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Note

Isolation of 3-*O*-α-D-gluco- and 3-*O*-β-D-galacto-pyranosyloxy-2-furyl methyl ketones from nonenzymic browning of maltose and lactose with secondary amino acids

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The chemistry of nonenzymic browning reactions involving the interaction between reducing sugars with primary and secondary aliphatic amines or amino acids has been studied extensively[1–8]. In many of these investigations, aliphatic amines were chosen for nonenzymic browning reactions rather than primary or secondary amino acids because less melanoidin, color, and volatile components are formed.

In view of the foregoing, it is thought that free amino acids and amino functional groups of peptides and proteins in bread dough react with maltose (liberated by diastatic enzymes on wheat starch) and lactose during baking to form maltol and isomaltol. These latter two enolic compounds have been detected in baked cereals, breads, and baked cereal products by their characteristic purple color-reaction with ferric chloride reagent[9–13].

Patton[14] demonstrated the formation of maltol from the interaction of lactose and maltose with glycine. Hodge and Nelson[15] showed that isomaltol β-D-galactoside, compound **2**, formed from the reaction of lactose with secondary amine salts, is hydrolyzed or pyrolyzed to isomaltol. However, isomaltol α-D-glucoside (**1**) was not isolated from the reaction of maltose with secondary amine salts, 1-deoxy-1-piperidino-maltulose, an Amadori rearrangement product, was obtained instead. Hodge *et al.*[16] postulated that the formation of compound **2**, maltol and isomaltol, proceeds through the methyl α-dicarbonyl intermediate formed by Amadori rearrangement of an initially formed, *N*-substituted glycosylamine.

This work demonstrates the preparation of compounds **1** and **2** from maltose and lactose with secondary amino acids and isolation by chromatography on an

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TABLE I

ANALYSES OF COMPOUNDS

Compounds[a] *and amino acids*	*Yield* (%)	*M.p.* (°C)	$[\alpha]_D^{20}$ (H_2O, *c*)	*Found* C	H
1 $C_{12}H_{16}O_8$					
(1) DL-Pipecolinic	3	158–160	60.0, *c* 1	49.73	5.63
(2) L-(−)-Proline	3	158–159.5	60.0, *c* 1	49.81	5.84
(3) Sarcosine	6	158.5–160	60.0, *c* 1	49.98	5.63
2 $C_{12}H_{16}O_8$					
(1) DL-Pipecolinic	5	197–198	4.75, *c* 2	49.73	5.84
(2) L-(−)-Proline	5	200–201.5	4.80, *c* 2	49.15	5.63
(3) Sarcosine	7	197–198	4.50, *c* 2	49.98	5.84
(4) Amine salt[b]	37	203–205	−4.50, *c* 2	50.17	5.63

[a] *Anal.* Calc. for $C_{12}H_{16}O_8$: C, 50.0; H, 5.6. [b] Ref. 15.

acidic cation-exchange resin. Compound **1**, which has not been reported previously, was characterized by conversion into its tetraacetate.

Several variations of the isolation procedure were investigated. The complexity of the reaction mixture for compound **1** was decreased by continuous extraction with ethyl acetate. Because water is slightly soluble (9%) in ethyl acetate, the ethyl acetate extract contained secondary amino acids as well as **1**, together with other components. The syrupy ethyl acetate extract was separated by column chromatography on an acidic cation-exchange resin; hence, the reaction residue for compound **2** was added directly to the column of acidic cation-exchange resin. As compared with the other secondary amino acids (Table I) tested, sarcosine gave the highest yields of 6 and 7%, respectively. No change in the structure of compound **1** occurs when it is left in contact with Dowex-50W-X4 for 48 h, as disclosed by t.l.c. analysis. Compounds **1** and **2** resulted also from the reaction of maltose and lactose with the primary amino acids, alanine and glycine, in <3% yield.

3-*O*-α-D-Gluco- (**1**) and -β-D-galacto-pyranosyloxy-2-furyl methyl ketones (**2**) prepared from maltose and lactose with secondary amino acids show strong i.r. absorption at 1660 cm^{-1} for the carbonyl group with four absorption bands[14], attributed to C=C vibrations, which correspond closely to frequencies for the isomaltol moiety reported by Hodge and Nelson[15].

