THERMAL DEGRADATION OF 1-AMINO-1-DEOXYKETOSES AND THEIR ROLE IN FLAVOUR DEVELOPMENT

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by
Edward John Birch

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ABSTRACT

Sugars undergo caramelisation reactions at relatively high temperatures but when amino compounds are present, Maillard browning reactions are possible and these occur under less severe conditions. The reaction conditions and the basic character of the amino compounds determine the range of flavour compounds formed. The first step during Maillard browning is the condensation of a reducing sugar with an amine to form a glycosylamine and this compound may then undergo the Amadori rearrangement to form a 1-amino-1-deoxyketose.

The pyrolysis of two 1-amino-1-deoxyketoses (1-deoxy-1-glycino-D-fructose and 1-β-alanino-1-deoxy-D-fructose) was studied in this investigation to examine their participation in a low energy route to aroma formation. Thermal analysis and parallel chemical investigations showed that the formation of these Amadori compounds facilitates the thermal degradation of their sugar and amino acid moieties. In addition increased quantities of various aroma compounds are produced, compared with the controls. In particular, the toxic compound protoanemonin is formed and a degradation pathway leading to its production is proposed.

Most of the work involving the elucidation of degradation pathways during Maillard browning have involved studies in aqueous systems. Browning reactions between glucose and amino acids were also observed during heating in the dry-state in this study. These reactions are very vigorous once initiated and this precluded the study of a glucose plus amino acid control by the techniques used to study the pyrolysis of the 1-amino-1-deoxyketoses. Such reactions occur

at temperatures below those required for the thermal degradation of the corresponding Amadori rearrangement compound thus questioning the involvement of these compounds in the lowest energy thermolysis pathway in the absence of moisture. The results of experiments designed to investigate the role of Amadori compounds during the browning of sugar-amino acid systems in the dry-state demonstrated however that the reactions reported to occur in aqueous systems can also account for the dry-state processes at temperatures up until the spontaneous decomposition of the 1-amino-1-deoxyketose can occur. That the 1-amino-1-deoxyketose does not brown by itself or in the presence of glucose as readily as a glucose plus amino acid system is presumably a basicity effect. The stronger base (the amino acid) may promote a solid-state enolisation of the glucose and hence initiate browning at a somewhat lower temperature. The results of these experiments also demonstrate the stability of the 1-amino-1-deoxyketoses and show that their formation is not a rate-limiting step during browning.

In the third section of this thesis the effect of changing the amine moiety on the degradation pattern of 1-amino-1-deoxyketoses is assessed. Previous research has indicated that glucose by itself and Amadori compounds formed from weak primary bases degrade via an initial 1,2 enolisation step to form mainly 2-furaldehydes and pyrrole derivatives while 1-amino-1-deoxyketoses containing a strong basic moiety (usually formed from a secondary base) degrade via a 2,3 enol intermediate and give rise to fragrant caramel aroma compounds. Several 1-amino-1-deoxyketoses were prepared using primary and secondary bases covering a range of pkb values. These compounds were pyrolysed and their decomposition characteristics monitored by thermal analysis methods. Parallel

analysis of the volatiles produced and a comparison of the results from previous investigations generally endorsed the reported hypotheses on the degradation of Amadori compounds. It was found that the structure of the base and functional groups present influenced the degradation phenomena as well as the basicity. The thermal decomposition of amino acid - derived Amadori compounds for instance, did not fit into the pattern of that observed for 1-amino-1-deoxyketoses derived from other bases. The amino acid influences the degradation traits by promoting 1,2 enolisation and charring rather than aiding 2,3 enolisation similar to bases of comparable pkb.

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INTRODUCTION

I.1. THE INVOLVEMENT OF AMADORI COMPOUNDS IN NON-ENZYMIC BROWNING - A REVIEW

I.1.1 The nature of non-enzymic browning

The characteristic appearance and flavour of a food depends not only on natural or added synthetic compounds but also on the compounds formed during the process of converting the food into a form acceptable for consumption. Enzymatic, chemical and physical changes are possible during each stage of processing and any of these reactions can have a favourable or unfavourable influence on the colour, taste, odour or texture of the final product.

Non-enzymic browning reactions arise during the processes of caramelisation, toasting, baking, roasting, concentration and storage of food and originate from carbonyl compounds or those compounds which yield carbonyl derivatives on degradation. Many comprehensive reviews have appeared on the subject in recent years (1-9) which recognize three mechanisms of non-enzymic browning. One of these, ascorbic acid oxidation, is oxygen dependent and since it is not a significant process when foods are heated, will not be discussed further. The other two processes are caramelisation and Maillard (10) browning which typically involve sugars as the source of carbonyls.

I.1.2 The chemistry of non-enzymic browning

I.1.2.1 Caramelisation: Caramelisation occurs when sugars are heated above their melting points, under low moisture conditions, in the absence of amino acids and proteins (6). Relatively high temperatures (11) are involved which promote a series of colour changes from colourless through amber to dark brown with consequent

changes in aroma ranging from sweet to fragrant caramel and finally to bitter acrid notes (4).

A wide variety of products from the pyrolysis of carbohydrates have been identified (11), but it seems that the carbohydrate source is not important (12). The following series of reaction steps for the thermal decomposition of glucose (4) can thus be generalised for most carbohydrates:

(i) Enolisation to produce the more reactive 2-ketoses

(ii) dehydration of the ketose without fission to 5-hydroxymethyl-2-furaldehyde

(iii) hydrolytic fission of 5-hydroxymethyl-2-furaldehyde or its intermediate precursors to give formic and levulinic acids

(iv) further reaction involving bond-fission, dehydration, dismutation, condensation, reversion, dimerisation and polymerisation of any of the key intermediates or their products.

1.1.2.2 Maillard browning: When sugars are heated in the presence of amino compounds, carbonyl-amino browning reactions occur. These are initiated more easily than caramelisation reactions under the same conditions (3). Maillard browning is both more rapid and more intense than caramelisation and yields a similar range of carbohydrate-derived products in addition to nitrogen containing compounds important in food flavour (9). Three pathways to flavour and brown pigment formation have been defined starting from an initial condensation reaction between a reducing sugar and an amine to yield an N-substituted glycosylamine (Fig. I.1) which isomerises to give a 1-amino-1-deoxy-2-ketose or Amadori (13) compound. The Amadori compound may rearrange via a 1,2 or 2,3 enol intermediate to initiate

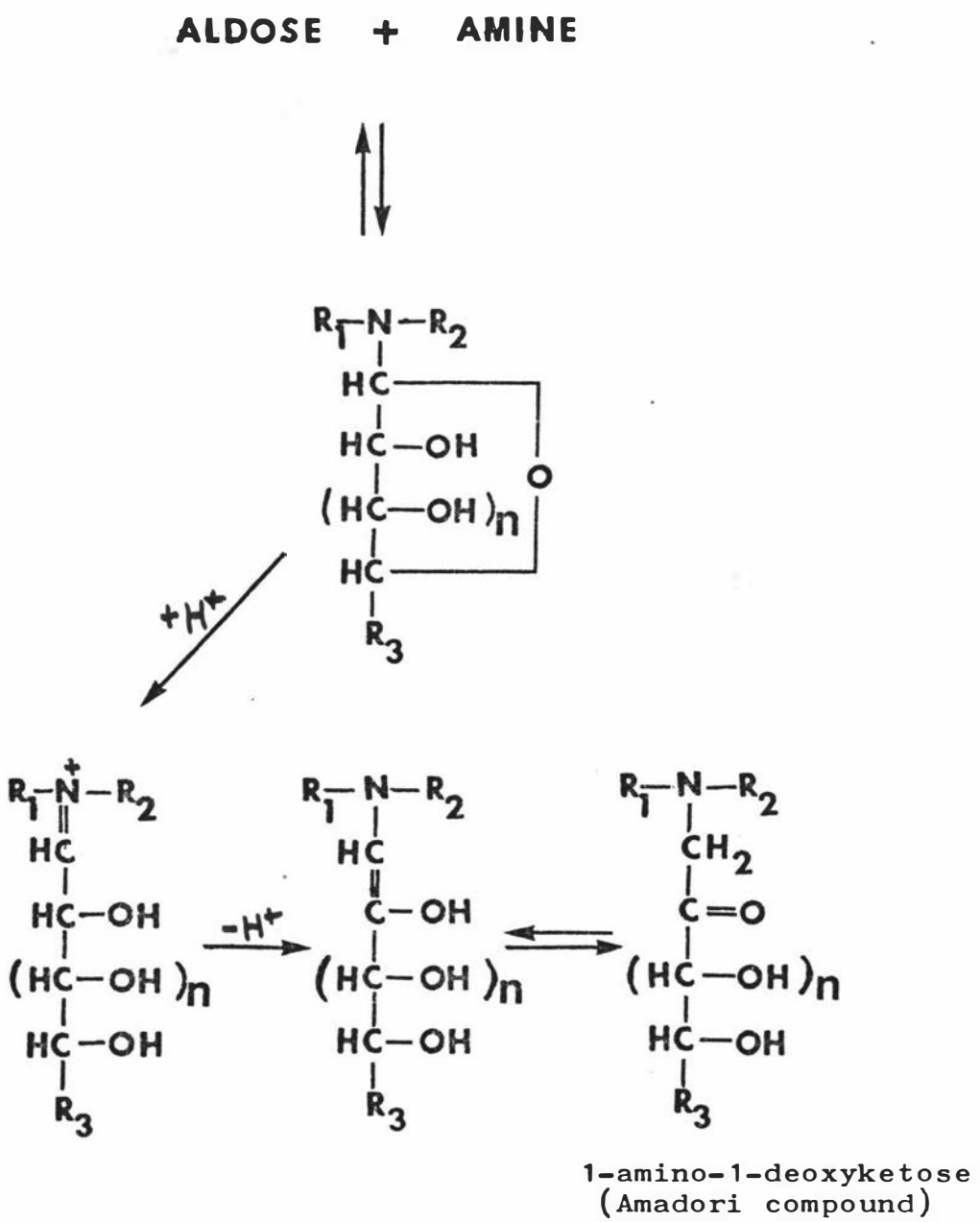


Fig I.1 Formation of Amadori compounds

two distinctive degradation pathways. The third pathway arises through reaction between α-dicarbonyl compounds produced by the first two pathways and α-amino acids by a Strecker degradation (14). These degradation pathways are discussed in the reviews (1-9).

1.1.3 Evidence for the involvement of Amadori compounds in Maillard browning

Many browning experiments have been conducted in aqueous solution at elevated temperatures using conditions similar to those of Maillard (10). These conditions lead to a large number of compounds which may be intermediates or merely products of side reactions. Under low moisture conditions sugars and amino acids brown to yield relatively simple mixtures (15) and 1-(N-amino acid)-1-deoxy-D-fructoses can be isolated from these (16). Hodge (3) integrated the early browning reaction theories and affirmed the involvement of 1-amino-1-deoxyketoses in carbonyl-amino browning reactions. Since then many Amadori compounds have been prepared (1-9, 16-21) and isolated from natural sources (22-24).

It has been shown that Amadori compounds derived from primary amines are able to condense with a further aldose molecule to form a diketoseamine (25, 26). Di-D-fructoseglycine (26) decomposes very readily to regenerate the monoketoseamine. This mechanism explains the conversion of aldoses to more reactive compounds during browning where the carbohydrate is present in a large excess, thus explaining the catalytic role of amines in browning reactions (7).

The browning of 1-deoxy-1-glycino-D-fructose, by itself, has been assessed under low moisture conditions and compared with the browning of other proposed

browning precursor compounds (7, 27, 28). These studies support the involvement of Amadori compounds as the first major intermediate in the non-enzymic browning reaction, although, in a kinetic analysis of the reaction between glucose and glycine (29) and a summary of the available evidence on browning (28), the completeness of current browning reaction schemes has been questioned. The suggested scheme of Spark (28) indicates the complexity of likely reactions involved during Maillard browning (see Fig. I.2).

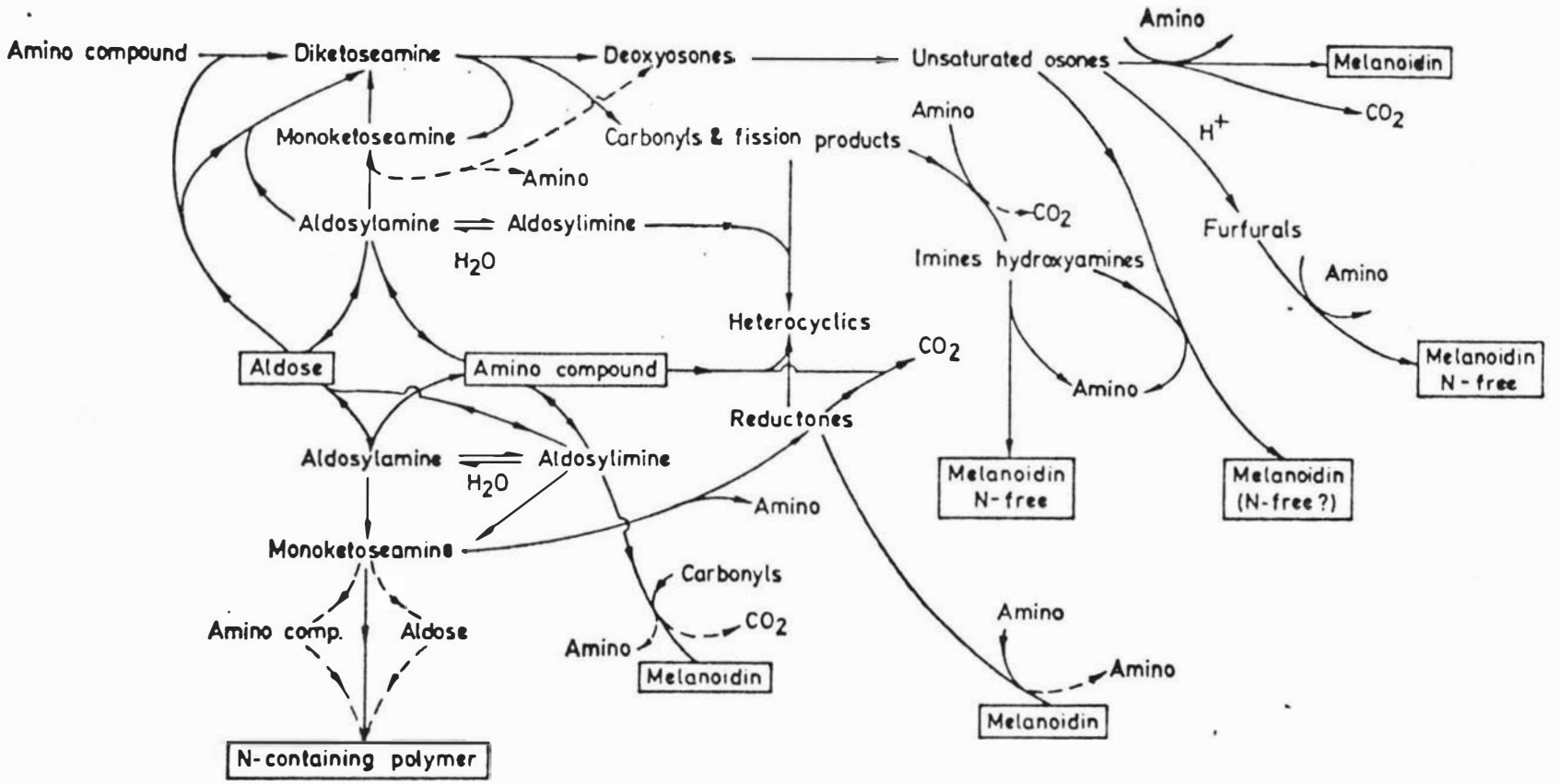
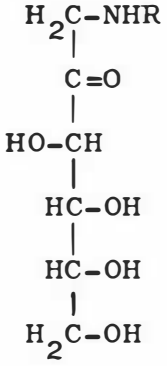


Fig I.2 Suggested scheme for the production of melanoidins in an aldose-amino system.

I.2 SCOPE OF THIS INVESTIGATION

Recent studies on the pyrolysis of Amadori compounds (19-21) support their involvement as precursors of organoleptically important volatiles in thermally processed foods. Despite the significance of Amadori compounds in non-enzymic browning however, there appears to be little information to show that 1-amino-1-deoxyketoses lie on a low energy route to volatile formation when sugars are heated above their melting points in the presence of amino compounds. Part A of this investigation discusses the pyrolysis of 1-deoxy-1-glycino-D-fructose (i) and 1-β-alanino-1-deoxy-D-fructose (ii) with reference to the question of a low-energy pathway to aroma formation and reports on the individual degradation products obtained and their likely routes of formation.



(i) R= $-CH_2COOH$

(ii) R= $-CH_2CH_2COOH$

Although it has been demonstrated that the formation of a 1-amino-1-deoxyketose represents the first step in the browning reaction between aldoses and amino acids in solution (3), this may not be the case when they are heated together in the dry-state at elevated temperatures. Furthermore the formation of an Amadori compound may not provide the lowest energy route to product formation. In Part B, the role of

the 1-amino-1-deoxyketose intermediate in the browning reaction between glucose and β-alanine in the solid-state is assessed.

The two accepted routes of decomposition of Amadori compounds, 1,2 and 2,3 enolisation, involve early elimination of the 1-amino group followed by the formation of labile carbonyl compounds whose further degradation and interaction determine the pattern of volatiles derived from the carbohydrate moiety. 2-Furaldehydes and pyrroles are distinctive end-products of the 1,2 enolisation pathway while the operation of the 2,3 enolisation pathway typically yields caramel compounds (4). It has been suggested that the extent to which 1,2 enolisation is favoured over 2,3 enolisation is a function of the basicity of the amine (6). The pyrolysis of six further Amadori compounds in Part C of this research adds to the conclusions of previous investigators.

I.3 EXPERIMENTAL APPROACH FOR STUDYING THE THERMAL DEGRADATION OF CARBOHYDRATES.

The use of thermal analysis methods combined with parallel chemical investigations has been widely applied to study the thermal degradation of sugars (30-34). The approach involves employing thermal analysis techniques to gain information on the characteristics of physical transformations of the sample and a general pattern of its succeeding degradation during pyrolysis. Subsequent chemical investigations allow an evaluation of the nature of the degradative reactions occurring.

In this study differential scanning calorimetry (d.s.c., (35)) was employed to investigate the phenomena of melting and to determine the temperature range over which degradation occurred. Thermogravimetric analysis (t.g., (36)), combined with derivative thermogravimetric analysis (d.t.g.) was then used to monitor the change in sample mass as the degradation proceeded and to distinguish between d.s.c. peaks due to mass loss or melting of the sample. Once the degradation pattern had been established, different stages of the thermal analysis curves could be investigated by analysis of the decomposition products obtained under isothermal conditions. This was achieved by using thermal treatments which corresponded to certain points on the t.g. curves (tested by weight-loss trials), and carrying out pyrolysis in a closed system where fractions of different volatility could be separated and collected. Although comparisons between the various techniques are likely to be complicated due to differences in operating conditions (35, 36), this approach has proved useful in the past (40). Quantification of the fractions making up the "mass balance" (for

definition and details see Appendix 1) and the use of gas chromatography (g.c.) and combined gas chromatography-mass spectrometry (g.c.-m.s.) for volatile analysis, allowed an evaluation of the degradative processes occurring.

PART A : Analysis of the thermal degradation of 1-deoxy-1-glycino-D-fructose and 1-β-alanino-1-deoxy-D-fructose with reference to the involvement of Amadori compounds in a low energy thermolysis pathway when sugars and amino acids are heated.

A.1. SUMMARY

Comparisons of the thermal analysis and pyrolysate data for the 1-amino-1-deoxyketoses with that for the sugar and amino acid controls demonstrate, in a quantitative fashion, that the formation of Amadori compounds represents a low energy thermolysis pathway. The thermal degradation of Amadori compounds leads to the production of volatiles which, in some cases, occur in high yields compared with controls. One such volatile is protoanemonin, a toxic vessicant.

Kinetic analysis of the t.g. curves and radioactive tracer studies on the formation of protoanemonin stress that a single route for the formation of individual volatiles is unlikely under pyrolytic conditions. Most of the volatiles formed from the decomposition of the glycosyl moiety of the Amadori compounds may be accounted for via an initial 1,2 enolisation of the 1-amino-1-deoxyketose in preference to a 2,3 enolisation.

A.2. INTRODUCTION

Many of the compounds isolated from browning reactions have been suggested to arise through amine-sugar condensations via the Amadori Rearrangement at lower temperatures and in higher yields than would occur by thermal degradation of the sugars in the absence of amines (4).

In this investigation the thermal decomposition of 1-deoxy-1-glycino-D-fructose and 1-β-alanino-1-deoxy-D-fructose was studied to evaluate the proposition that the formation of Amadori compounds facilitates the thermal degradation of carbohydrates when amino compounds are present. The experimental procedures outlined in section I.3 were employed to compare between the Amadori compounds and their parent sugar and amino acids with respect to the physical transformations and chemical reactions that occur during pyrolysis.

A.3 THERMAL ANALYSIS BY D.S.C., T.G. AND D.T.G.

A.3.1 Experimental

A.3.1.1 Amadori compounds: 1-deoxy-1-glycino-D-fructose and 1-β-alanino-1-deoxy-D-fructose were prepared according to published methods (25, 26). For details see Appendix 2.

Physical properties:

1-deoxy-1-glycino-D-fructose:
M.pt 144-146° (decomp), Litt. (26) 145-146° (decomp)
Elemental analysis: Found : C, 40.3%; H, 6.5%; N, 6.0%
Calc. for $C_8H_{15}NO_7$: C, 40.5%; H, 6.4%; N, 5.9%

The compound gave one spot by paper chromatography, R_G^* 0.54 in butanol-acetic acid-water (4:1:1 v/v) and displayed chromatographic homogeneity by electrophoresis at pH 2.1

I.R. : see Appendix 2

1-β-alanino-1-deoxy-D-fructose:
M.pt 150-152° (decomp), Litt. (26) 153°
Elemental analysis: Found : C, 42.5%; H, 6.9%; N, 5.5%
Calc. for $C_9H_{17}NO_7$: C, 43.0%; H, 6.8%; N, 5.6%
R_G : 1.05 in butanol-acetic acid-water
I.R. : see Appendix 2

A.3.1.2 Thermal analysis: Samples for analysis viz, 1-deoxy-1-glycino-D-fructose, 1-β-alanino-1-deoxy-D-fructose, D-glucose, glycine and β-alanine were ground to a fine powder and dried under vacuum at 60° for 24 hr.

D.s.c. traces were recorded on a Perkin-Elmer d.s.c.

* R_G = mobility of compound with respect to glucose

1B instrument. Samples (10 mg) were placed in crimped aluminium pans and kept under nitrogen while the temperature was increased at 4°/min over the range 25-400°C.

T.g. and d.t.g. data were obtained with a Mettler series No 21 recording vacuum thermoanalyser with samples (100 mg) in an open platinum crucible and under a nitrogen atmosphere (gas flow rate 100 ml/min). The temperature was raised at 0.5°/min or 4°/min and the range of 25-650°C examined.

Although the results for glucose and the amino acids have been recorded in the literature (38-41) they were repeated in this work to provide comparisons with the Amadori compounds treated under the same conditions.

A.3.2 Results and discussion

The d.s.c. endotherm for 1-deoxy-1-glycino-D-fructose (fig. A.1) shows an acute inflection corresponding to the melting of the sample (147°) which is accompanied by a second overlapping peak. The appearance of this peak, with an unstable line shape, indicates that the sample is melting with decomposition. Glucose melted in the manner typical of many organic compounds giving a sharp d.s.c. signal (146°) with decomposition following at a higher temperature. Glycine melted without visible degradation at 230°. At that temperature both glucose and the Amadori compound had been substantially degraded.

The t.g. results for 1-deoxy-1-glycino-D-fructose at a heating rate of 4°/min (fig. A.2) show that the decomposition of the sample commences near its melting point. The steep inflection indicates that the

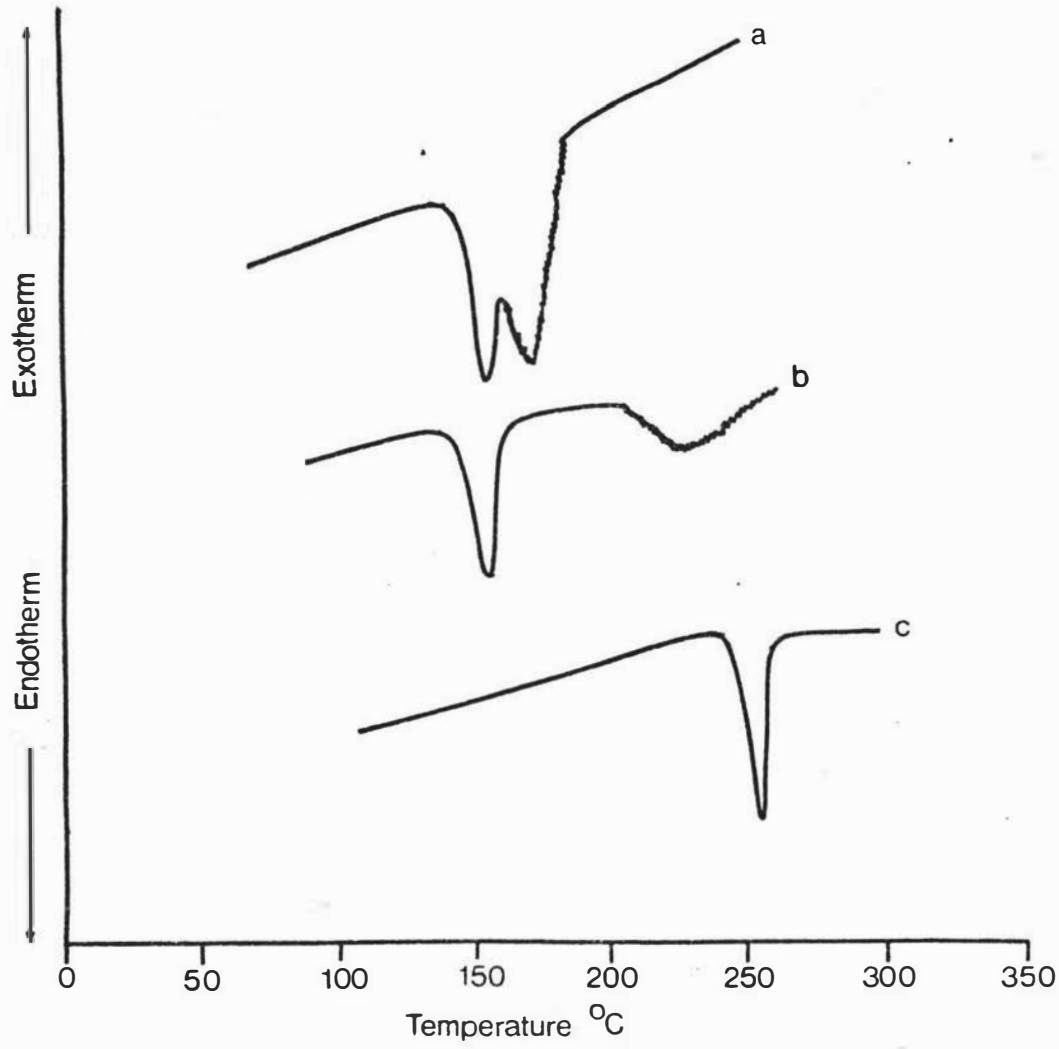


Fig A.1 Differential scanning calorimetric curves for (a) 1-deoxy-1-glycino-D-fructose, (b) glucose and (c) glycine.

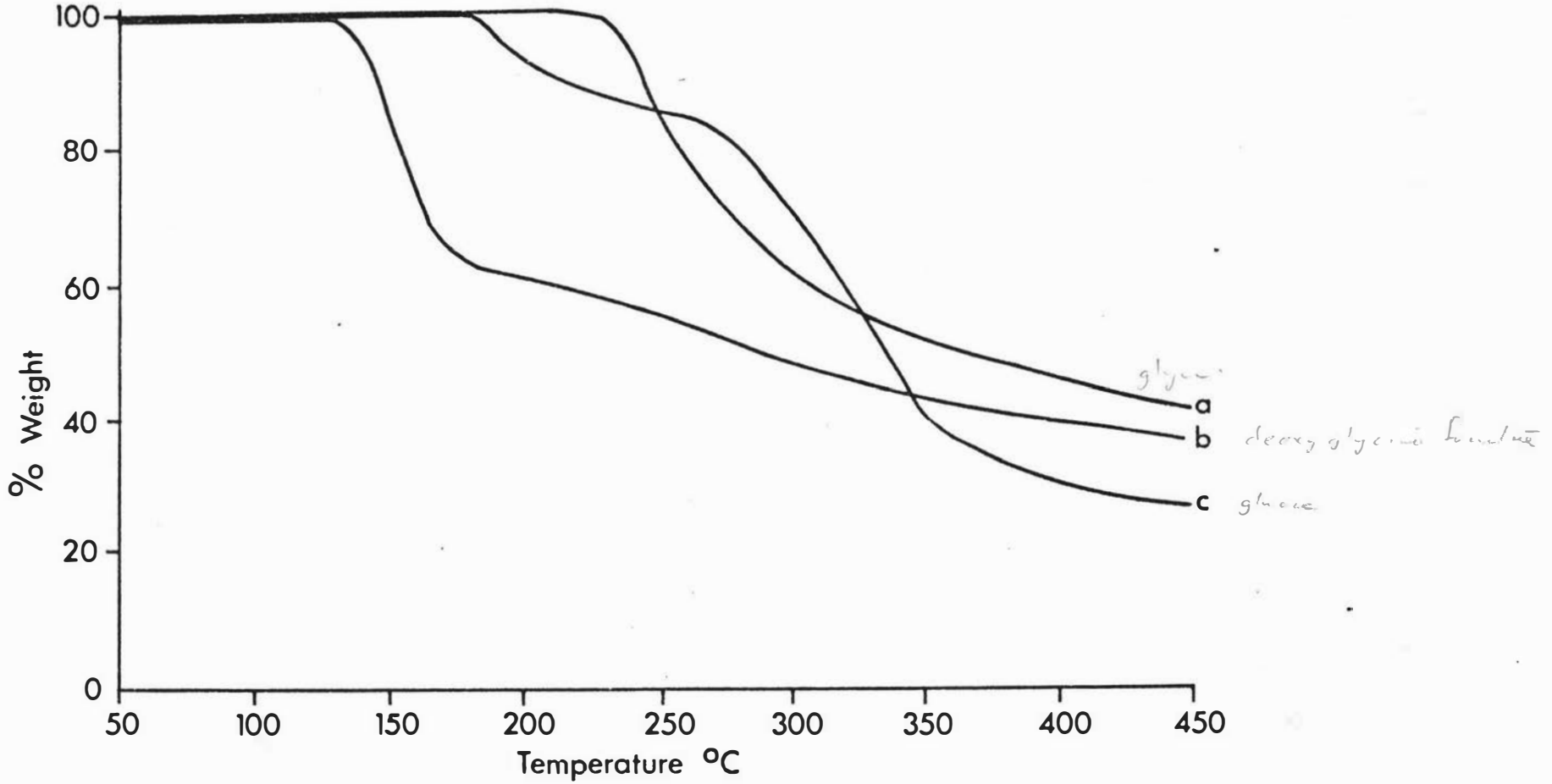


Fig A.2 Thermogravimetric analysis curves for (a) glycine, (b) 1-deoxy-1-glycino-D-fructose and (c) glucose, heating rate 4°/ min.

initial degradation proceeds comparatively rapidly to leave a substantial quantity of residue which then slowly decomposes. Thus pyrolysis is occurring in two main stages. The t.g. traces for glucose and glycine (fig. A.2) show that their degradation begins at temperatures where the Amadori compound has considerably degraded.

