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Note

The formation of 1,6-anhydro-3,4-*O*-[5-(hydroxymethyl)-2-furfurylidene]-β-D-galactopyranose from lactose during pyrolysis

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It is well recognized that sugars undergo pyrolysis under baking conditions, influencing the quality of baked foods. The reaction has therefore been extensively studied, interest being mainly focused on the formation of flavor compounds and on browning. About a hundred compounds produced from mono- and oligosacharides by pyrolysis have been identified [1–4].

Pyrolysis of lactose has been investigated in this laboratory[5–7], and we have recently reported the isolation of four oligosaccharides, four anhydro sugars, and various volatile compounds from pyrolyzates of lactose obtained by heating solid lactose.

In the present investigation, we have isolated from pyrolyzates of lactose a new cyclic acetal, 1,6-anhydro-3,4-*O*-[5-(hydroxymethyl)-2-furfurylidene]-β-D-galactopyranose, which tastes strongly bitter.

Compound **1** was characterized chemically. Two hydroxyl groups were identified in **1** by formation of the diacetate **2** and dimethyl eher **3**. Hydrolysis of the formolyzate of **3** gave 2-*O*-methylgalactose, which was reduced with $NaBD_4$ and then acetylated to give 1,3,4,5,6-penta-*O*-acetyl-2-*O*-methyl-(1-^{2}H)galactitol (**6**). Identification of **6** was performed by comparison of its g.l.c. and m.s. behavior with data for an authentic sample obtained similarly from 1,6-anhydro-3,4-*O*-isopropylidene-β-D-galactopyranose (**4**).

The crystalline **1** was treated with a solution of 2,4-dinitrophenylhydrazine in 3% HCl–MeOH to give the crystalline 2,4-dinitrophenylhydrazone of 5-(hydroxymethyl)-2-furaldehyde (HMF) and 1,6-anhydro-β-D-galactopyranose (levogalactosan). These results indicated the presence of HMF and levogalactosan residues in **1**.

Further evidence is provided by m.s., and by the assignments of ^{1}H- and ^{13}C-n.m.r. spectra of **1**, as shown in Table I.

The formation of **1** from galactose by heat treatment, and the condensation

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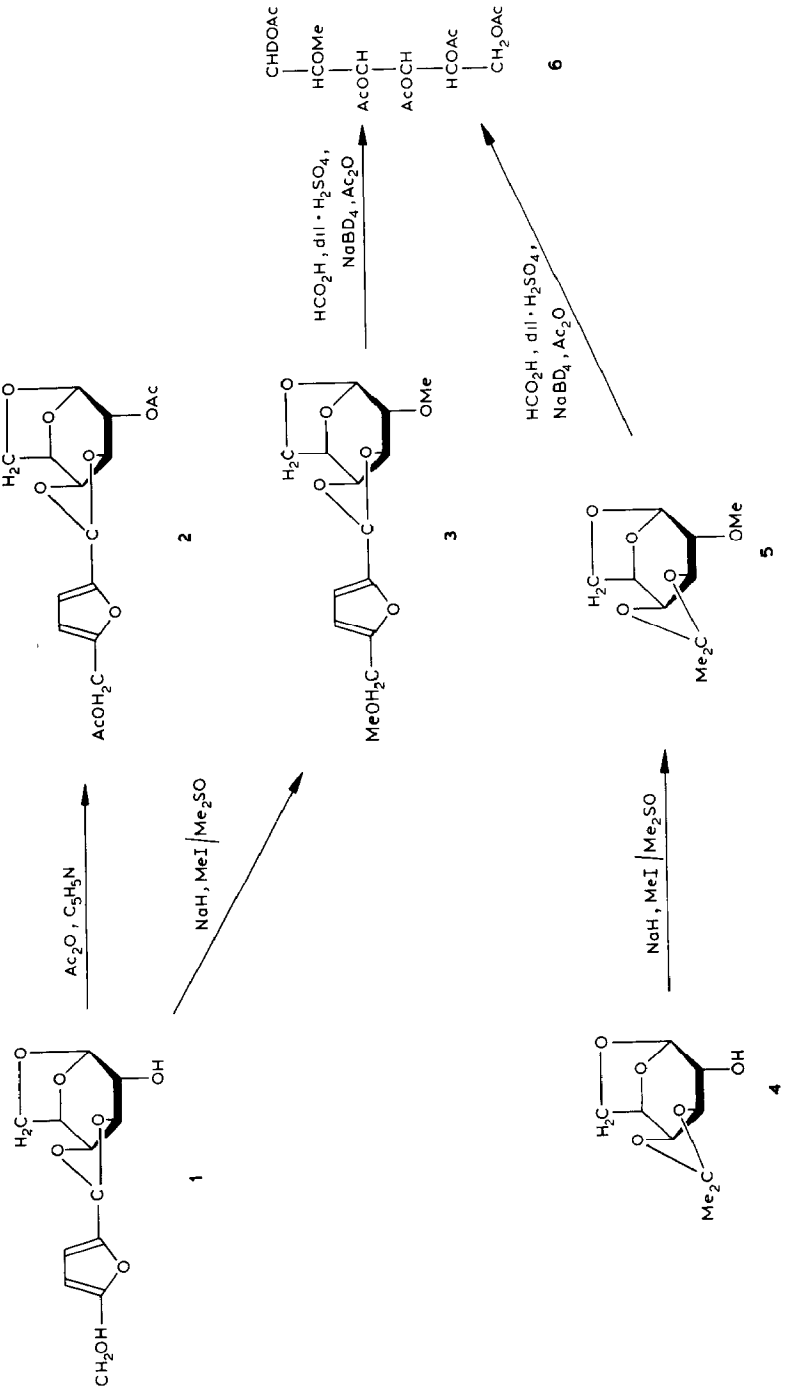
Ac2O , C5H5N
NaH, MeI/Me2SO
HCO2H , dil · H2SO4 , NaBD4 , Ac2O
NaH, MeI/Me2SO
HCO2H , dil · H2SO4 , NaBD4 , Ac2O
1
2
3
4
5
6

