A STUDY OF β-HYDROXY-α-KETO ACIDS*

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The preparation of hydroxypyruvic acid, CH₂OH·CO·COOH, has often been claimed in the older literature, but the proofs of structure put forward have left much in doubt. The demonstration that this keto acid is a product of the enzymatic oxidative deamination of dl-serine (1) has prompted the detailed study of the hydroxyketo acids corresponding to serine and threonine, i.e. of hydroxypyruvic and β-hydroxy-α-ketobutyric acids.

Hydroxypyruvic acid was presumed by Will, in 1891 (2), to be present in the products of the decomposition of cellulose nitrate by alkali. The principal evidence consisted in the precipitation from the acidified reaction mixture of the lead salt of an acid that was not oxidized by bromine and yielded the osazone of mesoxalic acid semialdehyde (I). These experiments were later confirmed (3) and descriptions of the isolation of what appears to be the same osazone can be found repeatedly in the literature; it was, for instance, obtained after the oxidation of glyceric acid with ferrous ion and hydrogen peroxide (4, 5) and from the products of the photochemical oxidation of aspartic (6) and tartaric (7) acids. Compound I also was isolated (8) after the treatment with phenylhydrazine of hiptagenic acid, C₃H₅O₄N (9), a product of the hydrolysis of two naturally occurring glucosides, hiptagin (9) and karakin (10). This acid is probably closely related to hydroxypyruvic acid, being an oximino or hydroxylamino derivative of this keto acid or of one of its tautomers.

The possibility of tautomerism between hydroxypyruvic acid (II),

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dihydroxyacrylic acid (III), and tartronic acid semialdehyde (IV) is obvious. Because of the stability of the product formed by alkali from cellulose nitrate toward bromine and alkali, Will assigned to it structure II. Further support was supplied to this assumption later (11), although evidence favoring structure IV was also produced (12). Compound III is known in the form of certain derivatives, e.g. ethyl \(\beta\)-hydroxy-\(\alpha\)-ethoxy-

\[
\begin{array}{ccc}
\text{CH}_2\text{OH} & \text{CHO} & \text{CHO} \\
\text{CO} & \Rightarrow & \text{COOH} \\
\text{COOH} & \text{COOH} & \text{COOH}
\end{array}
\]

acrylate, \(\text{HO-CH}=\text{C}(\text{OC}_2\text{H}_5)-\text{COOC}_2\text{H}_5\) (13). Solutions of tartronic acid semialdehyde (IV) have been made available by the work of Fischer, Baer, and Nidecker (14).

A simple synthetic method for the preparation of solutions of \(\beta\)-hydroxy-\(\alpha\)-keto acids was found in this laboratory. It consists in the careful hydrolysis of the corresponding \(\beta\)-bromo-\(\alpha\)-keto acids. Hydroxypyruvic acid (II), obtained in this manner from bromopyruvic acid, \(\text{BrCH}_2\cdot\text{CO-COOH}\), gave an insoluble lead salt and readily reduced Benedict’s solution. The results of the oxidation of freshly prepared solutions with periodic acid, to be discussed in the following paragraph, established beyond doubt that compound II actually had been obtained. In contrast to the products previously mentioned (2-5) this compound did not yield the osazone of mesoxalic acid semialdehyde (I). The hydroxypyruvic acid 2,4-dinitrophenylhydrazone, however, was obtained in a 90 per cent yield. The structure of this derivative was demonstrated by its reduction to \(dl\)-serine, apparently the first instance of the synthesis of an amino acid from a dinitrophenylhydrazone. Bromopyruvic acid itself gave a 2,4-dinitrophenylhydrazone which, with sodium hydroxide in aqueous ethyl alcohol, afforded the 2,4-dinitrophenylhydrazone of ethoxypyruvic acid (V).

Freshly prepared solutions of hydroxypyruvic acid are rapidly decomposed by periodic acid with the consumption of 1 mole of oxidizing agent and the production of about 0.9 mole each of formaldehyde and of oxalic
acid per mole of bromopyruvic acid used as the starting material. Taken together with the reduction of hydroxypyruvic acid 2,4-dinitrophenyl-hydrazone to dl-serine, this proves conclusively that 90 per cent of the hydrolysis product of bromopyruvic acid is indeed hydroxypyruvic acid (II). The reactions leading from bromopyruvic acid to serine on the one hand and to formaldehyde and oxalic acid on the other are summarized in Scheme 1.

