was obtained. This is 62% of the N-ethyl piperidinium benzylpenicillinate known to be present in the original material.

Decomposition of Benzylpenicillinic Acid Diisopropyl Etherate in Chloroform.—One gram of the crystalline acid was dissolved in 100 cc. of chloroform and allowed to stand about forty-three hours at 23°, during which time an amorphous precipitate formed. After filtering and washing with chloroform 270 mg. of dry solid was thus obtained. After crystallization from ethanol (in which the solid was initially easily soluble until crystallization took place) an examination of its ultraviolet and infrared absorption spectra gave ample evidence of its identity as benzylpenillic acid, the spectra of our isolation product being everywhere superposable upon those given by an authentic sample of benzylpenillic acid.

Summary

1. A crystalline form of benzylpenicillinic acid has been described.

2. Possible uses as a means of isolating benzylpenicillin from the other known penicillin homologs and as a primary standard of purity for benzylpenicillin have been indicated.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, NEW YORK STATE COLLEGE FOR TEACHERS]

A Spectrophotometric Study of the Reaction of Ferric Iron and Citric Acid

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Although it is well known that citric acid and ferric iron form a soluble complex and this fact is sometimes used in analytical separations,[1] a search of the literature indicates no systematic study of this reaction has been made. Although Bobtelsky and Semichen[2] have concluded that the reaction of solutions of ferric chloride and trisodium citrate produces two complexes of the types $\mathrm{Fe_2(citr)_3{}^{-3}}$ and $\mathrm{Fe_3(citr)_2{}^{+3}}$, the validity of the conclusion may be questioned inasmuch as their solutions contained chloride which also forms a complex with ferric ion.[3,4]

More recently Bobtelsky and Jordan[5] have claimed that citric acid and ferric iron form a complex in which the ratio of citric acid to iron is 2:3. Their statement is based on "conductimetric, photometric and thermometric" titrations. The end-point of these titrations is located graphically and appears to involve considerable uncertainty. These authors give no quantitative data except in graph form. However, even if the method employed by these authors is validated, their conclusions in this particular case still are subject to question since they used ferric sulfate as a source of ferric iron for the titration in question. We have established, in work to be described in a later paper, that ferric iron and sulfate ion themselves form a complex. Moreover, the solution in question undoubtedly contained appreciable quantities of the primary hydrolysis product of ferric ion, namely, $\mathrm{FeOH^{++}}$, since it has been shown[6,7] that even in solutions of high acidity considerable quantities of this ion are present. It would appear that the solutions employed by Bobtelsky and Jordan in this connection contained either mixtures of iron complexes or mixed iron complex or both. For these reasons one is inclined to question the validity of their conclusions.

Accordingly, the present investigation was undertaken, which established not only the composition of the complex but its dissociation constant as well.

By the method used it was shown that the reaction was

$$\mathrm{Fe^{+3} + H\,citr^{-2} \rightleftarrows Fe(H\,citr)^{+}} \qquad (1)$$

There was no evidence to indicate that there are any higher complexes formed between the reactants.

The ratio of iron to citrate in the complex was established by the method of continuous variations proposed by Job[8] and elaborated by Vosburgh and Cooper.[9]

Solutions of ferric nitrate and citric acid were mixed in various ratios so that the total moles of iron plus citric acid was constant throughout the series, as was also the volume of the solutions. The extinction coefficient of each solution was measured at various wave lengths between 325 and 485 mμ. The difference between the observed extinction coefficient and that calculated on the assumption of no reaction between iron and citric acid was plotted against that ratio M_{Fe} (M_{Fe} plus $M_{\text{citric acid}}$). The resulting curves show sharp maxima at $R = 0.5$ for all wave lengths employed. This indicates that the only complex formed in this solution is that in which the ratio of iron to citrate is unity.

The ionic state of the citrate which forms the complex was established by noting the effect of changes in concentration of hydrogen ion on the stability of the complex. From the above the indicated reaction is

$$\mathrm{Fe^{+3} + H_3citr \rightleftarrows Fe(H_xcitr)^{+x}} + (3 - x)\mathrm{H^+} \qquad (2)$$

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(1) H. A. Fales and F. Kenny, "Inorganic Quantitative Analysis," The Century Company, New York, N. Y., 1939, p. 345.