The ^{1}H-n.m.r.-spectral data for 3-*O*-(2,3,4,6-tetra-*O*-acetyl)-α-D-gluco- (**3**) and -β-D-galacto-pyranosyloxy-2-furyl methyl ketones (**4**) showed the following chemical shifts: δ 2.27 and 2.25 for the acetyl (CH_3CO) and δ 6.73 and 7.00 for the vinylic protons of the isomaltol moiety; δ 5.55 (H-1) for the α-D-*gluco* and δ 4.85 (H-1) for the β-D-*galacto* configurations. The respective n.m.r. data showed also $J_{1,2}$ values of 4.0 and 8.0 Hz, which support the α-D-*gluco* and β-D-*galacto* configurations, respectively. However, the n.m.r.-spectral data for the original 3-*O*-(2,3,4,6-tetra-*O*-

TABLE II

^{1}H-N.M.R. DATA AT 100 MHz FOR SOLUTIONS OF 3-*O*-(2,3,4,6-TETRA-*O*-ACETYL)-α-D-GLUCO- AND 3-*O*-β-D-GALACTO-PYRANOSYLOXY-2-METHYL KETONES IN BENZENE-d_6

Proton	*Chemical shifts*[a]			*Coupling constant (Hz)*	
	α-D-gluco	β-D-galacto		α-D-gluco	β-D-galacto
CH_3OCO	1.71[b]	1.78[b]	(1.77)[c]		
CH_3CO	2.27	2.25	(2.24)		
H-1	5.55	4.85	(4.85)	$J_{1,2}$ 4.0	$J_{1,2}$ 8.0
H-2	5.13	5.66	(5.67)		$J_{1,2}$ 8.0[c]
H-3	5.91	5.13	(5.13)		
H-4	5.31	5.43	(5.44)		
H-5	4.17	3.62	(3.62)		
H-6	4.17	4.09	(4.09)		
H-6′	4.17	4.09	(4.09)		
HC=	6.04	6.19	(6.18)		
HC=	6.73	7.00	(7.00)		

[a]In p.p.m. downfield from internal Me_4Si. [b]Integrated for four methyl groups. [c]Original stock sample prepared by Hodge and Nelson, recrystallized from ethanol[15].

TABLE III

ANALYSES OF COMPOUNDS

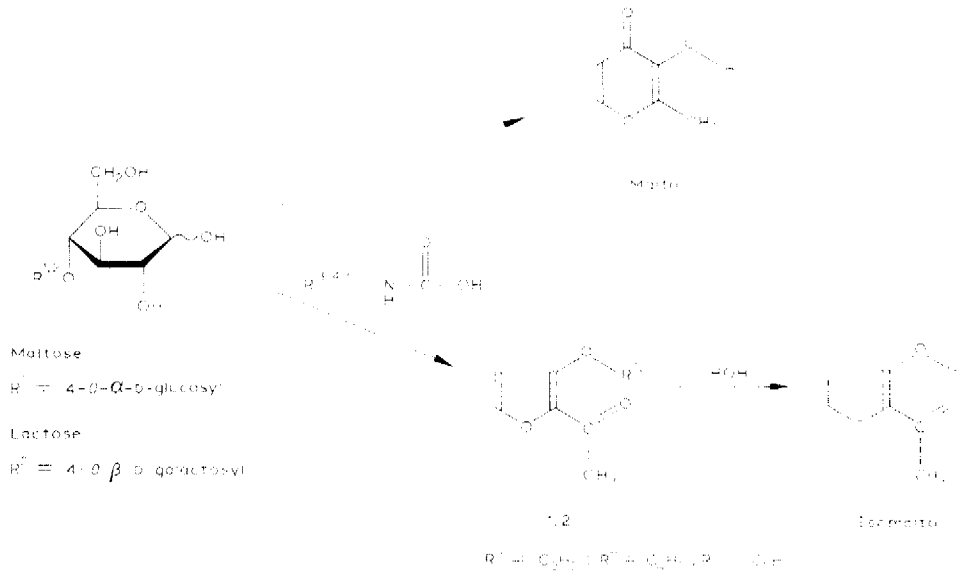
Compounds[a] *and amino acids*	*Yield* (%)	*M.p.* (°C)	$[\alpha]_D^{20}$ *(CHCl$_3$)*	*Found* *C*	*H*
3 $C_{20}H_{24}O_{12}$					
(1) DL-Pipecolinic	70	137–138	144.6, *c* 1	52.71	5.42
(2) L-(−)-Proline	67	137–138	144.5, *c* 1	52.64	5.45
(3) Sarcosine	73	136.5–138	144.5, *c* 1	52.67	5.32
4 $C_{20}H_{24}O_{12}$					
(1) DL-Pipecolinic	75	149.5–151	−14.50, *c* 1	52.70	5.46
(2) L-(−)-Proline	82	149.5–151	−14.40, *c* 1	52.76	5.49
(3) Sarcosine	80	150–151.5	−14.35, *c* 1	52.66	5.38
	95[b]	128.5–129.5	−14.60, *c* 5	52.81	5.31