**Scheme 1**

![Chemical reaction diagram](image)

**Behavior of Hydroxypyruvic Acid toward Strong Alkali (Table I)**

When hydroxypyruvic acid solutions in a nitrogen atmosphere are treated with sodium hydroxide (0.02 to 0.8 N) and are acidified after 1/4 hour, they show an ability to consume iodine (15) corresponding to the formation of appreciable amounts (10 to 64 per cent) of enediol. This solution reduces Benedict’s reagent at room temperature. That this strong reducing agent is dihydroxyacrylic acid (III) is shown by its behavior. After oxidation with iodine, treatment of the solution with phenylhydrazine acetate at room temperature affords an almost quantitative yield, based on the enediol content, of the osazone of mesoxalic acid semialdehyde (I), mentioned previously. Moreover, when a solution of hydroxypyruvic acid in 0.8 N sodium hydroxide is shaken with oxygen, about 0.7 mole of oxygen is consumed and equivalent amounts of oxalic and formic acids are formed for each mole of starting material. The reactions involved are presented in Scheme 2.
Formation of Enediol from Hydroxypyruvic Acid

In each experiment the calculated amount of 0.16 N sodium hydroxide was added to 0.01 mole of sodium hydroxy pyruvate in 200 cc. of water, in order to obtain the indicated alkali concentration. The solutions were kept for 30 minutes in a nitrogen atmosphere before removal of aliquots, which were then acidified and titrated with 0.1 N iodine solution.

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Normality of solution with respect to NaOH</th>
<th>Iodine consumption per 0.01 mole hydroxy- pyruvic acid</th>
<th>Enediol formed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.01</td>
<td>0.72</td>
<td>3.6</td>
</tr>
<tr>
<td>2</td>
<td>0.02</td>
<td>2.0</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>0.09</td>
<td>7.0</td>
<td>35</td>
</tr>
<tr>
<td>4</td>
<td>0.22</td>
<td>9.2</td>
<td>46</td>
</tr>
<tr>
<td>5</td>
<td>0.83</td>
<td>12.8</td>
<td>64</td>
</tr>
<tr>
<td>6</td>
<td>1.5</td>
<td>12.5</td>
<td>63</td>
</tr>
</tbody>
</table>

Scheme 2

CHO \[\rightarrow\] \[\text{I}_{2}\] \[\rightarrow\] COOH
\[\rightarrow\] C\[\text{H}_3\text{NHNH}_2\] \[\rightarrow\] C\ [=N\text{NH}_2\text{CH}_3\] \[\rightarrow\] COOH

When phenylhydrazine acetate at room temperature is added to the acidified solution before oxidation with iodine, amorphous bulky precipitates result from which pure glyoxalosazone (VI) can be secured in yields roughly dependent on the amount of enediol formed. This derivative probably arises as a result of the decarboxylation of compound III. Compound IV, possibly also present in the alkaline solutions of hydroxypyruvate, is known to give glyoxalosazone with phenylhydrazine at 60° (14).
course of the reaction between enediols and phenylhydrazine is not entirely clear. Ascorbic acid yields the osazone of dehydroascorbic acid (16, 17), while reductone (dihydroxyacrylic aldehyde) does not yield a crystalline derivative with phenylhydrazine unless it is first oxidized with iodine to dehydroreductone (18).

Behavior of Hydroxypyruvic Acid toward Weak Alkali

The complex transformations of this acid induced by 0.01 N alkali were studied in detail. The difficulties experienced in the interpretation of the results were not unexpected in view of the reactivity of this hydroxyketo acid and the multiplicity of reaction products to which it could give rise.

Hydroxypyruvic acid consumes 1 mole of periodic acid and yields 1 mole each of formaldehyde and oxalic acid; after treatment with 0.01 N sodium hydroxide under nitrogen for 2 to 4 hours it rapidly reduces 1.5 to 1.7 moles of periodate, but then yields only 0.4 mole of oxalic acid and less than 0.1 mole of formaldehyde. At the same time carbon dioxide is produced, irrespective of the duration of contact with alkali, in an amount corresponding to about 0.4 mole (Table II). Without previous contact with alkali hydroxypyruvic acid yields practically no carbon dioxide on treatment with periodate. (Compare Experiment 1 in Table II and the discussion in the following paper (19).) The disappearance of formaldehyde indicates the abolition of a primary alcohol group; the simultaneous production of roughly 0.5 mole of oxalic acid reveals the presence of a grouping such as \(-\text{CHOH-CO-};\) COOH. The production of carbon dioxide by periodate points, in the light of the discussion presented in the following communication (19), to the formation of a polyhydroxy acid \(-\text{(CHOH)}_2\text{COOH}.\)

The various findings outlined here suggest the occurrence of an aldol condensation between 2 or more molecules of II, or possibly between II and IV, analogous to the condensation of glyceraldehyde and dihydroxyacetone to \(dl\)-fructose and \(dl\)-sorbose (20, 21) or of \(d\)-glyceraldehyde to \(d\)-fructose and \(d\)-sorbose (22). The experimental results could be explained by the formation of a compound of the type of an \(\alpha\)-ketotrihydroxyadipic acid.

