Carbohydrate Research, 26 (1973) 377–384

THE REACTION OF ACYLATED KETOSES WITH AMMONIA. AMMONOLYSIS OF 1,3,4,5,6-PENTA-*O*-ACETYL-*KETO*-L-SORBOSE*

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(Received July 25th, 1972; accepted in revised form, November 3rd 1972)

ABSTRACT

Penta-*O*-acetyl-*keto*-L-sorbose reacted with aqueous ammonia to give rise to an unusual formation of melanoidins and to a complex mixture of degradation products. 2-(L-*xylo*-Tetrahydroxybutyl)-6-(L-*threo*-2,3,4-trihydroxybutyl)pyrazine and 2,5-bis(L-*xylo*-tetrahydroxybutyl)pyrazine have been isolated and a number of imidazole compounds identified. Intramolecular acyl-migration products were not detected.

INTRODUCTION

The reaction of acyl esters of aldoses and aldobioses with ammonia has been extensively investigated[1]. Noteworthy among the competitive mechanisms observed is the intramolecular O→N migration of acyl groups to afford crystalline, and in general easily isolable, 1-deoxy-1,1-bis(acylamido)alditols and *N*-acylglycosylamines. Deacylations and transesterifications, leading to variable amounts of free sugar, are concurrent reactions. Significant amounts of basic or polymeric substances were not produced from acetates or benzoates of aldoses.

Acyl esters of ketoses react with ammonia by a completely different pathway, and heterocyclic nitrogenated compounds and polymeric substances (melanoidins) are the principal products, together with fragments that indicate extensive rupture of the ketohexose molecule. This behavior, however, cannot be considered unique for the acylated ketoses, as the free ketose gives analogous substances to a certain extent, although the acyl group exerts a decisive influence on the reaction rate and yields of the products.

We have studied the reaction of 1,3,4,5,6-tetra-*O*-acetyl-*keto*-L-sorbose (**1**) with 24% aqueous ammonia, our first objective being the isolation of migration products, namely *N*-acetyl-L-sorbosylamine, and the hypothetical 2-deoxy-2,2-bis-(acetamido)-L-iditol. However, a very complex fragmentation process took place resulting in at least twenty different products, as indicated by paper chromatography.

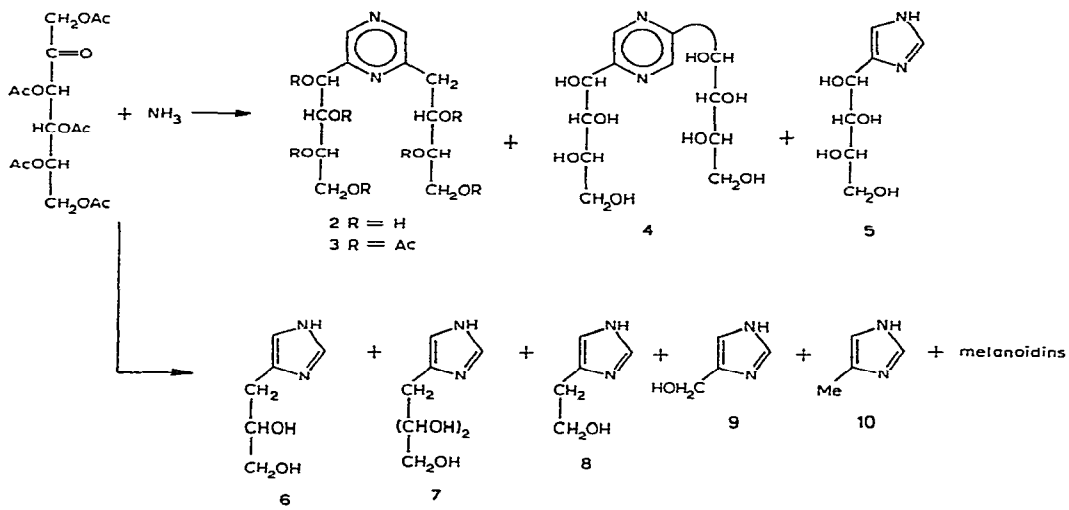
These products were resolved on columns of cellulose and charcoal, and by preparative paper chromatography but, except for the isolation of pyrazines and the

*Dedicated to Professor V. Deulofeu, in honor of his 70th birthday.

identification of imidazole compounds, the complex syrups could not be further resolved. By being kept several days at room temperature these syrups showed greater insolubility in methanol with increased formation of melanoidins, suggesting the presence of reactive fragments of low molecular weight originated by rupture of the hexose molecule that subsequently polymerized.

