetone mixture, and then from benzene alone, resulting in the separation of 3-phenylsalicylic acid from a high melting dicarboxylic acid impurity. Finally it was sublimed at 130°C and 0.3 mm. pressure. The melting point of the pure compound was 186-187°C (literature value[76], 180°C).

3-tert-butylsalicylic acid

A sample of 3-tert-butylsalicylic acid was sublimed twice at 120°C and 0.1 mm. pressure. The melting point was then 156-158°C.

salicyluric acid

A sample of this acid was purified by recrystallisation from chloroform-methanol mixture, followed by sublimation at 130°C and 0.2 mm. The final melting point was 166-167°C (literature value[76], 170-172°C)

gentisic acid

Gentisic acid was dissolved in water and precipitated from solution by the addition of concentrated hydrochloric acid. The precipitated crystals were recrystallized from chloroform-acetone mixture, and finally sublimed at 130°C and 0.1 mm. pressure. The melting point of the pure sample was 202-204°C (literature value[76], 200°C).

2,4-dinitrophenol

2,4-dinitrophenol was purified by recrystallisation from water, followed by sublimation at 80°C and 0.1 mm. pressure. The melting point of the final sample was 111-113°C (literature value[76], 113°C).

Measurements

The first dissociation constant of the acids was usually determined by titration of solutions of the acid in sodium hydroxide solution with perchloric acid, though with some of the more soluble acids the organic acid solution was titrated directly with alkali. The hydrogen ion concentration was measured with the glass electrode. Normally, the dissociation constant of each acid was calculated from the results of at least two titrations, preferably at concentrations of organic acid of 5mM or greater, and at an ionic strength of 0.10 M. With the less soluble acids, lower concentrations had to be used. Solubility difficulties became particularly important in the case of the cresotic acids in the 3 M medium, when the dissociation constants were calculated from the results of two titrations, both with concentrations of acid of only 2 mM. (More reproducible values of pK_a were apparently obtained at the higher concentrations of organic acid, e.g. 5 and 10 mM.)

The actual method of calculating[76a] the pK_a at each point on the titration curve involved substitution of the appropriate values in the equation:

$$- \log [H^+] = pK_a + \log \frac{[RH^-]}{[RH_2]} ,$$

the values of $[RH^-]$ and $[RH_2]$ being derived from the considerations that the solution must be electrically neutral and that these two species can be considered to be the only forms of the organic acid present at the pH's in question.

To determine the stabilities of the complexes formed by the acids with ferric ions, a solution of ferrous and ferric perchlorate containing sufficient perchloric acid to prevent the precipitation of ferric hydroxide was "titrated" with a solution of the organic acid in alkali. It was found that in the initial stages of the titration the addition of the solution of the organic acid in excess sodium hydroxide caused slight precipitation of ferric hydroxide, resulting in slow attainment of equilibrium. To overcome this, for measurements at hydrogen ion concentrations greater than $10^{-3.8}$ M, a solution of the organic acid in sodium hydroxide was prepared and the excess alkali converted to sodium bicarbonate by passing carbon dioxide through the solution. When the hydrogen ion concentration during a titration had dropped to $10^{-3.8}$ M, a solution of the organic acid in sodium hydroxide was used instead of the

bicarbonate solution. Passage of nitrogen through the solution in the titration vessel effectively mixed the contents and removed the carbon dioxide.

At the same time, an iron solution was added from a second burette so as to maintain constant values for the total concentrations of ferric and ferrous iron in the titration vessel.

After each addition the E.M.F.'s of the glass and platinum electrode cells were measured, it being usually found that equilibrium had been attained after five minutes.

This technique of using sodium bicarbonate solution to titrate ferric ion solutions without the formation of a precipitate of ferric hydroxide was used very successfully by Hedström in his investigation of the hydrolysis of the iron(III) ion[66].

For some of the less soluble acids this technique was slightly modified. To a solution of ferric and ferrous perchlorate with perchloric acid was added a solution of the sodium salt of the organic acid. The resultant solution was titrated with a solution of sodium bicarbonate or sodium hydroxide, depending on the pH, the total iron(II) and iron(III) concentrations being kept constant as before.

RESULTS

I. 3 M Perchlorate

As a result of the theoretical considerations mentioned earlier, the stabilities of the different complexes formed between the iron(III) ion and the derivatives of salicylic acid were investigated in a medium made 3 M in perchlorate ion by the addition of sodium perchlorate.

Difficulties, however, immediately arose caused by the abnormal behaviour of the glass electrode. Electrodes immersed in 3 M perchlorate solutions displayed drifts of potential of as much as 3 mV per hour. The variation in potential did not appear to involve the liquid junction, since very constant oxidation-reduction potentials were obtained simultaneously. Screening the electrode system and storing the glass electrode in an acid or alkaline solution did not lessen this drifting of potential. Different commercially available glass electrodes were tested, but of the limited range available, all displayed the same phenomenon.

Considerable time was spent in trying to overcome this difficulty, but to no avail.

Some results were obtained for o-cresotic

acid on two occasions when the drift in potential appeared to be minimal. These are described below.

o-Cresotic acid

The pK_a of o-cresotic acid in a 3 M perchlorate medium was determined as described earlier (see Table 3). The average value of pK_a under these conditions was 3.20.

In the investigation of the complexes formed between iron(III) ions and the acid, apparently acceptable results were obtained with two titrations, i.e. during these titrations the potential of the glass electrode remained reproducible, resulting in consistent values for the hydrogen ion concentration using the experimentally observed value for dE/d(log h) of the glass electrode instead of the theoretical value of 59.16 mV.

The difficulties caused by the lack of reproducibility of the glass electrode potential were aggravated in the complex formation titrations by the much greater time (usually three hours or more) taken by them, as against the half-hour required to carry out a simple acid-base titration. Hence the results of only two complex formation titrations in 3 M perchlorate are presented here.

One of the titrations was carried out

by titrating an iron(II)-iron(III) solution, made 4 mM in hydrogen ion with perchloric acid, with a solution of sodium o-cresotate containing a small amount of sodium bicarbonate. However, the low solubility of o-cresotic acid in 3 M perchlorate prevented values of $[RH]/h$ greater than 0.1 being obtained, and the second titration was performed by titrating an iron solution containing o-cresotic acid with sodium bicarbonate solution. By this method it was possible to obtain lower values of h and thus higher values of $[RH]/h$.

The results of the second of these two titrations are shown in some detail in Table 2. By plotting $(b-k')h/[RH]$ against $[RH]/h$ values for K_1 and K_2 can be obtained as described earlier. In this set of results dE/d(log h) of the glass electrode was 62.1 mV. The first value of E in the second column is the E.M.F. of the ferric-ferrous iron mixture, corrected for the diffusion potential, with no o-cresotic acid added but with sufficient perchloric acid present (70 mM) in the system to depress the hydrolysis of iron.

The values of K_1 and K_2 obtained are shown in Table 4. Each line in the table represents a single titration. The graphs are shown in Figure 5.

Table 2

Determination of the equilibrium constants of complex formation K_1 and K_2 of o-cresotic acid in 3M perchlorate*

$[R]_t$(mM)	E(mV)	E_{Gl}(mV)	h(mM)	b	$[RH]/h$	$(b-k')\frac{h}{[RH]}$
	-451.2					
1.9167	-379.5	+40.5	3.263	15.26	0.0722	206.9
1.7149	-371.2	+48.2	2.456	21.46	0.1036	202.7
1.6025	-365.2	+53.2	2.041	27.36	0.1284	208.6
1.4811	-357.2	+60.2	1.576	37.73	0.1773	208.4
1.3768	-348.0	+68.0	1.181	54.40	0.2550	208.9
1.3033	-339.6	+75.2	0.9051	75.98	0.3578	207.9
1.2373	-329.6	+83.2	0.6735	112.3	0.5111	215.0
1.1500	-310.9	+98.8	0.3784	233.7	1.0102	225.6

* The number of figures in each term in the Table is greater than that warranted in fact in order not to introduce an appreciable error into the calculations.

Table 3

The carboxylic dissociation constant of o-cresotic acid in 3M perchlorate medium

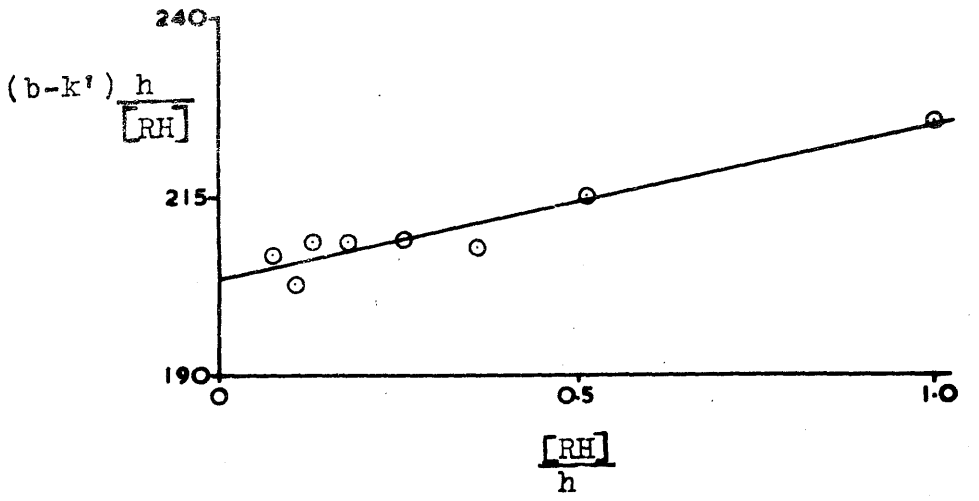
Initial concn. of o-cresotic acid	$[H^+]$ (mM)	$[RH^-]$ (mM)	$[RH_2]$ (mM)	pKa
1.963	0.1283	1.408	0.2838	3.20
	0.1574	1.342	0.3326	3.20
	0.1880	1.279	0.3783	3.20
	0.2204	1.220	0.4208	3.19
	0.2488	1.159	0.4658	3.21
	0.2840	1.106	0.5025	3.20
	0.3181	1.054	0.5389	3.21
	0.3563	1.008	0.5699	3.20
	0.3886	0.9572	0.6054	3.21
	0.4224	0.9096	0.6382	3.22
	0.4641	0.8715	0.6617	3.21
			Average	3.20
2.395	0.1426	1.756	0.3821	3.18
	0.2300	1.543	0.5284	3.17
	0.3151	1.346	0.6628	3.19
	0.3462	1.287	0.7019	3.20
	0.4509	1.131	0.7998	3.20
	0.5550	0.9897	0.8869	3.21
			Average	3.19

Table 4

The equilibrium constants of complex formation of

o-cresotic acid with iron (III) ions in 3M Sodium Perchlorate medium

Symbol	$[Fe^{II}]$ (mM)	$[Fe^{III}]$ (mM)	$[H^+]$ (mM)		K_1	K_2
X	1.5	0.56	4.0		182	31
O	1.5	0.56	4.0		204	21
				Average	193	26



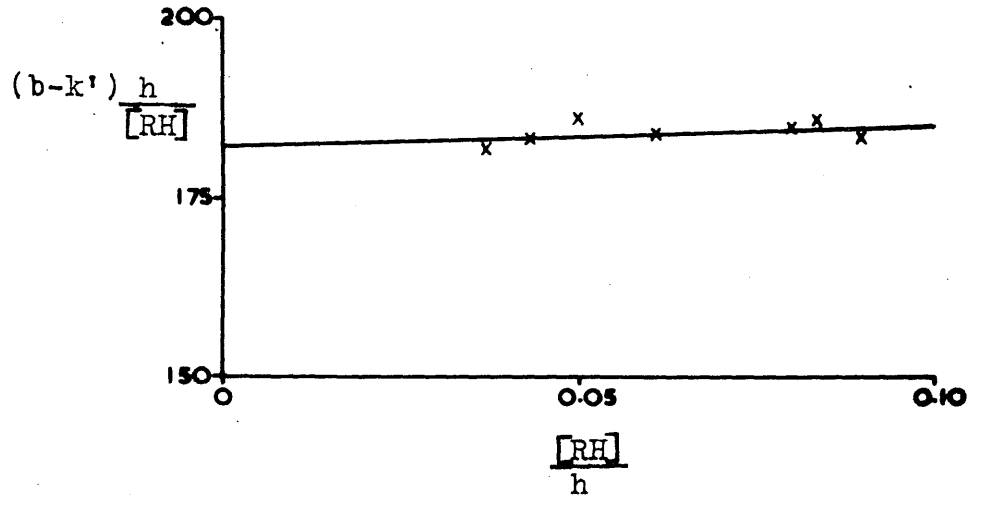


Fig. 5. o-Cresotic acid. Determination of K_1 and K_2 in 3 M perchlorate.

