[CONTRIBUTION FROM THE DEPARTMENT OF ANIMAL AND PLANT PATHOLOGY OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH]

A Study of Isocitric Acid from Blackberries

BY WILLIAM F. BRUCE

Although optically active isocitric acid was isolated from blackberries by Nelson[1] several years ago, its optical properties have not been sufficiently investigated. These properties are of particular interest because Nelson reports[2] that the sign and degree of rotation vary from one lot of blackberries to another. An explanation of this variation may well be important in considering the formation of this and other optically active substances in nature. Moreover, since the synthetic acid has not yet been resolved, a study of the optical constants of the natural acid is a necessary preliminary to any detailed study of the acid in a biochemical system. Since the acid can form a lactone, it should, like the sugar acids,[3] show a large change in optical rotation upon lactone formation. From the magnitude and direction of this change, as established in the following study, a new interpretation of the results obtained by Nelson has appeared.

This report contains a description of optically pure isocitric acid lactone (I) and some of its derivatives. In order to secure a crystalline solid, isolation as the methyl rather than the ethyl ester was undertaken. The excellent crystallizing ability of dimethyl isocitrate lactone (II), which was isolated, permitted demonstration of the optical purity of the material. This was accomplished by submitting the substance to different physical purification procedures in which the optical properties remained constant, and to chemical transformations in which the optical properties were greatly changed, but were reproduced when the material was returned to its original composition (Table I), and further by isolating material with the same activity from blackberries of different sources and different seasons.

The effect of treating dimethyl isocitrate lactone and isocitric acid lactone with alkali is shown in Table II. This table shows that in both the ester and the acid the rotation of the lactone form is strongly negative. This rotation is diminished upon addition of one mole of alkali, and is still less negative when two moles are added. The ester in fact becomes (+) while the acid remains (−). In both cases, the trisodium salt (III), which must be identical in each instance, has a negligible rotation. This involves a shift toward the negative in the ester and toward the positive in the acid. The configurations of the asymmetric carbon atoms must be the same in both compounds since the acid is made from the ester and can be transformed to the same ester again even after treatment with excess sodium hydroxide. This difference in behavior when alkali is added can be explained by assuming that the isocitric acid lactone ring is gradually opened concurrently with salt formation, whereas the hydrolysis of one methyl group and the opening of the lactone ring in dimethyl isocitrate lactone are rapid reactions, followed by the slower hydrolysis of the remaining methyl group. This interpretation is supported by the fact that determination of methoxyl upon addition of *two* moles of alkali shows the liberation of *one* mole of methyl alcohol.

The hydrolysis of the monomethyl ester which remains in the solution is suited to a kinetic study. The optical activity plotted against time after addition of one more mole of sodium hydroxide (or three moles of alkali to the dimethyl lactone ester) gives a hyperbolic curve (Fig. 1) and the reciprocal of the observed activity plotted against time gives a straight line (Fig. 2), for which the equation is

$$\frac{1}{\alpha} = Kt + C$$

where $\lambda = 546, K = 1.04, C = 0.92$
where $\lambda = 589, K = 1.50, C = 1.00$

The theoretical derivation of this equation depends on the following assumptions. (a) During the period of observation, the optical activity is proportional to the concentration of the monomethyl ester (IV) in solution, *i. e.*

$$\alpha = K_1E \qquad (1)$$

where E is the concentration of ester at any time. (b) The time rate of change of the ester into the salt is proportional to the concentration of ester and of sodium hydroxide present at any time, t, namely

$$-\mathrm{d}E/\mathrm{d}t = K_2E\,[\mathrm{NaOH}] \qquad (2)$$

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[1] E. K. Nelson, THIS JOURNAL, **47**, 568 (1925).

[2] E. K. Nelson, *ibid.*, **52**, 2929 (1930).

[3] C. S. Hudson, "Scientific Papers of the Bureau of Standards," No. 533, 1926, p. 281.

