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An investigation of the compounds produced by spray-drying an aqueous solution of glucose and glycine

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Spray-drying an aqueous solution of glucose and glycine results in the production of a brown powder that has an aroma reminiscent of chocolate powder. In this study, volatile components of the brown powder were extracted by Grob headspace stripping or solvent extraction techniques and analysed by gas chromatography–mass spectrometry (GCMS) or, in specific instances, gas chromatography fourier transform infrared detection (GC-FTIR). Solvent extracts were also derivatised with diazomethane to increase the amenability of many compounds to study by gas chromatography (GC) techniques. These approaches yielded the identification of a range of oxygen and nitrogen heterocyclic compounds.

In further experiments, coloured compounds that were extracted into an organic solvent were separated by high performance liquid chromatography (HPLC) and detected at a wavelength of 430 nm. Thermospray liquid chromatography–mass spectrometry (LCMS) techniques were also employed to measure the molecular mass of detected coloured species. Furthermore, desorption mass spectrometry was used to record an electron impact spectrum of a coloured compound that had been fractionated by HPLC. These data indicated the detection of this compound in GCMS and GC-FTIR studies and have led to a postulated identity of this and other related coloured species detected during these investigations.

INTRODUCTION

The compounds produced upon reaction of glucose with glycine, under Maillard conditions, have been the subject of many investigations. (See for example, Oh *et al.*, 1991; Fumitaka *et al.*, 1985; Olsson *et al.*, 1978; Ledl *et al.*, 1983; and Ingles & Gallimore, 1985). Reaction mixtures of these reagents are usually prepared by heating aqueous or organic solutions containing both components for many hours (Ledl *et al.*, 1983; Olsson *et al.*, 1978). Recently, a novel method for production of Maillard reaction mixtures was introduced (Baines *et al.*, 1989). This method involved spray-drying aqueous solutions of reducing sugars and amino acids (without prior reaction) and results in the preparation of coloured powders. The colour of the powder was found to be characteristic of the components of spray-drier feedstock, e.g. a maltose–glycine reaction mixture resulted in a salmon pink powder, whilst a glucose-glycine system led to the production of a dark brown powder.

Commercially, these powders exhibited potential in the area of food colouration. In particular, once redissolved in water, the glucose–glycine material produced a brown solution that showed an attractive red hue. We have been investigating the chemical composition of this powder so as to gain an understanding of the chemistry that had occurred during its formation.

This paper describes our approaches to the study of the low-molecular-weight components of the spray-dried glucose–glycine reaction mixture.

EXPERIMENTAL

Chemicals

All chemicals were of analytical reagent grade and purchased from Aldrich Chemical Company, Poole, UK. Ammonium acetate (AR grade) was obtained

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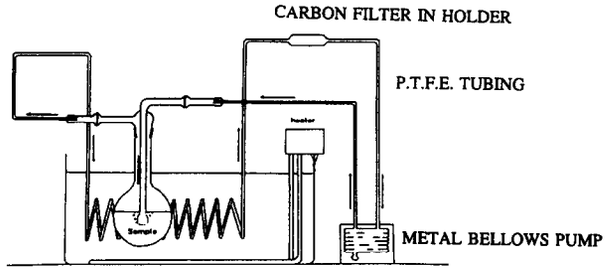


Fig. 1. A schematic of a Grob Headspace Stripping Apparatus.

from FSA, Loughborough, UK. All solvents were HPLC grade from Rathburns Chemicals, Walkerburn, UK.

Preparation of a spray-dried glucose-glycine model Maillard reaction mixture

Equimolar quantities of glucose and glycine were dissolved in deionised water to give a final concentration of 1M of each reactant. This mixture was spray-dried, according to the method of Baines *et al.*, (1989), using a Niro Mobile Minor Spray drier (Cornwell Products Ltd, UK). The drier inlet and outlet temperatures were 235 and 170°C, respectively. Flow rate of the aqueous feed stock was approximately 20 ml per minute.

Headspace stripping conditions [Grob & Zurcher, 1976]

A portion of the brown spray-dried powder (10 g) was dissolved in an aqueous solution of sodium sulphate (10 g in 50 ml distilled water). The volatile components of the resultant mixture were extracted (over a period of one hour) by the passage of air blown through the sample with a metal bellows pump and trapped onto a carbon filter (5 mg). An extract was prepared by washing the carbon filter with dichloromethane (3 × 20 μl). The apparatus used to prepare this extract is shown schematically in Fig. 1. Portions (0.6 μl) of the resultant extract were analysed by GCMS.

