
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

VOLUME 67

JANUARY 8, 1945

NUMBER 1

Some Salts of Aconitic Acid¹

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It has long been known that aconitic acid is the principal organic acid in sugar cane juice³ and is present in sorgo.⁴ There was no practical way of obtaining it from either of these natural sources until Ventre⁵ found that, during the evaporation of sorgo juice for the production of sugar, an insoluble calcium aconitate separates from the evaporator sirups in quantities sufficient to make its recovery feasible as a by-product of the sorgo sugar process. In order to ascertain the best method of eliminating this insoluble aconitate from sirups its properties were investigated and compared with those of tricalcium aconitate prepared from solutions of pure aconitic acid. A search of the literature revealed that tricalcium aconitate has been incompletely and confusingly described by previous workers. Amorphous forms of this salt have been reported by Baup,⁶ Guinochet,⁷ and Yoder.^{3c} The crystalline salt has been characterized as a hexahydrate by Baup,⁶ Behr,^{3a} v. Lippmann,⁸ and Prinsen-Geerligs,^{3f} and as a trihydrate by Guinochet.⁷ No one has stated

that both hydrates are obtainable. Parsons,^{4a} due to erroneous interpretation of his analytical method and data, described the insoluble aconitate from sorgo juice as the calcium acid salt which Guinochet⁷ stated is an amorphous water-soluble glass.

In addition to the methods previously described for the formation of amorphous tricalcium aconitate,^{3a,6,7} we have found that it separates in water-soluble form from alkaline solutions of the salt on standing at room temperature and from alkaline or acid solutions by precipitation with alcohol. At temperatures up to 70° solutions of tricalcium aconitate slowly deposit crystals of the hexahydrate, while at temperatures above 75° they yield the trihydrate. Solutions prepared by double decomposition of sodium aconitate and calcium acetate or chloride deposit at temperatures up to 60° crystals of calcium sodium aconitate dihydrate, $\text{CaNaAcon}\cdot 2\text{H}_2\text{O}$. Preparations of the trihydrate of tricalcium aconitate from solutions containing appreciable quantities of sodium ions were contaminated with this double salt.

The optical-crystallographic properties of these calcium aconitates were not those of the insoluble aconitates obtained by McCalip and Seibert⁹ and by Ventre⁵ from sugar plant products and processes. Published analyses of the material from sirups,^{4a,9} and all analyses (unpublished) of the material obtained by Ventre⁵ showed the presence of relatively large proportions of magnesium. Parsons^{4a} assumed the magnesium was present as the oxide or hydroxide which had been retained in colloidal suspension in the juice until occluded by the aconitate. Ventre and Paine⁵ refer to their material as a physical admixture of calcium and magnesium aconitates. However, the insoluble materials obtained by McCalip and Seibert⁹ and

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(4) (a) H. B. Parsons, *Am. Chem. J.*, **4**, 39 (1882); (b) H. W. Wiley and C. Maxwell, *ibid.*, **12**, 216 (1890).

(5) E. K. Ventre, *The Sugar J.*, **3**, No. 7, 23 (1940); *Sugar*, **35**, No. 1, 36 (1941); E. K. Ventre and H. S. Paine, U. S. Patent 2,280,085, Apr. 21, 1942.

(6) S. Baup, *Ann.*, **77**, 293 (1851).

(7) E. Guinochet, *Compt. rend.*, **94**, 455 (1882).

(8) R. O. v. Lippmann, *Ber.*, **12**, 1649 (1879).

(9) M. A. McCalip and A. H. Seibert, *Ind. Eng. Chem.*, **33**, 637 (1941); *The Sugar Bull.*, **19**, no. 17, 84 (1941).

by Ventre⁵ have the optical-crystallographic characteristics of dicalcium magnesium aconitate hexahydrate, $\text{Ca}_2\text{MgAcon}_2 \cdot 6\text{H}_2\text{O}$, although their magnesium content is generally less than that of this salt. Preparations of this substance have been made which contain magnesium in quantities ranging from almost theoretical down to approximately 25% of theory, and which, regardless of the magnesium content within these limits, are homogeneous and show identical optical-crystallographic properties. These preparations and the insoluble aconitates from the sirups may be solid solutions of tricalcium aconitate hexahydrate and either trimagnesium aconitate⁷ or dicalcium magnesium aconitate.