TABLE I

OPTICAL BEHAVIOR OF DIMETHYL ISOCITRATE LACTONE

Dissolved in dioxane for determination of rotation

		$[\alpha]^{26}_{589}$	$[\alpha]^{26}_{546}$
Crystallized from	CH_3OH	−65.3 ± 0.2	−78.2 ± 0.5
	H_2O	−64.9 ± .4	....
	C_6H_6	−66.3 in acetone	....
Fractionally distilled, b. p. 204–206° (26 mm.)		−65.7 ± 0.3	−78.0 ± 0.4
Three moles of NaOH, then 3 moles of HCl, isolation and methylation by CH_2N_2		−63.3 ± 3.0	−77.6 ± 1.7

TABLE II

BEHAVIOR OF OPTICALLY ACTIVE DIMETHYL ISOCITRATE LACTONE AND ISOCITRIC ACID LACTONE WITH SODIUM HYDROXIDE

	Dimethyl isocitrate lactone		Isocitric acid lactone	
	$[\alpha]^{26}_{589}$	$[\alpha]^{26}_{546}$	$[\alpha]^{26}_{589}$	$[\alpha]^{26}_{546}$
Substance	−65.3 ± 0.3[b]	−78.0 ± 0.3[b]	−62.0 ± 0.2[c]	−73.2 ± 0.3[c]
+1 Mole NaOH[a]	−23.1 ± 0.1[b]	−27.7 ± .5[b]	−36.7 ± .2[c]	−44.8 ± .2[c]
+2 Moles NaOH[a]	+17.9 ± 0.1[c]	+21.5 ± .5[c]	−22.6 ± .2[c]	−28.6 ± .7[c]
+3 Moles NaOH[a]	± 0[c]	− 1.3 ± .5[c]	− 1.7 ± .2[c]	− 3.3 ± .5[c]
+3 Moles HCl	+18.0 ± 1.2[c]	+24.3 ± 1.8[c]	+17.7 ± .1[c]	+25.3 ± .2[c]

[a] Calcd. to the appropriate sodium salt. [b] In dioxane. [c] In water.

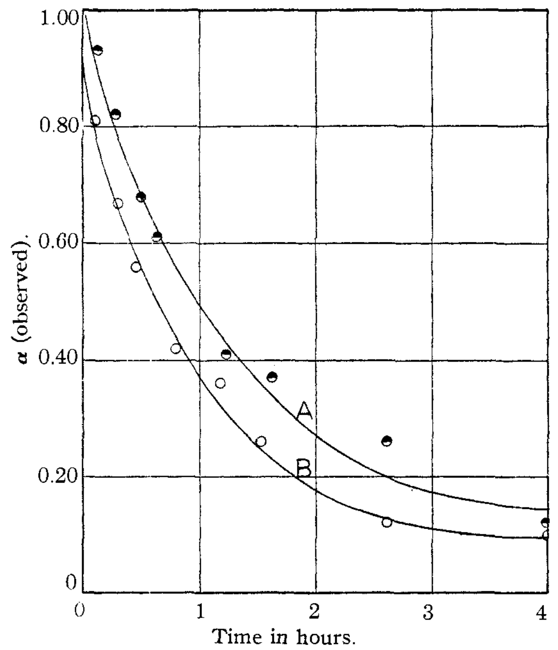


Fig. 1.—Rate of hydrolysis of monomethyl isocitrate (IV) by sodium hydroxide. A, λ = 546; B, λ = 589.

Since one molecule of ester on conversion into the salt removes one molecule of sodium hydroxide by the reaction IV + NaOH = III + CH_3OH, and since equimolecular amounts of both the ester and sodium hydroxide were originally taken, then at any time the concentration of E equals the concentration of sodium hydroxide and equation (2) becomes

$$-dE/dt = K_2E^2 \qquad (3)$$

which on integration becomes

$$1/E = K_2t + C_1 \qquad (4)$$

Substituting in equation (4) the equivalent of E, α/K_1, from equation (1), we get

$$\frac{1}{\alpha} = Kt + C \qquad (5)$$

which is identical with the equation representing the observed results.

