[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF BARNARD COLLEGE]

A STUDY OF BENZALPYRUVIC ACID AND ITS ESTERS. I. THE ACTION OF SUNLIGHT ON CRYSTALLINE METHYL BENZALPYRUVATE[1]

BY MARIE REIMER

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For a study of the action of sunlight on unsaturated organic compounds benzalpyruvic acid and its esters seem to present interesting possibilities.

In most of the unsaturated acids and esters previously investigated the doubly linked carbon atoms have been in the 1,2 positions to the carboxyl group as in maleic[2] and fumaric,[3] cinnamic,[4] and substituted cinnamic acids[5] or, where systems of "conjugated double linkages" have been present, in the 1,2 and 3,4 positions.[6] In the unsaturated ketones[7] studied the ethylenic linkage has also been in the 1,2 position to the carbonyl group. The only β,γ unsaturated compound thus far investigated as to its reaction in the light is phenyl*iso*crotonic acid.[8]

The reactions in all these cases have been found to be either an isomerization of one geometrical form to another, a reaction often reversible, or a polymerization to a dimolecular product where the substance is exposed in the dry condition, with an accompanying oxidation at the double linkage in many cases and, usually, a certain amount of decomposition.

In a study of the action of light on a series of cinnamylidene esters[9] it was pointed out that the behavior of these compounds was not what one would expect from their chemical relations and the suggestion was made at that time that the anomalous behavior was possibly due to a difference in geometrical configuration of the compounds. To test the validity of this assumption it would be necessary to prepare a large number of space isomers of unsaturated compounds and to investigate the action of sunlight upon the isomeric forms. This was done in this Laboratory with esters of α-cyanocinnamylidene-acetic acid.[9a].

The ethyl and the *iso*butyl esters were prepared in isomeric forms which were actually found to give quite different products in the light. This has also been noted

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[1] A portion of the work described in this paper was carried out in the Chemical Laboratories of Stanford University, through the courtesy of the Chemistry Department.

[2] Ciamician and Silber, *Ber.*, **36**, 4266 (1903).

[3] Stoermer, *Ber.*, **42**, 4870 (1909); **44**, 660 (1911).

[4] Stoermer, *Ber.*, **42**, 4865 (1909). de Jong, *Abh. Akad. Wetenschappen Amsterdam*, **20**, 55 (1911); *Rec. trav. chim.*, **31**, 258 (1912); *Ber.*, **55**, 463 (1922). Stobbe, *Ber.*, **52**, 666 (1919). Stoermer and Laage, *Ber.*, **54**, 79 (1921).

[5] Stoermer and Heymann, *Ber.*, **46**, 1249 (1913). Stoermer and Frederici, *Ber.*, **41**, 324 (1908). Stoermer, *Ber.*, **44**, 637 (1911). Stoermer, *Ann.*, **409**, 36 (1915).

[6] Liebermann, *Ber.*, **28**, 1440 (1895). Riiber, *Ber.*, **35**, 2411 (1902). Reimer, *Am. Chem. J.*, **45**, 417 (1911).

[7] Ruhemann, *J. Chem. Soc.*, **85**, 1453 (1904). Stobbe and Wilson, *Ann.*, **374**, 237 (1910); *J. Chem. Soc.*, **97**, 1722 (1910). Stobbe, *Ber.*, **44**, 869 (1911). Conant and Lutz, THIS JOURNAL, **45**, 1303 (1923).

[8] Stoermer and Stockmann, *Ber.*, **47**, 1793 (1914).

[9] (a) Reimer and Keller, *Am. Chem. J.*, **50**, 157 (1913). Compare (b) Stobbe, *Ber.*, **45**, 3396 (1912).

by Riiber[10] in the case of the isomeric cinnamylidene-acetic acids. The difference in the behaviors of cinnamic and of *allo*cinnamic acids in the sunlight[11] is well known.

