CEREAL CHEMISTRY

VOL. 38 MAY, 1961 NO. 3

PREPARATION AND PROPERTIES OF GALACTOSYLISOMALTOL AND ISOMALTOL[1]

J. E. HODGE AND E. C. NELSON

ABSTRACT

Isomaltol, an enolic compound ($C_6H_6O_3$), first isolated in trace amounts by Backe in 1910 from a steam distillate of bread, is now prepared from lactose in 25% over-all yield. Dehydration of the glucose moiety of lactose by reaction with secondary amine salt in tertiary amine-buffered alcoholic medium yields the new compound, isomaltol beta-D-galactoside, which is hydrolyzed or pyrolyzed to produce Backe's isomaltol. Reaction of maltose with piperidine acetate under the same conditions yields 1-deoxy-1-piperidino-maltulose. This new Amadori compound does not dehydrate to a glucoside of isomaltol analogous to the lactose reaction.

Comparison of the properties of isomaltol with those of maltol under identical conditions shows that isomaltol is not a pyrone but probably a furan derivative. Isomaltol and maltol probably are formed in bread by Maillard-type browning reactions, and not by fermentation, as Backe assumed.

Maltol and isomaltol are crystalline enolic compounds, $C_6H_6O_3$, volatile with steam and readily sublimed, that have been isolated from baked cereals, bread, and bread crusts (2,3,6,30). Both compounds are reported as trace constituents of bread; however, they may contribute to the fragrant aroma of freshly baked bread and to the final flavor of bread (15). Maltol is also obtained in low yields by extracting larch bark (25,32) or fir needles (10); pyrolyzing cellulose, starch, or wood (8,11); roasting malt (5,18), chicory (28), or coffee (2,30); heating milk (21,23,24,26); autoclaving lactose or maltose with glycine (22); and degrading streptomycin with alkali (29). Whereas considerable literature exists on maltol, isomaltol has been reported by only one investigator (2,3).

Backe extracted isomaltol from a bread distillate in 1910. Nestlé's "farine lactée," a powdered preparation of condensed milk and flour,

[1]Manuscript received July 29, 1960. Contribution from the Northern Regional Research Laboratory, Peoria, Illinois. This is a laboratory of the Northern Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture. Presented in part at the 45th annual meeting, Chicago, Illinois, May 1960.

Note added in proof: Galactosylisomaltol now fully named 2-acetyl-3-β-D-galactopyranosyloxyfuran.

had been condemned at the customhouse in Rio de Janeiro because extracts gave a red-violet color with ferric chloride, and this was interpreted as showing the presence of salicylic acid preservative in the biscuit powder (2). Backe proved that the color was caused by a new compound, melting at 98°C., which he steam-distilled from an acid suspension of the biscuit powder (0.07 g. from 1 kg.). Lack of a suitable preparative method hindered Backe's investigations, but he did prepare three crystalline derivatives and found that his compound was isomeric with maltol (I, Fig. 1) and had some properties closely resembling it. With meager evidence, some of it in contradiction, he proposed the 3-hydroxy-5-methyl-4-pyrone structure (II, Fig. 1) for isomaltol.

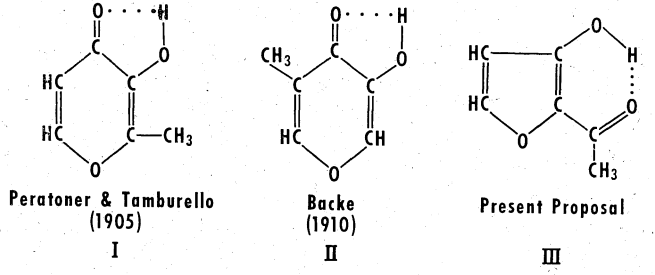


Fig. 1. Accepted structure for maltol (I) and proposed structures for isomaltol (II and III).

Results and Discussion

The present work shows that a simple, chemical dehydration of lactose by piperidine acetate (or other secondary amine salts) produces the *O*-galactoside of isomaltol in 35–40% yield. Isomaltol is then dry-distilled from the galactoside in 68% yield. Identity with Backe's isomaltol is established by preparing the same *O*-methyl ether, *O*-benzoyl ester, and moss-green copper salt that Backe reported. Discovery of this preparative method makes isomaltol available in quantity for flavor research and structural studies.

The galactoside of isomaltol, a new compound, is characterized by optical rotation and facile hydrolysis with almond emulsin at 25°C. as isomaltol beta-D-galactopyranoside. Alpha-D-Galactopyranose was isolated after both enzymatic and acid hydrolysis. Methanolysis gave methyl beta-D-galactopyranoside. Ring structure in the galactosyl radical is shown by formation of only a tetra-*O*-acetyl derivative upon acetylation of the galactoside in excess pyridine-acetic anhydride, together with the demonstration that the tetra-acetate readily forms the same *p*-nitrophenylhydrazone which is formed by acetylation of the preformed *p*-nitrophenylhydrazone of the galactoside. The mild reaction

conditions which dehydrate the glucose moiety of the disaccharide are assumed to leave the galactose moiety intact in pyranose form. Also, galactose probably remains linked to the fourth carbon atom of the glucose radical; otherwise, piperidino-hexose-reductone (35) would be formed as the major product from an unsubstituted glucose or galactose under these reaction conditions (14, 16,17).