As described by Guinochet,⁷ calcium acid aconitate, CaHAcon , is an amorphous glass extremely soluble in cold water. Attempts to prepare monocalcium aconitate, $\text{Ca}(\text{H}_2\text{Acon})_2$, resulted in mixtures of free aconitic acid and the calcium acid salt, CaHAcon .

The optical-crystallographic properties of the crystalline salts discussed above, of tricalcium aconitate hexahydrate and monopotassium aconitate, both described by Guinochet,⁷ and of the hitherto undescribed magnesium acid aconitate tetrahydrate and zinc acid aconitate tetrahydrate are recorded in Table I.

Experimental

Tricalcium Aconitate Hexahydrate, $\text{Ca}_3\text{Acon}_2 \cdot 6\text{H}_2\text{O}$,^{3a,7,6,8} and Trihydrate, $\text{Ca}_3\text{Acon}_2 \cdot 3\text{H}_2\text{O}$.⁷—Tenth-molar solutions of tricalcium aconitate were prepared by double decomposition of potassium or ammonium aconitate¹⁰ with an excess of calcium chloride or acetate and adjusted to pH 6.3 to 6.8 (glass electrode).¹¹ Since these solutions were supersaturated, only freshly prepared solutions were used. After the solution had been brought as rapidly as possible and without agitation to the temperature desired, crystallization was induced by rubbing the sides of the vessel with a glass rod. The crystals deposited in hard crusts which adhered to the sides of the vessel. The crusts were loosened and broken up several times during the heating in order to distribute the material throughout the solution. When the solutions were left undisturbed in new glassware, the deposition of crystals was often delayed for several hours.

At temperatures up to 70°, preferably at 60 to 65°, the crystals which deposited were the hexahydrate, and at temperatures above 80° the trihydrate. The latter deposited from solutions of a wide range of pH, crystals of high purity having been obtained from solutions of pH 4.5 and from solutions that were faintly ammoniacal. Maximum yields were obtained from solutions whose pH values were 6.5 to 6.8. Optical-crystallographic properties of both hydrates are given in Table I. Yields of hexahydrate were 2.2 to 2.8 g. per 100 ml. in twenty-four hours; of trihydrate, 2.6 to 3.6 g. per 100 ml. in four to five hours.

Anal. Calcd. for $\text{Ca}_3\text{Acon}_2 \cdot 6\text{H}_2\text{O}$: Ca, 21.06; H_3Acon , 61.0. Found, up to 70°: Ca, 21.06, 21.02, 21.19; H_3Acon

isolated,¹² 61.1, 60.4, 60.9. Calcd. for $\text{Ca}_3\text{Acon}_2 \cdot 3\text{H}_2\text{O}$: Ca, 23.25; H_3Acon , 67.4. Found: 80 to 95+°: Ca, 23.10, 23.26, 23.24; H_3Acon isolated, 67.1, 66.9, 67.1.

Crystals of the hexahydrate suspended in water in a loosely stoppered flask and heated at 80° for twenty-four hours were converted to the trihydrate: Ca found before heating, 21.16, 21.11%; Ca found after heating, 23.27, 23.01%.

The dry hexahydrate began to lose water of crystallization rapidly at 70° and came to constant weight at 110–120° with the loss of the equivalent of 4 molecules of water. There was no further loss of weight up to 140°. The trihydrate was stable when heated to 140° and began to lose water of crystallization at 150°.

Anal. Calcd. for $4\text{H}_2\text{O}$ lost from $\text{Ca}_3\text{Acon}_2 \cdot 6\text{H}_2\text{O}$: H_2O , 12.63; loss on heating to 120° to const. weight (24 hours), 12.65, 12.68, 12.38, 12.44, 12.60. Calcd. for $\text{Ca}_3\text{Acon}_2 \cdot 2\text{H}_2\text{O}$: Ca, 24.11. Found after heating to 120° for 24 hours: Ca, 23.96, 23.96.