TABLE I

^{1}H- AND ^{13}C-N M R. CHEMICAL SHIFTS FOR 1,6-ANHYDRO-3,4-*O*-[5-(HYDROXYMETHYL)-2-FURFURYLIDENE]-*β*-D-GALACTOPYRANOSE IN DIMETHYL SULFOXIDE-d_6

Position	*^{1}H-N.m.r. data*[a]	*Position*	*^{13}C-N.m.r. data*
H-1	5.23	C-1	100.7
H-2	3.69 d $J_{2,OH-2}$ 5.7	C-2	68.5 or 68.8
HO-2	5.57 d $J_{OH-2,2}$ 5.7	C-3	77.9
H-3	4.01 d $J_{3,4}$ 7.1	C-4	68.5 or 68.8
H-4	4.53 m	C-5	71.2
H-5	3.49 d $J_{5,6}$ 5.9	C-6	62.2
H-6	4.37 m	C-2′	147.3
H-3′	6.57 d $J_{3',4'}$ 3.2	C-3′	111.3
H-4′	6.30 d $J_{4',3'}$ 3.2	C-4′	107.3
H-6′	5.73 s	C-5′	156.6
H-7′	4.37 m	C-6′	96.1
HO-7′	5.28 t	C-7′	55.6

[a] *J* values in Hz.

of levogalactosan with HMF, were confirmed by model experiments. It is generally accepted that anhydro sugars are important intermediates in pyrolysis of carbohydrates leading to various pyrolyzates and polymers[8,9]. It was deduced from these facts that **1** was formed by reaction of levogalactosan with HMF, which are themselves formed from lactose and galactose by heat treatment.

The formation of cyclic acetals from sugars and carbonyl compounds may be accelerated at low moisture content. It may be assumed that the various carbonyl derivatives formed by heat treatment of lactose would condense with the *cis* 3,4-diol of the galactose residue to form cyclic acetals.

EXPERIMENTAL

General methods. — Melting points were measured on a micro melting point apparatus (Yanagimoto, Japan) and are not corrected. Optical rotation was measured with a JASCO DIP-4 digital polarimeter. U.v. and visible spectra were recorded with a JASCO UVIDEC-505X spectrophotometer. I.r. spectra (KBr disks) were recorded with a Hitachi 260-10 spectrometer. ^{1}H-N.m.r. spectra were recorded at 100 MHz, with Me_4Si as the internal standard, with a JEOL-FX-100 spectrometer, operated in the pulsed Fourier-transform mode. ^{13}C-N.m.r. spectra were obtained with a JEOL-FX-100 spectrometer, operated at 25 MHz. Mass spectra were measured with a Hitachi M-52G mass spectrometer; the ionization current and accelerating voltage were 100 μA and 25 eV. respectively. Field-desorption m.s. were recorded with a JEOL-JMS-OISG-2 mass spectrometer; the accelerating voltage was 8 kV and the cathode voltage −5 kV. High-resolution m.s. were obtained with a JEOL-JMS-OISG-2 mass spectrometer, with ionization current and voltage of 100 μA and 25 eV, respectively.