Solvent extraction and derivatisation conditions

A portion (5 g) of the brown powder was dissolved in distilled water (50 ml) and extracted with dichloromethane (3 × 100 ml). The organic fractions were combined and concentrated, to dryness, under vacuum using a cold-water bath to prevent the loss of volatile components, then reconstituted in dichloromethane (200 μl). A portion (100 μl) of this extract was methylated with diazomethane prepared by the distillation of a solution containing Diazald (2·1 g), anhydrous diethylether (30 ml), potassium hydroxide (0.42 g) and ethanol (10 ml). Excess ethereal diazomethane was added to the dichloromethane extract. After a suitable reaction time (10 mins), the reaction mixture was reduced to dryness under a stream of nitrogen and reconstituted in dichloromethane (50 μl). The second portion (100 μl) of this extract was deuterondiazomethylated with a reagent prepared by the distillation of a mixture containing Diazald (2·5 g), anhydrous diethylether (30 ml), 2-(2-ethoxyethanol)-d (25 ml), and sodium deutroxide (NaOD; 10 ml of a 30% solution). Again, an excess of deuterodiazomethane was used. The reaction mixture was left to stand at room temperature for 10 min. After this time, the reaction mixture was reduced to dryness under a stream of nitrogen and reconstituted in dichloromethane (50 μl).

Equal volumes (20 μl) of both methylated and deuteromethylated fractions were mixed and a portion (0·6 μl) was analysed by GCMS.

GCMS conditions

I. Analysis of a headspace extract of the spray-dried powder

Component separations were made on an OV351 phase fused silica capillary column (25 m × 0·32 mm i.d., 0·15 μm film thickness; ex Phase Separations Ltd, Deeside, UK), mounted in a Hewlett Packard 5790 gas chromatograph, and linked via a direct interface (at a temperature of 250°C) to a Hewlett Packard 5970 mass selective detector (MSD). Data capture was by a Hewlett Packard series 200 data station. Helium was used, at a head pressure of 10 psi, as the GC carrier gas. Sample introduction was via a splitless injector, maintained at a temperature of 220°C. The split vent was closed for a total of 30 seconds on sample injection. A temperature profile of 70°C held for 5 mins, then ramped at 10°C/min to a top temperature of 270°C and held for a further 20 mins, was used to separate components of the headspace extract. The MSD was used in electron impact ionisation mode with an electron energy of 70 eV. Scanned mass range was 450–25 Da at a rate of 1 scan/s. Instrument resolution was set to unit mass.

II. Analysis of a methylated dichloromethane extract

Component separations were made on an OV351 phase fused silica capillary column (25 m × 0·3 mm i.d. 0·15 μm film thickness; ex Phase Separations Ltd, Deeside, UK), mounted in a Hewlett Packard 5890 Series I gas chromatograph, and linked via a direct interface (at a temperature of 250°C) to a VG Analytical 70–250 SE double focusing mass spectrometer. Data capture was by a DEC PDP 11/73 dedicated data station. Helium was used, at a headpressure of 10 psi, as a GC carrier gas. Sample introduction was via a cold on-column injector. The temperature profile used for component separation was as described above. The mass spectrometer was used in electron impact mode with an electron energy of 70 eV. Scanned mass range was 650–25 Da at a rate of 1 second per mass decade (exponential down scan of magnet). Instrument resolution was maintained at 1000 ppm. Ion source temperature was 180°C.

Accurate mass measurements were made by employing the above conditions but using an instrument resolution of 5000 ppm. Perfluorokerosine (PFK) was also bled into the ion source during the duration of the experiment. Molecular formula predictions were made

with a maximum 5 ppm error tolerance on the measured accurate masses.

GC-FTIR conditions

All experiments were performed on either a Hewlett Packard 5890 series II gas chromatograph linked to a Hewlett Packard infrared detector (IRD) or a comparable GC-FTIR system as supplied by Nicolet (500 model FTIR bench). Data capture was on either Hewlett Packard or Nicolet developed data stations. In both cases, the detector was a liquid-cooled mercury-cadmium-tellurium (MCT) detector. Scan range was 700–4000 cm^{-1} at 1 scan per second. Instrument resolution was 4 cm^{-1}. GC conditions were as described above.

