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The Condensation of 5-(Hydroxymethyl)-2-Furfuraldehyde with Some Aldoses on Heating

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ABSTRACT

*The following condensation products of 5-(hydroxymethyl)-2-furfuraldehyde (HMF) with glucose, galactose or lactose were identified in the reaction mixtures heated under various conditions: HMF-β-*D*-galactofuranoside (**1**), HMF-α-*D*-galactofuranoside (**2**), HMF-*D*-galactopyranoside (**3**), HMF-*D*-glucopyranoside (**4**), HMF-*D*-glucofuranoside (**5**), 4′,6′-**O**-[5-(hydroxymethyl)-2-furfurylidene]-lactose (**6**), 4′,6′-**O**-[5-(hydroxymethyl)-2-furfurylidene]-HMF-lactoside (**7**).*

*Compounds (**3**) and (**5**) were detected in galactose and glucose, respectively, heated at 215°C for 10 h by thin-layer chromatography. Compound (**6**) was suggested to exist in lactose heated at 215°C for 10 h, by thin-layer chromatography.*

*Compound (**5**) was also detected in a commercial caramel prepared by heating of millet honey with ammonium sulfate and a commercial caramel candy.*

INTRODUCTION

In the processing of intermediate moisture foods such as candy confections or bakery products, saccharides would be degraded to the other compounds,

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accompanying a baked flavour and a brown colour. So, pyrolysis of saccharides has investigated as a part of nonenzymatic browning during heat-processing of foods (Fagerson, 1969; Theander, 1980; Houminer, 1973; Shaw, 1977). Anhydro sugars have been recognized extensively as initial products in the pyrolysis. (Shafizadeh, 1971; 1982). Needless to say, the pyrolysis reaction cannot be limited to the initial stage without simultaneous further dehydration and polymerization, as well as extensive degradation to produce 5-(hydroxymethyl)-2-furfuraldehyde (HMF) (Liskowitz & Carroll, 1967; Sugisawa & Edo, 1966; Kato & Komorita, 1968; Gardiner, 1966).

From lactose pyrolysates, we have recently reported the isolation of 1,6-anhydro-3,4-**O**-[5-(hydroxymethyl)-2-furfurylidene]-β-D-galactopyranose which had a bitter taste (Urashima *et al.*, 1985). As it has been assumed from the result that HMF would be condensed with remaining lactose and resulting monosaccharides in the pyrolysate during heating, we tried to study the condensation of HMF with these saccharides by heating, and then to detect the condensation products in pyrolysates of these saccharides.

In addition to this, the condensation products were also checked in the reaction mixtures of glycine and these sugars and also in market samples of caramel and caramel candy.

MATERIALS AND METHODS

General

Anhydrous glucose, galactose, lactose monohydrate, glycine and HMF were purchased from Wako Co. A caramel prepared by heating millet honey with ammonium sulfate and a caramel candy (Ezaki Glico Co.) were commercial products purchased from a local supermarket. Other chemicals were commercial products of reagent grade.

^{1}H-NMR spectra were recorded at 100 MHz with TPS as an internal standard on a JEOL-FX-100 spectrometer, operated in the pulsed Fourier transform-mode. ^{13}C-NMR spectra were obtained with a JEOL-FX-100 spectrometer, operated at 25 MHz. Mass spectra were measured on a JMS-DX 300 mass spectrometer; ionization current and voltage were 100 μA and 25 eV, respectively. Gas–liquid chromatography–mass spectrometry (GC–MS) were operated in the following GC condition; glass column (0·2 cm × 100 cm) packed with a 2% OV-1 on Chromosorb W, temperature gradient of 4°C/min from 220°C to 240°C, and 40 ml/min of carrier gas flow rate.

Gas–liquid chromatography (GC)

A Hitachi model 163 gas chromatograph equipped with a flame ionization detector and a steel column (0·2 cm × 100 cm) packed with 10% SE-30 on Chromosorb W was operated with a temperature gradient of 5°C/min from 100°C to 300°C. FID was used in the detection. Injection temperature was 350°C and carrier gas flow rate was 40 ml/min.