The d.s.c. and t.g. curves for 1-β-alanino-1-deoxy-D-fructose were identical in appearance to those for 1-deoxy-1-glycino-D-fructose except that the former compound left more residue at 400° (10%). β-alanine showed no sign of decomposing at temperatures below 195°.

These results point to a possible low energy route for the thermal degradation of glucose and amino acids through the formation of Amadori intermediates.

When a lower heating rate is used for the thermal degradation of 1-deoxy-1-glycino-D-fructose, the first stage of the t.g. curve indicates that a number of consecutive and concurrent degradative processes are occurring (see the d.t.g. curve in fig. A.3), and decomposition is seen to commence well before the nominal melting point is reached. The d.s.c. trace for this compound (fig. A.1) suggests that decomposition is occurring in the liquid phase. Thus, decomposition in the solid and liquid phases are both possible, the latter being aided by higher heating rates (42).

In the absence of competing reactions, decomposition in a homogeneous liquid phase is first order (43, 44). Therefore,

$$\frac{dx}{dt} = k(a-x), \quad \text{i.e.} \quad k = \frac{\left(\frac{dx}{dt}\right)}{a-x}$$

where x = fraction decomposed, a = initial weight.

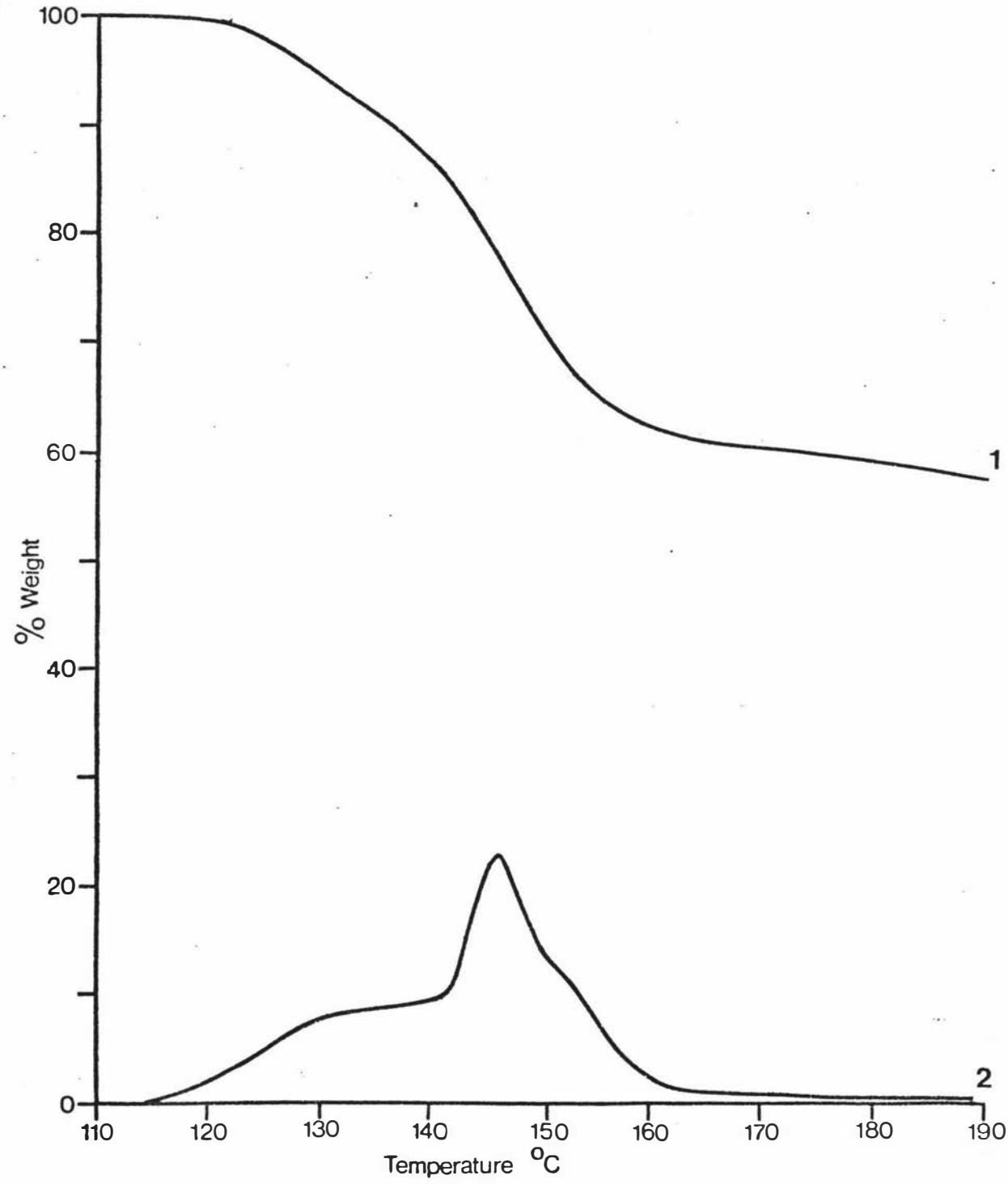


Fig A.3 Thermogravimetric analysis (1) and derivative thermogravimetry (2) curves for 1-deoxy-1-glycino-D-fructose, heating rate 0.5° /min., d.t.g. mode 5mg/min

Introducing the Arrhenuis equation one obtains :

$$k = Ae^{-Ea/RT} \quad , \quad \text{hence} \quad \log k = \log A - \frac{Ea}{2.303RT}$$

Using these relationships, a kinetic analysis of the t.g. curves for 1-deoxy-1-glycino-D-fructose was undertaken for both heating rates studied and plots of logk versus $\frac{1}{T}$ carried out (figs A.4 and A.5).

Fig. A.4 shows that three straight lines (A,B,C) are evident over the temperature range analysed for 1-deoxy-1-glycino-D-fructose at 4^{o}/min. Line A indicates decomposition in the solid phase and probably represents a nucleation growth phase of the decomposition which is autocatalytic. Once the nominal melting point is reached (145^{o}) dissolution occurs and the maximum rate of decomposition is also reached (42). Decomposition can now be treated as resulting from a homogeneous liquid phase and the data analysed to determine Ea values ignoring contributions from the solid-state processes (42). Two stages of the decomposition (lines B and C), corresponding to the two stages of the t.g. curve (fig. A.2), are indicated, giving Ea values of 3.6 kcal/mole and 22.2 kcal/mole respectively.

When the heating rate of 0.5^{o}/min is analysed (fig. A.5) the contribution of the decomposition via the liquid phase has been modified by the solid-state processes. The assumptions concerning first order decomposition in a homogeneous liquid phase no longer apply since the rate of decomposition appears to increase as the amount of reactant decreases and any derived rate constants would have no chemical significance (42). Clearly the heating rate has a significant influence on the degradation process and charring with resultant volatile formation is possible without the formation of an intermediate melt.

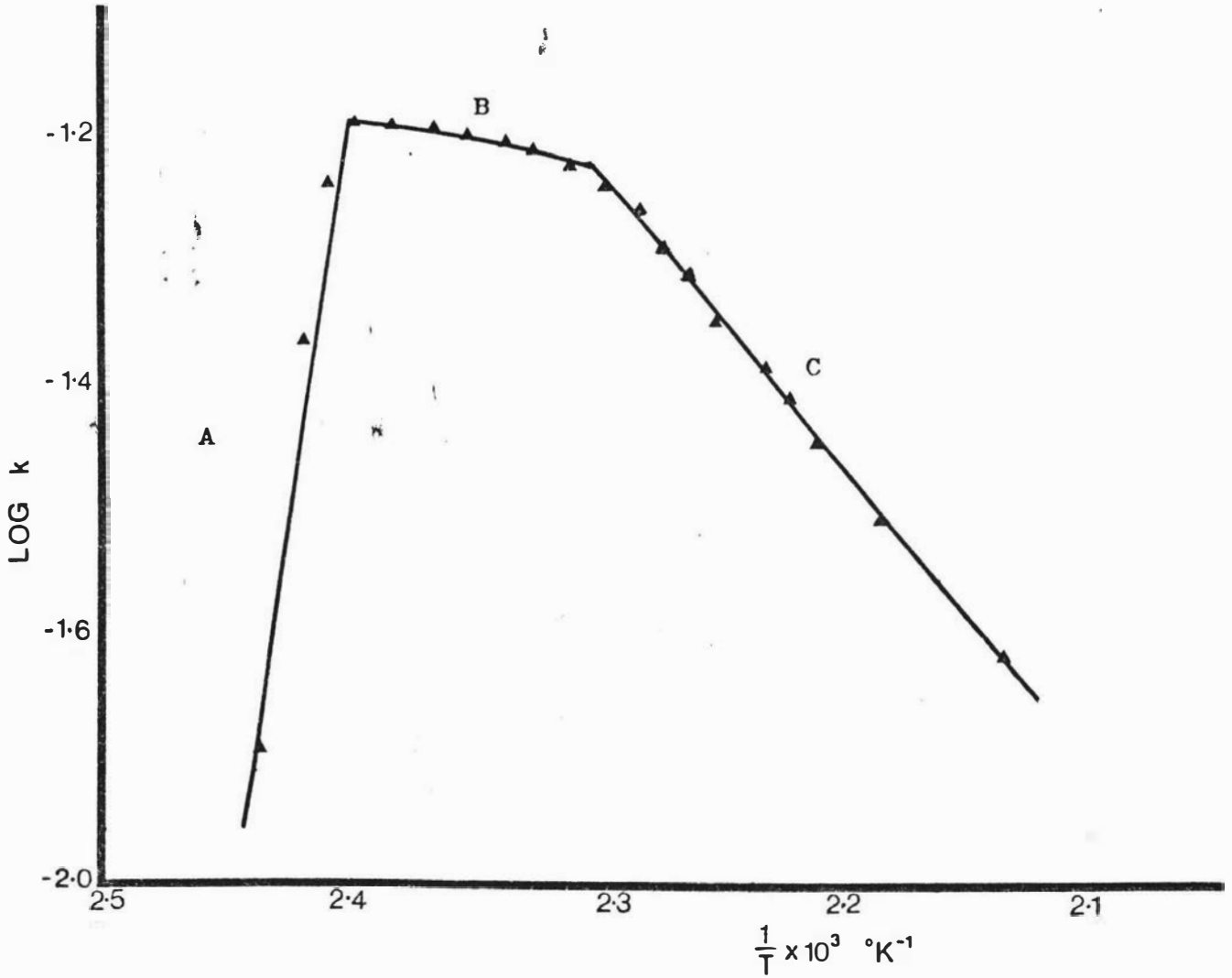


Fig A.4 Kinetic transformation of the t.g. curve for 1-deoxy-1-glycino-D-fructose, heating rate 4° /min.

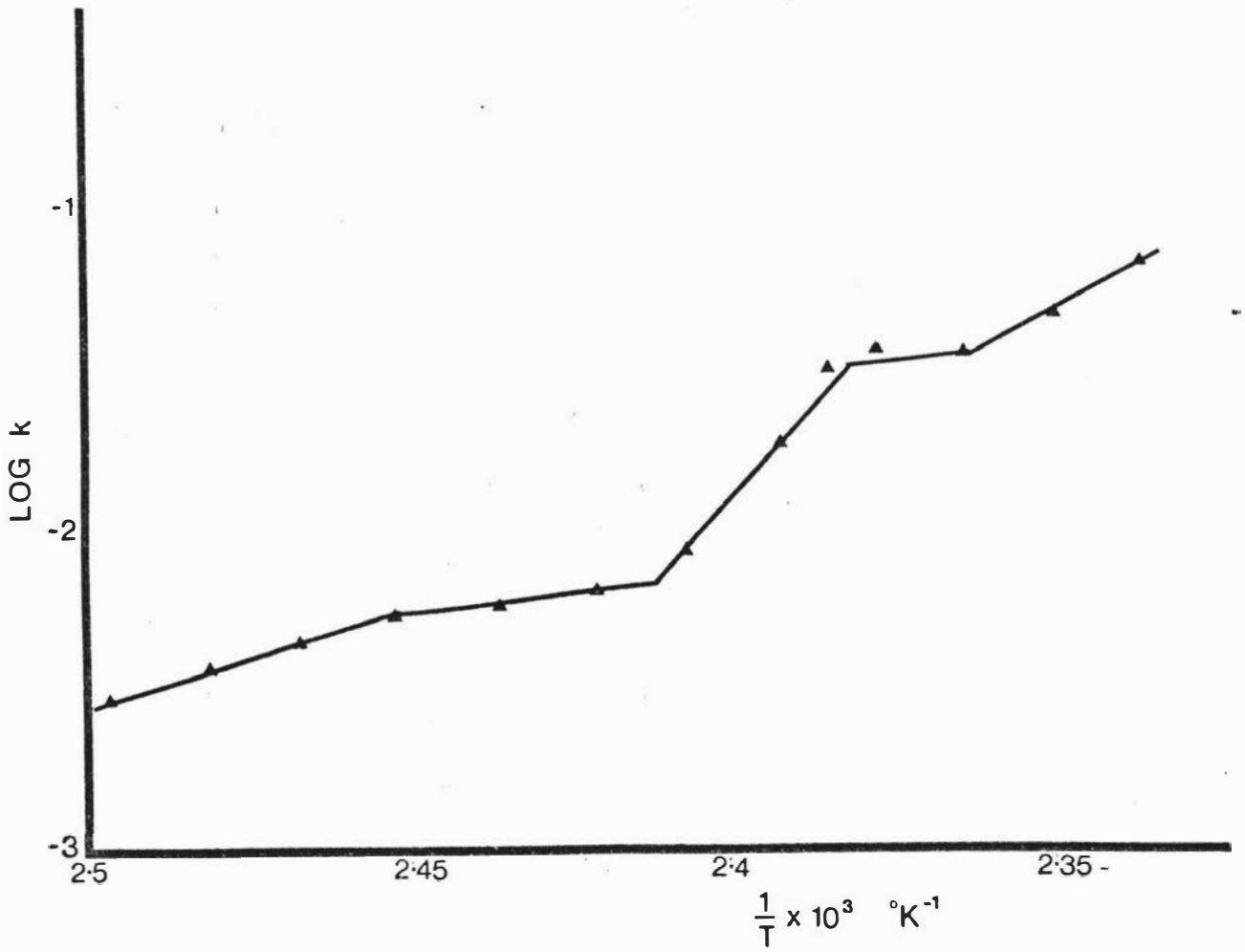


Fig A.5 Kinetic transformation of the t.g. curve for 1-deoxy-1-glycino-D-fructose, heating rate 0.5°/min.

A.4. ANALYSIS OF DECOMPOSITION PRODUCTS FROM THE 1-AMINO-1-DEOXYKETOSES AND CONTROLS

A.4.1. Pyrolysis conditions

The t.g. curves for 1-deoxy-1-glycino-D-fructose and 1-β-alanino-1-deoxy-D-fructose indicate that a thermal treatment of 195°/15 min may be appropriate to analyse the volatiles produced to the end of the first stage of decomposition. Separation of the volatiles by gas chromatography showed that a different pattern was obtained when the pyrolysis conditions were changed from less to more vigorous thermal treatments. The weight loss of a sample subjected to 195°/15 min corresponded to that obtained at the end of stage one according to the t.g. curve. A temperature of 400°/1 hr was used to study the products from the overall decomposition.

A.4.2 Experimental

A.4.2.1 Pyrolysis: All samples were dried to constant weight prior to pyrolysis and checked for purity by paper chromatography in butanol-acetic acid-water (4:1:1).

A quantitative isolation of the tar, char, water, organic volatile, carbon dioxide and carbon monoxide fractions formed during pyrolysis of the Amadori compounds and controls was obtained for the two selected thermal treatments as described in Appendix 1.

A.4.2.2 Gas chromatography: Volatiles from the polymer traps were separated on a 2.5 m x 0.32 cm outside dimension, stainless-steel column of Tenax-GC in a Hewlett-Packard 7620A gas chromatograph using a temperature program of 30-210° at 2°/min. The

chromatograph was fitted with thermal conductivity (employed for water determinations) and flame ionisation detectors. The volatiles were quantified by the use of external standards and comparison of the integrated peak areas. When the flame ionisation detector was employed, C-factors were used for correcting the observed response (peak area) with the carbon content of the component (= M.wt/N^{o} C atoms x 12), ignoring all oxygen-bonded carbon atoms. (91).

A.4.2.3 Gas chromatography-mass spectrometry : The contents of the polymer traps were identified using the g.c. column described in section A.4.2.2 fitted in a Pye 104 gas chromatograph which was coupled to an AEI MS-30 double beam mass spectrometer. A stream splitter enabled the simultaneous recording of g.c. and mass spectral data. G.c. traces were matched with those obtained in the Hewlett-Packard instrument and the mass spectra were compared to authentic standards run on the same instrument, or, where these were not available, to known reference spectra.

A.4.3 Results and discussion

Mass balances for the 1-amino-1-deoxyketoses and controls are summarised in table A.1. The occurrence of most of the degradation products in this table may be understood in terms of well established thermolysis pathways involving both the amino acid and sugar moieties of the Amadori compound (11,45-47). The char figures and the t.g. curves demonstrate that during the overall decomposition, the 1-amino-1-deoxyketoses form more char than the pyrolysis of the same amount of glucose under the same conditions. This increased charring may be explained by the interaction of amino groups with available carbonyl groups, such interaction being followed by a series of dehydration

TABLE A.1

PYROLYSIS PRODUCTS FROM AMADORI COMPOUNDS AND CONTROLS

PYROLYSIS PRODUCT	YIELD %									
	195° for 15 mins					400° for 1 hour				
	Glucose	Glycine	1-deoxy-1-glycino-D-fructose	β-alanine	1-deoxy-1-β-alanino-D- fructose	Glucose	Glycine	1-deoxy-1-glycino-D-fructose	β-alanine	1-deoxy-1-β-alanino-D-fructose
Char	96.4[a]	97.1[a]	58.2	77.8[a]	60.7	23.3	36.4	39.4	24.0	38.0
Tar			1.5		2.3	10.1	12.8	1.0	10.3	2.3
Carbon dioxide	0.1	0.5	16.1	1.0	2.5	4.5	5.3	17.8	3.7	9.6
Carbon monoxide	0	0	Tr	0.1	0	1.3	2.1	4.2	1.5	3.7
Water	2.5	2.2	20.2	20.6	29.7	42.7	33.0	25.3	41.5	36.3
Organic volatiles[b]	0.2	0	1.0	0.1	2.0	14.5	8.5	9.0	12.0	7.0
Unaccounted[c]	0.8	0.2	3.0	0.4	2.8	3.5	1.9	3.3	7.0	3.1

[a] *Distinct separation of residue into tar and char had not occurred*

[b] *Recovered from the volatile traps (see Experimental)*

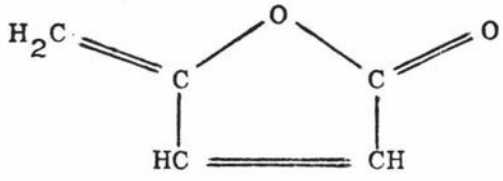
[c] *This fraction contains any untrapped nitrogenous gases*

and polymerisation reactions as established for amino sugars (48).

At 195° for 15 min substantial decomposition of the Amadori compounds has occurred while, under the same conditions, glucose and the amino acids remain thermally stable except that β-alanine has dehydrated. On a molar basis, 1-deoxy-1-glycino-D-fructose, on this thermal treatment, loses 90 mass units representing (from table A.1) 1 mole of carbon dioxide plus 3 moles of water per mole of Amadori compound. The most likely source of carbon dioxide is the amino acid moiety (46,47). It has been suggested that such a decarboxylation reaction is facilitated by 1,2 enol formation (20) during decomposition. Decarboxylation is of minor significance with 1-β-alanino-1-deoxy-D-fructose however, the evolution of water (4 moles per mole of Amadori compound) being the dominant reaction. Decarboxylation following 1,2 enol formation in this case would require a transition-state ring structure containing one more carbon atom than the ∝ structure while a dehydration step would have the same conformation. Dehydration is the initial decomposition step with β-amino acids (46,47).

On degradation at 400° for 1 hr all compounds produce increased percentages of carbon monoxide and organic volatiles showing that fragmentation reactions are becoming more important. The volatiles produced have possible importance in aroma production (4). It was therefore of interest to identify between the 1-amino-1-deoxyketoses and controls with respect to the types and quantities of these volatiles produced. Table A.2 summarises the relative concentrations of organic volatiles identified from the polymer traps for the Amadori compounds pyrolysed under the two sets of thermal treatments.

At 195° for 15 min, the volatiles produced are, in general, compounds expected from glucose at higher temperatures (11,49) with some nitrogen containing compounds also present. An unusual feature is the production of protoanemonin (iii) in relatively large quantities. Its formation is discussed in the next section.



(iii)

Pyrolysis of the Amadori compounds at 400° for 1 hour also leads to the production of volatiles expected from carbohydrate pyrolysis, with increased quantities of nitrogen compounds present. The formation of pyrazines is of particular significance. They are recognised as important contributors to food flavours (50) and are major components of the volatile fraction produced from 1-deoxy-1-glycino-D-fructose. Pyrazines have not been detected in glycine or β-alanine pyrolysates (51) but have been isolated from heating a mixture of fructose and glycine (52). Dimethylpyrazines have been identified from a glucose and glycine mixture at low temperatures (53). A suggested route for pyrazine formation is through an α-dicarbonyl induced Strecker degradation of α-amino acids (3).

TABLE A.2

VOLATILE ORGANIC PYROLYSIS PRODUCTS FROM 1-DEOXY-1-GLYCINO-D-FRUCTOSE AND 1-DEOXY-1-β-ALANINO-D-FRUCTOSE

COMPOUNDS [a]	*1-deoxy-1-glycino-D-fructose*		*1-β-alanino-1-deoxy-D-fructose*		*Mass Spectrum*	*M.S. Ref*
	195°/15 min	*400°/1hr*	*195°/15 min*	*400°/1hr*	*m/e*	
Propene		++		+	41,42,39,27	54
Methanol	+++	++		+	31,32,29	54
Acetaldehyde	++++	+++	++++	+++	29,44,43	54
Butene		++		+	41,56,39	54
1,3-Butadiene		+		+	41,56,55	54
Ethanol	++	+	++	+	31,45,27,29,46	54
Acetonitrile		++		+	41,40,39	54
Furan	+	+	+	+	39,68,38	54
Acetone	+++	++	++	+	43,58,15	54
Acrylonitrile		+			26,53,52,51	54
Acetic acid		++++	+	++++	43,45,60	54
Propionitrile		+		+	28,54,55,26	54
2-Methylfuran	++	++	++	+	82,53,81	54
Diacetyl	++	+	++		43,15,86	54
Propionic acid		++			28,29,74,27,45	54
Methylpropionate		+			29,57,27,59,88	54
Acrylic acid		+		+++	27,72,55,26	54
Acetol		++		++	43,31,15,74	54
Benzene	+	+		+	78,52,77,51,50	54
2,5-Dimethylfuran	+	+++		+	43,96,95,53,81	54
2,3-Pentanedione	+	+	++	+	43,29,57,27,100	54
Phenol	+	+			94,39,65,66	54
Pyrrole		+	++	+	67,39,41,40	54
1-Methylpyrrole	+	++			81,80,39,42	54
Pyridine	+			+	79,52,51,50	54

Cont...

COMPOUNDS[a]	1-deoxy-1-glycino-D-fructose 195°/15 min	1-deoxy-1-glycino-D-fructose 400°/1hr	1-β-alanino-1-deoxy-D-fructose 195°/15 min	1-β-alanino-1-deoxy-D-fructose 400°/1hr	Mass Spectrum m/e	M.S. Ref
Toluene		+			91,92,39,65	54
N-Methylformamide		+			59,30,29,58	54
2-Methylpyrazine		+			94,67,39,53,40	54
2-Methylpyrrole		+			80,81,53,27	54
N-Methylacetamide		+			73,43,40,58	54
2-Furaldehyde	++		++	+	96,95,39,29	54
α-Furfuryl alcohol		+			98,41,39,42,53	54
2,4 (5)-Dimethylpyrrole		+			94,95,80	54
Protoanemonin	+++	+	++++	+++	42,96,26,68,54	55
2,6 (6)-Dimethylpyrazine		++		+	108,42,40,39,81	56
2,3-Dimethylpyrazine		++		+	108,67,42,40	56
2-Furylmethylketone	+++	+	++	++	95,110,39,42	54
5-Methyl-2-furaldehyde	++	+	++	+	110,109,53	54
Trimethylpyrazine		++++			42,122,39,81	56
Tetramethylpyrazine		++	+		54,136,42,53	57
2-Acetylpyrrole				++	94,109,66,43	54

[a] ++++ = Major product; +++ = > 10% pryolysate; ++ = 1-10% of pyrolysate; + = < 1% of pyrolysate

A.5. THE FORMATION OF PROTOANEMONIN

A.5.1. Introduction

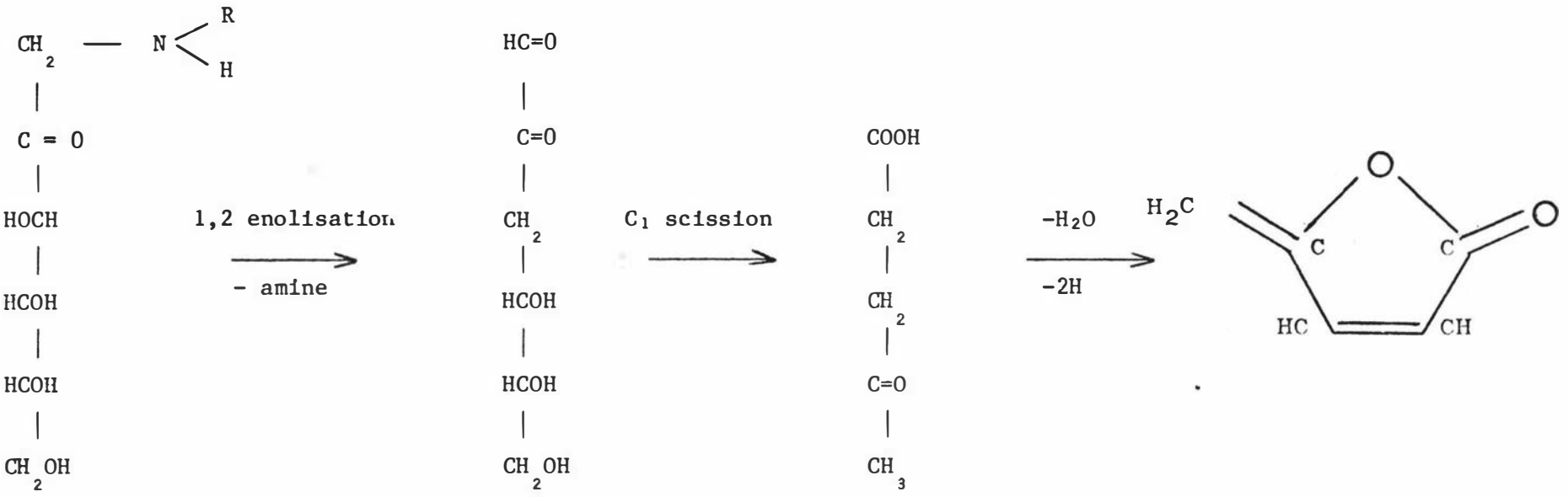
Of all the volatiles listed in table A.2, protoanemonin is the least readily accounted for. It has not been previously reported in carbohydrate pyrolysis but was shown to be formed in a lactose-casein browning system (55) although yields were not reported. Protoanemonin is the lactone of Y-hydroxy-vinylacrylic acid (58) and has been isolated as the crystalline glucoside "ranunculin" from buttercup and other ranunculaceae (59). It is known to have antimicrobial and antioxidant properties (60).

A pathway is outlined in fig. A.6 for the formation of protoanemonin. The first intermediate is 3-deoxy-D-erythro-hexosulose which is the major intermediate expected during the pyrolysis of Amadori compounds via a 1,2 enolisation mechanism (20). Levulinic acid is a standard end-product during the degradation of carbohydrates (11) and its formation from glucose involves a 1,2 carbon bond scission (61). Protoanemonin is known to be formed from levulinic acid under certain conditions (62,63).

This pathway is evaluated in this section using pyrolytic and radioactive tracer studies.

A.5.2 Experimental

A.5.2.1 Protoanemonin: Protoanemonin was synthesised by acid-catalysed lactonisation of acetylacrylic acid (62) which was prepared from glyoxylic acid (64). The U.V., mass spectrum and gas chromatographic retention times for the synthetic product were identical with those of the compound obtained from the pyrolysates.