Reversed phase HPLC conditions

All reversed phase HPLC separations were performed using Hewlett Packard 1090L liquid chromatograph connected to a Hewlett Packard 3393A integrator. The column used throughout was a 25 cm × 4·6 mm ODS-2 (ex Chromex, Manchester, UK) of particle size 5 μm. A mobile phase linear gradient of 80:20% 0·1 M aqueous ammonium acetate:methanol (held for 10 min) to 50:50% 0·1 M aqueous ammonium acetate:methanol was developed over 30 mins, after which the column was further eluted with 100% methanol. The mobile phase flow rate was 1·0 ml/min. Column eluent was monitored at 430 nm using the HP1090L in-built filter photometric detector. A sample size of 25 μl was injected in the column using the system's in built auto injector.

Thermospray LCMS conditions

A Hewlett Packard 1090L liquid chromatograph was linked via a VG Analytical thermospray/plasmaspray interface to a VG Analytical 70-250 SE double focusing mass spectrometer. Data capture was by a dedicated DEC PDP 11/73 data system. Interface temperature was 250°C. Scanned mass range 650–100 Da at a rate of 1 second per mass decade (exponential down scan of magnet). Instrument resolution was 1000 ppm. Ionisation mode was positive ion plasmaspray. Ion source temperature was 200°C.

RESULTS AND DISCUSSION

A modification of the Grob headspace stripping technique (Grob & Zurcher, 1976) was used to prepare a relatively non-polar extract of the spray-dried glucose–glycine reaction mixture. The total ion current (TIC) chromatogram resulting from GCMS analysis of this extract is shown in Fig. 2. Component identifications are listed in Table 1. Inspection of these data shows that the major volatile components extracted from the brown powder were a series of furans, pyrroles, and pyrazines. The formation of many of these oxygen and nitrogen heterocycles in Maillard reaction mixtures is documented (Olsson *et al.*, 1978; Nursten, 1981). The substituted pyrazines detected in the headspace extract are likely to be responsible for the chocolate-like aroma that was associated with this spray-dried powder.

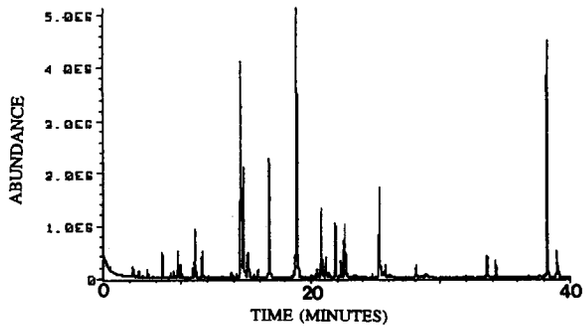


Fig. 2. TIC arising from the GCMS analysis of a headspace extract of the spray-dried glucose–glycine reaction mixture.

Whilst the analysis of a headspace extract of the spray-dried glucose–glycine reaction mixture yielded some useful information regarding this system, a limitation of such protocol is that extracted components must be reasonably volatile. This results in the detection of only a small window of components of complex mixtures. Further characterisation of the spray-dried glucose–glycine system was therefore achieved through extraction of an aqueous solution of the brown powder with dichloromethane. The resultant yellow-coloured extract constituted 0·1% (by weight) of the total powder. Initial GCMS studies (results not shown) of this extract indicated several components were of a polar nature and were not chromatographed satisfactorily. This characteristic was postulated to be a result of an acid residue originating from the incorporation of the glycine in reaction products or the presence of other acidic groups in produced moieties. It was also noted that molecules that possess amine functions would be poorly chromatographed.

During our investigations, those compounds that possessed acidic functionality were derivatised with diazomethane to reduce their polarity. The TIC-chromatogram arising from analysis of a diazomethylated dichloromethane fraction of the brown powder is

Table 1. Components tentatively identified in a headspace extract of a spray-dried glycose–glycine reaction mixture

2-(Hydroxymethyl)pyrrole
2-Furan carbonitrile
2-Furan carboxaldehyde
4-Hydroxy-4-methyl-pentan-2-one
2-Acetyl furan
2,3-Dimethylpyrazine
2,5-Dimethylpyrazine
2,6-Dimethylpyrazine
5-Methyl-2-furan carboxaldehyde
Trimethylpyrazine
N-Methyl-2-acetylpyrrole
2-Ethyl-5,6-dimethylpyrazine
1,5-Dimethyl-2-formylpyrrole

