Summary.

The work showed that the addition of a methyl group to the nitrogen atom of the condensation products of the *ortho*- and *para*-hydroxy aromatic aldehydes with quinaldin gave these compounds the properties of indicators, but that compounds not possessing these *ortho*- or *para*-phenol groups did not acquire this property. A little preliminary work was done to determine the suitability of these compounds to serve as indicators in volumetric analysis.

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THE C_4-SACCHARINIC ACIDS. I. THE RESOLUTION OF *dl*-2,3-DIOXYBUTYRIC ACID INTO THE OPTICALLY-ACTIVE COMPONENTS. THE DERIVATIVES OF THESE ACIDS.

BY J. W. E. GLATTFELD AND GEORGE E. MILLER.[1]

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The saccharinic acids are acids which would result from the oxidation of the $H - \underset{|}{C} = O$ group of an aldo-monosaccharide of the formula $C_nH_{2n}O_n$ to the $O = \underset{|}{C} - OH$ group at the expense of one of the $H - \underset{|}{\overset{|}{C}} - OH$ groups which would be reduced to the $H - \underset{|}{\overset{|}{C}} - H$ group. These acids have the same molecular formula as the corresponding aldo-monosaccharides and may thus be looked upon as the results of an internal oxidation-reduction reaction. The saccharinic acids formed from some of the pentoses and hexoses have been the direct subject of study[2] by Nef and his students at various times, and have also entered into consideration in connection with all the sugar-oxidation experiments conducted in this laboratory in recent years. These oxidations were carried out in alkaline solution, under which conditions the production of saccharinic acids was a possibility. In a recent paper from this laboratory[3] there was reported, in the oxidation of maltose in alkaline solution, the production of an acid, the phenylhydrazid of which gave a perfect analysis for that of a 4-carbon-atom saccharinic acid. The properties of the free acid furthermore were those which would be expected of one of these acids. Its configuration could not be reported because of lack of

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[1] The dissertation of which this paper is a condensation was presented by George E. Miller as part fulfilment of the requirements for the degree of Doctor of Philosophy in the University of Chicago. The original dissertation is on file in the University library.

[2] Nef, *Ann.*, 376, 1–120 (1910).

[3] THIS JOURNAL, 40, 973 (1918).

data as to the properties and constants of the 4-carbon-atom saccharinic acids.

Nef has referred[1] to the handicap which this lack of data has imposed on the work with sugars in alkaline solution. In order to supply some of this deficiency we have attempted the preparation of the saccharinic acids in a systematic way and have started with the C_4-saccharinic acids to throw light on the first question of interest in this connection, namely, which of the possible optically-active C_4-saccharinic acids is formed in the oxidation of maltose in alkaline solution. The present paper is a report on the preparation of the first pair of saccharinic acids, the 2,3-dioxy-butyric acids. Work on the preparation of the other 8 active acids is now in progress.

The *dl*-2,3-dioxy-butyric acid has been prepared by Hanriot.[2] The synthesis was repeated by Nef[3] who studied the acid in somewhat more detail and reported a few of its properties as well as those of the phenylhydrazid and brucine salt. The constitution of the *dl*-acid was proved by Nef[2] by the method of synthesis and by oxidation to malic acid. We prepared our acid in accordance with the procedure of these investigators. The analysis of the vacuum-dried barium salt showed the substance to be pure. The acid was finally obtained by precipitating the barium from an aqueous solution of the salt quantitatively by means of sulfuric acid. The solution of the acid was subjected to complete distillation *in vacuo* and the acid was then obtained as a slightly yellow oil. It was freed from inorganic salt by repeated solution in ethyl acetate, and was then used in the resolution described below.

The phenylhydrazid was made according to Nef's directions.[3] After many recrystallizations it was found to melt at 100–101° instead of at 99° as reported by Nef.

In order to prove that the acid was actually *dl*-2,3-dioxy-butyric acid it was prepared also from vinylacetic acid $CH_2 : CHCH_2COOH$ by oxidation with permanganate. The vinylacetic acid was prepared according to the directions of Hauber[4] and oxidized according to the directions of Penschuk.[5] The acid so prepared gave a phenylhydrazid which melted at 100–101°. A mixed melting-point determination of this phenylhydrazid with the one from the *dl*-acid above, showed them to be identical.

