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Detection of Maillard Browning Reaction Products as Trimethylsilyl Derivatives by Gas–Liquid Chromatography

Melville L. Wolfrom,[1] Naoki Kashimura,[2] and Derek Horton*

D-Glucosylamines and their Amadori rearrangement products, 1-deoxy-1-(N-substituted)amino-D-fructoses, formed in the Maillard browning reaction of D-glucose with various amines, are shown to be readily detectable, together with the reactants, by gas–liquid chromatography of the trimethylsilylated reaction mixture. The procedure affords a convenient method for monitoring the course and extent of the browning reaction. A mixture of *N,O*-bis(trimethylsilyl)acetamide, *N*-(trimethylsilyl)imidazole, and chlorotrimethylsilane was used for trimethylsilylation.

The detection of carbohydrate intermediates in the nonenzymic browning reaction has been achieved mainly by colorimetric methods (Hodge, 1955; Wolfrom and Rooney, 1953; Wolfrom *et al.*, 1955; Talley and Porter, 1968) and by paper chromatography (Kato, 1962; Anet, 1960a,b; Borsook *et al.*, 1955). The unstable dicarbonyl sugar intermediates have been converted into their crystalline osazones (Anet, 1960a,b; Kato, 1960; Machell and Richards, 1960). The gas–liquid chromatographic technique has had limited success in application to this problem because of involatility and instability of the initial sugar–amine derivatives and their subsequent transformation products (Kadunce, 1967). The development of newer reagents for trimethylsilylation (Pierce, 1968) aroused our interest in conducting a reinvestigation on the detection of the Maillard browning reaction products by gas–liquid chromatography (glc) of their trimethylsilyl derivatives. A mixture of *N,O*-bis(trimethylsilyl)acetamide (Klebe *et al.*, 1966), *N*-(trimethylsilyl)imidazole (Horning *et al.*, 1967), and chlorotrimethylsilane was found satisfactory for derivatizing D-glucosylamines, the Amadori products, and other compounds formed in the reaction of D-glucose with *p*-toluidine, 4-aminobutyric acid, and various amino acids, at ambient or at elevated temperatures. The derivatives of the products and initial reactants produce detectable and reproducible peaks on glc. A brief preliminary report of this work has been presented (Wolfrom and Kashimura, 1969). The technique has been applied to freeze-dried mixtures of sugars and amino acids, a model system for such dried food products as orange juice powder, the flavor deterioration of which is considered to be partly due to the nonenzymic browning reaction of reducing sugars and amino compounds (Hodge, 1953). This convenient method for simultaneous analysis of the initial reactants and the transformation products should be of value both for fundamental studies on the browning reaction and also in applied work as a quantitative monitor for product deterioration in stored or processed food products arising from the initial stages of the nonenzymic browning reaction.

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EXPERIMENTAL SECTION

Materials. *p*-Toluidine (mp 44–45°) was recrystallized twice from ether. *N*-*p*-Tolyl-D-glucopyranosylamine [mp 108–114°, $[\alpha]^{23}D$ −117° (*c* 1.0, pyridine)] was prepared by the method of Ellis and Honeyman (1952). 1-Deoxy-1-*p*-toluidino-D-fructose [mp 155–157°, $[\alpha]^{23}D$ −21.0° (*c* 1.0, pyridine)] was prepared by the method of Weygand (1940). *N,N*-Bis(1-deoxy-D-fructos-1-yl)glycine ("difructoseglycine") [amorphous powder, $R_{glucose}$ 0.12 (4:1:1 (v/v) butyl alcohol–acetic acid–water, silver nitrate–sodium hydroxide detection), N 3.67%, ν_{max}^{KBr} 1610–1630 cm^{-1}] and 3-deoxy-D-*erythro*-hexosulose [$R_{glucose}$ 2.6–2.9 (principal component, minor contaminants $R_{glucose}$ 1.20 and 1.75)] were prepared and purified by the method of Anet (1960b). Other carbohydrates used as internal standards for glc had physical constants in good agreement with literature values. Amino acids (Mann Research Laboratories) were used without further purification.