Materials. — Lactose monohydrate was purchased from Wako Co. Anhydrous D-galactose and 5-hydroxymethyl-2-furaldehyde (HMF) were purchased from Tokyo Kasei Co. Other chemicals were commercial products of reagent grade. Levogalactosan was prepared by the method[10] of Montgomery *et al.*

Chromatography. — G.l.c. was performed with a Hitachi model 163 instrument, equipped with a flame-ionization detector and a column (0.2 × 100 cm) packed with 10% SE-30 on Chromosorb W. The temperature was programmed from 100 to 300° at 5°/min. Preparative t.l.c. was conducted on plates coated with Silica Gel 60G (Merck) or plates precoated with silica gel 60 (Merck), employing as solvent systems 9:1 dichloromethane–acetic acid (solvent *A*) for the separation of acidic compounds or 3:2 dichloromethane–acetone (solvent *B*) for purification. Plates were then sprayed with 1% 2,4-dinitrophenylhydrazine in 3% HCl–MeOH. Qualitative t.l.c. used aluminum sheets precoated with Silica Gel 60 (Merck), employing as solvent systems solvent *B* or 10:5:4 butanol–2-propanol–water (solvent *C*) for the separation of levogalactosan. Plates were sprayed with 10% sulfuric acid–MeOH and then heated for 5 min at 110° to reveal the spots.

Pyrolysis of lactose and galactose. — Lactose monohydrate (800 g) in a round-bottom flask (4 L) having a reflux condenser attached was heated in an oil bath for 20 h at 200° (bath temperature). After heating for 8 h, the temperature of the reactor was maintained at 120°, while the sample melted and its color changed uniformly to brown. The pyrolyzate was obtained as a tar having a baked, sugary flavor. Anhydrous galactose (20 g) in a flask (100 mL) having a reflux condenser attached was heated for 20 h in an oil bath at 200° (bath temperature).

*Isolation of 1,6-anhydro-3,4-*O*-[5-(hydroxymethyl)-2-furfurylidene]-β-*D*-galactopyranose* (**1**) *from lactose pyrolyzates.* — The lactose pyrolyzate (800 g) was dissolved in 4 L of distilled water and the solution was extracted continuously with ethyl acetate for 10 h. The aqueous ethyl acetate fraction obtained was evaporated to a syrup (6 g) that was redissolved in 10 mL of ethyl acetate and then charged onto preparative t.l.c. (solvents *A* and *B*). The zone containing the title compound [R_F 0.22 (solvent *A*), and 0.61 (solvent *B*)] was extracted with ethyl acetate. Evaporation of the extract afforded **1** as crystals. Recrystallization from hot ethyl acetate gave 15.3 mg of prisms; m.p. 162–163°, $[\alpha]_D$ +33° (*c* 0.33, methanol); λ_{max}^{MeOH} 221 nm (ε 2860); ν_{max}^{KBr} 3250 (ν_{O-H}), 1670, 1650 ($\nu_{c=c}$), 1200, 1180, 1140, 1110, 1070, 1030, 1000, 980 (ν_{c-o}), and 810 cm^{-1} (γ_{C-H}); *m/z* (%) 270(M, 10), 253(5), 249(14), 211(24), 197(8), 177(8), 169(10), 144(7), 127(100), 126(16), 125(19), 123(15), 111(25), 110(34), 109(84), 98(26), 97(45), 81(35), 73(15), 69(39), 57(24), 43(13), and 41(19); field-desorption m.s. *m/z* 270; exact mass calc. *m/z* 270.0722, found 270.0739. Attempts were made to increase the yield of **1** by varying the experimental conditions. It was found that under the foregoing conditions, either longer or shorter reaction-periods gave lower yields of **1**. At shorter times of reaction, larger amounts of unreacted lactose were recovered. At longer reaction-times, less unpyrolyzed lactose was recovered, but greater amounts of polymer were obtained.

