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Studies of Metal Complexes of Radiobiological Interest

I. The Acidity Constants of Croconic Acid

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Potentiometric and spectrophotometric methods were used for the determination of the dissociation constants of croconic acid at 25°C.

The potentiometric method gave only approximate values of pK_1 0.3–0.5 and pK_2 1.4–1.6 (2 M (H, Na)Cl) and $pK_1 \simeq 0.6$ and $pK_2 \simeq 1.8$ (0.1 M (H, Na) Cl).

The ultraviolet spectra of croconic acid have been determined in mixed solutions of 2 M HCl and 2 M NaCl. The absorption data were treated by two methods:

(*a*) The graphical method for determining the four unknown parameters in eqn. (9) by testing reasonable values of the molar extinction coefficients of H_2A and HA^-. The slope and intercept of the best straight lines corresponded to $pK_1 = 0.2–0.3$ and $pK_2 = 1.3–1.5$.

(*b*) The least squares technique using a high-speed digital computer for determining a set of parameters giving the minimum standard deviation. This minimum was obtained for $pK_1 = 0.32$ and $pK_2 = 1.51$. In addition the spectra for the H_2A and HA^- forms of croconic acid were obtained by this method.

In recent years, the coordination chemistry of the alkaline earth metals has attracted special interest due to the occurrence of strontium and barium among the fission products of heavy atoms. Since soluble calcium or strontium chelates have proved to have little or no effect in eliminating strontium from the living body, attention has been turned to more insoluble complexes.

It has been known for a long time that strontium rhodizonate (SrC_6O_6) is less soluble than the calcium salt (see, *e.g.*, Weiss and Shipman [1]) and this fact has encouraged the use of sodium rhodizonate in biochemical investigations. Rhodizonate solutions (especially acid solutions) are, however, not very stable, thus preventing wider use of this reagent. One of the decomposition products is croconic acid, which is a homologous compound with a 5 membered ring. Croconate solutions seem to be more stable than rhodizonate solutions.

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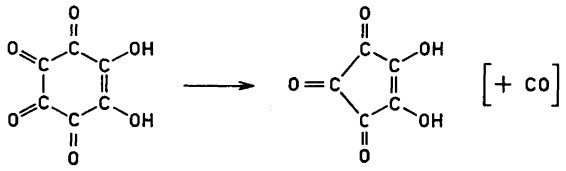

Rhodizonic acid *Croconic acid*

The acidity constants of croconic acid are not accurately known. Carpéni [2] has reported the values of pK_1 and pK_2 to be 2.17 and 4.0 from potentiometric data, but quite different from this, Yamada, Mizuno and Hirata[3] have roughly estimated $pK_1 < 1 < pK_2 < 2$ from spectrophotometric measurements. For a study of the complex chemistry of croconic acid a more accurate determination of these dissociation constants is therefore desirable. Both the potentiometric and the spectroscopic methods have been investigated here and the latter method has proved to give more reliable values of K_1 and K_2.

EXPERIMENTAL

Potassium croconate was prepared by oxidation of sodium rhodizonate in alkaline solution according to the method of Nietzki and Benckiser [4]. Either oxygen was bubbled through the rhodizonate solution in 1 M KOH at 60—70° for a few hours, or sodium rhodizonate was oxidized with MnO_2 in a solution of potassium carbonate (*cf.* Yamada, Mizuno and Hirata [3]). The crystals formed after cooling were filtered off. The filtrate was evaporated and more crystals were obtained. The salt was recrystallized twice from water and dried at 110°. Combustion with V_2O_5 as catalyst gave a carbon content of 27.8 % (no hydrogen). Calculated for $C_5O_5K_2$: 27.5 % C. Yield *ca.* 50 %.

The potentiometric titrations of $[H^+]$ were carried out at 25°C in a thermostated room. The emf was measured with a Radiometer pH Meter 4 using a Radiometer G 202 B glass electrode. The titrations of dipotassium croconate (K_2A) were carried out with 50—150 ml of 2 M or 0.1 M (H, Na)Cl in 2 M or 0.1 M NaCl solutions using a common saturated KCl/Hg_2Cl_2, Hg electrode as a reference. The cells were standardized against solutions of known $-\log [H^+]$ and constant ionic strength (0.1 M and 2 M, respectively).