On standing at room temperature, alkaline solutions of tricalcium aconitate slowly deposited an amorphous salt which was more soluble in cold water than the crystalline hexahydrate. When equal volumes of ethyl alcohol were added to solutions of calcium aconitate of pH ranging from 3.0 to 7.0, either at room temperature or on the steam-bath, voluminous precipitates of amorphous tricalcium aconitate hexahydrate were formed. These coagulated on heating, were very soluble in cold water, and changed to crystalline forms only on very prolonged digestion. On being heated, the dry material gradually and continuously gave up water of hydration until at 280° it became anhydrous and turned brown. It also lost water of hydration in a vacuum desiccator over calcium chloride at room temperature.

Anal. Calcd. for $\text{Ca}_3\text{Acon}_2 \cdot 6\text{H}_2\text{O}$: Ca, 21.06; H_2O , 18.94. Found: Ca, 21.27, 20.64, 21.07, 20.92; loss at 280°, 20.75, 20.87, 20.10, 19.05.

Calcium Acid Aconitates.—When evaporated without agitation, solutions of aconitic acid in which sufficient calcium carbonate to form calcium acid aconitate, CaHAcon , had been dissolved dried to an amorphous glass as described by Guinochet.⁷ However, when these solutions were heated in the steam-bath and the sides of the vessel were rubbed with a rod, crystals of tricalcium aconitate trihydrate were deposited. When an equal volume of alcohol was added, voluminous precipitates of water-soluble amorphous calcium aconitate were formed.

Solutions prepared from aconitic acid and calcium carbonate in the stoichiometric proportions for $\text{Ca}(\text{H}_2\text{Acon})_2$ dried at room temperature to a semi-crystalline mass of free aconitic acid and glassy calcium acid aconitate. This material, after being powdered and dried *in vacuo* at 70°, was extracted with ethyl acetate and lost 39.6% in weight, leaving a residue which contained 17.77% Ca; calcd. for CaHAcon , 17.39% Ca. Aconitic acid of melting point 188° was recovered from the extract. Solutions prepared for $\text{Ca}(\text{H}_2\text{Acon})_2$, when heated in the steam-bath and induced to crystallize, deposited tricalcium aconitate trihydrate; when alcohol was added to them, it precipitated soluble amorphous calcium aconitate, whose solution was acidic in reaction.

Calcium Sodium Aconitate Dihydrate, $\text{CaNaAcon} \cdot 2\text{H}_2\text{O}$.—On standing at room temperature for twenty-four hours or longer, 0.2 molar solutions of sodium aconitate (pH 6.5 to 6.8), in which an equivalent quantity, or an excess, of calcium acetate or chloride had been dissolved, deposited loosely adherent crystals of a double aconitate of calcium and sodium of the composition $\text{CaNaAcon} \cdot 2\text{H}_2\text{O}$. This salt was stable in water up to 60°, but slowly decomposed in water at higher temperatures leaving an in-

(10) The aconitic acid used was made from citric acid by Bruce's method, "Org. Syntheses," 17, 1 (1937), and recrystallized from hydrochloric or acetic acid until it melted at 184° or higher.⁹

(11) Solutions made by neutralizing with calcium hydroxide deposited poorly crystalline salts. Solutions of the concentration and pH used could not be prepared by neutralization with calcium carbonate.

(12) Values reported were obtained by extracting the acid from hydrochloric acid solution with ether for twenty-four hours in continuous extractor, volatilizing the ether, drying the residue *in vacuo* and weighing, dissolving the residue in water, titrating the solution with standard alkali, and averaging the value calculated from the weight with that calculated from the titration.