(2) M. M. Bobtelsky and A. E. Semichen, *Compt. rend.*, **208**, 646 (1939).

(3) H. E. Bent and C. L. French, THIS JOURNAL, **63**, 568 (1941).

(4) E. Rabinowitch and W. H. Stockmayer, *ibid.*, **64**, 335 (1942).

(5) M. Bobtelsky and J. Jordan, *ibid.*, **69**, 2286 (1947).

(6) A. B. Lamb and A. G. Jacques, THIS JOURNAL, **60**, 967, 1215 (1938).

(7) W. C. Bray and A. V. Hershey, *ibid.*, **56**, 1889 (1934).

(8) P. Job, *Ann. chem.*, [10] **9**, 113 (1928).

(9) W. C. Vosburgh and G. R. Cooper, THIS JOURNAL, **63**, 437 (1941)

TABLE I

DETERMINATION OF THE RATIO OF IRON TO CITRATE IN THE COMPLEX

Total Iron plus Citric Acid = 2×10^{-3} m./l. in each solution

$R = M_{\text{Fe}}/(M_{\text{Fe}} + M_{\text{citric acid}})$	ϵ,[a] mμ 325	345	365	385	405	425	445	465
0.2	0.425	0.448	0.403	0.322	0.218	0.128	0.065	0.030
.4	0.920	0.850	.770	.620	.425	.247	.125	.057
.5	1.030	1.035	.910	.720	.490	.285	.146	.067
.6	0.919	0.900	.760	.540	.328	.204	.098	.043
.8	.725	.608	.460	.272	.137	.068	.037	.014
1×10^{-3} m./l. citric acid—no $Fe(NO_3)_3$	.180	.012	.007	.006	.000	.000	.000	.000
1×10^{-3} m./l. $Fe(NO_3)_3$ in 0.1 M HNO_3[b] —no citric acid	.221	.033	.014	.008	.012	.012	.012	.012

[a] $\epsilon = \log I_0/I/S$ where S = cell thickness in cm. [b] Nitric acid is used to reduce hydrolysis and hence the absorption due to $FeOH^{++}$.

TABLE II

DETERMINATION OF THE RATIO OF IRON TO CITRATE IN THE COMPLEX

Total Iron plus Citric Acid = 2×10^{-2} m./l. in each solution

$R = M_{\text{Fe}}/(M_{\text{Fe}} + M_{\text{citric acid}})$	ϵ, mμ 445	465	485
0.2	0.670	0.307	0.125
.4	1.092	.532	.218
.5	1.220	.600	.270
.6	1.050	.485	.202
.8	0.555	.250	.107
1×10^{-2} m./l. citric acid—no $Fe(NO_3)_3$	0.000	0.000	0.000
1×10^{-2} m./l. $Fe(NO_3)_3$ in 0.1 M HNO_3—no citric acid	0.120	0.120	0.120

From reaction (2) one may write

$$\log K_c - \log \frac{[Fe(H_x citr)^{+x}]}{[Fe^{+3}][H_3 citr]} = (3 - x) \log [H^+] \quad (3)$$

where K_c has the usual significance and where the brackets refer to concentrations in moles per liter.

For a series of solutions of varying acidity and constant ionic strength the variable terms on the left side of the equation (3) were plotted against the log $[H^+]$. The slope of the resulting curve equals $(3 - x)$ in reaction (2). The data yielded a value of unity for x. Hence the reaction involved is written in (1) above.

The same data afford the value of the equilibrium constant for reaction (2).

Experimental

All reagents employed were of the highest purity obtainable. Ferric nitrate solutions were standardized by the Zimmerman–Reinhardt method,[10] after removing the nitrate ion by fuming with sulfuric acid. Solutions of sodium thiocyanate were prepared from purified sodium thiocyanate crystals previously dried at 160°. Citric acid solutions prepared from purified citric acid were standardized by acidimetric titration as were also other acids employed.

The extinction coefficients were measured with the commercial model Beckman photoelectric spectrophotometer.

All measurements herein reported were made at room temperature, 25 ± 2°.

Experimental Data and Discussion

Tables I and II contain the data from which the ratio of iron to citrate in the complex was established.

