attention to the possibility of cyclic nitrogen bases occurring in petroleum distillates of the pyrindine type. The structural relationship of pyrindine to pyrindacine is similar to that between quinoline and acridine.

The occurrence of 5,6-dihydropyrindine in shale oil distillates and the $C_{16}H_{25}N$ base in crude kerosene distillates contributes to the importance of the five-membered carbon ring and will direct the attention of the chemist toward a further search for natural products of these types. Furthermore, it is to be expected that further research in the Texas Laboratory on the kerosene bases will develop a relationship between petroleum bases and naphthenic acids, more especially as concerns cyclopentane structure.

A synthesis of 5,6-dihydropyrindine seemed desirable in confirmation of Eguchi's interpretation of his C_8H_9N base, although the proof of structure furnished by the Japanese chemist was apparently conclusive. It is realized that the method of preparing this substance, as described in the present paper, must be improved in several steps before 5,6-dihydropyrindine becomes available in quantity for further research.

AUSTIN, TEXAS

[CONTRIBUTION FROM THE KENT CHEMICAL LABORATORY, UNIVERSITY OF CHICAGO]

THE C_4-SACCHARINIC ACIDS. V. THE PREPARATION OF 2,3-DIHYDROXYBUTYRIC ACID LACTONE. 3-HYDROXYISOCROTONIC ACID LACTONE. AN ATTEMPT TO PREPARE 2,2'-DIHYDROXYISOBUTYRIC ACID[1]

BY J. W. E. GLATTFELD, GLADYS LEAVELL, GEORGE E. SPIETH AND DONALD HUTTON

RECEIVED JUNE 15, 1931 PUBLISHED AUGUST 5, 1931

The resolution of 2,3-dihydroxybutyric acid (hereafter called "2,3-acid") has already been reported in a paper from this Laboratory.[2] The acid used for the resolution experiments was prepared in accordance with the procedures of the two previous workers, Hanriot, and Nef.[3] As neither of these authors was interested specifically in this acid, they did not make a very extended study of its preparation. No mention is made by them, for instance, of the simultaneous formation of an unsaturated acid, 3-hydroxyisocrotonic acid, although the formation of this acid is mentioned by Carré.[4] This unsaturated acid was formed to some extent in every one of our runs, in some cases even to the exclusion of the desired 2,3-acid. It was isolated in the form of its lactone in large quantities during

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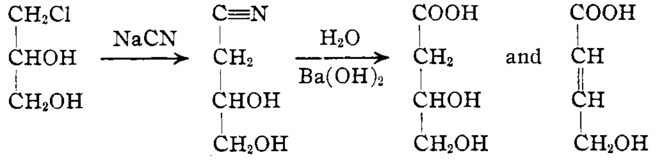
[1] This article is constructed largely from dissertations presented by Gladys Leavell, George E. Spieth and Donald Hutton in partial fulfilment of the requirements for the degree of Doctor of Philosophy in the University of Chicago.

[2] Glattfeld and Miller, THIS JOURNAL, **42**, 2314 (1920).

[3] Hanriot, *Ann. chim. phys.*, [5] **17**, 104 (1879); Nef, *Ann.*, **376**, 35 (1910).

[4] Carré, *Bull. soc. chim.*, [4] **3**, 834 (1908); *Compt. rend.*, **146**, 1282 (1908).

the work of preparation reported below. It is a very valuable by-product as it serves as a source for erythronic acid, which it yields when oxidized with permanganate,[5] or with barium or silver chlorate.[6] Although many experiments were carried out in an attempt to prevent the formation of the unsaturated acid, none of them was entirely successful. During this work, however, a satisfactory procedure for the preparation of the 2,3-acid and its separation from the unsaturated acid was developed and is given below because no detailed directions are at present available in the literature. By following these directions a reasonably good yield of the 2,3-acid (28%) may be obtained. This procedure does not differ in principle from that of Nef, but it does differ in some important details. It may be outlined as follows



$$\begin{array}{c} CH_2Cl \\ | \\ CHOH \\ | \\ CH_2OH \end{array} \xrightarrow{NaCN} \begin{array}{c} C{\equiv}N \\ | \\ CH_2 \\ | \\ CHOH \\ | \\ CH_2OH \end{array} \xrightarrow[Ba(OH)_2]{H_2O} \begin{array}{c} COOH \\ | \\ CH_2 \\ | \\ CHOH \\ | \\ CH_2OH \end{array} \text{ and } \begin{array}{c} COOH \\ | \\ CH \\ \| \\ CH \\ | \\ CH_2OH \end{array}$$