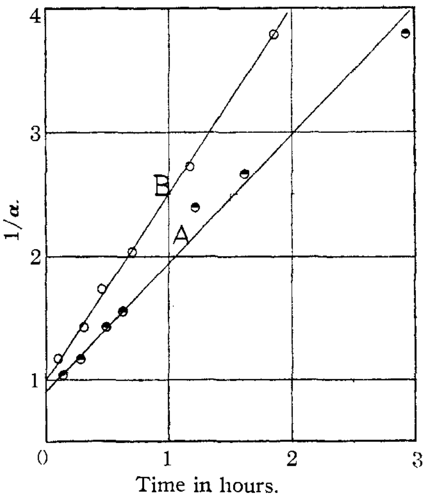
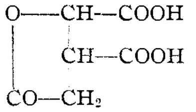
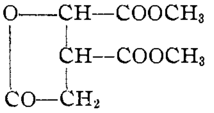


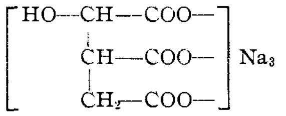
Fig. 2.—Reciprocal of α (observed) against time from Fig. 1. A, λ = 546; B, λ = 589.

The diethyl lactone ester, α^{26}_{589} −66°, was prepared from the acid by esterification with ethyl alcohol. Since the diethyl lactone ester ($C_{10}H_{14}O_6$) and the triethyl ester ($C_{12}H_{20}O_7$) have the same percentage of carbon and nearly the same percentage of hydrogen, it is not feasible to detect mixtures of one in the other by elementary analysis. A 20–30% decomposition of the triethyl ester, α_D + 14°, prepared by Nelson would therefore suffice to give the variation in rotation which has been reported, without requiring the assumption that the rotation of the acid in the fruit varies from one lot to another.

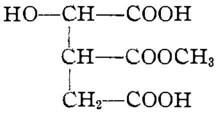
The structure of the monomethyl ester (IV) is still in doubt so far as the position of the methyl group is concerned. Because the methyl groups of the dimethyl lactone ester (II) differ widely in the ease with which they are removed by sodium hydroxide, the one more easily hydrolyzed has been assigned to the carboxyl on the carbon atom holding the hydroxyl at the end of the chain.


I


II


III

$$\left[\begin{array}{l}HO-CH-COO- \\ \qquad CH-COO- \\ \qquad CH_2-COO-\end{array}\right]Na_3$$


IV

$$\begin{array}{l}HO-CH-COOH \\ \qquad CH-COOCH_3 \\ \qquad CH_2-COOH\end{array}$$

Experimental

Isolation from Blackberries of Active Isocitric Acid as the Dimethyl Ester Lactone (II).—Canned blackberries were suggested by Nelson[4] as a convenient source of isocitric acid. Puyallup water pack blackberries, prepared by the Pacific N. W. Canning Co. of Puyallup, Washington, and Inavale water packed cultivated blackberries, packed by the Washington Berry Growers Packing Corp. of Sumner, Washington, were used as sources of blackberries. Lots packed in 1932 and in 1934 were secured. The juice from 2 gallons (5.9 kg.) was treated by the procedure given by Nelson[1] (p. 569) with the following modifications. The precipitated lead salts were decomposed by hydrogen sulfide. The acid residue was well dried by repeated evaporations with methyl alcohol, and methyl rather than ethyl alcohol was used for the esterification. Upon distillation in a Claisen flask in which the side-arm is modified to serve as receiver (Sabelkölbchen), the following fractions were collected at 20 mm. (1932 lot): 2 g. 100–150°, 10 g. 150–175°, 2 g. 175–220°. The main fraction, a mixture of sirup and crystals, was dried on a porous plate. The white crystals which resulted weighed 7 g. and melted at 101–104°. This substance, upon recrystallization from benzene, melted at 104–106°, $[\alpha]^{26}_{589}$ −66.3° ± 0.1 (in acetone); α −5.63°; $c = 8.47$. Recrystallized from methyl alcohol, the substance melted at 105–107°, $[\alpha]^{26}_{589(a),\,546(b)}$ = −65.5° ± 0.2(a), −78.2° ± 0.4(b); α −6.00°(a), −7.15°(b); $c = 9.15$ (in dioxane). Upon distillation the substance boiled at 204–206° at 26 mm. (bath 215°) and melted at 106–107°. $[\alpha]^{27}_{589(a),\,546(b),\,486(c)}$ −65.3° ± 0.3(a). −77.6° ± 0.3(b), −100.0° ± 0.5(c); α −7.08°(a), −8.41°(b). −10.82°(c); $c = 10.82$ in dioxane. Crystallized from water, the substance melted at 105–107°, $[\alpha]^{26}_{589}$ −64.9° ± 0.4; α − 5.18°; $c = 8.00$ in dioxane.