1 The reactivity of hydroxypyruvic acid may prove of biological interest. In animals known to synthesize ascorbic acid (e.g. the rat), it is possible that the pathway of the reaction is via an asymmetric aldol condensation between \(l\)-glyceraldehyde and hydroxypyruvic acid to \(2\)-keto-\(l\)-gulonic acid, followed by enolization and lactonization to the vitamin.
The prolonged contact of hydroxypyruvic acid with 0.01 N alkali causes a slow spontaneous decarboxylation accompanied by a drop in pH (Table II). After 72 hours 1 mole of II gives rise to almost 0.5 mole of carbon dioxide. This formation of carbon dioxide may be due to a gradual decarboxylation of a β-keto acid, which is in tautomeric equilibrium with the α-keto acid originally produced by weak alkali: 

\[ \text{CHOH·CO·COOH} \rightleftharpoons \text{COH＝COH·COOH} \rightleftharpoons \text{CO·CHOH·COOH} \rightarrow \text{CO·CH}_2\text{OH} + \text{CO}_2. \]

A simple tautomeric shift of II to the β-aldehydo acid (IV) appears unlikely, since under those circumstances 1 rather than 0.5 mole of carbon dioxide should be produced. The view that the spontaneous decarboxylation of the condensation product of hydroxypyruvic acid leads to the formation of the grouping \(-\text{CO·CH}_2\text{OH}, i.e.\) of a primary alcohol group, is borne out to a large extent by the trend in the amounts of formaldehyde and oxalic acid produced by the action of periodate on the condensation product at different stages of spontaneous decarboxylation (Fig. 1). In measure with the increase in spontaneously produced carbon dioxide, the formaldehyde values rise from 0.07 to 0.26 mole and the yields in oxalic acid drop from 0.4 to 0.15 mole (Table II). Attempts, briefly summarized in the experimental part, to determine in a more definite

<table>
<thead>
<tr>
<th>Experiment No.*</th>
<th>Time after alkalization</th>
<th>pH</th>
<th>Moles of carbon dioxide per mole of hydroxypyruvate produced by spontaneous decarboxylation</th>
<th>Products of oxidation with NaIO₄ mole per mole of hydroxypyruvate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>hrs.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>7.0</td>
<td>0.050</td>
<td>0.094</td>
</tr>
<tr>
<td>2a</td>
<td>4</td>
<td>11.1</td>
<td>0.066</td>
<td>0.37</td>
</tr>
<tr>
<td>2b</td>
<td>24</td>
<td>9.9</td>
<td>0.27</td>
<td>0.39</td>
</tr>
<tr>
<td>2c</td>
<td>48</td>
<td>9.4</td>
<td>0.40</td>
<td>0.40</td>
</tr>
<tr>
<td>2d</td>
<td>72</td>
<td>9.2</td>
<td>0.47</td>
<td>0.36</td>
</tr>
<tr>
<td>2e</td>
<td>96</td>
<td>9.0</td>
<td>0.50</td>
<td>0.38</td>
</tr>
<tr>
<td>2f</td>
<td>120</td>
<td>9.0</td>
<td>0.53</td>
<td>0.34</td>
</tr>
<tr>
<td>2g</td>
<td>144</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2h</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* The freshly prepared 0.05 M sodium hydroxypyruvate solution (Experiment 1) was made 0.01 N with respect to alkali (Experiment 2a). The progressive decrease in pH, increase in carbonate, and the products of oxidation with sodium periodate (carbon dioxide, formaldehyde, and oxalic acid) are recorded as Experiments 2b to 2h. The values of carbonate reported in the fourth column were subtracted from the total produced in the periodate oxidation to give the figures reported in the fifth column. Corrections are made for the values of carbon dioxide found in Experiment 1.
manner the type of carbon skeleton produced by the condensation of II have so far not led to conclusive results.2

Synthesis of β-Hydroxy-α-ketobutyric Acid—The keto acid corresponding to dl-threonine, dl-β-hydroxy-α-ketobutyric acid, CH$_3$·CHOH·CO·COOH, was also synthesized. β-Bromo-α-ketobutyric acid, prepared from α-ketobutyric acid, yielded, by careful hydrolysis, solutions of the desired sub-

![Graph](http://www.jbc.org/)

**Fig. 1.** Behavior of condensation product produced from hydroxypyruvic acid by 0.01 N alkali. Curve I, carbon dioxide formed by spontaneous decarboxylation. Curves II, III, and IV represent the products of periodate oxidation: Curve II, carbon dioxide; Curve III, formaldehyde; Curve IV, oxalic acid. Ordinate, moles of reaction products per mole of hydroxypyruvic acid; abscissa, time elapsed after addition of alkali.

stance. The 2,4-dinitrophenylhydrazone was obtained in good yield.3 Oxidation of the solution of this hydroxyketo acid by periodate produced

2 It is evident from the extreme lability of II to alkali that it cannot have arisen directly from the alkaline decomposition products of cellulose nitrate mentioned in the beginning of this paper. This agrees with the conclusions reached by Kenyon in a careful investigation of the reactions involved in the degradation of cellulose nitrate by alkali (23); compare (24).