Hydrolysis of the galactoside proceeds with mild catalysts, such as 20% acetic acid or 5% sodium bicarbonate, indicating enolic linkage of the galactosyl radical to the hydroxyl group of isomaltol. Moreover, a positive test for enol is obtained immediately after the addition of almond emulsin to an aqueous solution of the galactoside, but not before. One oxygen atom in the isomaltol radical, $C_6H_5O_3$, is therefore enolic, the second is carbonyl (formation of a *p*-nitrophenylhydrazone), and the third is not acylated in pyridine-acetic anhydride or pyridine-benzoyl chloride.

The molecular absorption coefficient (E_m, liters mole^{-1} cm^{-1}) of *O*-galactosylisomaltol in methanol is 18,300 at 276 mμ. Isomaltol in methanol gives E_m 16,000 at 280 mμ, and *O*-methyl isomaltol in methanol gives 17,400 at 281 mμ. The isomaltol radical in the galactoside therefore contains as much conjugated unsaturation as is present in

TABLE I

CHARACTERISTIC DIFFERENCES IN PROPERTIES OF ISOMALTOL AND MALTOL

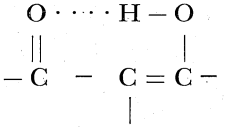
PROPERTY OR REAGENT	ISOMALTOL	MALTOL
Melting point	101° ± 2°C	162°C
Ultraviolet absorbance:		
λ_{max}; $E^{1\%}_{1\,cm}$ in 0.1*N* HCl	283 mμ; 1,366	274 mμ; 731
in 0.1*N* NaOH	318 mμ; 1,485	318 mμ; 754
IR absorbance in KBr disks:	3.23–3.43 μ;	3.11 μ (sharp)
O — H . . . O	3.50–3.77 μ[a]	
"Carbonyl"	6.20–6.45 μ[b]	6.03 μ (sharp)
Acidity:		
pH of 0.5% solution, 22°C	3.7	5.3
5% Sodium bicarbonate	CO_2 liberated	No CO_2
Diazomethane in ether, methylation	Fast, complete	Slow, incomplete
Concentrated hydrochloric acid	Decomposes[c]	Forms stable salt
Reducing power in alkali:		
Fehling solution at 100°C	Slow	Rapid
2,3,5-Triphenyltetrazolium chloride, 60°C., 5 minutes	Colorless	Red formazan
Hydrazone formation:		
p-Nitrophenyl (mono)	Forms on heating in neutral MeOH	Does not form
2,4-Dinitrophenyl (mono)	Forms readily in the cold	Does not form

[a] Two groups of rather strong, irregular, broad bands.
[b] The carbonyl band at 6.2 μ is merged with an adjacent strong band attributed to — C = C — in chelate conjugation with the carbonyl group (12).
[c] With formation of unidentified orange crystalline compound of higher molecular weight; m.p. 124°C.

isomaltol itself and in the *O*-methyl ether. Two double bonds in conjugation with the carbonyl group are indicated. For example, an isomer, 5-(hydroxymethyl)-2-furaldehyde, gives E_m 16,700 at 284 mμ (31).

Whereas Backe emphasized the similar properties of isomaltol and maltol, this work shows their similar properties (general solubilities, ferric chloride color reaction, iodoform reaction, and *C*-methyl analysis) are less important for structural considerations than their dissimilar properties. Important dissimilarities noted in parallel comparisons of physical and chemical properties appear in Table I.

Isomaltol is more volatile, acidic, ketonic, unstable to acid, and stable to alkali than maltol. These significant differences (Table I) suggest that isomaltol does not have a pyrone ring structure. From the acid-lability, alkali-stability characteristics, a 2-substituted furan structure is indicated. Pyrones do not form hydrazones by the usual methods. Not only does isomaltol readily form *p*-nitrophenyl- and 2,4-dinitrophenylhydrazones, but the *O*-methyl enol ether derivative also forms a *p*-nitrophenylhydrazone under neutral conditions. This demonstration of distinctly separate carbonyl and enolic hydroxyl functions, together with high volatility, displaced infrared bands for O − H · · · O and carbonyl (12,20,27); formation of a green copper-chelate salt soluble in organic solvents and insoluble in water (compare acetylacetone, acetoacetic ester) allows the assumption that isomaltol contains an enolized beta-diketone chelate structure:



Therefore isomaltol is tentatively formulated as III (Fig. 1), with work toward proof of structure still in progress.

Backe conducted many experiments to show the mode of isomaltol formation in bread. His biscuit powder, ether-extracted before baking, gave maltol, not isomaltol. After baking and steam-distilling an acidified suspension of the bread, he extracted much more isomaltol than maltol from the distillate. Ordinary wheat flour, heated at 150°C. without water, gave no isomaltol or maltol according to the ferric chloride test for enol on an ether extract of the flour. When the wheat flour was worked into a dough and heated at 150°C. in the same way, the ether extract gave a strong purple color with ferric chloride, which could have been caused by the presence of either isomaltol or maltol, or both. When the "dry" wheat flour was first heated at 150°C., then worked into a dough and rebaked for 2 hours at 150°C., the color reaction was weak; but when the preheated, enzyme-inactive flour was treated with

malt extract, then worked into a dough and rebaked, the color reaction was very strong. The malt extract alone, dried on an inert material, gave no color reaction after baking. Starch gave negative tests when heated dry and as a paste, but when pasted with malt extract before heating, the test was strongly positive. On the other hand, starch treated with a purified commercial diastate (alcohol precipitate of malt extract) gave no color reaction under the same conditions. Maltose (that contained some malt impurities) and pure sucrose gave the color reaction when heated with water alone at 150°C. in a sealed tube, but glucose did not. Backe concluded that wheat flour and malt, but not diastase, contained a "special enzyme" that, along with heat, is necessary for the formation of isomaltol in bread.