II. 0.1 M Ionic Strength Medium

The difficulties experienced through the abnormal behaviour of the glass electrode in 3M perchlorate solution caused the whole question of the medium to be used to be re-examined. It was decided that an accurate measure of the hydrogen ion concentration was of greater importance than minor variations in the liquid junction potential and activity coefficients, and hence it was decided to work in a medium containing a very much lower concentration of inert salt. This would, of course, introduce the difficulties with liquid junction potentials and activity coefficients mentioned above, but by working with low concentrations of ferric ion the changes in ionic strength resulting from the alterations in ferric ion concentration were minimised.

By using an ammonium nitrate bridge between the electrodes and the saturated calomel electrode it was assumed that the liquid junction potential of the cell was kept approximately constant, ammonium nitrate possessing approximately equal anion and cation conductances. However, at higher hydrogen ion concentrations deviations from this behaviour were noted. In Figure 6 is plotted (against the hydrogen

ion concentration) the observed values of the oxidation-reduction potentials of a solution of ferric, ferrous, hydrogen and perchlorate ions in which the ionic strength and the ratio of the concentrations of total ferric to ferrous iron were kept constant. It is seen that on increasing h, the observed E.M.F. initially rises to a maximum, and then decreases. The initial rise is caused by the increase in ferric ion concentration with h resulting from the decreased concentration of hydrolysis products of the Fe^{III} ion. The decrease in potential at values of h greater than 33 mM is probably caused by the increasing influence of the liquid junction on the observed E.M.F. The value of the maximum at h = 33 mM was taken to be E_o.

This assumption is open to question, since the liquid junction potential may well be significant at h = 33 mM, thus producing an apparent value of E_o less than the correct value. However, the value taken for E_o is not critical, an error of as much as 3 mV producing a deviation of only 11% in the calculated value for K_1. Furthermore, the values of K_1 obtained using this assumption agree well with values obtained by other methods.

In determining the contribution of

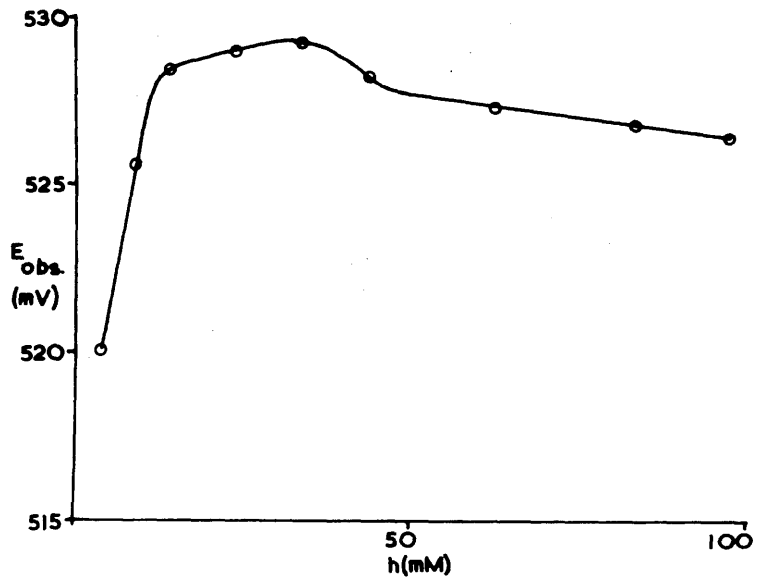


Fig. 6. The effect of hydrogen ion concentration on the apparent oxidation-reduction potential (versus the saturated calomel electrode) of a solution of iron(III) (0.36mM) and iron(II) (0.15mM) at ionic strength 0.1.

hydrolysis products of iron(III) to the oxidation-potential reduction, the values of k_{11} and k_{22} (2.9×10^{-3} M and 1.4×10^{-3} M respectively) obtained or derived from the work of Milburn and Vosburgh[70] were used. Hedström[66] had determined a value for k_{12} in 3 M perchlorate, and from this value an estimate was made of k_{12} in ionic strength 0.1 of 2.8×10^{-6} M^2.

Taking into account the above considerations, the complex formation between iron(III) and a number of phenolic compounds was investigated. The results obtained are presented in the following pages.

o-Cresotic acid

The pK_a of the carboxylic group of this acid was found to be 2.82 (see Table 5). Only two points in the titration of the 10 mM solution were obtained before precipitation of the acid occurred.

Average values of K_1 and K_2 of 383 and 2.9 respectively were found (see Figure 10 and Table 7).

In some earlier experiments at high hydrogen ion concentrations (10^{-2} to 10^{-3} M) in which ferrous-ferric solutions had been titrated directly with sodium o-cresotate, it had been noted that the results could be explained on the basis of the 1:1

complex alone being present, i.e. a straight line resulted on graphing (b-k') against $[RH]/h$. The value of K_1 obtained from these titrations was somewhat higher than that found at lower values of h (h less than 1 mM) but it was considered that this error might arise from the inconstancy of the liquid junction potential, although the glass electrode standardisation did not alter even up to hydrogen ion concentrations of 10 mM.

Salicylic acid

Two titrations of 2.5 and 20 mM solutions gave a value of 2.82 for the pK_a of the acid in a solution of ionic strength 0.1 (see Table 5). Part of the titration curve obtained in the titration of the 2.5 mM solution is shown in Figure 7.

The values of K_1 and K_2 for salicylic acid were determined in a similar way to those of o-cresotic acid, though, as a result of the greater solubility of salicylic acid in water, the iron solution was titrated directly with a solution of sodium salicylate containing an excess of alkali in the form of sodium bicarbonate. The results of three titrations carried out in this way are shown in Figure 11 and in Table 7. The average values K_1

equal to 820 and $K_2 = 17$ were found.

m-Cresotic acid

From the results of two titrations of 5 and 10 mM solutions of m-cresotic acid, a value for the pK_a of the acid of 2.97 was obtained (Table 5).

Average values of K_1 and K_2 of 980 and 17 respectively were found (see Table 7 and Figure 12).

p-Cresotic acid

Titration of 5 and 10 mM solutions of p-cresotic acid in excess alkali with perchloric acid gave an average value of pK_a of 2.90 (Table 5).

The average values of K_1 and K_2 obtained were 948 and 18 respectively (see Figure 13 and Table 7).

6-Methylsalicylic acid

This acid was markedly more soluble than the other methylsalicylic acids, considerably easing practical difficulties during the titrations caused by low solubility.

The average value of the pK_a of the carboxylate group was found to be 3.16 (see Table 5).

Average values for K_1 and K_2 of 381

and 4.6 respectively were obtained (see Figure 14 and Table 7).

3-iso-Propylsalicylic acid

The main difficulty with this particular compound lay in the low solubility of the acid itself in water, though solubility difficulties were not experienced with the sodium salt of the acid. As a result of this, it was possible to obtain values of the oxidation-reduction potential and hydrogen ion concentration over only a limited range of conditions before either a solid phase of ferric hydroxide or 3-iso-propylsalicylic acid was present in the solution. At the low pH's at which the measurements had to be made only the 1:1 complex was formed in significant amount, and the value of K_1 was obtained from the gradient of the graph of (b-k') against $[RH]/h$.

The dissociation constant of the acid was determined by titration of a 10 mM solution of the sodium salt with perchloric acid (Table 5). The value of the pK_a obtained, 2.76, was checked by titration of a 5 mM solution of the salt. Although this second titration confirmed the value of 2.76,

the pK_a values obtained in it were not as constant as in the first titration, and they are not reported.

Only a few values of h and the oxidation-reduction potential were measured in each titration of the ferric-ferrous solution with sodium 3-iso-propylsalicylate before ferric hydroxide was precipitated. Thus the value of K_1 obtained (350) was very approximate (see Figure 15 and Table 7).

γ-Resorcylic acid

The dissociation constant of the carboxylic group of this acid was determined by titration of 10 and 20 mM solutions of the sodium salt with perchloric acid. From these titrations the value of the pK_a was taken to be 1.07, this particularly low value showing the strong acidity of the carboxylic group of this acid (see Table 5).

In the complex formation titrations, average values of K_1 and K_2 of 573 and 16 respectively were obtained (see Figure 16 and Table 7).

β-Resorcylic acid

To determine the dissociation constant of the carboxylic group of β-resorcylic acid, 16,

20 and 27 mM solutions of sodium β-resorcylate were titrated with perchloric acid. An average value for the pK_a of 3.10 was obtained (see Table 5). By titration the pK_a of the more acid of the two phenolic groups was found to be approximately 8.5, but since this was well outside the range of pH in which measurements were made, and therefore the contribution of hydrogen ion from this source negligible, its value was not measured accurately. Part of the titration curve obtained in the titration of the 16.5 mM solution of sodium β-resorcylate is shown in Figure 8.

The equilibrium constants of complex formation were obtained in the usual way, From two titrations average values of K_1 and K_2 of 1340 and 58 respectively were obtained (see Figure 17 and Table 7).

Salicyluric acid

The pK_a of the carboxylic group was found by titration to have an average value of 3.41 (see Table 5). Similarly an approximate value of 8.2 was obtained for the pK_a of the phenolic group. Part of the titration curve of one of the titrations is shown in Figure 9.

Investigation of the purple complex of salicyluric acid with iron(III) ions by Job's Method of Continuous Variations spectrophotometrically indicated that it was essentially similar in type to those of the o-hydroxybenzoic acids, consisting of ferric ions and salicyluric acid ions in the molar ratio 1:1.

The complexes formed between ferric ions and this acid were also investigated potentiometrically using the same technique as was used with the other derivatives of salicylic acid. The results obtained fitted the same pattern, values of K_1 and K_2 of 121 and 3.8 respectively being obtained (see Figure 18 and Table 8).

At low values of $[RH]/h$ in the graph of $(b-k')h/[RH]$ against $[RH]/h$ deviations from linearity towards higher values were noted. These results are apparently explained on the assumption that a complex $FeRH^{2+}$ is being formed. In that case a further term $K_1'.h$ would have to be introduced into the expression:

$$(b-k')\frac{h}{[RH]} = K_1 + K_2\frac{[RH]}{h} + K_3\frac{[RH]^2}{h^2}$$

resulting in:

$$(b-k')\frac{h}{[RH]} = K_1'.h + K_1 + \frac{K_2[RH]}{h} + \frac{K_3[RH]^2}{h^2}$$

where

$$K_1' = \frac{[FeRH]}{[Fe][RH]}$$

At low pH's the last term in the above equation will be negligible, and in the event of the species $FeRH^{2+}$ being present, a straight line should result on graphing $\{(b-k')h/[RH] - K_2[RH]/h\}$ against h. A linear relation was observed in the case of this compound, giving an average value for K_1' of 7 mM^{-1} (see Figure 19 and Table 8 - the abnormally high value of the ordinate intercept of one of the graphs in Figure 19 cannot be explained).

2,4-Dinitrophenol

The dissociation constant of the phenolic group of this acid was measured in the usual way by titration of 10 mM solutions of the sodium salt of the acid with perchloric acid. An average value for the pK_a of 3.88 was obtained (see Table 6).

On titrating a ferrous-ferric solution with the sodium salt of 2,4-dinitrophenol, there was no evidence of complexing of the iron from the pH or oxidation-reduction potential measurements, nor from any obvious colour changes. It would

appear that any complexing of the phenol is masked by the formation of hydroxyl complexes with the iron. Errors in the values of the formation constants of the ferric hydroxyl complexes are of a similar or greater order to the degree of complexing of the ferric ions by the phenol.

