Chemical Studies on the Reaction Products of Methylpentose and Ammonia

Part I. 4(5)-(L-Erythro-2,3-Dihydroxybutyl)Imidazole from the Reaction of L-Rhamnose with Ammonia

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A new imidazolic compound isolated from the reaction mixture of L-rhamnose and ammonia was identified with 4(5)-(L-erythro-2,3-dihydroxybutyl)imidazole. Consequently, it was inferred that 3-deoxy-L-rhamnosone and formaldehyde were produced as the decomposition products of the reaction of L-rhamnose and ammonia.

4(5)-Methylimidazole had been long ago isolated by Windaus¹⁾ from the reaction mixture of glucose and ammonia, but, until recently, in spite of many efforts,²⁾ no other imidazolic compounds could be isolated and identified. Of late, one of the authors, Kômoto, has newly isolated four imidazolic compounds [2-hydroxymethyl-4(5)-methylimidazole,³⁾ 4(5)-(2-hydroxyethyl)imidazole,⁴⁾ 4(5)-(2,3-dihydroxypropyl)imidazole^{5,6)} and 4(5)-(2,3,4-trihydroxybutyl)imidazole⁵⁾] other than well-known 4(5)-methylimidazole.

From L-rhamnose-ammonia reaction system, the three imidazolic compounds, namely, 4(5)-methylimidazole,⁷⁾ 4(5)-methyl-5(4)-ethylimidazole⁸⁾ and 2,4(5)-dimethylimidazole⁹⁾

have been isolated. Lately, we could paper-chromatographically detect at least seven imidazolic compounds including the above three as shown in Fig. 1. Tentatively, the seven compounds were designated respectively No. 1, No. 2, No. 3, No. 4, No. 5, No. 6 and No. 7 according to the locations of their R_F values. From the R_F values¹⁰⁾ and the color reactions with Pauly's reagent,¹⁰⁾ it was inferred that No. 1 (R_F 0.95, yellow), No. 3 (R_F 0.75, yellow) and No. 4 (R_F 0.68, red) would respectively be 4(5)-methyl-5(4)-ethylimidazole, 2,4(5)-dimethylimidazole and 4(5)-methylimidazole, all of which have previously been reported. The picrate of the compound No. 2 (R_F 0.82, red) has the same melting point with that of 4(5)-ethylimidazole picrate (m.p. 116°C). The results indicate the possibility that the compound No. 2 would be identified with 4(5)-ethylimidazole. The formation of 4(5)-ethylimidazole should be very interesting in view of the fission mechanism of rhamnose in aqueous ammonia, as that of 4(5)-methyl-5(4)-ethylimidazole.

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FIG. 1. Paperchromatogram of Imidazolic Compounds Produced in the Reaction Mixture of Certain Sugars and Ammonia.

- a) Reaction mixture of L-rhamnose and ammonia
- b) Reaction mixture of glucose and ammonia
- c) 4(5)-Methyl-5(4)-ethylimidazole
- d) 4(5)-Ethylimidazole
- e) 2,4(5)-Dimethylimidazole
- f) 4(5)-Methylimidazole

Solvent mixture (I) was used as an irrigant.

In the present paper, we report only a new crystalline compound corresponding to No. 7 isolated from the reaction mixture. The new compound, $C_7H_{12}N_2O_2$, m.p. $151\sim152^\circ\text{C}$, $[\alpha]_D^{26} + 37.2^\circ$ (c 0.62, in water), gave a positive color reaction with Pauly's reagent (red) and had a R_F value of 0.29 on paperchromatogram using solvent mixture (I) as an irrigant. On periodate oxidation, one mole of this compound consumed approximately one mole of periodate and liberated one mole of acetaldehyde, whereas it gave neither formic acid

nor formaldehyde. Reduction of the periodate oxidation product of the new compound with sodium borohydride gave an imidazolic compound which was characterized as 4(5)-(2-hydroxyethyl) imidazole.

From all results described above, it was considered that the new compound would be 4(5)-(2,3-dihydroxybutyl) imidazole.