Patton and co-workers showed the presence of maltol in overheated skimmilk (21,23) and also in milk evaporated in the usual way (26). Milk containing lactose-l-C^{14} gave radioactive maltol upon evaporation (23,24). In model systems, lactose and maltose gave maltol upon autoclaving with glycine at pH 7, but glucose and galactose (also sucrose, starch, and cellulose) did not (22). Patton's work allows an interpretation of Backe's findings. Maltol present in the biscuit powder before baking was preformed in the condensed milk fraction. However, some of the maltol remaining after baking could generate from maltose (liberated by diastatic action in the dough) by its interaction with amino acids, amine salts, and peptides in Maillard-type browning reactions (13).

Patton and Flipse showed that maltol was generated from the glucose moiety of lactose in milk (24). Our work shows that isomaltol also is generated from the glucose moiety of lactose by interaction with secondary amine salts. In another analogy with Patton's work, our experimental conditions do not produce isomaltol from hexoses; crystalline amino-hexose-reductones are isolated instead (14,16). In contrast with Patton's experiments, *O*-glucosylisomaltol was not obtained from several maltose-piperidine acetate reactions under conditions that gave *O*-galactosylisomaltol from lactose. Instead, the new Amadori rearrangement product, l-deoxy-l-piperidino-maltulose crystallized out, and this surprisingly stable compound would not yield the isomaltol radical upon further heating with piperidine acetate.

It is now more reasonable to assume that maltol and isomaltol are formed nonenzymatically from maltose and/or lactose in bread. The amine reagent in Backe's experiments would be the free amino acids and peptides present in the malt extract which, however, would not likely be present in the precipitated diastase he used. Free amino acids (33) and the amino groups of peptides and proteins in doughs probably

act on the disaccharides in Maillard-type browning reactions during baking.

More research is needed to establish the presence, concentrations, and mode of formation of maltol and isomaltol in bread. Backe's claims for isomaltol (2) and Sherman's for maltol (30) in *ordinary* wheat breads should be substantiated; both men used only ferric chloride color reactions that do not distinguish between the two. Further investigations are needed because both compounds possibly contribute to bread flavor (15).

Experimental

Melting points, determined in capillary tubes, are corrected. Chemicals used are reagent grade or the best commercial grade unless otherwise specified.

O-Galactosylisomaltol. In a 2-liter, three-necked reaction flask, fitted with anchor-blade stirrer, reflux condenser, dropping funnel, and hemispherical electric heating mantle, 1 mole of alpha-lactose hydrate (360 g.) is stirred into 300 ml. absolute ethanol at room temperature until smoothly dispersed. One mole of piperidine (100 ml. practical grade) is added; then 1 mole of glacial acetic acid (58 ml.) is dropped, over several minutes, into the continually stirred mixture. After the dropping funnel is replaced by a thermometer, the reaction temperature is held at 78° ± 2°C. Triethylamine (50 ml.) is added to keep the reaction mixture alkaline over the first 12 hours. When solution occurs after 10 to 12 hours, more triethylamine (50 ml.) is added, and heating at 78°C. is continued for a total of 24 hours[2]. If the crystalline product does not separate spontaneously during the heating period, seed crystals are produced by diluting a sample of the mixture with ethanol.

The heating mantle is replaced with a crushed-ice bath, ethanol (300 ml.) is added with continued stirring, and the crystalline mush is held at 5°C. or below for 1 hour before filtering with suction. The filter cake is washed with ethanol, suspended and stirred in ethanol, refiltered, pulverized, and dried in a vacuum desiccator over anhydrous calcium chloride to constant weight. The crude yield is 105–107 g., 37% of theory; m.p. 203°–205°C., with boiling and distillation of isomaltol to the upper walls of the capillary tube. Processing of the mother liquor yields 5 to 10 g. piperidino-hexose-reductone (35) but very little more *O*-galactosylisomaltol.

The same yield of *O*-galactosylisomaltol is obtained when anhy-

[2] If the reaction mixture is boiled continuously, the temperature slowly increases from 81° to 83°C. More triethylamine is needed to buffer the mixture, and the yield is the same.

drous beta-lactose is substituted for alpha-lactose hydrate.

When the procedure is repeated with 0.75, 1.00, and 1.25 moles of piperidine acetate per mole of lactose, the yields are 25, 37, and 41% of theory based on the amount of lactose added, and 33, 37, and 33% based on piperidine, respectively. With 0.75 mole of piperidine acetate, the 24-hour product is contaminated with unchanged lactose (removed by the recrystallization from water before determining the yield). However, when the reaction time is extended to 32 hours to dissolve all the lactose, the yield is increased to 35% based on lactose and 47% based on piperidine.

When the procedure is repeated with 1 mole of morpholine instead of piperidine, except that the mixture is refluxed at 82°C. for 24 hours, the yield is 21% and is increased to 26% by refluxing for a second 24-hour period.