Amadori Compound — 3-deoxy-D-erythro-hexosulose — Levulinic Acid — Protoanemonin

Fig. A.6 *PATHWAY OF PROTOANEMONIN FORMATION*

R = $-CH_2COOH$, $-CH_2CH_2COOH$

A.5.2.2 3-Deoxy-D-erythro-hexosulose: This compound was prepared by the method of Kato (65). I.R., U.V. and optical rotation were consistent with the published data.

A.5.2.3 Radioactive 1-β-alanino-1-deoxy-D-fructoses: [1-^{14}C] glucose (~ 50 μCi, Radiochemical Center, Amersham) was dissolved with D-glucose (2 g, B.D.H. "Analar") in distilled water (5 ml). The solution was lyophilised and the residue dried at 60° in vaccuo over phosphorus pentoxide for 24 hr. Labelled glucose (1 g), β-alanine (400 mg), sodium metabisulphite (300 mg) and distilled water (0.17 ml) were ground, transferred to a flask (50 ml) and heated at 100° for ½ hr. The product was dissolved in water (5 ml) and passed through Dowex-50-X8 (20 gm). Ion exchange chromatography yielded 200 mg crystalline material which was recrystallised from methanol-water (2:1) m.pt. 150-152° (decomp).

The synthesis was repeated using [2-^{14}C] glucose and [6-^{14}C] glucose to yield the desired radioactive products.

A.5.2.4 Pyrolysis, g.c., g.c.-m.s.: Organic pyrolysis products from the degradation of 3-deoxy-D-erythro-hexosulose, levulinic acid and the 1-β-alanino-1-deoxy-D-fructoses were collected and analysed as described in section A.4.2. A thermal treatment of 280°/15 min was necessary for the radioactive Amadori pyrolyses since at 195°/15 min the yield of protoanemonin was too low to make radioactive tracer studies feasible, while at 400°/1 hr the estimation and separation of this compound was complicated by the presence of pyrazines.

A.5.2.5 Measurement of radioactive protoanemonin: Protoanemonin was collected from the g.c. outlet by

bubbling the effluent gas through distilled water (2 ml) in a cuvette. The absorbance of the resulting solution (λmax 260, log ε 4.15) was measured in a Unicam S.P. 800 spectrophotometer and the concentration of protoanemonin determined (62). The entire U.V. was recorded to confirm that conversion into anemonin had not occurred. As a check on the above procedures, 2-furaldehyde (λmax 276, log ε 4.18) was similarly collected for comparison with radioactive data determined for this compound from the pyrolysis of glucose (45). A known weight of pyrolysis residue (char) was also recovered for radioactivity determinations.

Activity of all samples was measured by transferring an aliquot of an aqueous solution of known concentration, or a suspension of the char, into a vial containing toluene omnifluor-triton (2:1, 20 ml) and counting in an Isocap/300 Liquid Scintillation System (Searle Analytical). Counting efficiencies were determined by using a set of quenched standards and the counts adjusted accordingly.

A.5.3 Results and discussion

In a study of the pyrolysis of 3-deoxy-D-erythro-hexosulose at 550°/8 min (66), protoanemonin was not reported. In the present study however, protoanemonin was found among the pyrolysis products of this osulose at 195°/15 min (see Table A.3). Protoanemonin was also detected at 400°/1 hr, in trace amounts, from 3-deoxy-D-erythro-hexosulose and glucose. Although the relative yields appear low, a comparison of the gas chromatograph peak heights for the pyrolysates arising from the osulose and 1-β-alanino-1-deoxy-D-fructose, subjected to 195°/15 min and standardised with respect to weight of starting material, showed that the formation of protoanemonin via

3-deoxy-D-erythro-hexosulose was sufficient to account for the yields of this compound amongst the Amadori pyrolysates. The preponderance of other low molecular weight volatiles (table A.3) indicates, however, that spontaneous decomposition of 3-deoxy-D-erythro-hexosulose cannot play an important role in the thermal degradation of Amadori compounds.

Pyrolysis of levulinic acid at 280°/15 min also gave traces of protoanemonin. As an explanation of the route to levulinic acid formation when glucose was pyrolysed (45), the accepted pathway (67), which is operative in solution and involves 5-hydroxymethyl-2-furaldehyde was discounted on the basis that it requires the addition of water which would be unfavourable under pyrolytic conditions. Indeed, neither levulinic nor formic acid was isolated when 5-hydroxymethyl-2-furaldehyde was pyrolysed (68). On this basis it was postulated (45) that glucose degrades to give an intermediate which can dehydrate

Table A.3. Pyrolysis products from 3-deoxy-D-erythro-hexosulose at 195°/15 min.

Compound	Relative Amount
Methanol	++++
Acetaldehyde	++++
Ethanol	++++[a]
Acetic Acid	++++
Diacetyl	++
2-Furaldehyde	++++
Protoanemonin	+
2-Furylmethylketone	+
5-Methyl-2-furaldehyde	++
5-Hydroxymethyl-2-furaldehyde	++++

[a]Assignment tentative since ethanol was used during the synthesis, however peak persisted after prolonged drying

further to form 5-hydroxymethyl-2-furaldehyde or, by a separate parallel process, lose water and one molecule of formic acid (involving C-1) to yield a residue which can then form levulinic acid via intramolecular disproportionation. This common intermediate seems likely to be 3-deoxy-D-erythro-hexosulose.

The carbon skeleton cleavage shown in fig A.6 can be tested from the results in table A.4. The specific activity of the protoanemonin from the Amadori compound synthesised using [2-^{14}C] glucose shows the almost complete retention of the label while the corresponding figures for the retention of the C-1 and C-6 labels are 10% and 70% respectively. Hence a scission between C-1 and C-2 is favoured to leave a 5-carbon fragment incorporating carbons 2 to 6. However C-6 is not incorporated at least 30% of the time while C-1 can be incorporated 10% of the time. These findings support the pathway outlined in fig A.6 but suggest that an alternative mechanism exists which can be explained by the recombination of fragments, one and only one of which contains C-2. This phenomenon is not unusual in pyrolytic reactions since stable free radicals (32) formed from the homolytic cleavage of carbon chain substituents during the charring process, are capable of forming new carbon-carbon bonds.

Table A.4 also shows that 2-furaldehyde is formed via the loss of C-6 about 75% of the time with the balance arising from the loss of C-1. These results are consistent with earlier studies (45) where the formation of 2-furaldehyde was shown to occur mainly through the elimination of formaldehyde (C-6) from the decomposition of 5-hydroxymethyl-2-furaldehyde, which is the normal end-product of carbohydrate decomposition via 1,2 enolisation and 3-deoxy-D-erythrohexosulose formation. The less favoured of the

Table A.4. Incorporation of radioactivity into pyrolysis products of 1-β-alanino--1-deoxy-D-fructoses derived from glucose labelled at various positions.

Compound	Specific activity (10^3 mCi/mmol)	dpm/mg
[1-^{14}C] Glucose	4.26	
1-β-alanino-1-deoxy-D-fructose	3.93	
Protoanemonin	0.39	
2-Furaldehyde	2.93	
Residual char		1698
[2-^{14}C] Glucose	4.42	
1-β-alanino-1-deoxy-D-fructose	4.19	
Protoanemonin	4.06	
2-Furaldehyde	4.44	
Residual char		45,756
[6-^{14}C] Glucose	4.49	
1-β-alanino-1-deoxy-D-fructose	4.37	
Protoanemonin	2.94	
2-Furaldehyde	1.17	
Residual char		29,266

two pathways involves loss of C-1 as formaldehyde from the decomposition of 2-furyl-hydroxymethylketone. The table further shows that the residual char left in the pyrolysis unit is largely devoid of compounds retaining C-1 of the original hexose. Almost thirty times more C-2 is retained, showing that a C_1-C_2 scission is very common during the pyrolytic reactions and supports previous tracer studies (45) where C-1 was found to

contribute heavily to the formation of carbon dioxide and carbon monoxide during carbohydrate pyrolysis.

PART B: Investigations of the thermal degradation of glucose-amino acid systems in the dry-state

B.1 SUMMARY

The role of Amadori compounds during the browning of sugar-amino acid mixtures in the dry-state at temperatures up to those where spontaneous decomposition of the 1-amino-1-deoxyketose occurs has been assessed. Even though a mixture of glucose plus an amino acid appears to brown more readily than glucose plus an Amadori compound, the results presented in this section show that the formation of a 1-amino-1-deoxyketose represents the first step on the lowest energy route to product formation. Furthermore, once initiated, the course of browning in the dry-state under the conditions considered, parallels the pathways demonstrated for browning in solution. The 1-amino-1-deoxyketose formed functions as a catalyst in the browning sequence and provides relatively few browning products directly via its own decomposition.

B.2 INTRODUCTION

The results presented in Part A of this thesis show that the thermal degradation of the Amadori compounds studied occurs at lower temperatures than those for their parent sugar and amino acids. To show that the formation of a 1-amino-1-deoxyketose represents the lowest energy route for browning in a glucose-amino acid mixture however, a study of the browning characteristics of a mixture of glucose and the parent amino acid is warranted. Such a study was excluded from Part A since difficulties were encountered in obtaining d.s.c., t.g. and pyrolysis data due to "foaming" of the samples.

In subsequent preliminary experiments, samples of glucose, glycine, β-alanine, 1-deoxy-1-glycino-D-fructose and 1-β-alanino-1-deoxy-D-fructose alone and in combinations as intimate mixtures were packed into capillary tubes and allowed to brown in a melting-point apparatus by raising the temperature at 1^{o}/min. These experiments demonstrated that a mixture of glucose plus an amino acid browns at lower temperatures than either of its components. The mixture also browned more readily than glucose plus a 1-amino-1-deoxyketose which in turn browned more readily than the Amadori compound alone. Thus the spontaneous decomposition of a 1-amino-1-deoxyketose, if formed in significant quantities in these systems, cannot of itself explain these observations.

Experiments were therefore designed to investigate the role of Amadori compounds in reactions where glucose and amino acids are heated together in the dry-state. In the first experiment the formation of a 1-amino-1-deoxyketose is examined over the temperature range $120-170^{o}$. The second experiment evaluates the contribution of the Amadori compound to

visual browning in systems where solid state effects are absent. The third experiment objectively determines the role of the 1-amino-1-deoxyketose during browning in the dry-state at 120° (where all components are in the solid state) and at 150° (where glucose is molten).

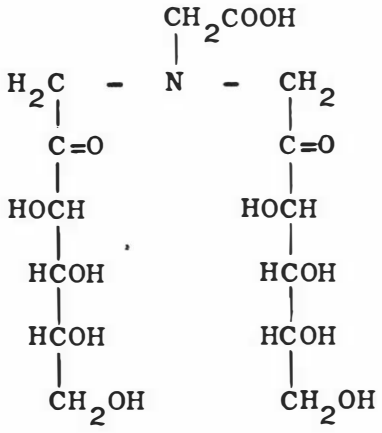
B.3 EVIDENCE FOR THE FORMATION OF AMADORI COMPOUNDS IN SUGAR-AMINO ACID SYSTEMS ON HEATING IN THE DRY-STATE

B.3.1 Experimental

Mixtures of glucose and glycine (1:1 mole ratio) were dried to constant weight and mulled with glycerol or paraffin at 60°. Examination of the dried mixture by paper chromatography in butanol-acetic acid-water (4:1:1) using triphenyltetrazolium chloride-sodium hydroxide showed no Amadori compounds had formed as a result of this treatment. The mulls were dispersed into glycerol (120° and 170°) or paraffin (150°). Samples were removed at appropriate intervals from the glycerol baths and tested for the formation of 1-deoxy-1-glycino-D-fructose as above. The paraffin mixture was allowed to heat for 15 sec then cooled in a dry-ice/ethanol bath and washed with water. The aqueous fraction was reduced to low volume and chromatographed in butanol-acetic acid-water (4:1:1). The spots were visualised using reagents described in the literature (23).

B.3.2 Results and discussion

Table B.1 summarises the experimental results. These demonstrate the formation of the 1-amino-1-deoxyketose over this temperature range and indicate its relative stability, since it is still present even when browning is well advanced. The aqueous extract of the mixture which had been heated in paraffin gave an Amadori positive spot with the expected mobility and colour reactions of difructose-glycine ((iv),(26)) as well as the monofructoseamine when examined by paper chromatography.



(iv)

Table B.1 Formation of 1-deoxy-1-glycino-D-fructose from glucose plus glycine mixtures heated in the dry-state

Medium used	Temp °C	Time	Test for Amadori*	Observations
Glycerol	120	2 min	N.S.	Very light brown
		4 min	+ve	" " "
		7 min	N.S.	Light brown
		12 min	N.S.	" "
		20 min	+ve	Mid-brown
		40 min	+ve	Dark brown
Paraffin	150	15 sec	+ve	Dark brown
Glycerol	170	0 sec	N.S.	Dark brown
		15 sec	+ve	" "

* N.S. = not sampled.

B.4 OBSERVATIONS ON THE RATE OF BROWNING OF MODEL SYSTEMS IN THE DRY-STATE IN HOT OIL, WHERE ONE COMPONENT IS MOLTEN

B.4.1 Introduction

Since the formation of Amadori compounds during browning in the dry-state has been indicated (see B.3), the results of the preliminary experiments (see B.2) may be evaluated in two ways. Either the formation of a 1-amino-1-deoxyketose is merely a side-reaction during browning or there is a requirement for it to be in a liquid phase before decomposition or interaction can proceed. Temperatures above the melting points of glucose and 1-β-alanino-1-deoxy-D-fructose were thus employed to monitor the browning of systems where at least one component was molten and hence eliminate solid state effects.

B.4.2 Experimental

Samples of glucose, β-alanine, 1-β-alanino-1-deoxy-D-fructose (fru-β-ala), 3-deoxy-D-erythro-hexosulose and various mixtures of these compounds (pre-dried to constant weight) were mulled in paraffin at 60° and dispersed into paraffin at 160° or 155°. The course of browning was visually monitored. The lower temperature was employed to indicate any differences in browning behaviour which could not be differentiated at 160°.

B.4.3 Results and discussion

The results (table B.2) demonstrate that reactions between glucose and β-alanine in a melt, where intimacy of contact between reactants is achieved, are not greatly aided by the presence of the Amadori compound.

Table B.2 Rate of browning of various systems in paraffin

Mull	Component	Time to end of reaction (secs)*	Observation
Paraffin at 160°	Glucose	120	molten - no colour change
	β-alanine	120	solid - no colour change
	Fru-β-Ala	80	dark brown mass
	3-deoxy-D-erythro-hexosulose	Immediate	reddish-brown tar
	Glucose + β-alanine	15	dark brown mass
	Glucose + Fru-β-Ala	15	dark brown tar
	Fru-β-Ala + β-alanine	120	brown, some black patches, still solid
	β-alanine + 3-deoxy-D-erythro-hexosulose	Immediate	black tar
	Fru-β-Ala + 3-deoxy-D-erythro-hexosulose	Immediate	black tar
Paraffin at 155°	Glucose + β-alanine	30	dark brown
	Glucose + Fru-β-Ala	40	dark brown
	Glucose + Fru-β-Ala + β-alanine	25	dark brown
	Fru-β-Ala + β-alanine	120	brown

* 120 secs or when colour change was no longer significant.

Browning is very rapid if 3-deoxy-D-erythro-hexosulose is present initially. The spontaneous decomposition of 1-β-alanino-1-deoxy-D-fructose to yield 3-deoxy-D-erythro-hexosulose plus β-alanine, the expected products via 1,2 enolisation and degradation (9), is not a dominant process, otherwise the Amadori compound would brown at least as rapidly by itself as in the presence of glucose. Rather, the Amadori compound may react with glucose to produce 3-deoxy-D-erythro-hexosulose via difructose-β-alanine which then takes part in browning reactions with any amines present (69). Evidence for the formation of a diketoseamine during solid-state browning was presented in section B.3. Hence the scheme ((7), fig B.1) for browning in solution, appears to be directly applicable to the dry-state at temperatures up to those required for spontaneous thermal decomposition reactions to occur. Reaction between 1-β-alanino-1-deoxy-D-fructose and β-alanine appears to be of minor significance at these temperatures.

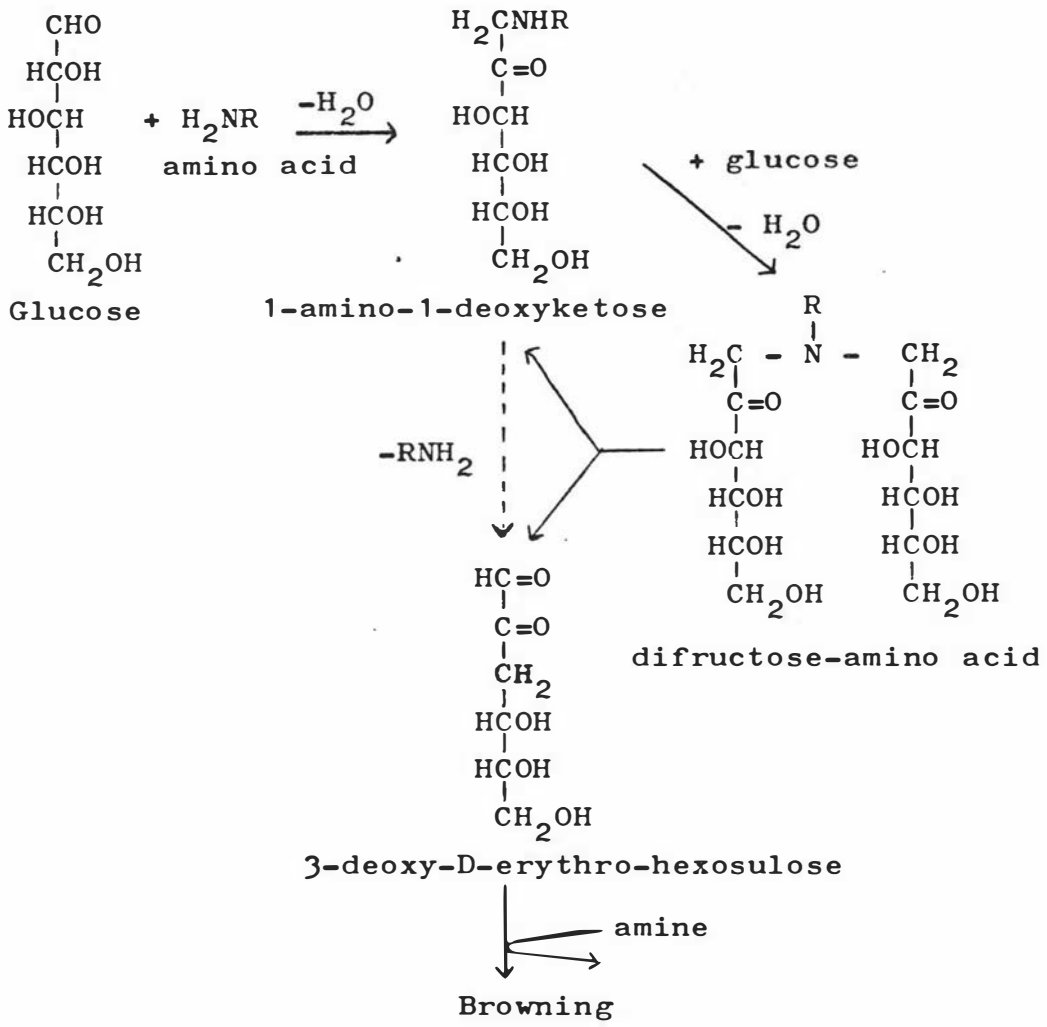


Fig B.1 Browning route for sugars and primary amino acids

B.5 STUDY OF THE INVOLVEMENT OF 1-β-ALANINO-1-DEOXY-D-FRUCTOSE IN THE LOWEST ENERGY ROUTE TO PRODUCT FORMATION DURING THE BROWNING OF A GLUCOSE-β-ALANINE MIXTURE IN THE DRY-STATE.

B.5.1 Introduction

The preceeding sections (B.3 and B.4) infer the importance of Amadori formation during browning in the dry-state. However the contribution of Amadori formation to the reaction between glucose and an amino acid at temperatures well below the melting point of glucose remains obscure. Such systems brown at lower temperatures than glucose plus an Amadori compound (section B.2).

The role of the Amadori compound in the lowest energy pathway to browning in the solid state is objectively evaluated in this section where the disappearance of glucose and β-alanine and the formation of the 1-amino-1-deoxyketose are monitored at 120°, where the browning reaction between glucose and β-alanine occurs readily with a minimum of heat treatment, and also at 150°, where glucose is molten, allowing greater intimacy of contact between reactants.

B.5.2 Experimental

B.5.2.1 Browning reactions: In parallel experiments, intimate mixtures of powdered glucose and β-alanine (1:1 mole ratio) or glucose, 1-β-alanino-1-deoxy-D-fructose and β-alanine (1:1:1 mole ratio) were weighed into melting point tubes (approx 20 mg sample) which were placed into holders immersed in an oil bath maintained at 120° or 150°. Constant stirring of the oil ensured an even temperature and a test using thermocouples placed inside the packed tubes showed

that the desired temperature was reached in 0.5 sec. Individual tubes were removed during the course of browning and immediately cooled by placing in an ice-bath. After cooling and drying the tubes were reweighed and crushed into vials of distilled water (3 ml). U.V. and visible spectra of the resultant solutions were recorded on a Unicam SP 800 recording spectrophotometer. A paper chromatographic survey as outlined in section B.3.1 was also performed.

B.5.2.2 Glucose Analysis: (Glucose oxidase)

Reagent -

$Na_2HPO_4.H_2O$	2.1 g
$NaH_2PO_4.2H_2O$	1.1 g
2,2'-Azino-di-(3-ethyl benzthiazoline sulfonic acid (A.B.T.S.))	75 mg
Glucose oxidase (E.C. 1.1.3.4)	100 mg
Peroxidase (E.C. 1.11.1.7)	100 mg
Distilled water	150 ml

Method -

Aliquots of the glucose samples (0.1 ml containing up to 0.2 mg/ml glucose) were added to the reagent (3 ml) in a screw cap vial. The solution was incubated for 30 min at 25° and the absorbance read at 420 nm against a blank containing 0.1 ml distilled water in place of the sample. The glucose concentration was determined from a standard curve.

B.5.2.3 Analysis of β-alanine and 1-β-alanino-1-deoxy-D-fructose: The levels of these compounds were monitored using an automatic amino acid analyser (70). (Methods were developed for more rapid analysis during preliminary experiments (see Appendix 3)). The samples for analysis were diluted if necessary so that an injection (0.1 ml containing approx

100 nmoles) gave on-scale peaks from which concentrations could be determined. Standard solutions were first run from which colour factors were determined for each compound.

B.5.3 Results and discussion

Data showing the levels of glucose, β-alanine and 1-β-alanino-1-deoxy-D-fructose for the systems glucose plus β-alanine (1:1) and glucose plus β-alanine plus 1-β-alanino-1-deoxy-D-fructose (1:1:1) as a function of time for the two chosen temperatures are plotted in figs B.2 to B.5. In each case glucose is consumed much more rapidly than β-alanine. The initial loss of β-alanine parallels the build-up of the 1-amino-1-deoxyketose, thus suggesting the formation of an Amadori compound as the primary step during browning in the solid-state under these conditions. The rapid consumption of glucose once the Amadori concentration reaches an appreciable level is consistent with the 1-amino-1-deoxyketose performing a catalytic role in the conversion of glucose to 3-deoxy-D-erythro-hexosulose via the formation of difructose-β-alanine, in accordance with fig B.1. Evidence for the formation of the difructose-β-alanine was seen in reactions at 120°. A paper chromatographic survey revealed a faint Amadori positive compound having half the r.f. value of 1-β-alanino-1-deoxy-D-fructose (26) in the glucose-β-alanine system over the time interval 30 to 80 seconds.

The relative stability of the monoketoseamine is clearly demonstrated. At 120° it builds up to a steady-state concentration which persists long after all the glucose has reacted and, even though some degradation is evident at 150°, the same steady-state level is attained at this temperature. The loss of

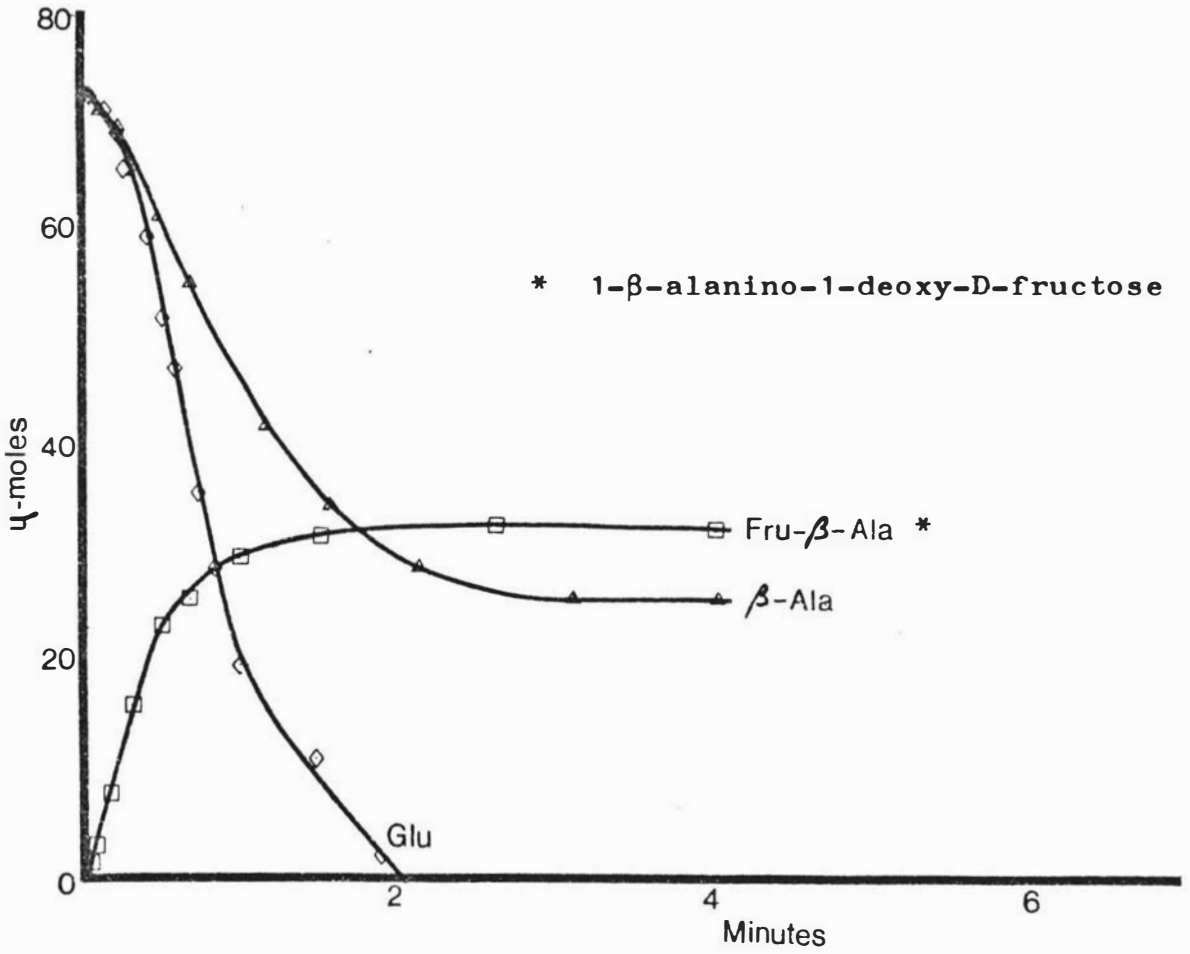


Fig B.2 Progress of the browning reaction between glucose and β-alanine (1:1) at 120° in the solid state.

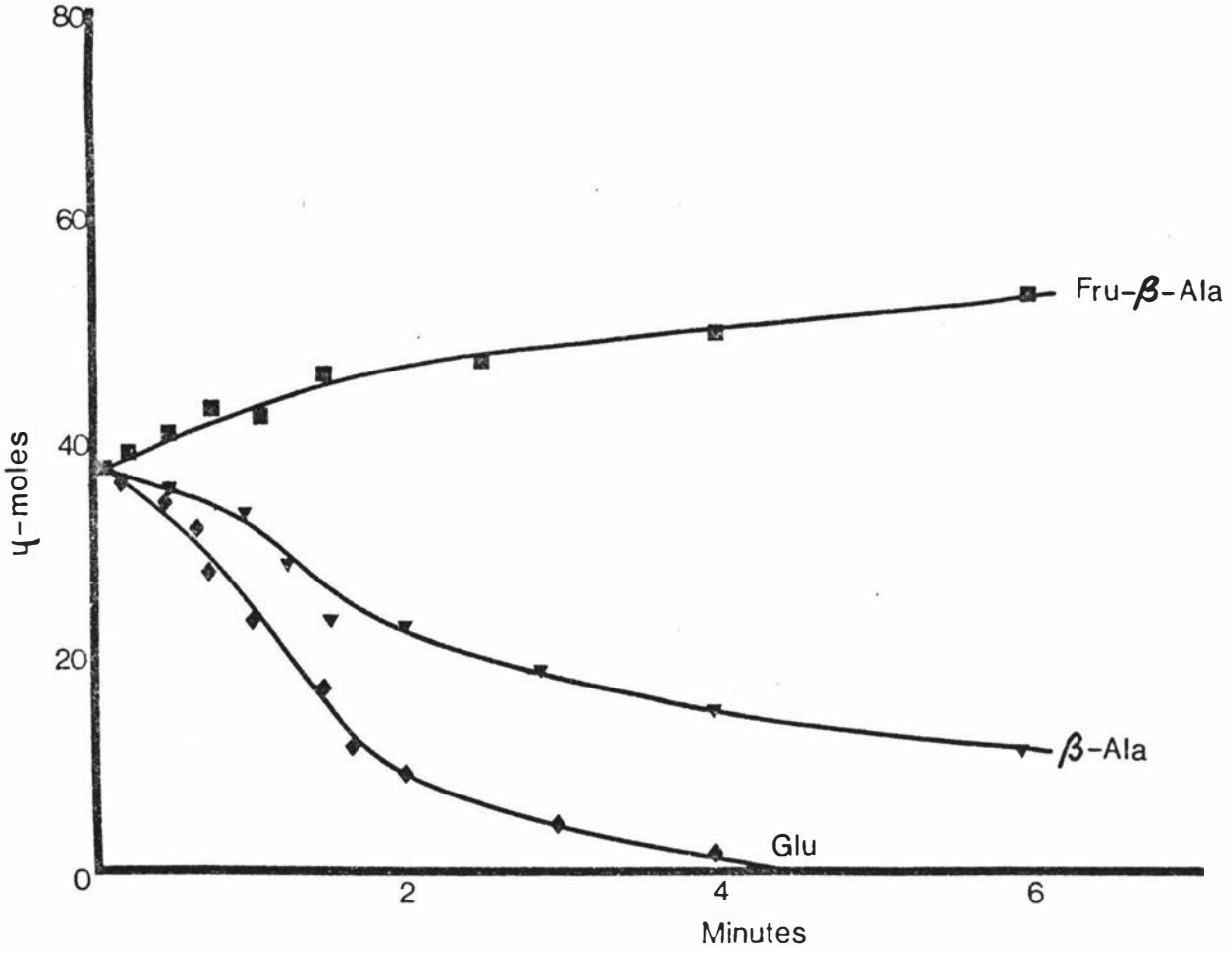


Fig B.3 Progress of the browning reaction between glucose and β-alanine in the presence of 1-β-alanino-1-deoxy-D-fructose (Fru-β-ala) (1:1:1) at 120° in the solid state.