Thin-layer chromatography (TLC)

TLC was performed with silicagel 60 or silicagel 60G and 2:1:1 ethyl acetate–acetic acid–water as developing solvents. 2,4-Dinitrophenylhydrazine (1% in 3% HCl-MeOH) solution was the spray reagent.

Acetylation and methylation

The acetylation was carried out with acetic anhydride and pyridine in the usual way (Wolfrom & Thompson, 1963). Methylation was performed by the Hakomori method (1964).

Condensation of HMF and sugars during heating, and isolation of compounds (1)–(7) from the reaction mixtures

The mixtures of HMF (1 g), D-glucose or -galactose (1 g) and 1,4-dioxane (1 ml) in a test tube with a cap were heated at 150°C for 6 h. 1,4-Dioxane was added in order to unify the reaction condition. After heating, a sample was dissolved in 10 ml alkaline water ($NaHCO_3$) and extracted with 10 ml ethyl acetate, three times. Then, the water fraction was evaporated to dryness in a rotary evaporator and extracted with 30 ml ethanol, followed by concentration to 5 ml. The compounds (**1**)–(**5**), which had R_fs of 0·51, 0·44 and 0·36 (galactose) and 0·43 and 0·39 (glucose), were isolated from the ethanol extracts by preparative TLC.

The mixture of HMF (5 g) and α-lactose (2 g) in a test tube with a cap was heated at 100°C for 6 h. Then the sample was dissolved in 20 ml water and extracted with 20 ml ethyl acetate, three times. The compounds (**6**) and (**7**) which have R_fs of 0·2 and 0·36, respectively, were isolated from the water fraction by preparative TLC.

The compounds (**1**)–(**7**) were subjected to ^1H and ^{13}C-NMR, and their acetyl derivatives were subjected to MS.

Detection of the condensation products of HMF and saccharides in the pyrolysates of saccharides

Anhydrous D-glucose (150 g) or D-galactose (150 g) in a round bottom flask attached to a reflux condenser, was heated at 215°C (heater's temperature) for 10 h. The samples immediately melted and became brown. Then, they were dissolved in 300 ml alkaline ($NaHCO_3$) hot water, followed by addition of 1·5 litres of ethanol. The supernatants were evaporated to 100 ml in a rotary evaporator, and followed by readdition of 800 ml ethanol. The supernatants were also evaporated to dryness in a rotary evaporator, and extracted with 500 ml ethanol. The ethanol-soluble fractions were concentrated to 10 ml. The components which had an R_fs of 0·39 (glucose pyrolysates) and an R_f of 0·36 (galactose pyrolysates) in TLC, respectively, were isolated, acetylated and subjected to GC.

α-Lactose monohydrate (400 g) was heated at the above condition. The sample was gradually melted. Then it was extracted with methanol using the above methods. The component which had an R_f of 0·20 in TLC was isolated and subjected to GC of its methylate.

Detection of compounds (3), (5) and (7) in the reaction mixtures of glycine and galactose, glucose or lactose during heating

The mixture of D-galactose (20 g) and glycine (2 g) in 10 test tubes with caps was heated at 120°C for 4 h in an oven. Then, it was dissolved in 600 ml alkaline ($NaHCO_3$) water, and concentrated to 200 ml, followed by addition of 1 litre of ethanol. The ethanol-soluble fraction was evaporated to dryness and extracted with 500 ml of ethanol, then concentrated to 10 ml. The component, which had an R_f of 0·36 in TLC, was collected and subjected to GC of its acetate.

The mixture of D-glucose (20 g) and glycine (2 g) was heated at 140°C for 1 h in an oven. The ethanol-soluble component which had an R_f of 0·39 in TLC was collected and subjected to GC of its acetate.

The mixture of α-lactose monohydrate (20 g) and glycine (2 g) was heated at 130°C for 3 h in an oven. Then, it was extracted with methanol. The component which had an R_f of 0·20 in TLC was collected and subjected to GC of its methylate.

Detection of compound (5) in a commercial caramel and a caramel candy

Two kilograms of commercial caramel syrup were extracted with 3 litres of ethanol, and then the solution was condensed to 1 litre, followed by the addition of 4 litres of ethanol. The supernatant was evaporated to dryness and extracted with 1·5 litres of ethanol, then concentrated to 20 ml. The component which had an R_f of 0·39 in TLC was isolated from the fraction and subjected to GC of its acetate.