Nonenzymic Browning Reaction. *Reaction of D-Glucose with p-Toluidine.* A mixture of D-glucose (180 mg),

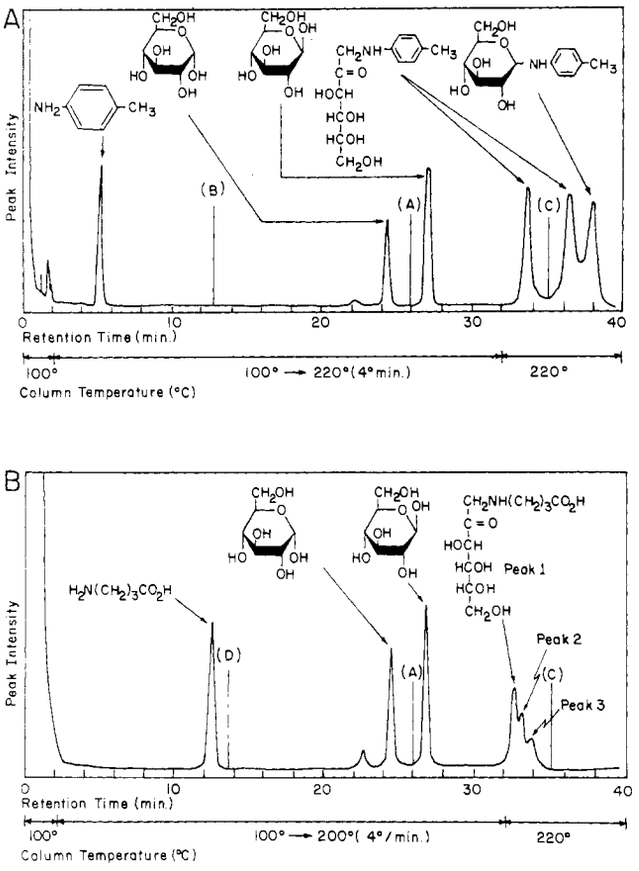

Figure 1. Gas–liquid chromatograms of initial products of the nonenzymic browning reaction between D-glucose and (A, upper trace) *p*-toluidine or (B, lower trace) 4-aminobutyric acid. Internal standards: (A) D-glucitol; (B) L-malic acid; (C) phenyl 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranoside; (D) methyl β-L-arabinopyranoside.

D-glucitol (internal standard, 60 mg), *p*-toluidine (108 mg, 1 molar equivalent to D-glucose), and acetic acid (60 mg) in *N,N*-dimethylformamide (5 ml) was heated at 100°. Aliquots of the mixture, after various times of reaction, were subjected directly to trimethylsilylation. Analytical results are shown in Figures 1A and 2 (upper left), and Table I.

Reaction of N-p-Tolyl-D-glucosylamine in the Presence of Acetic Acid (the Amadori Rearrangement). Equimolar amounts of *N*-*p*-tolyl-D-glucosylamine and acetic acid in *N,N*-dimethylformamide (0.2 *M* solution with respect to each reactant) were heated at 100° and aliquots of the mixture were subjected at various times to direct trimethylsilylation. Analytical results are shown in Figure 2 (upper right).

Reaction of D-Glucose with 4-Aminobutyric Acid. An aqueous solution (20 ml) of D-glucose (1.80 g), 4-aminobutyric acid (1.03 g, 1:1 molar ratio sugar–amine), and D-glucitol (0.600 g) was freeze dried overnight to give a white powder containing ~2.5% of water. Aliquots (10 mg) of this powder were placed in small vials and heated in an oven at either 35 or 100°. The powder turned into a brown or black syrup. These products were trimethylsilylated directly at various time intervals. Analytical results are shown in Figures 1B and 2 (lower left and lower right), and in Table I.

In preparative experiments a small amount of sodium hydrogen sulfite (0.1 mol/mol of sugar) was added to the initial reaction mixture. This procedure decreased the extent of discoloration of the reaction mixture and increased the yield of the intermediate reaction product (Anet, 1957).

Table I. Gas–Liquid Chromatographic Data on the Trimethylsilyl Derivatives of Products of Reaction of D-Glucose with Amines

Compound	Elution temp,[a] °C	Retention time, min[c]
α-D-Glucopyranose	192	24.4
β-D-Glucopyranose	204	27.0
p-Toluidine	112	5.2
4-Aminobutyric acid	148	13.5
N-*p*-Tolyl-D-glucosylamine	220	38.0
1-Deoxy-1-*p*-toluidino-D-fructose	220	33.8, 36.4
1-(3-Carboxypropyl)amino-1-deoxy-D-fructose (peak 1)[b,c]	220	32.6
Internal standards		
D-Glucitol (A)[b]	200	26.0
L-Malic acid (B)[b]	144	13.0
Phenyl α-D-galactopyranoside tetraacetate (C)[b]	220	35.0
Methyl β-L-arabinopyranoside (D)[b]	152	14.5

[a] Temperature programming: 100° (2 min), 100–200° (30 min, 4°/min), 220° (8 min); SE-30 column. For other conditions, see Experimental Section. [b] See Figure 1. [c] See Figure 2.