With diethanolamine in place of piperidine, the yield is only 13% of theory. Monoethanolamine gives a dark, hygroscopic product that yields no isomaltol on hydrolysis. Glycine, in place of piperidine acetate, is scarcely soluble and does not react in 29 hours at 75°C. When triethylamine replaces piperidine, 91% of the lactose is recovered unchanged. When sterically hindered di-isopropylamine is used instead of triethylamine as the nonreactant buffer, the yield does not change.

One mole of anhydrous dimethylamine acetate, 1 mole alpha-lactose hydrate, 100 g. trimethylamine, and 500 ml. methanol when refluxed at the boiling point (60° to 72°C.) for 24 hours yields 21% of the theoretical amount of *O*-galactosylisomaltol. Reheating the mixture at 76°–78°C. for 8 hours increases the yield to only 22% of theory, and 8 g. of dimethylamino-hexose-reductone (16) is recovered from the filtrate. When dimethylamine acetate is used in dimethylformamide solvent with heating at 90°C. for 15 hours and with vacuum distillation of solvent during the last 3 hours, the yield is 18% of theory.

Recrystallization of the crude products from methanol yields analytically pure *O*-galactosylisomaltol that melts and decomposes at 204°–205°C. Recrystallization from water yields a bulky, hydrous precipitate that melts at about 195°C. when dry. However, this low-melting form gives the same specific optical rotation, $[\alpha]_D^{25}$ −4.5° ($c = 2$, $l = 2$, in water) and the same crystalline tetra-*O*-acetyl derivative as the more dense high-melting form. Calculated for $C_{12}H_{16}O_8$: 50.00% C, 5.60% H, 288.3 molecular weight. Found: 50.17% C, 5.63% H, 0.00% N, 0.96% *C*-methyl per 288.3 molecular weight (4).

The ultraviolet absorption spectrum between 220 and 400 mμ shows a single, strong maximum at 278 mμ, E (1%, 1 cm.) = 632, in

water; at 276 mμ, E = 634, in methanol. The infrared spectrum of either methanol- or water-recrystallized forms in potassium bromide disks shows no absorption bands in the range 3.5 to 6.0 μ. A very strong absorption appears at 6.11 μ (1,637 cm^{-1}) with a shoulder (strong) at 6.06 μ (1,650 cm^{-1}). Other strong bands between 6 and 7 μ are placed at 6.28 (1,592 cm^{-1}) and 6.83 μ (1,464 cm^{-1}) with a medium band at 6.70 μ (1,493 cm^{-1}).

O-Galactosylisomaltol is neutral, moderately soluble in water (about 3 g. per 100 ml. at 25°C.), and sparingly soluble in ice water or neutral organic solvents at room temperature. It is moderately soluble in pyridine, *N*,*N*-dimethylformamide, and boiling methanol. It does not reduce 2,6-dichlorophenolindophenol in 0.1*N* sodium hydroxide at 25°C., but it does reduce hot Fehling solution. It gives no deep color with ferric chloride in aqueous or alcoholic solution.

Hydrolysis and Methanolysis of O-Galactosylisomaltol. Fifty millimoles of *O*-galactosylisomaltol (14.4 g.) in 200 ml. of 2*M* orthophosphoric acid are steam distilled. One liter of distillate is collected and exhaustively extracted with chloroform until a negative test for enol is obtained in the aqueous layer. Concentration of the combined, dried chloroform extracts, with trituration of the distillation residue in 1:1 ether-petroleum ether, yields 2.5 g. crude isomaltol, 41% of theory. Recrystallization from benzene in three crops gives 2.2 g. of pure isomaltol, m.p. 98°–98.5°C., agreeing in all properties with those reported by Backe (3). Sublimation at 1 mm. mercury pressure gives white, opaque crystals, m.p. 100°–101°C. Calculated for $C_6H_6O_3$: 57.14% C, 4.80% H, 126.1 molecular weight. Found: 57.18% C, 4.80% H, 124 neutral equivalent, 0.98 *C*-methyl per 126.1 molecular weight (4).

A solution of 1.7 g. *O*-galactosylisomaltol in 20% aqueous acetic acid is refluxed for 8 hours and distilled at atmospheric pressure for 4 hours. Dilution of the still-pot residue with alcohol and ether gives, on long standing at 1°C., 0.54 g. (51% of theory) of reducing sugar identified as alpha-D-galactose by optical rotation $[\alpha]_D^{25}$ + 150° (initial, by extrapolation) → + 78.7° (final, $c = 3.0$, $l = 1$, in water); by melting point, 165°–167°C.; and by paper chromatography parallel with an authentic sample.

A solution of *O*-galactosylisomaltol in 5% sodium bicarbonate, heated at 100° for 20 minutes and then neutralized, gives a deep purple with a drop of ferric chloride solution.

O-Galactosylisomaltol (2.88 g.) in 100 ml. water is treated with 75 mg. almond emulsin and stored at 25°C. Immediately after addition of the emulsin, a sample of the solution gives a deep purple color with

a drop of ferric chloride solution. After 1 day, the mixture is extracted with 250 ml. ether, and on four successive days thereafter with 150 ml. ether. The combined extracts, dried and evaporated, gave a crude crystalline residue which, when dissolved in dry ether and cooled to −17°C., deposited 1.0 g. (79% of theory) of crystalline isomaltol. The aqueous phase remaining after the ether extractions gave (in three crops upon evaporation and dilution with ethanol) 78% of the theoretical amount of crystalline alpha-D-galactose, identified by melting point, optical rotation, and paper chromatography parallel with an authentic sample.