The reaction of **1** (2 mg) with 5 mL of 1% 2,4-dinitrophenylhydrazine in 3% HCl–MeOH for 30 min at room temperature gave orange needles of the 2,4-dinitrophenylhydrazone of HMF; $\lambda_{max}^{CHCl_3}$ 388, and (alkaline $CHCl_3$) 420 nm; t.l.c. and m.s. identical with those of an authentic sample. The solution remaining was treated with an excess of heptanal for 30 min at room temperature to remove the excess of 2,4-dinitrophenylhydrazine. After addition of 10 mL of water, heptanal and its hydrazone derivative were removed by extraction 3 times with ethyl ether. The aqueous acid fraction was made neutral with M NaOH, evaporated to dryness with a rotary evaporator, and then extracted with abs. ethanol. A small amount of syrup obtained from the ethanol extract gave one spot in t.l.c. (R_F 0.50, solvent *C*) and one peak by g.l.c. of its Me_3Si derivative (R_T 13.5 min). In admixture with authentic levogalactosan, the material gave the same chromatographic results.

Detection of **1** *from the pyrolyzate of galactose.* — The pyrolyzate (20 g) from galactose was dissolved in 100 mL of distilled water and then extracted with ethyl acetate by using a continuous liquid extractor. The ethyl acetate-soluble fraction was evaporated to a syrup and then examined by g.l.c. The formation of **1** by pyrolysis of galactose was confirmed by comparison of its R_T value (23.5 min) with that of **1**.

Formation of **1** *by condensation of levogalactosan with HMF.* — A mixture of levogalactosan (10 mg) and HMF (60 mg) in a test tube (1.5 × 13 cm) was heated for 5 h at 100°. The brown product was dissolved in 1 mL of methanol and then examined by t.l.c. and g.l.c. The formation of **1** was confirmed by comparison of its R_F value (0.61, solvent *B*) in t.l.c. and its R_T value (23.5 min) in g.l.c. with those of **1**.

2-O-Acetyl-1,6-anhydro-3,4-O-[hydroxymethyl)-2-furfurylidene]-β-D-galacto-pyranose (**2**). — Acetylation[11] of **1** with acetic anhydride and pyridine gave material that was extracted with chloroform, and then evaporated to dryness in a stream of nitrogen to afford amorphous **2**; R_T 26.5 min (g.l.c.); λ_{max}^{MeOH} 222 nm; *m/z* (%) 354(M, 7), 312(60), 281(30), 219(23), 207(23), 187(20), 169(27), 165(27), 151(23), 126(53), 110(23), 109(90), 97(23), 95(23), 85(27), 81(40), 73(30), 69(30), 55(23), and 43(100); ^{1}H-N.m.r. ($CDCl_3$): δ 2.09 (3 H s), 2.14 (3 H s), 3.69 (1 H m), 4.09 (1 H dm), 4.55 (6 H m), 5.43 (1 H s), 5.77 (1 H s), 6.41 (1 H d, *J* 3.2 Hz), and 6.55 (1 H d, *J* 3.2 Hz). Calc. *m/z* 354.0927; found 354.0950.

1,6-Anhydro-3,4-O-[5-(methoxymethyl)-2-furfurylidene]-2-O-methyl-β-D-galactopyranose (**3**). — Compound **1** (2 mg) was permethylated according to the method[12] of Hakomori. The permethylated product was purified through a column of Wakogel S-1 with methanol, and dried to afford amorphous **3**; R_T 21.5 min (g.l.c.); λ_{max}^{MeOH} 221 nm; *m/z* (%) 298 (M, 10), 267(20), 225(96), 183(24), 168(19), 158(31), 155(23), 149(18), 141(69), 140(49), 139(29), 137(23), 126(34), 125(31), 113(53), 112(94), 111(71), 109(79), 99(19), 97(29), 95(30), 87(80), 85(36), 82(38), 81(100), 71(20), 69(27), 58(51), 57(49), 55(31), 45(40), and 41(29); ^{1}H-n.m.r. ($CDCl_3$): δ 3.38 (3 H s), 3.50 (3 H s), 3.68 (1 H m), 4.18 (1 H m), 4.50 (6 H m), 5.48 (1 H s), 5.78 (1 H s), 6.33 (1 H d, *J* 3.2 Hz), and 6.53 (1 H d, *J* 3.2 Hz). Calc. *m/z* 298.1057; found 298.1051.