Reaction of D-Glucose with Amino Acids. Solutions of D-glucose (9.0 mg), D-glucitol (3.0 mg), and an α-amino acid (1 molar equiv/mol of sugar) in *N,N*-dimethylformamide (1 ml) were heated for various time intervals at 100° and aliquots were subjected to direct trimethylsilylation. Glc data on the products, at reaction times when most of the D-glucose had reacted, are given in Table II.

Trimethylsilylation. Hexamethyldisilazane, chlorotrimethylsilane, *N,O*-bis(trimethylsilyl)acetamide, *N,O*-bis(trimethylsilyl)trifluoroacetamide (Stalling *et al.*, 1968), and *N*-(trimethylsilyl)imidazole (pyridine solution, 1.5 mequiv/ml) were products from Pierce Chemical Co., Rockford, Ill. Reaction conditions evaluated in preliminary experiments with compounds described here were: 2:1 (v/v) hexamethyldisilazane–chlorotrimethylsilane (Sweeley *et al.*, 1963); *N,O*-bis(trimethylsilyl)acetamide in pyridine, *N,N*-dimethylformamide, or acetonitrile at various temperatures; *N,O*-bis(trimethylsilyl)acetamide and chlorotrimethylsilane in *N,N*-dimethylformamide at various temperatures; *N*-(trimethylsilyl)imidazole in pyridine at room temperature; *N,O*-bis(trimethylsilyl)trifluoroacetamide in acetonitrile, *N,N*-dimethylformamide, or pyridine at various temperatures; and 5:5:1 (v/v) *N,O*-bis(trimethylsilyl)acetamide–*N*-(trimethylsilyl)imidazole–chlorotrimethylsilane at room temperature.

The last noted mixture gave optimum results and was used routinely for analysis of the reaction mixtures (Figure 1) to provide data for the reaction time–composition curves presented in Figure 2: the freeze-dried sample (10 mg) or organic solution containing about 10 mg of substrates was treated with *N,O*-bis(trimethylsilyl)acetamide (0.5 ml), *N*-(trimethylsilyl)imidazole (0.5 ml), and chlorotrimethylsilane (0.1 ml) for at least 1 hr at room temperature.

Gas–Liquid Chromatography. A Beckman GC-5 instrument equipped with a flame ionization detector was used. A stainless steel column (6 ft × ⅛ in., packed with 3% SE-30 on Chromosorb G; helium flow rate, 40 ml/min) and a copper column (8 ft × ⅛ in., packed with 15% SE-52 on Chromosorb W; helium flow rate, 30 ml/min) were used. Packing materials were obtained from Varian Aerograph Co., Walnut Creek, Calif. Temperature programming as noted in Figure 1 was adopted as a general procedure. Internal standards other than D-glucitol were added immediately before introduction into the gas chromatograph.

Measurement of Color Formation. The optical densities at 490 nm of appropriately diluted aqueous solutions