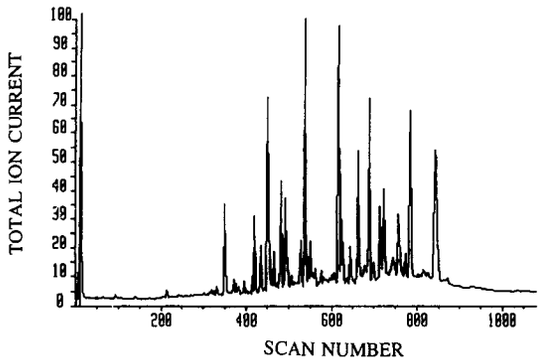


Fig. 3. TIC arising from the analysis of a methylated dichloromethane fraction of the spray-dried glucose–glycine reaction mixture.

shown in Fig. 3. This profile was seen to be more complex than that arising from analysis of a headspace extract of the same material (see Fig. 2).

The information yielded by the above study was increased through the preparation of deuteromethylated analogues of acidic compounds. During these experiments, CHD_2 esters were generated. GCMS studies of samples prepared by mixing equal portions of methylated and deuteromethylated dichloromethane fractions of the brown powder enabled the determination of the number of sites of methylation (hence, the number of acidic groups) of a molecule. This is demonstrated by the electron impact spectra shown in Fig. 4. Examination of these data shows that both molecules exhibited molecular ions of m/z 181. These responses were confirmed by chemical ionisation mass spectrometry (data not shown). In both spectra, there was also an equal abundance ion of m/z 183, which led to the conclusion that both molecules possessed one acidic group. Furthermore, although there were many similarities between these spectra, detection of the ester function in fragment ions aided the deduction of fragmentation pathways for both molecules (see Fig. 5). This in turn allowed the unequivocal identification of both species. Figure 4(a) therefore represents 2-formyl-5-methyl pyrrole-1-acetic acid methyl ester and Fig. 4(b) was identified as 2-acetyl pyrrole-1-acetic acid methyl ester. As these esters were generated after extraction, it was concluded that both were free acids in the spray-dried powder (compounds **1** and **2**). The mechanism of formation of these acids under Maillard conditions has been previously documented (Olsson *et al.*, 1978; Olsson & Pernemalm, 1979).

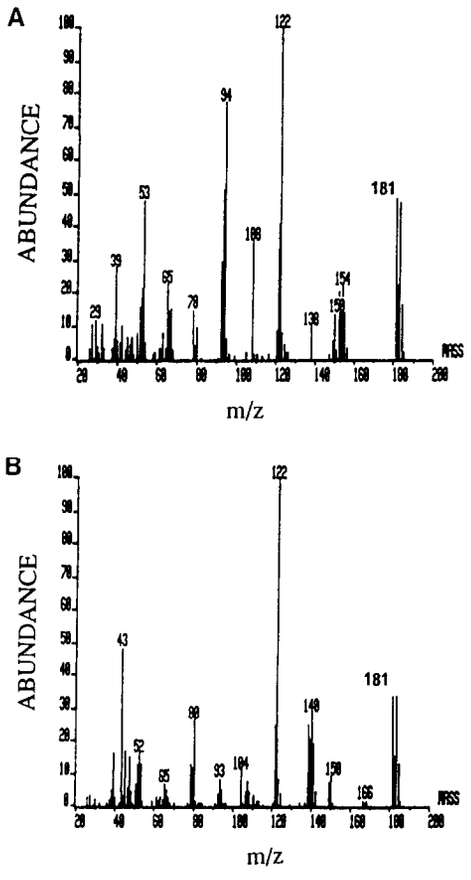


Fig. 4. An electron impact spectra of mixed methyl and deuteromethyl esters. (a) 2-Formyl-5-methylpyrrole-1-acetic acid; and (b) 2-Acetylpyrrole-1-acetic acid.