On the assumption that the formation of significant amounts of migration products could be hindered by a high rate of deacylation to give L-sorbose or by rupture of the carbon chain provoked by the strong concentration of ammonia, the ammonolysis of 1,3,4,5,6-penta-*O*-acetyl-*keto*-L-sorbose (**1**) was conducted also with 1% aqueous ammonia and 1% ammonia in 2-propanol. However, the general scheme leading to melanoidins and unresolvable syrups did not change.

Pyrazines occur (see Scheme I) with retention of the carbon chain of the sugar, but imidazoles and melanoidins involve C–C rupture and dehydrations. The definite influence of the acyl groups is demonstrated by the recovery of the major part of the sugar and the slight formation of melanoidins and imidazole compounds in the reaction of aqueous, methanolic, or 2-propanolic ammonia with L-sorbose[2].



Scheme I

The reaction of 1,3,4,5,6-penta-*O*-acetyl-*keto*-L-sorbose (**1**) with 24% aqueous ammonia gave 2-(L-*xylo*-tetrahydroxybutyl)-6-(L-*threo*-2,3,4-trihydroxybutyl)pyrazine (**2**) in 11% yield, and a low yield (0.4%) of 2,5-bis(L-*xylo*-tetrahydroxybutyl)pyrazine (**4**). The imidazole compounds were separated on ion-exchange resins from the bulk of the syrup and identified by paper chromatography as 4(5)-(L-*xylo*-tetrahydroxybutyl)imidazole (**5**), 4(5)-(2,3-dihydroxypropyl)imidazole (**6**), 4(5)-(2,3,4-trihydroxybutyl)imidazole (**7**), 4(5)-(2-hydroxyethyl)imidazole (**8**), 4(5)-hydroxymethylimidazole (**9**), and 4(5)-methylimidazole (**10**). The formation of these substances from L-sorbose

can be explained by extending to this sugar known patterns of fragmentation in ammoniacal media[3].

The yield of melanoidins was expressed on the basis of the free sugar (L-sorbose) involved in the reaction. Thus, in the ammonolysis of 1,3,4,5,6-penta-*O*-acetyl-*keto*-L-sorbose (**1**), the melanoidins amounted to 20%; this value is aproximate since these substances are present in different stages of polymerization which have varying solubilities in methanol. It is the ubiquitous presence of these soluble melanoidins that render difficult the isolation of the low-molecular-weight fragments described in the preceding paragraph.

The pyrazine ring in 2-(L-*xylo*-tetrahydroxybutyl)-6-(L-*threo*-2,3,4-trihydroxybutyl)pyrazine (**2**) was demonstrated by the characteristic u.v. absorption for pyrazine[4,5] at 267 nm. The composition, nonreducing character, and resistance to hydrolysis of the product suggested the condensation of two molecules of L-sorbose in the presence of ammonia. The position of the substituents on the pyrazine ring was determined by oxidation of **2** with hydrogen peroxide in alkaline medium, and the previously described 2,6-pyrazinedicarboxylic acid (**12**) was unambiguously identified by paper chromatography[6] and by the preparation of a dimethyl ester[7] (**13**).