TABLE I
OPTICAL-CRYSTALLOGRAPHIC PROPERTIES OF ACONITATES

Salt	Crystal form	Indices of refraction ^a (immersion method)				Extinction	Interference figures
		n_α	n_β or n_i ^b	n_γ	Double refraction ($n_\gamma - n_\alpha$)		
$\text{Ca}_3\text{Acon}_2 \cdot 6\text{H}_2\text{O}$	Rhomb-like or 6-sided prisms	1.497	1.545	1.620	0.123	Optic axis figs.
$\text{Ca}_3\text{Acon}_2 \cdot 3\text{H}_2\text{O}$	Rods and plates	1.520	indet.	1.639	.119	Parallel	None
$\text{Ca}_2\text{MgAcon}_2 \cdot 6\text{H}_2\text{O}^c$	Rhombs, prisms	1.504	1.560	1.643	.139	Partial figs.
$\text{CaNaAcon} \cdot 2\text{H}_2\text{O}$	Irreg. fragments, wedge-shaped forms	1.457	1.572 ^b	1.626	.169	None
$\text{Cd}_3\text{Acon}_2 \cdot 6\text{H}_2\text{O}^7$	Prisms	1.590	1.602	1.620	.030	Optic axis figs.
$\text{MgHAcon} \cdot 4\text{H}_2\text{O}$	Small prisms	1.447	indet.	1.660	.213	None
$\text{ZnHAcon} \cdot 4\text{H}_2\text{O}$	Small prisms	1.468	indet.	1.690	.222	None
KH_2Acon^7	Thin plates	1.459	^d	1.657	.198	None

^a All indices ± 0.002 . ^b n_i = an intermediate refractive index. ^c Also insoluble aconitates from sugar plant products and aconitates containing Ca and Mg in atomic ratios between 2.75:0.25 and 2:1. ^d Not determined, probably shown only when plates are tipped on edge.

soluble mixture richer in calcium. The same double salt was formed from solutions of tricalcium aconitate to which an excess of sodium chloride or acetate had been added. Neither of the hydrates of tricalcium aconitate could be prepared in pure form from solutions containing appreciable quantities of sodium ions. The double salt was insoluble in dilute acetic acid, but soluble in dilute hydrochloric and nitric acids. It was slightly hygroscopic. Samples dried at 100° to constant weight began to lose water of crystallization at 110° and became anhydrous at 170° with slight decomposition and discoloration. The optical-crystallographic properties are given in Table I.

Anal. Calcd. for $\text{CaNaAcon} \cdot 2\text{H}_2\text{O}$: Ca, 14.81; Na, 8.52; H_2Acon , 64.4; H_2O , 13.33. Found: Ca, 14.76, 14.86, 14.81, 14.71; Na, 8.24, 8.39; H_2Acon isolated, 62.2, 62.9; loss at 170°, 13.92, 13.28, 13.32.

Dicalcium Magnesium Aconitate Hexahydrate, $\text{Ca}_2\text{MgAcon}_2 \cdot 6\text{H}_2\text{O}$, and Solid Solutions of Calcium Aconitate Containing Magnesium.—Sufficient calcium carbonate was dissolved in a 0.2 M solution of aconitic acid to form the hypothetical salt $\text{Ca}(\text{H}_2\text{Acon})_2$ and the resulting solution was neutralized to pH 6.4 (glass electrode) with magnesium oxide and filtered. A portion of the filtrate was heated for twenty-four hours at 60 to 65° and another portion for four hours in the steam-bath (95+°). Crystallization was induced as in the tricalcium aconitate preparations. The precipitates, which separated rather easily, were more coarsely crystalline and less adherent than the hexahydrate of tricalcium aconitate and approximated dicalcium magnesium aconitate hexahydrate, $\text{Ca}_2\text{MgAcon}_2 \cdot 6\text{H}_2\text{O}$, in composition. The same salt was formed at 95+° from solutions of tricalcium aconitate containing magnesium acetate in such quantities that the atomic ratios of Mg:Ca were approximately 2:3 and 4:3. The preparation of crystals having the exact composition of dicalcium magnesium aconitate was difficult; generally the precipitates contained less than the theoretical quantity of magnesium although they were homogeneous and showed the same optical-crystallographic properties as those of dicalcium magnesium aconitate (Table I). Preparations of homogeneous crystals ranging in composition from $\text{Ca}_2\text{MgAcon}_2 \cdot 6\text{H}_2\text{O}$ to $\text{Ca}_{2.75}\text{Mg}_{0.25}\text{Acon}_2 \cdot 6\text{H}_2\text{O}$ were obtained (1) from solutions prepared as for tricalcium aconitate to which widely varying proportions of magnesium acetate or chloride were added, (2) from solutions of trimagnesium aconitate to which varying quantities of calcium acetate were added, and (3) from solutions containing calcium and magnesium in varying proportions to each other but in quantities the sum of which was stoichiometrically equivalent to the aconitic acid present. No crystals having the optical-crystallographic properties of either hydrate of tricalcium aconitate could be detected in preparations having compositions within the range given above.