470 g of commercial caramel candy was dissolved in 800 ml of water and defatted with 1·6 litres of ethyl ether. 4 litres of ethanol were added into the water fraction and then the solution was concentrated to 500 ml, followed by the addition of 3 litres of ethanol. The supernatant was evaporated to dryness and extracted with 1 litre of ethanol and concentrated to 10 ml. The component which had an R_f of 0·39 was isolated and subjected to GC of its acetate.

RESULTS AND DISCUSSION

Identification of compounds (1)–(7)

The thin-layer chromatogram of the reaction mixture of HMF and galactose or glucose showed 3 and 2 spots with R_fs of 0·51 (compound **1**), 0·44 (compound **2**), 0·36 (compound **3**) and 0·43 (compound **4**), 0·39 (compound **5**), respectively. After isolation by preparative TLC, they were subjected to acetylation and the resulting acetates to ^{1}H- and ^{13}C-NMR and MS analyses.

As shown in Fig. 1, compound (**1**) had chemical shifts at δ 9·48, 7·53 and

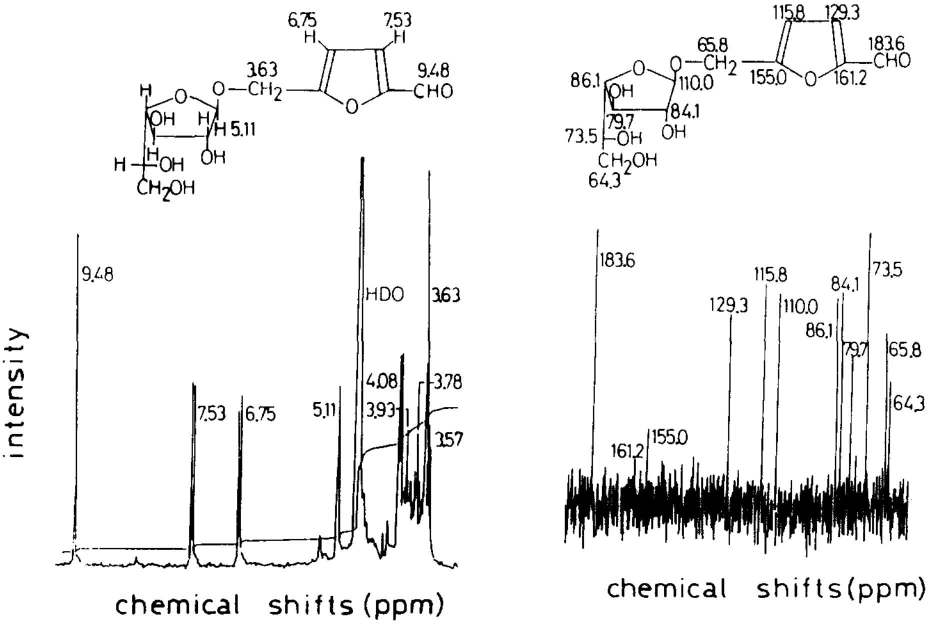

Fig. 1. ^{1}H-NMR and ^{13}C-NMR assignments of compound (1).

6·75 corresponding to aldehyde proton and H-3, H-4 of HMF. The signal shift at δ 5·11 was assigned as the anomer proton of a β-galactoside, so compound (**1**) was assumed to be HMF-galactoside. From the ^{13}C-NMR signals, it was identified as HMF-β-D-galactofuranoside, because the chemical shifts at δ 110·0, 84·1, 79·7, 86·1, 73·5 and 64·3 corresponded to the C1–C6 atoms of methyl β-D-galactofuranoside.

As compounds (**2**)–(**5**) also had chemical shifts at δ 9·5, 7·5 and 6·8 in their ^{1}H-NMR spectra, they were assumed to be HMF-glycoside. From the assignment of their ^{13}C-NMR chemical shifts (Fig. 2), compound (**3**) and compound (**5**) were identified as HMF-α and β-D-galactopyranoside and HMF-α and β-D-glucopyranoside, respectively.

