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AMINE-CATALYZED TRANSFORMATION OF ENOLIC NONENZYMIC BROWNING PRODUCTS, ISOMALTOL GLYCOPYRANOSIDES INTO 1,6-ANHYDRO-β-D-HEXOPYRANOSES

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ABSTRACT

The nonenzymic browning products, isomaltol D-galacto- and D-gluco-pyranosides, are transformed by 5:1 (v/v) triethylamine–pyrrolidine into 1,6-anhydro-β-D-galactopyranose (41%) and 1,6-anhydro-β-D-glucopyranose (<3%), respectively. The amines, designed to simulate the amino functionality in proteins, peptides, and ammonia (eliminated by decomposition of amino acids, proteins, and peptides) relative to nonenzymic browning during the baking process, catalyzed the transformations through the production of alkoxide ions formed from deprotonation of the ring hydroxyl groups in 1:1 (v/v) aqueous ethanol.

INTRODUCTION

The formation of 1,6-anhydro-β-D-galactopyranose (**3**) and other 1,6-anhydro-β-D-hexopyranoses by action of hot alkali on some aliphatic, vinyl, and aromatic D-glycopyranosides, and D-glycopyranosyl fluorides, has been studied extensively[1–7]. This chemistry showed that 1,6-anhydro-β-D-hexopyranoses are not formed from D-glycopyranosides on treatment with hot alkali if the aglycon has a complicated structure, involving an allylic, γ-carbonyl, or enolic group; hence, reducing hexoses are liberated[1,4,5].

The preparation of 1,6-anhydro-β-D-glucopyranose by Karrer and Smirnoff[2], from tetra-*O*-acetyl-β-D-glucopyranosyltrimethylammonium bromide was applied also successfully by Micheel[3] to D-galactose derivatives.

Previous studies[8] have shown that maltose and lactose interact with secondary amino acids to form such nonenzymic browning-products as maltol, isomaltol, isomaltol β-D-galactopyranoside (**1**), and isomaltol α-D-glucopyranoside (**2**), confirming the view that free amino acids and the amino groups of peptides and pro-

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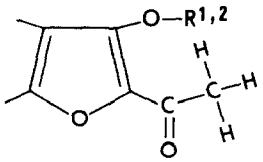
teins in bread dough act on maltose and lactose in Maillard-type browning reactions during the baking process.

In view of the foregoing, the present report is concerned with further transformation of the nonvolatile, water-soluble, enolic nonenzymic browning products, isomaltol β-D-galactopyranoside (**1**) and its α-D-*gluco* isomer (**2**), during the baking process. The hydrogen atoms of the active methyl group on the isomaltol aglycon[9] of isomaltol α- and β-D-glycopyranosides are available for H-bonding with the oxygen atom of the carbonyl group, permitting enolization (Scheme 1).



Maltose
R^1 = 4-*O*-α-D-Glucosyl-
(3-*O*-α-D-Glucopyranosyloxy-2-furyl methyl ketone)

Lactose
R^2 = 4-*O*-β-D-Galactosyl-
(3-*O*-β-D-Galactopyranosyloxy-2-furyl methyl ketone)

Scheme 1

RESULTS AND DISCUSSION

This work demonstrates conversion of the water-soluble, nonvolatile, enolic nonenzymic-browning products, 3-*O*-β-D-galacto- (**1**) and 3-*O*-α-D-glucopyranosyloxy-2-furyl methyl ketones[8,10] (**2**), into 1,6-anhydro-β-D-galactopyranose (**3**) and 1,6-anhydro-β-D-glucopyranose (**4**), respectively. The work also shows that alkoxide ions can be formed through deprotonation of ring hydroxyl groups by a mixture of triethylamine and pyrrolidine in hot aqueous ethanol for 1,6-*O*-substitution. Compounds **1** and **2** were treated with 5:1 (v/v) triethylamine–pyrrolidine in (1:1, v/v) aqueous ethanol under reflux for 24 h at 79–80° to give the highest yield (41%) for compound **3**. These conditions did not increase the yield (<3%) for compound **4**. Prolonged reaction-time increased the quantity of the brown polymeric substance and the complexity of the reaction solutions. However, the reaction mixtures were purified significantly by three extractions with chloroform. Thin-layer chromatography (t.l.c.) disclosed two major components in the aqueous extract for compound **3**, identified as 1,6-anhydro-β-D-galactopyranose (**3**) and unreacted compound **1**. Compound **3** was separated from the dark-brown, syrupy, aqueous extract by dry-column chromatography on Silica Gel G. 1,6-Anhydro-β-D-galactopyranose (**3**) thus obtained was identified by comparison of its properties with those of an authentic sample and by conversion into the triacetate **5**.