The observed extinction coefficient of each solution in Table I, minus the extinction coefficient at the same wave length calculated on the assumption of no reaction between the iron and citrate, was plotted against R. The result is shown in Fig. 1. The sharp maxima indicate the ratio of iron to citrate in the complex is unity. The fact that the maxima occur at the same value of R for all wave lengths makes it exceedingly improbable that there are any other iron–citrate complexes in these solutions.[9]

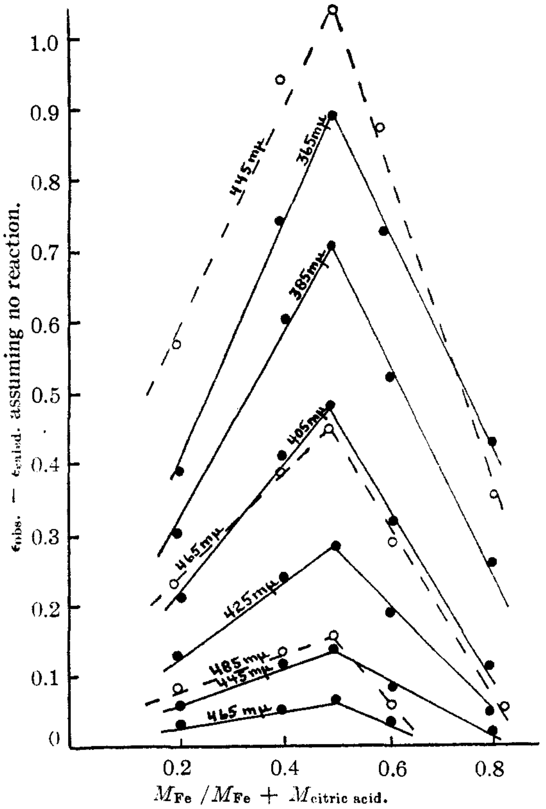

Fig. 1.—●, Data from Table I; ○, data from Table II.

However, it is possible that additional complexes of ratios of citrate to iron greater than

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(10) Reference 1, p. 426.

TABLE III

THE EFFECT OF VARIATION IN M_{H^+} ON THE STABILITY OF FERRIC CITRATE COMPLEX

Total Fe = 1×10^{-3} m./l.; total KSCN = 1×10^{-3} m./l.; total citric acid = 5×10^{-3} m./l. Ionic strength adjusted to 1.00 by addition of $NaNO_3$. Based on K_c (reaction 4) = 127.3. Molar extinction coefficient $FeSCN^{++}$ = 1875

1 M_{HNO_3}	2 ϵ 525mμ	3 $M_{\text{FeSCN}^{++}} \times 10^5$	4 $M_{\text{SCN}^-} \times 10^4$	5 $M_{\text{Fe}^{+3}} \times 10^4$	6 M_{Fe} citrate complex $\times 10^5$	7 M molecular citric acid $\times 10^3$	8 $[FeH_xcitr^{+x}]/[Fe^{+3}][H_4citr]$	9 K_c (Eq. 7)
0.08	0.162	8.64	9.13	7.42	15.6	4.84	43.5	0.278
.09	.167	8.91	9.11	7.68	12.8	4.87	34.2	.277
.12	.177	9.44	9.06	8.18	7.6	4.92	18.9	.272
.15	.182	9.71	9.03	8.43	5.0	4.95	12.0	.270
							Av.	0.274±0.003

unity are formed in more concentrated solutions. To test this possibility we prepared another series of solutions similar to those described in Table I except in each case the molarity of iron and citrate was ten times as great as the corresponding solution in Table I. The extinction coefficients were measured as above and the data treated in the same way. The data are given in Table II and plotted in Fig. 1.

The sharp maxima at $R = 0.5$ indicate no tendency to form higher complexes at these higher concentrations.

As stated above, the value of x in equation (3) was obtained by measuring the effect of changes in concentration of hydrogen ion on the equilibrium point of reaction (2).