The light absorption measurements were made with a Zeiss PMQ II spectrophotometer with 1 cm quartz cells. In neutral or alkaline solutions the absorption was fairly stable but it decreased slowly in weakly acid solutions. This decrease was especially rapid in strong $HClO_4$, whereas 89 % of the initial absorption still remained after 7 days in 5 M HCl.

LIST OF SYMBOLS

[ ]	concentrations
$[A]_{tot}$	total concentration of croconic acid (3)
H_2A, HA^-, A^{2-}	croconic acid and croconate ions
D	optical density = extinction (6)
E	electromotive force in mV
I	ionic strength
K_1, K_2	stoichiometric acid dissociation constants (7)
K_{av}	average dissociation constant (5)
m	number of different wave-lengths (14)

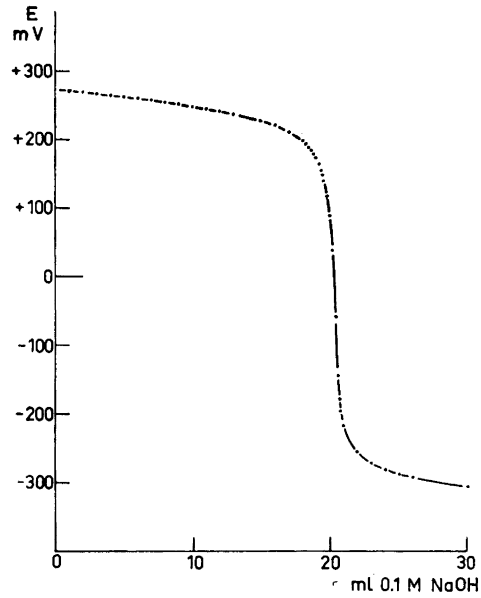

Fig. 1. Potentiometric titration of 1 mmole of croconic acid (obtained by ion-exchange of the dipotassium salt) with 0.1 M NaOH.

n	number of experimental points in least squares calculation (13)
$\bar{n}$	formation function according to Bjerrum [5]
Z	average number of H bound per A (1)
ε	overall molar extinction coefficient (9)
ε_0, ε_1, ε_2	molar extinction coefficients (6)
λ	wave-length in mμ
σ_λ	standard deviation in ε at a certain wavelength (13)
σ_{tot}	standard deviation in ε for nine wave-lengths (14)

RESULTS

The determination of Z function from potentiometric titrations. From the potentiometric titration curve (Fig. 1) it is quite evident that croconic acid is highly dissociated even at very low pH values. The acid is dibasic but the two dissociation steps are so close that they do not appear in Fig. 1. The average number of hydrogen atoms bound to the croconate ion is defined by:

$$Z = \frac{2[H_2A] + [HA^-]}{[A]_{tot}} \qquad (1)$$

The concentration of H_2A and HA^- were computed using the law of electroneutrality (ionic medium not included):

$$[K^+] + [H^+] = [Cl^-] + [HA^-] + 2[A^{2-}] \quad (2)$$

where $[K^+] = 2[A]_{tot}$ and $[Cl^-]$ is the amount of HCl added.

The total concentration of croconic acid in all its forms is

$$[A]_{tot} = [H_2A] + [HA^-] + [A^{2-}] \quad (3)$$

From (1), (2) and (3) it follows that

$$Z = \frac{[Cl^-] - [H^+]}{[A]_{tot}} \quad (4)$$

There is a close relation between the formation function, $\bar{n}$, for the complex formation described by Bjerrum [5] and the Z function for polyvalent acids (*cf.* Sillén [6]). Thus the ligands of a complex correspond to the protons and the central metal atom to the anion of the acid. If $\bar{n}$ is plotted *versus* the logarithm of the ligand concentration curves are obtained with the same form as plots of Z against log $[H^+]$. The "average" constant may be calculated from the midpoint of this curve. Regardless of the number of ligands or the difference between the individual constants, Bjerrum [5] has shown that the average formation constant is equal to the reciprocal ligand concentration at the midpoint provided the formation curve is symmetrical.