Preparation of 1,6-anhydro-3,4-O-isopropylidene-β-D-galactopyranose (**4**). — Conventionally prepared[13] from levogalactosan, compound **4** had m.p. 146–148° (lit.[14] m.p. 151–152°); *m/z* (%) 188(6), 187(M − 15, 100), 143(14), 127(6), 115(7), 101(14), 100(11), 99(14), 98(10), 97(17), 85(17), 81(23), 73(15), 71(19), 70(18), 69(23), 59(68), 57(44), 55(26), 45(18), 43(65), and 41(12); ^{1}H-N.m.r. ($CDCl_3$): δ 1.35 (3 H s), 1.53 (3 H s), 3.53 (1 H d), 3.63 (1 H m), 3.84 (1 H m), 4.16 (2 H t), 4.47 (2 H m), and 5.38 (1 H s).

1,6-Anhydro-3,4-O-isopropylidene-2-O-methyl-β-D-galactopyranose (**5**). — Compound **4** (2 mg) was permethylated according to the Hakomori method[12] to afford amorphous **5**; *m/z* (%) 217(M + 1, trace), 216(M, trace), 201(55), 149(27), 143(32), 129(36), 113(32), 101(40), 97(28), 95(15), 87(18), 85(33), 81(32), 71(24), 69(26), 59(85), 58(32), 57(59), 55(56), 45(35), 43(98), 41(45), and 29(100); ^{1}H-n.m.r. ($CDCl_3$): δ 1.37 (3 H s), 1.53 (3 H s), 3.48 (3 H s), 3.58 (1 H m), 3.76 (1 H s), 4.13 (2 H t), 4.47 (2 H m), and 5.43 (1 H s). Calc. *m/z* 216.1020; found 216.0997.

1,3,4,5,6-Penta-O-acetyl-2-O-methyl-(1-^{2}H)galactitol (**6**). — The alditol acetate derivative (**6**) of 2-*O*-methyl-(1-^{2}H)galactitol obtained from **3** and **5** was prepared by the method[15] of Stellner *et al.*; *m/z* (%) 408(M + 1, 1), 334(2), 333(5), 259(3), 202(3), 188(4), 172(3), 160(5), 145(3), 139(15), 129(8), 119(6), 118(100), 115(5), 103(3), 97(11), 87(4), 86(4), 85(4), 59(7), and 43(52). Calc. *m/z* 408.1637 (M + 1); found 408.1614.

REFERENCES

1 I. S. Fagerson, *J. Agr. Food Chem.*, 17 (1969) 747–750.
2 O. Theander, in P. Koivistoinen and L. Hyvonen (Eds.), *Carbohydrate Sweeteners in Food and Nutrition*, Academic Press, London, 1980, pp. 185–199.
3 Y. Houminer, in G. G. Birch and L. L. Green (Eds.), *Molecular Structure and Function of Food Carbohydrate*, Applied Science Publishers Ltd., London, 1973, pp. 133–154.
4 P. E. Shaw, J. H. Tatum, and R. E. Berry, in G. G. Birch and R. S. Shallenberger (Eds.), *Developments in Food Carbohydrate*, Vol. 1, Applied Science Publishers Ltd., London, 1977, pp. 91–111.
5 H. Hohno and S. Adachi, *J. Dairy Sci.*, 65 (1982) 1421–1427.
6 H. Hohno, K. Suyama, and S. Adachi, *J. Dairy Sci.*, 66 (1983) 11–16.
7 T. Urashima, K. Suyama, and S. Adachi, *Nippon Nogeikagaku Kaishi*, 57 (1983) 641–647; *Chem. Abstr.*, 99 (1983) 103837b.
8 F. Shafidazeh, *J. Polym. Sci., Part C*, 36 (1971) 21–51.
9 F. Shafizadeh, *J. Anal. Appl. Pyrolysis*, 3 (1982) 283–305.
10 E. M. Montgomery, N. K. Richtmyer, and C. S. Hudson, *J. Am. Chem. Soc.*, 65 (1943) 3–7.
11 M. L. Wolfrom and A. Thompson, *Methods Carbohydr. Chem.*, 2 (1963) 212.
12 S. Hakomori, *J. Biochem. (Tokyo)*, 55 (1964) 205–208.
13 O. T. Schmidt, *Methods Carbohydr. Chem.*, 2 (1963) 319.
14 D. McCreath and F. Smith, *J. Chem. Soc.*, (1939) 387–391.
15 K. Stellner, H. Saito, and S. Hakomori, *Arch. Biochem. Biophys.*, 155 (1973) 464–472.