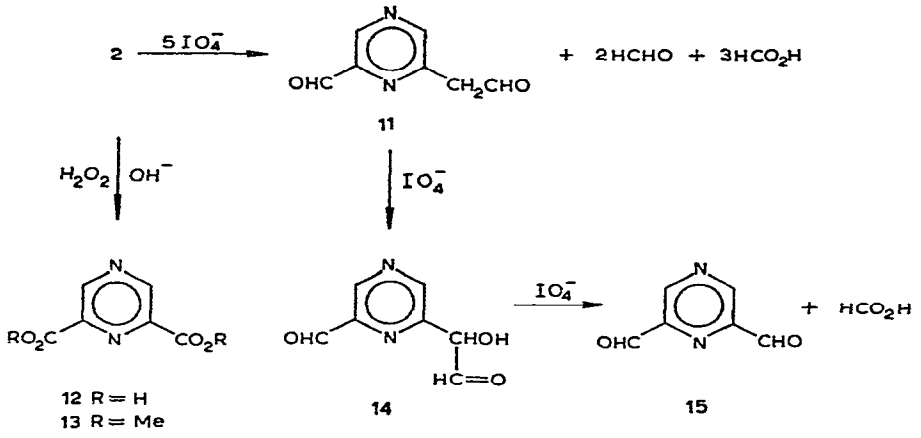
The n.m.r. spectrum of **2** in deuterium oxide agrees with that of the isomer "deoxy-D-fructosazine", whose structure was demonstrated by Kuhn and coworkers[8]. The aromatic protons resonated at τ 1.36 and 1.47; the C-1″ methylene (τ 6.86) and H-1′ (τ 4.94) bands were well separated from those of the rest of the protons (multiplet from τ 5.84 to 6.46).

The u.v. and n.m.r. spectra of the heptaacetate (**3**) of **2** confirmed these results and agreed with published data for the corresponding protons of the acetate of "D-fructosazine"[5] and of 2-(tetrahydroxybutyl)quinoxaline[9], with the exception of the C-1″ methylene doublet (τ 6.87).

The location of hydroxyl and methylene groups was determined by periodate oxidation of **2**. An immediate uptake of 5 moles of periodate per mole of **2**, with production of 3 moles of formic acid and 2 moles of formaldehyde, agrees with the presence of two primary hydroxyl groups and two secondary hydroxyl groups in one side chain, and three secondary hydroxyl groups in the other. The subsequent uptake of 2 moles of periodate and the production of 1 mole of formic acid can be interpreted on the basis of hydroxylation of the activated methylene group in the intermediate **11** to give the hydroxyaldehyde **14**, whose rupture would lead to the dialdehyde **15** (see Scheme II).

Formation of compound **2** can be rationalized on a basis, applied here to L-sorbose, similar to that postulated in a previous paper[7] for the formation of 2-(D-*arabino*-tetrahydroxybutyl)-6-(D-*erythro*-2,3,4-trihydroxybutyl)pyrazine.

Together with compound **2**, a small proportion of a second pyrazine (λ_{max} 273.8 nm) was isolated, whose structure was determined by hydrogen peroxide oxidation to 2,5-pyrazinedicarboxylic acid. Based on the configuration of the starting sugar (L-sorbose), the formation of 2,5-bis(L-*xylo*-tetrahydroxybutyl)pyrazine (L-sorbosazine, **4**) was suggested, and this structure was confirmed by the n.m.r.



Scheme II

spectrum. It showed two aromatic resonances at τ 1.27; H-1′ and H-1″ resonated at τ 4.94, and the rest of the protons (eight) as a multiplet between τ 5.84 and 6.54. No methylene proton on a carbon atom linked to the aromatic ring was observed. The formation of 3 can be interpreted in a way similar to that of fructosazine[8].

The melanoidins were purified by repeated precipitation from water with methanol. A brown precipitate, insoluble in methanol, was obtained which is being further studied.