Anal. Distilled sample. Calcd. for $C_8H_{10}O_6$ (II): C, 47.51; H, 5.0. Found: C, 47.6, 47.3; H, 5.5, 5.6.

Titration.—0.2348 g. used 19.7 cc. of 0.100 *N* sodium hydroxide fairly rapidly, and on warming to 70–80° a total of 21.63 cc.; apparent equivalent weight, 108.

Material with these same properties was secured from the other lots of blackberries in yields ranging from 2 to 8 g. per gallon. The 1932 lots gave the best yields. No dimethyl isocitrate lactone with less optical activity was isolated.

Isocitric Acid Lactone (I).—Two and one-half grams of pure dimethyl isocitrate lactone was boiled with 30 cc. of 1 *N* hydrochloric acid for two and one-half hours. The water was then removed in a vacuum at 45° and the colorless sirup which remained was dried over sodium hydroxide in a Fischer pistol at 100°. It solidified gradually after being rubbed with a glass rod. It was crystallized from ethyl acetate and melted at 153–154°. $[\alpha]^{26}_{589(a),\,546(b),\,486(c)}$ −62.0° ± 0.2(a), −73.2° ± 0.3(b), −93.7° ± 4.0(c); α −7.90°(a), −9.34°(b), −11.96°(c); $c = 12.75$ in water.

Anal. Calcd. for $C_6H_6O_6$: C, 41.35; H, 3.48. Found: C, 41.3, 41.7; H, 3.9, 3.6.

Diethyl Isocitrate Lactone.—A solution of 4 g. of isocitric acid lactone (I) in 50 cc. of ethyl alcohol containing 1.5% hydrochloric acid was boiled for four hours. The alcohol was removed in a vacuum and the residue was fractionated in a vacuum. The chief fraction weighed 3.8 g. and distilled at 167–169° at 2.5 mm.; $[\alpha]^{26}_{589(a),\,546(b),\,486(c)}$ −54.2° ± 0.1(a), −64.4° ± 0.1(b), −83.6° ± 0.2(c); α −66.57°(a), −79.18°(b), −103.75°(c); $d = 1.230$.

Anal. Calcd. for $C_{10}H_{14}O_6$: C, 51.16; H, 6.08. Found: C, 51.13; H, 6.1.

Amide.—A solution of 1.5 g. of dimethyl isocitrate lactone (II) in 5 cc. of warm methyl alcohol was saturated with dry ammonia gas. Heat was spontaneously evolved, and upon standing overnight the solution deposited a crystalline precipitate very slightly soluble in dioxane or methyl alcohol. The substance dissolved slowly in 10 cc. of water at 45°, and precipitated on cooling to 0°. The yield was 0.8 g. When the substance was dissolved in boiling water, no precipitate appeared on cooling even when seeded. After the substance was dried in a Fischer pistol at 80°, it melted with decomposition at 204°, becoming yellow at 190°; $[\alpha]^{23}_{589(a),\,546(b)}$ −5.02° ± 0.8(a), −5.85° ± 0.4(b); α −0.12°(a), −0.14°(b); $c = 2.39$ in water.

Anal. Calcd. for $C_6H_{11}N_3O_4$: C, 38.09; H, 5.88; N, 22.20. Found: C, 37.66, 37.71; H, 6.7, 6.0; N, 22.18.

Hydrazide.—To a solution of 241 mg. of dimethyl isocitrate lactone (II) in 1 cc. of anhydrous dioxane at 45° was added 0.2 cc. of hydrazine hydrate. The solution became warmer and a white solid at once began to separate. After ten minutes the solid was collected on a filter and washed with dioxane. The substance weighed 300 mg. and melted at 201–202° with decomposition; when the bath was preheated to 200°, it melted at 208–209° with decomposition. It is insoluble in the usual solvents, but can be dissolved in glycerol or in ethylene glycol on heating.

Hydrolysis of Dimethyl Isocitrate Lactone (II) by Alkali.—To 0.1484 g. of II suspended in a small amount

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(4) Private communication.

of water was added 2 portions (each being 0.733 cc. (1 mol)) of 1 *N* sodium hydroxide as follows.