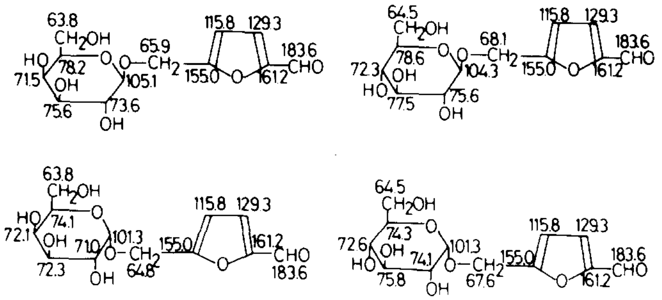

Fig. 2. ^{13}C-NMR assignments of compound (**3**) and compound (**5**).

The MS of their acetates are given in Fig. 3. The patterns of compounds (**1**) and (**2**) were essentially identical, indicating that compound (**2**) would be HMF-α-D-galactofuranoside. The compound (**4**), which had two peaks in its GC chromatogram, was assumed to be HMF-α and β-D-glucofuranoside from the MS.

From the above results, the mechanism in Fig. 4 was proposed for the condensation of HMF and galactose or glucose during heating, because the furanoses of glucose and galactose are very few in their normal state.

The mixture of lactose and HMF was heated at the more mild condition, because polymers were formed when it was heated at 150°C. The high content system of HMF, in which 1,4-dioxane was absent, was used in order to activate the addition of HMF to lactose. The thin-layer chromatogram of the condensation products of HMF and lactose showed two spots of R_f 0·20 (compound **6**) and 0·36 (compound **7**). The ^{1}H-NMR of compound (**6**) in Fig. 5 showed signal shifts at δ 6·67, 6·55 and 5·95, which arose from protons bound to the furfurylidene residue and acetal carbon, respectively, as presented in our previous paper (Urashima *et al.*, 1986). From the ^{13}C-NMR assignment, it was identified to be 4′,6′-**O**-[5-hydroxymethyl)-2-furfurylidene]-lactose.

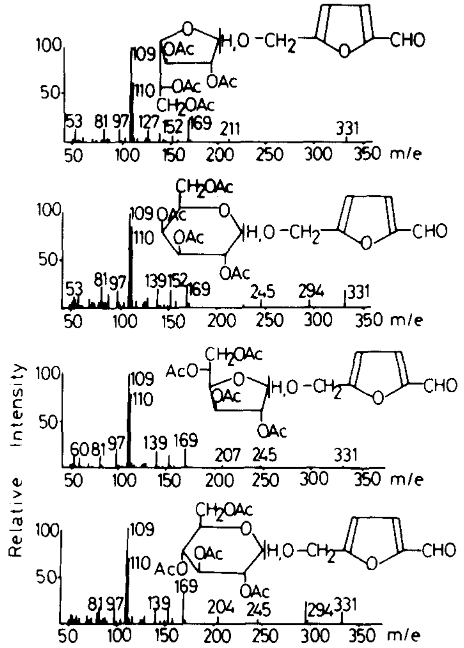

Fig. 3. MS of the acetates of HMF-galactoside and HMF-glucoside.

Compound (7) was assumed to be 4′,6′-O-[5-hydroxymethyl)-2-furfurylidene]-HMF-lactoside, because its ^1H-NMR signal had chemical shifts due to both furfurylidene residue at δ 6·55, 6·50 and 5·76 and HMF-glycoside residue at δ 9·44, 7·49 and 6·70.

From the formation of furfurylidene acetal prior to HMF-glycoside from lactose and HMF during heating treatment, it was deduced that the reactivity of the Cl aldehyde of lactose is less than that ofgalactose or glucose in heating.