3 The synthesis of a compound described as β-hydroxy-α-ketobutyric acid was reported recently (25). There are, however, several points of difference between this product and the compound described here.
oxalic acid and acetaldehyde almost quantitatively, with the consumption of 1 mole of oxidant.

EXPERIMENTAL

Bromopyruvic Acid

Preparation—This compound was prepared according to a modification of the procedure described by Ward (26). 1 mole (88.1 gm.) of pyruvic acid (m.p. 13.6-13.8°) was heated to 50° in a 3-neck flask equipped with ground glass joints, and 160 gm. (1 mole) of bromine, previously dried by shaking with concentrated H₂SO₄, were added dropwise with stirring and exclusion of moisture. The heat of the reaction was usually sufficient to keep the temperature at 50°; external temperature control was resorted to when necessary. The thick fuming sirup was immediately poured into a large crystallizing dish, the flask washed with a little hot benzene, and the washing added to the main product. Sometimes the material set in the flask to a fuming crystal mass which was dissolved in a small amount of hot benzene. The mixture was placed in a vacuum desiccator over moist NaOH pellets, and the solvent removed by suction. On the next day the material was ground to a fine powder¹ and kept in vacuo for 48 to 72 hours, with frequent renewal of the alkali, until no more fumes of HBr were given off. The yield was 164 gm. (98 per cent) of white crystals melting at 70°. Crystallization from dry chloroform (1 cc. per gm.) with the aid of mechanical stirring to prevent caking yielded 135 to 140 gm. of hexagonal prisms melting at 74°.⁵

C₃H₃O₃Br. Calculated. C 21.6, H 1.8, Br 47.9
167.0 Found. “ 21.4, “ 1.6, “ 47.8

Bromopyruvic Acid 2,4-Dinitrophenylhydrazone—5 gm. (0.03 mole) of bromopyruvic acid dissolved in 100 cc. of water were treated with a solution of 5.9 gm. (0.03 mole) of 2,4-dinitrophenylhydrazine in 400 cc. of 2 N HBr with vigorous stirring and cooling in ice water. After 1 hour the precipitate was filtered off, washed with cold 2 N HBr and cold water, and dried in vacuo over P₂O₅. The product weighed 9.8 gm. (95 per cent) and melted at 180°. It was analytically pure. Crystallization from dioxane gave fine yellow needles of unchanged melting point.


¹ The unstable intermediate compound (26) as well as the HBr-free bromopyruvic acid is a strong vesicant.

⁵ The melting points, reported without correction, were determined with the Fisher-Johns apparatus. It is noteworthy that bromopyruvic acid, when observed under these conditions, forms a transparent glass at about 60° (which may account for the reported melting point of 59° (26)) and melts to an oil at 74°.
Ethoxypyruvic Acid 2,4-Dinitrophenylhydrazone—1 gm. (2.9 mm) of the bromopyruvic acid hydrazone was dissolved in 100 cc. of 80 per cent alcohol and titrated electrometrically with 0.2 N aqueous NaOH. When a pH of 8 was reached, 2 equivalents of alkali had been consumed and the titration was stopped. Acidification with 2 N HCl gave 0.82 gm. (91 per cent) of the ethoxypyruvic acid 2,4-dinitrophenylhydrazone, melting at 153-155° (the solidified melt had a melting point of 163-166°). Crystallization from ethyl acetate yielded yellow needles melting at 154° (second m.p. 164-167°). The mixture of this compound with hydroxypyruvic acid 2,4-dinitrophenylhydrazone (see below) melted at 138-140°. A solution of the hydrazone in 80 per cent ethanol was titrated electrometrically with 0.1 N alkali.

C₁₂H₁₂O₇N₂ (312.2)
Calculated. C 42.3, H 3.9, N 18.0 —OC₂H₅ 14.4, neutralization equivalent 312
Found. " 42.1," 3.9, " (Dumas) 18.0, —OC₂H₅ 13.4, neutralization equivalent 305

Hydroxypyruvic Acid

Preparation—The following procedure, out of several tried (e.g. treatment with silver carbonate or with potassium acetate in acetic acid-acetic anhydride), proved the most successful. A solution of 4.175 gm. (0.025 mole) of bromopyruvic acid in 30 cc. of water was placed in a 500 cc. volumetric flask and 465 cc. of 0.107 N NaOH or KOH (2 equivalents) were added in such a way as to keep the pH below 8.5. The first 400 cc. could be added slowly in 1 portion with shaking to give a pH of 6.8. The remainder was added in small volumes at intervals, additions being made whenever the pH fell from 8.5 to 7. These solutions, 0.05 M with respect to hydroxypyruvic acid after adjustment to volume, were used in all experiments.