Thus, by comparison with the previous phenolic acids, the complexing power of 2,4-dinitrophenol with ferric ions is negligible.

3-Phenylsalicylic acid, 3-tert-butylsalicylic acid, and gentisic acid

It had been hoped to obtain data concerning the complexing power of the above acids towards ferric ions, all three forming the typical purple or blue colour of solutions of o-hydroxybenzoic acids with ferric ions. However, the very low solubilities of the 3-phenyl- and 3-tert-butyl- acids in water prevented quantitative examination of either their strengths or their complexing power.

Gentisic acid was found to be too readily oxidised by ferric ions for any measurements of its complexing power to be made.

Summary

The results obtained in a medium of ionic strength 0.1 M are shown in Table 9.

While there was evidence that the complex FeR_3^{3-} was formed by most of the acids, it was not possible to make any assessment of the value of K_3. It should be noted that the accuracy of measurement of K_2 was considerably less than K_1.

Table 5

The carboxylic dissociation constants of the acids in 0.1 M ionic strength

Initial concn. of organic acid (mM)	$[H^+]$ (mM)	$[RH^-]$ (mM)	$[RH_2]$ (mM)	pKa
	o-cresotic acid			
2.396	0.3936	1.6906	0.4018	2.78
	0.4842	1.5874	0.4691	2.79
	0.5888	1.5048	0.5170	2.77
	0.6761	1.4111	0.5771	2.78
	0.7674	1.3273	0.6285	2.79
	0.8610	1.2514	0.6730	2.80
	0.9550	1.1813	0.7126	2.80
	1.047	1.1143	0.7502	2.81
	1.175	1.0881	0.7478	2.77
			Average	2.79
2.934	0.2163	1.8692	0.2892	2.86
	0.2818	1.7794	0.3502	2.84
	0.3673	1.6643	0.4281	2.85
	0.4519	1.5551	0.5014	2.85
	0.5433	1.4593	0.5625	2.85
	0.7328	1.2927	0.6631	2.85
	0.8318	1.2222	0.7022	2.84
	0.9226	1.1489	0.7450	2.85
	1.019	1.0863	0.7782	2.85
			Average	2.85

Table 5 (contd.)

Initial concn. of organic acid (mM)	$[H^+]$ (mM)	$[RH^-]$ (mM)	$[RH_2]$ (mM)	pKa
	o-cresotic acid (contd.)			
10.11	0.3981	7.6441	2.0036	2.82
	0.4875	7.2630	2.3389	2.82
			Average	2.82
	salicylic acid			
2.538	1.355	1.355	1.183	2.81
	1.169	1.408	1.069	2.81
	0.9954	1.461	0.9560	2.81
	0.9016	1.499	0.8854	2.82
	0.8110	1.536	0.8143	2.82
	0.7245	1.573	0.7470	2.82
	0.6412	1.611	0.6757	2.82
	0.5610	1.647	0.6077	2.82
	0.4159	1.729	0.4684	2.81
			Average	2.82
20.07	0.3034	15.49	3.180	2.83
	0.3784	14.79	3.745	2.83
	0.5070	13.66	4.663	2.83
	0.6622	12.59	5.532	2.82
	0.8318	11.55	6.364	2.82
	1.019	10.56	7.154	2.82
	1.236	9.630	7.894	2.82
	1.496	8.764	8.571	2.82
	1.778	7.944	9.206	2.81
			Average	2.82

Table 5 (contd.)

Initial concn. of organic acid (mM)	$[H^+]$ (mM)	$[RH^-]$ (mM)	$[RH_2]$ (mM)	pKa
	o-cresotic acid (contd.)			
10.11	0.3981	7.6441	2.0036	2.82
	0.4875	7.2630	2.3389	2.82
			Average	2.82
	salicylic acid			
2.538	1.355	1.355	1.183	2.81
	1.169	1.408	1.069	2.81
	0.9954	1.461	0.9560	2.81
	0.9016	1.499	0.8854	2.82
	0.8110	1.536	0.8143	2.82
	0.7245	1.573	0.7470	2.82
	0.6412	1.611	0.6757	2.82
	0.5610	1.647	0.6077	2.82
	0.4159	1.729	0.4684	2.81
			Average	2.82
20.07	0.3034	15.49	3.180	2.83
	0.3784	14.79	3.745	2.83
	0.5070	13.66	4.663	2.83
	0.6622	12.59	5.532	2.82
	0.8318	11.55	6.364	2.82
	1.019	10.56	7.154	2.82
	1.236	9.630	7.894	2.82
	1.496	8.764	8.571	2.82
	1.778	7.944	9.206	2.81
			Average	2.82

Table 5 (contd.)

Initial concn. of organic acid (mM)	$[H^+]$ (mM)	$[RH^-]$ (mM)	$[RH_2]$ (mM)	pKa
		m-cresotic acid		
5.039	0.2512	3.686	0.8737	2.97
	0.3273	3.446	1.054	2.97
	0.4046	3.218	1.221	2.97
	0.4898	3.005	1.376	2.97
	0.5781	2.800	1.529	2.98
	0.6761	2.617	1.653	2.97
	0.7762	2.440	1.779	2.97
	0.8810	2.276	1.888	2.97
	0.9772	2.110	2.003	2.99
			Average	2.97
9.916	0.2891	6.761	1.824	2.97
	0.3483	6.421	2.090	2.97
	0.3802	6.255	2.220	2.97
	0.4150	6.094	2.345	2.97
	0.4467	5.931	2.472	2.97
			Average	2.97
		p-cresotic acid		
5.2136	0.2951	3.837	0.9028	2.90
	0.3784	3.602	1.073	2.90
	0.4345	3.452	1.183	2.90
	0.4943	3.308	1.285	2.90
	0.5508	3.165	1.389	2.90
	0.6486	2.969	1.525	2.90

Table 5 (contd.)

Initial concn. of organic acid (mM)	$[H^+]$ (mM)	$[RH^-]$ (mM)	$[RH_2]$ (mM)	pKa
		p-cresotic acid (contd.)		
	0.7499	2.785	1.652	2.90
	0.9594	2.444	1.883	2.90
	1.079	2.299	1.975	2.90
			Average	2.90
10.11	0.3373	7.206	1.945	2.90
	0.4027	6.855	2.220	2.90
	0.4786	6.512	2.476	2.90
	0.5495	6.176	2.733	2.91
	0.6745	5.705	3.089	2.90
	0.8110	5.259	3.420	2.90
	0.9550	4.836	3.733	2.91
	1.122	4.451	4.011	2.90
	1.486	3.750	4.504	2.91
			Average	2.90
		6-methylsalicylic acid		
5.093	0.1730	3.684	0.9242	3.16
	0.2254	3.422	1.124	3.16
	0.2851	3.176	1.310	3.16
	0.3467	2.940	1.488	3.16
	0.4169	2.720	1.651	3.16
	0.4932	2.513	1.802	3.16
	0.5754	2.320	1.942	3.16

Table 5 (contd.)

Initial concn. of organic acid (mM)	$[H^+]$(mM)	$[RH^-]$(mM)	$[RH_2]$(mM)	pKa
	6-methylsalicylic acid (contd.)			
	0.6683	2.126	2.079	3.17
	0.7603	1.974	2.184	3.16
			Average	3.16
9.9842	0.1995	7.012	1.983	3.15
	0.2649	6.455	2.420	3.15
	0.3388	5.923	2.835	3.15
	0.4236	5.417	3.228	3.15
	0.5188	4.937	3.597	3.15
	0.6281	4.486	3.939	3.15
	0.7516	4.066	4.254	3.14
	0.8913	3.668	4.549	3.14
	1.227	2.976	5.043	3.14
			Average	3.15
	3-iso-propylsalicylic acid			
9.954	0.3228	7.645	1.420	2.76
	0.3614	7.470	1.554	2.76
	0.4188	7.231	1.736	2.76
	0.4592	7.062	1.865	2.76
	0.4977	6.893	1.994	2.76
			Average	2.76
	γ-resorcylic acid			
20.81	2.767	18.80	0.610	1.07
	3.296	18.58	0.695	1.06

Table 5 (contd.)

Initial concn. of organic acid (mM)	$[H^+]$ (mM)	$[RH^-]$ (mM)	$[RH_2]$ (mM)	pKa
	γ-resorcylic acid (contd.)			
	3.776	18.32	0.821	1.07
	4.236	18.05	0.958	1.10
	4.764	17.86	1.018	1.08
	5.272	17.66	1.091	1.07
			Average	1.07
10.21	2.897	8.681	0.3162	1.10
	3.350	8.538	0.3418	1.08
	3.758	8.366	0.3995	1.10
	4.188	8.230	0.4239	1.09
	4.624	8.115	0.4304	1.06
			Average	1.09
	β-resorcylic acid			
20.30	0.2291	14.61	4.187	3.10
	0.2938	13.63	4.997	3.10
	0.3631	12.67	5.787	3.10
	0.4467	11.74	6.547	3.10
	0.5370	10.84	7.285	3.10
	0.6383	9.970	7.998	3.10
	0.7586	9.133	8.677	3.10
	0.8913	8.324	9.331	3.10
	1.047	7.556	9.947	3.10
	1.233	6.833	10.52	3.10
			Average	3.10

Table 5 (contd.)

Initial concn. of organic acid (mM)	$[H^+]$ (mM)	$[RH^-]$ (mM)	$[RH_2]$ (mM)	pKa
	β-resorcylic acid (contd.)			
16.47	0.2291	11.59	3.523	3.12
	0.2884	10.82	4.772	3.12
	0.3606	10.07	4.772	3.12
	0.4385	9.340	5.365	3.12
	0.5224	8.635	5.940	3.12
	0.6194	7.956	6.491	3.12
	0.7328	7.306	7.015	3.12
	0.8550	6.677	7.521	3.12
	1.000	6.083	7.993	3.12
			Average	3.12
26.79	0.2553	17.23	5.280	3.08
	0.3388	15.86	6.370	3.07
	0.4315	14.53	7.422	3.07
	0.7907	10.89	10.29	3.08
			Average	3.08
	salicyluric acid			
10.52	0.1151	7.742	2.302	3.41
	0.1535	7.158	2.815	3.41
	0.1963	6.588	3.315	3.41
	0.2466	6.034	3.800	3.41
	0.3034	5.494	4.271	3.41
	0.3724	4.975	4.722	3.41
	0.4571	4.480	5.151	3.40

Table 5 (contd.)

Initial concn. of organic acid (mM)	$[H^+]$ (mM)	$[RH^-]$ (mM)	$[RH_2]$ (mM)	pKa
		salicyluric acid (contd.)		
	0.5495	4.001	5.565	3.40
	0.6622	3.549	5.951	3.40
	0.7980	3.129	6.308	3.40
			Average	3.41
21.01	0.1567	13.39	5.529	3.42
	0.1941	12.40	6.317	3.42
	0.2393	11.43	7.080	3.42
	0.2891	10.49	7.820	3.41
	0.3467	9.573	8.535	3.41
	0.4150	8.691	9.224	3.41
	0.4932	7.840	9.886	3.41
	0.5902	7.027	10.51	3.40
	0.7047	6.250	11.11	3.40
	0.8414	5.513	11.67	3.40
			Average	3.41

Table 6

The dissociation constant of

2, 4-dinitrophenol in 0.1 M ionic strength

Initial concn. of 2, 4-dinitrophenol (mM)	$[H^+]$ (mM)	$[R^-]$ (mM)	[RH] (mM)	pKa
10.04	0.02679	7.444	2.120	3.88
	0.05152	6.849	2.646	3.88
	0.06887	6.256	3.173	3.87
	0.08872	5.674	3.689	3.87
	0.1096	5.101	4.197	3.88
	0.1274	4.748	4.510	3.87
	0.1387	4.545	4.689	3.87
	0.1738	4.003	5.168	3.87
			Average	3.87
9.939	0.04864	6.804	2.572	3.89
	0.07047	6.017	3.271	3.89
	0.09863	5.257	3.953	3.88
	0.1321	4.505	4.612	3.89
	0.1816	3.790	5.245	3.88
	0.2483	3.105	5.849	3.88
	0.3451	2.464	6.410	3.88
			Average	3.88

Table 7

The equilibrium constants of complex formation of the substituted salicylic acids with iron (III) ions in ionic strength 0.1M

Symbol	$[Fe^{II}]$ (mM)	$[Fe^{III}]$ (mM)	$[H^+]$ (mM)	K_1	K_2
		o-cresotic acid			
+	0.75	0.28	3.9	390	3.9
x	0.076	0.18	2.7	375	1.9
			Average	383	2.9
		salicylic acid			
□	0.15	0.36	3.6	900	18
o	0.76	0.28	3.9	740	10
x	0.15	0.36	3.6	820	22
			Average	820	17
		m-cresotic acid			
x	0.15	0.36	3.6	950	14
o	0.15	0.36	3.6	1010	22
□	0.76	0.28	3.9	980	15
			Average	980	17
		p-cresotic acid			
x	0.15	0.36	3.6	950	17
o	0.76	0.28	3.9	945	18
			Average	948	18

Table 7 (Contd.)