Glycerol-α-chlorohydrin

Nitrile of 2,3-dihydroxybutyric acid

2,3-Dihydroxybutyric acid

3-Hydroxyisocrotonic acid

2,3-Dihydroxybutyric Lactone

Glycerol-α-chlorohydrin.—As indicated above, glycerol-α-chlorohydrin is the starting product in this synthesis. It was prepared by the action of 1350 cc. of hydrochloric acid of sp. gr. 1.18 on 1 kg. of glycerol in the presence of 48 cc. of glacial acetic acid. The reaction mixture was introduced into pressure bottles, heated in an oil-bath at 120° for seven hours, and then fractionated at 15 mm. The 110–160° fraction was refractionated at 3–4 mm. The average yield of the fraction of b. p. 101–105° at 3–4 mm. in five runs was 707 g., or 59% (Cl, calcd., 32.08; found, 32.24, 32.14).

Preparation of the Lactone.—A solution made by the addition of 32.6 g. of potassium cyanide (or the equivalent in sodium cyanide) in 50 cc. of water, to 55 g. of glycerol-α-chlorohydrin in 225 cc. of 95% alcohol, was kept with occasional shaking in a closed towel-wrapped pressure bottle (tested citrate of magnesia bottle) in a water-bath at room temperature for thirty-six hours. The water-bath was then heated to boiling and so maintained for two and one-half to three hours, the bottle cooled and contents subjected to filtration to remove salts. The filtrates in two such experiments were combined and the solvents removed as completely as possible with water pump (15–20 mm.) and water-bath at 60°. The residue was treated with 200 cc. of 95% alcohol, the mixture warmed, subjected to filtration and the salts washed with alcohol. The filtrate was again concentrated under the conditions mentioned above. The residue was dissolved in 200 cc. of water and added to a mixture of 160 g. of hydrated barium hydroxide in 800 cc. of water. The mixture was then kept in a boiling water-bath for four hours while a stream of air, washed by passage through sodium hydroxide solution, was drawn through it (evaporated water not replaced). The hot mixture was then added to a hot solution made up of 166 cc. of 6 *N* sulfuric acid in 2 liters of water, and the mixture kept hot while

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[5] Lespieau, *Bull. soc. chim.*, [4] **1**, 1113 (1907).

[6] Géza Braun, THIS JOURNAL, **51**, 235, 246 (1929).

the final quantitative precipitation of the barium was effected by the use of a dilute solution of sulfuric acid. The barium sulfate was removed and the filtrate and washings from the barium sulfate, which contained a trace of barium ion, were subjected to distillation (at 15–20 mm. and water-bath finally at 90°) to remove the water completely; the residue was extracted with absolute alcohol, the alcohol solution decanted and the salts thoroughly washed by decantation. The alcohol was removed from the extract and washings by distillation at 15–20 mm. with water-bath finally at 90°. The residue was then transferred to a Claisen flask and distilled at 3–8 mm., from an oil-bath by the use of an oil pump. The material which boiled below 60° was discarded and two fractions 60–140° and 140–200° collected. The average yields of these two fractions in thirty experiments were 21 g. and 55 g., respectively. These two fractions were twice refractionated and the final fractions, 70–110° and 135–160° at 2–5 mm., averaged in twenty-six experiments, 19.6 g. and 33.5 g. The 70–110° fraction (Fraction I) consisted chiefly of 3-hydroxyisocrotonic lactone and the 135–160° fraction (Fraction II) chiefly of 2,3-dihydroxybutyric lactone; 23 and 28% of the theoretical, respectively.