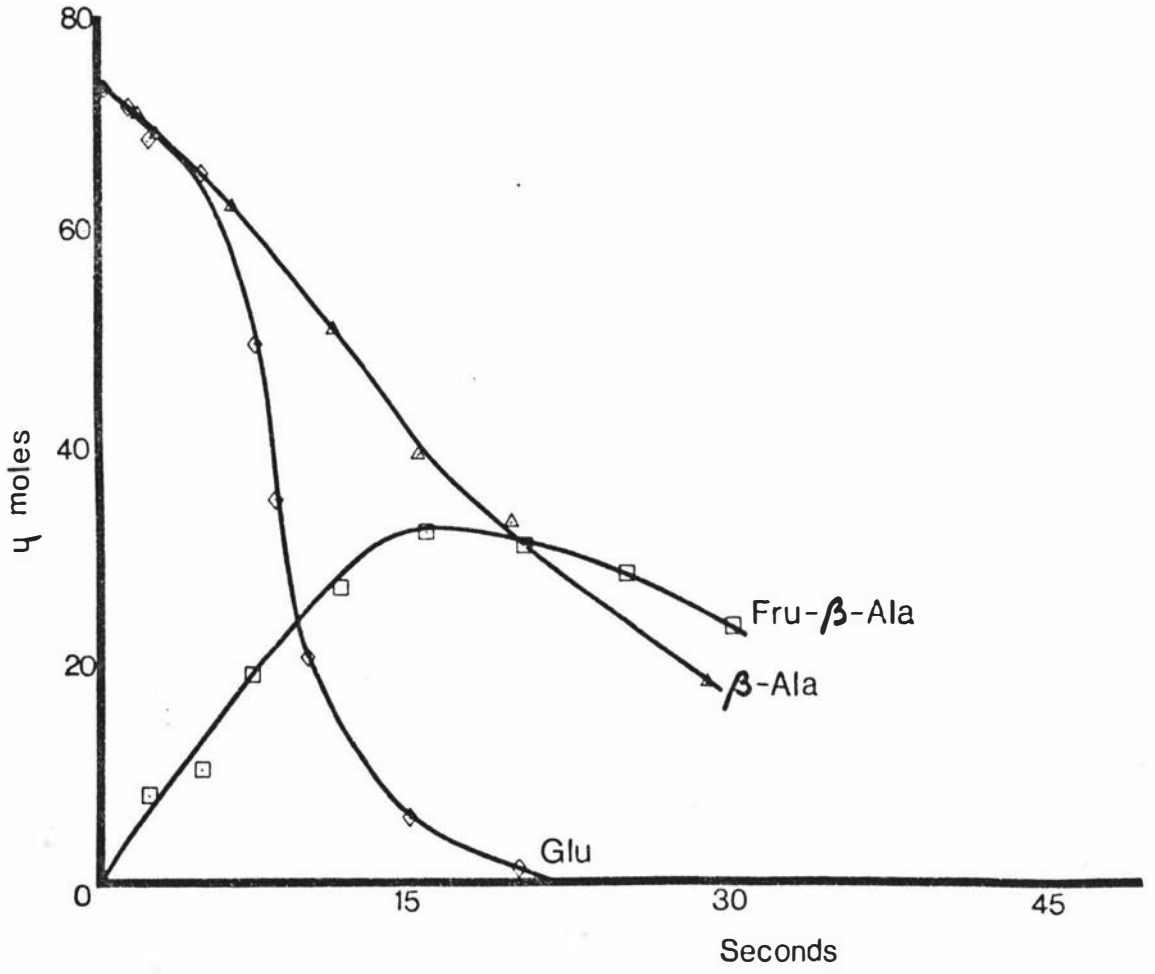


Fig B.4 Progress of the browning reaction between glucose and β-alanine (1:1) at 150° in the solid state.

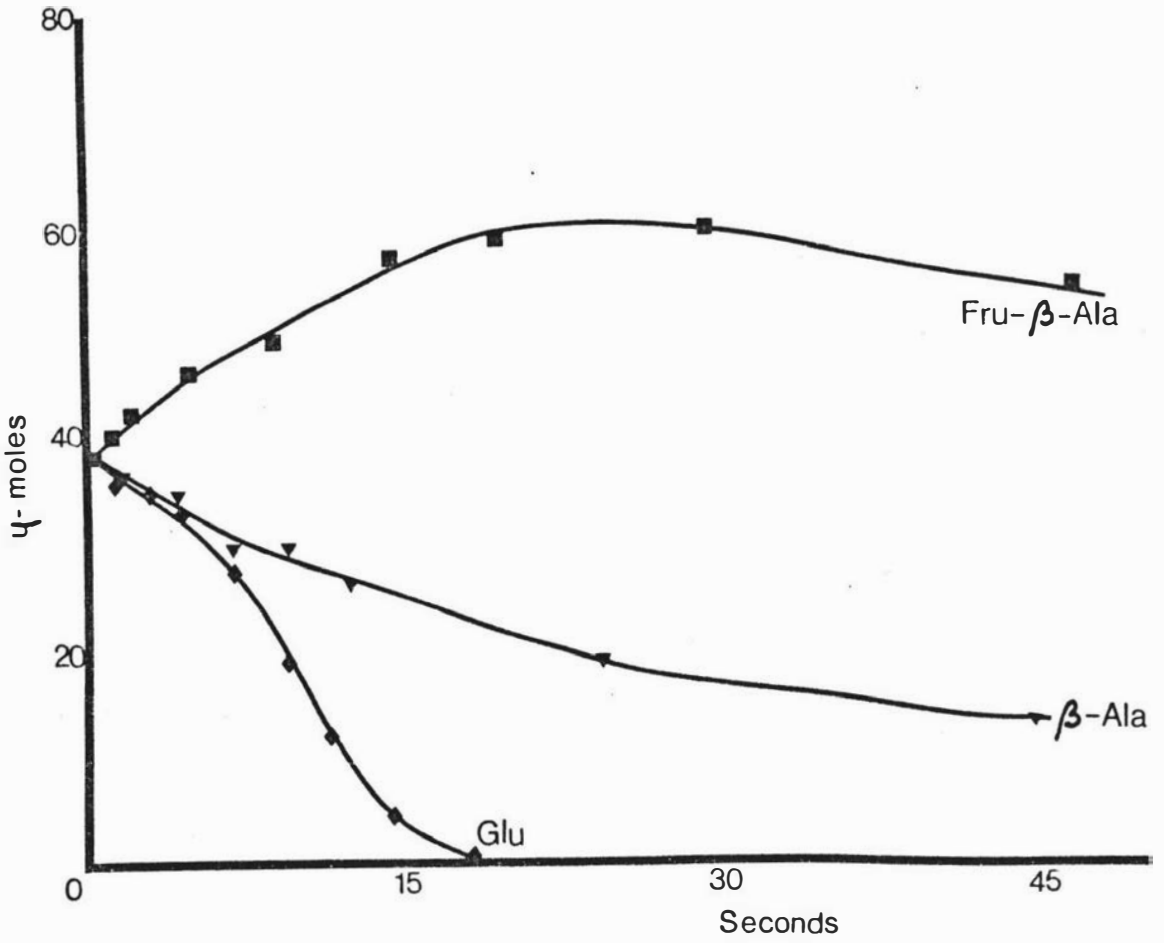


Fig. B.5 Progress of the browning reaction between glucose and β-alanine in the presence of 1-β-alanino-1-deoxy-D-fructose (1:1:1) at 150° in the solid state.

β-alanine is almost linear with respect to time while there is glucose left for reaction. A similar situation has been found in solution (28) and explained by suggesting that a rate-limiting step, with respect to the browning reaction as a whole, occurs early in the reaction pathway. 3-Deoxy-D-erythro-hexosulose has been demonstrated to react rapidly with amines (see table B.2), and difructose-amino acids are very labile compounds (26). Hence the rate limiting step during browning seems likely to be the reaction between glucose and the monoketoseamine to yield the difructose compound.

The initial reaction in the browning pathway is the reaction between glucose and β-alanine to form the monoketoseamine. The effect of adding pre-formed 1-β-alanino-1-deoxy-D-fructose on the rate of browning at the two temperatures can be seen by comparing the percentage of glucose remaining as a function of time for the two systems (Figs B.6 and B.7). At 120°, when the reactants are all initially present in the solid state, the presence of added Amadori compound appears inhibitory. When solid state effects have been eliminated (150°) the rate of glucose loss is equivalent in both systems which suggests that the formation of monoketoseamine is not a rate-limiting step in these browning reactions. This evidence is consistent with the reaction between glucose and the 1-amino-1-deoxyketose being the slow step. The reaction between glucose and an amino acid (e.g. β-alanine (pkb 10.24), glycine (pkb 9.78) is likely to be more rapid than that between glucose and a 1-amino-1-deoxyketose (e.g. 1-deoxy-1-glycino-D-fructose has pkb 8.4 (26)) due to the different basicities of the amines, an effect previously noted in solution (71). The isomerisation of D-glucose to D-fructose on heating at 110° in the presence of a basic catalyst in the

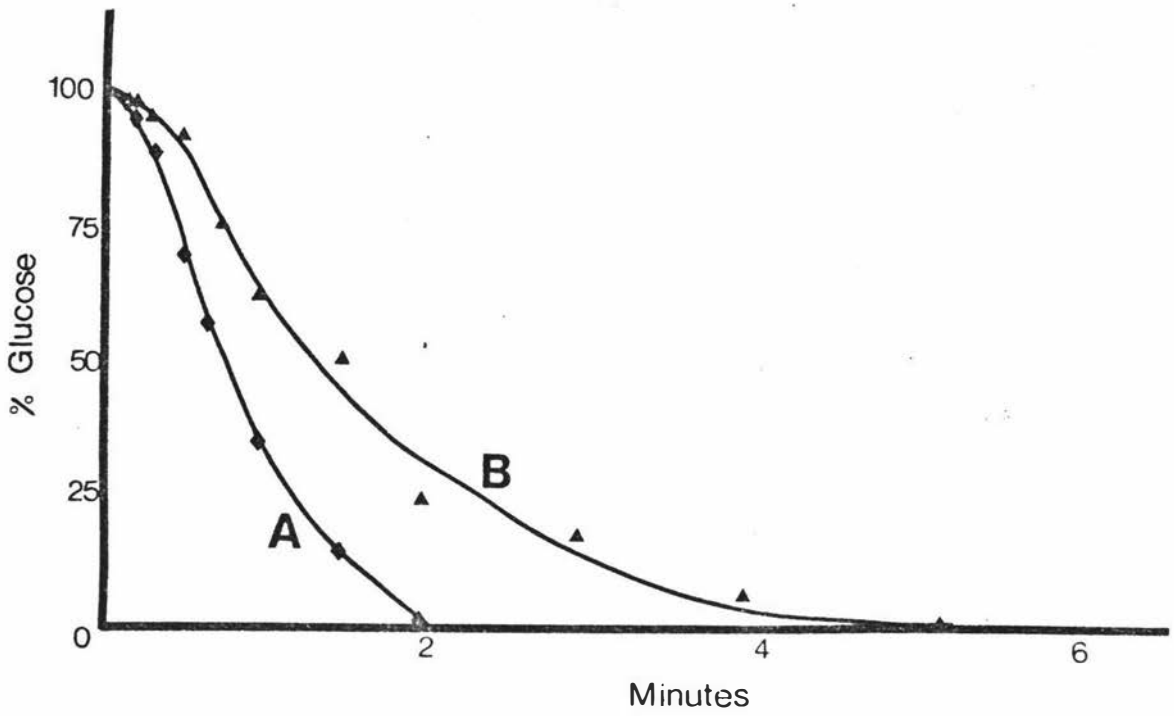


Fig. B.6 Disappearance of Glucose during browning at 120° in the solid state: A, glucose + β-alanine (1:1): B, glucose + β-alanine + 1-β-alanino-1-deoxy-D-fructose (1:1:1).

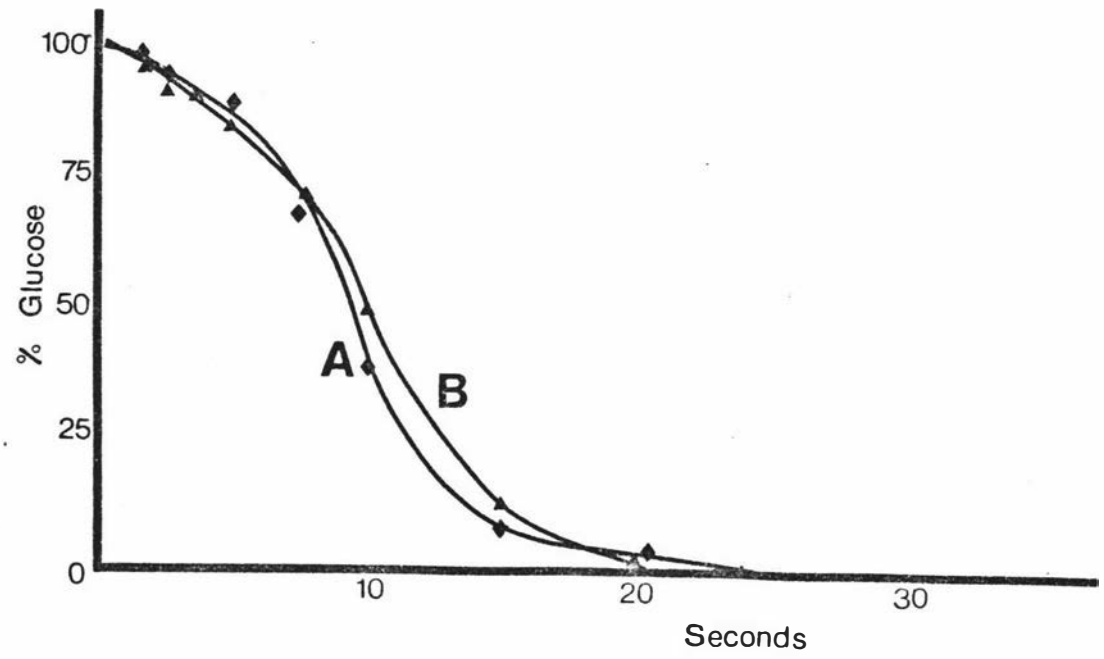


Fig B.7 Disappearance of Glucose during browning at 150° in the solid state: A, glucose + β-alanine (1:1): B, glucose + β-alanine + 1-β-alanino-1-deoxy-D-fructose (1:1:1).

solid state has been reported (40). In a similar manner a base-catalysed enolisation may promote the reaction between glucose and an amino acid or a 1-amino-1-deoxyketose at temperatures below the melting point of glucose. From preliminary experiments the observed ease of browning of the amino acids with glucose followed the order: β-alanine > glycine > 1-β-alanino-1-deoxy-D-fructose i.e. of greater to lesser basicity.

Colour development (measured as the change in absorbance at 490 nm) along with the increase in the most intensely absorbing U.V. species (figs. B.8 and B.9) display a definite lag or "induction" phase before browning becomes significant (when glucose is 50% degraded). An increase in the relative amount of 1-β-alanino-1-deoxy-D-fructose leads to a decrease in absorbance in all cases, showing its relative stability in the browning reaction. The maximum U.V. absorbance (a typical spectrum is reproduced in fig B.10) occurred at 295 nm instead of the normally observed 283 nm (caused by a build-up of 5-hydroxy-methyl-2-furaldehyde (72, 73)). A maximum at 295 nm has been observed previously in a glucose-glycine derived browning system (69) where it was considered due to an addition reaction between 1-deoxy-1-glycino -D-fructose and glycine. Substituted pyrroles having U.V. absorbance maxima at 295 nm, have also been observed in browning reactions (74). These compounds are produced via a 1,2 enolisation step during the degradation of Amadori intermediates (9).

Weight losses associated with the reactions (figs B.11 and B.12) show the same trends as the absorbance curves, in that they reflect the stability of the 1-amino-1-deoxyketose. If one considers that the losses arise principally from glucose itself until such

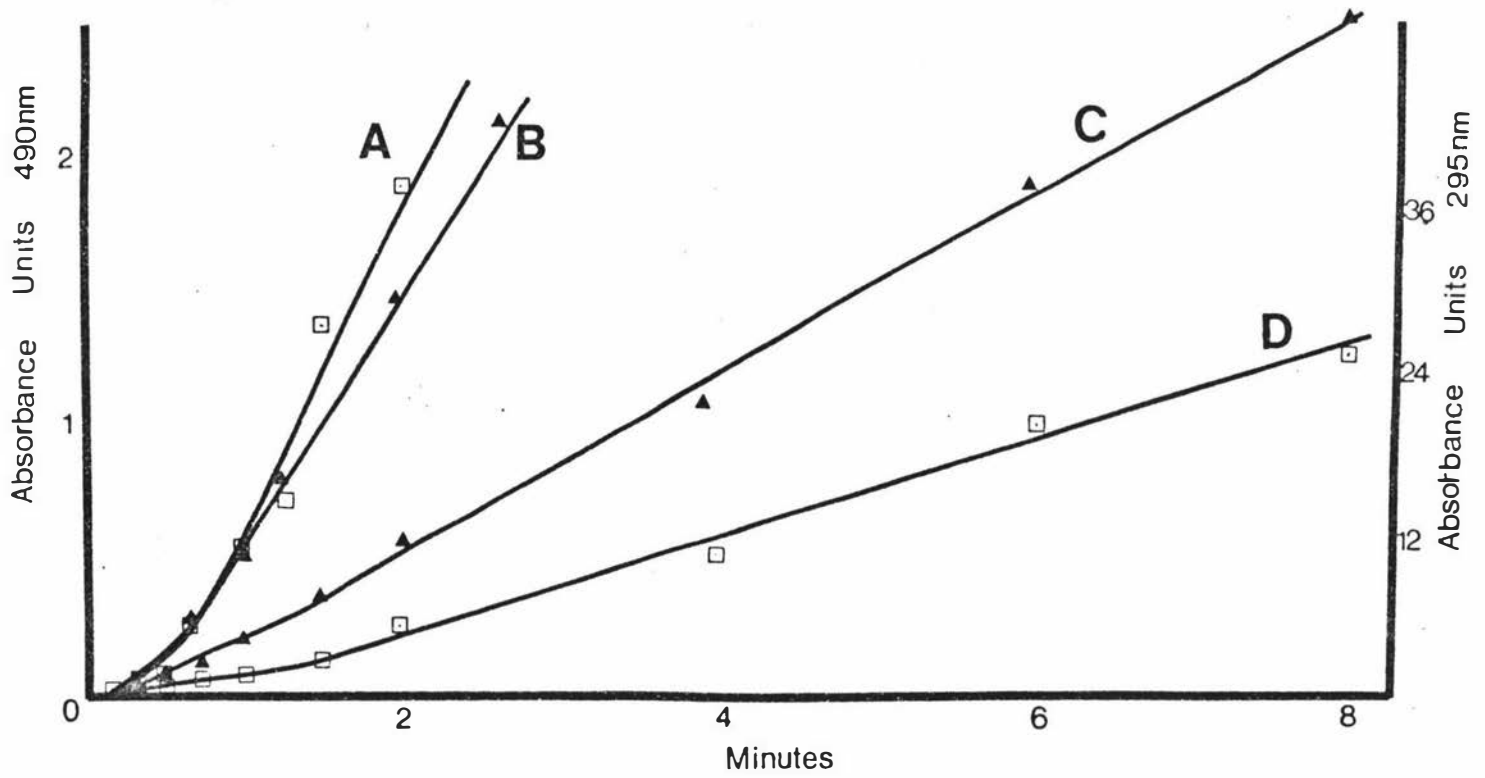


Fig B.8 Change in absorbance during browning at 120°: A, glucose + β-alanine (1:1) 295 nm: B, glucose + β-alanine (1:1) 490 nm: C, glucose + β-alanine + 1-β-alanino-1-deoxy-D-fructose (1:1:1) 490 nm: D, glucose + β-alanine + 1-β-alanino-1-deoxy-D-fructose (1:1:1) 295 nm.

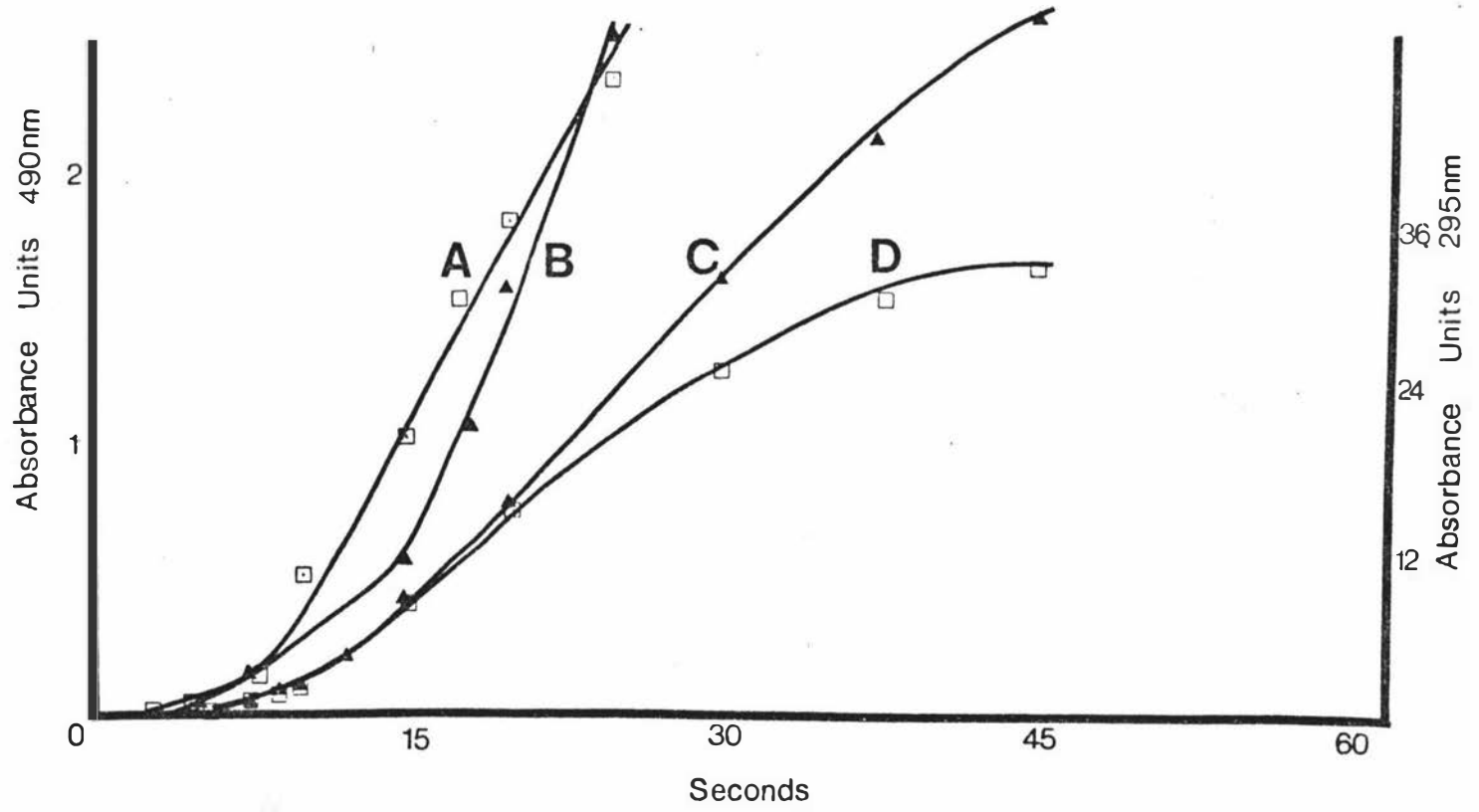


Fig B.9 Change in absorbance during browning at 150°: A, glucose + β-alanine (1:1) 295 nm: B, glucose + β-alanine (1:1) 490 nm: C, glucose + β-alanine + 1-β-alanino-1-deoxy-D-fructose (1:1:1) 490 nm: D, glucose + β-alanine + 1-β-alanino-1-deoxy-D-fructose (1:1:1) 295 nm.

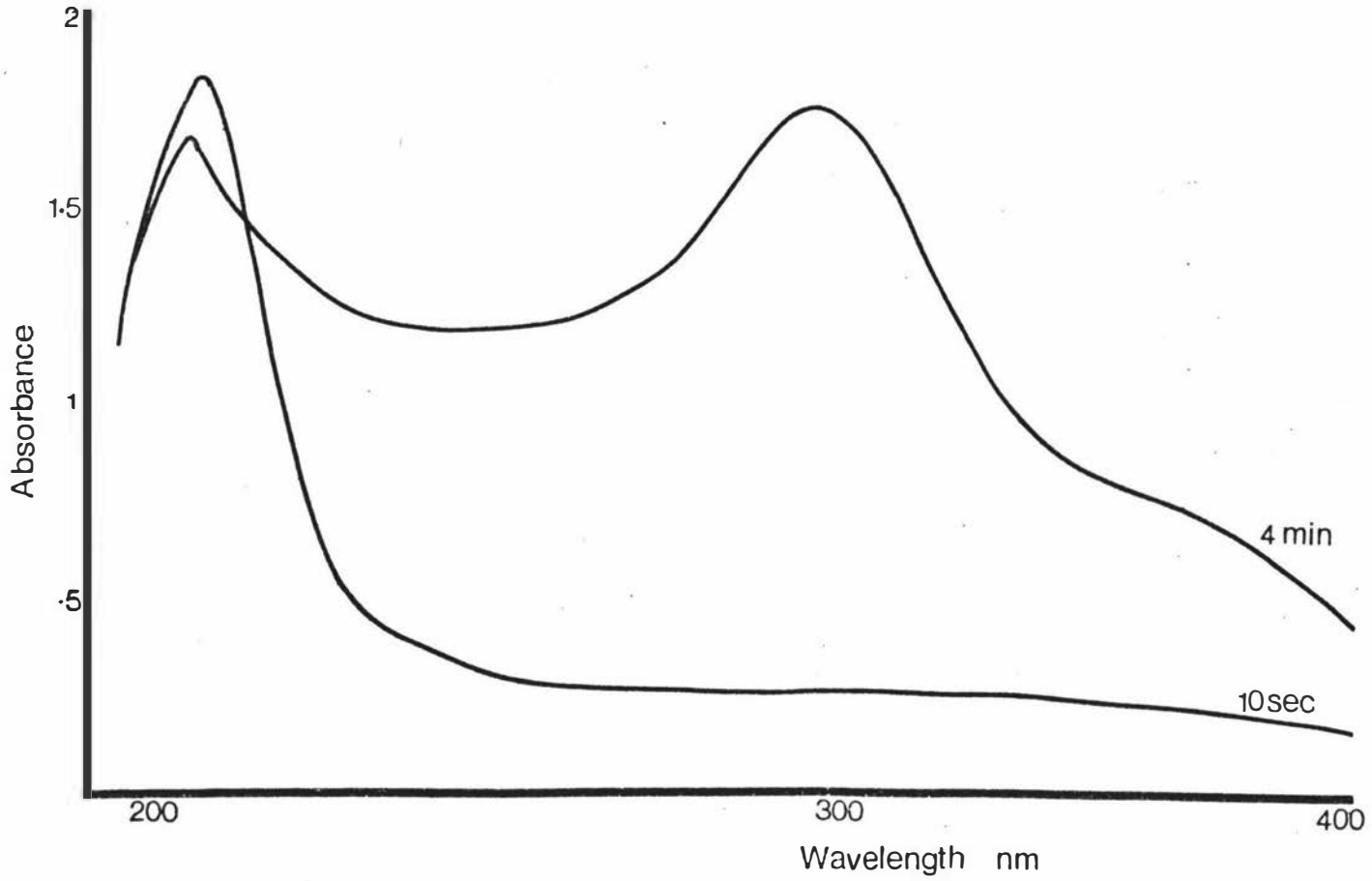


Fig B. 10 U.V. spectrum of browning products obtained from glucose plus β-alanine (1:1) at 120°.

time as all the glucose is consumed, then such losses are explicable in terms of the pathways outlined in fig. B.1. For example, in the system glucose plus β-alanine at 120°, the build-up of 1-β-alanino-1-deoxy-D-fructose reaches a steady-state phase after one minute. At this time 5% of the overall weight has been lost which represents a 10% loss with respect to glucose. This is equivalent to the loss of one molecule of water per molecule of glucose. In this system, visual browning becomes more intense at this point with concurrent gas evolution (the sample displayed considerable frothing from this stage until the reaction was stopped, at which time the sample volume had increased at least fourfold). The "induction" period of the browning reaction may be represented by a build-up of the intermediates to a steady-state phase leading to the formation of colour precursors (reactive intermediates which may be generally represented by the osuloses). The overall reaction during this phase (see fig B.1) can thus be represented as:

Glucose $\xrightarrow{-H_2O}$ 3-deoxy-D-erythro-hexosulose.