Symbol	$[Fe^{II}]$ (mM)	$[Fe^{III}]$ (mM)	$[H^+]$ (mM)	K_1	K_2
		6-methylsalicylic acid			
x	0.76	0.28	5.4	394	4.6
o	0.76	0.28	3.9	373	4.2
□	0.15	0.36	4.1	377	4.7
			Average	381	4.6
		3-iso-propylsalicylic acid			
x	0.15	0.36	4.1	370	
o	0.76	0.28	3.9	340	
			Average	350	
		γ-resorcylic acid			
o	0.76	0.28	3.9	565	17
x	0.15	0.36	4.1	580	15
			Average	573	16
		β-resorcylic acid			
o	0.76	0.28	3.9	1360	57
x	0.15	0.36	4.1	1320	59
			Average	1340	58

Table 8

The equilibrium constants of complex formation of salicyluric acid with iron (III) ions in ionic strength 0.1M

Symbol	$[Fe^{II}]$ (mM)	$[Fe^{III}]$ (mM)	$[H^+]$ (mM)		K_1'	K_1	K_2
o	0.76	0.28	3.9		9	128	3.8
x	0.15	0.36	4.1		6	114	3.8
□	0.15	0.36	5.6		6		
				Average	7	121	3.8

Table 9

Summary of the results obtained at 25° and in an ionic strength of 0.1 M.

Acid	K_a(mM)	pK_a	$K_1'(mM^{-1})$	K_1	K_2
Salicylic acid	1.51	2.82		820	17
o-Cresotic acid	1.51	2.82		383	2.9
m-Cresotic acid	1.07	2.97		980	17
p-Cresotic acid	1.25	2.90		948	18
3-iso-Propylsalicylic acid	1.74	2.76		350	
6-Methylsalicylic acid	0.69	3.16		381	4.6
γ-Resorcylic acid	84	1.07		573	16
β-Resorcylic acid	0.79	3.10		1340	58
Salicyluric acid	0.389	3.41	7	121	3.8
2, 4-Dinitrophenol	0.132	3.88			

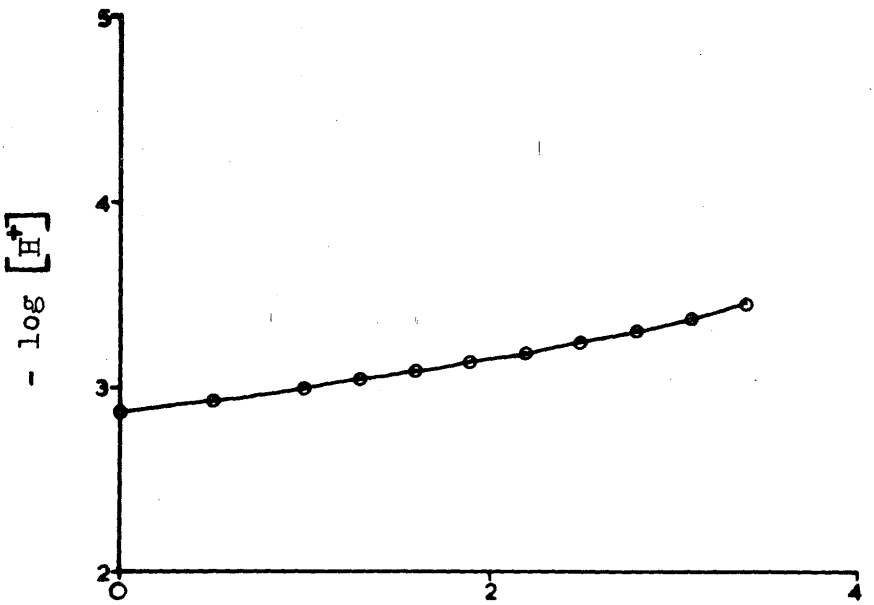


Fig. 7. Partial curve obtained in the titration of 20 ml 2.538 mM salicylic acid with 9.79 mM sodium hydroxide (Ionic strength 0.1).

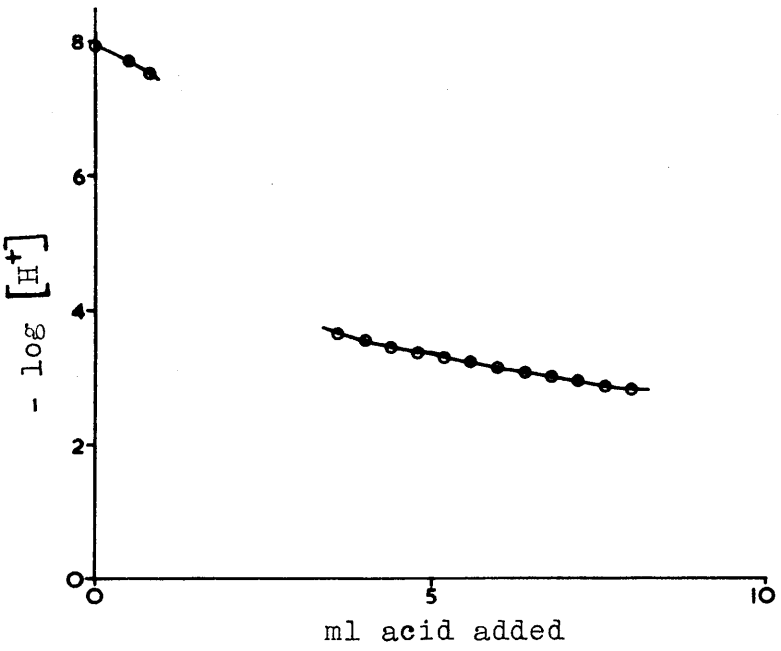


Fig.8. Partial curve obtained in the titration of 40 ml 16.47 mM β-resorcylic acid in 19.57 mM sodium hydroxide with 79.9 mM perchloric acid (Ionic strength 0.1).

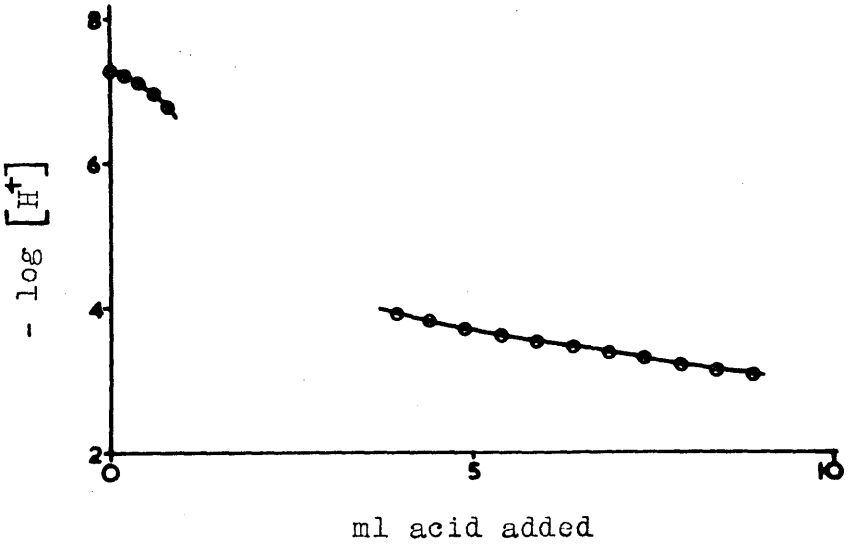


Fig. 9. Partial curve obtained in the titration of 40 ml 21.01 mM salicyluric acid in 23.48 mM sodium hydroxide with 79.9 mM perchloric acid (Ionic strength 0.1).

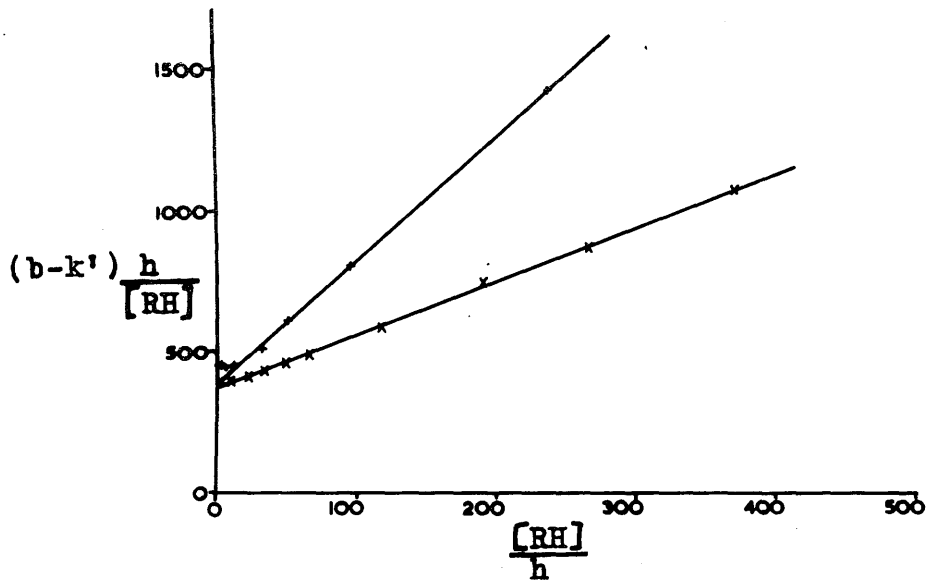


Fig. 10. o-Cresotic acid. Determination of K_1 and K_2.

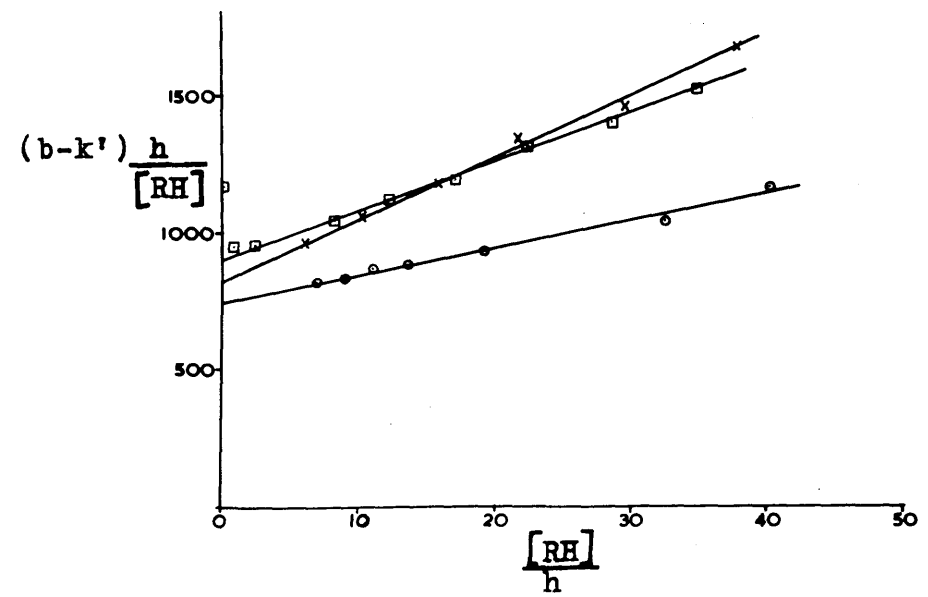


Fig. 11. Salicylic acid. Determination of K_1 and K_2.