In a similar way, one can prove that the average dissociation constant, K_{av}, is equal to the hydrogen ion concentration at the midpoint of the Z function. In the special case of a dibasic acid we thus have

$$pK_{av} = \frac{1}{2}(pK_1 + pK_2) = -\log [H^+]_m \quad (5)$$

where $[H^+]_m$ refers to the midpoint value of $Z = 1$. When the ratio between K_1 and K_2 is large (about 10 000 or more) the two steps are separated and

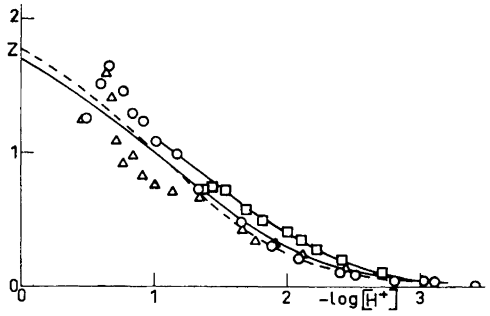

Fig. 2. Z-data (see text) as a function of $-\log [H^+]$ for 2 M (H,Na)Cl: O (5 mmoles of K_2A), △ (1 mmole of K_2A), and 0.1 M (H,Na)Cl: □ (1 mmole of K_2A). The normalized curve $Y = (pv + 2v^2)(1 + pv + v^2)^{-1}$, $X = \log v$ with $p = 4$ (full-drawn curves) and $p = 3$ (dashed curve) are fitted to the data (*cf.* Sillén [6]).

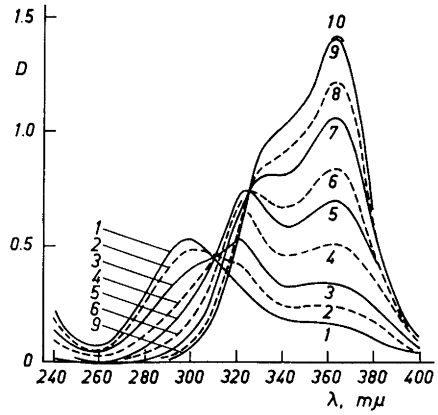

Fig. 3. Spectra of 4×10^{-5} M croconic acid in 2 M (H,Na)Cl. The following numbers refer to curves at different $[H^+]$: 1, 1.98 M; 2, 0.98 M; 3, 0.50 M; 4, 0.22 M; 5, 0.11 M; 6, 0.050 M; 7, 0.022 M; 8, 0.01 M; 9, 0.001 M; 10, 10^{-6} M. Curve 1 has maximum at 299 mμ and curve 9 at 363 mμ.

under these conditions, pK_1 and pK_2 are equal to $-\log [H^+]$ at $Z = 1.5$ and 0.5, respectively. However, if the constants are close to each other two steps cannot be seen in the Z-curve (*cf.* Sillén [6]).

From the titration without croconate, all other conditions being unchanged, the calibration curve of E *versus* $-\log[H^+]$ was obtained. Fig. 2 shows the Z function at 0.1 M and 2 M ionic strength. It was impossible to obtain the complete curves due to the inaccuracy of the Z values in strong acid solutions.

By curve-fitting (*cf.* Sillén [6]) we could estimate: $pK_1 \simeq 0.6$, $pK_2 \simeq 1.8$ in 0.1 M (H,Na)Cl, and $pK_1 = 0.3-0.5$ and $pK_2 = 1.4-1.6$ in 2 M (H,Na)Cl. The latter values agree with the spectrophotometric measurement below.