Purification of the Lactone.—Fraction II gave a test for chloride ion which was due largely, but apparently not entirely, to the presence of a small amount of ammonium chloride. It was found that solution in ten parts of dry ethyl acetate (or 5 parts ethyl acetate and 2–3 parts ether) and allowing to stand overnight caused the precipitation of the salt. The salt was removed by filtration through a folded filter, and the ethyl acetate by distillation (water-bath finally at 100°)*. The residue was dissolved in water and portions of a suspension of pure silver oxide in water added until a white precipitate ceased to form. The filtered solution now failed to give a chloride ion test. The water was removed by distillation with the water pump (15–20 mm. and water-bath finally at 100°), the residue fractionated with the oil pump and the 135–160° fraction at 2–5 mm. again collected. This fraction again tested for chloride ion. The process from the point indicated by the asterisk above was repeated three or four times. While it was easy to get a product that gave only a faint test for chloride, the complete removal of the impurity required ten to fourteen repetitions of the process. Evidently chloride ion is freed during the distillations and a perfectly pure lactone can be obtained only when the source of contamination is destroyed.

The pure lactone boiled under the conditions here used (Claisen flask with long arms, oil-bath and 20 drops of distillate per minute) at 148–150° at 2–4 mm.

Anal. Subs., 0.2190, 0.2639: H_2O, 0.1234, 0.1472; CO_2, 0.3775, 0.4522. Calcd. for $C_4H_6O_3$: H, 5.92; C, 47.08. Found: H, 6.31, 6.24; C, 47.03, 46.82. *Molecular weight* determinations gave the following: 104.1, 106.9, 102.2, 104.6, average 104.4. Calculated for $C_3H_6O_3$, 102.05.

Crystallization of the Lactone.—A sample of the pure lactone in a test-tube was cooled in a carbon dioxide snow–acetone bath. Stirring of the sample and scratching of the sides of the test-tube soon caused the entire sample to solidify. This solid sample was saved for inoculation purposes. It was soon found that the lactone could be recrystallized. Thus one volume of lactone in three volumes of a one to one acetone–ether mixture was cooled to about −35° by immersion in a carbon dioxide–acetone mixture, inoculated with the solid mentioned above, and thoroughly stirred for ten minutes. The solution had then set to a pasty mass of crystals. After a half hour the mother liquor was drawn off by the insertion of a glass tube with cotton plug to the bottom of the flask and the application of suction to the tube. There was left in the flask a beautiful mass of white crystals which melted at 22.5–26°. The crystals were three times subjected to the crystallization process outlined above except that only one-half volume of acetone–ether mixture was used and the mixture of crystals and mother liquor was kept in an ice–salt mixture for one-half hour before removal of the mother liquor. The crystals still melted over the same range. They were allowed to melt and the liquid subjected to distillation at 5 mm.; the temperature rose at once to 150° and practically all of the sample distilled between 150 to 151°.

Titration. Subs., 0.2174, 0.2028: 21.38, 19.98 cc. of 0.1 *N* alkali (phenolphthalein). Calcd. for C_4-saccharinic acid lactone: 21.28, 19.84 cc.

The Phenylhydrazide.—The melting point of this compound was found to be higher than that reported by Nef (99°), and by Glattfeld and Miller (100–101°).

Apparently the pure phenylhydrazide is not always formed when the usual procedure for hydrazide formation (addition of phenylhydrazine to the lactone in ethyl acetate solution followed by short heating and letting the reaction mixture stand for several days) is followed. A product, recrystallized from ethyl acetate until the melting point no longer rose, of m. p. 105.5° was obtained by this usual procedure. Analysis of this product showed it not to be the pure phenylhydrazide (calcd. for $C_{11}H_{14}O_3N_2$: N, 13.33; found: 14.64, 14.63, 14.43). The true phenylhydrazide was obtained, however, when a mixture of 12 g. of lactone (b. p. 150–155° at 6 mm.) and 13 g. of phenylhydrazine, without solvent, was heated for two hours on the boiling water-bath and then allowed to stand for forty-eight hours; yield 13 g. Three recrystallizations from ethyl acetate yielded 7 g. of lustrous white plates with a constant melting point of 109°.