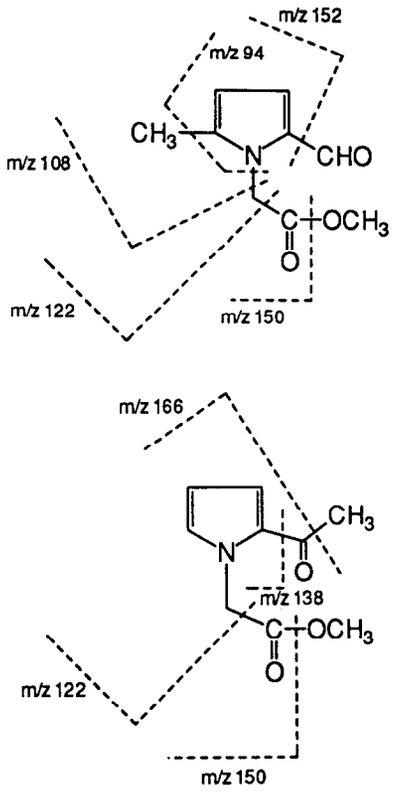
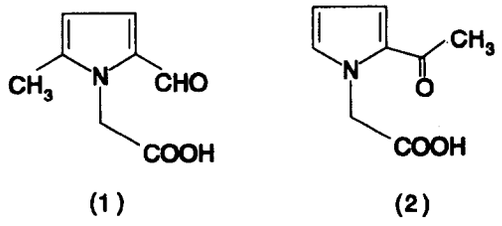


Fig. 5. Fragmentation pathways of 2-formyl-5-methylpyrrole-1-acetic acid methyl ester and 2-acetylpyrrole-1-acetic acid methyl ester under electron impact ionisation.

Throughout these investigations, similar strategies were applied to the identification of other components of the dichloromethane fraction of the brown powder.