Consequently, a synthesis of this compound was attempted according to the synthesis of 4(5)-(2,3-dihydroxypropyl) imidazole previously reported by the authors.⁶⁾ The synthetic method is shown in the following scheme.

The synthesized crystalline compound, $C_7H_{12}N_2O_2$, m.p. $151\sim152^\circ\text{C}$, $[\alpha]_D^{26} + 38.2^\circ$ (c 0.55, in water), showed the same R_F value with that of the new compound. Its melting point was not depressed by admixture with the new compound, and the infrared spectra of the two substances were identical as shown in Fig. 2.

The results confirmed strongly that the structure of the new compound is the same as that of 4(5)-(L-erythro-2,3-dihydroxybutyl) imidazole, and it is also possible to infer that 3-deoxy-L-rhamnosone and formaldehyde

FIG. 2. Infrared Spectra of a New Compound (I) and Synthetic 4(5)-(L-Erythro-2,3-Dihydroxybutyl) Imidazole (II).

would be formed as decomposition products in the interaction of L-rhamnose and ammonia.

EXPERIMENTAL

Infrared spectra were determined as potassium bromide tablets using the Hitachi EPI-S2 type infrared spectrometer. Absorption spectra in visible region were measured with the Hitachi EPS-2 type recording spectrometer. Paperchromatographic data were obtained by ascending development on Tōyō Roshi No. 50 filter paper using acetone—chloroform—water—28% aqueous ammonia (30 : 5 : 4 : 0.2 v/v/v/v) (I) or *n*-butanol—ethanol—1% aqueous ammonia (2 : 1 : 1 v/v/v) (II) as irrigant, and Pauly's reagent as spray reagent. Unless otherwise stated, the irrigant (I) was exclusively applied to paperchromatography. Melting points were not corrected. L-Rhamnose used as a starting material was the reagent grade chemicals of the Wakō Pure Chemical Industries, Ltd. Cellulose powder used for column chromatography was Tōyō Roshi cellulose powder (100~200 mesh). The authentic 4(5)-methyl-5(4)-ethylimidazole, 2,4(5)-dimethylimidazole, 4(5)-methylimidazole and 4(5)-ethylimidazole were respectively synthesized as previously described.¹⁰⁾

I. Isolation of imidazolic compounds from reaction mixture of L-rhamnose and ammonia

A) Preparation of reaction mixture. Zinc chloride (42.5 g) was dissolved in distilled water (400 ml) and to the solution was added 28% aqueous ammonia (22 ml). The zinc hydroxide precipitated was filtered off and washed with water until Cl⁻ was not detected. The zinc hydroxide obtained was dissolved in 100 ml of 28% aqueous ammonia, and L-rhamnose (50 g) was added to the solution. The clear solution was stored at 44~5°C in a stoppered bottle for 130 hrs. The dark brown precipitate de-

FIG. 3. Paperchromatogram of Imidazolic Compounds Produced in the Reaction Mixture of L-Rhamnose.

- 1) Ether extract of the reaction mixture.
- 2) Unextracted portion of the reaction mixture.

posited was filtered off and washed thoroughly with water, and the filter cake was suspended in 250 ml of warm water followed by bubbling with H₂S gas through the solution. The precipitate of zinc sulfide produced was filtered from the mixture, and the brown clear filtrate was obtained. The filtrate was evaporated to a sirup (8 g) under reduced pressure.

B) Isolation of the new compound from the sirup. The dark brown sirup was diluted with 30 ml of water followed by extracting continuously with ether for 40 hrs. The unextracted portion was again evaporated to a sirup (4 g). The chromatograms of the ether extract and the unextracted portion are shown in Fig. 3. Four grams of the above sirup were separated on a cellulose column using solvent mixture (I) as irrigant. The fractions corresponding to new compound were collected and evaporated to a sirup (1.2 g). The sirup was dissolved in 2 ml of *n*-butanol on warm water bath. After standing at room temperature for 2 days, the light brown crude crystals were obtained in a yield of 203 mg. They were recrystallized from water containing *n*-butanol as white needles, m.p. 151~2°C, $[\alpha]_D^{20} + 37.2^\circ$ (*c* 0.62, in water). *Anal.* Found: C, 54.10; H, 7.81; N, 17.62%. *Calcd.* for C₇H₁₂N₂O₂: C, 53.83; H, 7.74; N, 17.94%.