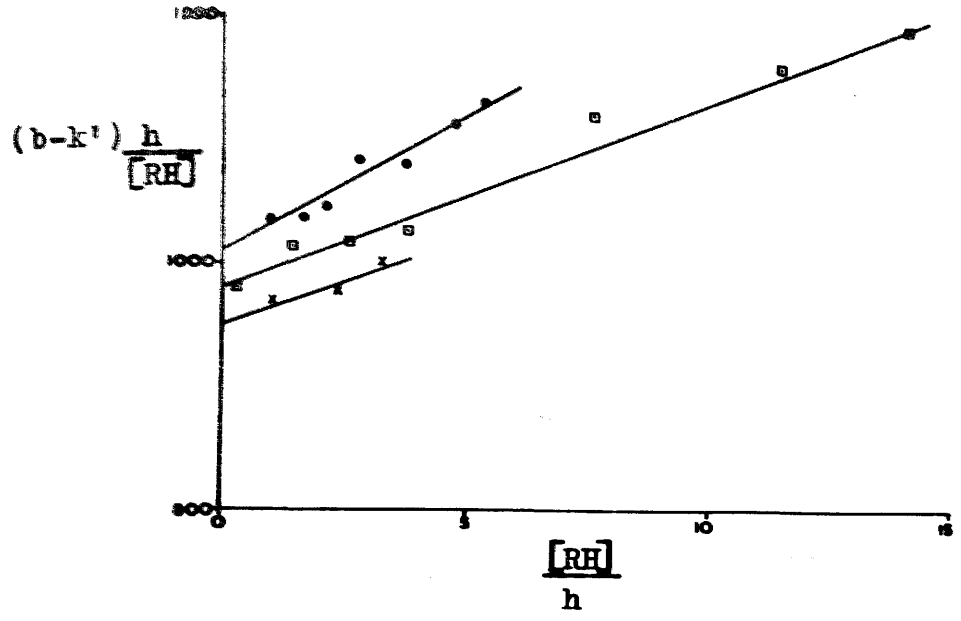


Fig. 12. m-Cresotic acid. Determination of K_1 and K_2.

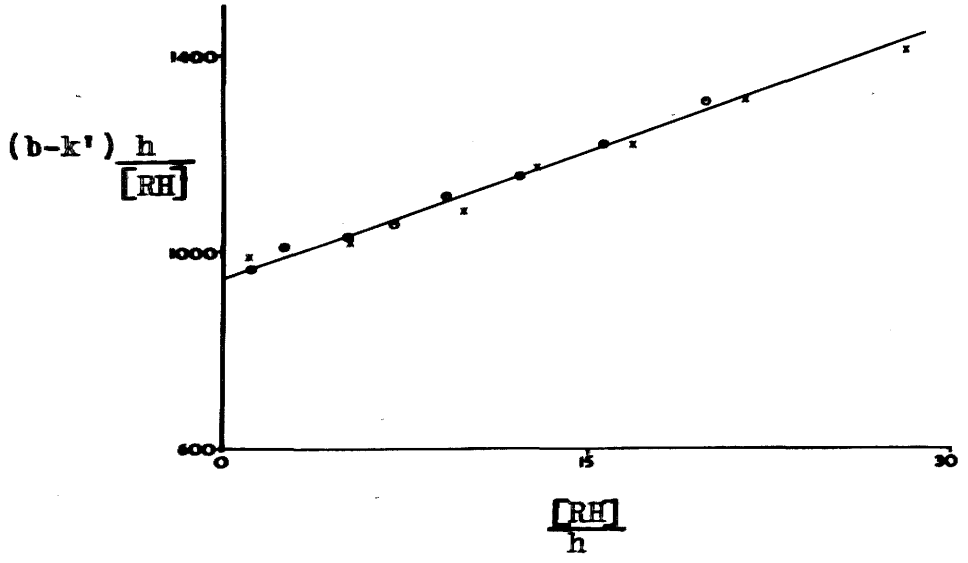


Fig. 13. p-Cresotic acid. Determination of K_1 and K_2.

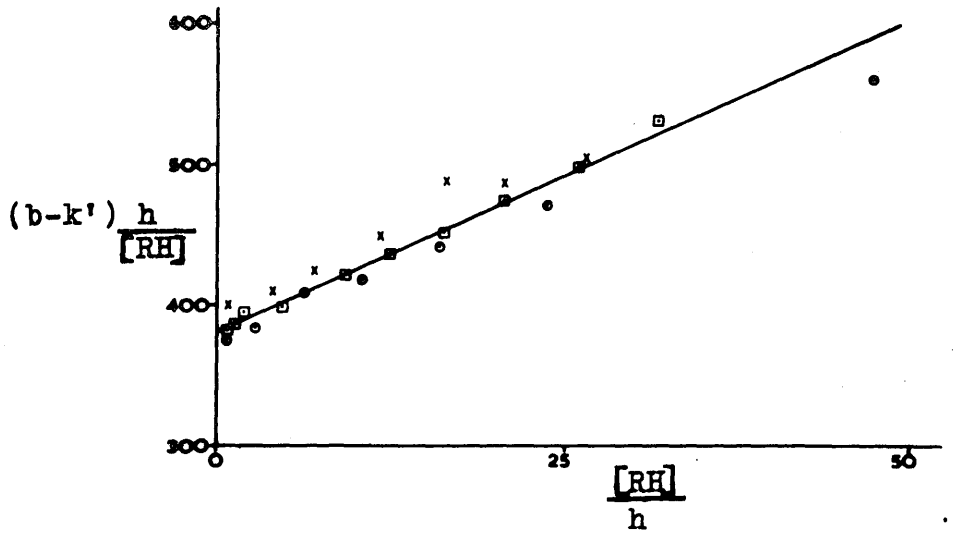


Fig. 14. 6-Methylsalicylic acid. Determination of K_1 and K_2.

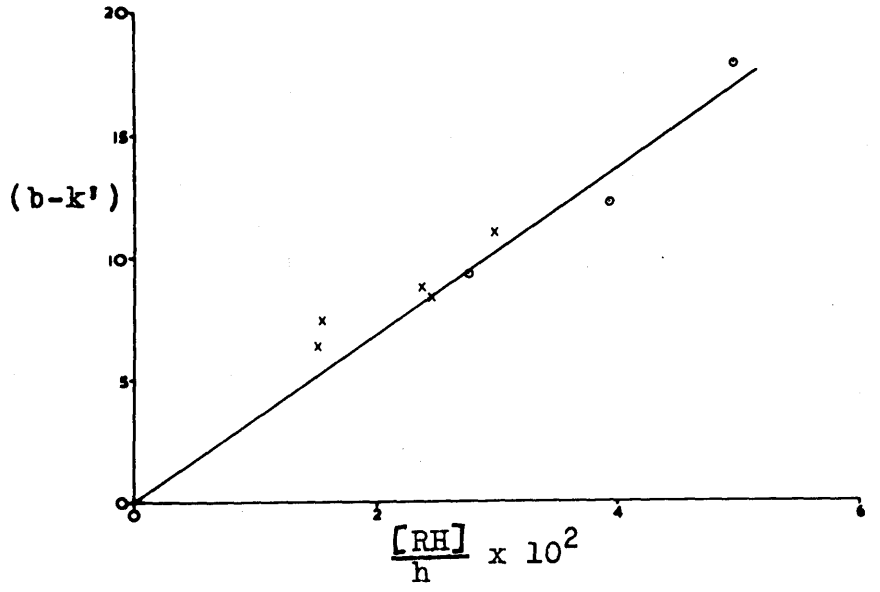


Fig. 15. 3-iso-Propylsalicylic acid. Determination of K_1.

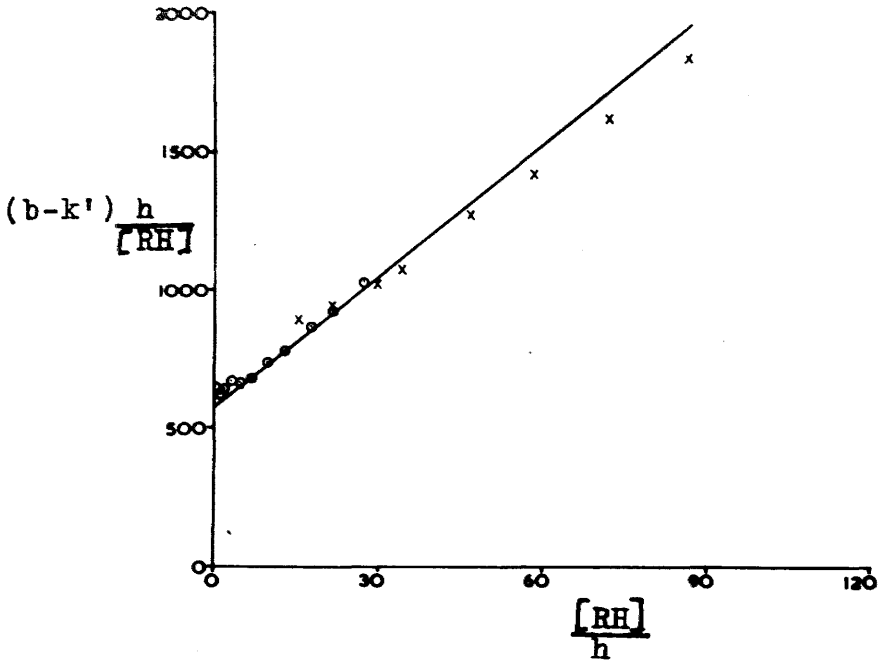


Fig. 16. γ-Resorcylic acid. Determination of K_1 and K_2.

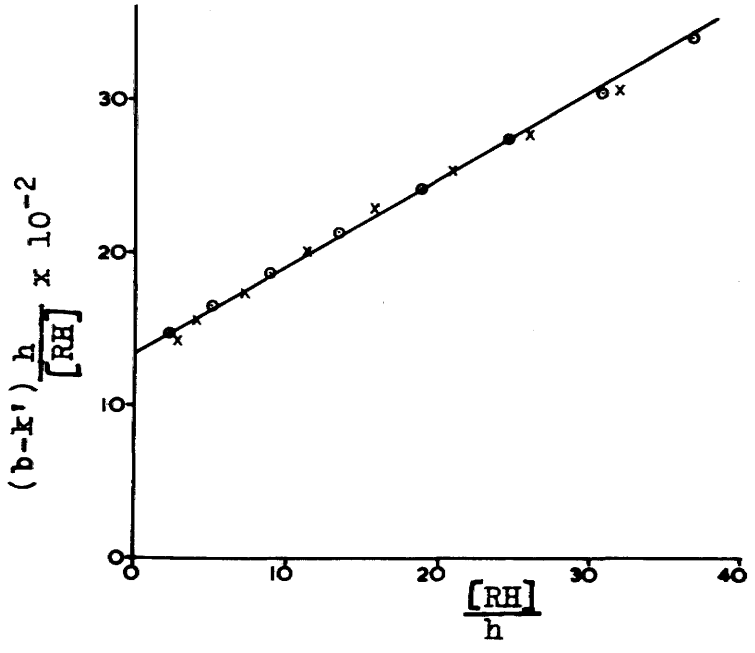


Fig. 17. β-Resorcylic acid. Determination of K_1 and K_2.

