Food Chemistry **33** (1989) 81–91

Intramolecular Cyclizations of Lysine Amadori Rearrangement Products

Varoujan Yaylayan

Department of Food Science and Agricultural Chemistry,
Macdonald College of McGill University,
21 111 Lakeshore Road, Ste. Anne de Bellevue,
Quebec, Canada H9X 1C0

&

Peter Sporns

Department of Food Science, University of Alberta,
Edmonton, Alberta, Canada T6G 2P5

(Received 29 July 1988; revised version received and accepted 30 September 1988)

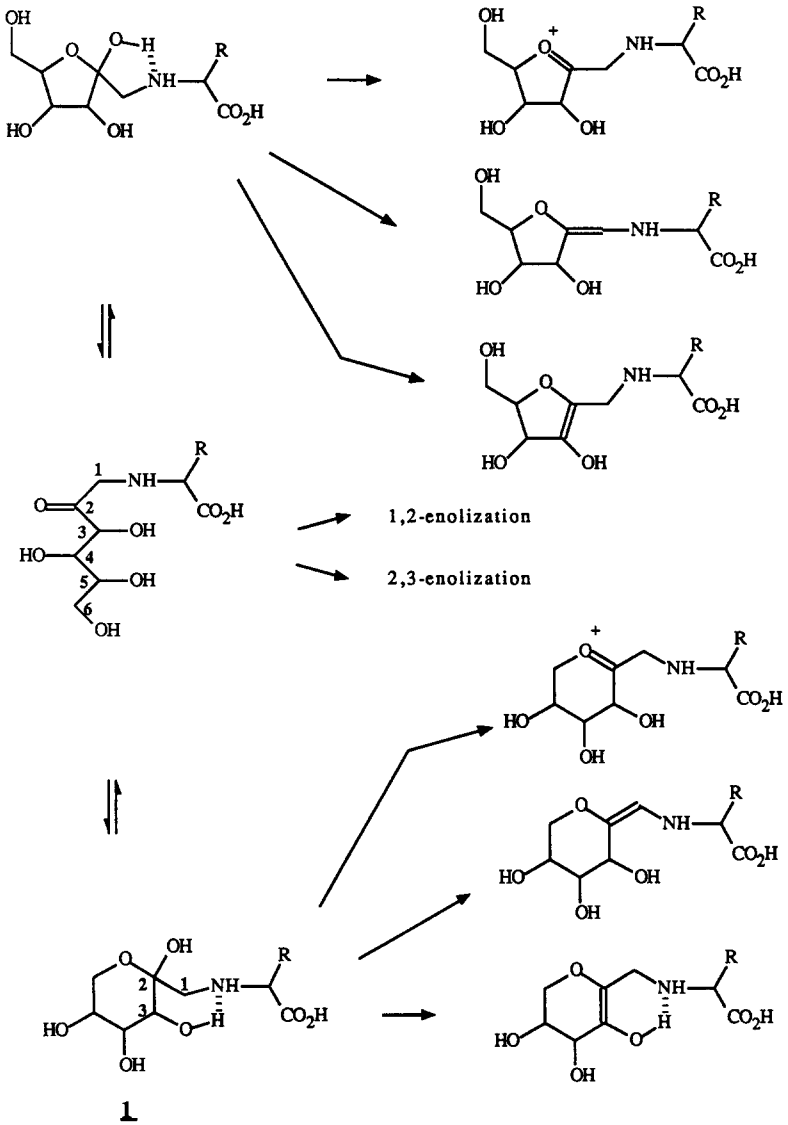
ABSTRACT

Based on the high resolution electron impact mass spectrometric studies of lysine Amadori rearrangement products and the fragments identified in their mass spectra, the effect of mono and di-glycosylation on the fragmentation pathways was studied. According to this study monoglycosylated lysines having a free amino group decompose by intramolecular cyclization reactions leading to six, five and four membered nitrogen-containing rings, whereas, disubstituted lysine decomposes through elimination of the sugar moieties with the production of pyrans and sugar fragments.