EXPERIMENTAL

General procedures. — Melting points are not corrected. The following eluents were used for paper chromatography on Whatman No. 1 paper and for cellulose-column chromatography: (A) 20:3 (v/v) butyl alcohol–water; (B) 10:2:3 (v/v) butyl alcohol–ethanol–water; (C) 5:2:2 (v/v) butyl alcohol–ethanol–water; (D) 7:1:2 (v/v) 2-propanol–butyl alcohol–water; (E) 4:1:1 (v/v) butyl alcohol–acetic acid–water; (F) 4:1:1 (v/v) butyl alcohol–formic acid–water. T.l.c. was performed on Silica Gel G (Merck) with solvent (G) 97:3 (v/v) benzene–methanol as the mobile phase. The spots were detected with the following spray reagents: (a) silver nitrate–sodium methoxide[10]; (b) aniline hydrogen phthalate[11]; (c) 1% ninhydrin in acetone; (d) diazo reagent for imidazole compounds[12]; (e) ferrous ammonium sulfate for pyrazinecarboxylic acids[13]; (f) alkaline hydroxylamine–ferric nitrate for esters[14]. The fractions from the chromatographic columns were evaporated separately *in vacuo* with an outside bath temperature below 60°, and suitably combined after comparison by paper chromatography. N.m.r. spectra were recorded at 20–25° at 60 MHz with a Varian A-60 spectrometer; tetramethylsilane (τ 10.00) was used as the internal standard. The following abbreviations are used: s (singlet), d (doublet), m (multiplet).

*Reaction of 1,3,4,5,6-penta-*O*-acetyl-*keto-L-*sorbose* (**1**) *with aqueous ammonia.* —

1,3,4,5,6-Penta-*O*-acetyl-*keto*-L-sorbose[15] (**1**, 39 g) was shaken with 24% aqueous ammonia until dissolved (2.5 h). The solution was immediately evaporated to dryness, and the syrup obtained was dried in a vacuum desiccator and extracted with hot ethyl acetate (5 × 100 ml) to eliminate the acetamide. The residual syrup (26 g) was dissolved in boiling methanol and kept for three weeks at room temperature. A brown product (melanoidins, 1.9 g) was collected and, after subsequent spontaneous evaporation of the solvent to one half, a further amount of precipitate (1.7 g) was filtered off. The solution was evaporated to dryness, the residue was again extracted with hot ethyl acetate (5 × 60 ml), and the residual syrup (16 g) was dried in a vacuum dessicator. On paper chromatogram in solvent B, the precipitate remained at the starting point, whereas the residual syrup gave with reagents a, b, and c a complex scheme of spots.

Isolation of 2-(L-xylo-*tetrahydroxybutyl*)-*6-*(L-threo-*2,3,4-trihydroxybutyl*)*pyrazine* (**2**). — The residual syrup (16 g) was chromatographed on a column of cellulose (4.5 × 70 cm). Elution was performed with solvents A (fractions 1–183), B (fractions 184–243), C (fractions 250–273), D (fractions 274–285), methanol, and finally water. Fractions of 50 ml each were collected. From fractions 87–153 compound **2** (770 mg) was obtained and crystallized from abs. ethanol, m.p. 122°, $[\alpha]_D^{20}$ −93.0° (*c* 0.2, water); paper chromatography (solvent B, reagent a): one spot, $R_{Sorbose}$ 0.69; reagents b and c did not reveal spots; u.v. data: $\lambda_{max}^{H_2O}$ 267 nm (ε 11,700) (lit.[4,5]: pyrazine ring λ_{max} 274 nm); n.m.r. data (D_2O): τ 1.36 1.47 (2 s, H-3 and H-5 of pyrazine ring), 4.94 (d, H-1′), 5.84–6.46 (m, 8H, H-2′, H-2″, H-3′, H-3″, C-4′, and C-4″ methylenes), and 6.86 (m, C-1″ methylene).

Further amounts of **2** were isolated (see following paragraphs) to give a total yield of 11%.

Anal. Calc. for $C_{12}H_{20}N_2O_7$: C, 47.36; H, 6.63; N, 9.21. Found: C, 47.21; H, 6.73; N, 9.20.