As a substance for a continuation of this study benzalpyruvic acid was chosen, since as an α-ketonic acid, a β,γ-unsaturated acid and an α,β-unsaturated ketone it seemed to be of particular interest. It bears also a very close relationship to benzoylacrylic acid, the reactions of which have been for some time an object of study in this Laboratory.[12]

Although but one benzalpyruvic acid has been described, a review of the literature seems to show that an isomeric acid exists. The substance was prepared by Claisen[13] by condensing pyruvic acid and benzaldehyde with hydrochloric acid as condensing agent and described as an oil. More than 20 years later Erlenmeyer[14] repeated Claisen's experiment, using sodium hydroxide instead of hydrochloric acid, and obtained a solid acid. He suggests that the oily nature of Claisen's acid may have been due to impurity or to admixture of an isomeric form. In repeating Erlenmeyer's experiments it is conspicuous that the yield of solid acid obtained from the alkaline condensation product is not quantitative, but that a considerable portion of the acid stays in solution from which it separates as an oil on spontaneous evaporation of the solvent. This oil is like that described by Claisen and is probably a mixture of isomeric acids.

Since the separation of these acids in pure condition has not yet been accomplished, the isomeric methyl esters of the acids have been studied. The present paper deals with the preparation of materials and with the behavior of the crystalline methyl ester in the sunlight.

The products formed by action of the sunlight on methyl benzalpyruvate exposed in the dry condition are a polymeric ester, melting at 154°, obtained in small quantity and an oil, probably a mixture of isomers and decomposition products. On exposure in benzene solution, a reaction which in other cases almost invariably gives an isomeric compound, the product of reaction was found to be a polymer, melting at 117°. Reactions with these two polymeric substances have shown that the higher, melting has the structure of the truxillic acid series, while the lower, melting is related to the truxinic acids. The formation of the latter compound may be explained on the assumption that in solution isomerization had taken place and that the isomeric ester had polymerized. It is expected that reactions with the isomeric ester, now being studied, will make it possible to decide this point. The reaction is an unusual one as but few cases of polymerization of this type of substance in solution have been described.[15]

A comparison of this ester, I, with that of benzoylacrylic acid, II,

$$\underset{\text{I}}{C_6H_5CH{=}CHCOCOOCH_3} \qquad \underset{\text{II}}{C_6H_5COCH{=}CHCOOCH_3}$$

is of interest. The system of "conjugated double linkages" in the two

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[10] Riiber, *Ber.*, **46**, 335 (1913).

[11] Stobbe and Steinberger, *Ber.*, **55**, 2225 (1922).

[12] Rice, THIS JOURNAL, **45**, 222 (1923).

[13] Claisen, *Ber.*, **14**, 2472 (1881).

[14] Erlenmeyer, *Ber.*, *36*, 2527 (1903).

[15] Ciamician and Silber, Ref. 2; **42**, 1388 (1909). Stobbe and Rücker, *Ber.*, **44**, 869 (1911). Ref. 9 a.

is the same but stands in a different relation to the phenyl and the carboxyl groups. The products obtained from I are polymers, while on exposure of II to the sunlight no polymers are formed but isomerization takes place with the greatest ease.[12] Whether the difference in reaction of these two substances is due to difference in the chemical effect of the neighboring groups on the unsaturated part of the molecule or to a difference in the physical state of the compounds during exposure is still to be determined.