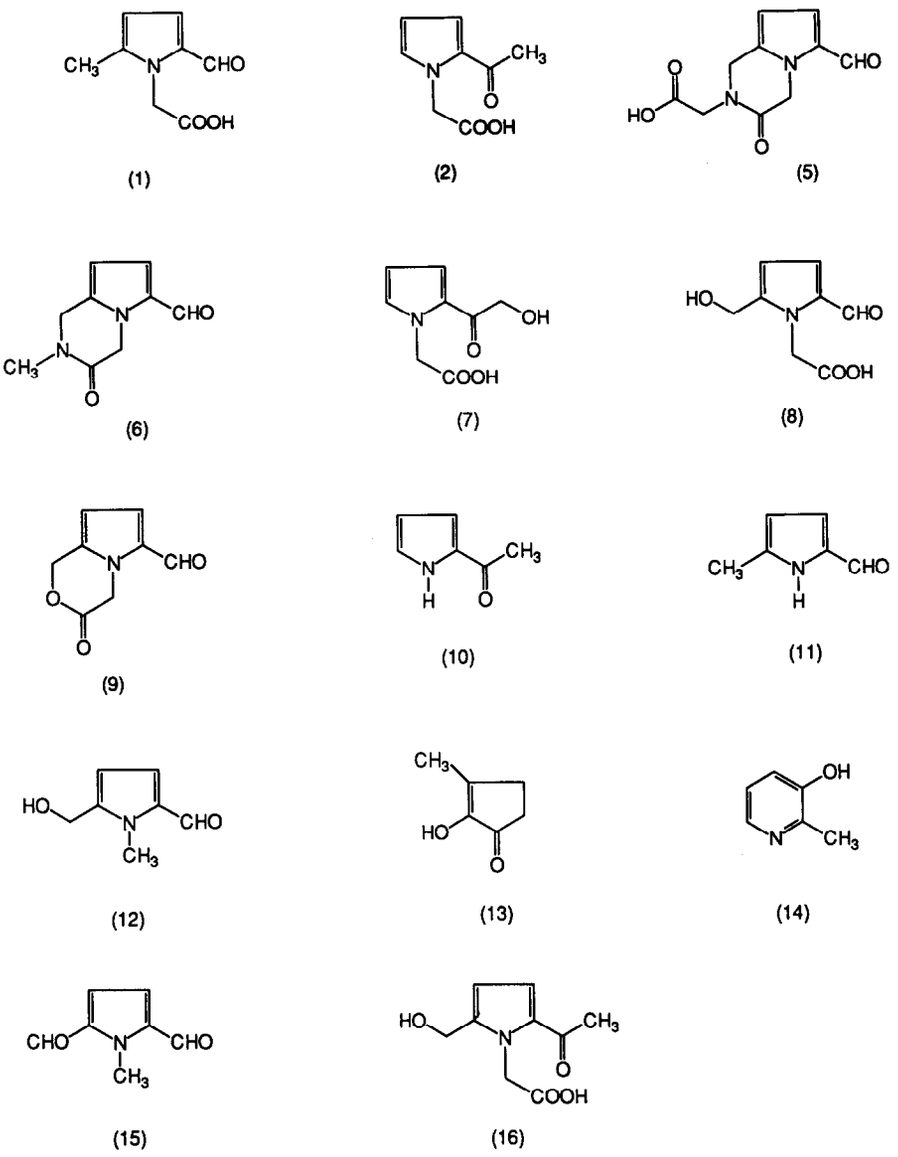


Fig. 6. Identified components of the spray-dried glucose–glycine reaction mixture.

Those species that were positively identified are shown in Fig. 6, and mass spectral data of these compounds are given in Table 2. As expected, it was found that this extract was dominated by oxygen- and nitrogen-containing heterocyclic compounds, with pyrrolic compounds particularly evident.

Chemicals that are responsible for the colour of Maillard reaction mixtures were of particular interest during these studies. Those chemicals that coloured the dichloromethane extract of the brown powder and remained after diazomethylation of this fraction were separated by reversed phase HPLC and monitored at a wavelength of 430 nm. Component characterisation, by mass, was achieved by analysis of the methylated fraction by thermospray LCMS. A typical HPLC profile arising from these experiments is shown in Fig. 7. The most abundant coloured species detected in this chromatogram was characterised by a protonated molecular ion (MH^+) of m/z 249. In further experiments, this yellow-coloured compound was collected as it eluted from the HPLC and analysed by desorption electron impact mass spectrometry. The spectral data recorded during this study (Fig. 8) indicated that this compound had also been detected during the GCMS investigations of the derivatised dichloromethane

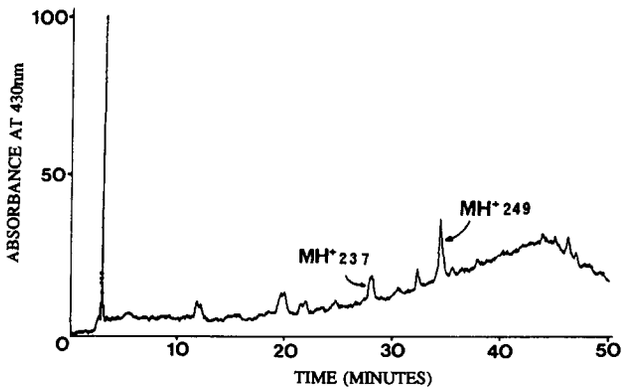


Fig. 7. Reversed phase HPLC profile arising from the analysis of a methylated dichloromethane extract of the spray-dried glucose–glycine reaction mixture (detection at a wavelength of 430 nm).

Table 2. Mass spectral data of compounds identified in a dichloromethane extract of the spray-dried glucose–glycine reaction mixture. (x) is the ions relative abundance

Compound	Prominent ions of mass spectrum (m/z)
1[a]	See Fig. 1(a)
2[a]	See Fig. 1(b)
5[a]	See Fig. 11.
6	See Fig. 13.
7[a]	138(100), 45(70), 166(69), 197(25;M^+), 27(20), 80(18), 52(18), 39(17).
8[a]	108(100), 163(55), 197(50;M^+), 80(49), 140(42), 120(34), 39(30), 45(30).
9	165(100;M^+), 93(42), 52(28), 108(22), 121(14), 136(13), 66(10), 65(9).
10	94(100), 109(82;M^+), 66(50), 39(30), 43(12) 53(8).
11	109(100;M^+), 108(82), 80(45), 53(40), 43(15), 27(14), 29(10).
12	139(100;M^+), 97(45), 122(40), 39(21), 82(17), 67(15), 110(15), 53(12), 124(12).
13	112(100;M^+), 41(50), 69(49), 43(45), 27(35), 39(35), 82(29), 55(28).
14	109(100;M^+), 80(60), 39(33), 27(15), 81(10), 53(10), 60(9), 38(9).
15	137(100;M^+) 107(32), 79(20), 52(18), 108(17), 78(17), 51(16), 93(16).
16[a]	122(100), 150(85), 209(84; M^+, 177(55), 68(23), 39(23), 82(15), 153(13).