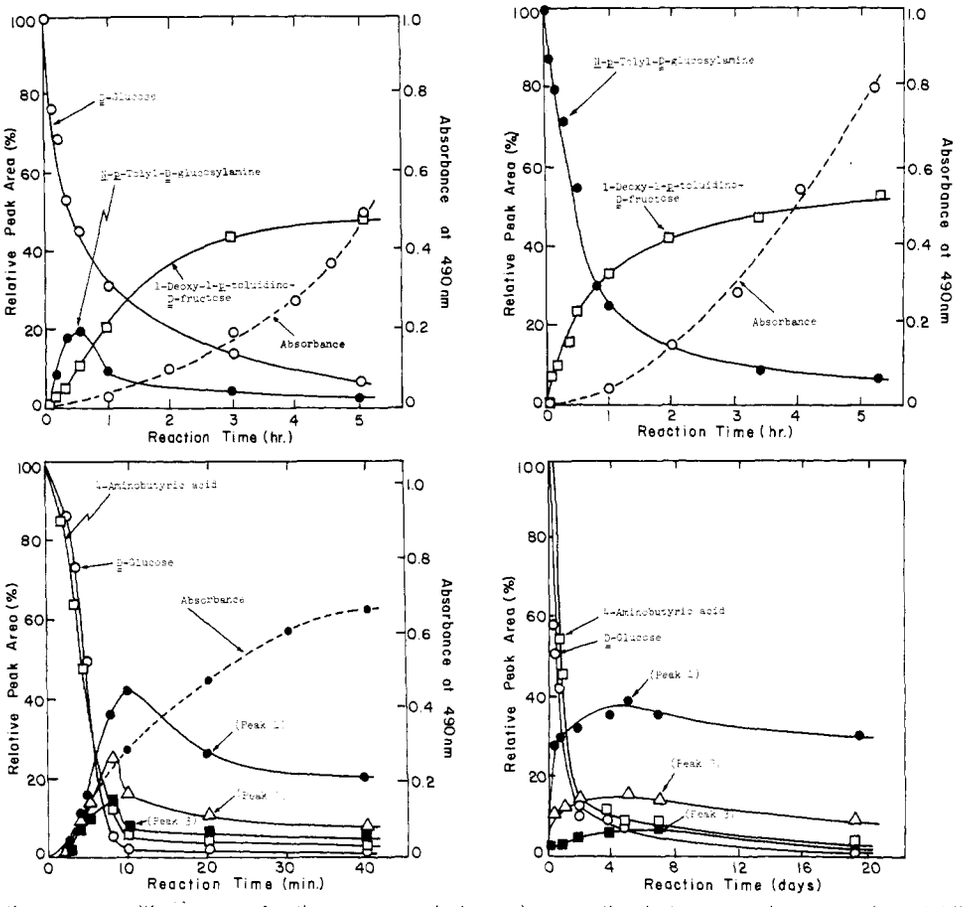

Figure 2. Reaction time–composition curves for the nonenzymic browning reaction between D-glucose and *p*-toluidine at 100° (upper left); the decomposition of *N*-*p*-toly-D-glucosylamine at 100° (upper right); the nonenzymic browning reaction between D-glucose and 4-aminobutyric acid at 100° (lower left) and at 35° (lower right). The dotted curves denote color development (absorbance at 490 nm).

of the reaction mixtures were determined in a 1-cm cell with a Beckman DU spectrophotometer, Model 4200 (see Figure 2).

Isolation of Trimethylsilyl Derivatives for Mass Spectrometry. The trimethylsilylated samples in solution were evaporated *in vacuo* and the residues were placed on top of small columns (1 × 12 cm) of silica gel (Davison, grade 950, Grace Chemical Co.) that had been wetted with benzene. Developers used were generally benzene, benzene–methanol mixtures, pyridine, and methanol, in that order, and monitoring by glc was employed to guide the pooling of appropriate fractions and to verify that the products isolated were homogeneous. Unreacted D-glucose was eluted by methanol.

Mass spectra were recorded with an AEI-MS-902 mass spectrometer equipped with a direct insertion probe. The ionizing potential was 70 eV, the accelerating potential 8 kV, and the source temperature 250°.

The isolated per(trimethylsilylated) derivatives were examined by infrared spectroscopy as well as by mass spectrometry; principal data of diagnostic value (compare Kochetkov and Chizhov, 1966; Lönngren and Svensson, 1974) are recorded in the following section.

Penta-O-trimethylsilyl-D-glucopyranose. The compound (α,β anomeric mixture) had: $[\alpha]^{23}D$ +69° (c 1, cyclohexane); ν_{max}^{film} 2940, 1395, 1250, 760, and 850 cm^{-1}; nmr data (60 MHz, chloroform-*d*) δ 4.98 (d, $J_{1,2}$ = 3.0 Hz, H-1); mass spectrum *m/e* 540 ($M^{\cdot+}$), 525 ($M^{\cdot+}$ − 15), 435 ($M^{\cdot+}$ − 15 − 90), 305, 217, 204, 191, 147, 103, 75, and 73.

N-p-Tolyltetra-O-trimethylsilyl-D-glucopyranosylamine. The compound showed: ν_{max}^{film} 2920, 1620, 1510, 1250, 840, 820, and 760 cm^{-1} (no OH bond was present); mass spectrum *m/e* 557 ($M^{\cdot+}$), 542 ($M^{\cdot+}$ − 15), 467 ($M^{\cdot+}$ − 90), 454 ($M^{\cdot+}$ − 103), 361, 348, 305, 271, 221, 217, 208, 204, 147, 117, 106, 103, 91, 89, 75, and 73.