Dicalcium magnesium aconitate hexahydrate and the

aconitates containing less magnesium were less soluble than either of the hydrates of tricalcium aconitate, since they, rather than tricalcium aconitate, deposited at all temperatures up to 100°. They were insoluble in dilute acetic acid, but soluble in dilute hydrochloric and nitric acids. The dry salt lost but a slight amount of water of crystallization at 70–80° and came to constant weight at 140° with the loss of 4 molecules of water. Some specimens of the series of solid solutions came to constant weight at 110 to 130° with losses equivalent to between 2 and 3 molecules of water.

Anal. Calcd. for $\text{Ca}_2\text{MgAcon}_2 \cdot 6\text{H}_2\text{O}$: Ca, 14.43; Mg, 4.39; H_2Acon , 62.8. Found: Ca, 14.07, 14.57, 14.11, 15.05; Mg, 4.53, 4.27, 4.63, 4.07; H_2Acon isolated, 61.3, 61.2, 61.7. Calcd. for loss of 4 H_2O from $\text{Ca}_2\text{MgAcon}_2 \cdot 6\text{H}_2\text{O}$: H_2O , 12.99. Found: loss at 140° to const. weight (20–24 hours), 13.16, 12.66, 12.90, 13.16.

Magnesium Acid Aconitate Tetrahydrate, $\text{MgHAcon} \cdot 4\text{H}_2\text{O}$.—A suspension of 30 parts of aconitic acid in 100 parts of water was neutralized with magnesium oxide and the resulting solution was filtered. To the filtrate 15 parts of aconitic acid were added and dissolved by heating. The solution was again filtered and concentrated on the steam-bath until a scum formed on top. Concentration was continued with slow stirring until a thick paste had formed. The solid was removed by filtration while hot, washed with a small amount of cold water, and dried for forty-eight hours over calcium chloride. By concentrating the mother liquors with stirring, second and third crops were obtained. The total yield was 64 parts. It was recrystallized from boiling water by concentration with slow stirring. It is soluble in cold water, forming a solution which resembles magnesium citrate in taste. The salt began to lose water rapidly at 110° and decomposed at 120°. The optical-crystallographic properties are given in Table I.

Anal. Calcd. for $\text{MgHAcon} \cdot 4\text{H}_2\text{O}$: Mg, 9.06; H_2Acon , 64.9; neutralization equivalent, 268.2. Found: Mg, 8.98, 9.03; H_2Acon isolated, 63.0; neutralization equivalent, 275.2, 273.2.

Zinc Acid Aconitate Tetrahydrate, $\text{ZnHAcon} \cdot 4\text{H}_2\text{O}$.—To prepare this salt it is essential that the solution be kept cold to avoid separation of the very insoluble trizinc salt as a viscous liquid described by Guinocet.⁷ Ten parts of aconitic acid were covered with 50 parts of water, and 4.7 parts of zinc oxide were added slowly and dissolved at room temperature. The solution was filtered and the filtrate was allowed to concentrate at room temperature in a vacuum desiccator over sulfuric acid or calcium chloride. When crystals started to form the solution was placed in a refrigerator overnight. The salt was filtered off, washed with a small amount of cold water, and dried over calcium chloride. The yield was 12 parts. The salt was very soluble in cold water. It began to lose water of crystal-

lization very rapidly at 60° and decomposed at 110°. Optical-crystallographic properties are given in Table I.

Anal. Calcd. for $ZnHAcon \cdot 4H_2O$: Zn, 21.14; H_3Acon , 56.2; neutralization equivalent, 309.5. Found: Zn, 21.13, 21.10; H_3Acon isolated, 55.1; neutralization equivalent, 324.

Tricadmium Aconitate Hexahydrate, $Cd_3Acon_2 \cdot 6H_2O$.⁷—This salt, described by Guinochet,⁷ was prepared by double decomposition from hot solutions of sodium aconitate and cadmium chloride or acetate. The optical-crystallographic properties are given in Table I.

Anal. Calcd. for $Cd_3Acon_2 \cdot 6H_2O$: Cd, 42.72; H_3Acon , 44.2. Found: Cd, 42.43, 42.63; H_3Acon isolated, 43.9, 41.8.