The production of 1-β-alanino-1-deoxy-D-fructose involves one molecule each of glucose and β-alanine with the concurrent loss of one molecule of water. The contribution of glucose to the production of 1-β-alanino-1-deoxy-D-fructose (equivalent to the β-alanine consumed (see fig B.2))can be subtracted from the overall glucose lost to yield the relationships shown in fig B.13 for the system glucose and β-alanine at 120°.

The figure shows that, during the first minute of the reaction, for every mole of glucose which is used in the production of 1-β-alanino-1-deoxy-D-fructose there is another mole consumed. As shown earlier (fig B.1) this mole may react with the 1-amino-1-

deoxyketose to eventually form 3-deoxy-D-erythro-hexosulose. After one minute the 1-amino-1-deoxyketose has reached a steady-state concentration (Fig B.2) and, until all the glucose is consumed, the amounts of glucose and β-alanine lost are comparable. During this phase the reactive carbohydrate-derived compounds (represented by 3-deoxy-D-erythro-hexosulose) promote the "secondary" phase of the browning reaction and the rate of browning increases (see Fig B.8). Thus: 3-deoxy-D-erythro-hexosulose + amines ⟶ browning products.

A plot of log α against time (Fig B.14) for β-alanine gives a straight line over the interval 10 to 120 seconds, showing that the loss of β-alanine follows a first order rate law. In a kinetic appraisal of the browning reaction between glucose and glycine in solution (72), the reaction was found to be first order with respect to glycine and contained a half-order relationship with respect to glucose during the "induction phase".

All of the evidence presented in this section thus parallels the findings for the browning of glucose-amino acid systems in solution and indicates the relative stability of the 1-amino-1-deoxyketose in the dry-state at temperatures up until its spontaneous decomposition can occur.

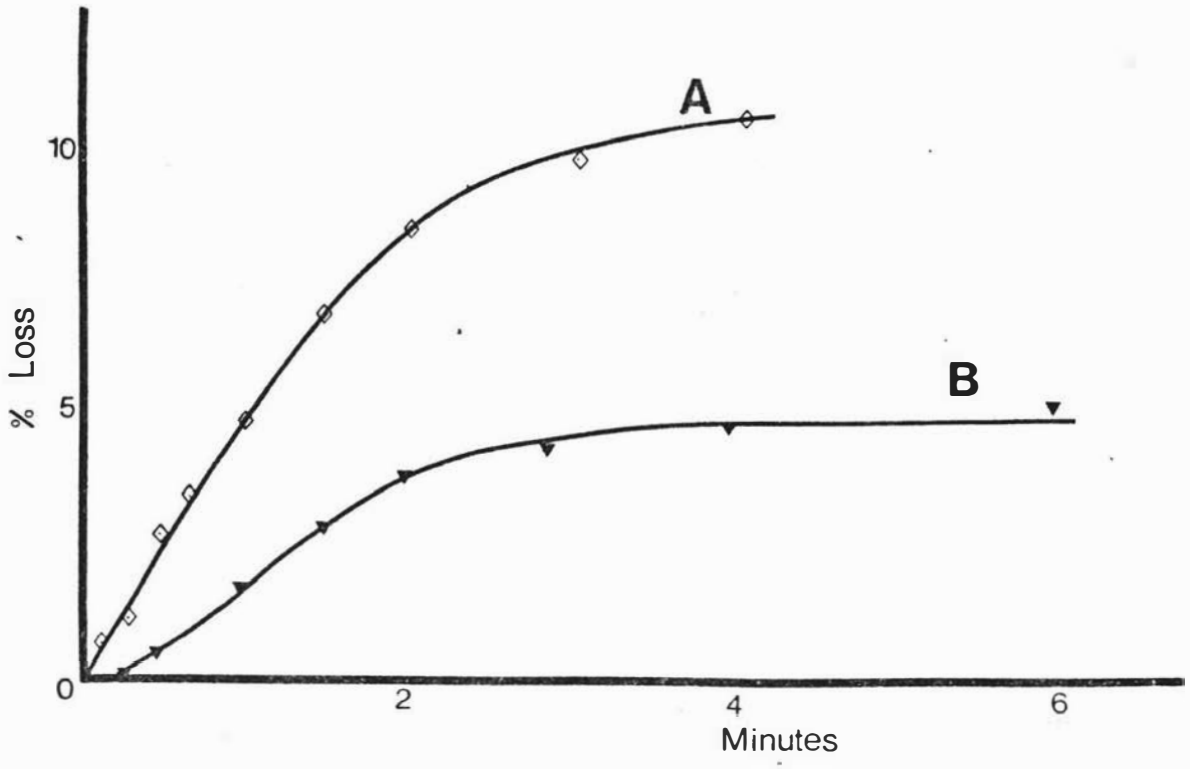


Fig B.11 Weight losses during browning at 120° in the solid state for A, glucose + β-alanine (1:1) and B, glucose + β-alanine + 1-β-alanino-1-deoxy-D-fructose (1:1:1).

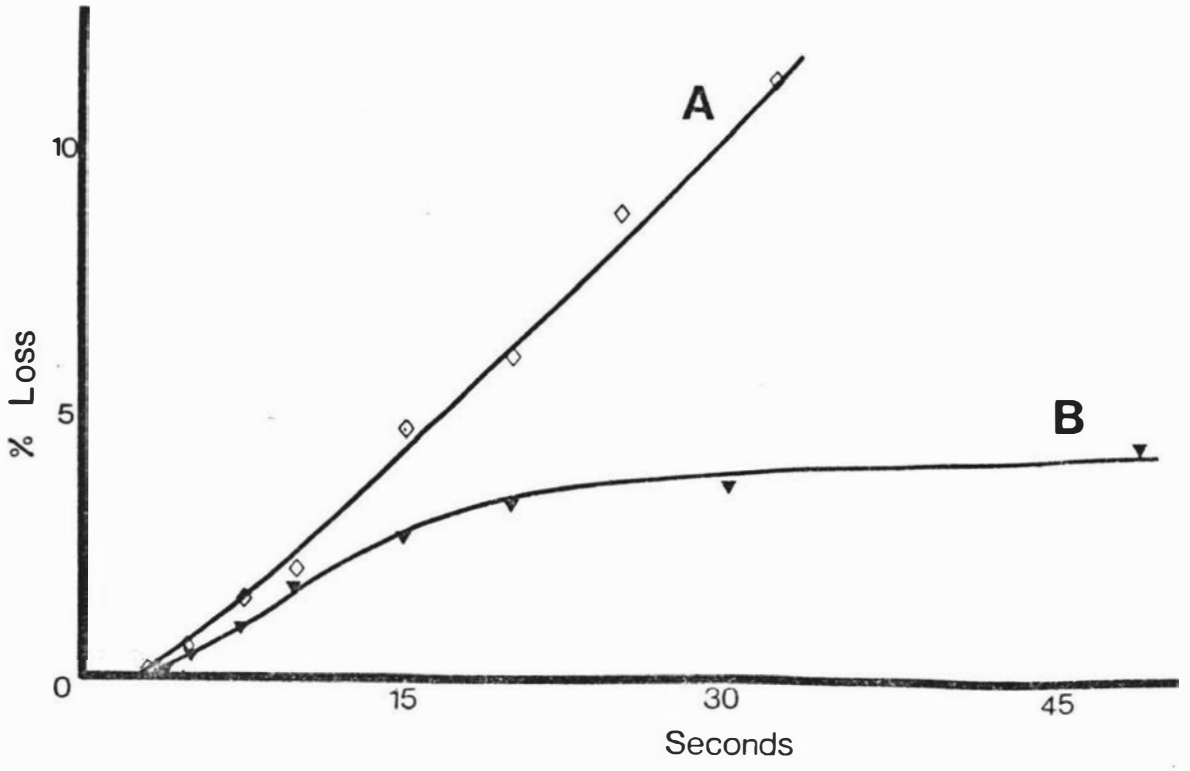
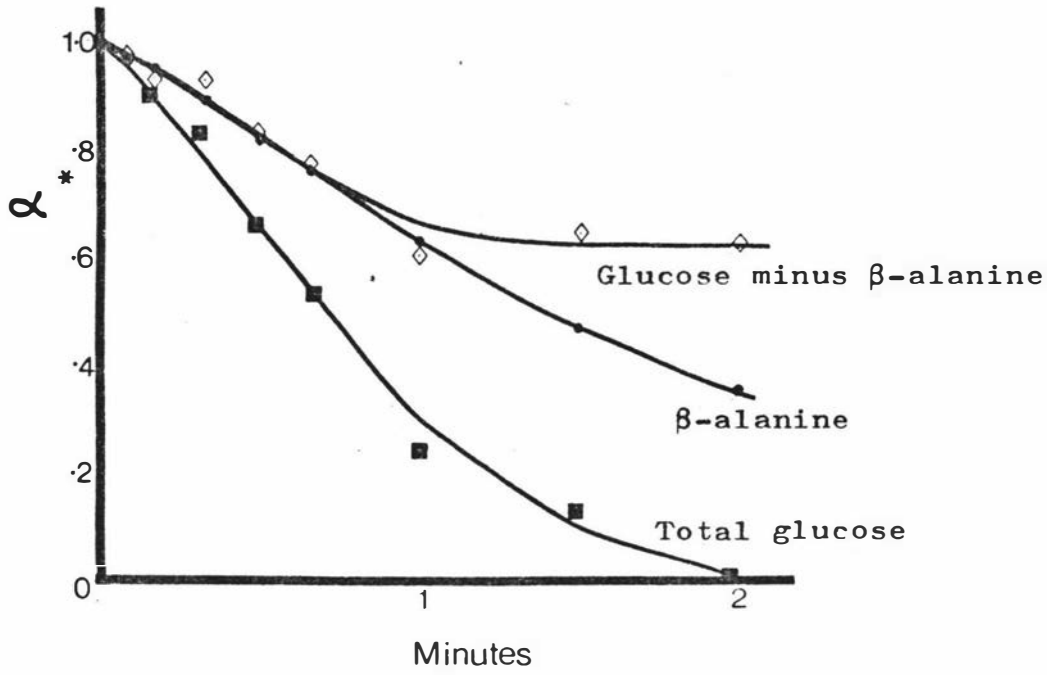


Fig B.12 Weight losses during browning at 150° in the solid state for A, glucose + β-alanine (1:1) and B, glucose + β-alanine + 1-β-alanino-1-deoxy-D-fructose (1:1:1).



* α = (a-x) where a=1, x=fraction reacted

Fig B.13 Relationship between the disappearance of glucose and β-alanine during browning at 120°C in the solid state.

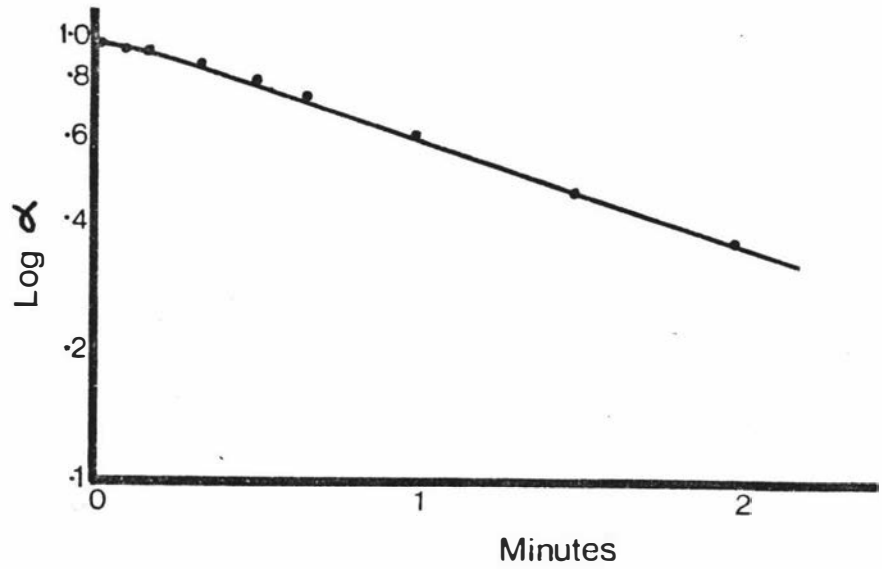


Fig B.14 First order plot for the disappearance of β-alanine during browning of glucose + β-alanine (1:1) at 120° in the solid state.

PART C: Studies on the effect of changing the amine moiety on the degradation patterns of 1-amino-1-deoxyketoses derived from D-glucose

C.1 SUMMARY

The thermal degradation of 1-amino-1-deoxy-fructoses derived from different classes of amines is reported. The nature of the amino moiety was found to influence the types of degradation products in two ways. Firstly the base contributes in that it is itself the source of some products. Secondly the base influences the decomposition of the carbohydrate moiety. This influence depends on both the basicity of the amine and on its reactivity towards the carbohydrate-derived intermediates. 1,2 enolisation and rearrangement reactions leading to considerable charring are dominant when Amadori compounds are derived from weak bases. Fragmentation reactions and the 2,3 enolisation pathway are more important in the degradation of Amadori compounds derived from strong bases. An exception is that with amino acids the presence of the carboxyl group strongly influences the degradation processes by promoting 1,2 enolisation and charring.

C.2 INTRODUCTION

Glucose, in the absence of amines, undergoes thermal decomposition principally via a 1,2 enolisation mechanism to yield furfurals and scission products derived from 3-deoxy-D-erythro-hexosulose (45, 66). In this case the 2,3 enolisation pathway only operates to a minor extent (7).

The degradation of monoketoseamines (Amadori compounds) derived from primary amines has been reported to commence with a 1,2 enolisation (6), see fig C.1, and the range of volatiles isolated in the present study from the pyrolysis of 1-deoxy-1-glycino-D-fructose and 1-β-alanino-1-deoxy-D-fructose supports this. 2,3 enolisation can occur however, as is shown by the formation of pyrones (21), when certain Amadori compounds derived from primary amino acids were pyrolysed.

It has been suggested that decomposition of monoketoseamines derived from secondary bases commences with a 2,3 enolisation (8), see fig C.2. In the pyrolysis of 1-deoxy-1-prolino-D-fructose, (20) evidence was also found for the operation of the 1,2 enolisation pathway. 2,3 enolisation and amine elimination are highly favoured with protonated, strongly basic tertiary amino groups at C-1, as would be provided, for example, by Amadori compounds of piperidine, pipecolic acid, pyrrolidine and proline (75). It has been suggested (76) that the yield of the 2,3 enolisation-derived fragrant caramel O-heterocyclics may be limited by the recondensation of amines at C-3 of the hexosone produced by 2,3 enolisation of the Amadori compound. This suggestion has been adopted (19, 73) as a modification to the 2,3 enolisation pathway, operative when strongly basic

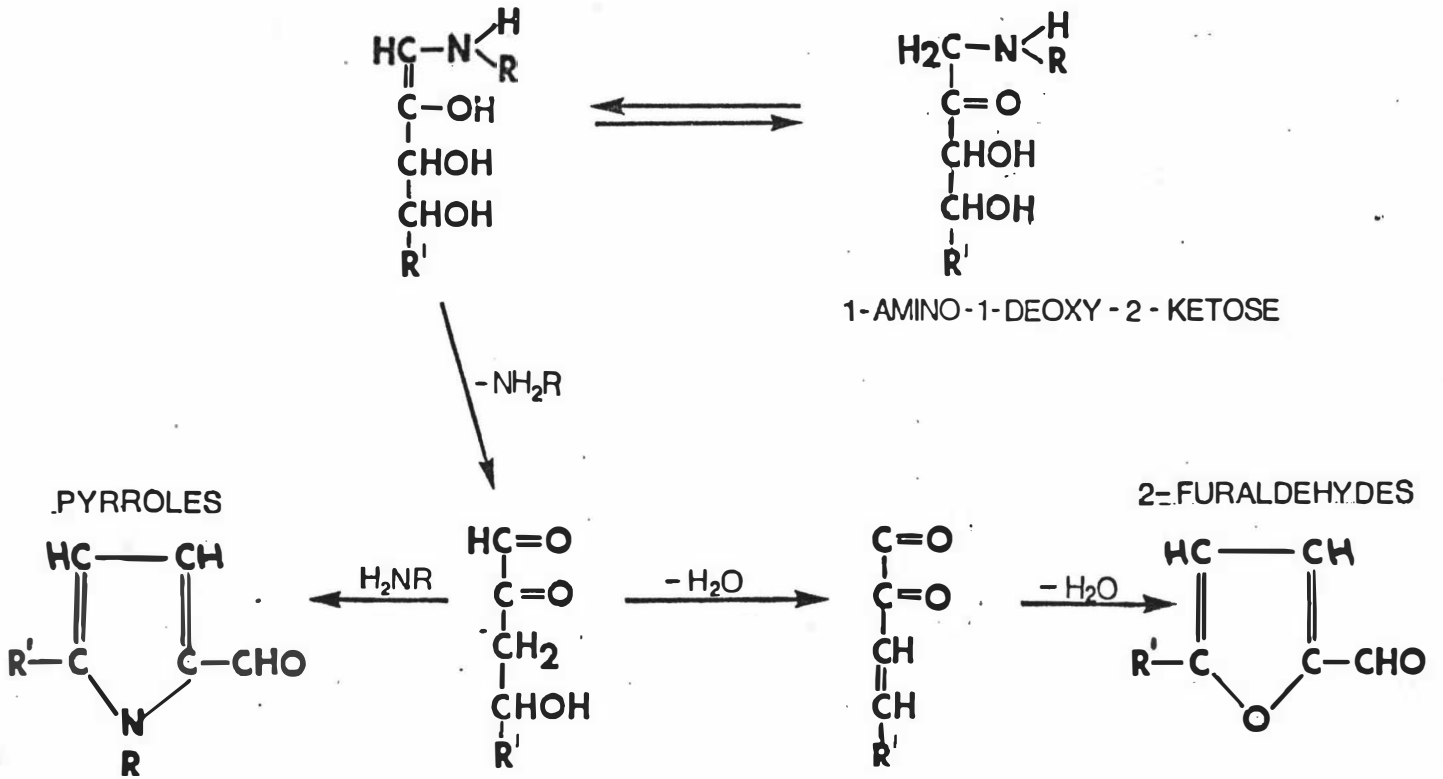


Fig C.1 Degradation of monoketoseamines via 1,2 enolisation

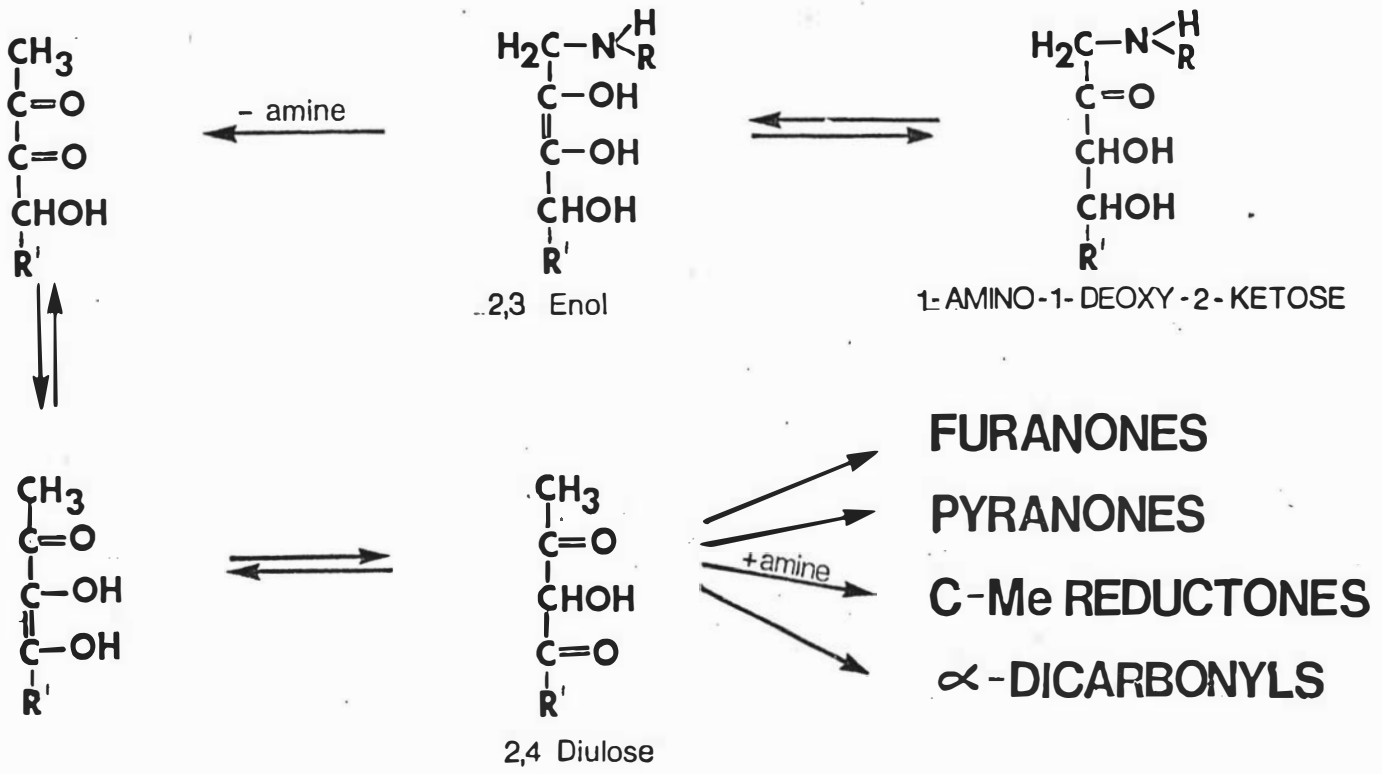


Fig C.2 Degradation of monoketoseamines via 2,3 enolisation.

Amadori compounds decompose, to account for the formation of the less fragrant 4-carbon reductones isolated from the pyrolysis of 1-deoxy-1-piperidino-D-fructose. From a comparison of volatile products from the decomposition of this compound and 1-deoxy-1-prolino-D-fructose (20) the authors observed that the more weakly basic secondary-amine derivative (proline) produced smaller amounts of fragmentation products and more 6-carbon enolones. They found no 4-carbon amino reductones from 1-deoxy-1-prolino-D-fructose.

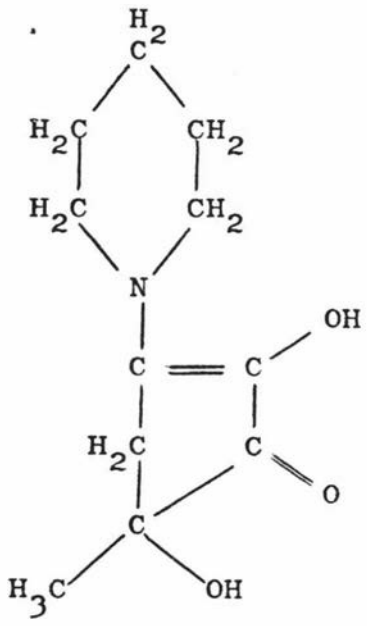
The literature thus points to two areas that merit further investigation:

i) Do the degradation pathways of monoketoseamines follow a simple relationship dependent on the basicity of the amine substituent?

ii) Do differences in the class of amines play an important role apart from their basicities? In this context differences between primary and secondary amines and amino acids may be evident.

In this study the pyrolysis of five Amadori compounds derived from glucose are described using the experimental approach employed in Part A. Bases employed in their synthesis were morpholine, dibenzylamine and N-methylaniline (secondary bases), p-toluidine (a primary base) and the amino acid, proline. These bases extend the range of basicities and types of amine substituents investigated to date. Data on general aspects of the thermal decomposition of the 1-amino-1-deoxyfructoses is presented as well as comparisons of a more specific nature based on organic volatile analysis. In addition the thermal degradation of piperidino-hexose-reductone (v) produced from 1-deoxy-1-piperidino-D-fructose by the elimination of two molecules of water (77), is

described. This reductone was included since it is readily isolated from a browning mixture of glucose and piperidine and is the major 2,3 enolisation product isolated from the low temperature pyrolysis of 1-deoxy-1-piperidino-D-fructose (73, 77). Analysis



(v)

of 1-deoxy-1-piperidino-D-fructose itself was not included in this study as difficulties were encountered in isolating a product sufficiently pure for pyrolytic purposes.

C.3 THERMAL ANALYSIS BY D.S.C., T.G. AND D.T.G.

C.3.1 Experimental

C.3.1.1 Amadori compounds: Previously published synthetic procedures were employed to prepare 1-deoxy-1-morpholino-D-fructose (78), 1-deoxy-1-dibenzylamino-D-fructose (78), 1-deoxy-1-p-toluidino-D-fructose (79), 1-deoxy-1-N-methylanilino-D-fructose (80), 1-deoxy-1-prolino-D-fructose (20) and piperidino-hexose-reductone (77). These preparations are detailed in Appendix 2.

Physical properties (for I.R.'s see Appendix 2):

1-deoxy-1-morpholino-D-fructose:

M.pt 146-148° (decomp)

Found	: C, 48.2%;	H, 7.9%;	N, 5.8%
Calc. for $C_{10}H_{19}NO_6$	: C, 48.2%;	H, 7.6%;	N, 5.6%

1-deoxy-1-dibenzylamino-D-fructose:

M.pt 159-161°

Found	: C, 66.5%;	H, 7.2%;	N, 4.0%
Calc. for $C_{20}H_{25}NO_5$	: C, 66.9%;	H, 7.0%;	N, 3.9%

1-deoxy-1-p-toluidino-D-fructose:

M.pt 153-154°

Found	: C, 58.0%;	H, 7.4%;	N, 5.3%
Calc. for $C_{13}H_{18}NO_5$	: C, 58.0%;	H, 7.4%;	N, 5.2%

1-deoxy-1-N-methylanilino-D-fructose:

M.pt 148-152°

Found	: C, 58.1%;	H, 7.3%;	N, 5.2%
Calc. for $C_{13}H_{18}NO_5$	: C, 58.0%;	H, 7.4%;	N, 5.2%

1-deoxy-1-prolino-D-fructose:

M.pt 113-115° (decomp)

Found : C, 46.8%; H, 7.4%; N, 4.8%

Calc. for $C_{11}H_{19}NO_7.CH_3OH$: C, 46.9%; H, 7.5%; N, 4.6%

piperidino-hexose-reductone:

M.pt 228-230°, λ max (H_2O) 309, zero optical activity

Found : C, 62.6%; H, 8.1%; N, 6.8%

Calc. for $C_{11}H_{17}NO_3$: C, 62.6%; H, 8.1%; N, 6.6%

C.3.1.2 Thermal analysis: Samples for analysis were prepared as described in section A.3.1.2 and the d.s.c. traces were similarly recorded.

T.g. and d.t.g. data were obtained on a Stanton-Redcroft t.g. -750 Thermobalance using shallow platinum crucibles. Samples (1-4 mg) were heated at 10°/min under a nitrogen atmosphere (flow rate 2 ml/min).

C.3.2 Results and discussion

D.s.c., t.g. and d.t.g. data for the thermal decomposition of the compounds under study are presented in figs C.3 to C.7. The d.s.c. traces show that for the proline and morpholine derivatives decomposition accompanies melting, while for the other three 1-amino-1-deoxyketoses a distinct lag exists between the melt and decomposition peaks. The proline Amadori compound has a different decomposition pattern from the others in that once decomposition has commenced, endothermic reactions appear to be balanced by exothermic ones (these reactions may have low ΔH values) and the trace returns to the baseline, whereas decomposition is clearly endothermic for the p-toluidine, N-methyl-aniline and dibenzylamine derivatives.