Spectrophotometric determination of K_1 *and* K_2. In the main series, the $[H^+]$ covered the range from 2 M HCl to 2 M NaOH and the ionic strength was kept constant at 2.0 M with NaCl. In Fig. 3 the spectra are shown for 4×10^{-5} M total croconic acid in 2 M (H,Na)Cl. There is very little change in the spectra below $[H^+] = 0.001$ M, which shows that croconic acid is a rather strong acid.

The two isosbestic points (at *ca.* 310 and *ca.* 325 mμ) are not sharp, and they indicate that there are three different species of croconic acid. In strong acid (5 or 12 M HCl) or alkali solvent effects seem to occur; these spectra are not included in Fig. 3 (*cf.* Fig. 4).

At a certain wave-length the optical density, D, is the sum of the absorption of all forms of croconic acid

$$D = \varepsilon_0[H_2A] + \varepsilon_1[HA^-] + \varepsilon_2[A^{-2}] \qquad (6)$$

where ε_0, ε_1, and ε_2 are the molar extinction coefficients of the forms H_2A, HA^- and A^{2-}, respectively (cell of unit length = 1 cm).

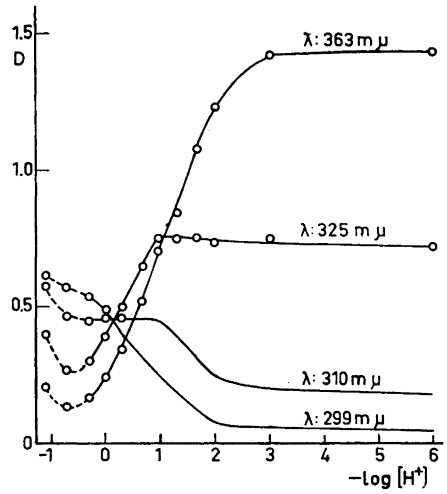

Fig. 4. Variation of optical density, D, for 4×10^{-5} M croconic acid with acidity at four different wave-lengths (the two maxima and the two isosbestic points).

The stoichiometric dissociation constants are

$$K_1 = \frac{[H^+]\,[HA^-]}{[H_2A]} \text{ and } K_2 = \frac{[H^+]\,[A^{2-}]}{[HA^-]} \tag{7}$$

Combining eqns. (3), (6) and (7) one can derive the relation

$$D[A]_{tot}^{-1} = \frac{[H^+]^2\varepsilon_o + K_1[H^+]\varepsilon_1 + K_1K_2\varepsilon_2}{[H^+]^2 + K_1[H^+] + K_1K_2} \tag{8}$$

Eqn. (8) can be rearranged to a form more suitable for numerical calculations

$$\frac{(\varepsilon_o - \varepsilon)}{(\varepsilon - \varepsilon_2)}[H^+]^2 = \frac{(\varepsilon - \varepsilon_1)}{(\varepsilon - \varepsilon_2)}[H^+]K_1 + K_1K_2 \tag{9}$$

where $\varepsilon = D[A]_{tot}^{-1}$ is obtained experimentally. There is no direct way in which ε_1 can be obtained and ε_o is not accurately known since some HA^- still exists in the most acid solution, 2 M HCl (Fig. 4). A graphical method was used for evaluating K_1 and K_2 from eqn. (9) which may be written as linear equation

$$Y = K_1X + K_1K_2 \tag{10}$$

$$\text{where } Y = \frac{\varepsilon_o - \varepsilon}{\varepsilon - \varepsilon_2}[H^+]^2 \text{ and } X = \frac{\varepsilon - \varepsilon_1}{\varepsilon - \varepsilon_2}[H^+].$$

Plots were made of Y *versus* X for combinations of reasonable values of ε_o and ε_1 for the two peaks at 363 mμ and 299 mμ. The best fit with a straight line was obtained for $\varepsilon_o = 1.0 \times 10^3$ and $\varepsilon_1 = (13\text{—}15) \times 10^3$ at 363 mμ and

Table 1. Graphical solutions of eqn. (9)