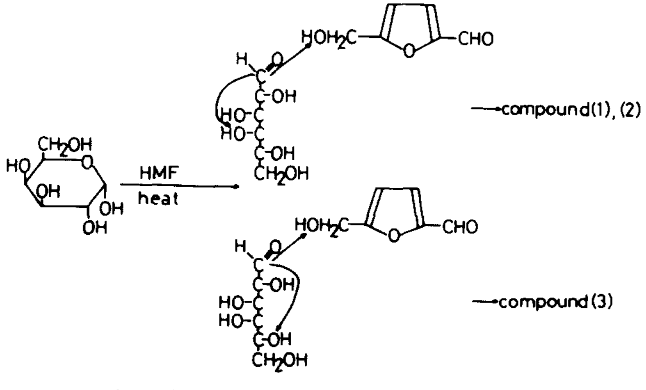

Fig. 4. The route of condensation of galactose and HMF during heat processing.

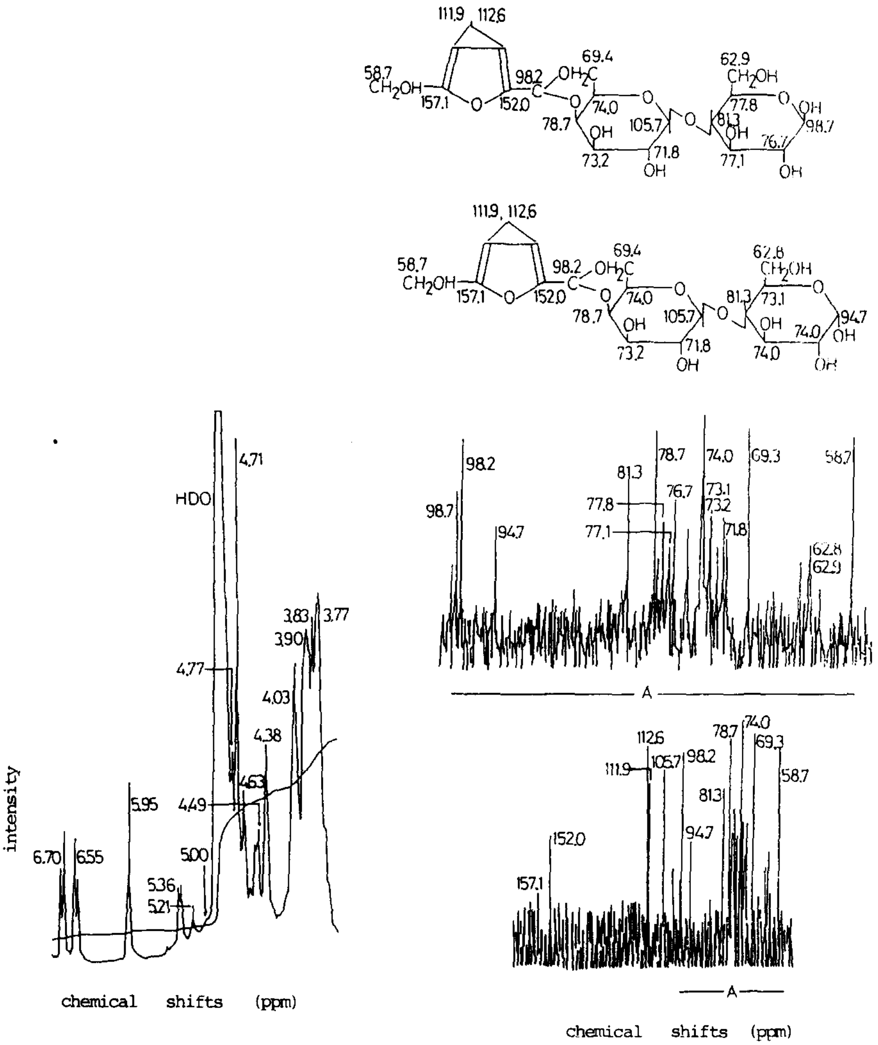

Fig. 5. ^{1}H-NMR and ^{13}C-NMR assignments of compound (**6**).

Detection of compounds (3), (5) and (7) in products of heated sugars

Compound (**3**) was detected in the pyrolysate of galactose and the reaction mixture of galactose and glycine by heating as shown in Fig. 6. It is deduced from the result that compound (**3**) was formed by heating galactose and glycine under less severe conditions.

Compound (**4**) was also detected in the pyrolysate of glucose and the reaction mixture of glucose and glycine by heating. Moreover, the compound was detected in a commercial caramel and a caramel candy.