Experimental Part

Preparation of Benzalpyruvic Acid and its Methyl and Ethyl Esters

According to Erlenmeyer's procedure, the acid was prepared by mixing equimolecular quantities of pyruvic acid and benzaldehyde and treating the mixture with an excess of 10% sodium hydroxide solution. He makes the statement that poor yields are obtained when more than 4 g. of the acid is used in each condensation. In this investigation it has been found that the poor yields are due to the extraordinary ease of reversal of the condensation reaction in alkaline solution, this reversal being greatly accelerated by increased temperature. As most of the heat developed during the reaction is heat of neutralization of the pyruvic acid, the temperature during the condensation can be controlled by first neutralizing the acid with the requisite amount of 10% sodium hydroxide solution, adding the benzaldehyde to the cooled solution and then the sodium hydroxide necessary for the condensation. In this way amounts of material eight times as large as recommended by Erlenmeyer have been used with a yield of 80% of condensation product. The procedure is as follows: 35.2 g. of pyruvic acid is added to 160 cc. of 10% sodium hydroxide and the solution cooled to 0°, 42.4 g. of benzaldehyde is added and then slowly 80 cc. of 10% sodium hydroxide while the whole is vigorously stirred. The temperature must not rise above 12° to insure good results. After six minutes the reaction mixture curdles, and after nine minutes a mass of fine granular sodium salt has separated. This is allowed to stand not longer than ten minutes at 0° to complete the condensation, and then rapidly filtered off. As the salt, wet with sodium hydroxide solution, is very soluble in water it cannot be washed but is surface-dried as effectively as possible by pressing between filter papers and then air-dried. In the dry condition the salt dissolves so slowly that a considerable part of it is hydrolyzed to benzaldehyde and sodium pyruvate by the sodium hydroxide with which it is contaminated. To hasten solution and thus diminish the hydrolysis the dry salt is ground to a fine powder and shaken for a few moments in an electric shaking machine with water, 500 cc. for each 40 g. of salt. The slightly cloudy solution is filtered, cooled to 0° and at once acidified with hydrochloric acid and diluted by pouring the concentrated acid upon crushed ice. The benzalpyruvic acid separates as an oil, but when the mixture is stirred vigorously during the acidification the oil quickly changes to a mass of shining, plate-like, yellowish crystals; m. p., 57°.

The yields of acid thus obtained rarely exceeded 60% of the amount calculated from the sodium salt used. The remainder is deposited on spontaneous evaporation of the filtrates as an oil, mixed with fine crystals. This is evidently the oil described by Claisen and is probably a mixture of isomers. Work on the separation of these acids is now in progress. Since crystallization of the acid from boiling water, as recommended by Erlenmeyer, led to considerable loss, the crude acid was used for preparation of the ester.

THE METHYL ESTERS.—Fifty g. of benzalpyruvic acid was dissolved in 500 cc. of methyl alcohol, the mixture saturated with hydrogen chloride and allowed to stand overnight. Two-thirds of the alcohol was then distilled. The ester separated in long, bright yellow needles, melting at 73–74° after one recrystallization from methyl alcohol. It is readily soluble in ether, acetone, benzene, ligroin, ethyl alcohol.

Analysis. Subs., 0.2094: CO_2, 0.5328; H_2O, 0.1019. Calc. for $C_{11}H_{10}O_3$: C, 69.47; H, 5.26. Found: C, 69.39; H, 5.35.

The strongly acid residues from which this ester separated were heated to expel most of the alcohol, poured into sodium carbonate solution and the solution was extracted with ether. On evaporation, the ether deposited a considerable quantity of heavy, reddish oil mixed with the crystalline ester. Analysis of the oil indicates that it is an isomeric ester.

Analysis. Subs., 0.1683: CO_2, 0.4273; H_2O, 0.0902. Calc. for $C_{11}H_{10}O_3$: C, 69.47; H, 5.26. Found: C, 69.25; H, 5.95.

From 135 g. of acid, 116 g. (80%) of crystalline ester and 7.59 g. (5%) of the oil were obtained. A small quantity of crystalline acid was regained from the sodium carbonate washings.

THE ETHYL ESTER.—The procedure for preparation of the methyl ester was duplicated for the ethyl ester. It is a lemon-yellow oil boiling at 169–171° (7 mm.).

Analysis. Subs. 0.1836: CO_2, 0.4742; H, 0.0999. Calc. for $C_{12}H_{12}O_3$: C, 70.58; H, 5.88. Found: C, 70.43; H, 6.04.