λ	$\varepsilon_0 \times 10^{-3}$	$\varepsilon_1 \times 10^{-3}$	$\varepsilon_2 \times 10^{-3}$	K_1	K_1K_2	K_2
363	1.0	13.0	35.9	0.64	0.025	0.035
»	»	15.0	»	0.50	0.015	0.03
299	15.0	5.0	1.1	0.49	0.015	0.03
»	»	6.0	»	0.54	0.025	0.04
»	»	7.0	»	0.62	0.035	0.05

$pK_1 = 0.2 - 0.3$
$pK_2 = 1.3 - 1.5$

for $\varepsilon_0 = 15.5 \times 10^3$ and $\varepsilon_1 = (5-7) \times 10^3$ at 299 mμ. The slope of these lines corresponds to K_1. The intercept on the horizontal axis is $-K_2$ and that on the vertical axis is K_1K_2. The results are summarized in Table 1. On the whole this method is rather time-consuming even if a desk-calculator is employed.

Thamer and Voigt [7] have developed a method for determining overlapping dissociation constants for dibasic acids if ε_0 and ε_2 can be obtained by direct measurement at low and high pH values. This method fails, however, in our case since the absorption does not reach a limiting value in the strong acidic region.

In order to obtain more accurate results, the data were treated by the method of least squares. These calculations are very time-consuming unless a high-speed digital computer is employed. In cooperation with Mr. Kurt Eriksson at the FOA division of applied mathematics a program [8] was developed for the FACIT EDB computer to calculate the best values of the 4 constants K_1, K_2, ε_0 and ε_1 from eqn. (8). The input data were the values of $[H^+]$, ε_2 and the "over all" molar extinction coefficient, $\varepsilon = D[A]_{tot}^{-1}$, for 9 different wave-lengths.

In the first place we investigated the possibility of a direct estimation of all four parameters. At certain wavelengths, however, some of the parameters turn out to be negative.

The program was changed so that the values of ε_0 and ε_1 would be calculated by the method of least squares for a series of reasonable values of K_1 and K_2. This approach is in a way closely related to the "error-pit" calculation method by Dyrssen, Ingri and Sillén [9]. Since the dissociation constants are independent of the wave-length they were used as input data. Eqn. (8) can be written in the form

$$D[A]_{tot}^{-1} = \varepsilon = f([H^+]) \qquad (11)$$

where the dependent variable, ε, is subject to random errors; the error in $[H^+]$ can be neglected. The least squares method requires that a set of parameters be determined which minimizes

$$\Delta \varepsilon = [\varepsilon - f([H^+])] \qquad (12)$$

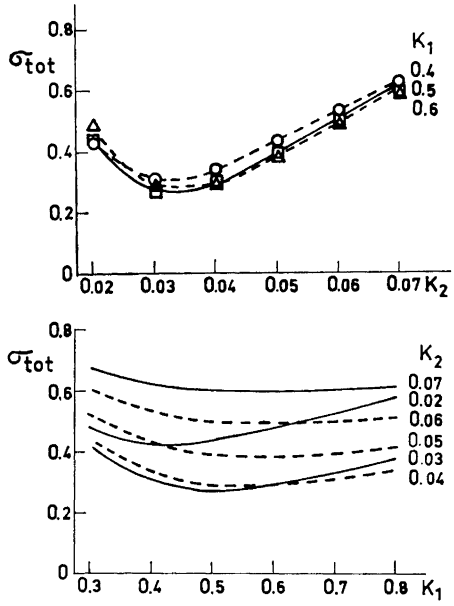

Fig. 5. High-speed computing of ε_0, ε_1, K_1 and K_2. 36 combinations of K_1 (0.3—0.8) and K_2 (0.02—0.07) were tested. The vertical axis is σ_{tot} for nine different wave-lengths.