Methanolysis of *O*-galactosylisomaltol in anhydrous methanol containing 1.1% (wt/vol) hydrogen chloride for 5 days at 25°C. produced 74% of the theoretical amount of crude methyl beta-D-galactopyranoside. After two recrystallizations from methanol, the melting point was 177°–177.5°C. Calculated for $C_7H_{14}O_6$: 43.30% C; 7.28% H. Found: 43.30% C, 7.33% H.

O-*Galactosylisomaltol Tetra-Acetate.* Fifty millimoles (14.4 g.) of *O*-galactosylisomaltol are suspended in 75 ml. pyridine and 30 ml. acetic anhydride (300 mmoles). After standing 40 hours at 25°C., the yellow solution is filtered and poured into 600 ml. ice water; yield, 21.7 g., 95% of theory, m.p. 127°–128°C. Recrystallization from 150 ml. ethanol gives 21.5 g. of glittering crystals; m.p. 128.5°–129.5°C.; $[\alpha]_D^{25}$ − 14.6° ($c = 5.0$, $l = 2$, in chloroform). Calculated for $C_{20}H_{24}O_{12}$: 52.63% C, 5.30% H, 37.73% $COCH_3$. Found: 52.81% C, 5.31% H, 37.53% $COCH_3$ by method of Kunz (19).

O-*Galactosylisomaltol* p-*Nitrophenylhydrazone.* Methanol solutions of 6.7 mmoles *p*-nitrophenylhydrazine and 5.0 mmoles *O*-galactosylisomaltol are combined and refluxed for 2 hours on the steam pot. The deep red hydrazone is filtered off, extracted several times with boiling methanol, and dried under vacuum at 100°C.; yield 1.4 g., 65% of theory; m.p. 217°–218°C. with decomposition. Calculated for $C_{18}H_{21}N_3O_9$: 51.06% C, 5.00% H, 9.93% N. Found: 50.6% C, 5.11% H, 9.90% N (Dumas).

O-*Galactosylisomaltol* p-*Nitrophenylhydrazone Tetra-Acetate.* Acetylation of *O*-galactosylisomaltol *p*-nitrophenylhydrazone in pyridine-acetic anhydride in the usual way gives a mixture, the major component of which is the orange-yellow tetra-*O*-acetyl derivative, m.p. 143.5°–144.5°C. Calculated for $C_{26}H_{29}N_3O_{13}$: 52.79% C, 4.94% H, 7.10% N. Found: 52.3% C, 5.05% H, 6.98% N (Dumas).

On treating the tetra-*O*-acetyl derivative of *O*-galactosylisomaltol with *p*-nitrophenylhydrazine acetate in methanol, the same compound, m.p. 143.5°–144.5°C. (mixed melting point unchanged) was obtained.

Isomaltol. Crude *O*-galactosylisomaltol (40 g.) is placed in an alembic flask constructed with annular receiver immediately above the spherical cucurbit of 250-ml. capacity. Carbon dioxide is slowly passed through a side-arm in the cucurbit; then the flask is lowered into a preheated Wood's metal bath at 240°C. Distillation of isomaltol begins with caramelization of the galactoside and continues for 10 minutes at atmospheric pressure with bath temperature in the range 240°–250°C. The delivery arm is irradiated with a heat lamp to keep the distillate liquid until it drops into the cooled receiver. Crystallization occurs immediately. The crude cake is broken up under 15 ml. water, and the dispersion is cooled to 2°C., after which it is filtered and the filter cake is washed with ice water. After drying over anhydrous calcium chloride at atmospheric pressure, 12 g. of pale-yellow crystalline powder (68% of theory) melting at 101°–102°C. with sublimation are obtained. Recrystallization from water or ether gives the pure compound, which melts rather sharply in the range 98°–103°C. The melting point varies with crystalline form and density of packing in the capillary tube. The compound is identical with isomaltol obtained by hydrolysis (analysis above).

Isomaltol is optically inactive. In the ultraviolet, a single absorption band appears at 280 mμ, E (1%, 1 cm.) = 1,270 in absolute methanol. In 0.1*N* hydrochloric acid, the maximum is at 283 mμ, E = 1,366. In 0.1*N* sodium hydroxide, the major peak shifts to 318 mμ, E = 1,485, and a minor peak becomes visible at 258 mμ, E = 500.

Isomaltol is soluble in alcohols, acetone, ether, chloroform, benzene, ethyl acetate, and hot water; it is difficultly soluble in petroleum ether and cold water. Aqueous solutions are acidic (pH 3.60 at 25°C., $c = 1.00$); they liberate carbon dioxide from 5% sodium bicarbonate and titrate to a definite end point at pH 8.7 with sodium hydroxide. Aerated neutralized and weakly alkaline solutions are yellow; these turn colorless upon acidification. Isomaltol can be recovered from heated 5*N* sodium hydroxide solutions in high yield if air is excluded. In contrast, strong acids readily decompose isomaltol. Hot 25% aqueous *p*-toluenesulfonic acid in the Elek-Harte acetyl determination (7) liberates steam-volatile acid that is not unaltered isomaltol. The distillate contains 34.4% acetyl equivalent by titration with base (7) and 3.3% formyl equivalent by bromine oxidation (1). Cold, concentrated hydrochloric or phosphoric acid solutions of isomaltol turn yellow, red, and deep greenish brown before depositing a crystalline precipitate. Recrystallization of the precipitate gives bright-orange crystals, yet unidentified, that melt sharply at 124°C. Calculated for $C_{12}H_{10}O_5$: 61.53% C, 4.30% H, 234.2 molecular weight. Calculated for

$C_{10}H_8O_4$: 62.50% C, 4.20% H, 192 molecular weight. Found: 62.42% C, 4.31% H, 209 molecular weight (Rast), 0.89 *C*-methyl per 192 molecular weight (4).