Reaction of Sunlight on Crystalline Methyl Benzalpyruvate when Exposed in the Dry Condition

The ester in 30g. portions was ground to a fine powder, spread as evenly as possible over white porcelain trays and exposed to the direct sunlight. After a few hours the mass became slightly sticky. There was no odor of benzaldehyde at any time during the exposure, showing that there was no oxidation at the double linkage, as is the case with many cinnamylidene esters.

After about 30 days' exposure[16] the product was a heavy, yellow paste mixed with white solid. The mass was scraped from the plates and treated with cold methyl alcohol which dissolved the pasty substance leaving the colorless solid. On spontaneous evaporation of the alcoholic solution, crystals of unchanged methyl benzalpyruvate slowly separated. The residue was a heavy, yellow oil, probably a mixture of isomers with some decomposition products, since analysis gave results varying by several per cent. from the calculated formula for the esters. About one-half of this oil distilled at 155–158° (13 mm.) and solidified in the receiver to crystals of the original ester. The residue was a charred, gummy mass from which no pure substance could be obtained. From 27 g. of light product 3 g. of the original ester (11%), 3 g. of colorless solid (11%) and 21 g. of oily product (78%) were obtained.

Dimethyl Diphenyl-diketo-tetramethylene-bismethyleneformate.—The colorless solid, insoluble in cold methyl alcohol, was dissolved in a very small quantity of boiling acetone from which it separated in thick, shining crystals melting at 154°. Analysis and molecular-weight determinations prove that the substance is a dimeric form of methyl benzalpyruvate.

Analysis. Subs., 0.1914: CO_2, 0.4860; H, 0.1056. Subs., 0.2604, 0.9269: $(CH_3)_2CO$, 30, 30; T_B 0.040°, 0.145°. Calc. for $(C_{11}H_{10}O_3)_2$: C, 69.47; H, 5.26; mol. wt., 380. Found: C, 69.25; H, 5.51; mol. wt., 376, 364.

The compound is very slightly soluble in boiling ether, methyl and ethyl alcohols, readily soluble in benzene and boiling acetone.

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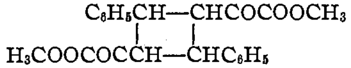
[16] In the California sunlight the time of exposure could be very much shortened. The products were the same.

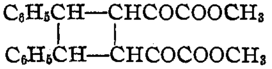
It does not react with bromine and is affected by a cold solution of potassium permanganate only after standing for several hours. It is not depolymerized on exposure to the sunlight in benzene or in methyl alcohol solution. When it melts a yellow product is formed.

To determine the structure of this ester the acid obtained by its saponification was oxidized. If the formation of the dimeric product in the light had taken place in the usual way, one would expect a tetramethylene compound.



I

or



II

Careful oxidation of the acid obtained from saponification of the polymeric ester would give a truxillic or a truxinic acid according to whether the structure of the polymer were I or II.

Saponification of the ester was carried out as follows. To a suspension of 1.6 g. of the finely powdered ester in 15 cc. of ethyl alcohol, 5 cc. of 10% potassium hydroxide in ethyl alcohol was added slowly while the mixture was kept cool. This was shaken vigorously for ten minutes, the small quantity of undissolved ester removed and the alcohol evaporated in a current of air. The residue was dissolved in water and the solution acidified. The heavy white precipitate was filtered and air-dried. The substance is an acid readily soluble in the usual organic solvents. It separates from solution in boiling water in fine, white, shining needles. The melting point of the acid is about 187°, with decomposition between 190° and 200°. In some preparations the acid melted with evolution of gas between 110° and 116°, solidified in the melting-point tube and melted again at 187°, indicating that the acid crystallized with various amounts of water. All attempts to obtain the acid free from water were unsuccessful. In a desiccator over sulfuric acid or warmed to expel the water, the acid became brown and gummy.[17]

Analysis. Air-dried subs., 0.1615, 0.1376: CO_2, 0.3720, 0.3154; H_2O, 0.0814, 0.0691. Calc. for $(C_{10}H_8O_3)_2$: C, 68.18; H, 4.54. Calc. for $(C_{10}H_8O_3)_2.2H_2O$: C, 61.85; H, 5.15. Found: C, 62.82, 62.51; H, 5.56, 5.58.