II. Periodate oxidation of the new compound

In a 50-ml volumetric flask, 11.1 mg of the com-

pound in 25 ml of 95% ethanol and 1 ml of 0.1 M-sodium metaperiodate solution were diluted to 50 ml with water and the solution was kept at room temperature in the dark. At intervals of 30 min., 1 hr., 2 hrs., 4 hrs. and 6 hrs., each portion of 2.5 ml was withdrawn to follow the progress of the oxidation, by estimation of periodate consumption as well as acetaldehyde-, formic acid- and formaldehyde production. The blank solution was also prepared with the same method.

A) Periodate consumption.¹¹⁾ To each portion (2.5 ml) of the withdrawn oxidation mixture were immediately added 5 ml of saturated solution of sodium hydrogen carbonate, 10 ml of 0.01 N sodium arsenite solution and 2 ml of 20% solution of potassium iodide, respectively. After being set aside for 15 min. in the dark, the excess of sodium arsenite solution was titrated against 0.01 N iodine solution, using 0.5 ml of 1% solution of starch as an indicator. At the same time, the blank test was also carried out. After 1 hr., periodate consumption was 1.25 moles per mole of the new compound, and, after 2 hrs., 1.34 moles.

B) Determination of formic acid.¹²⁾ To 2.5 ml of the oxidation mixture 1.0 ml of ethylene glycol was mixed and allowed to stand for 30 min. in the dark. The solution was titrated with 0.001 N sodium hydroxide solution to methyl-red end point, but presence of formic acid was not detected.

C) Determination of formaldehyde. Although the determination of formaldehyde was carried out according to the chromotropic acid method described by O'dea and Gibbons,¹³⁾ the presence of formaldehyde could not be detected.

D) Determination of acetaldehyde. The determination of acetaldehyde was performed as described by Nicolet and Shinn,¹⁴⁾ and 0.85 moles of acetaldehyde per mole of the new compound were estimated.

E) Sodium borohydride reduction of an imidazolic aldehyde produced from periodate oxidation of the new compound. Sodium borohydride

reduction was performed essentially as described by Fujii and Kōmoto.¹⁵⁾ The solution of the new compound (2.2 mg) in 5 ml of 95% ethanol and 1 ml of 0.1 N sodium periodate solution were diluted to 10 ml with water. After the mixture was stored at room temperature for 2 hrs. in the dark, 0.05 ml of the aqueous solution saturated with barium chloride were added to 5 ml of the oxidation mixture, and the solution was allowed to stand for a few min. to give precipitate. The precipitate was filtered off from the solution, and to the filtrate was added a sufficient quantity of diluted solution of sodium hydroxide until the solution became alkaline. After a few min., the precipitate produced was filtered off from the solution and to the filtrate was added 0.07 ml of 5% sodium borohydride solution containing a few drops of sodium hydroxide solution. After the solution was allowed to stand at room temperature for 30 min., the excess of sodium borohydride was destroyed by adding acetic acid, followed by neutralizing the solution with barium hydroxide and by evaporating the neutralized solution to dryness.

The reduced imidazolic compound was extracted with 95% ethanol. The extract was concentrated and paperchromatographed, using solvent mixture (I) or (II) as irrigant. The R_F value and the color reaction of the imidazolic compound were identical with those of authentic 4(5)-(2-hydroxyethyl) imidazole.

III. Synthesis of 4(5)-(L-erythro-2, 3-dihydroxy-butyl) imidazole

A) Preparation of 3-deoxy-L-rhamnosone. The preparation was performed as in the case of preparation of 3-deoxy-D-glucosone described by Kato.¹⁶⁾ A mixture of finely powdered L-rhamnose (20 g), *n*-butylamine (8.8 g) and methanol (35 ml) was heated at 62~5°C for 3.5 hrs. with occasional stirring to give a browning solution. To the solution which was cooled to room temperature were added 7.5 ml of glacial acetic acid, and the mixture was warmed at 55°C for 2 hrs. The resulting dark solution was cooled and diluted to a volume of about 280 ml with water.

