228. Shichiro Akiya, Seiichi Okui and Shigeo Suzuki : Studies on the Degeneration Process of Sugars. IV*. Reaction of Periodic Acid on α -Methyl-D-glycoside

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The oxidation of methyl hexoside (I) with periodic acid has been shown by Fleury and co-workers¹⁾ to result in the liberation of the carbon atom at 3, and oxidation of the alcohol groups at 2 and 4 to carbonyl groups, producing a dialdehyde (II) (D'-methoxy-D-hydroxymethyldiglycolic aldehyde).

Jackson and Hudson²⁾ applied this reaction to several kinds of methyl pentosides and methyl hexosides, and prepared strontium D'-methoxy-D-hydroxymethyl-diglycolate (IV) or its barium salt by bromine oxidation in the presence of strontium or barium carbonate.

Periodic acid oxidation, furthermore, has been developed to be utilized for the determination of structure and molecular weight of sugars, or polysaccharides, especially of oligosaccharides, but in previous reports, derivatives of aldehyde compounds which were oxidized by periodic acid were not described.

We tried the periodic acid oxidation of α -methyl-D-glycopyranoside to prepare aldehyde derivatives and succeeded in the isolation as the *p*-nitrophenylhydrazone derivative, D'-methoxy-D-hydroxymethyl-glycolic aldehyde *p*-nitrophenylhydrazone.

The method consists in the oxidation of α -methyl-D-glycopyranoside with sodium metaperiodate (NaIO_4) in an aqueous solution at 10° , followed by the addition of alcoholic solution of *p*-nitrophenylhydrazine after the removal of IO_3^- and IO_4^- with lead acetate, selected to yield the hydrazone.

In this case, when the free periodic acid (HIO_4) is used as the oxidation reagent or the reaction is carried out in acid solution, the hydrazone formed is rapidly destroyed to fragments by the hydrogen anion, as will be described later.

This is a remarkable difference from the case of the preparation of Sr salt (IV), for which the use of free periodic acid is favorable and is disturbed by alkaline ion. The use of phenylhydrazine and 2,4-dinitrophenylhydrazine are unsuitable for both preparations and purifications of hydrazones.

The *p*-nitrophenylhydrazone (III) is an yellow, unstable powder, changing to orange yellow color in the air, to brown in abs. alcohol and acetone, and to red over 60° , melting at 125° with decomposition.

* Previous report : This Journal **71**, 865 (1951).

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The structure of (III) has been determined as containing one mole of *p*-nitrophenylhydrazine group in the molecule, as a result of microanalyses and methoxyl determination, while a by-product having two moles of *p*-nitrophenylhydrazine groups is obtained in a small yield by precipitation from ether solution of the crude hydrazone by the addition of benzene, but it is hard to obtain as a pure compound.

When this hydrazone is treated with mineral or organic acid, glyceraldehyde, methanol, free glyoxal and glyoxal *p*-nitrophenylosazone are formed, from which glyceraldehyde can be distilled as methylglyoxal in sulfuric acid solution and methanol is also detected in this distillate. On the other hand glyoxal *p*-nitrophenylhydrazone, considered to be formed as an intermediate in first step in the tartaric acid solution, is also detected as a slight spot by paper chromatography_{Rf} (R_f=0.83, acetone+water (2:1)).

According to the above results, it is presumed that the hydrazine residue is combined with the aldehyde of glyoxal side in the hydrazone molecule, but it is doubtful whether the hydrazine combined with glyceraldehyde residue could rearrange to the other aldehyde side in acid solution.

In fact, when the dialdehyde (II) is heated with the *p*-nitrophenylhydrazone of monoacetone-glyceraldehyde in hydrochloric acid solution, glyoxal *p*-nitrophenylosazone is easily formed, but in tartaric acid solution this reaction does not proceed completely.

It is a confirmatory evidence in the structure of (III) that the hydrazone (III) is cleaved immediately to yield glyoxal *p*-nitrophenylhydrazone even in a tartaric acid solution.

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Experiment

1) Preparation of D'-methoxy-D-hydroxymethyldiglycolic aldehyde *p*-nitrophenylhydrazone.

A solution of 1.9 g. of α -methylglycoside in 10 cc. of water is added to a cooled solution of 5.0 g. of NaIO₄ and 30 cc. of water. After mixing and being allowed to stand for 30 mins. at 10°, the solution of 6.5 g. of lead acetate in 40 cc. ethanol is added to the reaction mixture, and centrifuged to remove IO₃' and IO₄'. To the liquid layer separated from the precipitate, ethanolic solution of 1.5 g. of *p*-nitrophenylhydrazine is added at a stretch, warmed to 40° for a few minutes, and cooled to 10°, and a small quantity of water is added dropwise to the solution until it becomes turbid, and then the mixture is treated with charcoal. The filtrate is poured into 200 cc. of water, by which the crude product separates as orange yellow precipitate, washed by 200 cc. of water and dried in vacuo.