In order to characterize this acid further it was esterified with methyl alcohol. One g. of acid melting at 187° was dissolved in 10 cc. of methyl alcohol saturated with hydrogen chloride. Almost immediately a mass of fine, colorless needles separated. They were filtered, washed with methyl alcohol and air-dried. The same product was formed on esterification of the acid which melted first at about 110°, resolidified and melted again at 187°. The new ester melted at 155°; when mixed with the ester formed in the light reaction which melted at 154° the melting point was lowered 20°. This ester is then different from the light product. Analysis proved the two to be isomeric.

Analyses. Subs., 0.1566, 0.1657: CO_2, 0.4027, 0.4221; H_2O, 0.0770, 0.0792. Calc. for $(C_{11}H_{10}O_3)_2$: C, 69.47; H, 5.26. Found: C, 69.55, 69.47; H, 5.46, 5.31.

The ester is readily soluble in benzene, chloroform, acetone and boiling alcohol, slightly soluble in boiling ether and insoluble in ligroin. It is much less stable in the light than the isomeric ester. Exposed in the dry condition to the sunlight it quickly becomes yellow, a reaction which takes place slowly in diffused daylight. This decomposition serves to distinguish it readily from its isomer.

It can be obtained directly from the isomeric ester, melting at 154°, by suspending the latter in methyl alcohol saturated with hydrogen chloride, in which it slowly dis-

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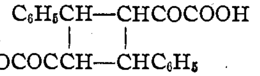
[17] It is interesting to note that Fittig [*Ann.*, **299**, 28 (1898)] describes exactly parallel behavior in the case of benzylpyruvic acid, $C_6H_5CH_2CH_2COCOOH$.

solves. The solution deposits needle-shaped crystals of the ester melting at 155°. After two weeks the transformation is complete.

On saponification of the ester melting at 155° the acid melting at 187° was obtained. The transformation of this ester to the acid and the reverse reaction were repeated several times. No trace of any other product was obtained.

Oxidation of the acid melting at 187° was accomplished by adding a slight excess of aqueous permanganate to the sodium carbonate solution of the acid and heating the mixture to boiling. The excess of permanganate was then decolorized with alcohol and the oxides of manganese removed. The clear solution, evaporated to small volume, deposited on acidification a colorless acid which separated from hot methyl alcohol in clear needle-shaped crystals which became opaque in the air and melted at 280°. The properties of this substance characterize it as α-truxillic acid. The acid oxidized had then the structure



$$\begin{array}{l} C_6H_5CH\text{—}CHCOCOOH \\ \qquad\quad | \qquad | \\ HOOCOCCH\text{—}CHC_6H_5 \end{array}$$

and, probably, the space configuration of α-truxillic acid.

As no cases are known in which isomerization of a truxillic acid to a truxinic acid or *vice versa* has taken place, it is safe to conclude that the isomeric esters have the same structure, the difference between them being due to a different space arrangement. Experiments so far do not make it possible to draw conclusions as to these space configurations.

Reaction of Light on Crystalline Methyl Benzalpyruvate when Exposed in Benzene Solution

A saturated solution of the ester in benzene was exposed to the sunlight in a series of 12 mm. quartz test-tubes. After about 60 hours white crystals began to separate. As the quantity of the solid product did not increase with longer exposure the solvent was almost completely removed by a current of dry air. The yellow residue was then stirred rapidly with a small quantity of cold methyl alcohol and the solution filtered. After the crystalline product was washed with cold methyl alcohol it was perfectly colorless and melted at 117°; yield, 75%.