Table II. Retention Times of Products of Reaction of D-Glucose with Amino Acids

Trimethylsilyl derivatives detected in the reaction of D-glucose with[a]	Retention times,[b] min, at column temp of	
	130–230° [c]	210°
L-Alanine	10.8, 11.2	5.2, 3.2
2-Aminobutyric acid	11.3, 12.0	5.5, 4.7
L-Isoleucine	12.8, 13.7	7.0, 8.1
L-Proline	13.0, 13.5, 15.2	7.6, 8.6, 10.4

[a] For details see Experimental Section. [b] SE-30 column. [c] 130° (3 min), 130–230° (17 min, 5.88°/min).

1-Deoxy-1-p-toluidinotetra-O-trimethylsilyl-D-fructose. The compound showed: ν_{max}^{film} 2920, 1735, 1620, 1520, 1250, 840, 810, and 752 cm^{-1}; mass spectrum *m/e* 557 ($M^{\cdot+}$), 542 ($M^{\cdot+}$ − 15), 452 ($M^{\cdot+}$ − 15 − 90), 437 ($M^{\cdot+}$ − 120), 377 ($M^{\cdot+}$ − 180), 362 ($M^{\cdot+}$ − 195), 347 ($M^{\cdot+}$ − 103 − 107), 319 ($M^{\cdot+}$ − 15 − 120 − 103), 306, 305, 271, 257, 217, 205, 204, 147, 120, 117, 106, 103, 89, 75, and 73.

1-Deoxy-1-[3-(trimethylsilyloxycarbonyl)propyl]amino-tetra-O-trimethylsilyl-D-fructose. The compound showed: ν_{max}^{film} 3300, 2920, 1750 (shoulder), 1720 (shoulder), 1685, 1670, 1420, 1250, 840, and 750 cm^{-1}; mass spectrum *m/e* 520 ($M^{\cdot+}$ − 105), 448 ($M^{\cdot+}$ − 59 − 105), 437 ($M^{\cdot+}$ − 188), 432 ($M^{\cdot+}$ − 90 − 103), 376 ($M^{\cdot+}$ − 59 − 90 − 100), 349 ($M^{\cdot+}$ − 59 − 90 − 100 − 27), 330 ($M^{\cdot+}$ − 90 − 205),

Table III. Gas–Liquid Chromatographic Determination of D-Glucose in Reaction Mixtures of D-Glucose and Amino Acids

AA (1 mol/mol of original D-glucose)	Wt % of D-glucose remaining[a]	
	Procedure A[b]	Procedure B[c]
4-Aminobutyric acid	88.7	81.5
L-Arginine free base	47.0	19.2
Glycine	97.0	98.0
L-Lysine-HCl	88.0	99.5

[a] Original amount 360 mg (see Experimental Section), relative error ±1.5%. [b] Heated for 10.5 hr at 65° in 5 ml of water under nitrogen (D-glucitol added before heating); amino acid removed by IRA-400 (OH^-) resin before trimethylsilylation. [c] Heated for 4 hr at 100° in 4 ml of water in a sealed tube.

319 ($M\cdot^+ - 188 - 103 - 15$), 314 ($M\cdot^+ - 59 - 195 - 59 - 58$), 305, 301, 288, 268, 266, 217, 204, 147, 117, 103, 89, 75, and 73.

Reaction Time–Product Composition Curves. D-Glucitol was used as the principal internal standard and a linear calibration curve of weight per cent D-glucose/weight per cent D-glucitol plotted *vs.* total peak area of D-glucose/peak area of D-glucitol was obtained over the range of relative wt % 0.1–0.9. The product composition (as relative peak areas) is expressed as peak area of product/peak area of D-glucose at zero reaction time. The peak areas were determined as peak height multiplied by peak width at half-height.

Quantitative Determination of D-Glucose. In a typical experiment (procedure A), a mixture of D-glucose (360 mg), D-glucitol (hydrate, mp 90°, 180 mg, internal standard), and an amino acid (1 molar equivalent to D-glucose) in 5 ml of water was heated for 10.5 hr at 65°. The reaction mixture was diluted with 40 ml of water and 1 ml of the diluted solution was passed through a small column of Amberlite IRA-400 (OH^-) resin and the column was washed with 20 ml of water. The eluate was then freeze dried and the further dried residue trimethylsilylated. Results are given in Table III. In additional experiments (procedure B), the heating period was 4 hr at 100° and the ion-exchange procedure was omitted. By reference to the standard, the relative error for procedure A was ±1.1% and for B 1.5%.