Fichter and Sonneborn[6] also prepared the 2,3-dioxy acid from vinylacetic acid by the above method and state that the acid is identical with that made by Hanriot from the α-chlorohydrine. As they base their

[1] Nef, *Ann.*, **376**, 9 (1910).

[2] Hanriot, *Ann. chim. phys.* [5] **17**, 62 (1879).

[3] Nef, *Ann.*, **376**, 35 (1910).

[4] Hauber, *Ber.*, **36**, 2897 (1903).

[5] Penschuk, *Ann.*, **283**, 109 (1894).

[6] Fichter and Sonneborn, *Ber.*, **35**, 942 (1902).

statement only on the analysis of the barium salt for barium oxide, which would obviously be the same for the barium salts of all dioxy-butyric acids, they cannot be said to have proved the identity of the acids from the two sources. It was, therefore, considered necessary to make the proof of the identity absolute by preparing the phenylhydrazids and proving them identical before proceeding with further studies of the acid.

The Resolution of the *dl*-2,3 Dioxy-butyric Acid into the Active Components.

Brucine, cinchonine, quinine and strychnine were tried and brucine found to be best for the resolution. The brucine salt was made in the usual way[1] by adding a slight excess of the alkaloid to an aqueous solution of the acid and heating the mixture on the water-bath. The use of an electrically-driven mechanical stirrer greatly hastened the solution of the alkaloid.

Eighty-three g. of the *dl*-acid and 305 g. of brucine were heated in about 2 liters of water on the boiling water-bath until the solution reacted alkaline to litmus. Three g. of brucine did not go into solution. After extraction with benzol and subjection of the extracted solution to complete vacuum distillation, 337 g. of crude brucine salt was obtained. This was treated with 750 cc. of boiling absolute alcohol. Fifteen g. of flocculent material remained undissolved. This material was separated by hot filtration. The clear filtrate was allowed to stand overnight and deposited crystals which weighed 58.4 g. The mother liquor was subjected to complete vacuum distillation at 60°. The residue, weighing 257.5 g., was dissolved in 300 cc. of boiling absolute alcohol. This solution deposited crystals which weighed 13.55 g. The mother liquor from the second crop was subjected to complete vacuum distillation at 70°. The residue weighed 204 g. This was treated with 140 cc. of boiling alcohol. This solution deposited a third crop of crystals which weighed 54.8 g. A fourth crop of crystals which weighed 17 g. was obtained in the same way by treating the residue, which weighed 141 g., with 72 cc. of hot absolute alcohol. The mother liquor from the fourth crop was subjected to complete vacuum distillation. The residue weighed 130 g. No more crystals could be obtained.

The rotations of these 4 crops of brucine salts were taken in exactly 4% aqueous solution. The density of the solution was considered to be 1.011. The following results were obtained.

Crop.	$[\alpha]_D^{20}$.	α in one dcm. tube.
I	—29.42	—1.19
II	—27.95	—1.12
III	—27.95	—1.12
IV	—26.95	—1.09

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[1] THIS JOURNAL, 40, 976 (1918). Footnote.

The rotation of the successive crops of brucine salts convinced us that a partial separation of the *dl*-acid into its optical components had been effected. Our next effort was, therefore, to obtain the less soluble brucine salt in its purest form. Another quantity of crude brucine salt was prepared and recrystallized as above. Again the specific rotation of the first crop was —29.42°. The successive crops had approximately the same rotations as the corresponding ones in the first experiment. The salt with the specific rotation of —29.42° was now dissolved in the smallest possible quantity of hot absolute alcohol. The solution deposited a crop of crystals whose specific rotation was taken. This crop was again dissolved in the smallest possible quantity of absolute alcohol and allowed to stand overnight during which time it deposited a crop of crystals, which was separated by filtration and dried to constant weight *in vacuo* over sulfuric acid. The rotation of this crop was taken. This process was repeated 7 times. The rotations of the successive crops were invariably between —29.18° and —29.42°. It was, therefore, concluded that the *pure* brucine salt of one of the optical isomers had the specific rotation of approximately —29.42°.