	1	2
Wt. of soln., g.	3.073	4.408
d	1.02	1.02
α_{589}	-0.99	$+0.76$
α_{546}	-1.14	$+ .90$
$[\alpha]^{26}_{589}$	-23.1 ± 0.1	$+17.9 \pm 0.1$
$[\alpha]^{26}_{546}$	$-27.7 \pm .5$	$+21.5 \pm .2$

Addition of a third mole of sodium hydroxide produced no marked change in α, but after the solution stood several hours, the rotation was found to have decreased. A more detailed study of the hydrolysis was therefore made by adding to 0.9411 g. of II suspended in 10 cc. of water 1 mol (4.66 cc. 1 *N*) of sodium hydroxide. The liquid was warmed to 50° to secure complete solution, but at room temperature a white crystalline solid melting at 105–107° separated. It was unchanged II. A second mol of sodium hydroxide was then added, a clear solution was secured and allowed to stand overnight. The solution weighed 20.36 g., *d* 1.03, $[\alpha]_{589(a),\ 546(b)}$ $+0.58°$(a), $+0.73°$(b); $[\alpha]^{26}_{589(a),\ 546(b)}$ $+17.5° \pm 0.6$(a), $+22.0° \pm 0.6$(b). These values did not change on further standing. A third mol of alkali was added and readings taken at intervals showed a gradual decrease in rotation from α $+0.62°$ to α $-0.05°$ after twenty-four hours. A plot of these readings against time gave a smooth hyperbolic curve like that in Fig. 1. This change was more easily and accurately studied by adding 3 mols of alkali directly to II, since the first two mols are used within a few minutes. To 0.1109 g. of II was added 1.64 cc. of 1 *N* sodium hydroxide (3 mols) and a little water. The solution weighed 3.59 g., *d* 1.03. The observed rotations are plotted against time in Fig. 1. After twenty hours α was 0.05° or less, and the *p*H 9.5. In a similar experiment in which 3.1 mols of sodium hydroxide was added, the rotation decreased to 0° within an hour, and in another trial in which 2.5 mols was used, the rotation slowly decreased to a constant value.

Upon addition of 3.1 mols of hydrochloric acid to the practically inactive solution resulting from the action of 3.1 mols of sodium hydroxide on 0.1475 g. of II, 8.69 g. of solution resulted, *d* 1.03, with a rotation $[\alpha]_{589(a),\ 546(b)}$ $+0.31°$(a), $+0.42°$(b); $[\alpha]^{26}_{539(a),\ 546(b)}$ $+18.0° \pm 1.2$(a), $+24.3° \pm 1.8$(b). This was evaporated to dryness, extracted with dioxane and the extract was evaporated. The sirupy residue was treated with excess diazomethane and crystallized from water. The product melted at 105–107° and gave no depression in a mixed melting point determination with II. A solution of 22.0 mg. of this product in dioxane, weighing 1.267 g., *d* 1.03, gave $[\alpha]_{589(a),\ 546(b)}$ $-1.13°$(a), $-1.39°$(b); $[\alpha]^{26}_{589(a),\ 546(b)}$ $-63.3° \pm 3.0$(a), $-77.6° \pm 1.7$(b).

The number of methyl groups liberated by 2 mols of sodium hydroxide was determined by the micro Zeisel procedure modified in the following way. In a 25-cc. distilling flask was put 10.95 mg. of II. A 1-mm. capillary leading to the bottom of this flask, which was closed with a one-hole rubber stopper, was connected through a calcium chloride tube with a carbon dioxide generator. The side-arm was connected by a tight rubber connection directly to the inlet of the micro Zeisel apparatus with its boiling hydriodic acid. To the sample was added 0.180 cc. (2 mols) of 1 *N* sodium hydroxide and a slow current of dry carbon dioxide was bubbled through the solution. A water-bath kept at 60° was placed around the flask. The stream of carbon dioxide was continued for an hour. The residue in the distilling flask appeared dry, and no further precipitate was observed when the silver nitrate receiver was changed at the end of this time. The precipitate, collected in the usual way[5] weighed 9.35 mg., equivalent to 18.5% methoxyl. The residue in the distilling flask dissolved in 1.0 cc. of water gave α_{589} $+0.35°$. The accuracy of the result is limited by the precision with which the sodium hydroxide is measured out. This volume must be kept small to avoid condensation of water on the sides of the distilling flask and to keep the time required for the determination within reasonable limits. Calcd. for $C_7H_7O_5OCH_3$, 15.4% methoxyl.