Isolation of 1-(3-Carboxypropyl)amino-1-deoxy-D-fructose. The reaction mixture from D-glucose and 4-aminobutyric acid (previous experiment) was decolorized twice with carbon and then placed on a column (40 × 4.5 cm) of Dowex 50-8W (H^+) resin. Elution with aqueous pyridine gave the title compound contaminated with a small amount of 4-aminobutyric acid. On paper chromatography (4:1:1 butyl alcohol–ethanol–water) it gave a spot having $R_{glucose}$ 0.72, ninhydrin and silver nitrate positive; in 4:1:1 butyl alcohol–acetic acid–water it had $R_{glucose}$ 1.48 (Anet and Reynolds (1957) gave $R_{glucose}$ 1.30). The dried effluent was trimethylsilylated and subjected to glc, when it gave the peak corresponding to peak 1 in Figure 1 (retention time 5.4 min at 220° isothermal, retention time 3.54 relative to the average retention time of trimethylsilylated α,β-D-glucose).

RESULTS AND DISCUSSION

Mixtures from the nonenzymic browning reaction of D-glucose with various amines, containing D-glucosylamines, 1-deoxy-1-(N-substituted)amino-D-fructose, and other carbohydrate transformation products, were subjected to trimethylsilylation with various reagents and the resultant mixtures were analyzed by glc on columns of methylsiloxane and methylphenylsiloxane. No single silylating reagent proved totally satisfactory, but a 5:5:1 (v/v) mixture of *N,O*-bis(trimethylsilyl)acetamide, *N*-(trimethylsilyl)imidazole, and chlorotrimethylsilane gave reproducible peaks for both reactants and products, and was the best of numerous combinations of reagents that were examined. By use of these reagents for 1 hr at room temperature, *N*-*p*-tolyl-D-glucosylamine and *N*-*p*-tolyl-D-mannosylamine (either formed *in situ* by reaction of the aldose with *p*-toluidine in the presence of acetic acid or used as isolated crystalline compounds) gave comparable and reproducible results on gas chromatographic analysis (Figure 1A and Table I). Two peaks having shorter retention times than the derivative of *N*-*p*-tolyl-D-glucosylamine were identified as the trimethylsilyl derivative of 1-deoxy-1-(*p*-toluidino)-D-fructose, as a crystalline preparation of the latter derivative also gave the same result. The two peaks presumably arise because of the opportunity for tautomerism in the sugar chain; one peak probably corresponds to a cyclic form and the other to the acyclic form.

Mass spectrometry of the trimethylsilylated glycosylamine and its Amadori rearrangement product, from the reaction of D-glucose with *p*-toluidine, indicated that the products in each instance were tetra-*O*-trimethylsilyl derivatives. The spectra of both products showed weak molecular ion peaks, together with stronger ones at $M\cdot^+ - \cdot CH_3$ and families of fragmentations that can be interpreted on the basis of established (see Kochetkov and Chizhov, 1966) schemes for fragmentation of *O*-trimethylsilyl derivatives of sugars; the assignment as a glycosylamine or as the Amadori rearranged product could readily be deduced from the spectra.

Similar reactions between D-glucose and other amines (4-aminobutyric acid, 2-aminobutyric acid, L-alanine, L-isoleucine, and L-proline), either in *N,N*-dimethylformamide solution or as freeze-dried solids (~2.5% water) likewise gave rise to peaks in the trimethylsilylated product that had retention times longer than those of the trimethylsilylated starting materials (Table II). The reaction of D-glucose with 4-aminobutyric acid was studied in detail, as the latter amino acid is a frequent constituent of fruits and vegetables (Greenstein and Winitz, 1961), is abundant in citrus fruits, and reacts with reducing sugars more readily than do the common α-amino acids (Lento *et al.*, 1958). A typical gas–liquid chromatogram of the reaction mixture is shown in Figure 1B (see also Tables I and II) at a reaction time when some of the starting materials were still present; peaks corresponding to three components, closely spaced and eluted more slowly than the reactants, can be observed. The component denoted as peak 1 in Figure 1B was shown to correspond to the trimethylsilylated Amadori rearrangement product; ion-exchange chromatographic resolution of the reaction mixture gave the Amadori product almost homogeneous by paper chromatography and upon trimethylsilylation it gave a peak congruent with peak 1 on the gas chromatogram. The mass spectrum of this product suggested that it was a penta(trimethylsilyl) derivative of 1-deoxy-1-(3-carboxypropyl)-amino-D-fructose; the molecular ion was not observed but characteristic fragmentation peaks including $M\cdot^+ - Me_3SiOH - CH_3$ were present.