INTRODUCTION

The established importance of the decomposition products of 1-(amino acid)-1-deoxy-D-fructoses or Amadori rearrangement products (ARPs, **1**, in Scheme 1) in food (Nursten, 1980) and biological systems (Monnier *et al.*, 1984), led us to investigate their fragmentations under high resolution electron impact conditions (Yaylayan & Sporns, 1988). Since EIMS

Food Chemistry 0308-8146/89/$03·50



Scheme 1.

fragmentations are based on ground state solution chemistry, as supported by a wide body of experimental evidence (Budzikiewicz *et al.*, 1967), the fragmentation schemes obtained could be extrapolated to actual decompositions of Amadori products taking place in food systems, at high temperatures. Such extrapolations have been carried out and published elsewhere (Yaylayan & Sporns, 1987).

ARPs are the key intermediates in the non-enzymatic interaction of reducing sugars with amino acids. The process is called non-enzymatic

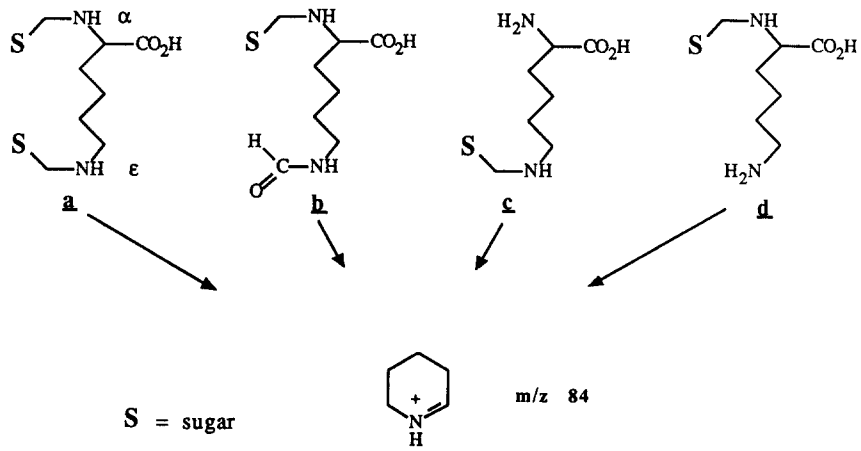
browning or the Maillard reaction (Hodge, 1953). With time, ARPs are decomposed to form various heterocyclic compounds, with important flavor characteristics, and intermediates that can cross-link proteins in food as well as *in vivo*, thus leading to the toughening of food products and, in the case of biological systems, the stiffening of tissue (joint stiffness, atherosclerosis, etc.), symptoms associated with ageing (Monnier *et al.*, 1984).

Some of the reactive intermediates formed from the decomposition of ARPs, can also undergo polymerizations leading to brown polymers. In biological systems, it has been shown that the pigmented cross-links in human senile cataracts have brownish color and fluorescent characteristics similar to that of food polymers (Monnier *et al.*, 1984).

The importance of the decomposition of Amadori products is not restricted to flavor, color and polymer production alone but extends to the formation of carcinogens, antioxidants and, in the case of tryptophan, to the formation of psychoactive compounds such as β-carbolines.

Lysine can form three different Amadori rearrangement products with glucose (Scheme 2); one at the C-2 nitrogen (**d**) the other at the C-6 nitrogen (**c**) and the third at both positions simultaneously (**a**).