Anal. Subs., 0.1025, 0.1053: N_2, 12.25 cc. (24°, 738 mm.), 12.4 cc. (23°, 748 mm.). Calcd. for $C_{10}H_{14}N_2O_3$: N, 13.33. Found: 13.04, 13.08.

3-Hydroxyisocrotonic Acid Lactone

This lactone does not seem to have been studied extensively. Lespieau[7] prepared it by heating 2,3-dichlorobutyric acid and also[8] by the treatment of 2,3-dichlorobutyric acid with sodium carbonate. Lespieau reports the boiling point as 95–96° at 13 mm. and melting point as +4°. As mentioned above, Carré[4] reports that it was formed in his reduced pressure distillation of 2,3-dihydroxybutyric acid.

This unsaturated lactone appeared in all of our distillations of the crude 2,3-dihydroxybutyric acid. We confirmed the observation of Carré that the unsaturated lactone is not formed in the distillations of the pure 2,3-acid lactone; this latter lactone is perfectly stable once it is formed and distils at 2–4 mm. with no apparent decomposition. The unsaturated lactone must therefore be formed at some stage before the 2,3-acid has changed to its lactone.

Purification of Lactone.—About 150 g. of Fraction I (see above) was twice fractionated and the lower fractions (one-tenth of whole) discarded both times. The portion of b. p. 70–75° at 2–3 mm. pressure was cooled in a freezing mixture until about three-fourth of it was solid. The solid was separated by rapid suction filtration, melted, again partly frozen, the solid separated, etc. This process was carried out seven times; 10 g. of material which contained only 0.04% chlorine was obtained. It melted at +5°.

Anal. Subs., 0.2071, 0.1716: H_2O, 0.0925, 0.0750; CO_2, 0.3232, 0.3592. Calcd. for $C_4H_4O_2$: H, 4.79; C, 57.13. Found: H, 5.00, 4.89; C, 56.95, 57.11.

Attempts to titrate the purified lactone indicated that it contained no free acid; the first drop of 0.1 *N* alkali colored the solution (phenolphthalein). The results of ti-

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[7] Lespieau, *Compt. rend.*, **138,** 1050 (1904); *Bull. soc. chim.*, [3] **33,** 466 (1905).

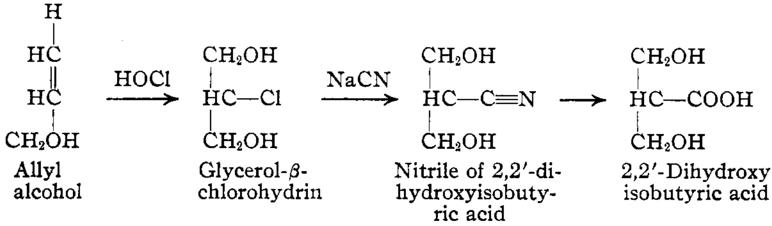
[8] Lespieau, *Compt. rend.*, **141,** 43 (1905); *Bull. soc. chim.*, [4] **1,** 1113 (1907).

trations carried out in the usual manner (boiling out carbon dioxide after addition of excess acid) gave varying results; apparently the lactone undergoes some change in structure during titration in the hot solution.

Compound Formed with Phenylhydrazine.—The lactone reacts with phenylhydrazine but the yield of product is small if a solvent is used. A mixture of 14.5 g. of lactone (b. p. 75–77° at 3–4 mm.) and 22 g. of phenylhydrazine, heated for three hours on the water-bath, however, yielded (transferred with ether) 18 g. of crude product which, after repeated recrystallization from ethyl acetate, melted at 183°. Crystals from other preparations also melted at the same point. The substance was very stable (one sample did not show signs of decomposition in eighteen months) and seemed to be a characteristic derivative of 3-hydroxyisocrotonic acid. Analysis showed that it was not the simple phenylhydrazide, however; figures for carbon, hydrogen and nitrogen were all high (calcd. for $C_{10}H_{12}N_2O_2$: C, 62.48; H, 6.29; N, 14.57. Found: C, 68.16, 68.24; H, 6.79, 6.79; N, 17.44, 17.24).