Reaction mixtures in which other amino acids were used gave similar results, with several peaks having longer retention times than the reactants. The reactions were generally slower, except in the case of arginine (Table III). As with 4-aminobutyric acid, the principal intermediate observed appeared to be the 1-deoxy-1-(N-substituted)amino-D-fructose rather than the glycosylamine that is presumably formed initially. The isolated trimethylsilyl derivatives were less stable than those from D-glucose and *p*-toluidine or 4-aminobutyric acid.

In addition to providing a useful method for examining the products of sugar–amine reactions, this gas–liquid chromatographic technique also affords a convenient procedure for rapid analytical monitoring of the browning reaction, both in model systems and in food products, by

quantitative assay of the sugar and amine concentration as a function of progress of the reaction. The amines used give reproducible gas chromatographic peaks having retention times much shorter than D-glucose (Table I). Two stable and reproducible peaks, corresponding to penta-*O*-trimethylsilyl-α- and -β-D-glucopyranose, were given by D-glucose, and by use of an internal standard (Alexander and Garbutt, 1965) of D-glucitol it was possible to quantitate the disappearance of D-glucose with a reproducibility of $\pm 1.5\%$ (Table III).

Some illustrations of the application of this technique are shown in the reaction time–composition curves shown in Figure 2, which present the course of the initial stages of the Maillard reaction between D-glucose and *p*-toluidine or 4-aminobutyric acid, together with color development (absorbance at 490 nm). Over a 5-hr period at 100°, some 95% of the D-glucose in the D-glucose–*p*-toluidine system had reacted and the principal product detected was 1-deoxy-1-*p*-toluidino-D-fructose; the glycosylamine formed rapidly initially reached a maximum after 0.5 hr but was also transformed rapidly into the rearranged product. Similar heating of the latter, *N*-*p*-tolyl-D-glucosylamine (in the presence of acetic acid), and analysis of the product showed the progress of the Amadori rearrangement with disappearance of one-half of the glycosylamine after ~0.5 hr and concomitant formation of 1-deoxy-1-*p*-toluidino-D-fructose. The concentration of the latter did not exceed 40–50%, presumably because of further decomposition with progress of the browning reaction; the plot of color formation provides an index of the generation of final browning products. Similar behavior for the disappearance of reactants is observed in the reaction between D-glucose and 4-aminobutyric acid. The reaction is much faster than that with *p*-toluidine; at 100° >90% of the reactants had disappeared after 10 min and the Amadori rearrangement product [1-deoxy-1-(3-carboxypropyl)amino-D-fructose, peak 1] attained a maximum after 10 min and decreased thereafter, with progressive development of color in the reaction mixture. The *N*-alkylglycosylamines rearrange more readily than do the *N*-aryl analogs, and the anticipated initial glycosylamine was not identified among the reaction products. In addition to peak 1, two other components (peaks 2 and 3) were observed; their concentration reached a maximum after ~8 min and decreased thereafter. These products remain to be identified. The same reaction, performed at 35°, showed loss of ~90% of the original D-glucose and 4-aminobutyric acid after 2 days.

The conditions of the glc procedure described here did not give well-defined reproducible peaks with "difructoseglycine" (Anet, 1960b) nor with 3-deoxy-D-*erythro*-hexosulose (Anet, 1960b; Kato, 1960), so that additional analytical procedures will be required if the formation and disappearance of these later intermediates in the browning reaction are to be monitored. The glc procedure of El-Dash and Hodge (1971) for 3-deoxy-D-*erythro*-hexosulose was not available at the time this work was performed.

The present technique can be used to examine the sugar–amine reactions in model browning reactions and in actual food products, and also to examine the effect on the browning reaction of such agents as sulfites, ascorbic acid, and carbonyl compounds; the results of such studies with special reference to flavor deterioration of dried citrus juices, will be the subject of a separate report.

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