Compound **2** was oxidized with sodium metaperiodate, the uptake of oxidant being determined[16] at 222.5 nm with a Beckman DU spectrophotometer and the formaldehyde[17] at 570 nm; formic acid was titrated with 0.1M sodium hydroxyde. Oxidations were performed in duplicate with samples (5–12 mg) of **2** dissolved in 0.1M sodium metaperiodate (10 ml) and kept at 30° during the oxidation. The results are given in moles per mole of **2**:

Time (h)	0.25	0.75	2.5	4	5	24	48
Periodate	5.0	5.1	5.4	5.5	5.8	7.0	7.0
Formic acid	3.0	3.1	3.1	3.3	3.3	4.1	4.1
Formaldehyde	2.0	2.0	2.0	2.0	2.0	2.0	2.0

2-(L-xylo-*Tetraacetoxybutyl*)-*6-*(L-threo-*2,3,4-triacetoxybutyl*)*pyrazine* (**3**). — Compound **2** (100 mg) was dissolved in 1:1 acetic anhydride–pyridine (3 ml). The solution was kept for 24 h at room temperature, and then evaporated to dryness in a vacuum dessicator. The syrup obtained (170 mg) was slightly yellow and did not crystallize from the usual solvents. T.l.c. (solvent G, reagent f) revealed one spot of R_F 0.40 and another spot at the origin. The syrup was chromatographed on a column

(1.2×28 cm) of Silica Gel Davison with benzene and increasing concentrations of methanol in benzene as eluent. Fractions of 5 ml each were collected. Fractions 63–86 (1.5% methanol) gave the acetate as a colorless syrup (54 mg); t.l.c. (solvent G, reagent f): only one spot, R_F 0.42; $[\alpha]_D^{20}$ −51.7° (*c* 0.5, chloroform); n.m.r. data (chloroform-*d*): at τ 1.60 and 1.50 (2 s, H-3 and H-5 of pyrazine ring), 3.90 (d, H-1′), 4.14–4.97 (m, H-2′, H-2″, H-3′, and H-3″), 5.77 (m, C-4′ and C-4″ methylenes), 6.87 (d, C-1″ methylene), and 7.80–8.04 (acetate methyl groups).

Anal. Calc. for $C_{26}H_{34}N_2O_{14}$: C, 52.17; H, 5.68; N, 4.68. Found: C, 53.03; H, 6.44; N, 4.40.

Isolation and structure identification of 2,5-bis(L-xylo-*tetrahydroxybutyl*)*pyrazine* (L-*sorbosazine*, **4**). — From fractions 184–195 a brown product was isolated (30 mg) that was recrystallized from abs. ethanol (charcoal). Needles (15 mg) were obtained that on paper chromatography (solvent B, reagent a) gave only one spot: $R_{Sorbose}$ 0.44; m.p. 175–177°; $[\alpha]_D^{20}$ −27° (*c* 0.1, water); u.v. data: $\lambda_{max}^{H_2O}$ 273.8 nm (ε 8,000); n.m.r. data (D_2O): τ 1.27 (s, H-3 and H-6, pyrazine nucleus), 4.94 (m, H-1′ and H-1″) and 5.84–6.54 (m, 8 H, H-2′, H-2″, H-3′, H-3″, C-4′ and C-4″ methylenes).

This compound (6 mg) was dissolved in 6% hydrogen peroxide (0.25 ml) previously made alkaline with sodium hydroxyde (10 mg), and the solution was heated for 40 min at 80°. Hydrogen peroxide (100%, 0.02 ml) was added to the cooled solution which was kept for 1 h at 80°. After being cooled to room temperature, the solution was acidified to pH 1 with hydrochloric acid, and evaporated in a vacuum dessicator. The residue obtained was chromatographed[6] on paper (solvent F, reagent e) with authentic samples of 2,5- and 2,6-pyrazinedicarboxylic acids; it showed a blue-violet spot of R_F 0.46, coincident with that of 2,5-pyrazinedicarboxylic acid.

Identification of imidazole compounds. — Paper chromatography (solvent B) of the fractions of the cellulose column, revealed with reagent (a), showed a complex scheme of spots having $R_{Sorbose}$ ranging from 0.06 to 4.60. These fractions did not yield pure products by subsequent preparative paper chromatography on Whatman 3 MM paper.

Fractions 35–153 of the cellulose column revealed, on paper chromatography (solvent E, reagent d) imidazole compounds. These fractions were applied to a sulfonic resin column Zeo-Karb 225 (H) which was eluted successively with water, 0.5M, M, and 2M aqueous ammonia.

Water (0.6 l) eluted no imidazole compounds and the residue gave a complex mixture of spots on paper chromatography.