Attempted Preparation of 2,2′-Dihydroxyisobutyric Acid

This acid has not yet been reported in the literature. As it is one of the C_4-saccharinic acids according to our definition,[9] its preparation was attempted by the process indicated below.



The experimental procedures followed for preparation and purification were exact duplicates of those outlined above for the preparation and purification of 2,3-dihydroxybutyric lactone except that the β- instead of the α-chlorohydrin of glycerol was used. Again two products were formed, a low and a high boiling product. The former was shown to be 3-hydroxyisocrotonic acid and the latter was at first thought to be the desired 2,2′-iso acid[10] mainly because of the fact that reduction of the product yielded some isobutyric acid. Further study, however, showed that if the 2,2′-iso acid was present at all, it was much contaminated by the 2,3-acid lactone. This raised the question as to the source of the 2,3-acid lactone. The most obvious explanation of the presence of the latter was that the β-chlorohydrin that had been used in the preparation work was contaminated with α-chlorohydrin. A search of the literature showed that the β-glycerol chlorohydrin prepared from allyl alcohol contains a large quantity of the α-chlorohydrin.[11] The pure α- and β-glycerol chlorohydrins

[9] Glattfeld and Sherman, THIS JOURNAL, **47**, 1742 (1925).

[10] Spieth, University of Chicago, Abstracts of Theses, Science Series, **IV**, 165 (1925).

[11] Smith, *Z. physik. Chem.*, **92**, 727 (1918); **94**, 723 (1920).

were therefore prepared and these then used in the preparation procedures. Both yielded the same products, 3-hydroxyisocrotonic and 2,3-dihydroxybutyric acid lactones. This shows that the reaction between the chlorohydrins and sodium cyanide is not a simple double decomposition reaction. A possible explanation is that both chlorohydrins under the experimental conditions used give the same glycidol, which then adds hydrogen cyanide to form the nitrile of the 2,3-acid practically exclusively.

Pure α- and β-Glycerol Chlorohydrins.—It has long been known[12] that the α-chlorohydrin of glycerol forms a stable condensation product (isopropylidene-α-chlorohydrin) with acetone. It is also known[13] that if the two hydroxyl groups necessary for condensation with acetone are in the 1,3 (as in β-chlorohydrin of glycerol) and not the 1,2 position (as in α-chlorohydrin of glycerol), the condensation proceeds with great difficulty if at all. These facts were taken advantage of in effecting the separation of the α- from the β-chlorohydrin of glycerol and the purification of the two compounds. The mixture of chlorohydrins obtained by the addition of hypochlorous acid to allyl alcohol was repeatedly condensed with acetone until no more condensation occurred. The purified condensation product then yielded the pure α-chlorohydrin and the uncondensed residue the pure β-chlorohydrin of glycerol as shown below.

The only property known to us by means of which the purity of given samples of α- and β-chlorohydrins of glycerol can be determined is their reaction rate with alkali. In dilute aqueous solution, alkali decomposes both into glycidol but at remarkably different rates. Smith[14] showed that the α-isomer is 50% decomposed in much less than one-tenth the time it takes the β-isomer to become 50% decomposed. He used the bimolecular reaction rate equation in his calculations and found that with the pure α-isomer the equation gave a fairly good "constant" but that when mixtures of α- and β-isomers were used the "constant" dropped rapidly with time. He showed, however, that the "constants" at 50% decomposition (K 50) are very good measures of purity and that K 50 for the pure α-isomer at 25° in 0.015 N barium hydroxide solution was 11.1 and that for the pure β-isomer about 0.81. For the work in hand it was not necessary to use 100% pure α- and β-isomers. No difficulty was experienced in obtaining samples of the two isomers that were at least 90% pure.