Lysine, being the most reactive essential amino acid during the Maillard reaction, is readily destroyed or deactivated, during processing of foods, through the formation of Amadori rearrangement products; the acid



	% Int.	% Σ
a	3.35	1.25
b	4.81	1.30
c	100	19.26
d	100	24.18

Scheme 2.

hydrolysis of *N*-epsilon fructosyl lysine produces indicator compounds such as furosine and pyridosine; furosine can be easily detected on the basic column of the amino acid analyzer where it appears after arginine (Finot & Mauron, 1972). The peptide-bound lactolusyl-lysine in milk powders was subsequently found on acid hydrolysis to consistently give 40% of its lysine content as lysine and 32% as furosine. This finding formed the basis of the furosine test for Maillard damage to milk powders (Bujard & Finot, 1978).

The facile formation of Amadori products of lysine in food necessitates the study of their decomposition products and the elucidation of their structures since some of these compounds contribute to the color, flavor and safety of the food products.

In this paper, based on the high resolution EIMS studies and the intermediates identified in the mass spectra, we propose possible mechanistic pathways of decomposition of lysine ARPs and the effect of the substituents on the decomposition products

MATERIALS AND METHODS

Instrumentation

The high resolution electron impact mass spectra of ARPs were determined on an Associated Electrical Industries (AEI, Manchester, England) MS-50, high performance double-focusing mass spectrometer with Nier-Johnson geometry. The ionization energy was 70 eV and the peak measurements were made by comparison with perfluorotributylamine at a resolving power of 15 000. The temperature in the ion source was varied between 150–250°C depending on the volatility of the particular compound. The samples were introduced directly in the ion source (quartz probe) through a vacuum lock system; the pressure inside was 2×10^{-7} torr and the accelerating voltage was 8000 V.

The data were analyzed using a DS-55 (Kratos), a computer-based data acquisition and analysis system for mass spectrometry. The system consisted of a minicomputer with a custom, high speed data acquisition interface and a set of programs for collecting, analyzing and reporting mass spectrometric data. The structures of fragment ions were determined, according to mass spectrometric ion-fragmentation mechanisms explained in detail by McLafferty, 1980).

Synthesis of the ARPs

Details of the ARP synthesis are given elsewhere (Yaylayan & Sporns, 1987).

RESULTS AND DISCUSSION

One of the consequences of our systematic study of 18 Amadori rearrangement products (ARPs) by high resolution EIMS was the proposition of a novel mechanism for their decomposition.

According to this mechanism (Scheme 1) (Yaylayan & Sporns, 1987), acyclic Amadori products may decompose by enolization at two positions, 1,2- and 2,3- whereas cyclic forms decompose by 1,2- or 2,3-dehydrations and O,2-dehydroxylation at the anomeric hydroxyl group. O,2-dehydroxylation can be initiated in the mass spectrometer by the formation of the molecular ion at the ring oxygen and in food systems by highly polar conditions. On the other hand, 1,2-dehydrations might be initiated in the mass spectrometer by the formation of the molecular ion in the amino acid nitrogen due to the electron withdrawing effect of the positively charged nitrogen atom. However, the 1,2-dehydration product would probably isomerize into the more stable 2,3-dehydration product in the pyranose form, to restore the six-membered ring hydrogen bond. In the mass spectrometer, amino acid nitrogens with relatively low ionization potentials and protonated amino acids species might prefer the dehydration paths relative to other ARPs.

Many of the decomposition products of ARPs cannot be explained by the classical, 1,2- and 2,3-enolization mechanisms; however, dehydration and dehydroxylation mechanisms of the cyclic forms can shed light on the formation of some of these compounds.