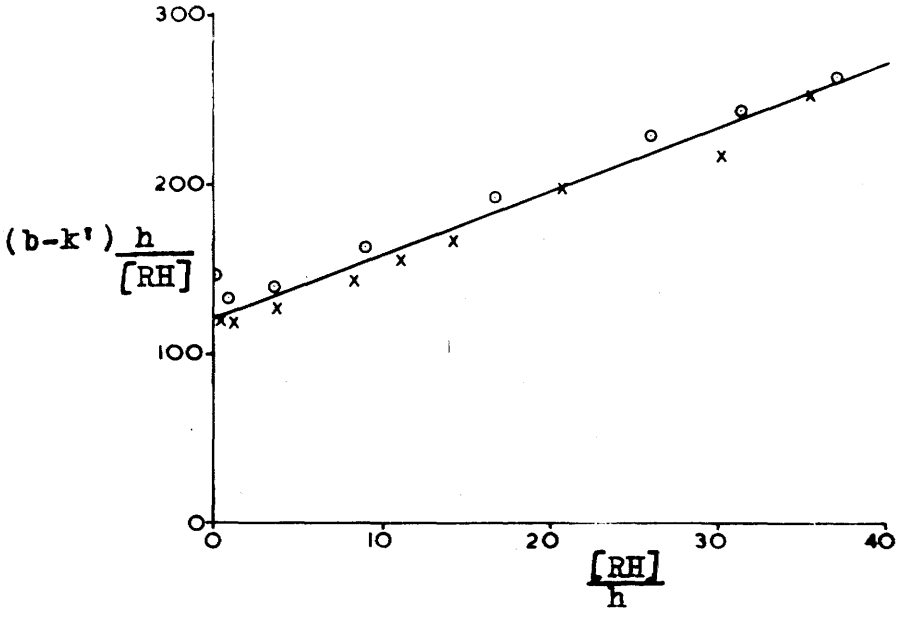


Fig. 18. Salicyluric acid. Determination of K_1 and K_2.

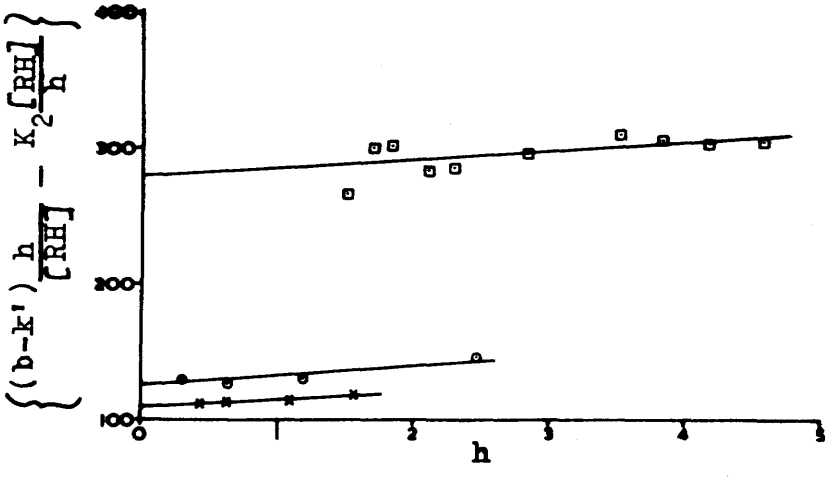


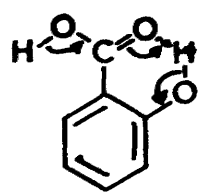
Fig. 19. Salicyluric acid. Determination of K_1'.

DISCUSSION.

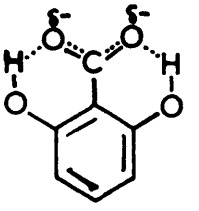
The ionisation constants of the acids studied.

The importance of structural and electronic effects on the dissociation constants of organic acids is by now well established. In that connection the values of the carboxylic dissociation constants of the acids studied relative to that of salicylic acid follow the expected pattern, though a few points of interest arise from a consideration of the 3- and 6- substituted salicylic acids.

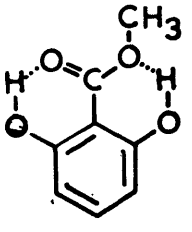
The methyl group in the position *para* to the carboxyl in *m*-cresotic acid increases the electron density in the carboxyl group, with a resultant stabilisation of the unionised form of the acid and decrease in its strength relative to salicylic acid. Electron repelling substituents in the 5-position would be expected to weaken the acid by opposing the ionisation of the proton by the mechanism indicated:



This effect is noted with 5-methylsalicylic acid (p-cresotic acid), and is even more marked where the substituent is a hydroxyl group, as in β-resorcylic acid. The abnormally high strength of γ-resorcylic acid is explained if it is assumed that the anion is stabilised by hydrogen bonding of both carboxyl oxygen atoms to the phenolic hydrogens[34], resulting in considerable resonance stabilisation:



It is perhaps of interest to note that the I.R. spectrum of methyl γ-resorcylate in carbon tetrachloride indicates the presence of two types of hydrogen bond, presumably those indicated below[77]:



The abnormal results in the cases of the 3- and 6- alkylsalicylic acids can only be

explained when steric considerations are taken into account. The 3-methyl substituent might have been expected to have a similar weakening effect on the acid strength to 5-methylsalicylic acid. In fact, it has the same dissociation constant as salicylic acid, and the bulkier 3-iso-propyl group in 3-iso-propylsalicylic acid results in an even stronger acid. It would appear that in the 3-methyl compound there are two opposing effects which balance each other out: the electronic effect of the methyl group ortho to the phenolic group tending to weaken the acid, and the same substituent's steric effect tending to strengthen the acid. In 3-iso-propylsalicylic acid the steric effect of the larger group is much greater than the opposed electronic effect, the nett result of these being a strengthening of the acid. Comparison of the 4- and 6-methylsalicylic acids indicates that electronic effects alone do not explain the stabilisation of the unionised acid in the 6-methyl-derivative: here, too, a steric effect would appear to be active, in this case causing a weakening of the acid.

It is at this point pertinent to consider investigations of the infra-red spectra of these

sterically hindered compounds. Hunsberger and co-workers[78] in a study of some compounds containing the salicylic acid grouping found that there was a decrease in the carbonyl frequency in those compounds in which a methylene or methyl group was adjacent to the carboxyl. Apparently hydrogen bonding is enhanced in these compounds, and the authors suggested that an explanation for the phenomenon was that the bulk of the adjacent methylene or methyl group pushed the carbonyl oxygen closer to the hydroxyl group than was the case in the other compounds. They used the term "steric facilitation of chelation" to describe this. Brooks, Eglinton and Morman[79] examined the infra-red spectra of a considerable number of substituted salicylic acids and found that alkyl substitution in the 3- and particularly in the 6-position led to lower carbonyl frequencies relative to that of salicylic acid itself, the displacement increasing with bulk of substituent (see Table 10).

Table 10.

pK_a and carbonyl stretching frequencies (cm^{-1}) (CCl_4) in some substituted salicylic acids.

Acid	pK_a *	ν_{CO}(monomer)[79]
Salicylic acid.	2.82	1698
4-Methylsalicylic acid.	2.97	1697
5-methylsalicylic acid.	2.90	1698
3-Methylsalicylic acid.	2.82	1695
3-iso-Propylsalicylic acid.	2.76	1693
3-tert-Butylsalicylic acid.	-	1691
3-Phenylsalicylic acid.	-	1695
6-Methylsalicylic acid.	3.16	1686
6-Ethylsalicylic acid.	-	1683

* this work

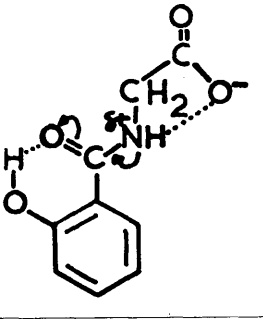
\- not measured

They ascribed the effect of the 3-alkyl groups to compression of the phenolic hydroxyl group with probable reduction in the O--H...O distance. This should cause strengthening of the hydrogen bond and resultant stabilisation of the anionic form of the acid. The observed pK_a's are in agreement with this.

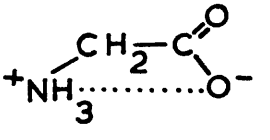
However, with 6-methylsalicylic acid, although the carbonyl frequency is markedly lower than with salicylic acid, the pK_a is greater. Thus, in this case we have the apparently anomalous result of enhanced hydrogen bonding, which would be expected to stabilise the anion, coupled with a weaker acid. Peltier[80] and Dippy and his collaborators[81] had previously measured the dissociation constant of 6-methylsalicylic acid, and had suggested that its abnormally low value resulted from inhibition of intramolecular hydrogen bonding through steric factors. However, it should be noted that the infra-red measurements were made on the unionised acid dissolved in carbon tetrachloride, whereas the dissociation constants were measured in aqueous solution, and Brooks and co-workers suggested that "in the solvated resonance stabilised anion small

deviations from coplanarity could lead to relative destabilisation with consequent weakening of the acid".

As might be expected, the pK_a of salicyluric acid, 3.41 in an ionic strength of 0.1M, is intermediate in magnitude between those of acetic acid (4.8)[82] and glycine (2.35)[82]. In solution the salicylurate ion will be stabilised to some extent by electrostatic attraction between the partial positive charge on the nitrogen atom and the negatively charged carboxylate group:



The effect of this stabilisation will not, of course, be nearly as great as that obtained in glycine, where the negative charge on the carboxylate group is stabilised by a whole positive charge on the nitrogen atom:



In these cases it is unlikely on steric grounds that there is any marked hydrogen bonding between the negatively charged oxygen atom and the positively charged NH_3 group, strong hydrogen bonds not being formed with completion of a five-membered ring[83].

The dissociation constant of hippuric acid (pK_a = 3.64)[82] is close to that for salicyluric acid found in this work (pK_a = 3.41), and correction of the latter to the thermodynamic value would bring it even closer to that for hippuric acid. Apparently, therefore, the presence of the phenolic group in salicyluric acid has little influence on the dissociation constant of the carboxylic group.

The high acidity of the last compound of this series, 2,4-dinitrophenol, does, of course, arise from the stabilisation of the phenolate ion by the electron-withdrawing nitro-groups in the 2- and 4- positions.

Comparison of the equilibrium constants of complex formation with those of other workers

As stated earlier, only the complexes of salicylic acid, β-resorcylic acid, and m-cresotic acid with iron(III) have been studied quantitatively before in aqueous solution, though the complexes of some of the other acids have been studied in alcohol-water mixtures[58,84].

In recent years a number of workers have investigated the equilibria in solutions of ferric ion and salicylic acid, mainly using photometric methods. All the papers listed below in Table 11, with the exception of Babko's, published in 1945, have been published since 1952. There have, of course, been a number of other workers who have investigated the system in a more qualitative way, but their results are not included.

It should be noted that the equilibrium constants calculated from Perrin's[59] work are in fact equivalent to the function

$$\frac{[FeR_n]\{H\}^n}{[Fe][RH]^n}$$

where n = 1 or 2, since his glass electrode was used to measure hydrogen ion activity rather than

Table 11

Previous investigations of equilibrium constants of complexes of iron (III) with salicylic acid in aqueous solution

Ref.	K_1	K_2	K_3	Method	Temp (^{o}C)	Ionic Strength
51	1000	11	2×10^{-7}	photometric	?	0.01
52	1120			photometric	15	3.6×10^{-3}
53	500	18	8×10^{-4}	photometric	25	3.0
53	470	15	1.5×10^{-4}	potentiometric	25	3.0
54	320	0.4	1.6×10^{-6}	photometric	18	0.25
59	550	11		potentiometric	20	0.15
56	1200	52	5×10^{-5}	photometric	20	0.25
57	1070	24		photometric	?	?
58	1100			photometric	?	0.1
This Work	820	17		potentiometric	25	0.1

Note:- Some of these values have been recalculated in terms of K_1, K_2 and K_3 from the author's data

concentration. Thus, to make his results strictly comparable with those of this work, an activity correction should be introduced. At an ionic strength of 0.15 M, the activity coefficient of the hydrogen ion is approximately 0.72, and hence more comparable values of K_1 and K_2 are 760 and 21 respectively.

On the whole, considering the wide range of conditions in which the measurements have been made, agreement between the different values of K_1 and K_2 is quite good.

The values obtained by previous workers of the constants for β-resorcylic acid and m-cresotic acid are given in Tables 12 and 13.

It is seen that the constants for β-resorcylic acid and m-cresotic acid recently reported by Tsin Jao, Sommer and Okáč[58] are in particularly good agreement with those obtained in this work. These workers investigated a number of substituted salicylic acids, including salicylic acid, β-resorcylic acid, γ-resorcylic acid and m-cresotic acid, as regards their complexing power with iron(III), but unfortunately most of their measurements were made in aqueous ethanol and cannot be directly compared with results in aqueous solution.