Analysis. Subs., 0.1731: CO_2, 0.4429; H_2O, 0.0825. Calc. for $C_{11}H_{10}O_3$: C, 69.47; H, 5.26. Found: C, 69.78; H, 5.32.

Molecular-weight determinations and subsequent reactions of the substance proved it to be a polymeric ester. In boiling benzene the compound decomposed to give a yellowish solution, but in acetone the decomposition was slight.

Analysis. Subs., 0.4518, 0.6241: $(CH_3)_2CO$, 28.5, 28.5; T_B, 0.078°, 0.113°. Calc. for $(C_{11}H_{10}O_3)_2$; mol. wt., 380. Found: 347, 331.

The behavior of this ester toward methyl alcohol was peculiar. After standing for some minutes with cold methyl alcohol it rapidly dissolved and could not be obtained in crystalline form from the solution. When all the methyl alcohol had evaporated, addition of a small quantity of benzene or glacial acetic acid to the oily residue caused it to solidify.

The substance is slightly soluble in ether and insoluble in ligroin. It can be crystallized from a small volume of benzene or glacial acetic acid in the form of very fine, soft, white needles. When it melts there is no decomposition, but when kept in the molten condition for several minutes it becomes yellow and does not again solidify, indicating depolymerization. This decomposition to a bright yellow compound takes place more rapidly in benzene solution in the sunlight.

Hydrolysis of the Ester Melting at 117°.—Two g. of ester was stirred rapidly into 10 cc. of a 10% solution of aqueous potassium hydroxide, in which it at once dissolved. On acidification of this solution an oily acid separated but was immediately redissolved. The clear acid solution was extracted with ether, and the ether extract dried with sodium sulfate. A pale yellow oil was the product left on evaporation of the ether. After many attempts to cause this acid to crystallize the solid was obtained by letting the oil stand in a strongly acid solution for several days. With these crystals as seed, several oily preparations, made by saponification in aqueous sodium carbonate and in alcoholic potassium hydroxide solutions, could be made to crystallize. The solid is a colorless acid readily soluble in ether, acetone, methyl and ethyl alcohols, water and glacial acetic acid. On standing in acetic acid solution it is partially decomposed. It is insoluble in ligroin and cold benzene. It separated from a large quantity of boiling benzene in very fine, white needles, melting at 180° with slow evolution of a gas above the melting point.

Analysis. Subs., 0.1524: CO_2, 0.3847; H_2O, 0.0645. Calc. for $(C_{10}H_8O_3)_2$: C, 68.18; H, 4.54. Found: C, 68.84; H, 4.70.

The acid was esterified by standing overnight in methyl alcohol, saturated with hydrogen chloride. The ester obtained melted at 117° and was proved by mixed melting point to be the original light product. To make sure that there had been no isomerization due to the hydrogen chloride the ester was also prepared through the silver salt with methyl iodide. The product was the same.

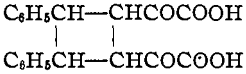
Oxidation of the Acid Melting at 180°.—A solution of 1.5 g. of the pure potassium salt of the acid in water was treated with an aqueous solution of a slight excess of potassium permanganate and the mixture allowed to stand for eight hours. The excess of permanganate was then decolorized with methyl alcohol, the oxides of manganese filtered out and washed with hot water, the aqueous extracts evaporated to small volume and acidified. An oil separated which gradually solidified. This was dissolved by addition of sodium carbonate, reprecipitated and crystallized from hot water. The acid separated in fine, white needles; m. p., 174–175°. It is difficultly soluble in hot water and benzene, and readily soluble in alcohol. On neutralization of a dilute solution with ammonium hydroxide and addition of calcium chloride, there is at first no precipitation, but after one-half hour clumps of fine needles of the calcium salt separate. These properties are characteristic of δ-truxinic acid.[18] That the product was δ-truxinic acid was proved by analysis and preparation of the methyl ester.