A product (**4**) was isolated in low (<3%) yield from the dark-brown syrupy residue. Thin-layer chromatography disclosed one component in the isolated,

syrupy residue. Thus, one major component was observed in the gas–liquid chromatogram with ~3% of impurities. Compound **4** was identified as 1,6-anhydro-β-D-glucopyranose (**4**) by comparison of its gas–liquid chromatographic retention value (12.6 min) with that (12.6 min; 12.5 min[11]) of an authentic sample, and by comparison of the gas chromatography–mass spectral (g.l.c.–m.s.) data (Table I) of its triacetate (**6**) with those of an authentic sample of 2,3,4-tri-*O*-acetyl-1,6-anhydro-β-D-glucopyranose. The mass-spectral data for compound **6** and the authentic 2,3,4-tri-*O*-acetyl-1,6-anhydro-β-D-glucopyranose are identical except that product **6** did not show an M^+ peak; however, the authentic triacetate showed a low peak corresponding to $M^+ + 1$, at *m/z* 289. Behavior of this type is observed in the spectra of ethers, esters and in cases where M^+ has low stability and fragments readily[12]. The loss of the species $[C_2H_4O_2]^+$, *m/z* 60, from M^+ (*m/z* 289) resulted in the formation of peaks at *m/z* 229 in both spectra. The elimination of fragments *m/z* 186 from the low-intensity peak at *m/z* 229 gives rise to the acetyl radical $[CH{\equiv}O]^+$, *m/z* 43 (base peak).

The ^{1}H-n.m.r. data for compound **3** and 2,3,4-tri-*O*-acetyl-1,6-anhydro-β-D-galactopyranose (**5**) agree closely with corresponding data of Buděšínský *et al.*[13] and Hall and Hough[14]; both appear to have the $^1C_4$(D) conformation. The three acetyl groups in **5** in benzene-d_6 are nonequivalent.

Alkaline degradation of phenyl β-D-glycopyranosides has not been successfully rationalized on the basis of any single mechanism. However, McCloskey and Coleman[15] pointed out that the double Walden-inversion mechanism involving a 1,2-anhydro-α-D-hexopyranose may be applied, without inconsistency, only to the 1,2-*trans* phenyl β-D-glycopyranosides. Treatment of tri-*O*-acetyl-1,2-anhydro-α-D-glucopyranose with alkali gives 1,6-anhydro-β-D-glucopyranose in either aqueous

TABLE I

GAS–LIQUID CHROMATOGRAPHIC AND MASS-SPECTRAL DATA FOR 1,6-ANHYDRO-β-D-GLUCOPYRANOSE (**4**) AND 2,3,4-TRI-*O*-ACETYL-1,6-ANHYDRO-β-D-GLUCOPYRANOSE (**6**)

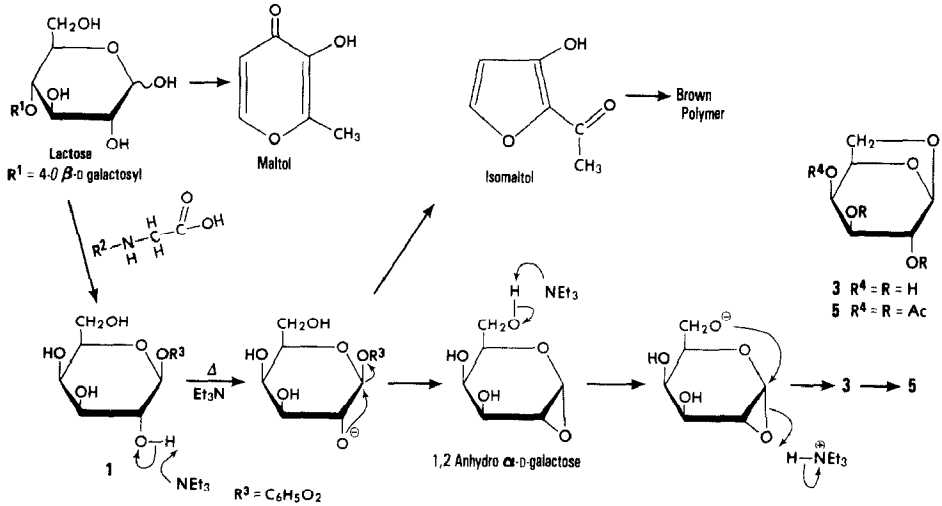
Compound	*G.l.c. retention time (min)*	*Mass spectral data (m/z and relative %)*
4	12.6	
	12.6[a]	
	12.5[b]	
6	24.2	29(1), 41(1), 42(1), 43(100), 44(2), 45(1), 60(1), 69(3), 70(4), 71(1), 73(2), 80(1), 81(9), 97(3), 98(7), 99(2), 101(1), 102(4), 103(2), 112(3), 115(5), 127(1), 140(2), 141(1), 144(1), 145(1), 157(6), 186(1), 229(1)
	24.3[c]	29(1), 41(1), 42(1), 43(100), 44(3), 45(1), 60(1), 69(4), 70(4), 71(1), 73(2), 80(1), 81(10), 97(3), 98(8), 99(2), 101(1), 102(4), 103(3), 112(3), 115(6), 127(1), 140(1), 141(1), 144(1), 145(1), 157(6), 186(1), 229(1), 289(1)