[a]*Anal.* Calc. for $C_{20}H_{24}O_{12}$: C, 52.6; H, 5.3. [b]Ref. 15 (secondary amine salts).

acetyl)-β-D-galactopyranosyloxy-2-furyl methyl ketone prepared by Hodge and Nelson[15] are in close agreement with compound **4** (Table II). The specific rotations for compound **4** prepared from lactose and secondary amino acids correspond closely with the specific rotation reported by Hodge and Nelson[15] for 3-*O*-(2,3,4,6-tetra-*O*-acetyl)-β-D-galactopyranosyloxy-2-furyl methyl ketone, which was prepared from lactose and secondary amine salts; however, compound **4** had a much higher m.p. than that reported by Hodge and Nelson[15] (Table III). The tetraacetate of isomaltol

β-D-galactoside prepared by these workers had m.p. 151–152°, before and after recrystallization[15]. Subsequently, the higher m.p. was observed also when the tetra-acetate was prepared and recrystallized under the same conditions described by Hodge and Nelson[15]; therefore, it appears that an incorrect melting point (128–129°) may have been reported for their compound, 3-*O*-(2,3,4,6-tetra-*O*-acetyl)-β-D-galactopyranosyloxy-2-furyl methyl ketone. The ^{1}H-n.m.r. data for compound **4** are original (Table II) (Hodge and Nelson[15] did not report ^{1}H-n.m.r. data).

T.l.c. disclosed maltol and isomaltol in the reaction mixtures for dehydration of disaccharides by secondary amino acids. Apparently, the Amadori-rearrangement product formed with secondary amino acids is unstable; therefore, the dehydration of maltose and lactose with secondary amino acids to 3-*O*-α-D-gluco- (**1**) and 3-*O*-β-D-galacto-pyranosyloxy-2-furyl methyl ketones (**2**) (Scheme 1) corresponds to the formation of isomaltol β-D-galactoside from lactose and secondary amine salts postulated by Hodge *et al.*[16]. By hydrolysis of compounds **1** and **2** with sodium methoxide[18] in dry methanol for 72 h at 25°, isomaltol was isolated in 37 and 40% yields, respectively. However, no attempt was made to determine the yields of D-galactose and D-glucose because the hydrolyzed solutions were highly colored, because of dehydration of the reducing sugars, and probably of isomaltol, by sodium methoxide. Isomaltol is also dry-distilled from 3-*O*-α-D-glucopyranosyloxy-2-furyl methyl ketone (**1**) at 175–180°; hence, compound **1** may now be considered as a new source for isomaltol during the baking process. Dehydration of disaccharides by secondary amino acids produced 3-*O*-α-D-gluco- (**1**) and 3-*O*-β-D-galacto-pyranosyl-oxy-2-furyl methyl ketones (**2**), maltol and isomaltol (Scheme 1).



This work confirms the view[9–13] that free amino acids, as well as amino functional groups of peptides and proteins in bread dough, act on maltose and lactose in nonenzymic browning-reactions during baking to form maltol and isomaltol.

EXPERIMENTAL

General methods. — Commercial α-lactose hydrate, DL-pipecolinic acid, L-(−)-proline, sarcosine, triethylamine (Aldrich Chemical Co., Milwaukee, WI), β-maltose hydrate (Pflanstiehl Laboratories, Waukegan, IL), Amberlite IR-120 (Rohm & Haas Co., Philadelphia, PA), and Dowex 50W-X4 acidic cation-exchange resin (Bio Rad Laboratories, Richmond, CA) were used.