Solutions of hydroxypyruvate (II) reduced Benedict’s solution on gentle warming and silver nitrate at room temperature. Bromide could be removed by adding a solution of the required amount of AgNO₃, although not without some reduction. The filtrate gave an insoluble, amorphous lead salt. The acid could not be extracted from its aqueous solution by ether, although it is soluble in it as shown below. No significant reaction with ferric chloride was observed, unless alkali was added, when a deep violet solution resulted.

The addition of phenylhydrazine acetate to solutions of II gave, at room temperature, brown amorphous precipitates which were ill defined and bore little resemblance to the well characterized osazone I. Attempts to prepare an osazone at a higher temperature led to deeply colored products.

Crude preparations of the free acid could be made by adding 1 equivalent of 10 N HBr to the concentrated aqueous solution of potassium hydroxy-
pyruvate and evaporating to dryness in vacuo in a current of N₂ at room temperature. Ether was added to the residue and the insoluble salts were repeatedly washed with ether, when a colorless ethereal solution was obtained. The evaporation residue of the ether solution formed a straw-colored sirup which, dissolved immediately in water, yielded solutions which showed a 90 per cent recovery by acidimetric titration and 80 per cent by periodate consumption.

**Hydroxypyruvic Acid 2,4-Dinitrophenylhydrazone**—5 gm. (0.03 mole) of bromopyruvic acid were hydrolyzed with 600 cc. of 0.1 N KOH, as described above, and 5.5 gm. (0.028 mole) of 2,4-dinitrophenylhydrazine in 500 cc. of 2 N HCl were added. After being chilled overnight the semicrystalline precipitate was filtered off, washed with 2 N KC1 and water, and dried in vacuo over P₂O₅. The material, which was free of halogen, weighed 7.5 gm. (95 per cent on the basis of the 2,4-dinitrophenylhydrazine added) and melted at 158-160°. Crystallization from ethyl acetate, followed by the thorough removal of the solvent at 0.1 mm. of Hg and 80°, gave 4.4 gm. of orange needles, melting at 162°. An additional amount of equally pure substance could be obtained from the mother liquor by crystallization from 3 volumes of ethyl acetate and 1 volume of ligroin (b.p. 60-90°). A solution of the hydrazone in 80 per cent alcohol was titrated electrometrically with 0.01 N alkali.

C₆H₄O₇N₄ (284.2)
Calculated. C 38.0, H 2.8, N 19.7, neutralization equivalent 284
Found. " 38.0, " 2.8, " (Dumas) 19.8, neutralization equivalent 282

**Reduction of Hydroxypyruvic Acid 2,4-Dinitrophenylhydrazone to dl-Serine**—The procedure used was patterned after the method for the reduction of phenylhydrazones (27). To 3 gm. (0.0105 mole) of the hydrazone dissolved in 100 cc. of 80 per cent ethanol was added aluminum amalgam (28) prepared from 10 gm. of aluminum turnings. The reaction mixture became warm and was cooled in ice water with occasional shaking for 30 minutes, followed by shaking at room temperature for 16 hours. The precipitate was centrifuged off and extracted three times with boiling water; the solution was evaporated in vacuo to dryness and the residue extracted with hot water. The deep brown color of the combined aqueous extracts (250 cc.) was removed by continuous extraction with ether for 3 days. The water layer was treated with norit and the filtrate evaporated in vacuo to a very small volume. Addition of absolute alcohol produced 300 mg. (27 per cent) of slightly colored crystals decomposing at 240°. When a small sample was heated with p-nitrobenzoyl chloride in pyridine, followed by the addition of 10 per cent Na₂CO₃, the strong pink color characteristic for serine in this general test for α-amino acids was observed (29). The crude material, which was almost analytically pure, was dissolved in 20 cc. of water and treated with charcoal (Darco G-60) in the
cold. The filtrate was concentrated in vacuo to a very small volume and an excess of absolute alcohol added when dl-serine was obtained in the form of white crystals weighing 260 mg.

C₆H₁₂O₃N (105.1)
Calculated. C 34.3, H 6.7, N 13.3
Found. " 33.9, " 6.9, " (Dumas) 13.3, amino N 13.3, amino acid N (30) 13.3

3-Phenyl-5-hydroxymethylhydantoin—The attempt to effect an additional characterization of the dl-serine by conversion to α-hydroxymethyl-5-phenylhydantoic acid (31) led in our hands directly to the corresponding hydantoin (32). The same observation was made with an authentic specimen of dl-serine. Analyses of both hydantoin preparations (respectively designated Hydantoins 1 and 2) are reported below. To 100 mg. of dl-serine in 0.5 cc. of water 1 cc. of N NaOH and 0.10 cc. of phenyl isocyanate (in two portions) were added with cooling in an ice-salt mixture. The mixture was kept at 0° for 1 hour, acidified with concentrated HCl, cleared by centrifugation, and concentrated to a small volume in an evacuated desiccator over P₂O₅. The precipitate was recrystallized from a small volume of water when crystals melting at 162-163° (Hydantoin 1) and 166-167° (Hydantoin 2) were obtained. A mixture of both specimens melted at 163-166°.