Aqueous solutions of isomaltol at pH 3.5 reduce permanganate readily and decolorize small amounts of 2,6-dichlorophenolindophenol quickly in the cold. With the latter reagent, turbidity soon develops, and unreduced red dye is later adsorbed on the initially colorless precipitate. Isomaltol does not reduce 2,6-dichlorophenolindophenol in 0.1 to 1.0*N* sodium hydroxide solutions at 20°C., but reduction occurs slowly upon heating. Also, Tollens reagent is not reduced even upon warming to 50°C. Fehling solution is slowly reduced upon heating at 100°C.

Highly dilute aqueous or alcoholic solutions of isomaltol give stable purple colors with ferric chloride. The hue changes with pH and is virtually the same for maltol and isomaltol at the same pH — violet at pH 3, cherry-red at pH 6. Titanium trichloride gives deep violet in methanol; however, in the Weygand-Csendes test in 95% methanol-pyridine (3:1), upon shaking with air, the initial pure blue lightens to greenish blue and then becomes a light orange-red; on standing, a bright yellow precipitate forms (34).

Isomaltol-O-Methyl Ether. A cold solution of 1.4 g. diazomethane in 50 ml. ether is added, 5 ml. at a time over 10 minutes, to 50 ml. of a cold ether solution containing 3.6 g. isomaltol. The methylated product soon crystallizes. The mixture is stored at 2°C. for several hours, the supernatant solution is decanted, and the crystalline precipitate is washed with cold ether and collected on a filter and dried (1.65 g.). A second crop (0.75 g.) is obtained by concentrating the decanted solution to 20 ml. volume, cooling, and filtering. A third, reddish crop (0.30 g.) separates from the filtrate after dilution with light petroleum ether and cooling. The total yield is 2.70 g., 68% of theory. Recrystallization from ether or water gives hard, dense, colorless prisms, m.p. 101.5°–103°C., that sublime on further heating. Backe reported a melting point of 101°–102°C. for isomaltol-*O*-methyl ether (3). The aqueous solution is neutral and gives no trace of purple with ferric chloride. Calculated for $C_7H_8O_3$: 60.00% C, 5.75% H, 22.15% OCH_3, 140.1 molecular weight. Found: 59.89% C, 5.75% H, 22.59% OCH_3, 154 molecular weight (Rast).

Isomaltol-*O*-methyl ether in methanol gives an absorption maximum at 281 mμ, E (1%, 1 cm. = 1,240, E_m = 17,400) that does not shift in acid or alkali. In carbon tetrachloride, no significant bands appeared in the infrared below 3.3 μ (above 3030 cm.$^{-1}$). A very strong band at 5.92–5.94 μ (1687 cm.$^{-1}$) corresponds to the carbonyl group.

Isomaltol O-Methyl Ether p-Nitrophenylhydrazone. Isomaltol-*O*-methyl ether (60 mg.) in 1 ml. warm methanol, *p*-nitrophenylhydrazine (70 mg.) in 2.5 ml. warm methanol, and 15 drops of 3*N* acetic acid are mixed. After boiling 4 minutes, the combined solution turns red. On cooling and standing, deep red needles (45 mg.) separate, m.p. 176°–177.5°C. The same compound is produced, without acetic acid, by boiling the alkaline solution of methyl ether and hydrazine for 35 minutes. Calculated for $C_{13}H_{13}N_3O_4$: 56.72% C, 4.76% H, 15.27% N, 11.27% OCH_3. Found: 56.81% C, 4.80% H, 15.53% N (Dumas), 11.66% OCH_3.

Isomaltol O-Benzoyl Ester. Isomaltol (0.21 g.) in 2 ml. dry pyridine is added to a cold mixture of benzoyl chloride (0.4 g.) in 3 ml. dry pyridine. The clear solution is allowed to stand at 25°C. for 18 hours before it is poured into 50 ml. of ice water. The crystalline precipitate (0.26 g.) melts at 98°–99°C. without sublimation on further heating to 200°C. After recrystallization from 1:1 benzene-toluene, the benzoate melts at 100°–101°C. and gives no trace of purple with ferric chloride in 95% ethanol. Calculated for $C_{13}H_{10}O_4$: 67.82% C, 4.38% H. Found: 67.90% C, 4.36% H. Backe reported a melting point of 99°C. for the benzoate of isomaltol (3).

Copper Salt of Isomaltol. A solution of 1.00 millimole copper sulfate is added to 0.95 millimole isomaltol in 20 ml. water. The translucent, hydrated green crystals that form slowly at 0°C. effloresce to an opaque, dull green powder when dried in air. No decomposition is evident on heating to 200°C. Calculated for Cu $(C_6H_5O_3)_2$: 45.94% C, 3.21% H. Found: 45.93% C, 3.29% H.