Bobtelsky and Kertes[84] examined the

Table 12

Previous investigations of equilibrium constants of complexes of iron (III) with β-resorcylic acid in aqueous solution

Ref.	K_1	K_2	K_3	Method	Temp (°C)	Ionic Strength
62	720	13	3×10^{-4}	photometric	?	?
58	1550	41		photometric	?	0.1
This Work	1340	58		potentiometric	25	0.1

Table 13

Previous investigations of equilibrium constants of complexes of iron (III) with m-cresotic acid in aqueous solution

Ref.	K_1	K_2	Method	Temp (°C)	Ionic Strength
58	1050	-	photometric	?	0.1
This Work	980	17	potentiometric	25	0.1

complexes of 2,4-dinitrophenol with iron(III) in aqueous alcohol. Two complexes, FeR and FeR_3, were detected. However, even in alcoholic solution their stabilities were not great.

The relative stabilities of the complexes of the substituted salicylic acids.

Many authors have attempted to correlate the proton dissociation constants of ligands with the dissociation constant of the corresponding metal-ligand complex. In 1934 Larsson[85] showed that there was a linear relation between the logarithm of the stability constant of some silver(I)-diammines (in aqueous ethanol) and the pK_{NH} of the ammine ligand (in water). Following Calvin and Wilson's paper[86] in 1945, a number of workers[87] have studied this relationship , and the conclusion can be drawn that only an approximate linear relation holds, and that only between structurally similar ligands forming structurally similar metal ion complexes.

Ågren[88] showed that a plot of pK_{a2} against pk_n, where

$$k_n = \frac{[Fe]\,[R]^n}{[FeR_n]}, \quad n = 1 \text{ or } 2,$$

for the compounds salicylic acid, 5-sulphosalicylic acid, and p-aminosalicylic acid (4-aminosalicylic acid), resulted in straight lines of gradient 1 and 0.5 respectively with equation

$$n.pK_{a2} = pk_n + A'$$

where K_{a2} is the phenolic dissociation constant and is defined by

$$K_{a2} = \frac{[R][H]}{[RH]} \qquad (RH \equiv \text{salicylate ion})$$

and A' a constant.

$$\text{Hence } K_{a2}^{n} = k_n \times \text{constant}$$

$$\text{or constant} = \frac{K_{a2}^{n}}{k_n} = K_n$$

Thus he concluded that substances falling on the same straight line have about the same value of K_n and the same standard free energy change,

$$"-\Delta G^{o}" = RT \ln K_n$$

for the reaction.

$$Fe + nRH \rightleftharpoons nH + FeR_n$$

It would be expected, therefore, that all the compounds studied in this work, with the exception of 2,4-dinitrophenol and salicyluric acid, would possess a similar relationship between the phenolic dissociation constant and the dissociation constant of the complex, i.e. equal values of K_n. However, although most of the values of K_1 and K_2 are of a similar order of magnitude, there are differences which are greater than experimental error. These differences between the values of ~~K_1 and K_2~~ corresponding constants for salicylic acid and those for the

substituted salicylic acids can be explained in terms of resonance or steric effects.

Duncan[89,91] derived a thermodynamic relation

$$\log K_s = pK_a - \frac{\overline{G_{ML}}^o - \overline{G_{HL}}^o}{2.303RT} + B$$

where K_s is the stability constant of a series of 1:1 complex ions, ML, derived from one metal, M, with a set of similar ligands, L, and pK_a is the negative logarithm of the dissociation constant of HL. $\overline{G_{ML}}^o$ and $\overline{G_{HL}}^o$ are the partial molar free energies of the species ML and HL respectively, and B is a constant independent of L.

This can be rearranged to

$$\log (K_s.K_a) = \frac{\overline{G_{HL}}^o - \overline{G_{ML}}^o}{2.303RT} + B$$

i.e.
$$\log K_1 = \frac{\overline{G_{HL}}^o - \overline{G_{ML}}^o}{2.303RT} + B \quad \text{if}$$

$$K_s.K_a = K_1$$

This last assumption is not, of course, strictly correct, since K_1 is not a thermodynamic constant. However, this does not affect the argument which follows.

In the event of the dependence of $\overline{G_{HL}}^o$ upon the ligand being similar to that of $\overline{G_{ML}}^o$, log K_1

will be constant. However, should the metal ion complex be stabilised to a greater extent than the proton-ligand complex, $\overline{G_{ML}}^{o}$ will be lowered relative to $\overline{G_{HL}}^{o}$ and the value of log K_1 will be greater. Similarly the converse will be true.

In a similar way it can be shown that for the reaction

$$Fe^{3+} + nRH^{-} \rightleftharpoons FeR_n^{(3-2n)} + nH^{+},$$

the general equation is

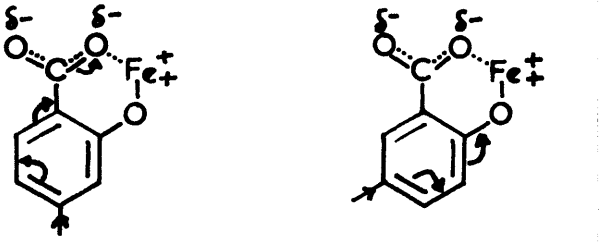
$$\log K_n = \frac{n\overline{G_{RH}}^{o} - \overline{G_{FeR_n}}^{o}}{2.303RT} + B_n ,$$

and the same arguments apply.

From a study of the absorption spectra of complex ions Williams[90] concluded that many ferric complexes are associated with partial charge transfer from the ligand to the cation. In a later paper[91] he and his co-workers suggested that the enhancement or depression of the stability of ferric complexes relative to the stability of the corresponding proton-ligand complex can be correlated with the increase or decrease of π bond acceptance by the ferric ion from the ligand. Thus substituents increasing the π electron density on the ferric ion would be expected to increase the stability of the cation-ligand complex to a greater extent than

that of the proton-ligand complex, resulting in an increase in the value of K_n, whereas those withdrawing π electrons from the ferric ion would be expected to have the opposite effect.

This effect is illustrated well in the case of those salicylic acids possessing substituents in the 4- and 5- positions. Both m- amd p-cresotic acid have values of K_1 significantly higher than that of salicylic acid. It will be noted that the methyl substituents possess +I inductive effects, and so will indirectly increase the π electron density in the chelated ring:



Where the substituent possesses a +M mesomeric effect, as does the hydroxyl group in β-resorcylic acid, the π electron density in the chelated ring is increased even more, with corresponding marked increases in the values of K_1 and even in K_2 for that acid.

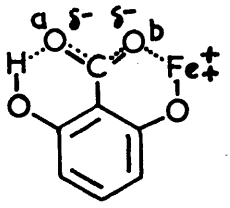
A different explanation must be found for the substantial reduction in K_1 and K_2 for the acids containing alkyl substituents in the 3- and 6- positions. This type of phenomenon, namely, that in which a bulky substituent adjacent to a chelating group reduces the stability of a metal complex, has been noted by a number of workers. Irving, Cabell and Mellor[92] found that although 2-methyl-1, 10-phenanthroline was a stronger base than 1,10-phenanthroline, it formed weaker complexes with ferrous ions, while the complexing ability of 2,9-dimethyl-1,10- phenanthroline with ferrous ion was negligible. This result they ascribed to steric hindrance to coordination. Luz, Fallab, and Erlenmayer[93] investigated the complex formation of cupric and nickelous ions with α-pyridinyl carbinols. Whereas pyridyl-2-carbinol was almost as strong a complexing agent as oxine, introduction of a methyl group in the α' position heavily decreased complex formation. Similar reductions in stability have been noted in 2-substituted 8-hydroxyquinolinates, these also being ascribed to steric hindrance[94].

This argument can be used to explain the relative decrease of K_1 and K_2 in the 3- and 6-alkylsalicylic acids. It was earlier stated that

evidence from infra-red and dissociation constant measurements indicated that it was possible that 3-alkyl groups caused reduction in the O-H....O distance. While this resulted in greater stabilisation of the hydrogen bonded 3-alkylsalicylate ion, it is very probable that this reduction in the O....O distance would destabilise the complex with the much bulkier ferric ion (ionic radius 0.6 Å; cf. normal covalent radius of hydrogen ion: 0.3 Å [83]) through, for example, distortion of valency angles. On the evidence available, it is likely that the relative lowering of K_1 and K_2 in 6-methyl-salicylic acid results from inhibition of mesomerism through distortion of the chelate ring, this distortion being caused by the interaction of the methyl group with the bulky carboxyl group. This conclusion would be in agreement with the view that the higher pK_a of 6-methylsalicylic acid relative to that of salicylic acid itself arises from distortion of the chelate ring in the molecule.

In γ-resorcylic acid an alternative explanation of the low values of K_1 and K_2 is possible, apart from the phenomenon of steric hindrance. The effect of the 6-hydroxyl group will be to lower the π electron density on the oxygen atom b through

hydrogen bonding of oxygen atom a with the phenolic group:



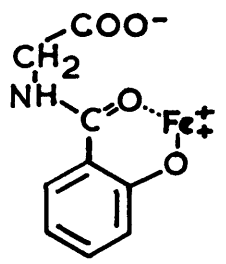
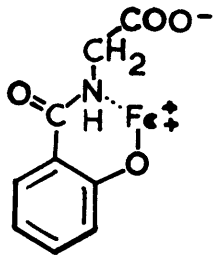
This will discourage charge transfer from the ligand to the cation, so destabilising it relative to the salicylate complex.

All the remarks so far have applied only to the 1:1 and 1:2 complexes. The accuracy of measurement of K_3 was not sufficiently great to draw conclusions regarding the influence of substituents.

The complexes of salicyluric acid with ferric ion.

Salicyluric acid forms a similar series of complexes with ferric ion to salicylic acid. However, in view of its somewhat different structure it is here considered separately from the other, simpler, derivatives of salicylic acid.

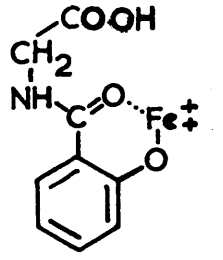
The 1:1 complex of salicyluric acid with ferric ion, FeR, poses an interesting problem as regards its structure. Two ~~probable~~ structures are possible, depending on whether the ferric ion is bonded to the carbonyl and phenolic groups, or the amide nitrogen and phenolic groups,

i.e.  or 

Ågren, in his study of salicylamide, concluded that the bonding to the ferric ion was most probably through the carbonyl and phenolic groups in view of the similarity of the wavelengths of maximum absorption of the complexes of salicylamide and salicylic acid with iron(III)[88]. If this argument can be accepted, it can be concluded that the metal ion is bonded as in the first structure above, since the wavelength of maximum absorption

of the 1:1 complex of salicylic acid is 525 mμ while that for the corresponding complex of salicyluric acid is 515 mμ. This is also the more likely structure on electronic considerations, since the presence of the carbonyl group adjacent to the amido-nitrogen would result in a partial positive charge on the nitrogen atom, so discouraging bonding by donation of electrons from it to the iron atom.

The most probable structure of the protonated complex would be the following:



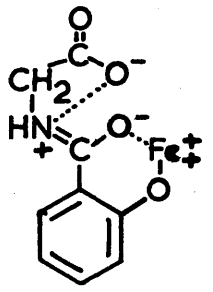
It is of interest to note that the values of K_a and K_1' indicate that the dissociation constant of the carboxylic group in the 1:1 complex, i.e. the equilibrium constant of the reaction,

$$FeRH^{2+} \rightleftharpoons FeR^{+} + H^{+},$$

is abnormally high ($K_a = K_1/K_1' = 17$ mM; cf. K_a for salicyluric acid, 0.389 mM). This could indicate

an interaction between the carboxyl group and the complexed ferric ion. The proximity of the positively charged iron atom to the carboxyl group would be expected to stabilise the carboxylate ion. Models of the molecule indicate that the carboxylate group can be brought close enough to the ferric atom for there to be a strong electrostatic interaction at least.