Analysis. Subs., 0.1796: CO_2, 0.4735; H_2O, 0.0854. Calc. for $C_{18}H_{16}O_4$: C, 72.97; H, 5.45. Found: C, 72.95; H, 5.36.

When the substance was treated with methyl alcohol saturated with hydrogen chloride, stiff, white needles of the methyl ester separated; m. p., 77–78°.

Analysis. Subs., 0.1796: CO_2, 0.4900; H_2O, 0.1000. Calc. for $C_{20}H_{20}O_4$: C, 74.07; H, 6.17. Found: C, 74.40; H, 6.18.

The formation of δ-truxinic acid proves that the acid oxidized had the structure



$$\begin{array}{l} C_6H_5CH\text{—}CHCOCOOH \\ \qquad\;\; | \qquad\;\; | \\ C_6H_5CH\text{—}CHCOCOOH \end{array}$$

and probably, a configuration corresponding to that of δ-truxinic acid. The ester formed in the light reaction has then also this structure.

Summary

Methods for the preparation of benzalpyruvic acid and its esters have been described.

Three polymeric products have been obtained from crystalline methyl benzalpyruvate; two of these correspond in structure to the truxillic acids

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[18] Liebermann, *Ber.*, **22**, 2250 (1889).

and the third to the truxinic acids. This third polymer is formed by the action of sunlight on methyl benzalpyruvate in solution, a reaction that usually gives an isomeric product.

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[CONTRIBUTION FROM THE OTHO S. A. SPRAGUE MEMORIAL INSTITUTE, LABORATORY OF CLINICAL RESEARCH, RUSH MEDICAL COLLEGE]

THE ACTION OF GUANIDINE UPON GLUCOSE IN THE PRESENCE AND ABSENCE OF OXYGEN

BY EDGAR J. WITZEMANN

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Nef[1] divided the effects of alkali upon sugars into two groups, (1) the more profound reactions involving the destruction or oxidation of the sugars and (2) the less profound effects involving merely transformations, such as the isomerization phenomena described by Lobry de Bruyn and Alberda van Ekenstein.[2] Woodyatt[3] has pointed out the close parallelism between these two groups of effects and the effects exerted on sugars in the normal and diabetic organism, respectively. The diabetic accomplishes transformations of Type 2 very effectively, but fails to a greater or less extent in accomplishing effects of Type 1. When the diabetic is supplied with insulin his lost capacity for transformations of Type 1 can be restored. From this it might appear that insulin acts upon glucose *in vivo* as strong alkalies act upon it *in vitro*. There is, however, no reason for thinking that insulin is a strong alkali like potassium hydroxide.

Recent experiments on the behavior of ammonium hydroxide, in comparison with potassium and sodium hydroxides, on the oxidation of butyric acid with hydrogen peroxide,[4] give a basis for the belief that catalyses attributed to the hydroxyl-ion concentration of the alkali used are also influenced under suitable conditions by other factors such as the nature of the positive ion. It was in pursuit of the idea that somewhat similar relations might hold for the alkali effects of Type 1 that some preliminary fundamental experiments upon the action of guanidine were made.

The experiments here described were carried out before insulin became known and are offered in the present form because they may have considerable interest in other ways than those suggested above. The results in general were indecisive because the effects of guanidine on glucose proved to be much like those of potassium hydroxide.

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[1] Nef, *Ann.*, **403**, 204 (1914). See also *Ann.*, **357**, 214 (1907); **376**, 1 (1910).

[2] de Bruyn and van Ekenstein, *Rec. trav. chim.*, **14**, 158, 203 (1895); **15**, 92 (1896); **16**, 257, 262 (1897).

[3] Woodyatt, *J. Biol. Chem.*, **20**, 129 (1914).

[4] Witzemann, *ibid.*, **49**, 123 (1921).