Monopotassium Aconitate, KH_2Acon .⁷—The optical-crystallographic properties of this salt, described by Guinochet,⁷ are given in Table I.

Anal. Calcd. for KH_2Acon : K, 18.43; H_3Acon , 82.1; neutralization equivalent, 106.1. Found: K, 18.23, 18.22; H_3Acon isolated, 81.3; neutralization equivalent, 106.3, 106.4.

Acknowledgment.—Thanks are due to A. L. Curl, Bureau of Agricultural and Industrial Chemistry, U. S. Department of Agriculture, for his aid in making the isolations and determinations of aconitic acid.

Summary

1. The insoluble aconitates which separate from sugar cane and sorgo sirups have been identified as calcium magnesium aconitates having the optical-crystallographic properties of dicalcium magnesium aconitate hexahydrate, although they generally contain less than the theoretical proportion of magnesium. It is suggested that they are solid solutions of tricalcium aconitate hexahydrate with either trimagnesium aconitate or dicalcium magnesium aconitate hexahydrate.

2. The preparation and properties of crystalline tricalcium aconitate hexahydrate, tricalcium aconitate trihydrate, calcium sodium aconitate dihydrate, dicalcium magnesium aconitate hexahydrate, magnesium acid aconitate tetrahydrate, and zinc acid aconitate tetrahydrate are described.

3. Optical-crystallographic properties and indices of refraction are given for the above salts and for tricadmium aconitate hexahydrate and monopotassium aconitate.

RECEIVED MARCH 13, 1944

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, NORTHWESTERN UNIVERSITY MEDICAL SCHOOL]

Monolayers of Egg Albumin on Concentrated Salt Solutions¹

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An examination of the literature^{2,3,4,5} reveals a disconcerting lack of agreement between the results of various workers on the force area curves of spread films of such well defined proteins as egg albumin. To anyone who has done experiments on spread films of proteins this lack of agreement is not surprising. It is by no means an easy task to obtain complete spreading of a protein on a dilute buffer solution; some protein is very apt to be lost in the underlying buffer solution. In addition to the difficulty of obtaining complete spreading is the complication due to surface active contaminants to which sufficient attention has not always been paid. There is another and possibly more important reason for such divergent results as have been obtained; the peptide chains in the protein molecules may spread apart on the surface and the extent to which this spreading occurs would be expected to be a function of a number of factors. This point will be discussed later.

The author feels that he has improved the tech-

nique of protein spreading and of the measurement of force area curves in two respects. First, a Wilhelmy balance employing a good analytical balance has been substituted for the conventional mica float thus avoiding all question of leakage around the mica float. The use of the Wilhelmy balance was suggested by the work of Harkins and Anderson.⁶ Second, a concentrated solution of ammonium sulfate has been used as the underlying solution upon which the protein film is spread. It had been noted by Gorter⁷ that the spreading of proteins into surface monolayers appeared to be much more rapid and complete as the electrolyte concentration of the underlying solution was increased. The results of our investigations along this line were so encouraging that 35% ammonium sulfate solution has been used as the underlying solution in all the experiments reported in this paper. It was found very helpful to enclose the entire apparatus in a cabinet which protected the surface of the ammonium sulfate solution from accidental contaminants from the air.

Experimental

Egg albumin was prepared from fresh hen's eggs by the method of Kekwick and Cannan.⁸ It was recrystallized three times and dialyzed against distilled water until

(1) Presented at the Symposium on Surface Active Agents and their Application to Biological Systems held by the Division of Physical Chemistry of the American Chemical Society at Cleveland, Ohio, April 4, 1944.

(2) Gorter and Philippi, *Proc. Acad. Sci. Amsterdam*, **37**, 788 (1934).

(3) Gorter, Van Ormondt and Dom, *ibid.*, **35**, 838 (1932).

(4) Philippi, On the Nature of Proteins. Thesis, University of Leyden, 1936.

(5) Bull, *J. Biol. Chem.*, **125**, 585 (1938).

(6) Harkins and Anderson, *THIS JOURNAL*, **59**, 2189 (1937).

(7) Gorter, *Proc. Acad. Sci. Amsterdam*, **37**, 20 (1934).

(8) Kekwick and Cannan, *Biochem. J.*, **30**, 227 (1936).