Decolorization with active carbon (5 g) followed by vacuum filtration was repeated four times until a pale yellow solution could be obtained. The solution of 500 ml was passed through a column of

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Dowex 50×4 (H-form, 100 g), and then the column was washed with water. Effluent combined with the washings was evaporated to a sirup under reduced pressure. Plenty of the unreacted L-rhamnose in the sirup were removed by fractional crystallization using 95% ethanol. The solution freed, as much as possible, from L-rhamnose was evaporated to a sirup (2.2 g.) under reduced pressure. 3-Deoxy-L-rhamnose in the sirup was separated by a cellulose column chromatography using *n*-propanol—ethyl acetate—water (7:1:2 v/v/v) as an irrigant. The fractions containing only 3-deoxy-L-rhamnosone were evaporated to dryness, and pure 3-deoxy-L-rhamnosone was obtained in a yield of 712 mg.

1) **Color reaction of 3-deoxy-L-rhamnosone with 2-thiobarbituric acid.** The color reaction was carried out as described by Kato. The absorption spectrum of the color reaction mixture is shown in Fig. 4.

FIG. 4. Absorption Spectrum of the Color Reaction Product of 3-Deoxy-L-Rhamnosone with 2-Thiobarbituric Acid.

(1 ml. of 0.0035% 3-deoxy-L-rhamnosone solution and 9 ml of 2-thiobarbituric acid reagent were heated in boiling water bath for 15 min.)

2) **Preparation of 2,4-dinitrophenylosazone from 3-deoxy-L-rhamnosone.** Three milliliters of a saturated solution of 2,4-dinitrophenylhydrazine in 2N hydrochloric acid were added to 4 ml of aqueous solution of 3-deoxy-L-rhamnosone (96.5 mg). After standing for 20 min. at room temperature, the re-

sulting osazone was filtered off by suction. The osazone was washed in turn with 2N hydrochloric acid, water and 50% ethanol, and then the washed osazone was dried in vacuum. The dried osazone was at first recrystallized from ethyl acetate and then three times from acetone, and the recrystallized osazone was obtained in a yield of 51 mg. However, by thin-layer chromatography using unactivated alumina as an adsorbent and ethyl acetate—toluene (1:1, v/v) as an irrigant, it was found that the recrystallized osazone was not yet perfectly purified. In order to purify the osazone, adsorption column chromatography was carried out by using unactivated alumina as adsorbent and ethyl acetate—toluene (1:1, v/v) as an irrigant. The pure osazone of red needles, m.p. 273~4°C, $[\alpha]_D^{21} - 1075^\circ$ (*c* 0.11 in dioxane), was obtained in a yield of 21 mg. *Anal.* Found: C, 42.39; H, 3.60%. Calcd. for $C_{18}H_{18}N_6O_{10}$: C, 42.69; H, 3.58%.

B) **Synthesis of 4(5)-(L-erythro-2,3-dihydroxy-butyl) imidazole.** To 3-deoxy-L-rhamnosone (500 mg) were added 28% aqueous ammonia (8 ml) and 37% formaldehyde solution (8 ml). After being stored at room temperature for 30 hrs., the mixture was evaporated to a sirup under reduced pressure. A large quantity of the hexamine produced were removed by fractional crystallization using absolute ethanol (5 ml). The solution almost freed from hexamine was partition-chromatographically separated on a cellulose column (4×60 cm) using solvent mixture (I) as an irrigant. The fractions containing solely the new compound were collected and evaporated to a sirup (1.11 g) under reduced pressure. To the sirup were added a few drops of *n*-butanol. After standing for 2 days, the crude crystals were obtained in a yield of 93 mg and were recrystallized by dissolving in a little quantity of water and adding a few drops of *n*-butanol to the solution. The pure crystals were obtained in a yield of 71 mg and were white needles, m.p. 151~2°C, $[\alpha]_D^{16} + 38.2^\circ$ (*c* 0.55 in water). *Anal.* Found: C, 53.62; H, 7.74; N, 17.71%. Calcd. for $C_7H_{12}N_2O_2$: C, 53.83; H, 7.74; N, 17.93%.

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