C₁₀H₁₀O₃N₂. Calculated. C 58.2, H 4.9, N 13.6
206.2 Found, Hydantoin 1. " 58.0, " 5.0, " (Dumas) 13.3
" " 2. " 58.0, " 4.9, " (" ) 13.5

Oxidation with Periodic Acid—5 cc. of a freshly prepared 0.05 M solution of sodium hydroxypyruvate were treated either with periodate, according to procedures (a) and (b), as described in the following paper (19), or under strongly acid conditions6 with 1 cc. of 0.5 M periodic acid in the presence of 10 cc. of water. In the last mentioned procedure the unused reagent was determined according to the method of Malaprade (33). In all experiments the consumption of periodate was 0.95 to 1.0 mole per mole of hydroxypyruvic acid, when acted upon by the reagent for 10 to 150 minutes. The oxidation products were 0.9 mole of formaldehyde (as the dimedon derivative melting at 189-191°) and 0.86 mole of oxalic acid. (For the isolation procedures, compare (19).)

dl-β-Hydroxy-α-ketobutyric Acid
dl-β-Bromo-α-ketobutyric Acid—α-Ketobutyric acid, which served as starting material, was prepared by hydrolysis (34) of ethyl ethoxalyl-

6 Since the 0.05 M hydroxypyruvate solutions were also 0.05 M with respect to bromide, small amounts of bromine were liberated by the periodic acid in the strongly acid solutions. These amounts were not sufficient, however, to influence the results.
propionate (35). Bromination of 51 gm. (0.5 mole) of keto acid, as described above, gave 87 gm. (96 per cent) of crude material. By crystallization from a mixture of 40 cc. of chloroform and 120 cc. of ligroin (b.p. 60-90°), 71 gm. of strongly hygroscopic white plates were obtained, which melted at 60° (determined in a closed tube).

\[
\text{C}_9\text{H}_6\text{O}_2\text{Br}. \quad \text{Calculated.} \quad \text{C} 26.6, \text{H} 2.8, \text{Br} 44.1
\]

181.0
\[
\text{Found.} \quad " 26.8, " 2.8, " 43.7
\]

\textit{dl-\(\beta\)-Hydroxy-\(\alpha\)-ketobutyric Acid—}Solutions of this acid were prepared by the careful addition (with electrometric control) of 200 cc. of 0.1 N alkali (2 equivalents) to a solution of 1.81 gm. (0.01 mole) of \textit{dl-\(\beta\)-bromo-\(\alpha\)-ketobutyric acid} in 20 cc. of water in such a manner as to maintain the pH between 7 and 8.5.

\textit{dl-\(\beta\)-Hydroxy-\(\alpha\)-ketobutyric Acid 2,4-Dinitrophenylhydrazone—}A solution of the acid (0.01 mole in a volume of 220 cc.), freshly prepared as described in the preceding paragraph, was cooled to 5° and a cold solution of 2.0 gm. (0.01 mole) of 2,4-dinitrophenylhydrazone in 300 cc. of 2 N HCl was added. The mixture was cooled overnight and the yellow precipitate washed with cold 2 N HCl and cold water. It weighed 2.6 gm. (87 per cent yield). Crystallization from 50 cc. of ethyl acetate gave 1.3 gm. of yellow needles, melting at 157-158°.

\[
\text{C}_{13}\text{H}_{18}\text{O}_7\text{N}_2 \quad (298.2)
\]

\begin{align*}
\text{Calculated.} & \quad \text{C} 40.3, \text{H} 3.4, \text{N} 18.8, \text{neutralization equivalent} 298 \\
\text{Found.} & \quad " 40.4, " 3.4, " (\text{Dumas}) 18.7, \text{neutralization equivalent} 297
\end{align*}

\textit{Oxidation with Periodic Acid—}The oxidation of 5 cc. of a 0.05 M hydroxyketobutyric acid solution in the presence of 15 cc. of M sodium bicarbonate, with 1 cc. of 0.43 M sodium periodate, resulted in the consumption of 0.25 mm of the oxidant in 30 minutes. A similar oxidation of 1.25 mm of the keto acid yielded, by the isolation procedure described in the following paper (19), 0.181 gm. of calcium oxalate monohydrate (1.24 mm), which required 23.8 cc. of 0.1 N potassium permanganate for oxidation.