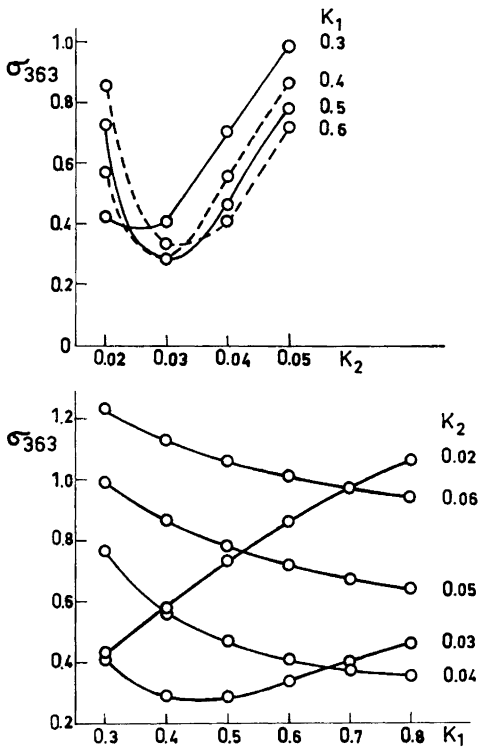

Fig. 6. High-speed computing of ε_0, ε_1, K_1 and K_2. Selected wave-length 363 mμ.

Table 2. Final computation of ε_0 and ε_1 at various wave-lengths. $K_1 = 0.48$ and $K_2 = 0.031$.

λ mμ	$\varepsilon_2 \times 10^{-3}$	$\varepsilon_0 \times 10^{-3}$	$\varepsilon_1 \times 10^{-3}$	σ_λ
280	0.05	9.29	0.73	0.1088
299	1.08	15.31	5.45	0.2250
310	4.30	10.81	12.87	0.2641
320	12.22	5.71	20.95	0.3083
325	17.78	4.01	21.20	0.3214
330	22.75	3.09	17.77	0.2941
340	25.75	2.26	13.72	0.1940
363	35.72	1.19	15.02	0.2763
380	15.45	0.03	12.34	0.3680
				$\sigma_{tot} = 0.2721$

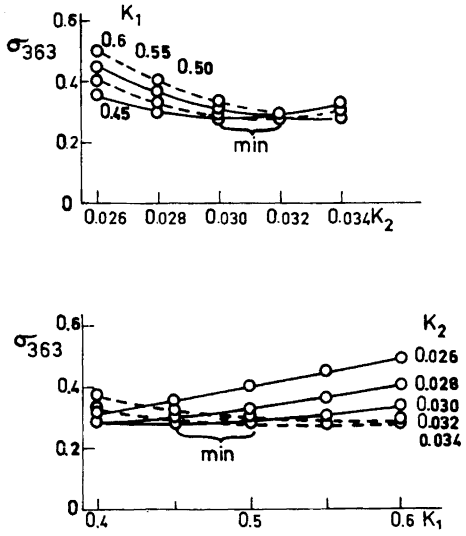

Fig. 7. High-speed computing of ε_0, ε_1, K_1 and K_2. Final step in the successive approximation of K_1 and K_2 (*cf.* Figs. 5 and 6). Selected wave-length 363 mμ.

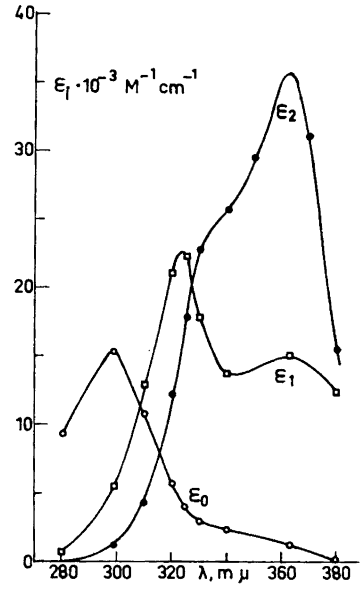

Fig. 8. Croconic acid (H_2A). Spectra of the species H_2A and HA^- calculated by high-speed computing using the values of $K_1 = 0.48$ and $K_2 = 0.031$. It may be seen that the intensity of the peak at 363 mμ is considerably increased for each proton that is removed from H_2A. At the same time the peak at 299 mμ shows a bathochromic shift.