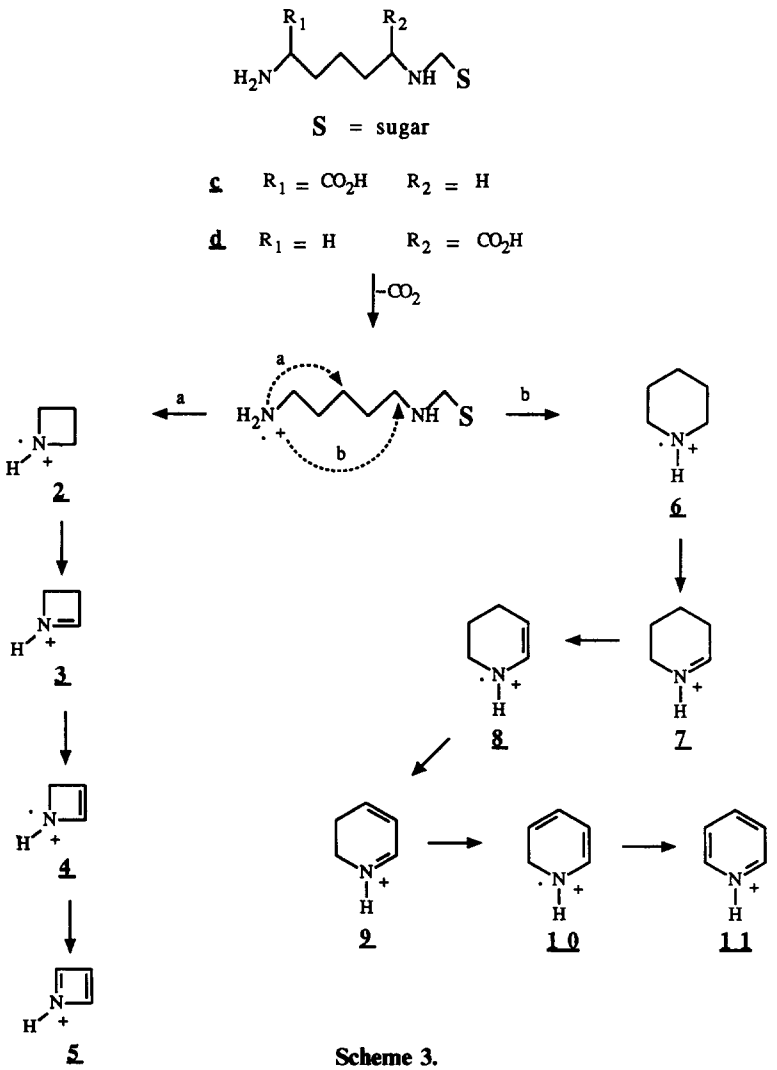
Intramolecular cyclization reactions of lysine ARPs

The four derivatives of lysine studied by high resolution EIMS are shown in Scheme 2.

Unlike other amino acids, lysine has two potential sites to react with a reducing sugar thus forming two monofructosyl (**c** and **d**) and one difructosyl (**a**) derivatives (ARPs) (Scheme 2). The epsilon nitrogen is more reactive than alpha, hence, in food systems *N*-epsilon-substituted monofructosyl ARP (**c**) will form first and then the difructosyl derivative (Kato *et al.*, 1982); the *N*-alpha substituted ARP can only be formed by synthesis since it requires selective protection of the more reactive nitrogen.

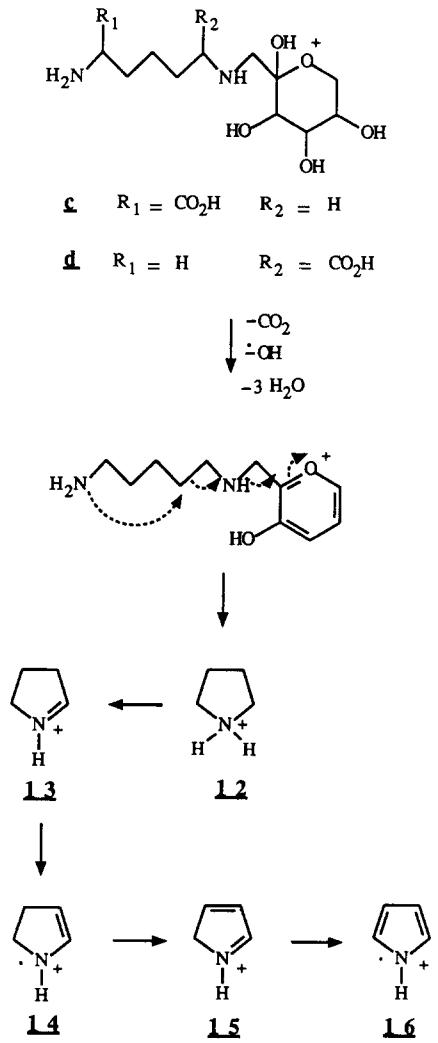
The rate of formation of the di- and mono-fructosyl derivatives can greatly affect the nature of the subsequent high temperature degradation products; as will be shown below, the mono-substituted ARPs tend to produce nitrogen-containing heterocyclic compounds (pyrroles, pyridines, piperidines and azetidine derivatives), whereas the difructosyl derivative produces mainly sugar fragments and substituted pyran derivatives. The