A mixture of 300 g. (K 50 = 5.26; % Cl = 36.54) of the chlorohydrin made by the method of Reed and Hurst (allyl alcohol + HOCl),[15] 200 g. of anhydrous sodium sulfate, 1200 cc. of acetone and 18 cc. of concd. sulfuric acid was introduced into a bottle which was then corked and placed in a "revolving" machine. This machine kept the sodium sulfate at all times suspended in the liquid. The machine was operated for twenty-two hours continuously, at the end of which time 75 g. of lead carbonate and 50 g. of sodium carbonate were added to the reaction mixture, the bottle put back on the machine and again rotated for six hours. The bottle was then opened, the liquid decanted and the salts washed by decantation with acetone. The acetone was removed by distillation at ordinary pressure with the water-bath finally at 100°. The residue was thoroughly extracted three or four times with low-boiling ligroin. The combined extracts were dried over sodium sulfate, fractionated at ordinary pressure, and the fraction of boiling range 145–165° saved. This was practically pure isopropylidene-α-chlorohydrin

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[12] Fischer and Pfähler, *Ber.*, **53**, 1606 (1920).

[13] Speier, *ibid.*, **28**, 2531 (1895); Irvine, Macdonald and Soutar, *J. Chem. Soc.*, **107**, 337 (1915).

[14] Smith, *Z. physik. Chem.*, **92**, 717–740 (1918); **94**, 691–738 (1920).

[15] Reed and Hurst, *J. Chem. Soc.*, **121**, 989 (1922).

(209 g.). The residues insoluble in ligroin from two such experiments were now combined and put through exactly the same condensation process again; the quantities of reagents noted above were used. Again a quantity of isopropylidene-α-chlorohydrin (70 g.) and a chlorohydrin residue insoluble in ligroin were obtained. The residue was again condensed as before but proportionately smaller quantities of reagents now used. The process was repeated twice more; quantities of isopropylidene-α-chlorohydrin obtained were 60 g. and 33 g. The final crude residue weighed about 90 g. It was added to corresponding material from other runs and then fractionated with oil pump and oil-bath. Usually three or four fractionations were sufficient to give a colorless product whose boiling range was 110–114° at 3–4 mm. The yield of this purified product was about 10–15%; *K* 50, 0.70–0.80 and % Cl, 31.30–31.80. The combined isopropylidene-α-chlorohydrin weighed 372 g., a 45% yield. Almost half of the chlorohydrin started with is unavoidably lost in the many experimental manipulations necessary in this process.

The experiment outlined above was repeated many times. The isopropylidene-α-chlorohydrin thus prepared was twice fractionated at ordinary pressure and samples then hydrolyzed by addition of three parts of dilute sulfuric acid (0.1 to 0.15 *N*) to one part of product. The mixture was placed on the revolving machine overnight, during which time the oily condensation product completely dissolved. The sulfuric acid was quantitatively removed by means of barium hydroxide solution (an excess of base must be avoided). The reaction mixture (slightly acid) was then subjected to distillation (barium sulfate not removed) from a water-bath and with a water pump until no more distillate came over, then fractionated with oil pump and oil-bath. The product thus obtained usually boiled at about 111–114° at 7–8 mm. and had *K* 50 about 9 and percentage of Cl, 31.5–31.8. Repeated fractionation gave a product which was perfectly colorless and which possessed a *K* 50 of between 9.5 and 10.00.

Phenylurethans of α- and β-Glycerolchlorohydrins.—Rider and Hill[16] have recently prepared these compounds and report the melting points as 128–129° for the α-isomer and 133° for the β-isomer. We prepared them from our purified products according to the directions of Rider and Hill with results as follows: 17 g. of each chlorohydrin with 13 g. of phenyl isocyanate gave 2.5 g. of crude product in the case of the α-isomer and 3 g. in the case of the β-isomer. Recrystallization from acetone–high boiling ligroin (1 to 10) yielded finally, in the case of the α-isomer, 0.6 g. of m. p. 127–129, and, in the case of the β-isomer, 2 g. of m. p. 133–136°.