The pure hydrazone is obtained by means of repeating the precipitation by water from the alcoholic solution of the hydrazone. m.p. 125° (decomp.). *Anal.* Calcd. for C₁₂H₁₅O₆N₃: C, 48.47; H, 5.05; N, 14.14. Found: C, 48.42; H, 4.86; N, 14.85. CH₃O- group. Calcd.: 10.47; Found: 10.23. $[\alpha]_D^{15} = 5.05-78.2^\circ$ (Substance: 0.0460 g. in 10 cc. 60% ethanol, *l*=10 cm.)

2) Decomposition of the hydrazone in acid solution.

i) Glyoxal *p*-nitrophenylosazone: 0.3 g of the hydrazone is heated in diluted hydrochloric acid or tartaric acid solution, by which red glyoxal *p*-nitrophenylosazone is immediately precipitated and is recrystallized from pyridine to crystals of m.p. 314° (decomp.). In admixture with an authentic sample, no depression of m.p. is observed. Above reaction also proceeds even in neutral, boiled water solution.

ii) Methylglyoxal: A mixture of 1 g. of dried hydrazone, 2 g. of conc. sulfuric acid and 10 cc. of water is distilled to yield 30 cc. of distillate. The distillate gives a characteristic odor, and a yellow precipitate by the addition of a small quantity of iodine and caustic soda (iodoform reaction).

To 20 cc. of the distillate is added a solution of 0.3 g. of *p*-nitrophenylhydrazine in 30 cc. of 3% hydrochloric acid, and the mixture allowed to stand for 2 hrs. The precipitate is filtered off and recrystallized from nitrobenzene and toluene, to long, bright red needles, m.p. 304° (decomp.).

iii) Methanol: A mixture of a small quantity of the hydrazone (about 5 mg.), 10 cc. of water and 3 drops of conc. sulfuric acid is distilled, and to 1 cc. of this distillate 0.1 cc. of 5% phosphoric acid and 0.2 cc. of 5% potassium permanganate solution are added, warmed for 10 mins., and permanganate is decolorized with 2.5% sodium sulfite solution. This colorless solution changes to violet upon the addition of 3 cc. of conc. sulfuric acid and a few drops of 2% solution of sodium chromotrope. The coloration of violet is almost quantitative as compared with the standard tube.

3) The reaction of dialdehyde compounds (II) with monoacetone-glyceraldehyde *p*-nitrophenylhydrazone in acid solution.

An alcoholic solution of 0.2 g. of monoacetone-glyceraldehyde *p*-nitrophenylhydrazone and 1 cc. of conc. hydrochloric acid is added to a solution of dialdehyde compound (II) prepared from 0.3 g. of α -methylglycoside as described in method (1) and heated for 10 mins., by which the red glyoxal *p*-nitrophenylosazone precipitated, m.p. 314° (decomp.). This reaction did not proceed in tartaric acid solution.

4) Monoacetone-glyceraldehyde *p*-nitrophenylhydrazone.

A solution of 0.5 g. of 1,2:5,6-diacetone-mannitol in a few cc. of water is added to a cooled solution of 0.5 g. NaIO₄ and 30 cc. of water. After 30 mins., IO₃' and IO₄' are removed by the addition of 0.65 g. of lead acetate as described before. To the filtrate which contains monoacetone glyceraldehyde, ethanolic solution of 0.3 g. of *p*-nitrophenylhydrazone is added and warmed to 50°. Evaporation of a part of alcohol in vacuo affords a crude product, which can be recrystallized from alcohol to m.p. 122°. *Anal.* Calcd. for C₁₂H₁₅O₄N₃: C, 54.33; H, 5.66; N, 15.84. Found: C, 54.14; H, 5.59; N, 15.63.

Summary

By periodic oxidation of α -methyl-D-glycopyranoside, D'-methoxy-D-hydroxymethyl-diglycolic aldehyde *p*-nitrophenylhydrazone, m.p. 125° (decomp.), is obtained. This hydrazone is decomposed in acid solution to glyoxal *p*-nitrophenylosazone, free glyoxal, glyceraldehyde and methanol.

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229. Keijiro Takagi and Shigeo Iwamoto: Test Method for Analgetic Activity. I.

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In a search for new analgesics, an efficient and reliable method, which can detect even a weak analgesic action, is necessary. For that purpose, the intensity and duration of pain stimulus must be susceptible to control at will.

The Hardy-Goodell-Wolff's apparatus has been applied on man¹⁾, dogs²⁾ and rats³⁻⁶⁾ in the past decade.

Wolfe and McDonald⁷⁾ stimulated mice by placing them on a metal plate maintained at a constant temperature between 55° and 70° under a round glass cylinder. The animal kicked about on hind legs trying to jump out from the heat. We utilized this method, for reasons of economy, and examined its availability.

I. Reactions of normal mice

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