The standard deviation in ε at a certain wave-length for eight different $[H^+]$ (Fig. 3) is

$$\sigma_\lambda = [1/(n-1)]^{1/2} \left[\sum_1^n (\Delta\varepsilon)^2 - \frac{1}{n} \left(\sum_1^n (\Delta\varepsilon) \right)^2 \right]^{1/2} \quad (n=8) \qquad (13)$$

The corresponding standard deviation taken over all wave-lengths is

$$\sigma_{tot} = (1/m)^{1/2} \left[\sum_1^m \sigma_\lambda^2 \right]^{1/2} \quad (m=9) \qquad (14)$$

Input data: $\varepsilon = D[A]_{tot}^{-1}$, $[H^+]$, ε_2, combinations of K_1 and K_2.
Output data: ε_0, ε_1, σ_λ, σ_{tot}.

In the first computation, the values of K_1 were 1, 0.5 and 0.1 and those of K_2 were 0.1, 0.05 and 0.01 (nine combinations). A lowest value of σ_{tot} (nine wave-lengths, *cf.* Table 2) was obtained for the pair $K_1 = 0.5$ and $K_2 = 0.05$.

Table 3. Values of the dissociation constants for croconic acid.

Reference	pK_1	pK_2	Ionic strength M	Method
Carpéni [2]	2.93	4.05	0.1	Potentiometric
	2.17	4.0	0.1	corrected values
Yamada *et al.*[3]	<1	1—2	1	Spectrophotometric
This work	0.3—0.5	1.4—1.6	2	Potentiometric
	~0.6	~1.8	0.1	»
				Spectrophotometric
	0.32	1.51	2	least squares calculation with digital machine.
	0.2—0.3	1.3—1.5	2	graphical evaluation

Three other computations were made with the following K-values:

(II) K_1: 0.3, 0.4, 0.5, 0.6, 0.7 and 0.8
K_2: 0.02, 0.03, 0.04, 0.05, 0.06 and 0.07
36 combinations
(III) (only for $\lambda = 363$ mμ)
K_1: 0.40, 0.45, 0.50, 0.55 and 0.60
K_2: 0.026, 0.028, 0.030, 0.032 and 0.034
25 combinations
(IV) (nine wave-lenghts)
K_1: 0.48
K_2: 0.031.

Fig. 5 shows the relation between σ_{tot} and the various combinations of K_1 and K_2. The minima corresponded to $K_1 \simeq 0.5$ and $K_2 \simeq 0.03$. The plots of the standard deviations for a specific wave-length may give sharper minima, in this case at $\lambda = 363$ mμ (Fig. 6). The results from the computation III with narrow intervals of K_1 and K_2 at $\lambda = 363$ mμ are shown in Fig. 7. The least standard deviation from these curves corresponded to $K_1 = 0.48$ and $K_2 = 0.031$. Finally, the calculations were made with this pair of K values over the whole spectrum in order to get the spectra for the three forms of croconic acid (Table 2 and Fig. 8).

In Table 3 we have collected values of the dissociation constants of croconic acid. Both our work and the investigation of Yamada, Mizuno and Hirata [3] show that the K-values found by Carpéni [2] are probably erroneous.

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REFERENCES

1. Weiss, H. V. and Shipman, W. H. *Anal. Chem.* **29** (1957) 1764.
2. Carpéni, G. *J. chim. phys.* **35** (1938) 193, 233.
3. Yamada, K., Mizuno, N. and Hirata, Y. *Bull. Chem. Soc. Japan* **31** (1958) 543.
4. Nietzki, R. and Benckiser, Th. *Ber.* **18** (1885) 499, 1833; **19** (1886) 293.
5. Bjerrum, J. *Diss.* Copenhagen 1941.
6. Sillén, L. G. *Acta Chem. Scand.* **10** (1956) 186.
7. Thamer, B. J. and Voigt, A. F. *J. Phys. Chem.* **56** (1952) 225.
8. Eriksson, K. and Strahl, M. *FOA 2 report C 2052—2990* (Nov. 1960).
9. Dyrssen, D., Ingri, N. and Sillén, L. G. *Acta Chem. Scand.* **15** (1961) 694

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