Titration of Isocitric Acid Lactone.—An aqueous solution of 0.165 g. of isocitric acid lactone in 1.37 g. of solution, *d* 1.05, showed $\alpha_{589(a),\ 546(b),\ 486(c)}$ $-7.90°$(a), $-9.34°$(b), $-11.96°$(c); $[\alpha]^{26}_{589(a),\ 546(b),\ 486(c)}$ $= -62.0° \pm 0.2$(a), $-73.2° \pm 0.3$(b), $-93.6° \pm 4.0$(c). To this was added 0.952 cc. (1 mol) of 1 *N* sodium hydroxide, making the weight of solution 3.52 g., *d* 1.03. For this solution $\alpha_{589(a),\ 546(b),\ 486(c)}$ $-2.04°$(a), $-2.45°$(b), $-3.69°$(c); $[\alpha]^{26}_{859(a),\ 546(b),\ 486(c)}$ $-36.7° \pm 0.2$(a), $-44.8° \pm 0.2$(b), $-66.7° \pm 1.8$(c). Addition of a second mol of sodium hydroxide gave 4.91 g. of solution, *d* 1.03, $\alpha_{589(a),\ 546(b),\ 486(c)}$ $-0.98°$(a), $-1.24°$(b), $-2.07°$(c); $[\alpha]^{26}_{589(a),\ 546(b),\ 486(c)}$ $-22.6° \pm 0.2$(a), $-28.6° \pm 0.7$(b), $-47.8° \pm 4.5$(c). A third mol of sodium hydroxide gave 5.92 g. of solution, *d* 1.03, $\alpha_{589(a),\ 546(b)}$ $-0.07°$(a), $-0.14°$(b); $[\alpha]^{26}_{589(a),\ 546(b)}$ $-1.7° \pm 0.2$(a), $-3.3° \pm 0.5$(b). This solution was slightly alkaline to phenolphthalein and was neutralized by adding 3 drops of 1 *N* hydrochloric acid. This did not measurably alter the rotation. A total of 3 mols (2.86 cc.) of 1 *N* hydrochloric acid was then added, giving 8.71 g. of solution, *d* 1.03, $\alpha_{589(a),\ 546(b)}$ $+0.37°$(a), $+0.53°$(b); $[\alpha]^{26}_{539(a),\ 546(b)}$ $+17.7° \pm 0.01$(a), $+25.3° \pm 0.2$(b).

Acknowledgment is made of the valuable assistance of M. Kunitz in the preparation of the manuscript.

Summary

Dimethyl isocitrate lactone has been prepared in optically pure state from isocitric acid in blackberries. From this pure compound isocitric acid lactone, its triamide, hydrazide and diethyl ester lactone have been prepared and their properties studied. A comparison has been made of the behavior of the acid lactone and the dimethyl ester lactone upon treatment with alkali. A new explanation is given for results which have previously seemed to indicate that the naturally occurring acid varies markedly in optical purity

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[5] Pregl, "Die quantitative organische Mikroanalyse," 1930, pp. 149, 203.

from one sample to another; the formation of some of the lactone in the process of isolating triethyl isocitrate accounts for all the observed results.

PRINCETON, NEW JERSEY RECEIVED JULY 2, 1935

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF WASHINGTON UNIVERSITY]

Intermediate States of Oxidation of Stannous Chloride[1]

BY T. R. BALL, WALTER WULFKUEHLER AND R. E. WINGARD

In studying the reduction of chromic acid by various reducing agents, including stannous chloride, Ball and Crane[2] found evidence for the existence of tin trichloride in solutions partially oxidized. The magneto-optic apparatus of Dr. Fred Allison was used in the previous and the present investigation.