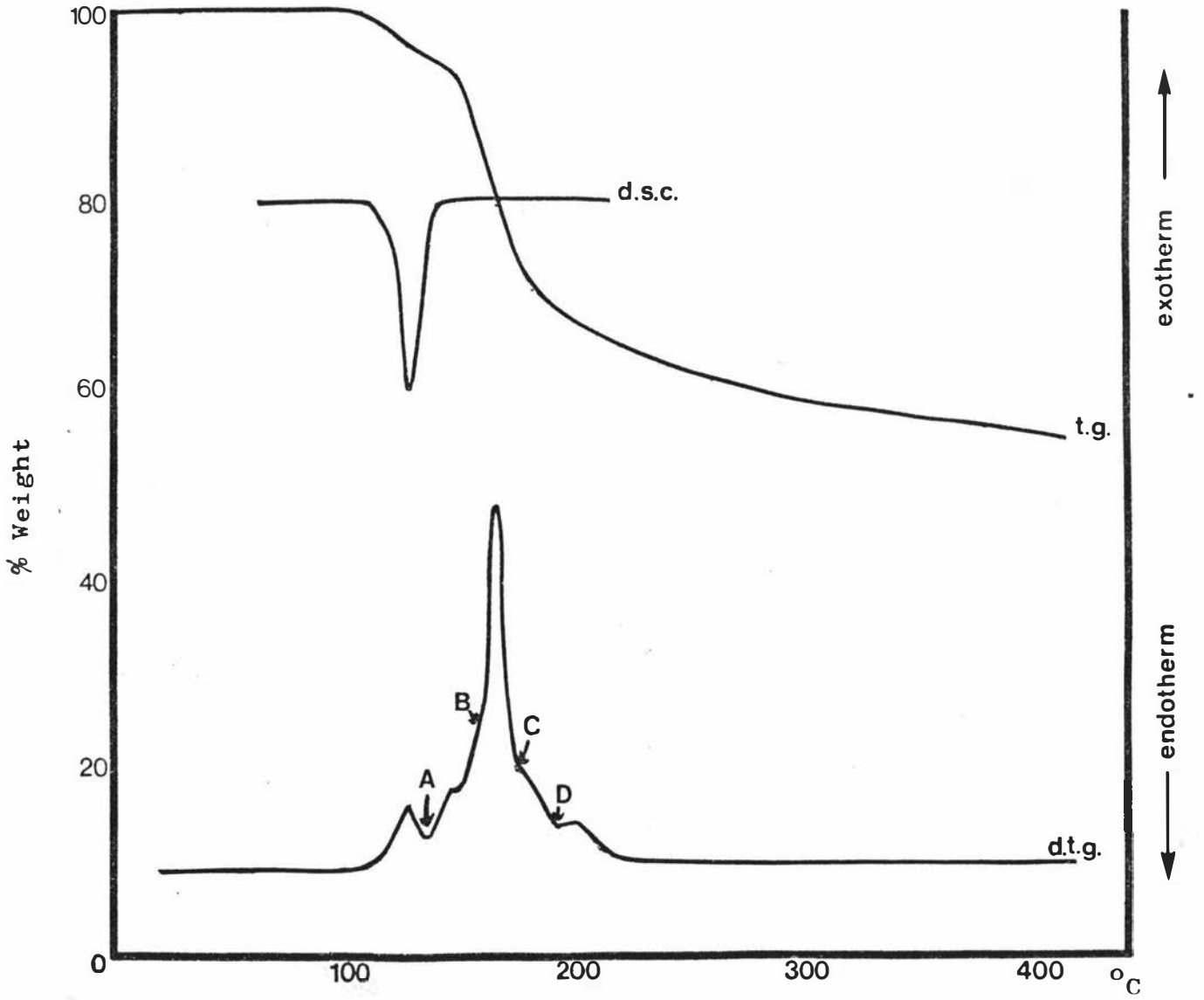


Fig C.3 Thermogram for the decomposition of 1-deoxy-1-prolino-D-fructose

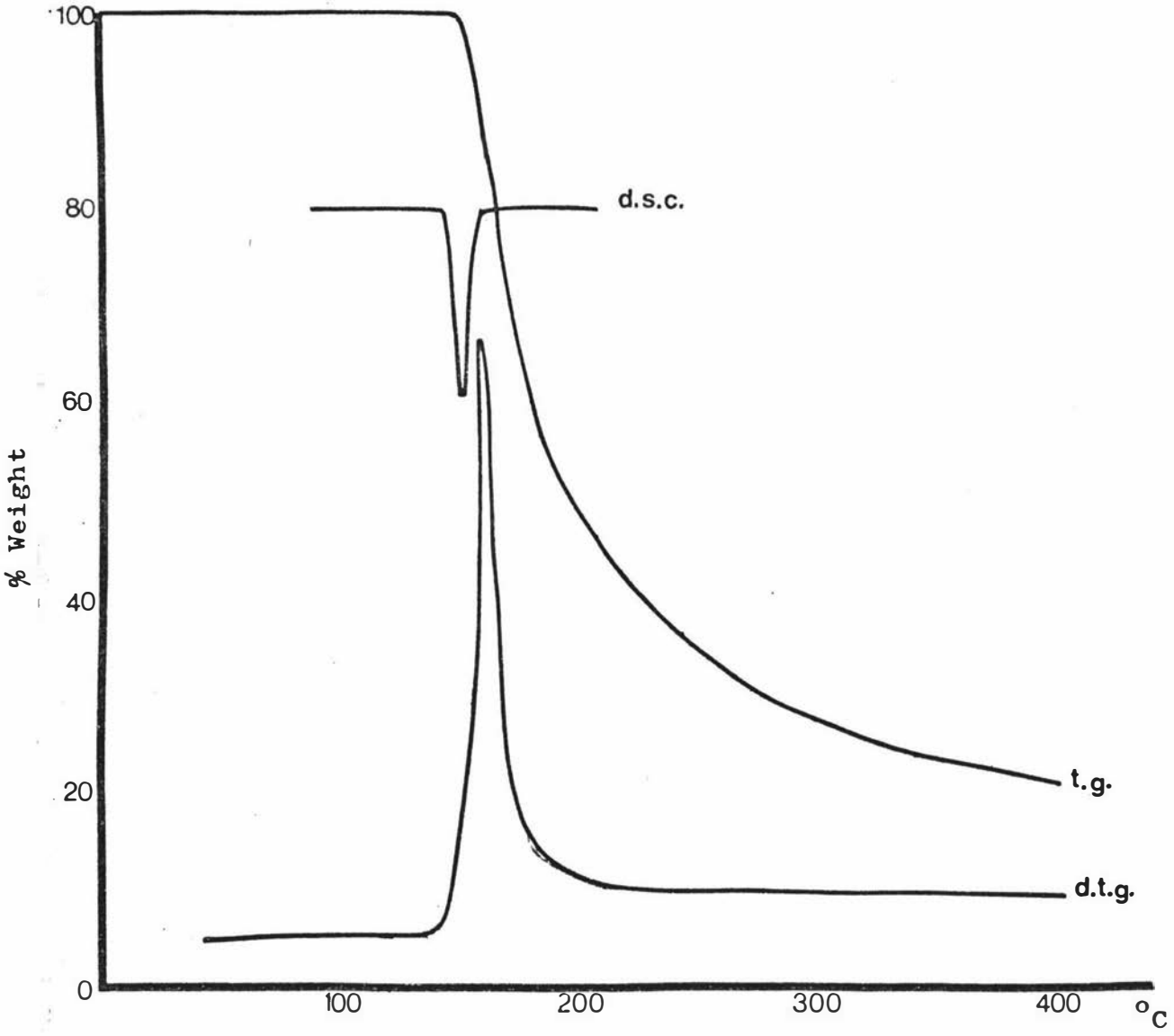


Fig C.4 Thermogram for the decomposition of 1-deoxy-1-morpholino-D-fructose

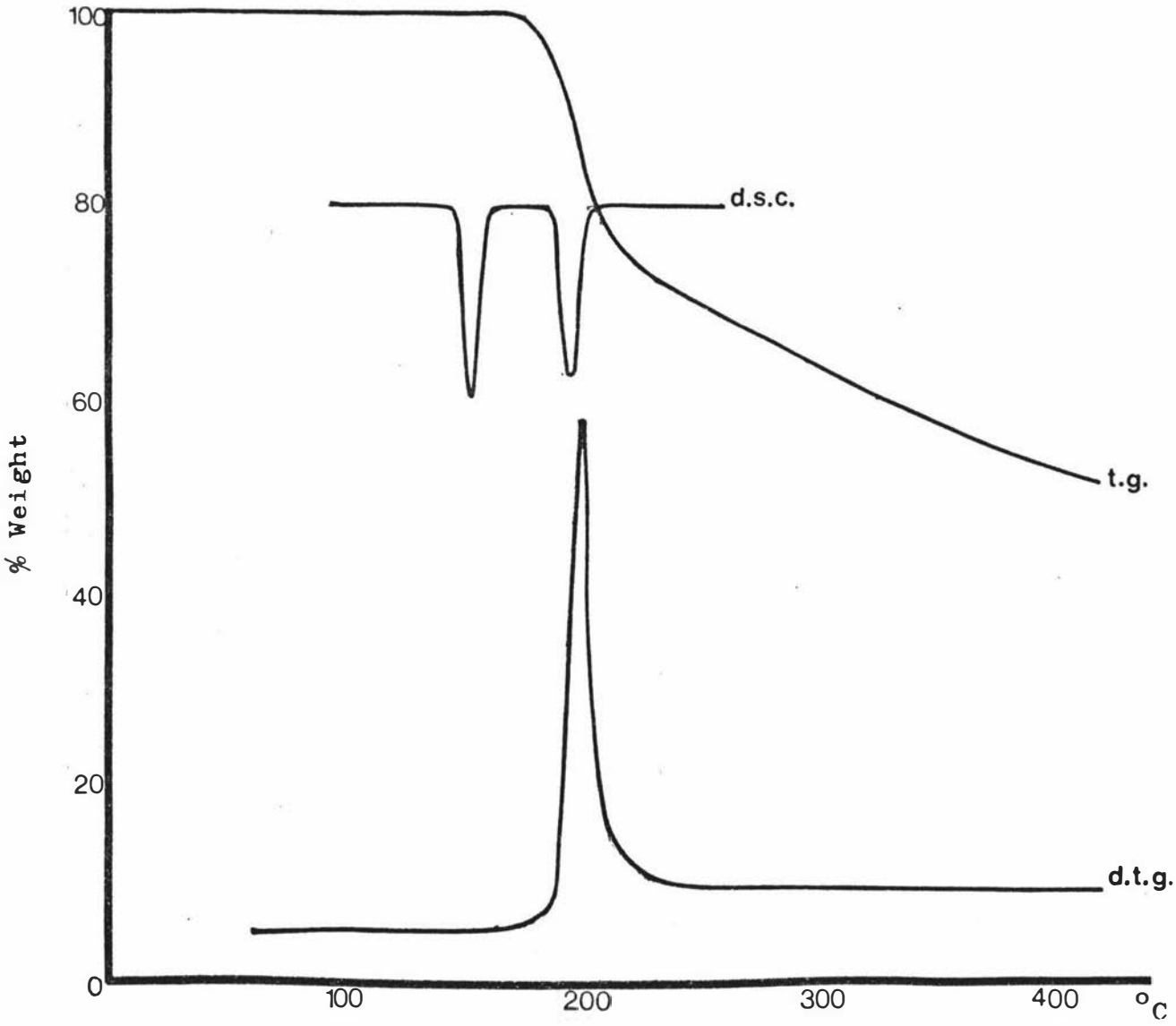


Fig C.5 Thermogram for the decomposition of 1-deoxy-1-p-toluidino-D-fructose

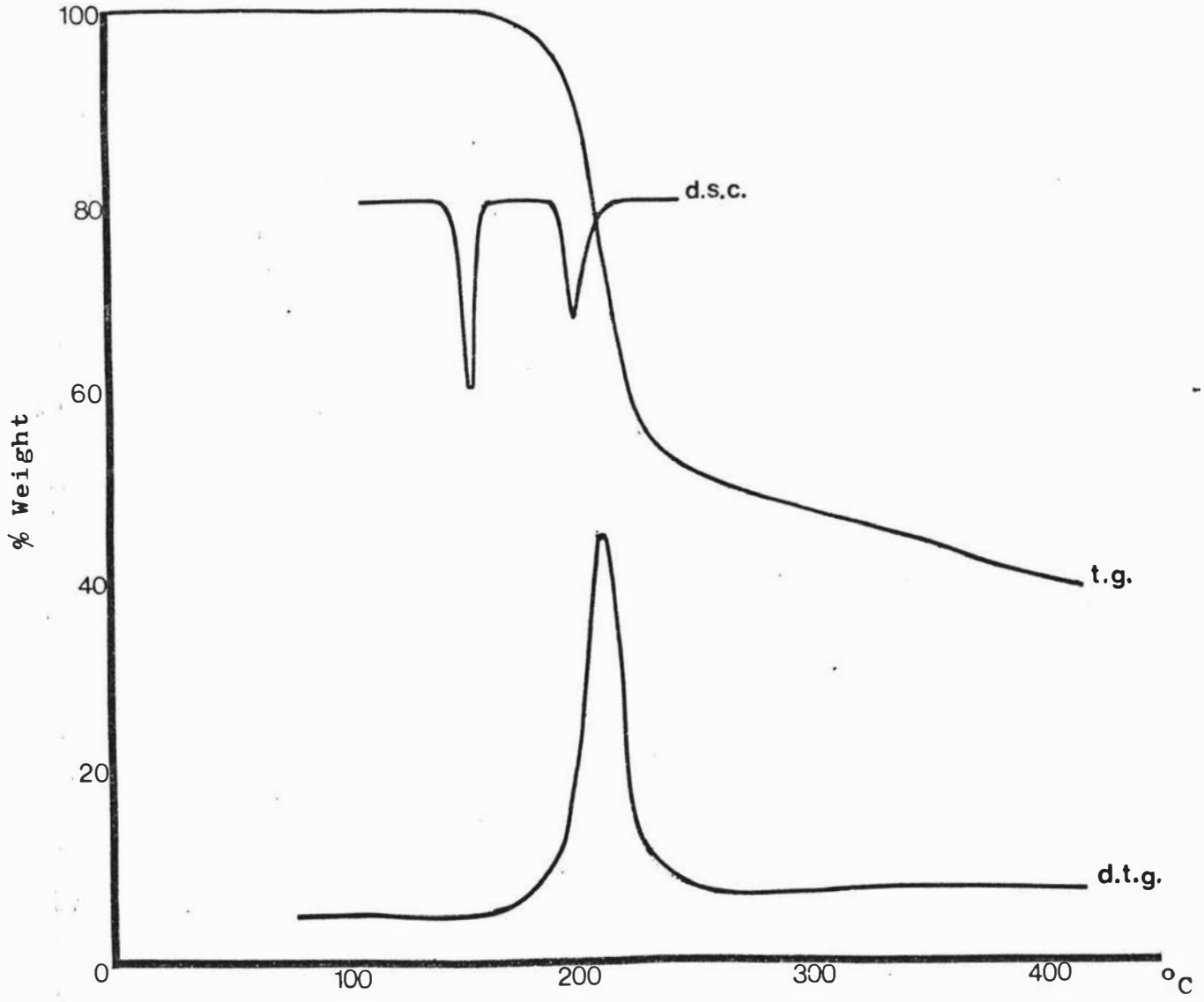


Fig C.6 Thermogram for the decomposition of 1-deoxy-1-N-methylanilino-D-fructose

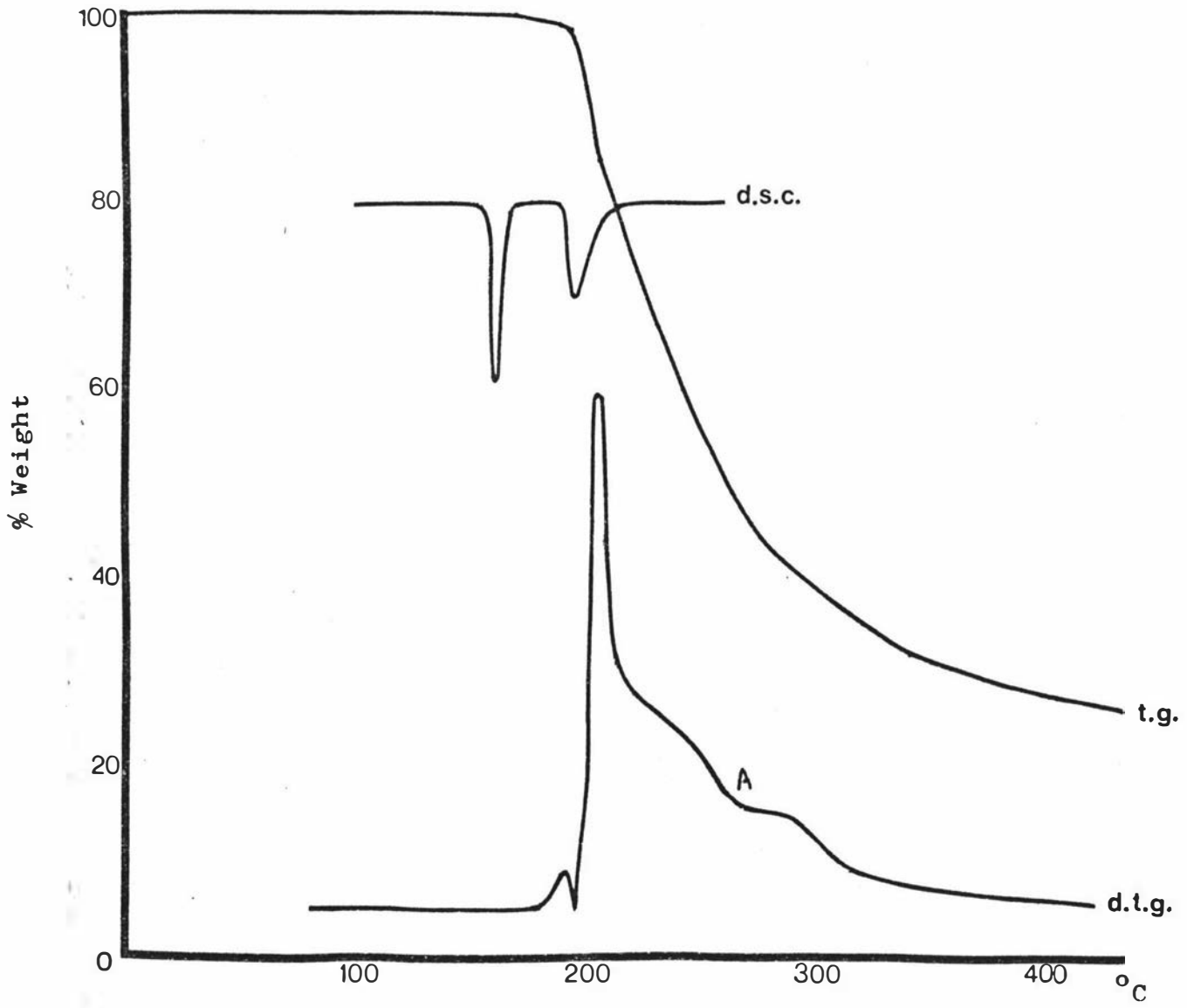


Fig C.7 Thermogram for the decomposition of 1-deoxy-1-dibenzylamino-D-fructose

The t.g. curves for the Amadori compounds all have a shape similar to those of 1-deoxy-1-glycino-D-fructose and 1-β-alanino-1-deoxy-D-fructose (see section A.3.2), displaying a rapid initial degradation which levels out to leave a residue which decomposes comparatively slowly. The first stage appears to have two components in the prolino case. The d.t.g. trace for this compound shows the decomposition pattern is complex with several contributing processes. This trace is similar in shape to the d.t.g. trace for 1-deoxy-1-glycino-D-fructose shown in fig A.3.

T.g. and d.t.g. data for piperidino-hexose-reductone (fig C.8) shows that decomposition accompanies melting, with a sharp initial degradation, to yield less than 20% of residue by 400°. This suggests that fragmentation reactions are likely to play a major part during the thermal decomposition and charring processes are of less importance.

A substantial amount of residue remains in the case of 1-deoxy-1-prolino-D-fructose (fig C.3). The derivatives of amines of intermediate basicity, morpholine and dibenzylamine, leave about half this amount of residue. Of the amines of low basicity, the p-toluidino derivative leaves over 50% residue while the N-methylaniline Amadori compound leaves 40%.

Basicities of the amines chosen for this study and those employed in previous studies (19, 20, 21) are presented in table C.1 along with the decomposition temperatures for their respective 1-amino-1-deoxyfructoses, taken from the d.s.c. curves for the compounds in this study or from the indicated references.

When the percentages of residue remaining at 400°

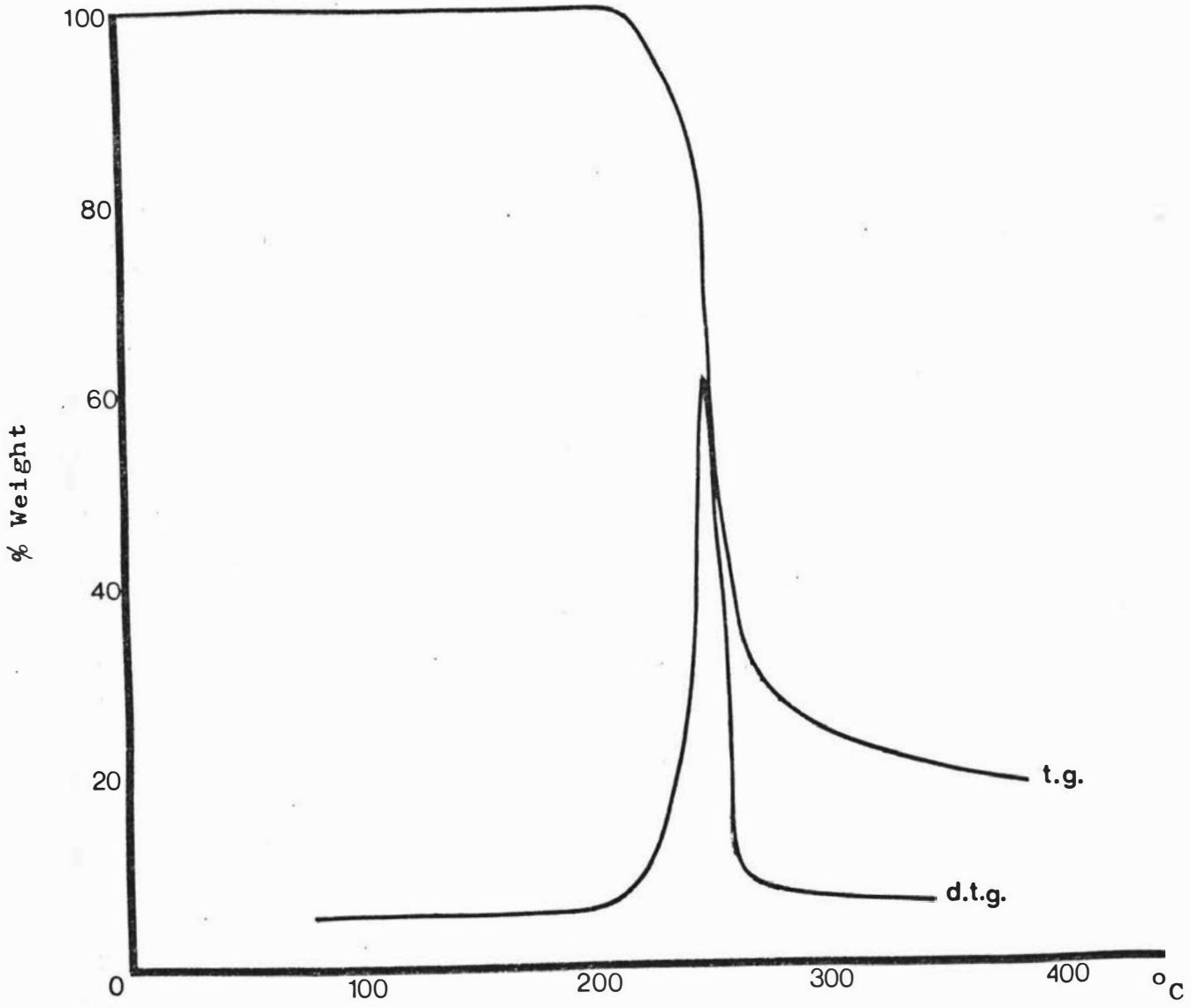


Fig C.8 T.g. and d.t.g. curves for the decomposition of piperidino-hexose-reductone

Table C.1 Physical data for amines under study

Base	pkb at 25°(81)	Nature of base	Amadori decomposition temperature °C
Piperidine	11.12	2°	127 (80)
Proline	10.64	2°†	120-125
N-Y-Amino-butyric acid	10.57	1°†	130 (21)
β-alanine	10.24	1°†	165
Alanine	9.87	1°†	178 (21)
Glycine	9.78	1°†	155
Valine	9.72	1°†	157 (21)
Dibenzylamine	8.52	2°	190
Morpholine	8.33	2°	150
p-Toluidine	5.08	1°	190
N-Methylaniline	4.84	2°	180

† = amino acid

for the 1-amino-1-deoxyfructoses employed in this study are plotted against the basicities of the parent bases (fig C.9), the amounts of residue left by the amino acid-derived Amadori compounds are seen to form a series distinct from the other bases. A more basic amino acid substituent leads to more charring while the reverse is true for non amino acid-derived Amadori compounds.

In fig C.10 the relationship between the decomposition temperature of the Amadori compounds and the basicity of the parent bases is shown. The decomposition temperature of the Amadori compound is lowered with increasing basicity of the amine substituent. With the exception of dibenzylamine, the amino acid-derived Amadori compounds again form a series distinct from the other bases.

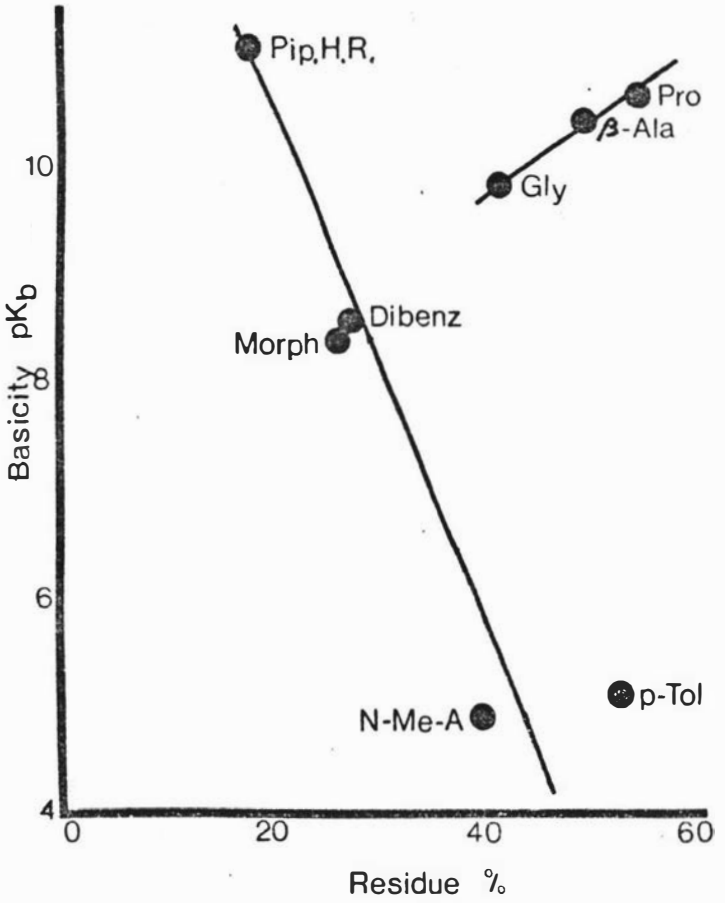


Fig C.9 Relationship between basicity of amine substituent and residue remaining at 400° from the pyrolysis of 1-amino-1-deoxy-2-fructoses

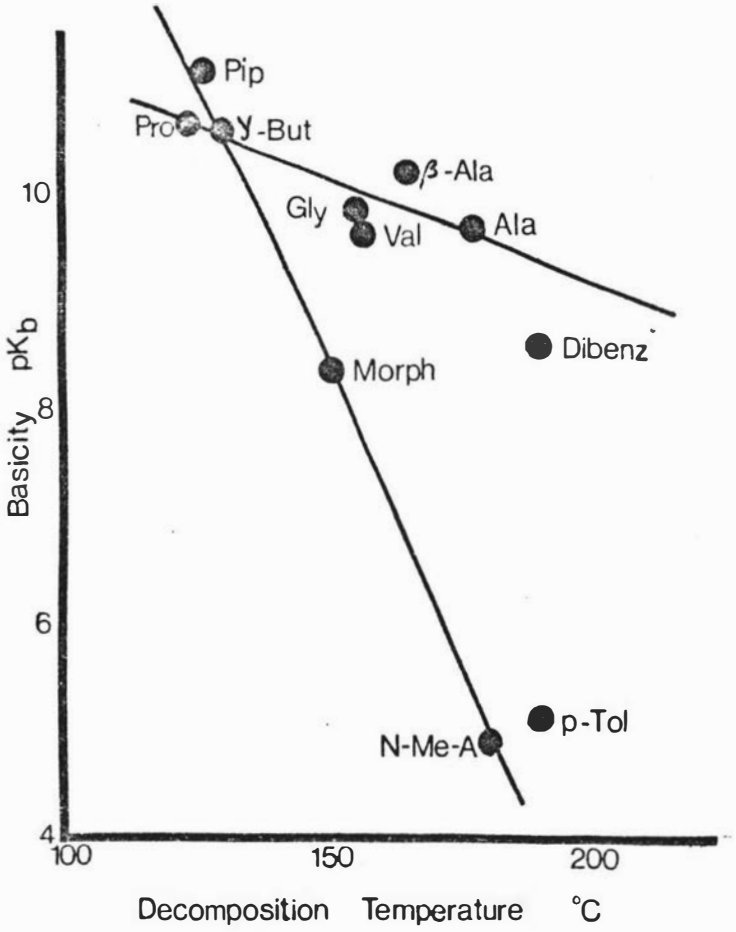


Fig C.10 Relationship between basicity of amine substituent and decomposition temperature of 1-amino-1-deoxy-2-fructoses

C.4 ANALYSIS OF PYROLYSIS PRODUCTS FROM THE 1-AMINO-1-DEOXYFRUCTOSES

C.4.1 Experimental

The experimental procedures were the same as those described in section A.4.2 for the pyrolysis of 1-deoxy-1-glycino-D-fructose and 1-β-alanino-1-deoxy-D-fructose. Pyrolysis conditions of 250°/15 min were chosen to allow a comparison of all the products from the Amadori compounds once the first stage of decomposition was complete (weight losses after this treatment showed that the first stages were complete according to the t.g. curves). Other temperatures were selected to investigate mass balance data corresponding to certain points on d.t.g. traces of interest.

In addition 1-deoxy-1-morpholino-D-fructose, 1-deoxy-1-dibenzylamino-D-fructose and 1-deoxy-1-p-toluidino-D-fructose were pyrolysed at 195°/15 min to sample the organic volatiles at a somewhat earlier stage. These compounds were also subjected to "Method A" of Shigematsu *et al.* (21), involving heating at 200°/5 min and extracting the organic volatiles into dichloromethane.

C.4.2 Results and discussion

The amounts of char formed at 250° (see table C.2) fall within a narrow range with the exception of the morpholino derivative which is somewhat lower. Comparisons of the quantity of tar recovered shows a reversal of the trend seen by comparing the residues remaining at 400° from the t.g. traces. The more basic amino derivatives leave relatively large tar fractions while the prolino and less basic amino compounds have low tar values. Percentages of tar

Table C.2 Pyrolysis products from the 1-amino-1-deoxyfructoses at 250°/15 min

Pyrolysis Product	Yield %					
	1-deoxy-1-morpholino-D-fructose	1-deoxy-1-N-methyl anilino-D-fructose	1-deoxy-1-p-toludino-D-fructose	1-deoxy-1-dibenzyl-amino-D-fructose	1-deoxy-1-prolino-D-fructose	piperidino-hexose-reductone
Char	31.5	50.4	60.4	55.1	59.5	49.4
Tar	29.4	8.4	5.3	22.8	2.0	32.6
Carbon dioxide	3.8	3.7	3.1	4.3	13.1	3.1
Carbon monoxide	0.4	0.8	0.3	1.0	1.0	0
Water	28.2	23.8	21.6	12.7	17.0	8.7
Organic volatiles	7.0	10.2	9.2	2.2	6.8	5.8
Unaccounted	0	2.9	0.1	1.8	0.6	1.5

recovered from 1-deoxy-1-glycino-D-fructose and 1-β-alanino-1-deoxy-D-fructose pyrolysates were also low (Table A.1) and this indicates that proline behaves more as an amino acid rather than a secondary base having a high pkb value. Consideration of the amount of CO_2 released suggests that decarboxylation of proline, an α-amino acid, is an important decomposition reaction. This phenomenon was also observed with 1-deoxy-1-glycino-D-fructose (Table A.1).