For the isolation of the dimedon derivative of acetaldehyde, 25 cc. of the keto acid solution (1.25 mm) were oxidized with 4 cc. of 0.43 M sodium periodate, in the presence of 15 cc. of M sodium bicarbonate, in a tightly stoppered flask. Half an hour after the addition of 1 cc. of 2 M sodium arsenite, in order to destroy unused oxidant, the solution was made acid to methyl red with glacial acetic acid and 200 cc. of a 0.4 per cent dimedon solution were added. The acetaldehyde dimedon derivative weighed 0.345 gm. and melted at 139°. The yield corresponded, after correction for solubility of the dimedon derivative, to 96 per cent of the expected amount.
Action of Strong Alkali on Hydroxypyruvic Acid

Formation of Enediol—To 200 cc. of 0.05 M hydroxypyruvate varying amounts of 0.16 N NaOH were added with shaking in a nitrogen atmosphere. After 30 minutes 5 cc. samples were withdrawn, cooled, acidified with 2 N HCl, and titrated with a 0.1 N iodine solution. The formation of dihydroxyacrylic acid (III), progressing with the strength of the alkali until an enediol concentration of about 64 per cent is reached, is presented in Table I. The same results were obtained with solutions permitted to remain alkaline for 2 hours.

Isolation of Glyoxalosazone—Portions (180 cc.) of the alkaline solutions, represented as Experiments 2 to 5 in Table I, were acidified with glacial acetic acid and treated with 3 cc. of phenylhydrazine in 50 per cent acetic acid at room temperature. Bulky brown precipitates were obtained in a few minutes and were filtered off after 1 hour. The crude dry products weighing 0.4, 0.9, 0.96, and 1.0 gm., respectively, were taken up in 25 cc. of absolute ethanol, decolorized with charcoal (Darco G-60), and crystallized by the addition of sufficient water to produce a slight turbidity. The yields of the light yellow rectangular plates of pure glyoxalosazone melting at 169° were 0.14, 0.30, 0.30, and 0.37 gm., respectively.

<table>
<thead>
<tr>
<th>C_{10}H_{14}N_{4}</th>
<th>Calculated. C 70.6, H 5.9, N 23.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>238.3</td>
<td>Found. 70.4, 5.8, 23.5 (Dumas) 23.5</td>
</tr>
</tbody>
</table>

Oxidation of Enediol with Iodine; Isolation of Osazone of Mesoxalic Acid Semialdehyde—A 0.05 M solution of hydroxypyruvic acid was made 0.8 N with respect to NaOH, as described above (Experiment 5, Table I). After the oxidation with iodine, a portion of the solution, corresponding to 0.75 mM of enediol, was treated with 3 cc. of phenylhydrazine in 15 cc. of 50 per cent acetic acid. After 2 hours at room temperature the precipitate was filtered off and dried; it weighed 200 mg. and melted at 214-215°. Crystallization from chloroform gave 110 mg. of the orange osazone of mesoxalic acid semialdehyde (I) melting with decomposition at 222-223° (36). A mixture with a specimen obtained by the alkaline decomposition of cellulose nitrate (2) melted at 221-222°.

<table>
<thead>
<tr>
<th>C_{12}H_{14}O_{2}N_{4}</th>
<th>Calculated. C 63.8, H 5.0, N 19.9</th>
</tr>
</thead>
<tbody>
<tr>
<td>282.3</td>
<td>Found. 63.6, 5.1, 19.9 (Dumas) 19.9</td>
</tr>
</tbody>
</table>

Action of Oxygen on Enediol—200 cc. of a 0.05 M solution of sodium hydroxypyruvate (10 mm) were placed in a hydrogenation flask, cooled in an ice bath, and made 0.85 N with respect to alkali by the addition, in an atmosphere of nitrogen, of 20 cc. of 9.4 N sodium hydroxide (which, as shown in Table I, brought about the formation of about 65 per cent of

The alkaline solutions became golden yellow after a few minutes. Acidification discharged the color.
enediol). After 30 minutes the flask was connected to a large gas burette
filled with oxygen, the nitrogen was displaced by oxygen, and the flask
was shaken for 45 minutes. The consumption of oxygen ceased after 10
minutes when about 7 mm of oxygen had been taken up and the originally
yellow color of the solution had disappeared. The remaining solution,
which was now inert to periodic acid, contained 16 milliequivalents of
newly formed acids.

The contents of the hydrogenation flask were made up to 250 cc. and 25
cc. aliquots (corresponding to 1 mm of hydroxypyruvate) were acidified to
methyl red with acetic acid. Oxalic acid was precipitated as the calcium
salt, of which 90 mg. (0.70 mm) were obtained, requiring 12.9 cc. of 0.1 n
KMnO₄ (calculated 14.0 cc.). A 100 cc. aliquot was brought to pH 1.5
with sulfuric acid and extracted continuously for 40 hours with ether, the
solvent in the boiling flask being placed over a few cc. of 10 per cent sodium
carbonate. The alkaline layer was analyzed for formic acid by means of
mercuric chloride (37, 38); it contained an amount corresponding to 0.62
mole of formic acid per mole of hydroxypyruvate.