[a] Mass spectral data measured as methyl esters

fraction. In addition, the results of analysis of a deuteromethylated analogue of this species showed that it possessed one acidic residue. The accurate mass of this molecule was also determined and led to a predicted molecular formula of $C_{12}H_{12}N_2O_4$. An assignment of the structure of this molecule was, however, difficult from these data alone. Complementary structural evidence was achieved by analysis of the methylated fraction by GC-FTIR. Thus, considering both IR (Fig. 9) and mass spectra data, it was postulated that the structure of this molecule was the methyl ester of compound **3** (note the free acid was prepared by reaction of glucose with glycine). This compound, however, remains to be synthesized; hence, the proposed structure must at present be regarded as tentative. Further inspection of the GCMS data highlighted a compound that appeared to be related to **3**. The electron impact spectrum of this species, as its methyl ester (Fig. 10), indicated a molecular ion of m/z 234, and from accurate mass determination, a chemical formula of $C_{11}H_{10}N_2O_4$ was predicted. The postulated structure for this species is shown as compound **4**. As the structure of this compound is similar to that of **3** it is also suspected to be coloured. Again, this chemical to be synthesised before it can be fully characterised.

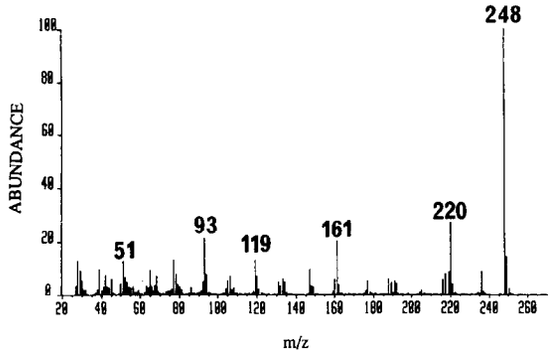


Fig. 8. An electron impact spectrum arising from the mass spectral analysis of compound **3**; recorded as its methyl ester.

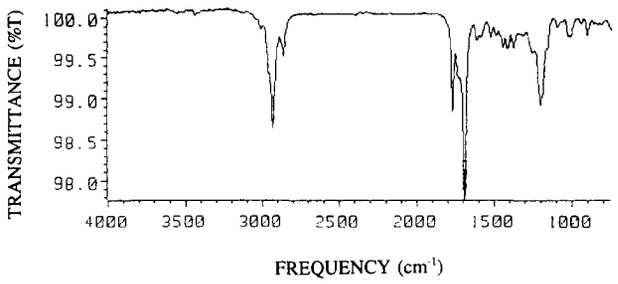


Fig. 9. A gas-phase FTIR spectrum arising from the analysis (by GCFTIR) of compound **3**; recorded as its methyl ester.

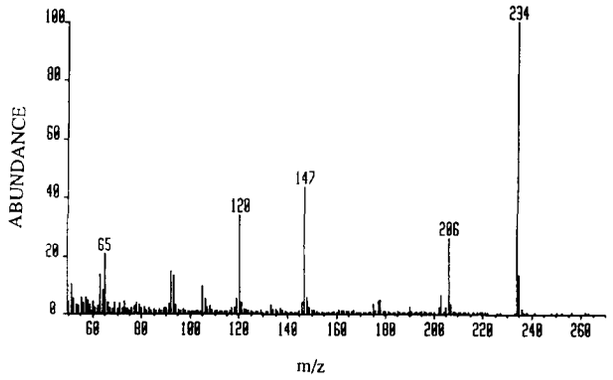
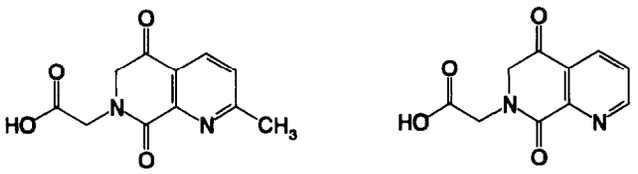


Fig. 10. An electron impact spectrum arising from the mass spectral analysis of compound **4**; recorded as its methyl ester.