[a]Authentic 1,6-anhydro-β-D-glucopyranose. [b]Reported, ref. 17. [c]Authentic 2,3,4-tri-*O*-acetyl-1,6-anhydro-β-D-glucopyranose.

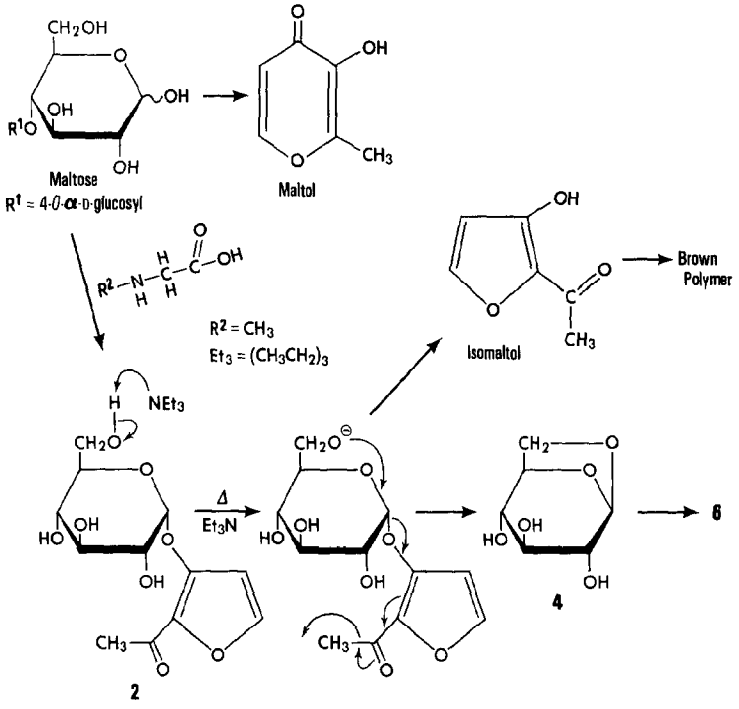
or ethanolic alkali, providing strong support for the double-inversion mechanism[16,17]. A single-inversion mechanism is suggested for the alkaline degradation of phenyl α-D-galactopyranoside, which is 1,2-*cis* substituted[4].

Isomaltol β-D-galactopyranoside (**1**) and its α-D-*gluco* (**2**) isomer were transformed into 1,6-anhydro-β-D-galacto- (**3**) and α-D-gluco-pyranoses (**4**) by a 5:1 mixture of triethylamine and pyrrolidine, which produced alkoxide ions formed from ring hydroxyl groups in hot aqueous ethanol. These conversions are probably nucleophilic substitution involving neighboring-group displacement by HO-2 in **1** and by HO-6 in **2** (ref. 4). The acidic properties and aromaticity[9] of the ring for the isomaltol aglycon is a direct correlation of the phenolic aglycon in the phenyl α- and β-D-glycopyranosides. Therefore, these transformations are in accordance with conditions for the postulated double-inversion for compound **1** and single-inversion mechanisms for **2**. Consequently, a double-inversion mechanism is suggested for the formation of compound **3** (Scheme 2), and a single-inversion for compound **4** (Scheme 3).

It is now reasonable to assume that the formation of the water-soluble 1,6-anhydrides from isomaltol β-D-galacto- (**1**) and α-D-gluco-pyranosides (**2**) occurs in bread and other baked cereal products. The alkoxide ions in this transformation may result when compounds **1** and **2** react with amino groups (free amino acids, proteins, peptides) and ammonia (eliminated by decomposition of proteins, peptides, and amino acids) during the baking process. Free amino acids and amino groups of proteins and peptides in dough probably act on lactose and maltose in Maillard-type browning reactions to produce compounds **1** and **2**, isomaltol, and maltol[8,10], whereupon the isomaltol β-D-galacto- (**1**) and α-D-gluco-pyranosides (**2**) undergo further degradation on contact with the amino groups to form the 1,6-



Scheme 2



Scheme 3.

anhydrides during the baking process, as shown in Schemes 1 and 2.