The Free Acid from the Brucine Salt, $[\alpha]_D^{20}$, —29.42°.—One hundred and thirty-seven g. of the brucine salt, $[\alpha]_D^{20}$ —29.42°, was dissolved in about 4 liters of hot water and treated with a hot solution of 118 g. of crystallized barium hydroxide in the usual way[1] to remove brucine from its salts. After the removal of brucine by filtration and extraction of the filtrate with benzol, the barium was removed with sulfuric acid and the filtrate from the barium sulfate was subjected to complete vacuum distillation. The residue was taken up in absolute alcohol and the solution was filtered to remove any inorganic salts present. The alcohol was completely removed by distillation *in vacuo* finally at 100°, and the acid was left as a clear yellow and very mobile oil.

The rotation of a portion of this acid was then taken and another portion was titrated. Other portions were converted into the barium and calcium salts and the phenylhydrazid.

Rotation.—The specific rotation of the acid in approximately 4% solution was found to be —8.29°, *i. e.*, 2.32 g. acid dissolved in 45.78 g. water gave α in a one dcm. tube —0.40°. The density of the solution was assumed to be 1.00 and the temperature was approximately 20°.

Titration.—The titration showed that the acid was present largely as lactone. The amounts of free acid and lactone were determined[2] and the following results obtained.

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[1] THIS JOURNAL, **40**, 981 (1918), footnote 2.

[2] The method of determining the free acid and lactone in a mixture of the two is as follows. The weighed sample is dissolved as rapidly as possible in cold water and immediately titrated to pink with 0.1 *N* sodium hydroxide solution, using phenolphthalein as indicator. A quantity of 0.1 *N* sodium hydroxide solution in excess of

	Weight of sample. G.	0.1 *N* NaOH for free acid. Cc.	0.1 *N* NaOH for lactone. Cc.	Total NaOH used. Cc.	Calc. for C_4 saccharinic acid.[a] Cc.
I........	0.4765	5.72	39.75	45.47	45.70
II.......	0.4843	5.83	40.76	46.59	46.47

[a] In calculating the theoretical cc. account must, of course, be taken of the amounts of free acid and lactone present in the samples as indicated by the titration figures.

These titrations show that about 14.4% of the sample in water solution exists as free acid and the rest as lactone. This agrees with Nef's observations.[1]

The Barium Salt.—A mixture of one g. of acid. 3 g. of barium carbonate and 75 cc. of water was heated to make this salt. The salt was gummy and could not be made to crystallize except by triturating with absolute alcohol. There was obtained 0.4 g. of the vacuum-dried salt; $[\alpha]_D^{20}$ +1.48°, *i. e.*, 0.4 g. salt in 9.6 g. water gave α +0.03° in a half-decimeter tube.

The Calcium Salt.—A mixture of 1.2 g. of acid, 3 g. of calcium carbonate and 75 cc. of water was heated to make this salt. It became crystalline only when triturated with absolute alcohol. There was obtained 0.4 g. of vacuum-dried salt; rotation, $[\alpha]_D^{20}$ +2.47°, *i. e.*, 0.4 g. of salt in 9.6 g. water gave α +0.05° in a half-decimeter tube.

The Phenylhydrazid.—A mixture of 1.75 g. of acid, 2 cc. of phenylhydrazine and 2 cc. of ethyl acetate gave, in the usual way 3.15 g. of the crude phenylhydrazid. This was recrystallized from 18 cc. of ethyl acetate and gave one g. of phenylhydrazid with the melting point of 102–103°. This was recrystallized from 3.7 cc. of ethyl acetate and gave 0.75 g. of crystals with a melting point of 102–103°. The specific rotation of this compound was found to be +1.71°, *i. e.*, 0.54 g. in 12.06 g. of water gave α +0.07° in a one dcm. tube.

The Free Acid from the Non-crystallizable Brucine Salt.—The mother liquor from the 4 crops of crystalline brucine salts was subjected to complete vacuum distillation at 60° and gave a residue which weighed 130 g. as mentioned above. The brucine was set free in the usual way and 15.25 g. of a light brown ether-soluble oil was obtained. The specific rotation of this acid was determined as +7.18°, *i. e.*, 1.65 g. of acid in 39.6 g. of water gave α +0.287° in a one dcm. tube. As the specific rotation of the optical isomer of the acid from the brucine salt rotating —29.42° should be +8.29°, the acid now under consideration was evi-

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the calculated amount is then added and the mixture heated on the boiling water-bath for 15 minutes. It is then cooled and a measured quantity of 0.1 *N* hydrochloric acid is added to acid reaction and the mixture boiled to expel carbon dioxide. It is again cooled and the excess hydrochloric acid is determined with 0.1 *N* sodium hydroxide. solution. The quantity of sodium hydroxide solution added at first in the cold determines the amount of free acid present and the remainder added determines the quantity of lactone.