The moss-green copper salt is insoluble in water, soluble in ammonia (deep blue) and glycine solutions (blue), and in methanol (green), chloroform (green), and pyridine (green). Upon addition of aqueous sodium hydroxide to the green methanol solution, the color changes to blue as cupric hydroxide is precipitated. The properties of this copper salt agree wholly with those reported by Backe (3).

Isomaltol p-Nitrophenylhydrazone. A solution of isomaltol (0.64 g., 5.0 mmoles) in 5 ml. methanol is added to a solution of 0.93 g. (6.0 mmoles) *p*-nitrophenylhydrazine in 30 ml. hot methanol. The weakly alkaline solution is heated at the boiling point for 1 hour and allowed to stand at 25°C. overnight. The first crop, recrystallized twice from nitrobenzene, yields 15 mg. of a slightly soluble, orange-red compound that melts at 287°–289°C. Other crops isolated from the filtrate, 0.75 g., melt at 181°–183°C. Recrystallization from nitrobenzene yields a deep red monohydrazone, melting at 187°–188°C. with decomposition. Calculated for $C_{12}H_{11}N_3O_4$: 55.17% C, 4.24% H, 16.09% N. Found:

55.15% C, 4.14% H, 16.9% N (Dumas).

Preparation with addition of acetic acid in the usual way gave the same hydrazone in a shorter reaction time.

Isomaltol 2,4-Dinitrophenylhydrazone. A solution of isomaltol (0.21 g., 1.67 mmoles) in 10 ml. ethanol is added to a solution of 0.40 g. (2.0 mmoles) of 2,4-dinitrophenylhydrazine in 2 ml. concentrated sulfuric acid, 3 ml. water, 10 ml. ethanol. Crystallization begins after 4 minutes at 25°C., yielding 0.44 g. (86% of theory) of deep-red hydrazone. Recrystallization from ethanol-ethyl acetate yields the pure monohydrazone melting at 216°C. with decomposition. Calculated for $C_{12}H_{10}N_4O_6$: 47.06% C, 3.29% H, 18.30% N. Found: 47.0% C, 3.28% H, 19.18% N (Dumas).

1-Deoxy-1-Piperidino-Maltulose. When beta-maltose hydrate was substituted for alpha-lactose hydrate in the procedure to prepare *O*-galactosylisomaltol, an intermediate compound crystallized and remained nearly insoluble over 12 hours of heating at 78°–80°C. This intermediate, identified as the Amadori rearrangement product, is prepared in higher yield as follows:

Maltose hydrate, 45.0 g. (0.125 mole), is mechanically stirred in 37 ml. absolute ethanol and 13 ml. triethylamine while 21.3 g. (0.25 mole) of piperidine (practical grade) and 15 g. (0.25 mole) of glacial acetic acid are added dropwise to the mixture. A heating mantle is applied to provide constant reaction temperature of 75°C. The mixture darkens, and all maltose is dissolved after 20 minutes. After 1 hour, crystallization of the product begins. After 2 hours at 75°C., the reaction is stopped by adding 100 ml. absolute ethanol and cooling in an ice water bath for 1 hour. The crystalline product is filtered with suction, washed until white with ethanol, and dried in a vacuum desiccator over calcium chloride. Yield, 33.3 g., 66% of theory; melting point, 173°C., with decomposition. A sample recrystallized from 100 parts boiling methanol melted and decomposed sharply at 174°C. and gave specific optical rotation $[\alpha]_D^{25}$ +48.0° ($c = 0.5$, $l = 4$, pH 10.1 in water). Calculated for $C_{17}H_{31}NO_{10}$: 49.87% C, 7.63% H, 3.42% N. Found: 49.9% C, 7.69% H, 3.43% N.

The compound is strongly basic and nearly insoluble in organic solvents. It reduces 2,6-dichlorophenolindophenol (also 2,3,5-triphenyltetrazolium chloride) quickly in 0.1*N* sodium hydroxide at 20°C. Also, *o*-dinitrobenzene is reduced to a purple dye under Fearon and Kawerau's ascorbic acid test conditions (9) within less than 1 minute, another distinction between Amadori compounds and *N*-glycosides (17). The aqueous solution at 23°C. ($c = 2.0$, pH 10.25) turns golden yellow on standing in the absence of air and develops reductonelike

reducing power toward indophenol, *o*-dinitrobenzene, and tetrazolium salts, like 1-deoxy-1-piperidino-D-fructose (17).

Acknowledgments

We are indebted to Mrs. Clara E. McGrew and Mrs. Bonita Heaton for the microanalyses and to Curtis Glass for determining the ultraviolet and infrared spectra. The work was done under the general supervision of R. J. Dimler and C. E. Rist. Anhydrous beta-lactose and maltol were gifts from Whitson Products Division of The Borden Company, Elgin, Illinois, and The Dow Chemical Company, Marquette, Michigan, respectively.