The amine-catalyzed formation of 1,6-anhydro-β-D-galactopyranose (**3**) and 1,6-anhydro-β-D-glucopyranose (**4**) from D-glycopyranosides supports the formation of 1,6-anhydro-β-D-hexopyranoses as degradation products of the nonvolatile, water-soluble, nonenzymic-browning substances, isomaltol β-D-galacto- (**1**) and α-D-gluco-pyranosides (**2**), during the baking process. This work demonstrates that 1,6-anhydro-β-D-hexopyranoses are formed from the enolic D-glycopyranosides, isomaltol α- and β-D-glycopyranosides on treatment with a mixture of triethylamine and pyrrolidine in hot aqueous ethanol.

EXPERIMENTAL

General methods. — Commercial pyrrolidine and triethylamine (Aldrich Chemical Co., Milwaukee, WI) were used. 3-*O*-α-D-Glucopyranosyloxy-2-furyl methyl ketone (**2**) was prepared by the method of Goodwin[8], and 3-*O*-β-D-galacto-pyranosyloxy-2-furyl methyl ketone (**1**) by the method of Hodge and Nelson[10].

Reactions were monitored by t.l.c. Purity of the products was established by t.l.c., g.l.c., m.p., and elemental analyses. T.l.c. was conducted on 0.25 mm of EM Reagent Silica Gel G (Brinkman Instruments, Inc.) with air-dried plates. The spots were detected by spraying with 5% ethanolic sulfuric acid and charring. Dry column

chromatography on Silica Gel G (5% water) and t.l.c. were performed with 23:2 butanone–water azeotrope–abs. ethanol (v/v) for unsubstituted compounds and 60% hexane–ethyl acetate for acetylated compounds. G.l.c. analyses of trimethylsilyl ethers[18] were recorded on a Tracor Model 560 chromatograph with a flame-ionization detector, which was fitted with a 3.175-mm × 3.08-m stainless-steel column containing 10% OV-1 silicone gum on 80–100 mesh Gas-Chrom W support (Anspec, Ann Arbor, MI). Single, symmetrical peaks were obtained for 1,6-anhydro-β-D-galactopyranose (**3**); however, product **4** showed one major peak with ~3% impurities for the Me_3Si derivative. The Me_3Si ethers were analyzed by programming the gas chromatograph at 120 to 250° with a temperature rise of 5°/min; injection-port temperature, 200°; detector temperature, 300°; helium pressure 60 lb/in^2; rotometer setting, 2. The retention values were determined with use of a digital computer. G.l.c.–m.s. analyses of the triacetates was performed with a gas chromatograph operating in tandem with a Kratos MS-30 mass spectrometer equipped with a combined chemical-ionization (c.i.)–electron impact (e.i.) source. E.i.–m.s. was generated at 70 eV. The g.l.c. column for separating the acetate derivatives was temperature-programmed from 125–250° at 4°/min. The gas chromatograph was equipped with a flame-ionization detector, which was fitted with a 3.175 mm × 3.08 m stainless-steel column packed with 3% SP-2340, on 100–120 mesh Gas Chrom Q support (Supelcoport). The specific rotation was recorded with a Perkin–Elmer Model 241 polarimeter (automatic). ^{1}H-N.m.r. spectra were recorded in 10-mm tubes with a Bruker WH-90 Fourier-transform n.m.r. spectrometer at 90-MHz sweep widths of 6024 Hz, with 4096 plot data-points to give chemical-shift values accurate to within ±1.5 Hz (±0.1 p.p.m.). Products were vacuum-dried in the presence of phosphorus pentaoxide for 24–48 h at room temperature before analysis. Melting points, measured in capillary tubes, are not corrected. Microchemical analyses were performed by Galbraith Laboratories, Inc.

Degradation of isomaltol D-*glycopyranosides.* — 3-*O*-β-D-Galacto- (**1**) and 3-*O*-α-D-glucopyranosyloxy-2-furyl methyl ketones (**2**) (5.0 g; 17.3 mmol), triethylamine (10 mL), and pyrrolidine (2 mL) in aq. ethanol (1:1, v/v; 200 mL) were stirred under reflux for 24 h at 79–80°. Solvents were removed under diminished pressure.