Results for water released reflect the degree to which the carbohydrate moiety has been dehydrated. The release of three molecules of water, for instance, would yield weight losses of 22.4, 20.1, 20.1, 15.0 and 18.6 for the Amadori derivatives of morpholine, N-methylaniline, p-toluidine, dibenzylamine and proline respectively, in reasonable agreement with the values in table C.2 and consistent with those discussed earlier for the glycine and β-alanine Amadori compounds. Piperidino-hexose-reductone has lost one molecule of water per molecule pyrolysed. Its formation involves the loss of three molecules of water from glucose. The higher loss in the case of 1-deoxy-1-morpholino-D-fructose may reflect some degradation of morpholine itself, which is susceptible to hydrolysis at temperatures above 200° (82) or to further dehydrations as a result of the increased fragmentation of the carbohydrate moiety reflected in the low char and high tar figures. The values for water released suggest that the sharp signals seen in the d.t.g. traces represent the regions where these dehydrations are occurring. The formation of rearrangement products, which cannot dehydrate further, involve the loss of three molecules of water from glucose and two from the 1-amino-1-deoxyfructoses. The extra molecule lost from all compounds studied must be involved in fragmentation reactions.

The carbon dioxide produced during this thermal treatment shows that with the exception of 1-deoxy-1-prolino-D-fructose approximately 4% of the Amadori compounds is recovered as CO_2. This fraction arises from the secondary degradations of the carbohydrate moiety as reflected in the figure for CO_2 released during the pyrolysis of glucose alone (table A.1). The carbon monoxide values may be similarly accounted for.

The irregular shape of the d.t.g. curve for 1-deoxy-1-dibenzylamino-D-fructose was investigated by pyrolysing this compound at $280^{o}/15$ min . This thermal treatment corresponds to point A on fig C.7. Values of 4.5% and 1.1% for CO_2 and CO respectively were obtained while the tar plus char and water plus organic volatiles accounted for 53.0% and 38.5% respectively. These figures on comparison with those in table C.2, show that decarboxylation and decarbonylation reactions are not occurring in this region and suggest that the decomposition pattern seen in the trace is due to volatilisation of dibenzylamine derivatives from the pyrolysis zone.

Pyrolysis of 1-deoxy-1-prolino-D-fructose at lower temperatures was undertaken in an attempt to explain the nature of the various thermal degradative processes which are evident from the d.t.g. curve for this compound.

Consideration (table C.3) of the values of char recovered show that the chosen thermal treatments correspond to the points A, B, C and D on the d.t.g. curve in Fig C.3. The evolution of both carbon dioxide and water prior to point A implies that more than one process has occurred and may represent a localised decomposition due to incomplete heat

Table C.3 Pyrolysis products from 1-deoxy-1-prolino-D-fructose under various thermal treatments (15 min duration)

Pyrolysis product	Yield %			
	135°	165°	195°	210°
Char	94.4	85.5	79.7	66.7
Tar	0	0	0	1.1
Carbon dioxide	1.3	4.4	4.5	10.5
Carbon monoxide	0.2	0.7	0.6	0.7
Water plus organic volatiles	3.5	8.1	13.1	20.6
Unaccounted	0.6	2.0	1.8	1.4

transfer. The amount of carbon dioxide released levels out at 4.5% until point C and there is a large increase between C and D, during which period the tar production commences. It is unclear where the carbon dioxide has originated from up until point C. The sharp weight loss between B and C does not involve carbon dioxide or carbon monoxide loss and probably represents a dehydration of the carbohydrate moiety. After this point Strecker degradation (9) of proline by α-dicarbonyl compounds produced from the dehydrated hexose unit could account for the carbon dioxide released. This evidence suggests that an initial step in the reaction series in the case of 1-deoxy-1-prolino-D-fructose is the release of proline from C-1 of the carbohydrate followed by dehydration, with little scission of the hexose unit, to produce reactive intermediates which in turn interact with the free amine in a Strecker type reaction. Part of the carbon dioxide released prior to point C could be produced via a competing process involving decarboxylation while the proline is still attached and while the Amadori compound is locked into a 1,2 enol structure (20). Dehydration and scission of the resultant pyrrolidine derivative would then follow.

Compounds constituting the organic volatile fractions from the pyrolysis of the 1-amino-1-deoxyketoses at 250°/15 min (table C.2) and the other selected pyrolysis conditions for the compounds noted (section C.4.1) are listed in tables C.4 to C.7.

The organic volatiles from the dibenzylamino, p-toluidino and N-methylanilino derivatives are all dominated by the presence of the free base. Morpholine is the major product from 1-deoxy-1-morpholino-D-fructose except in the dichloromethane extract where morpholine-derived compounds are the major products.

Table C.4 Volatile organic pyrolysis products from 1-deoxy-1-morpholino-D-fructose

Compounds[a]	Thermal Treatment 195°/15 min	250°/15 min	200°/5 min (CH_2Cl_2)	Hexose C atoms	Mass spectrum m/e	M.S. ref.
Methanol	+	+		1	31,32,29	54
Acetaldehyde	+	+		2	29,44,43	"
Ethanol	++	++		2	31,45,27,29,46	"
Acetone	+	++		3	43,58,15	"
Acetic Acid	+++	+++	++	2	43,45,60	"
2-Methylfuran	+	+		5	82,53,81	"
2,3 Butanedione	+	+	+	4	43,15,86	"
Propanoic acid	+	+		3	28,29,74,27,45	"
Benzene		+		6	78,52,77,51,50	"
Acrylic Acid		++		3	27,72,55,26	"
2,5-dimethylfuran		+		6	43,96,95,53,81	"
2,3-pentanedione	+	+		5	43,29,57,27,100	"
Toluene	+			6	91,92,39,65	"
Morpholine	++++	++++	+		57,29,87,86,56	"
2-furylmethylketone	+	+	+	5	95,110,39,42	"
Compound I	+++	+	+++	3	128,143,100,70	b
2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyrane-4-one	+		+	6	43,44,144,101	54
N-formylmorpholine	+++	++	+++	1	56,57,115,86,100	c
N-acetylmorpholine	++	++	++	2	57,56,86,114,129	c
Compound II			+	3	157,100,70,42	b
Morpholino-C-methyl-reductone			++++	4	171,128,100,154	d
iso-morpholino-C-methyl-reductone			++	4	171,128,100,142	d
4-hydroxy-2-morpholino-butanolacetone		+	++	4	112,113,127,171	d
Compound III			+	6	100,70,154,213	b

a ++++ = major product; +++ = > 10% of pyrolysate; ++ = 1-10% of pyrolysate; + = < 1% of pyrolysate
b Tentative assignment deduced from mass spectral breakdown pattern
c Identical with spectrum of authentic sample run on same instrument
d Tentative assignment, spectrum consistent with that of the piperidino analogue (19)

Table C.5 Volatile organic pyrolysis products from 1-deoxy-1-p-toluidino-D-fructose

Compound[a]	Thermal Treatment 195°/15 min	250°/15 min	200°/5 min (CH_2Cl_2)	Hexose C atoms	Mass spectrum m/e	M.S. ref
Methanol	+	+		1		54
Acetaldehyde	+	+		2		"
Ethanol	++	+		2		"
Furan	+			4		"
Acetone	+			3		"
Acetic acid	+	++	+	2		"
2-Methylfuran	+			5		"
2,3 Butanedione	+	+		4		"
Propanoic acid		+		3		"
2,5 dimethylfuran	+			6		"
2,3 pentonedione	+			5		"
Toluene	+	+		6		"
2-furaldehyde		+		5		"
2-furfurylalcohol		+		5		"
Protoanemonin	+	+		4		"
2-furylmethylketone	+	+		5		"
Dimethylpyrrole		+		6	94,95,80	"
2,4 hexadienoic acid		+		6	97,112,67,41,39	"
p-toluidine	++++	++++	++++		106,107	"
2,3 dihydro-3,5-dihydroxy-6-methyl-4H-pyrane-4-one	+			6	43,44,144,101	"
p-methyl-N-phenylpyrrole	+	++	++	5	157,156,115	"
N-formyl-p-toluidine		++	++	1	106,135,107	c
N-acetyl-p-toluidine		++	++	2	106,149,107	c
N-(5-methylfuryl)-p-toluidine		++	+	5	81,157,156	b

a,b,c : for explanation see table C.4

Table C.6 Volatile organic pyrolysis products from 1-deoxy-1-dibenzylamino-D-fructose

Compound[a]	Thermal Treatment 195°/15 min	250°/15 min	200°/5 min (CH_2Cl_2)	Hexose C atoms	Mass spectrum m/e	M.S. ref
Methanol	+	+		1		54
Acetaldehyde	+	+		2		"
Ethanol	+++	+		2		"
Acetone	+	+		3		"
Acetic acid	++	++	+	2		"
2,3 butanedione	+	+		4		"
Propanoic acid		+		3		"
Acetol	+			4		"
2,3 pentanedione	+	+		5		"
Toluene	+	+		6		"
2-furaldehyde	+			5		"
2-furfuryl alcohol		+		5		"
Acetol acetate		+		6		"
Protoanemonin	+	+		5		"
2-furylmethylketone	+	+	+	6		"
Benzaldehyde	++++	+++	++		77,106,105,51,50	"
Benzyl alcohol		+	+		79,108,107,77	"
Benzylamine		+			106,107,30,79	"
2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyrane-4-one		++	+	6		"
Benzoic acid		+		6	105,77,122	"
N-benzylpyrrole		++	+	4	91,157,65	"
N-benzylacetamide		+		2	43,30,15,106,149	"
N-methyldibenzylamine		+++	+	1	91,134,120,211	b
N-formyldibenzylamine		+	+++	1	134,91,106,225	c
Dibenzylamine		++++	++++		91,106,196,197	c
Tribenzylamine			+		91,106,196,287	b

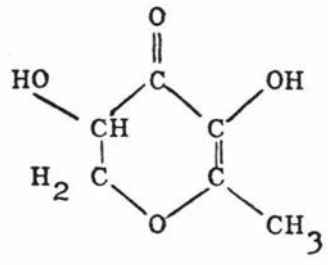
a,b,c : for explanation see Table C.4

Table C.7 Volatile organic pyrolysis products from 1-deoxy-1-prolino-D-fructose and 1-deoxy-N-methylanilino-D-fructose at 250°/15 min

Compound[a]	1-deoxy-1-prolino-D-fructose	hexose carbon atoms	1-deoxy-1-N-methyl anilino-D-fructose	hexose carbon atoms	Mass spectrum m/e	M.S. ref.
Methanol	+	1	+	1		54
Acetaldehyde	+	2	+	2		"
Ethanol	++	2	++	2		"
Furan	+	4				"
Acetone	+	3	+	3		"
Acetic acid	+++	2	++	2		"
2-methylfuran	+	5	+	5		"
2,3 butanedione			+	4		"
Propanoic acid	+	3	+	3		"
Pyrrolidine	++				71,70,43	"
Acetol			+	4		"
2,5 dimethylfuran			+	6		"
2,3 pentanedione	+	5	+	5		"
Acetoin			+	4		"
2,5 hexanedione			+	6		"
2-furaldehyde			++	5		"
2-furfuryalcohol			+	5		"
Acetol acetate			+	6	43,15,42,86,116	"
2-furylmethylketone	+	5				"
N-methylaniline			++++		106,107,78	"
2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyrane-4-one	+	6	++	6		"
N-formyl-pyrrolidine	++	1			99,43,70	20
N-acetyl-pyrrolidine	++++	2			43,70,113	"
N-proponyl 2-pyrrolidine	++	3			70,127,43	"
N-5-methyl-2-furfuryl-pyrrolidine	+	6			95,165,164	"
N-methyl-N-formylaniline			++	1	120,106,107,135	c
N-methyl-N-acetylaniline			+	2	107,106,120,149	c

a,c : for explanation see table C.4

These findings suggest that the primary pyrolytic event in the decomposition of 1-amino-1-deoxyketoses derived from heterocylic amines is a scission of the carbon-nitrogen bond at C-1. Whether or not the amine directs the fructose moiety to degrade via a 1,2 or 2,3 enolisation may also be inferred from the volatiles observed. The formation of 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyrane-4-one (vi) in each of these systems is evidence for the operation of the 2,3 enolisation pathway (20), while the isolation of furfuraldehydes and N-substituted pyrrole derivatives along with protoanemonin in the cases of 1-deoxy-1-N-methyl-anilino-D-fructose, 1-deoxy-1-p-toluidino-D-fructose and 1-deoxy-1-dibenzylamino-D-fructose indicate that 1,2 enolisation (9) is occurring in these systems.



(vi)

The relative importance of the two pathways is not fully clear because of the presence of one to four carbon fragments which could arise from either route. Other than the compounds discussed above and the free bases themselves, the remainder of the organic volatiles are derivatives of the corresponding base and are most likely formed via secondary reactions with reactive carbohydrate-derived carbonyls rather than formed directly from cleavage of the Amadori compound since they are more predominant at the higher temperature. The chain lengths and yields of the products from these 1-amino-1-deoxyfructoses suggest that the secondary degradation of the six-carbon

carbohydrate-derivatives occurs via C_4, C_2 scission rather than C_3, C_3 splitting observed when hexoses degrade under basic conditions (83), and that the free base appears to be little involved, if at all, in these degradation processes.

The pyrolysis of 1-deoxy-1-morpholino-D-fructose shows an entirely different pattern to the other three Amadori compounds discussed so far. C_4-amino reductones, not observed for the other 1-amino-1-deoxyfructoses are the major products at 200°. 1,2 enolisation is not directly evident, although 2-methylfuran is produced when 3-deoxy-D-erythro-hexosulose, a key intermediate in the 1,2 enolisation pathway, is pyrolysed (66). Morpholine is a much weaker base than piperidine and has a similar pkb value to dibenzylamine, yet the volatiles formed from 1-deoxy-1-morpholino-D-fructose are similar to those formed from 1-deoxy-1-piperidino-D-fructose and may be accounted for by the same pathways, the only difference being the isolation of the pyranone (v_i) in the former case. Hence the structural similarities between morpholine and piperidine are more important than their relative basicities in this context. These major degradative reactions are shown in Fig C.11. Interpretation of the mass spectral breakdown patterns for morpholino-C-methyl-reductone, iso-morpholino-C-methyl-reductone and 4-hydroxy-2-morpholino-butanolactone were based on the similarity to those of their piperidino analogues (19). Similarly the structures of compounds I, II and III were deduced on the basis of their mass spectra. An attempted synthesis of morpholino-C-methyl-reductone by the procedure for the piperidino analogue (84) did not yield a product pure enough for mass spectral conformation, so all assignments are tentative only. Compound III is the morpholino derivative of diacetylformoin which has a fruity-caramel aroma (9)

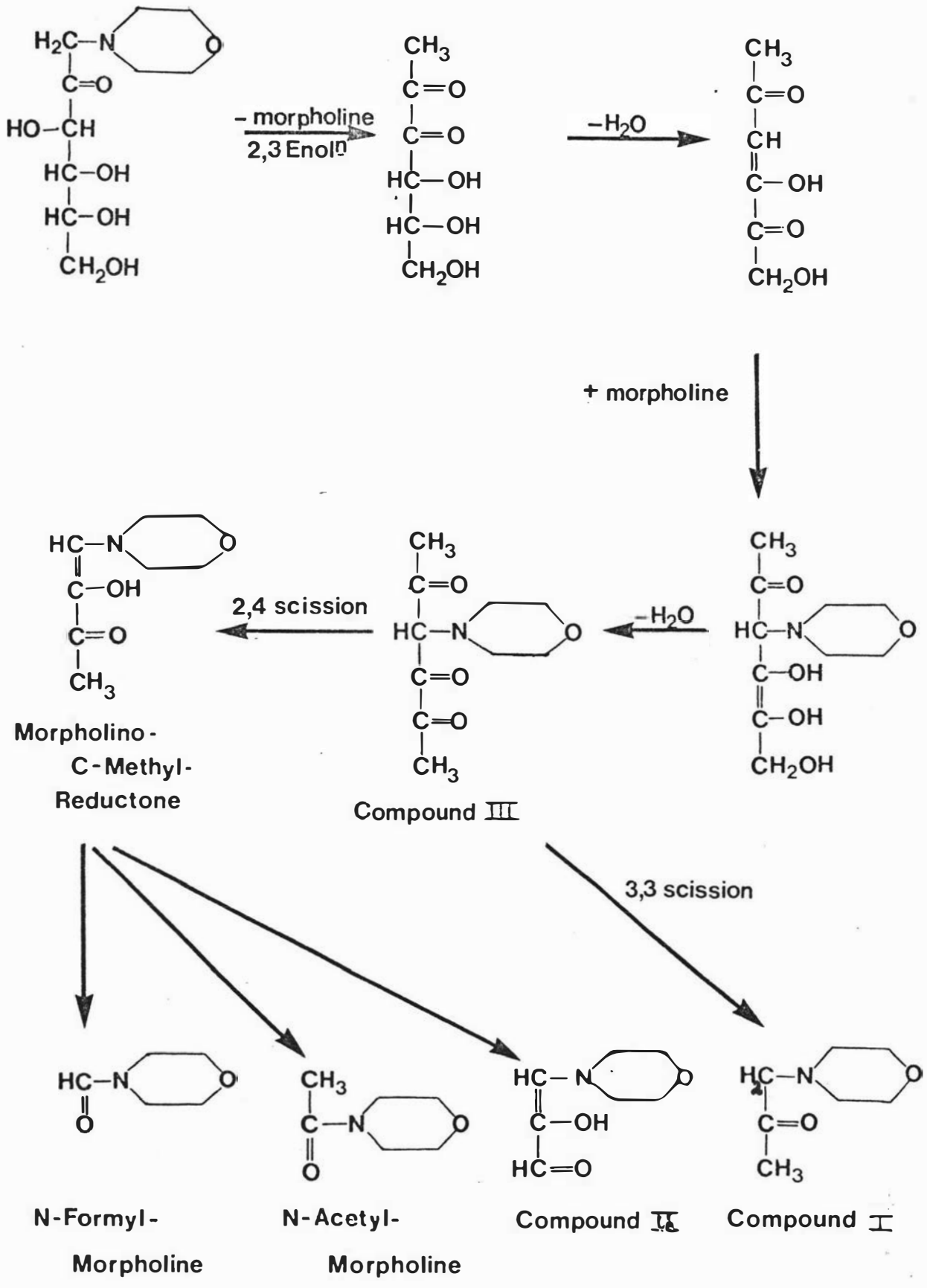


Fig C.11 Proposed decomposition reactions of 1-deoxy-1-morpholino-D-fructose

and has been implicated as an intermediate on the 2,3 enolisation pathway leading to the formation of 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyrane-4-one (vi) and related C_6 enolones (8). The amino-reductones, having burnt sugar aromas (8) are produced when the pathway via diacetylformoin is blocked by the addition of the base at C-3 of the dehydrated 2,3 enol intermediate.

The pyrolysis of 1-deoxy-1-prolino-D-fructose gives essentially the same range of volatiles found by previous researchers (20, 21). The operation of the 1,2 and 2,3 enolisation pathways is apparent along with the production of fragrant proline-derived compounds. No C_4-amino-reductones were observed although 2,4 scission was predominant over a 3,3 split. Proline is a stronger base than morpholine and should therefore direct the Amadori compound to decompose via a 2,3 enolisation pathway even more readily. However its propensity to decarboxylate rather than add back to the 2,3 enol intermediate dictates the decomposition pattern. There is evidence to support the decarboxylation of proline while it is still attached to the hexose unit and while the Amadori compound is locked into a 1,2 enol structure as proposed by Mills and Hodge (20). This process would give rise to the amides observed whereas the decarboxylation of the free base in a Strecker type reaction with the α-dicarbonyl compounds would be expected to yield the dehydrated pyrroline derivatives responsible for bread-like aromas (9). These compounds were, with the exception of pyrroline itself (20), not isolated in any of the three studies yet are evident when mixtures of proline and sugars are heated (75).

Most of the degradation products from 1-deoxy-1-

glycino-D-fructose and 1-β-alanino-1-deoxy-D-fructose could be accounted for via a 1,2 enolisation pathway and the furaldehyde and pyrrole derivatives isolated from the pyrolysis of Amadori compounds containing primary α-amino acid moieties in the study by Shigematsu *et al.* (21) shows that this pathway is of major significance. No C_4-amino-reductones were isolated in their study even though the amino acids are all stronger bases than morpholine and the 2,3 enolisation pathway is operative. The degradation of the Amadori compounds derived from primary amino acids in their study, as with 1-deoxy-1-glycino-D-fructose in this study (Part A), involves the formation of relatively large amounts of pyrazines which are not isolated when the amino acid alone is pyrolysed (47). It is unclear whether these compounds arise from interactions of the free amino acid itself or are fragmentation products of the charring process as they are not formed unless drastic conditions are employed.

Table C.8 broadly summarises the relationships discussed in this investigation for the Amadori compounds under study.

Table C.8 General relationship between the basic moiety of 1-amino-1-deoxyfructoses and the observed pyrolysis products

Amine substituent	Extent of polymerisation or charring	Carbohydrate derived products 1,2 enolisation ⟵ 2,3 enolisation ⟶	Characteristic amine-derived product	Aroma profile
Primary α-amino acids	High	furans, pyrroles ⟷ C_6 enolones	Pyrazines	↕ flowery, bready, corny, nutty, musty
Secondary α-amino acids	High	⟶ C_6 enolones	Amides	
Non α-amino acids	High	furans, pyrroles ⟷ C_6 enolones	Amides	
Primary bases	High	furans ⟷ pyrroles C_6 enolones	Amides	
Secondary bases	High to Low	furans, pyrroles ⟵ C_6 enolones ⟶ C_4 reductones	Amino-reductones (if high pkb)	
Glucose alone	Medium	furans ⟵ heat ⟶ C_6 enolones		
Aroma Profile		⟵ background, tangy, bitter — fragrant, caramel — burnt, sugar ⟶		

APPENDICES

APPENDIX 1 - PYROLYSIS PROCEDURES

A1.1 Pyrolysis Unit design

The design of the pyrolysis unit was based on that of Simmonds et al. (85) and consisted of a removable stainless-steel tube that was sleeved by an insulated furnace containing a nichrome heater. The furnace could be maintained at the desired temperature through the use of a variable rheostat placed in series with the unit. One end of the furnace contained a gas inlet and a "swagelok" port through which a thermometer could be inserted and the position adjusted to record the temperature anywhere within the unit. It was desirable to allow an equilibration period of at least 2 hours to stabilise the temperature at the desired level under the chosen gas flow (typically dry nitrogen at 8 ml/min). The other end of the furnace was threaded to allow introduction of the sample and give a gas-tight seal once the nut had been secured. This nut was drilled-out in the middle and had a short length of steel tubing (approx 3 cm x 0.64 cm outside diameter) brazed onto the outer surface, which was fitted with a "swagelok" reducing adapter to which a trap comprising a stainless-steel tube (15 cm x 0.32 cm outside diameter) containing a porous polymer (Chromosorb 105, 80-100 mesh; Applied Science Labs, State College, Pa; 200 mg) could be attached. The trap was adapted for direct insertion into a gas chromatograph (86). Such traps required "conditioning" consisting of heating up to 200^{o} under a low nitrogen gas flow, until they produced negligible background peaks as tested by gas chromatography.

A1.2 Operation of the Pyrolysis Unit and definition of Mass Balance fractions

For organic volatile analysis, a platinum boat containing the sample (10-30 mg) was placed at the appropriate position inside the removable stainless-steel tube with the gas flow turned off. The removable end containing the trap was quickly screwed into place and the gas turned on. Temperatures were maintained at given levels for the required times and the volatiles formed were carried into the trap, which was packed in dry-ice. At the conclusion of the pyrolysis, the stainless-steel tube together with the sample boat could be removed for cleaning.

Mass balance measurements were made by pyrolysing the sample (100-200 mg) packed into the wide end of a "Pasteur" pipette. The sample was contained using two glass-wool plugs and the length of the pipette was made such that the sample sat at the desired position within the pyrolysis unit while the capillary end of the pipette extended along the pyrolysis tube and into the end of the polymer trap. An adjustable swagelok fitting on the trap allowed it to be slid through the nut sufficiently far to enable the capillary end of the sample pipette to be secured to the end of the trap, employing teflon tape to make a gas-tight seal. The trap was then pulled back and the swagelok nut secured so the end of the trap sat exactly at the end of the pyrolysis unit. After the sample pipette had been attached to the trap and adjusted to the desired position the trap could be packed in dry-ice and when equilibrium had been attained, the gas flow was momentarily turned off and the sample moved into the pyrolysis zone and the outer nut secured. Once pyrolysis had commenced, tar collected on the glass surface between the sample and the polymer trap from

where it could be recovered. Char was defined as that material remaining in the immediate sample area while the organic volatiles plus water were condensed in the trap. Uncondensed material passing through the trap was carried into a series of U-tubes attached to the outer end of the trap. Carbon dioxide and carbon monoxide were recovered from the gas stream and determined as barium carbonate (31), the carbon monoxide being first converted to carbon dioxide by passage over iodine pentoxide at 150°.

APPENDIX 2 - PREPARATION OF AMADORI COMPOUNDS

A2.1 Synthetic procedures

1-deoxy-1-glycino-D-fructose:

D-Glucose (144 g) was dissolved in water (150 ml) and the solution was concentrated in vaccuo to 20 g water content. Glycine (15 g) and sodium metabisulphite (19 g) were added to the stirred glucose solution, held at 90°C over a boiling water bath (1 hr). The mixture was cooled and diluted with aqueous ethanol (1:1 v/v) to 500 ml. The synthesis was repeated and the reaction products combined and chromatographed on "Dowex 50-X8" (H^+, 400 g). The column was eluted with aqueous ethanol (3 l) and water (1 l) and the washings discarded. Basic products were eluted from the column with aqueous ammonia (0.2 N). The effluent was collected in 15 ml fractions and each fraction examined by paper chromatography ("No 1 Whatman") with butanol-acetic acid-water (4:1:1 v/v) as solvent. The spots were visualized by (a): aniline hydrogen phthalate (87), (b): alkalone triphenyltetrazolium chloride (88) and (c): ninhydrin. Fractions containing the Amadori compound were combined and concentrated in vaccuo to give a brown syrup which gave a white granular precipitate on storing at 4° for 10 days. The precipitate was filtered and washed with methanol-water (2:1 v/v) to remove the brown colouration and the crystals were recrystallised from methanol-water to give 1-deoxy-1-glycino-D-fructose, 9 gm.

1-β-alanino-1-deoxy-D-fructose:

The same synthetic procedure as that described for 1-deoxy-1-glycino-D-fructose was employed using

β-alanine (18 g) in place of glycine to yield 1-β-alanino-1-deoxy-D-fructose, 12 gm.

1-Deoxy-1-morpholino-D-fructose:

D-Glucose (50 g) was added to trimethylamine (50 ml) in a 250 ml 3-necked flask equipped with a condensor.

Morpholine (23 ml) and glacial acetic acid (20 ml) were added with vigorous stirring and the mixture heated to 85° and allowed to react for half an hour. At this time the solution was light amber. The mixture was cooled with stirring for 4 hours and stored for 24 hours at -20°. Subsequent stirring at room temperature for 6 hours afforded a white precipitate which was filtered and the extract (22 gm) washed with ethanol-acetone (1:1, v/v).

The product was recrystallised from methanol.

1-Deoxy-1-dibenzylamino-D-fructose:

D-Glucose (18 g) was stirred into absolute ethanol (A.R. 100 ml) in a 250 ml flask fitted with a reflux condensor. Dibenzylamine (19.8 g) and glacial acetic acid (6 ml) were added and the mixture refluxed for 3 hours. On cooling overnight a precipitate formed which was filtered and washed with ethanol until colourless to yield 24 gm of the required product which was rewashed with ethanol and dried in vaccuo over calcium chloride.

1-Deoxy-1-p-toluidino-D-fructose:

D-Glucose (20 g), p-toluidine (16 g), acetic acid (1N, 2 ml) and distilled water (4 ml) were heated in a boiling water bath for half an hour. Ethanol (200 ml) was added and a precipitate formed immediately. The mixture was left to stand for 48 hours when the

precipitate (20 gm) was filtered, washed with ethanol-ether (2:3, v/v) and dried over calcium chloride in vaccuo.

1-Deoxy-1-N-methylanilino-D-fructose:

D-Glucose (50 g) and N-methylaniline (47 ml) were stirred with absolute ethanol (100 ml) at 70-80° for 4 hours. Glacial acetic acid (25 ml) was added dropwise and heating continued for 2½ hours, at which time the solution was deep-amber. The solution was cooled with stirring and left 24 hours at -20°. Subsequent stirring failed to produce the product, as expected (3), however a precipitate was obtained after reducing the volume by half and prolonged storage at room temperature. The precipitate was filtered and washed with cold ethanol until colourless and recrystallised from ethanol to yield 20 g of product.

1-Deoxy-1-prolino-D-fructose:

D-Glucose (26 g) was dissolved in methanol (200 ml) and proline (18 g) was added. The solution was refluxed for 3 hours and malonic acid (3.8 g) added. Refluxing was continued for an additional 3 hours. The mixture was cooled and concentrated to half its original volume. Prolonged storage at room temperature afforded a white precipitate which was washed with methanol and filtered. After recrystallisation from methanol 15 g of white crystals were obtained which were shown to contain no glucose or proline by paper chromatography in butanol-acetic acid-water (4:1:1) and pyridine-water (4:1 v/v).

1-Deoxy-1-piperidino-D-fructose and piperidino-hexose-reductone:

Anhydrous D-glucose (30 g) and piperidine (19 g) were stirred at 70° for 20 minutes to give an amber syrup. Heating was stopped while malonic acid (6 g)

was added by small amounts over a 10 minute period. The colour changed to a reddish-brown. The mixture was stirred for 10 minutes and ethanol (20 ml) was added. Heating was recommenced and after half an hour acetone (20 ml) was added and the solution cooled to 0^{o}. The expected crystals (77) did not form although a tarry product was produced on prolonged standing, which gave a positive colour test for Amadori compounds with triphenyltetrazolium chloride. Purification of this product by preparative paper chromatography on "No 3 Whatman" paper in butanol-acetic acid-water (4:1:1) yielded a light brown syrup, chromatographically homogeneous, which failed to crystallise although various solvents and techniques were employed.