[1] *Ann.*, 376, 35 (1910).

dently still contaminated with some of the racemic acid. It was, therefore, converted into the barium salt. The 15.25 g. of acid yielded 14.8 g. of the vacuum-dried barium salt. This salt had a specific rotation of —1.48°, *i. e.*, one g. of salt in 24 g. of water gave α —0.03°. This indicates that the salt was pure, as this rotation is equal and opposite to that of the barium salt from the crystalline brucine salt. The gum was set free from this salt and purified in the usual way. The weight of free acid was 6 g. It was a light yellow mobile oil.

The rotation of a portion of this acid was taken, another portion was titrated and another portion was converted into the phenylhydrazid with the following results.

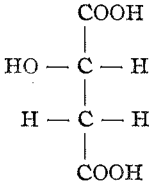
The Rotation.—The specific rotation of the acid was found to be +8.00°, *i. e.*, 0.83 g. of acid dissolved in 19.92 g. of water gave α +0.32° in a one dcm. tube.

The Titration.—A titration of another portion of the acid gave the following results. Only one titration was made because of the small amount of acid available.

Weight of sample. G.	0.1 *N* NaOH for free acid. Cc.	0.1 *N* NaOH for lactone. Cc.	Total. Cc.	Calc. for C_4 saccharinic acid. Cc.
0.4014	6.18	31.93	38.11	38.18

The Phenylhydrazid.—This compound was obtained only as a gum, very soluble in ethyl acetate.

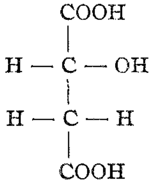
Determination of the Configuration of the Two Optical Isomers.—The foregoing data prove that the *dl*-2,3-dioxy-butyric acid had been separated into the pure dextro- and levo-rotating components. The next step in the problem was to assign the proper configuration to each of the new acids. It was shown that the dioxy acid which rotated —8.29° could be oxidized to natural, levo-rotating, malic acid. The malic acids were shown by Fischer[1] to have the configurations



$$\begin{array}{c} COOH \\ | \\ HO - C - H \\ | \\ H - C - H \\ | \\ COOH \end{array}$$

l-malic or natural malic acid.

and



$$\begin{array}{c} COOH \\ | \\ H - C - OH \\ | \\ H - C - H \\ | \\ COOH \end{array}$$

d-malic acid.

Of the two 2,3-dioxy-butyric acids under consideration only one could give *l*-malic acid by oxidation, namely the one with the configuration I (see below). The acid which rotates —8.29° then must have this configuration as it gives *l*-malic acid and must, therefore, be called *l*-2,3-dioxy-butyric acid while the acid which rotates +8.00° must have the configuration II (see below) and must be called *d*-2,3-dioxy-butyric acid.

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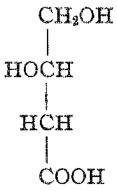
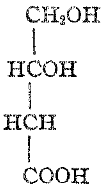
[1] *Ber.*, **29**, 1378 (1896).



$$\begin{array}{c} CH_2OH \\ | \\ HOCH \\ | \\ HCH \\ | \\ COOH \end{array}$$

(I).



$$\begin{array}{c} CH_2OH \\ | \\ HCOH \\ | \\ HCH \\ | \\ COOH \end{array}$$

(II).

The experimental evidence for these conclusions follows.

Nef[1] has reported on the oxidation of *dl*-2,3-dioxy-butyric acid to *dl*-malic acid. We repeated Nef's work. As our procedure differed somewhat from Nef's it is given below.