*1,6-Anhydro-α-*D-*galactopyranose* (**3**). — The dark-brown, syrupy residue was dissolved in water (100 mL) and extracted three times with chloroform (200 mL). A dark-brown, insoluble, polymer (0.133 g) was collected after the chloroform extraction. Evaporation of the aqueous extract gave a syrup (4.2 g) that was passed through a column of dry Silica Gel G (5% water) and eluted with 23:2 (v/v) butanone–water azeotrope–abs. ethanol. Fractions were collected and combined to yield 0.98 g of pure, crystalline **3**; recrystallization was from abs. ethanol–ethyl acetate; yield of **3**, 41%: m.p. 223.5–225°, $[\alpha]_D^{20}$ −22° (*c* 1, water); reported[3] m.p. 223–224°, $[\alpha]_D^{20}$ −21.9° (*c* 1, water); ^{1}H-n.m.r. data (Me_2SO-d_6): δ 5.11 (H-1; $J_{1,2}$ 1.45 Hz), 3.40 (H-2; $J_{2,3}$ 1.45 Hz), 3.40 (H-3), 3.70 (H-4), 4.18 (H-5), 3.67 (H-6), and 3.40 (H-6′), 4.99 (OH; *J* 3.9 Hz), 4.85 (OH; *J* 6.5 Hz), and 4.57 (OH; *J* 3.1 Hz).

The gas chromatogram for compound **3** showed one major peak for the Me_3Si derivative; the relative retention-value (16.6 min) for compound **3** was equal to that for an authentic sample of 1,6-anhydro-β-D-galactopyranose.

Anal. Calc. for $C_6H_{10}O_5$: C, 44.45; H, 6.22. Found: C, 44.54; H, 6.28.

1,6-Anhydro-α-D-glucopyranose (**4**). — A product (50.3 mg, <3%) was isolated from the dark-brown aqueous extract residue (4.2 g). This product (25 mg), after trimethylsilylation, was examined by gas–liquid chromatography. The chromatogram showed one major peak with ~3% of impurities for the Me_3Si derivatives; the relative retention-value (12.6 min) for the product was equal to that (12.6 min; reported[10] 12.5 min) for an authentic sample of 1,6-anhydro-β-D-glucopyranose (**4**). The residue (25 mg) was dissolved in dry pyridine (5.0 mL) and treated with acetic anhydride (1.0 mL), and the solution was kept overnight at 25°. The solution was evaporated with use of toluene under diminished pressure and the residue (20.5 mg) was injected directly into the gas chromatograph. One major peak was observed in the g.l.c. trace with ~3% impurities; the relative retention time (24.2 min) for the major peak was equal to that of authentic 2,3,4-tri-*O*-acetyl-1,6-anhydro-β-D-glucopyranose (24.3 min). The 2,3,4-tri-*O*-acetyl-1,6-anhydro-β-D-glucopyranose (**6**) in the product-mixture was confirmed by a comparison of its g.l.c.–m.s. data with that of an authentic sample of the triacetate derivative.

2,3,4-Tri-O-acetyl-1,6-anhydro-β-D-galactopyranose (**5**). — 1,6-Anhydro-β-D-galactopyranose (**3**, 0.1 g) was acetylated conventionally with pyridine–acetic anhydride to give 2,3,4-tri-*O*-acetyl-1,6-anhydro-β-D-galactopyranose (**5**) as crystals (0.1 g). Recrystallization was from ethyl acetate–hexane; yield of **5**, 56%: m.p. 73.5–75°, $[\alpha]_D^{20}$ −5.9° (*c* 1, $CHCl_3$); lit.[3] m.p. 73–75°, $[\alpha]_D$ −5.7° (*c* 1, $CHCl_3$); ^{1}H-n.m.r. data (chloroform-*d*): δ 5.43 (H-1), 4.76 (H-2), 5.27 (H-3), 5.27 (H-4), 4.46 (H-5), 4.34 (H-6), 3.69 ($J_{5,6'}$ 5.1 Hz, $J_{6,6'}$ 7.6 Hz, H-6'), and 2.19, 2.19, and 2.03 (Ac); (benzene-d_6): δ 5.50 ($J_{1,2}$ 1.6 Hz, H-1), 4.93 ($J_{2,3}$ 1.6 Hz, H-2), 5.41 (H-3), 5.41 (H-4), 4.06 (H-5), 4.17 (H-6), 3.42 ($J_{6,6'}$ 7.2 Hz, H-6'), and 1.60, 1.62 and 1.67 (Ac).

Anal. Calc. for $C_{12}H_{16}O_8$: C, 50.00; H, 5.59. Found: C, 49.88; H, 5.72.

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