reason for this difference is the fact that the nitrogen heterocyles are produced by the intramolecular cyclization reactions of the free amino group of monofructosyl derivatives whereas a similar cyclization is greatly hindered by the presence of the bulky sugar residue on the C-6 nitrogen, or by deactivation of the same nitrogen with formylation. The ability of each derivative to undergo cyclization reactions was measured by the relative intensity of the piperidinium cation produced at *m/z* 84·0816; this ion was chosen since it is the most stable cyclization product (six-membered ring vs. four or five-membered) and because this particular compound has been isolated from the reaction mixture of lysine and glucose (Miller *et al.*, 1984).



Scheme 3.

The two derivatives shown in Scheme 2, having free amino groups (**c** and **d**), undergo extensive intramolecular nucleophilic substitution reactions producing six, five and four membered heterocyclic compounds (Schemes 3 and 4). The free amino group can cyclize by nucleophilic attack either at C-4 producing azetidine derivatives or at C-6 producing piperidine derivatives, in both cases two stable neutral species are also produced (Scheme 3; see also Table 1). A third nucleophilic substitution reaction is possible at C-5 followed by two concerted *trans*-elimination reactions shown in Scheme 4 (see also Table 2); this cyclization produces pyrrolidine derivatives. The



Scheme 4.

TABLE 1
Ions Identified in Scheme 3

Compound	*m/z*	*% Base peak*	$\%\Sigma_{50}$[a]
2c	57·055 4	1·65	0·32
2d	57·059 8	7·19	1·74
3c	56·052 2	57·91	11·15
3d	56·052 0	67·65	16·36
4c	55·044 0	14·61	2·81
4d	55·044 1	7·41	1·78
5c	54·036 1	11·27	2·17
5d	54·035 7	6·93	1·67
6c	85·086 2	7·32	1·41
6d	85·086 1	5·74	1·39
7c	84·081 6	100·0	19·26
7d	84·081 7	100·0	24·18
8c	83·073 7	15·93	3·07
8d	83·073 7	10·94	2·64
9c	82·065 8	14·39	2·77
9d	82·066 0	12·84	3·10
10c	81·057 6	1·44	0·27
10d	—	—	—
11c	80·050 3	4·37	0·84
11d	—	—	—

[a] % of the total ion current of all the peaks greater than 50 atomic mass units.