The *dl*-dioxy-butyric acid is treated with 10 times its weight of nitric acid of sp. gr. 1.305 and heated for 2 hours at 65° in a water-bath. The reaction mixture is subjected to complete vacuum distillation at a temperature not exceeding 65°. The residue—a yellow gum—is dissolved in 10 to 12 times its weight of cold water. This solution is treated with an excess of calcium carbonate and this mixture shaken for 10 to 15 minutes. The excess of calcium carbonate is then removed by suction filtration. The filtrate is now concentrated by boiling to about $^1/_2$ its volume during which process the normal calcium malate separates.[2] To obtain the free *dl*-malic acid the vacuum-dried salt is suspended in a large volume of hot water and the necessary quantity of oxalic acid, calculated on the basis of an analysis of the vacuum-dried salt, is added in hot solution. The mixture is kept on the boiling water-bath for 3 hours and frequently shaken. It is then cooled and the calcium oxalate is separated by filtration. The filtrate is subjected to complete vacuum distillation finally at 100°. The residue is extracted 3 times with boiling ether. The combined filtered ether solution is completely evaporated, finally on the water-bath. The residue is placed *in vacuo* over sulfuric acid until the weight is constant when the malic acid remains as white scales. It was proved by experiment that malic acid itself is not appreciably oxidized under the above procedure.

Five and two-tenths g. of the 2,3-dioxy-butyric acid $[\alpha]_D^{20}$ —8.29, was now oxidized, following the above procedure in every detail, and a total of 2.15 g. of insoluble calcium salt was obtained. Part of this insoluble calcium salt was used for analysis for calcium oxide (see below) and the acid was obtained from the rest. The weight of acid obtained was 0.5127 g. This will be referred to hereafter as "free acid."

The proof of the identity of this insoluble salt with the calcium salt of pure natural malic acid and of the identity of the "free acid" with pure natural malic acid was brought by comparing the analyses of the 2 corre-

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[1] *Ann.*, 376, 36 (1910).

[2] *Ibid.*, 233, 168 (1886).

sponding vacuum-dried calcium salts, and the melting points, the specific rotations and the titration of the 2 corresponding acids. The natural malic acid was recrystallized from ether. Part of it was then dried *in vacuo* over sulfuric acid and part was converted into the normal calcium salt by the procedure described above.

Analysis of the Calcium Salts.—The first 2 analyses were of the salt obtained by oxidizing the 2,3-dioxy-butyric acid, the last 2 of the salt of pure natural malic acid.

Subs., 0.2025, 0.2197, 0.2070, 0.1639: CaO, 0.661, 0.0712, 0.0670, 0.0530.
Calc. for $C_4H_4O_5Ca$: CaO, 32.56. Found: 32.65, 32.40, 32.36, 32.28.

Melting Points.—The melting points of the "free acid," the pure natural malic acid, and a mixture of the two are as follows: Melting point "free acid," 96°; natural malic acid, 95°; mixture, 95–96°.

The Rotations.—The specific rotations of the "free acid" and of the natural malic acid were taken with the following results.

0.5117 g. of "free acid" in 12.3931 g. water gave α —0.13° in a one dcm. tube.

0.6588 g. of natural malic acid in 15.8939 g. water gave α —0.126 in a one dcm. tube.

$[\alpha]_D^{20}$ of "free acid."	$[\alpha]_D^{20}$ of natural acid.
—3.46°	—3.18°

The Titrations.—Samples of the "free acid" and of the natural acid were titrated with the following results.

	Weight of sample. G.	0.1 *N* NaOH for neutralization. Found. Cc.	Calc. Cc.
I	0.1533	22.91	22.83
II	0.1177	17.38	17.57
III	0.1671	24.77	24.94

The first and second titrations were made with the "free acid" and the third titration was made with the natural acid.

Summary.

dl-2,3-Dioxy-butyric acid from glycerol-α-monochloro-hydrine has been prepared. Proof of the structure of *dl*-2,3-dioxy-butyric acid is given by its preparation from vinyl-acetic acid, $CH_2 : CH.CH_2COOH$. The resolution of the *dl*-acid into the optical components by the use of brucine is reported. Configurations are assigned to the 2 forms of the acid and proofs of the correctness of these configurations given. The following specific rotations for the *d*- and *l*-forms of the acid and some of the derivatives are given.

	Free acid.	Barium salt.	Calcium salt.	Phenylhydrazid.
d-form	+8.00°	—1.48°	....	....
l-form	—8.29°	+1.48°	+2.47°	+1.71°

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