(3) (4)

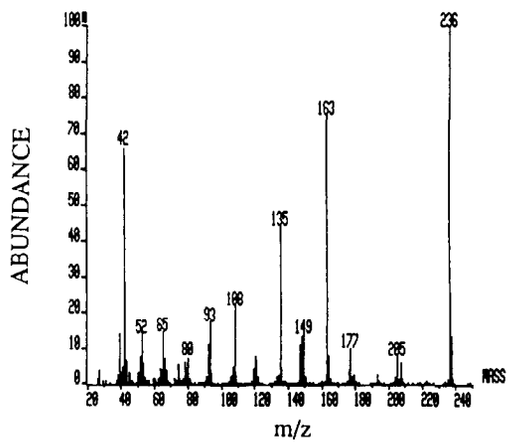


Fig. 11. An electron impact spectrum arising from the mass spectral analysis of compound **5**; recorded as its methyl ester.

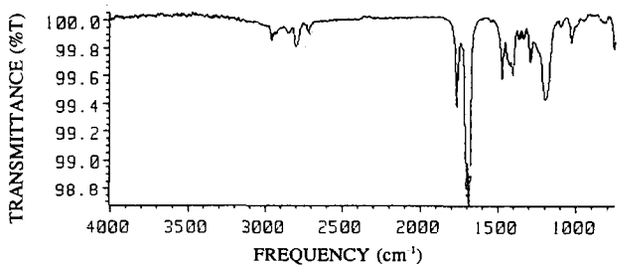
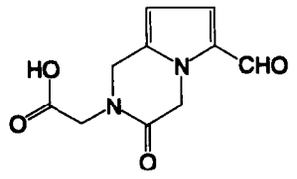
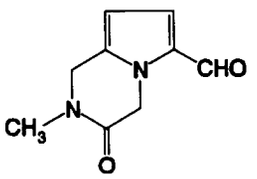


Fig. 12. A gas-phase FTIR spectrum arising from the analysis (by GC-FTIR) of compound **5**; recorded as its methyl ester.

A second coloured species detected in the HPLC profile (Fig. 6) was characterised by a protonated molecular ion of m/z 237. The electron impact spectrum recorded for this molecule (as its methyl ester) is given in Fig. 11. Consideration of these data and the gas phase IR spectrum (Fig. 12) arising from GC-FTIR studies of this species led to its identification as compound **5**. This molecule has been previously reported as a component of glucose–glycine Maillard reaction mixtures (Olsson *et al.*, 1978; Olsson & Pernemalm, 1979) but is not previously reported as being coloured. In addition, we have detected, during GCMS studies, a compound that is chemically similar to **5**. The electron impact spectrum of this species is shown in Fig. 13. Interpretation of these data led to the identification of this molecule as compound 6.



(5)



(6)

Comparison of the chemical structures of compounds **5** and **6** indicates that **6** could have been produced by loss of a molecule of carbon dioxide from **5**. Furthermore, it is suspected that compound **6** is weakly coloured due to the conjugation possible within this molecule and its similarities to **5**, a proven coloured species.

CONCLUSIONS

The usefulness of isotopically labelled derivatives of reaction products was demonstrated as an aid to structure elucidation of species of Maillard reaction mixtures, when mass spectrometry is used for their detection. Applying such strategy in conjunction with GCMS, GC-FTIR, and reverse phase HPLC techniques, we have shown that many of the low-molecular-mass products of a spray-dried glucose–glycine reaction mixture are substituted oxygen and/or nitrogen heterocycles. Pyrrole chemistry was particularly evident.

Finally, a strategy for the detection of low-molecular-mass coloured components of Maillard reaction mixtures was presented. This methodology was applied to the detection of two novel coloured components of the spray-dried glucose–glycine reaction mixture. Whilst these molecules require synthesis for their complete characterisation, structures in keeping with IR and MS data were postulated.

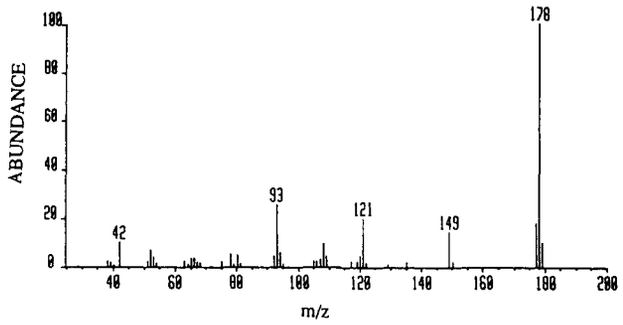


Fig. 13. An electron impact spectrum arising from the mass spectral analysis of compound **6**.

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