Action of Weak Alkali (0.01 n) on Hydroxypyruvic Acid

The solutions were prepared by adding 1 cc. of 10 n NaOH to 800 cc. of a
0.05 M hydroxypyruvate solution in a nitrogen atmosphere. The fresh
solutions had a pH of 11.6.

The consumption of periodate (determined in 5 cc. aliquots) and produc-
tion of oxalic acid and formaldehyde (determined in 20 cc. aliquots) were
followed by the methods described in the following paper (19).

Oxidative decarboxylation (19) was estimated from the amount of car-
bon dioxide produced when 1 cc. of solution, 1 cc. of water, and 1 cc. of 0.43
M sodium periodate were allowed to remain for 1 hour at room temperature
in the Van Slyke-Neill manometric apparatus. Spontaneous decarboxyla-
tion was determined in the same manner with the omission of oxidant.

When the 0.05 M hydroxypyruvate solutions were exposed at room t.em-
perature to 0.01 n alkali for 2 to 4 hours, the consumption of periodate
during 60 minutes rose to 1.5 to 1.7 moles per mole of keto acid initially
present and remained at about 1.5 moles throughout the period of the
experiment recorded in Table II, which summarizes the data on the course
of the transformation of hydroxypyruvic acid by weak alkali.

The alkalization of solutions of hydroxypyruvic acid with Ba(OH)₂,
the direct hydrolysis of bromopyruvic acid with an excess of baryta (pH

8 In contrast to the experiments carried out with strong alkali, no glyoxalosazonc
or any other definite derivative could be isolated upon the treatment of hydroxy-
pyruvate solutions, exposed to 0.01 n alkali, with phenylhydrazine or 2,4-dinitro-
phenylhydrazine. Enediol formation was minimal under these conditions. (Com-
pare Experiment 1 in Table 1.)
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or the addition of barium salts to weakly alkaline solutions of hydroxypyruvic acid yielded an insoluble amorphous barium salt containing Ba 45.7, C 18.7, H 1.3 (corrected for a water content of 9.4 per cent in the air-dried material).

A molar solution of hydroxypyruvic acid (50 cc.), prepared by concentration as described before, was held at pH 11 for 14 hours at room temperature. 1 m equivalent of sodium chlorite was then added (39) and the solution maintained for 7 days at pH 5 to 7 by the addition of acetic acid. The white precipitate produced by the addition of calcium chloride to the solution (freed of ClO₂ by evacuation at room temperature) weighed 1.5 gm. and contained Ca 18.9, C 26.6, H 2.3 (corrected for a water content of 13.2 per cent in the air-dried sample). The calcium salt reduced hot Benedict’s solution. This salt consumed, after the removal of calcium, 3 moles of sodium periodate and produced 0.8 mole of oxalic acid and 1.2 moles of carbon dioxide per atom of Ca. The oxidation and other analytical data are in fair agreement with those for the calcium salt of a 2-keto-3,4-dihydroxyglutaric acid (calculated, Ca 18.6, C 27.8, H 1.9).

Our indebtedness to Mr. W. Saschek for the microanalyses is gratefully acknowledged.

SUMMARY

The synthesis of β-hydroxy-α-keto acids was achieved by the careful hydrolysis of the corresponding β-bromo-α-keto acids. Bromopyruvic and dl β bromo α-ketobutyric acids yielded solutions of hydroxypyruvic and dl-β-hydroxy-α-ketobutyric acids respectively. The 2,4-dinitrophenylhydrazone of bromopyruvic acid could be converted into the corresponding derivative of ethoxypyruvic acid.

Both hydroxyketo acids gave crystalline 2,4-dinitrophenylhydrazones. The structure of synthetic hydroxypyruvic acid was proved through its cleavage by periodic acid to formaldehyde and oxalic acid and by the reduction of its dinitrophenylhydrazone to dl-serine. Similarly, periodic acid produced acetaldehyde and oxalic acid from hydroxyketobutyric acid.

In the presence of strong alkali (0.8 N) hydroxypyruvic acid quickly formed the enediol, dihydroxyacrylic acid, which could be made to yield glyoxalosazone or, after oxidation with iodine, the phenyllosazone of mesoxalic acid semialdehyde.

The exposure of hydroxypyruvic acid to weak alkali (0.01 N) produced a rapid and complex series of tautomeric changes and condensations. The nature of the reaction product is discussed in detail, and the evidence for its formation by an aldol condensation, perhaps analogous to that undergone by glyceraldehyde under similar conditions, is presented. Some im-
plications with respect to the biological significance of the reactivity of hydroxypyruvic acid are indicated.

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A STUDY OF $\beta$-HYDROXY-$\alpha$-KETO ACIDS
David B. Sprinson and Erwin Chargaff