Accordingly, a series of solutions was prepared each containing 1×10^{-3} m./l. $Fe(NO_3)_3$, 5×10^{-3} m./l. citric acid and sufficient sodium nitrate to bring the final ionic strength of each solution to unity. The nitric acid concentration of each solution differed, this component having a range of values from 0.08 to 0.15 m./l. for the series, the exact value for each solution being given in Column (1) Table III. Before making each solution up to the constant volume of the series sufficient potassium thiocyanate was added to give each solution a final concentration in this component of 1×10^{-3} m./l. The extinction coefficient of each solution was measured at 525 mμ with a Beckman spectrophotometer. The values obtained for the various solutions are shown in Column (2) Table III.

It has been shown[3,11] that thiocyanate ion may be used as a reliable indicator for ferric ion since it has been established that the reaction involved is

$$Fe^{+3} + SCN^- \rightleftarrows FeSCN^{++} \qquad (4)$$

The equilibrium constant (K_c) for this reaction has been evaluated by several workers. Bent and French[3] obtained for K_c reaction (4) a value of 30.3 at an ionic strength of 0.665. Edmonds and Birnbaum[12] obtained a value for K_c for this same reaction at an ionic strength of unity which, after estimating the probable effect on the constant produced by the change in ionic strength, does not appear to us to be in complete agreement with the value obtained by Bent and French. More recently, Frank and Oswalt[13] have determined the equilibrium constant for this reaction at ionic strengths less than unity and have obtained values which appear to be in complete agreement with the work of Edmonds and Birnbaum.

However, all the above workers agree that the red color produced by adding thiocyanate ion to ferric ion can, in sufficiently dilute solution, be ascribed completely to the formation of $FeSCN^{++}$ by reaction (4) and that the reaction involves a rapid mobile equilibrium between $FeSCN^{++}$ and the reacting ions.

We have therefore used $FeSCN^{++}$ as an indicator to determine the concentration of ferric ions in the solutions listed in Table III and hence to determine the value of x in equation (2). We have used 127.3 as the value of K_c reaction (4) at an ionic strength of unity. This value is based on the work of Edmonds and Birnbaum and Frank and Oswalt. The value used for the molar extinction coefficient of $FeSCN^{++}$ at 525 mμ is 1875. This was obtained by graphical interpolation of the molar extinction coefficients at other wave lengths given in the above paper[13] since these authors do not give any data at 525 mμ. At 525 mμ the extinction coefficient of the ferric citrate complex is negligible.

These values are used in the calculations of Table III in the following manner

Column (3): $M_{\text{FeSCN}^{++}} = \epsilon/1875$
Column (4): M_{SCN^-} = Total KSCN added $- M_{\text{FeSCN}^{++}}$
Column (5): $M_{\text{Fe}^{+3}} = M_{\text{FeSCN}^{++}}/(M_{\text{SCN}^-}\ K_c$ (reaction 4))
Column (6): $M_{\text{Fe-citrate complex}}$ = Total Fe $- M_{\text{Fe}^{+++}} - M_{\text{FeSCN}^{++}} - M_{\text{FeOH}^{+2}}$
Column (7): $M_{\text{Molecular citric acid}}$ = Total citric acid $- M_{\text{Fe-citrate complex}}$

In solutions as acid as these the ionization of the citric acid is negligible.

In evaluating $M_{\text{Fe-citrate}}$ (Column 6) it is necessary to correct for the hydrolysis of ferric ion, *i. e.*, $Fe^{+3} + H_2O \rightleftarrows FeOH^{++} + H^+$. For the equilibrium constant of this hydrolysis reaction (K_h) we have used 1.73×10^{-3} which is taken from the data of Bray and Hershey.[7]

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(11) O. E. Lanford and S. J. Kiehl, THIS JOURNAL, **64**, 291 (1942).

(12) S. Edmonds and N. Birnbaum, *ibid.*, **63**, 1471 (1941).

(13) H. S. Frank and R. L. Oswalt, *ibid.*, **69**, 1321 (1947).

Figure 2 shows the negative log $[H^+]$ plotted against

$$\log [Fe(H_x citr)^{+x}]/[Fe^{+3}][H_3 citr]$$

The result is a straight line as predicted by equation (3), the slope of which indicates x equals unity.

Having obtained the value of x in equation (3) one may use the same data to calculate K_c for equation (7). These are tabulated in Column 9, Table III. The average value of K_c is 0.274 ± 0.003.