TABLE 2
Ions Identified in Scheme 4

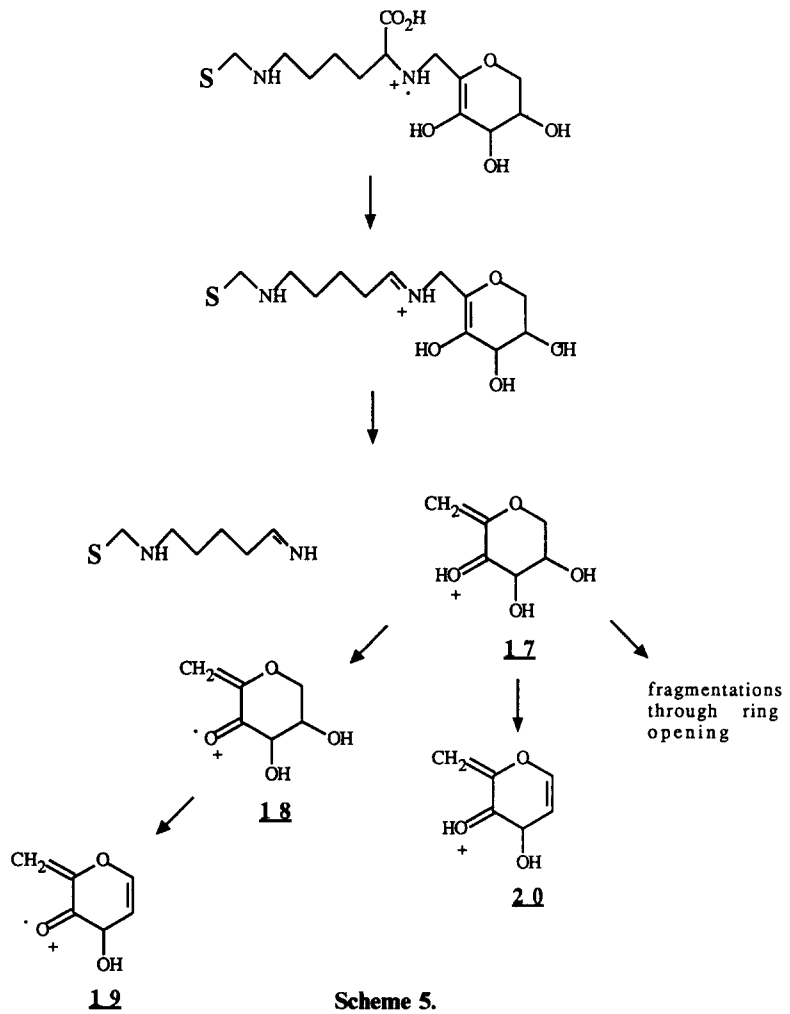
Compound	*m/z*	*% Base peak*	$\%\Sigma_{50}$[a]
12c	72·081 7	37·49	7·22
12d	72·081 8	69·29	16·75
13c	70·065 7	7·07	1·36
13d	70·065 6	7·94	1·92
14c	69·058 1	3·09	0·59
14d	69·058 1	4·37	1·06
15c	68·050 8	5·63	1·08
15d	68·050 6	2·56	0·62
16c	67·043 1	2·31	0·44
16d	—	—	—

[a] % of the total ion current of all the peaks greater than 50 atomic mass units.

driving force for this reaction is the neutralization of the positive charge on the oxonium ion and the loss of two stable molecules.

When the two amino groups are glycosylated, the bulky sugar residues prevent the intramolecular cyclization reactions and instead the sugars undergo *ortho* elimination and fragmentations through ring opening producing sugar fragments and pyran derivatives (Scheme 5; see also Table 3).

In considering therefore the type of products obtainable from the degradation of lysine Amadori products, especially for flavor effects, monofructosyl derivatives should be considered if nitrogen-containing heterocycles are desired, and the difructosyl derivative should be considered if oxygen-containing products are desired.



Scheme 5.

TABLE 3
Ions Identified in Scheme 5

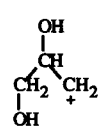
Compound	*m/z*	*% Base peak*	$\%\Sigma_{50}$[a]
17a	145·045 6	1·28	0·48
17b	145·045 5	1·22	0·33
18a	144·042 4	20·68	7·74
18b	144·042 4	19·51	5·25
19a	126·032 0	3·51	1·31
19b	126·033 1	1·24	0·33
20a	127·038 5	0·88	0·33
20b	—	—	—

[a] % of the total ion current of all the peaks greater than 50 atomic mass units.