Literature Cited

1. ALICINO, J. F. Microdetermination of the formyl group. Ind. Eng. Chem., Anal. Ed. **15:** 764 (1943).
2. BACKE, A. Sur un nouveau composé contenu dans les produits alimentaires. Compt. rend. **150:** 540–543 (1910).
3. BACKE, A. Recherches sur l'isomaltol. Compt. rend. **151:** 78–80 (1910).
4. BARTHEL, W. F., and LAFORGE, F. B. Determination of carbon-linked methyl groups. Ind. Eng. Chem., Anal. Ed. **16:** 434–435 (1944).
5. BRAND, J. Ueber Maltol. Berichte **27:** 806–810 (1894).
6. DREWS, E. Weitere Einsatzmöglichkeiten der Papierchromatographie auf dem Backereisektor. Brot u. Gebäck **12**(6)**:** 138–140 (1958).
7. ELEK, A., and HARTE, R. A. The microestimation of acetyl groups. Ind. Eng. Chem., Anal. Ed. **8:** 267–269 (1936).
8. ERDMANN, E., and SCHAEFER, C. Trockne Destillation von Cellulose. Berichte **43:** 2398–2406 (1910).
9. FEARON, W. R., and KAWERAU, E. The oxidation of ascorbic acid by *o*-dinitrobenzene, and the detection of dehydroascorbic acid. Biochem. J. **37:** 326–329 (1943).
10. FEUERSTEIN, W. Ueber das Vorkommen des Maltols in den Nadeln der Weisstanne (Abies alba Mill.). Berichte **34:** 1804–1806 (1901).
11. GOOS, A. W., and REITER, A. A. New products from wood carbonization. Ind. Eng. Chem. **38:** 132–135 (1946).
12. GORDY, W. Spectroscopic evidence for hydrogen bonds: Effects of chelation on the carbonyl frequency. J. Chem. Phys. **8:** 516–519 (1940).
13. HODGE, J. E. Chemistry of browning reactions in model systems. J. Agr. Food Chem. **1:** 928–943 (1953).
14. HODGE, J. E. Novel reductones and methods of making them. U. S. Patent 2,936,308 (May 10, 1960).
15. HODGE, J. E., and MOSER, HELEN A. Flavor of bread and pastry upon addition of maltol, isomaltol, and galactosylisomaltol. Cereal Chem. **38:** 221–228 (1961).
16. HODGE, J. E., NELSON, E. C., and FISHER, B. E. Reductones from reactions of sugars with amine sales. J. Org. Chem. (in preparation).
17. HODGE, J. E., and RIST, C. E. The Amadori rearrangement under new conditions and its significance for nonenzymatic browning reactions. J. Am. Chem. Soc. **75:** 316–322 (1953).
18. KILIANI, H., and BAZLEN, M. Ueber Maltol. Berichte **27:** 3115–3120 (1894).
19. KUNZ, A., and HUDSON, C. S. Relations between rotatory power and structure in the sugar group. XV. Conversion of lactose to another disaccharide, neolactose. J. Am. Chem. Soc. **48:** 1978–1984, see p. 1982 (1926).
20. MARTIN, A. E. O-H absorption in chelate compounds. Nature **166:** 474–475 (1950).
21. PATTON, S. The isolation of maltol from heated skim milk. J. Dairy Sci. **33:** 102–106 (1950).
22. PATTON, S. The formation of maltol in certain carbohydrate-glycine systems. J. Biol. Chem. **184:** 131–134 (1950).
23. PATTON, S. Review of organic chemical effects of heat on milk. J. Agr. Food Chem. **6:** 132–135 (1958).
24. PATTON, S., and FLIPSE, R. J. Carbon-14 activity of some heat degradation products of milk containing lactose-1-C^{14}. Science **125:** 1087–1088 (1957).
25. PERATONER, A., and TAMBURELLO, A. Sulla constituzione del maltolo. Gazz. Chim. Ital. **36:** 33–50 (1906).

26. POTTER, F. E., and PATTON, S. Evidence of maltol and hydroxymethylfurfural in evaporated milk as shown by paper chromatography. J. Dairy Sci. **39:** 978–982 (1956).
27. RASMUSSEN, R. S., TUNNICLIFF, D. D., and BRATTAIN, R. R. Infrared and ultraviolet spectroscopic studies on ketones. J. Am. Chem. Soc. **71:** 1068–1072 (1949).
28. REICHSTEIN, T., and BEITTER, H. Die Zusammensetzung der Aromastoffe von gerösteter Cichorie. Berichte **63:** 816–826 (1930).
29. SCHENCK, J. R., and SPIELMAN, M. A. The formation of maltol by the degradation of streptomycin. J. Am. Chem. Soc. **67:** 2276–2277 (1945).
30. SHERMAN, H. C. A source of error in the examination of foods for salicylic acid. J. Ind. Eng. Chem. **2:** 24–25 (1910).
31. SINGH, B., DEAN, G. R., and CANTOR, S. M. The role of 5-(hydroxymethyl)-furfural in the discoloration of sugar solutions. J. Am. Chem. Soc. **70:** 517–522 (1948).
32. STENHOUSE, J. Ueber Larixinsäure, einen krystallisirbaren flüchtigen Bestandtheil der Rinde des Lerchenbaums (Pinus larix L.). Ann. Chem. Liebigs **123:** 191–199 (1862).
33. SULLIVAN, BETTY, and PAYNE, W. E. The amino acids of the water extract of flour. Cereal Chem. **28:** 340–342 (1951).
34. WEYGAND, F., and CSENDES, E. Eine Nachweismethode für Endiole und Enole der 1,3-Diketone. Chem. Ber. **85:** 45–57 (1952).
35. WEYGAND, F., SIMON, H., and BITTERLICH, W.; HODGE, J. E., and FISHER, B. E. Structure of piperidino-hexose-reductone. Tetrahedron **6:** 123–138 (1959).