Since there is some disagreement in the literature about the value of K_c reaction (4) and since this constant is used in the calculations of Table III, it should be pointed out that if one uses the value of K_c from the data of Bent and French,[3] which differs by about 300% from the value which we have used, *and the corresponding value for the molar extinction coefficient of $FeSCN^{++}$* the calculated values of columns (8) and (9) are not greatly changed. Actually, the values for K_c (equation 7) so obtained differ from those given in Table III column (9) by only about 10%. It can be shown that this is not an altogether unexpected result since K_c reaction (4) and the molar extinction coefficient of $FeSCN^{++}$ are uniquely related in that the experimental determination of the latter involves first the determination of K_c or *vice versa*. In the calculations of Table III use is made of both constants, hence errors in one of the constants are largely cancelled by corresponding errors in the other.

Therefore if further studies of reaction (4) should indicate a somewhat different value of K_c from that which we have used in the calculations of Table III we believe such a change would not affect the validity of the latter calculations.

The linearity found in Fig. 2 and the constancy of column (9) Table III give added support to the conclusion previously drawn concerning the ratio of iron to citrate in the complex. Dividing equation (6)

$$[H^+]^2[Hcitr^{-2}]/[H_3citr] = (K_{1c},\ K_{2c})_{\text{citric acid}} \qquad (6)$$

by equation (7)

$$[FeHcitr^+][H^+]^2/[Fe^{+3}][H_3citr] = K_c \qquad (7)$$

one obtains

$$\frac{[Fe^{+3}][Hcitr^{-2}]}{[FeHcitr^+]} = \frac{(K_{1c},\ K_{2c})_{\text{citric acid}}}{K_c \text{ (eq. 7)}} = K_8 \qquad (8)$$

in which the brackets refer to concentrations in moles per liter.

In order to evaluate the dissociation constant of the ferric citrate complex it is necessary to know (K_{1c}, K_{2c}) for citric acid at an ionic strength of one.

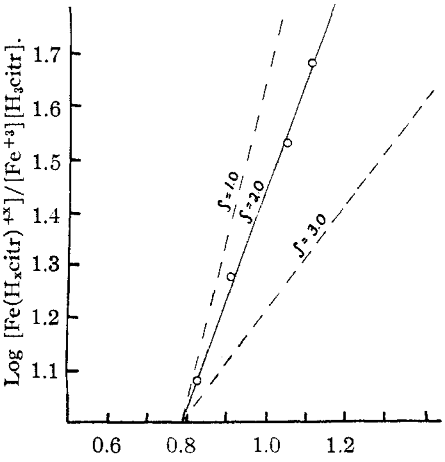

Fig. 2.

Adell[14] has measured the three stoichiometric ionization constants of citric acid at ionic strengths ranging from 0.1 to 3.0 at 18°. The temperature at which our measurements were made was 25° but according to Kolthoff and Rosenblum[15] these constants do not change more than about 3% between 18 and 25°. Therefore, we have used Adell's data, without attempt to correct them for temperature, to evaluate the constant for equation (8).

According to Adell[15] at an ionic strength of 1.0

$$(K_{1c},K_{2c})_{\text{citric acid}} = (1.66 \times 10^{-3})(8.11 \times 10^{-5}) = 1.35 \times 10^{-7}$$

The final value obtained for the dissociation constant of the ferric citrate complex (K_8) is therefore 4.92×10^{-7}.

The formula of the ferric citrate complex is of the same type as that for the complex formed by the reaction of ferric ion with another tribasic acid, orthophosphoric, which has previously been shown to be $FeHPO_4^+$.[11]

Summary

1. A study has been made of the reaction between ferric ion and citric acid. The formula of the complex, $FeHcitr^+$, was established.

2. The stoichiometric dissociation constant of this complex, at an ionic strength of 1.0, has been evaluated using ferric thiocyanate as an indicator for ferric ions.

ALBANY, N. Y.

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(14) Birger Adell, *Z. physik. Chem.*, **A187**, 66 (1940).

(15) I. M. Kolthoff and C. Rosenblum, "Acid Base Indicators," The Macmillan Co., New York, N. Y., 1937, p. 382.