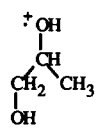
One fact worth mentioning in this respect is the behaviour of the formylated derivative (**b**); the open-chain conformation of the sugar predominates in this product, contrary to all other ARPs, giving rise to unique peaks at *m/z* 75 and *m/z* 76 (see Scheme 6); these two peaks are completely absent not only from the other three derivatives of lysine but also from all other mass spectra of ARPs.

This phenomenon can be explained by analogy to the catalytic effect of 1-hydroxypyridine on mutarotation of sugars (Lemieux *et al.*, 1971); the 1-hydroxypyridine can act as an acid through the hydroxyl group and as a base through the nitrogen atom and thus assists in the opening of the sugar ring. The tautomeric equilibrium between the hydroxyimine and the formyl amine, can catalyze, like 1-hydroxypyridine, the mutarotation of the sugar which is attached to it. A model of the molecule shows the proximity of the hydroxyimine to the catalytic site.

The high temperature decompositions therefore of lysine ARPs are greatly influenced by the substituents on the C-6 nitrogen; decompositions of lysine ARPs having a free amino group are characterized by intramolecular cyclization reactions whereas disubstituted ARPs mainly exhibit reactions leading to the elimination of the sugar moiety.



m/z 75.0454 (65%)



m/z 76.0531 (32%)

Scheme 6.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge funding for this research from the Natural Sciences and Engineering Research Council (NSERC) of Canada.

REFERENCES

Budzikiewicz, H., Djerassi, C. & Williams, D. H. (1967). *Mass Spectrometry of Organic Compounds.* Golden-Day Inc., San Francisco, CA.

Bujard, E. & Finot, P. A. (1978). Mesure de la disponibilite et du blocage de la lysine dans les laits industriels. *Ann. Nutr. Alim.*, **32**, 291–305.

Finot, P. A. & Mauron, J. (1972). Le blocage de la lysine par la reaction de Maillard II. Proprietes chimiques des derives *N*-(desoxy-1-D-fructosyl-1) et *N*-(desoxy-1-D-lactolusyl-1) de la lysine. *Helv. Chim. Acta.*, **55**, 1153–64.

Hodge, J. E. (1953). Chemistry of browing reactions in model systems. *J. Agric. Food Chem.*, **1**, 928–43.

Kato, H., Nakayama, T., Sugimoto, S. & Hayase, F. (1982). Volatile and nonvolatile Maillard reaction products between L-lysine and D-glucose. *Agric. Biol. Chem.*, **46**, 2599–600.

Lemieux, R. U., Anderson, L. & Conner, A. H. (1971). The mutarotation of 2-deoxy-β-D-erythropentose. Conformations, kinetics and equilibria. *Carb. Res.*, **20**, 59–72.

McLafferty, F. W. (1980). *Interpretation of Mass Spectra.* University Science Books, Mill Valley, CA.

Miller, R. Olsson, K. & Pernemalm, P-A. (1984). Formation of aromatic compounds from carbohydrates. IX. Reaction of D-glucose and L-lysine in slightly acidic, aqueous solution. *Acta Chemica Scandinavica B*, **38**, 689–94.

Monnier, V. M., Kohn, R. R. & Cerami, A. (1984). Accelerated age-related browning of human collagen in diabetes mellitus. *Proc. Natl. Acad. Sci. USA.* **81**, 583–87.

Nursten, H. E. (1980). Recent developments in studies of the Maillard reaction. *Food Chem.*, **6**, 263–77.

Yaylayan, V. A. & Sporns, P. (1987), Novel mechanisms for the decomposition of 1-(amino acid)-1-deoxy-D-fructoses (Amadori compounds): a mass spectrometric approach. *Food Chem.* **26**, 283–305.

Yaylayan, V. A. & Sporns, P. (1988). EI mass spectra of 1-(amino acid)-1-deoxy-D-fructoses. *Org. Mass Spectrom.* **23**, 849–50.