Preparative reactions were monitored by thin-layer chromatography (t.l.c.). Purity of the compounds were established by t.l.c., melting point (m.p.), and elemental analysis. 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. T.l.c. was performed with 80% methanol–ethyl acetate (v/v) for unsubstituted compounds and with 75% ethyl acetate–hexane for acetylated compounds. G.l.c. analyses of isomaltol was recorded by a Hewlett–Packard Model 5730A gas chromatograph equipped with a flame-ionization detector, which was fitted with a 3.175 mm × 3.08 m stainless-steel column packed with 80–100 mesh Chromosorb W coated with 15% Silicone SE-30 (Anspec, Ann Arbor, Michigan). Single, symmetrical peaks were obtained for isomaltol. ^{1}H-N.m.r. spectra were recorded with a Varian Model XL-100 spectrometer; chemical-shift peaks were assigned by spin-decoupling experiments, referred to internal tetramethylsilane. I.r. spectra were determined in potassium bromide pellets (1.22 mm thick containing 0.1M concentrations) with a Perkin–Elmer Model 621 spectrophotometer. Compounds **1** and **2** were vacuum-dried in the presence of phosphorus pentaoxide for 24–48 h at room temperature before microchemical analysis.

Method for nonenzymic browning of disaccharides with secondary amino acids. — α-Lactose and/or β-maltose hydrates (62.5 mmol), amino acids [0.125 mol; DL-pipecolinic, L-(−)-proline, sarcosine], and triethylamine (30 mL) in abs. ethanol (350 mL) were stirred under reflux for 24 h at 78–80°. Solvents were evaporated under diminished pressure.

3-O-α-D-Glucopyranosyloxy-2-furyl methyl ketone (**1**). — An aqueous solution of the residue from the reaction of maltose with the appropriate amino acids was continuously extracted with ethyl acetate (36 h). Evaporation of the extract gave a syrup that was passed through a column (2.5 × 73 cm) of Dowex 50W-X4 acidic cation-exchange resin, which was eluted with water (0.7 mL/min). The column was packed with a resin bed-volume of 150 mL. Compound **1** was isolated in the second bed-volume of eluant (300 mL). Fractions were collected and combined to yield pure crystals of **1**; recrystallization was from abs. ethanol (Table I); ν_{max} 1660 cm^{-1} (carbonyl group), 1650, 1590, 1455, and 1489 cm^{-1} (C=C vibrations).

3-O-β-D-Galactopyranosyloxy-2-furyl methyl ketone (**2**). — The residue from the reaction of lactose with secondary amino acids was added directly to the column of acidic cation-exchange resin, which was also eluted with water. Combined fractions yield pure crystals of **2**; recrystallization was from abs. ethanol (Table I); ν_{max} 1660 cm^{-1} (carbonyl group), 1635, 1592, and 1493 cm^{-1} (C=C vibrations); reported[15]

1650 cm^{-1} (carbonyl group), 1637, 1592, 1464, and 1492 cm^{-1} (C=C vibrations). The eluates from the isolation of compounds **1** and **2** were evaporated under diminished pressure.

Petacetylation. — 3-*O*-α-D-Gluco- (**1**) or -β-D-galacto-pyranosyloxy-2-furyl methyl ketones (**2**) (0.1 g) were dissolved in dry pyridine (10 mL) containing acetic anhydride (1.0 mL), and the solutions were kept overnight at 25 . The solutions were concentrated by evaporation with toluene under diminished pressure. 3-*O*-(2,3,4,6-Tetra-*O*-acetyl)-α-D-gluco- (**3**) and -β-D-galacto-pyranosyloxy-2-furyl methyl ketones (**4**) were obtained as crystals after evaporation. Crystallizations were from abs. ethanol. Physicochemical data are given in Tables II and III.

Hydrolysis of 3-O-α-D-gluco- (**1**) *and -β-D-galacto-pyranosyloxy-2-furyl methyl ketones* (**2**). — Sodium methoxide[18] (10 mL) was added to compounds **1** and **2** (0.2 g) dissolved in dry methanol (5 mL), and these solutions were kept for 72 h at 25°. Sodium ions were removed with a slight excess of Amberlite IR-120 ion-exchange resin until the solutions became neutral. An equal volume of water was added to the resin-free filtrate and the solution extracted with chloroform.

Gas–liquid chromatography of isomaltol. — The hydrolyzate residues (0.056 g) from the chloroform extractions were dissolved in dry methanol (0.2/mL), and this residue was injected directly (no trimethylsilylation) into the gas chromatograph. Three major peaks were observed in each chromatogram; hence, one relative retention-value (11.2 min) was equal to that of the reference isomaltol (11.3 min). Isomaltol was obtained from the hydrolyzates of compounds **1** and **2** in 37 and 40% yields (m.p. 96.5–98° after crystallization from hot water; reported[15] for compound **2**, 41% yield, m.p. 98–98.5°), respectively. The identities of the D-galactose and D-glucose moieties were established from the n.m.r. spectra of compounds **3** and **4**. The retention values were determined with use of a digital computer.

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