The evidence for the tervalent state of tin is very positive and conclusive in the field of organometallic compounds. Several compounds analogous to triphenylmethyl have been prepared. In dilute solutions in organic solvents, the molecular weights approach those of trialkyl tin compounds containing one tin atom. A few of the more important references are cited.[3]

In the inorganic field the evidence for tin sesquioxide appears to be quite strong. Von Fuchs[4] reduced ferric chloride with stannous chloride and as soon as the solution became green, added calcium carbonate and thus prepared tin sesquioxide. From this he got tin trichloride by solution in hydrochloric acid. However, Mellor[5] states that this is "probably a mixture of stannous and stannic chlorides." Berzelius[6] mixed solutions of stannous and ferric chlorides, each neutralized with ammonia just short of the point of precipitation. The mixture gradually lost its color and a precipitate formed which, upon drying in an atmosphere of carbon dioxide, yielded brownish-black Sn_2O_3. It was soluble in ammonia and was, therefore, not a mixture of stannous and stannic oxides.

Experimental

Procedure.—One-tenth normal stannous chloride was made by dissolving tin in concentrated hydrochloric acid and was kept in tightly stoppered bottles. By a fifty-fold dilution with 3 N hydrochloric acid, 0.002 N solutions were prepared; 250 ml. portions of the dilute solution were partially oxidized with 0.10 N potassium dichromate, added from a micro buret, care being taken to have the stannous chloride in excess at all times. In general, a fresh solution was prepared for each day's work. Observations were made on the magneto-optic apparatus and minima were found at the following positions on the scale (Allison units): 23.56, 23.67, 23.73, 23.83, 23.90, 24.03, 24.12, 24.24, 24.33, 24.39, 24.46. These are attributed to $SnCl_3$ as will be shown later. Blanks were run on the hydrochloric acid and the potassium dichromate and no minima were observed in this region of the scale. These results were obtained by Mr. Wulfkuehler and were later checked by Mr. R. E. Wingard with almost perfect agreement. He had no knowledge of the previous results, other than the fact that minima had been observed between 23 and 25 on the scale. This fact is mentioned because the statement has frequently been made that the minima are purely subjective. In order to ascertain the approximate concentrations of tin trichloride in the solutions studied, one of us (R. E. W.) made a sensitivity curve by the method of Nicol rotation such as was used by Bishop and Dollins[7] in their quantitative determination of calcium. A series of stannic chloride solutions was prepared and the angle through which the analyzing Nicol had to be rotated to cause the disappearance of the minima of the most abundant isotope was determined for each concentration. From these data a sensitivity curve was constructed in which log $1/c$ was plotted against the angular rotation. Solutions of trivalent tin were then prepared and the angle again determined. The concentration was read off on the curve, assuming that the curve would apply to the trivalent ion. The original unoxidized solutions had a total tin concentration of about 6 parts in 10^5 of solution. After partial oxidation with dichromate, the maximum concentration of $SnCl_3$ observed in any of the tests was about 2 parts in 10^9. From this it may be calculated that only about 0.01% of the tin was in the trivalent form.

Discussion

Slack[8] and MacPherson[9] have stated that the

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[1] This work was made possible by assistance to the senior author from a grant made by The Rockefeller Foundation to Washington University for research in science. It was presented at the Regional Meeting of the American Chemical Society in Kansas City, May, 1934.

[2] Ball and Crane, THIS JOURNAL, **55**, 4860 (1933).

[3] (a) Ladenburg, *Ber.*, **4**, 19 (1871); (b) Rügheimer, *Ann.*, **364**, 51 (1909); (c) Kraus, *Rec. trav. chim.*, **42**, 588 (1923); (d) Böeseken and Rutgers, *ibid.*, **42**, 1017 (1923); (e) Kraus and Sessions, THIS JOURNAL, **47**, 2361 (1925).

[4] Von Fuchs, *J. prakt. Chem.*, [1] **5**, 318 (1835).

[5] J. W. Mellor, "Comprehensive Treatise on Inorganic and Theoretical Chemistry," Longmans, Green and Co., New York, 1927, Vol. VII, p. 424.

[6] Berzelius, *Pogg. Ann.*, **28**, 443 (1833).

[7] Bishop, Dollins and Otto, THIS JOURNAL, **55**, 4365 (1933).

[8] Slack, *J. Franklin Inst.*, **218**, 445 (1934). This paper contains a complete bibliography of publications on the magneto-optic method.

[9] MacPherson, *Phys. Rev.*, **47**, 254 (1935).