An alternative explanation is that the resonance form



tends to stabilise the carboxylate group by analogy with glycine, However, if this were the case, since the amido-nitrogen bears only a partial positive charge, the strengthening of the acidity of the carboxyl group would be expected to be considerably less than in glycine. In fact, the species $FeRH^{++}$ is a stronger acid than glycine (K_a for $FeRH^{++}$ = 17 mM; K_{al} for glycine = ca. 5 mM).

Thus it would appear likely that the relative stabilisation of the carboxylate ion in the complex $FeRH^{++}$ arises through electrostatic interaction of the negatively charged carboxylate group with the positively charged iron atom, and may even involve some degree of covalent bonding between the two.

Comparison of biological and physical data

Although the physical properties studied have been explained qualitatively at least, no such outcome can be claimed for the comparison of biological with physical properties.

All the salicylic acid derivatives examined have been quantitatively investigated with regard to their metabolic stimulant properties by Andrews[26,95]. She obtained values of the molar potency ratios of the different compounds relative to salicylic acid from the gradient of the dose-response curve. These are shown in Table 18.

There would appear to have been little quantitative comparative work done on the anti-diabetic and anti-rheumatic properties of the salicylates: hence the paucity of information in the Table.

For comparison, results are also included in Table 18 of the proportion of salicylic acid, 3-methylsalicylic acid, and 3-iso-propylsalicylic acid bound to plasma protein at pH 7.4 with a total substituted salicylate concentration of 2 mM[96] (the therapeutically effective level); also the shift in carbonyl frequency of the simple molecule on substituting different alkyl groups in the salicylic

Table 14

Comparison of some physical and biological properties of salicylic acid and its derivatives

Acid	pK_a	K_1	M.S.	A-D.A.	A-R.A.	%P.B.	$\Delta\nu_{CO}$ (cm^{-1})
salicylic acid	2.82	820	1	+	+	70	0
o-cresotic acid	2.82	384	2.6	+	+	82	3
m-cresotic acid	2.97	980	1.8	*	*	*	1
p-cresotic acid	2.90	948	1.9	*	*	*	0
3-iso-propylsalicylic acid	2.76	350	6.5	*	*	85	5
6-methylsalicylic acid	3.16	381	no effect	*	*	*	12
γ-resorcylic acid	1.07	573	depressive	*	+	*	*
β-resorcylic acid	3.10	1340	depressive	*	*	*	*
salicyluric acid	3.41	121	depressive	*	*	*	*
2,4-dinitrophenol	3.87	-	stimulant	+	*	*	-

Abbreviations

M.S. metabolic stimulation: relative molar potency[26,95].

A-D.A. anti-diabetic activity[17,19,32].

%P.B. % bound to protein[96].

* not known.

\+ of therapeutic value.

acid nucleus[79]. This is denoted by $\Delta\nu_{CO}$ cm^{-1} and is equivalent to $[\nu_{CO}(\text{salicylic acid}) - \nu_{CO}(\text{substituted salicylic acid})]$.

On examining Table 18 it is seen that there is little correlation between pK_a and K_1 on the one hand, and the metabolic stimulant property. Within the series of 3-alkylsalicylic acids, it should be noted, however, that with increasing bulk of the 3-substituent, the acidity, degree of protein binding, metabolic stimulant activity, and carbonyl frequency shift all increase, whereas the value of K_1 decreases. Apparently the metabolic stimulant activity increases with increasing bulk of the substituent in the 3-position as does the degree of protein binding. The possibility of a correlation between biological activity and protein binding will be considered later. The values of pK_a, K_1, and $\Delta\nu_{CO}$ are of course also affected by the size of the 3-substituent, as discussed earlier.

This investigation was originally started to determine whether there was any simple correlation between the complexing power of salicylates towards ferric ions and their biological effects. In the light of the above results, this correlation would appear to be lacking, at least as far as its metabolic

stimulant property is concerned.

However, this fact does not necessarily mean that salicylate does not possess its biological activity by virtue of its ability to complex metal ions. This work was carried out using only one metal ion, viz., the ferric ion. It is possible, though unlikely, that using a different metal ion the stabilities of the different complexes might be correlated with the metabolic stimulant action of the different acids. Also, in the event of the salicylate molecule being biologically active through forming a protein-metal ion-salicylate complex, the relative stabilities of the metal ion-salicylate complexes may not be similar to those in which a protein molecule is also involved. The evidence reported here is therefore negative, and certainly lends no support to the idea of the salicylic acid molecule acting on biological systems through the formation of a metal complex.

Conclusion

As stated earlier, there would appear to be no simple relation between the complexing ability of the substituted salicylates towards ferric ions and their metabolic stimulant property. One is then still left with the unsolved problem of the mode of action of salicylate. A compound possessing the wide range of pharmacological actions of salicylate might either act in a large number of different ways, or possess one main property from which all the other actions stemmed.

The paper of Christensen[43] has already been mentioned. This indicated one mechanism by which a compound such as salicylic acid might produce a number of reactions through one main action. It would appear to be the first that suggested the biological significance of the binding of salicylate to protein. In this connection, it is of interest to note that the order of strength of binding to plasma protein is 3-*tert*-butylsalicylic acid > 3-*iso*-propylsalicylic acid > 3-methylsalicylic acid > salicylic acid[96], which is the same order as the molar potency ratios with respect to metabolic stimulation[26,95].

Even if valid, this explanation may account for only part of the action of salicylate, and the complete explanation is probably much more complex, and not reducible to one relatively simple main action.

Although it has not been possible to correlate the biological properties of the compounds in this work with the physical properties studied, in the course of the investigation the author was struck by the structural similarity between 2,4-dinitrophenol and salicylic acid on the one hand, and β-keto-acids important in lipid metabolism, on the other. Examination of the literature showed that there was some evidence for the interference of 2,4-dinitrophenol in lipid metabolism.

One action which has been reported for 2,4-dinitrophenol, though not for salicylic acid, is its ability to inhibit fatty acid metabolism[97-99] and also fatty acid biosynthesis[100]. Since 2,4-dinitrophenol interferes with carbohydrate metabolism through the uncoupling of oxidative phosphorylation, it was initially thought that its effect on lipid metabolism resulted from this property, which would reduce the concentration of

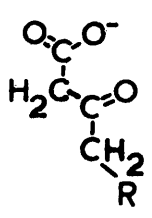
adenosine triphosphate (ATP) available. However, Judah[101] reported that added ATP did not reverse the inhibition of octanoate oxidation by 2,4-dinitrophenol. Witter, Newcomb and Stotz[102], studying preparations of liver particles, showed that the inhibition by 2,4-dinitrophenol of the oxidation of a model substrate, sorbic acid, was not overcome by maintenance of the level of ATP with the phosphocreatinine transphorylase of rabbitmuscle, or by the simultaneous addition of α-ketoglutarate, malate, heat stable factor, or Co-enzyme I, but was released by washing the enzyme free from inhibitor. These results they took to indicate that the action of 2,4-dinitrophenol on fatty acid metabolism did not arise indirectly through its interference with the Krebs Cycle, and that the presence of the phenol did not cause the destruction of a factor or enzyme necessary for the oxidation of fatty acids. Since they found that 2,4-dinitrophenol inhibited the formation of acetoacetate from sorbate but not from pyruvate, they concluded that the dinitrophenol might inhibit the oxidation of fatty acids at some stage prior to the formation of acetoacetate.

A possible mechanism for this is as follows. It is accepted that long chain fatty acids

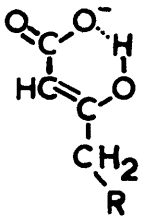
are degraded by a process of β-oxidation, the following scheme applying, where R is part of the fatty acid carbon chain.

$$R\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}COOH$$
$$\downarrow$$
$$R\text{-}CH_2\text{-}CH{=}CH_2\text{-}COOH$$
$$\downarrow$$
$$R\text{-}CH_2\text{-}CHOH\text{-}CH_2\text{-}COOH$$
$$\downarrow$$
$$R\text{-}CH_2\text{-}CO\text{-}CH_2\text{-}COOH$$
$$\downarrow$$
$$R\text{-}CH_2\text{-}COOH \; + \; CH_3COOH$$

At physiological pH the intermediate, $R.CH_2.CO.CH_2.COOH$, will be to a large extent ionised, and will consist of two forms in tautomeric equilibrium:



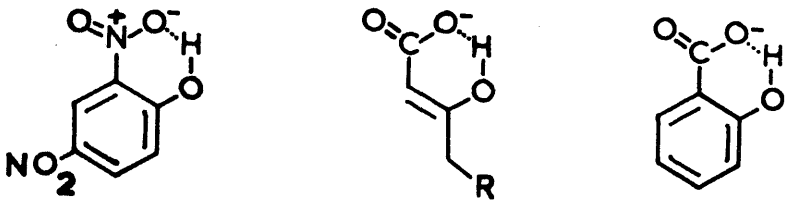
keto



enol

A significant part of the acid will be present in the enolic form, which will, of course, be stabilised by hydrogen bonding. Comparison of the structure of the enolic form of the acid with that of 2,4-dinitrophenol and of the salicylate ion indicates

that there are striking formal similarities in the structures of the three compounds:



It is possible that the 2,4-dinitrophenol may inhibit the stage in fatty acid metabolism involving hydrolysis of the keto-acid by a process of competitive inhibition. If the reactive species were the enolic form shown above, it should be possible for the dinitrophenol molecule to be adsorbed on to the enzyme surface but not to be metabolized, since the energy required to hydrolyse a double bond in a benzene nucleus is very much greater than that required for the hydrolysis of the double bond of the enolic acid.

It is noteworthy that Popják and Tietz[100] obtained evidence for the inhibition by 2,4-dinitrophenol of fatty acid biosynthesis (which is believed to occur by a mechanism essentially the reverse of β-oxidation) but no work seems to have been carried out to determine whether in this case the action is direct or mediated through the disruption of carbohydrate metabolism.

It would be of considerable interest to find out whether salicylic acid does have a similar action to 2,4-dinitrophenol in fatty acid metabolism. If so, it would indicate that the influence of salicylate on lipid metabolism might be a fruitful field of study.

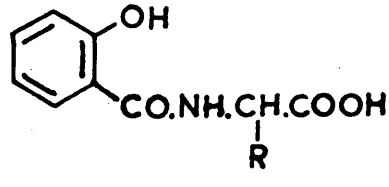
In conclusion, this work has not been successful in correlating biological effects of salicylates with their complexing power. It is perhaps of consolation to note that in few cases has any major correlation between the physical properties and biological actions of drugs been shown. Further work in elucidating the mechanism or mechanisms of action of salicylate at cellular level would, however, in all probability, lead to a greater understanding of the processes of metabolism in general, quite apart from any benefit it might have in indicating the types of derivative of salicylate which would be most suitable for clinical applications.

APPENDIX

In the early stages of the work described in this thesis, it was thought possible that the biological activity of the salicylates might arise from their ability to form complexes with metal ions.

In an attempt to stabilise these complexes of salicylic acid at higher pH's, e.g. physiological pH, it was thought of interest to synthesise a number of amino-acid conjugates of salicylic acid, and to examine whether the extra chelating group(s) introduced into the molecule resulted in stabilisation, or otherwise, of the complexes with ferric ion.

These conjugates had the general formula



where $R.CH(NH_2).COOH$ represents the structure of an amino-acid.

Following the general method of Fischer[72], the conjugates salicyloyl-α-alanine (m.p.150-151°) and salicyloyl-aspartic acid (m.p.173-174°) were readily synthesised, and were shown to be chromatographically

pure (benzene-propionic acid-water; 2:2:1) and to give satisfactory analyses. However, using the same methods, it was not found possible to isolate crystalline samples of either salicyloyl-histidine or salicyloyl-asparagine, the final products being intractable gums, even though a number of purification techniques, including chromatography, were used.

The two compounds obtained pure, along with samples of salicyloyl-β-alanine and salicyloyl-glutamic acid, supplied by Dr.C.J.W.Brooks, were all found to form violet complexes with ferric ions, similar to salicylic acid. Examination of these complexes spectrophotometrically by Job's method of Continuous Variations indicated that these four conjugates formed 1:1 metal ion : ligand complexes, in an analogous manner to salicylic acid.

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