Reaction of the Pure Chlorohydrins with Cyanide.—The highly purified α- and β-glycerol chlorohydrins prepared as indicated above were each now treated with sodium cyanide and the reaction mixtures put through the synthesis indicated above for the preparation of the 2,3-acid lactone. In each case 55 g. of the chlorohydrin was used and the corresponding quantities of reagents as used in the previous work. In the case of the β-isomer 18 g. (35%) of product of boiling range 148–150° at 4 mm. was obtained. A sample of this titrated correctly for a dihydroxybutyric lactone (subs., 0.3274: calcd. 31.87 cc. of 0.1 *N* alkali; found, 31.20). It solidified when cooled with carbon dioxide, could be recrystallized from acetone–ether and melted at 22.5–25.5°. These data show that it was the lactone of 2,3-dihydroxybutyric acid. In the case of the α-isomer, 20 g. of product (39%) of boiling range 146–149° at 4 mm. was obtained. It also solidified when sufficiently cooled, could be recrystallized from acetone–ether, after which it melted at 22.5–26°. It titrated for a dihydroxybutyric lactone (subs., 0.2174: calcd., 21.31 cc. of 0.1 *N* alkali; found, 21.28 cc.). These data offer convincing evidence that the products in the two cases were identical and that both were samples of 2,3 dihydroxybutyric lactone.

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[16] Rider and Hill, This Journal, **52,** 1525 (1930).

Summary

Directions are given for the preparation in about 28% yield of 2,3-dihydroxybutyric lactone from glycerol monochlorohydrin. The lactone has been obtained in crystalline form of melting range 22–26°. The phenylhydrazide melts at 109°.

A valuable by-product, 3-hydroxyisocrotonic lactone, was isolated in 23% yield. The compound which this lactone forms with phenylhydrazine (not the simple phenylhydrazide) melts at 183°.

2,2′-Dihydroxyisobutyric acid cannot be prepared by treatment of glycerol-β-chlorohydrin with sodium cyanide followed by hydrolysis. The product is 2,3-dihydroxybutyric lactone whether the α- or the β-chlorohydrin of glycerol is used in the synthesis.

CHICAGO, ILLINOIS

[CONTRIBUTION FROM THE CONVERSE MEMORIAL LABORATORY OF HARVARD UNIVERSITY]

STUDIES IN THE CHLOROPHYLL SERIES. VI. THE MECHANISM OF THE PHASE TEST

BY CATHERINE C. STEELE[1]

RECEIVED JUNE 20, 1931 PUBLISHED AUGUST 5, 1931

The essential change in allomerization and phase test saponification in the derivatives of chlorophyll *a* is the dehydrogenation of the grouping —CHOHCO— to —COCO—, and it has now been shown that the two hydrogen atoms are removed by oxygen from the air.[2] If the phase test saponification of methyl phaeophorbide *a* or chlorin *e* trimethyl ester is carried out in a modified Warburg apparatus[3] the amount of oxygen absorbed can be measured. Since the products are the "unstable chlorins," the oxidation is also shown to take place in the alkaline medium, and not in the subsequent acidification nor in the conversion of the chlorins to the phaeopurpurins.

The inherent difference between Willstätter's methods of "phase test" and "hot quick" saponification, whereby he obtained either phytochlorin *g* or chlorin *e* from phaeophorbide *a*, is now apparent.[4] Phase test saponification is an atmospheric oxidation process: with phaeophorbide *a* it also opens a ring but effects only partial saponification of the methyl group,

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[1] Commonwealth Fund Fellow at Radcliffe College.

[2] A preliminary statement of these results appeared in a Communication to the Editor, THIS JOURNAL, **53**, 1615 (1931), and the allomerization experiments are discussed in detail in Paper V, *ibid.*, **53**, 2382 (1931). In this present paper are discussed the corresponding experiments in the phase test.

[3] Hyde and Scherp, *ibid.*, **52**, 3359 (1930).

[4] Willstätter and Stoll, "Investigations on Chlorophyll," translated by Schertz and Merz, Science Printing Co., **1928**, p. 263.