Figure 7 shows that compound (**7**) is detectable in the pyrolysate of lactose

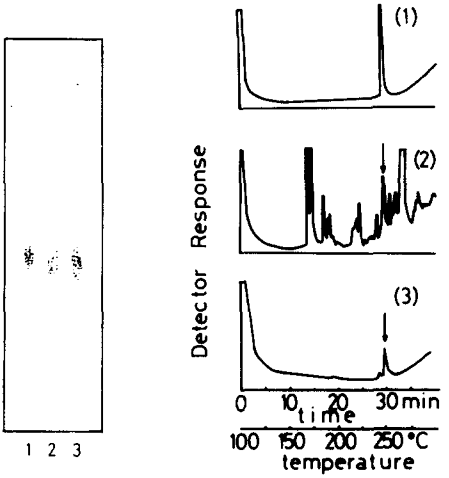

Fig. 6. TLC of HMF-galactopyranoside and GC of its acetate separated from galactose pyrolysate: (1) authentic sample of HMF-galactopyranoside, (2) compounds separated from galactose heated at 215°C of oil temperature for 10 h, (3) compounds separated from galactose and glycine mixture heated at 120°C for 4 h. Arrow indicates the peak of HMF-galactopyranoside.

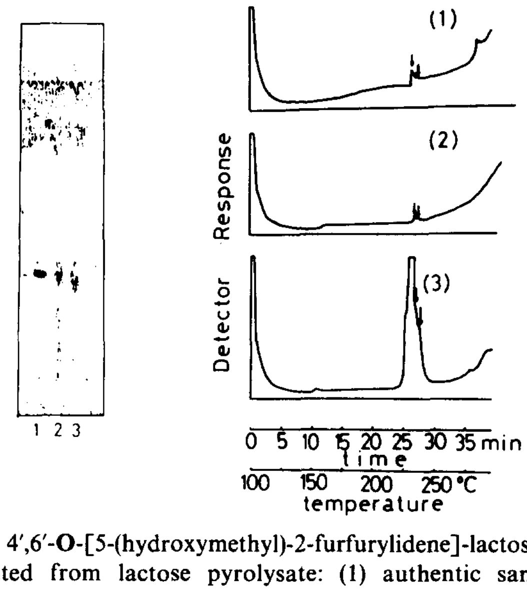

Fig. 7. TLC of 4′,6′-O-[5-(hydroxymethyl)-2-furfurylidene]-lactose and GC of the methylate separated from lactose pyrolysate: (1) authentic sample of 4′,6′-O-[5-(hydroxymethyl)-2-furfurylidene]-lactose, (2) compound separated from α-lactose monohydrate and glycine mixture heated at 130°C for 3 h, (3) compounds separated from α-lactose monohydrate heated at 215°C of oil temperature for 10 h. Arrows indicate the peaks of α and β anomers of 4′,6′-O-[5-(hydroxymethyl)-2-furfurylidene]-lactose.

and the reaction mixture of lactose and glycine by TLC. From the GC data of their methylates, compound (**7**) was also detected in the reaction mixture of lactose and glycine, though the presence of compound (**7**) was not clear in the pyrolysate of lactose since it overlapped other peaks. In spite of this fact, compound (**7**) was suggested to exist in the pyrolysate, because the formation of HMF in the pyrolysate of lactose has been elucidated (Gardiner, 1966; Urashima *et al.*, 1983).

It is deduced from the results that HMF would be condensed with sugars on the way of polymer formation from sugars in their pyrolysates. The presence of furfurylidene residue and HMF-glycoside in the polymer structure was suggested by Shafizadeh & Lai (1973). They reported that the polymers obtained from cellobiose and trehalose by heating show an IR absorption band at 1710 cm^{-1} and UV absorption maxima at 220 and 275 nm. According to the discussion described above, they seemed to be ascribed to the furfurylidene residue and HMF-glycoside, respectively in the polymers.

In Maillard type reaction systems at a low moisture, such condensation between HMF and sugars may also occur. The 5-(hydroxymethyl)-2-furfurylidene acetals of sugars would be considerably related to the quality of baked foods through their taste, nutritional and toxicological effects.

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