Ammonia (0.5M, 0.5 l) elution afforded, after evaporation, further amounts of **2** (900 mg), m.p. and mixed m.p. 122°, $R_{Sorbose}$ 0.68. Paper chromatography (solvent E, reagent d) and comparison with authentic samples showed the presence, in the mother liquors, of 4(5)-(L-*xylo*-tetrahydroxybutyl)imidazole (**5**, $R_{Imidazole}$ 0.37) (principal product), as well as of 4(5)-(2,3,4-trihydroxybutyl)imidazole (**7**, $R_{Imidazole}$ 0.66), and 4(5)-(2,3-dihydroxypropyl)imidazole (**6**, $R_{Imidazole}$ 0.74).

Elution of the resin with M aqueous ammonia gave a residue which showed, on paper chromatogram, a slight spot of compound **4**.

Elution with 2M ammonia gave a residue containing 4(5)-(2-hydroxyethyl)-imidazole (**8**, $R_{Imidazole}$ 1.01); 4(5)-(2,3-dihydroxypropyl)imidazole (**6**, $R_{Imidazole}$ 0.74) as the principal products, as well as traces of 4(5)-hydroxymethylimidazole (**9**, $R_{Imidazole}$ 0.85) and 4(5)-methylimidazole (**10**, $R_{Imidazole}$ 1.17).

Oxidation with hydrogen peroxide of **2**. — Compound **2** (150 mg) was dissolved in 6% hydrogen peroxide (6 ml) previously made alkaline with sodium hydroxide (240 mg); after being kept for 2 h at room temperature, the solution was slowly heated to 80° in a water bath (0.5 h), and 100% hydrogen peroxide (0.4 ml) was added. The solution was kept at 80° until it gave a negative reaction with the Fehling reagent (1 h) and, after being cooled to room temperature, the solution was acidified to pH 1 and kept for 48 h at 5°. The supernatant was decanted from the precipitate, which was washed with little 5M hydrochloric acid and water. The precipitate showed $\lambda_{max}^{H_2O}$ 277.5 nm and, on ascending paper chromatography[6] (solvent F, reagent e) a spot of R_F 0.67, which coincides with that of a pure sample of 2,6-pyrazinedicarboxylic acid. A sample of 2,5-pyrazinedicarboxylic acid showed R_F 0.49.

2,6-Bis(methoxycarbonyl)pyrazine (**13**). — The crude solid obtained in the oxidation just described was dissolved in a solution of diazomethane in ether[18] (8 ml). After 24 h at room temperature, the solution was evaporated, and the residue was sublimed at 90°/0.01 torr (12 mg) and crystallized from water, m.p. 126–127°; lit.[7]: m.p. 126–127°. 2,5-Bis(methoxycarbonyl)pyrazine[19] has m.p. 168°.

Purification of melanoidins. — The precipitate obtained by the reaction of **1** with 24% aqueous ammonia (3.6 g) was treated with boiling methanol (4 × 100 ml) to eliminate low-molecular-weight products. The methanol-insoluble portion was dissolved in water (10 ml) and reprecipitated by adding the same volume of methanol. The precipitate obtained was submitted four times to this treatment until the supernatant was free of soluble substances, and paper chromatography of the precipitate (solvent A, reagents a and c) did not reveal spots other than a brown one at the starting point; u.v. data: $\lambda_{max}^{H_2O}$ 287.5 nm; n.m.r. datum (D_2O): only complex multiplet at τ 5.50–6.77 (ethylenic protons).

Anal.: Found: C, 47.86; H, 5.85; N, 11.67.

ACKNOWLEDGMENTS

The authors are indebted to Dr. B. B. de Deferrari and to J. J. Ferrer (Departamento de Química Orgánica, Facultad de Ciencias Exactas y Naturales de Buenos Aires) for the microanalyses and the spectra, to Dr. M. Komoto (Hyogo University, Japan) and Dr. M. R. Grimmett (University of Otago, New Zealand) for kindly furnishing imidazole samples and to the Consejo Nacional de Investigaciones Científicas y Técnicas of Argentina for financial support.

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