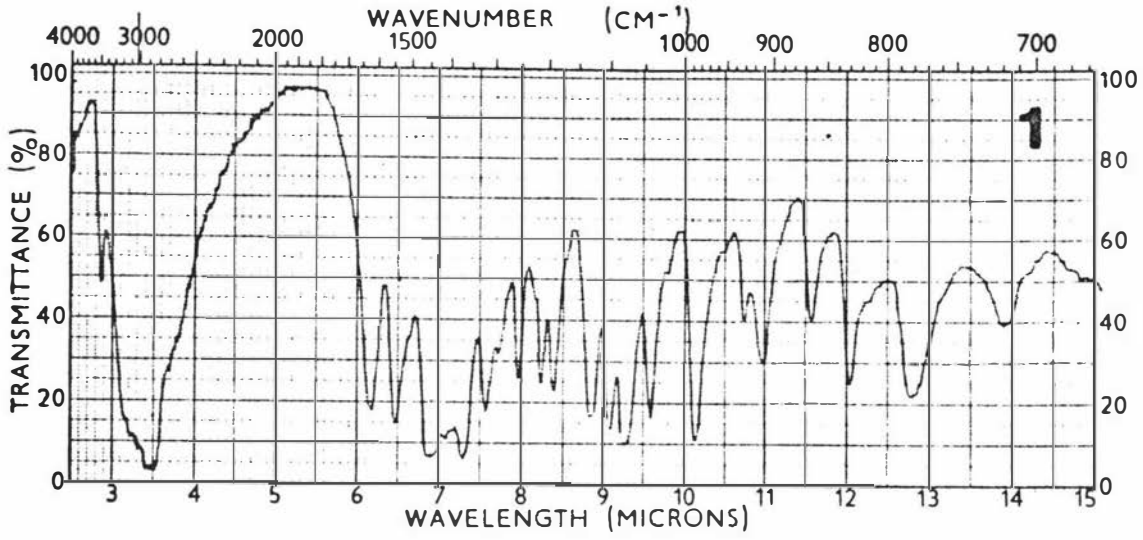
Reheating of the mother liquors from the above preparation for 6 hours at 70^{o} followed by standing at room temperature yielded optically inactive light-yellow crystals of piperidino-hexose-reductone which were washed, filtered and recrystallised from ethanol.

A2.2 Infrared Spectra

I.R. spectra of the Amadori compounds were obtained as nujol mulls on a Perkin-Elmer 137 (spectra 1-6) or a Beckman AccuLab 8 instrument with sodium chloride optics.

Key to spectra

1. 1-deoxy-1-glycino-D-fructose
2. 1-β-alanino-1-deoxy-D-fructose
3. 1-deoxy-1-morpholino-D-fructose
4. 1-deoxy-1-p-toluidino-D-fructose
5. 1-deoxy-1-dibenzylamino-D-fructose
6. nujol
7. piperidine-hexose-reductone
8. 1-deoxy-1-prolino-D-fructose
9. 1-deoxy-1-N-methylanilino-D-fructose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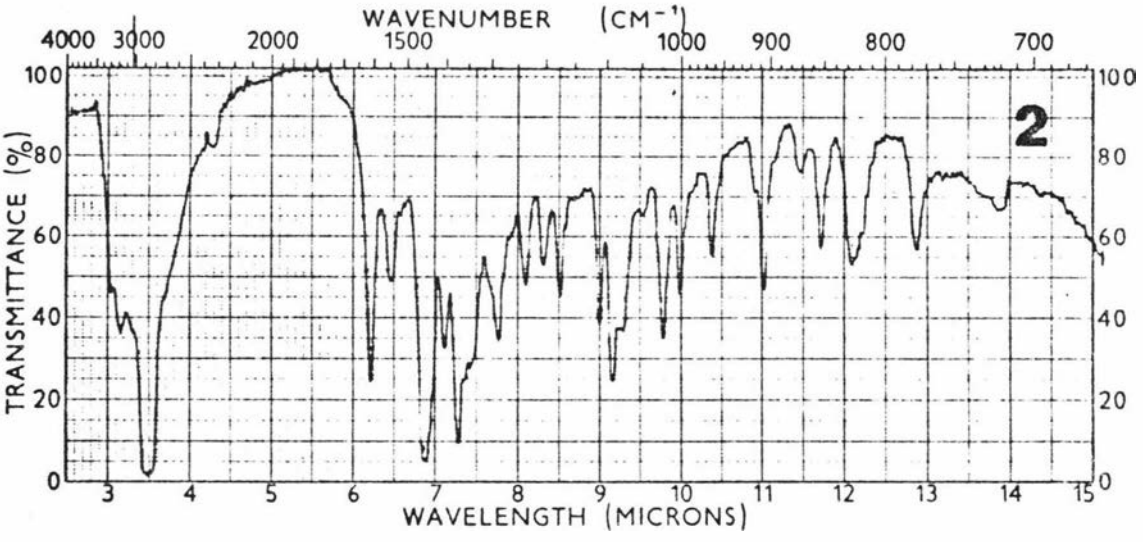
WAVELENGTH (MICRONS)
1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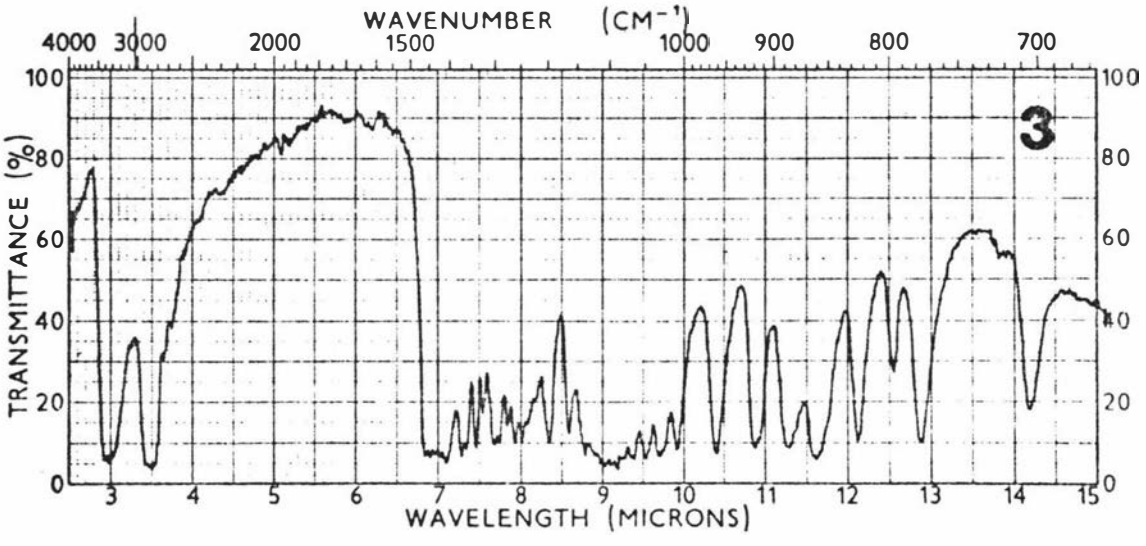
2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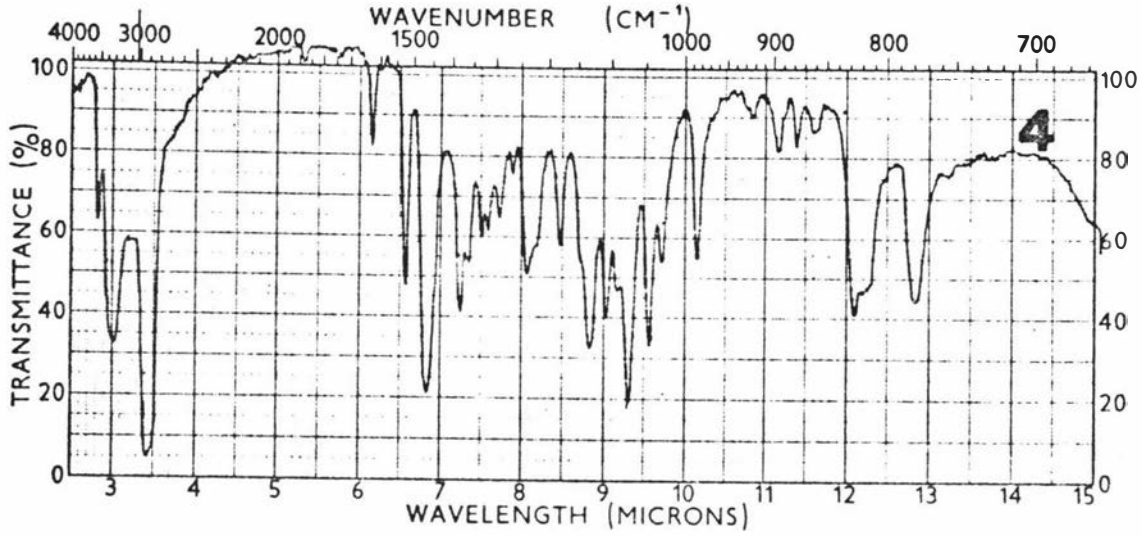
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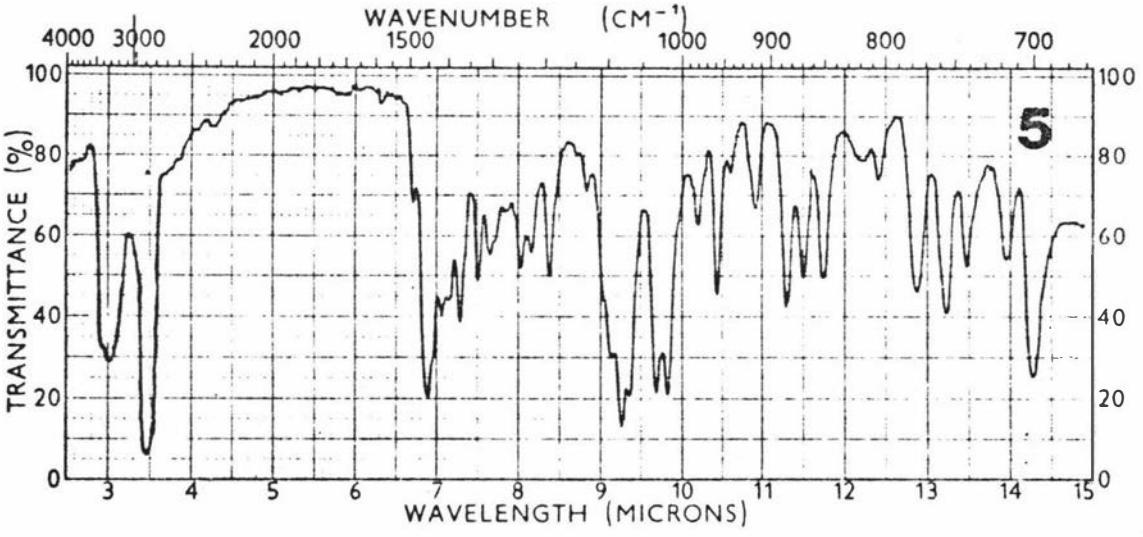
4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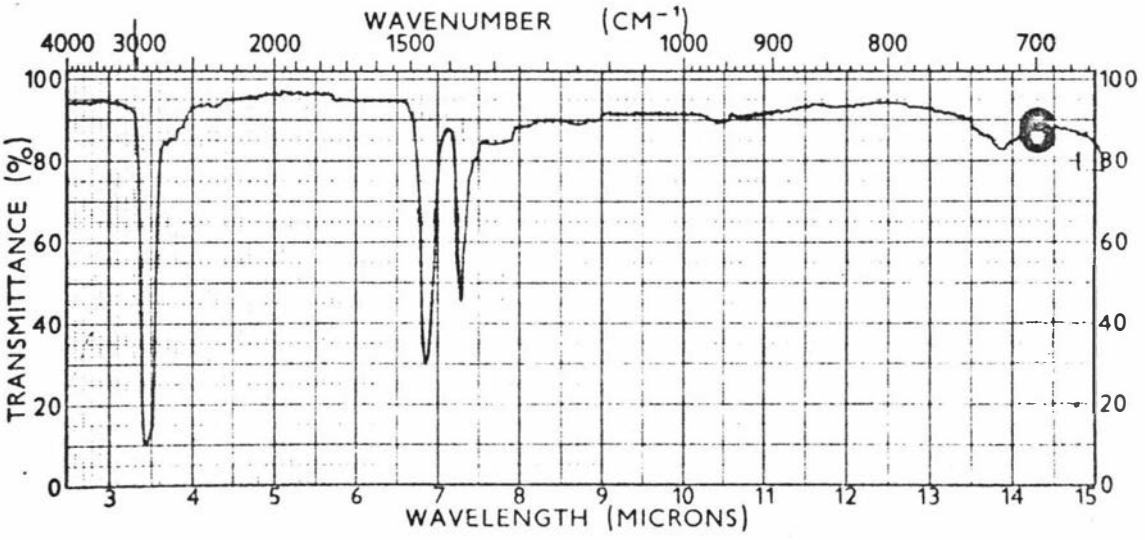
WAVELENGTH (MICRONS)
5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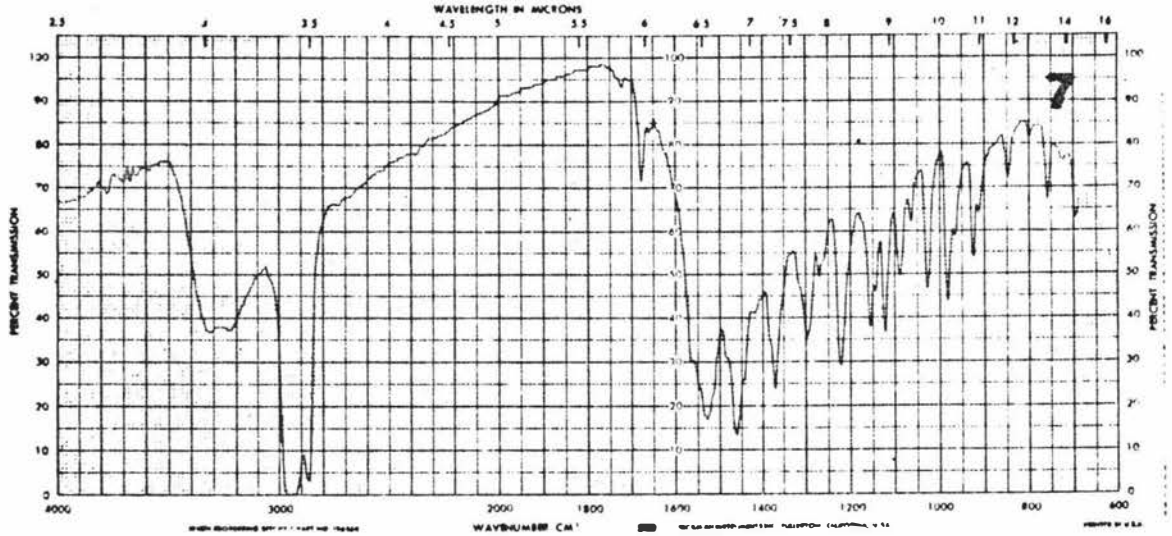
TRANSMITTANCE (%)
WAVELENGTH (MICRONS)
6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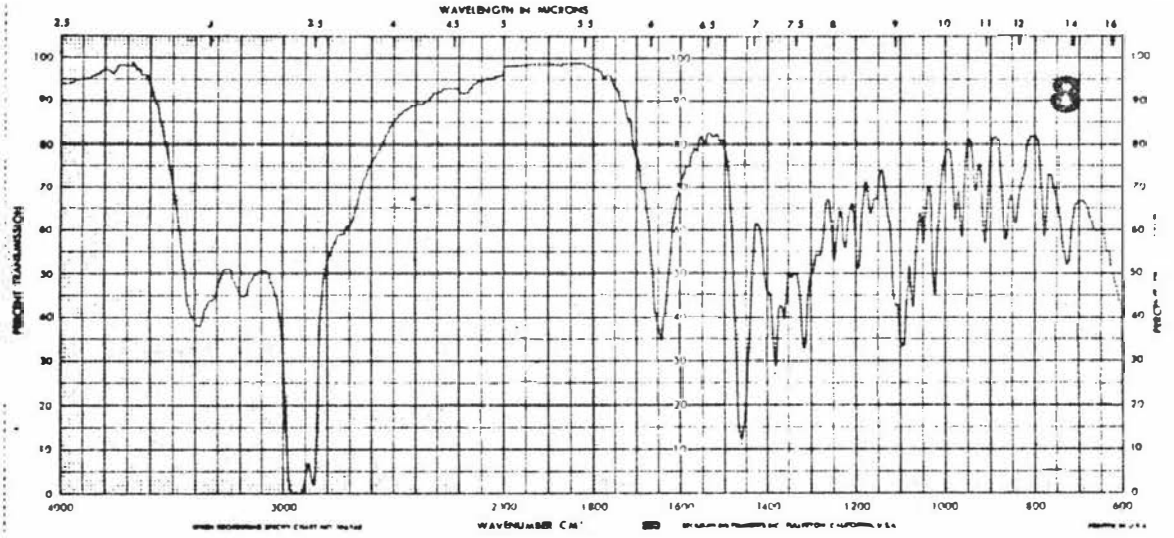
PERCENT TRANSMISSION
WAVENUMBER CM⁻¹
7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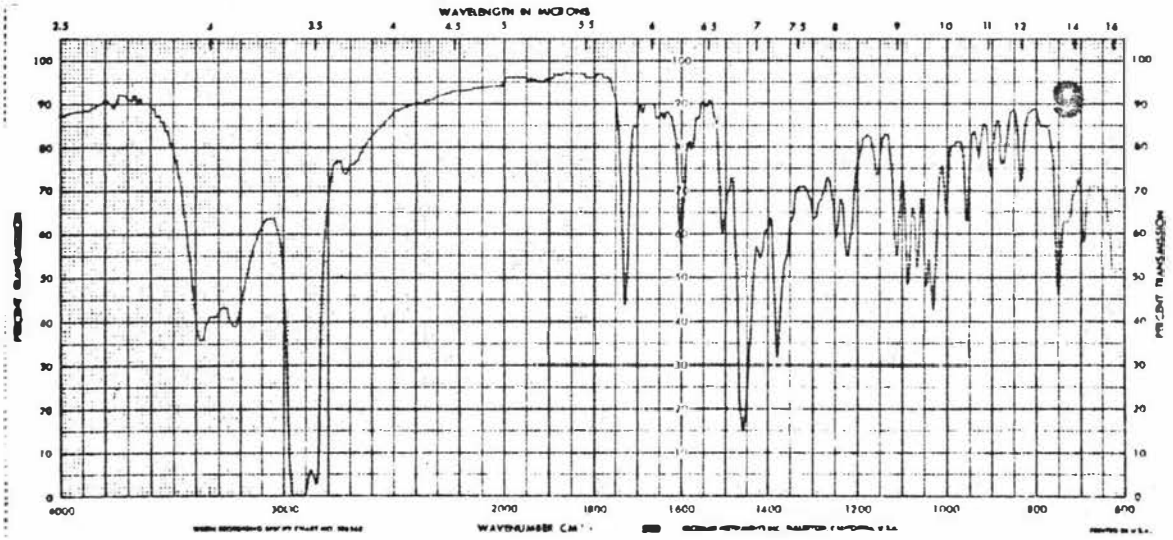
WAVELENGTH IN MICRONS
PERCENT TRANSMISSION
WAVENUMBER CM⁻¹
9

APPENDIX 3 - COLOURIMETRIC ANALYSIS OF BROWNING REACTION MIXTURES

Amino acid and Amadori compound levels may be determined using an automatic amino-acid analyser (see Part B.4.2.3) while glucose can be specifically monitored by the conventional enzymatic method employing glucose oxidase (see Part B.4.2.2) due to the relatively large amount of time taken for a single analysis on the amino acid analyser (7 hours), the waiting time involved before analysis and the large number of samples involved during preliminary screening runs to find the most appropriate experimental conditions, it became necessary to develop more rapid analytical techniques for the determination of the amino acid and Amadori compound.

Wolfrom *et al.* (89) developed a method of detecting browning reaction products as their trimethylsilyl (TMS) derivatives. This method was employed to follow the course of the browning reaction between glucose and β-alanine at 150° in the dry-state by monitoring the levels of the TMS peaks of glucose, β-alanine and 1-β-alanino-1-deoxy-D-fructose. Various TMS reagents and mixtures of these, as used by Wolfrom *et al.* were tested on two columns: 10% OV-101 on gaschrom Q (stainless steel) and 3% S.E.-30 on chromosorb G (all glass) using D-glucitol as an internal standard. Although the method allowed quantitative estimation of the three compounds individually no combination of reagents or columns was found to be satisfactorily reproducible for all compounds at once and hence analysis was tedious and the method was later abandoned in preference to analysis by U.V. spectrophotometry.

β-alanine was estimated as its 2:4

dinitrofluorobenzene derivative (90) by reading the absorbance at 380 nm against an appropriately diluted blank and glucose was estimated using glucose oxidase. 1-β-alanino-1-deoxy-D-fructose did not interfere with the β-alanine analysis and, even though the use of 2:4 dinitrofluorobenzene is not specific for β-alanine, comparison of the results with those later obtained using an amino acid analyser showed that the analyses were reliable. A method based on the reaction between Amadori compounds and triphenyltetrazolium chloride at room temperature to give a red pigment (26) was developed for analysis of the 1-amino-1-deoxyketose produced. Glucose did not interfere with the analysis providing the reading was taken within half an hour of mixing the reagents. The colour began to fade after this time in certain samples, but in others, where there was a high concentration of glucose, a secondary phase of colour development could be seen due to the effect of NaOH (required for colour development) catalysing the browning reaction between glucose and any β-alanine present. Glucose normally reacts with triphenyltetrazolium chloride after prolonged standing in the cold (24 hours) or when heat is supplied. It was necessary to run a fresh set of standards with each analysis and to allow at least ten minutes for colour development to occur. This is illustrated in fig A3.1 where a fixed wavelength of 574 nm was chosen with a sample concentration of 45 μg/ml. Although the method required care in application and appeared to have a narrow range within which concentration could be determined, it gave acceptable results when compared to those obtained using the TMS procedure, see fig. A3.2.

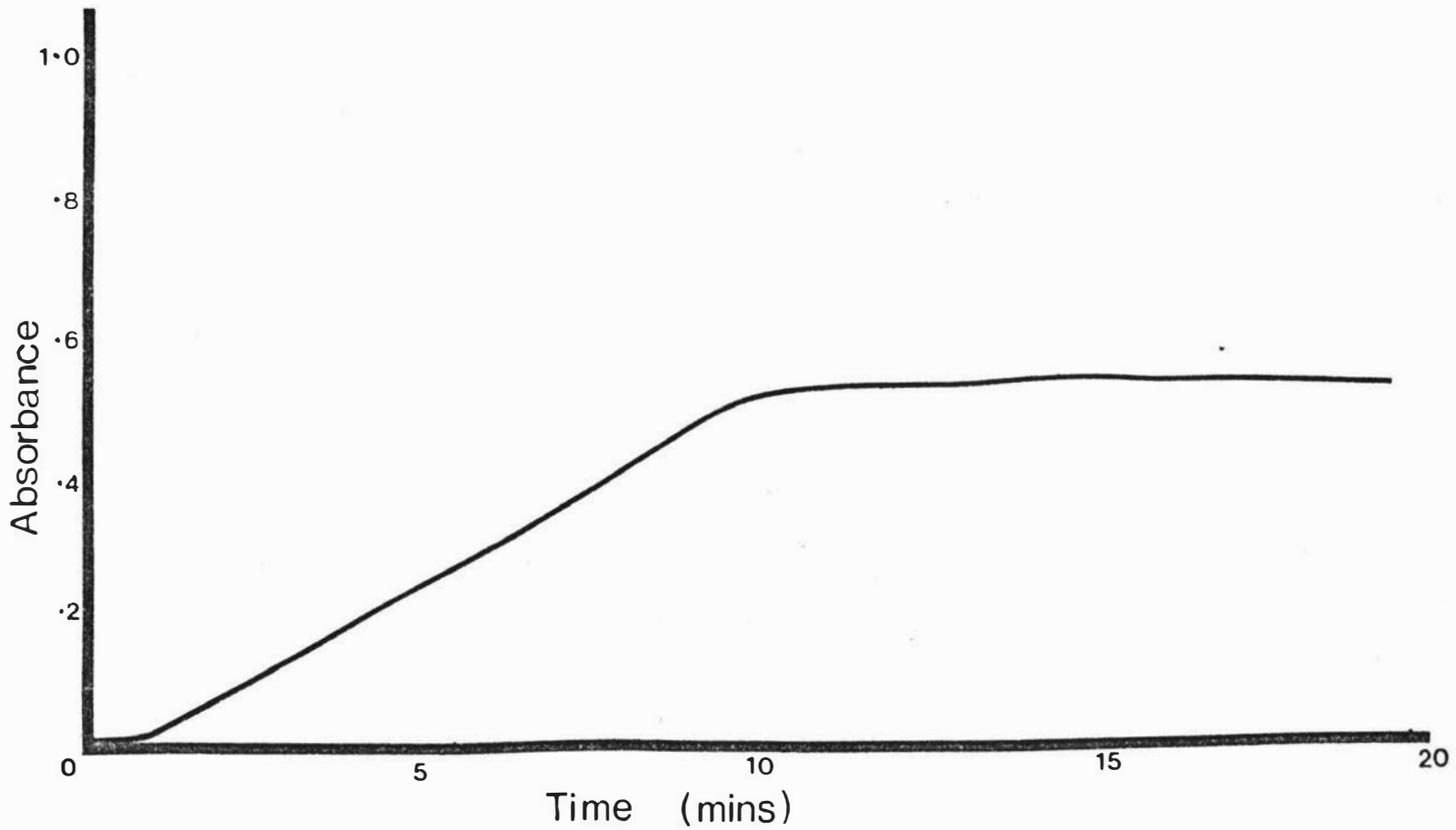

Fig A3.1 Change of absorbance with time for a 1-β-alanino-1-deoxy-D-fructose determination at 574 nm.

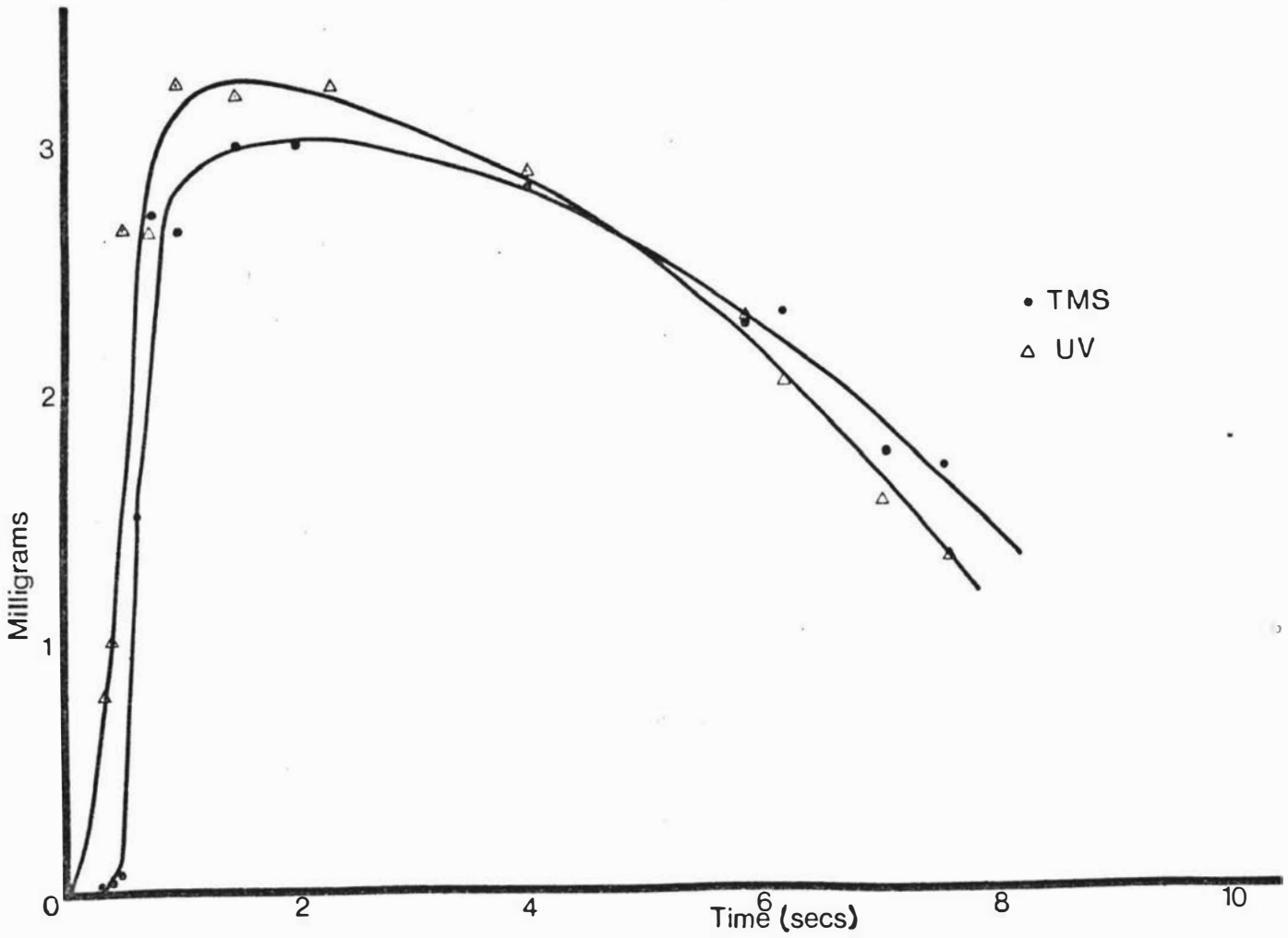

Fig A3.2 Comparison between U.V. and T.M.S. methods for monitoring 1-β-alanino-1-deoxy-D-fructose levels during browning.

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