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Coloured Compounds Formed by the Interaction of Glycine and Xylose

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ABSTRACT

Coloured compounds were isolated and characterised from the products of the reaction between aqueous xylose (1 M) and glycine (1 M), refluxed for 2h, initial pH, 6. The products were separated by extraction with light petroleum (boiling point, 60–80°C) followed by ethyl ether. The brown ether-solubles were fractionated by TLC using two systems and semi-preparative reversed phase HPLC in sequence. The twenty-two main peaks were collected and freed from adjacent peaks and shoulders by further HPLC before mass spectrometry. The seventeen compounds which gave orange or yellow solutions in methanol were of the following molecular weights: 149, 165, 178, 191, 192(a), 192(b), 192(c), 222(a), 222(b), 279, 281, 319, 364, 368, 385(a), 385(b) and 448 daltons. The compound of 319 daltons (B319) was characterised by pmr, electronic absorption spectroscopy and MS (low and high resolution). A structure is proposed for it. For the six coloured compounds over 300 daltons there is evidence for the presence of 3 and 4 linked heterocyclic rings. These may arise from reactions of 2-furfurylidene-4-hydroxy-5-methyl-3(2H)-furanone or a nitrogen-bearing analogue. Structures for some fragments have been proposed, the high resolution MS data for B319 helping indirectly, where compounds, for which only low resolution data were available, exhibited common ions.

INTRODUCTION

Colour formation is the primary characteristic of the Maillard reaction. Structural studies on the chromophores have been hindered in the past,

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because these products form complex mixtures of an unstable nature (Nursten, 1981). For simplicity, a xylose–glycine model system was used for all our studies (O'Reilly, 1982). The extreme complexity of the reaction products formed from even such a model system has been demonstrated by HPLC of fractions obtained by solvent extraction (O'Reilly, 1981).

In previous work, compounds soluble in light petroleum (boiling point, 60–80 °C), Fraction A, were progressively eluted on reversed phase HPLC, their molecular weights being 114, 346, 178, 194(a), 194(b), 194(c), 192, 272(a), 272(b), 371, 257, 326 and 342 daltons. With the exception of the first, 4-hydroxy-5-methyl-3-(2*H*)-furanone, all compounds were coloured (Nursten & O'Reilly, 1983). This paper deals with the compounds soluble in ethyl ether, Fraction B, after removal of Fraction A.

EXPERIMENTAL

A separation scheme for the xylose–glycine browning reaction products is given in Fig. 1. The light petroleum-solubles have already been described (Nursten & O'Reilly, 1983). Here we are solely concerned with the ether-solubles obtained as a brown oil in 0·08% yield, based on the weight of reactants. Fraction B is complex, HPLC analysis showing more than sixty peaks detected by absorbance at 260 nm and at least thirty-five at 450 nm (Fig. 2a). For clarity, the HPLC profile of Fraction B has been divided into three groups (1, 2 and 3), with each peak, or cluster of peaks, numbered. Reproducibility of HPLC separations requires care, a 0% to 100% methanol gradient helping, as did purging the column between injections with methanol and then with water for 5 min each. Although Fraction B could be stored in methanol at −20 °C without change in HPLC profile, it does alter after more than a day at room temperature.

Group 3 was chosen for further study because it was stable and contained most of the colour of Fraction B. Separation of Group 3 from Fraction B by HPLC and re-examination using the same solvent programme showed Group 3 to elute unchanged and with Groups 1 and 2 absent (see Fig. 2b). Group 3 was best obtained from Fraction B by preparative TLC (System 1) and further separated by HPLC (System 2) into at least thirty-six peaks detected at 450 nm (see Fig. 3). Twenty-one peaks from Group 3 were isolated by an elaborate procedure involving

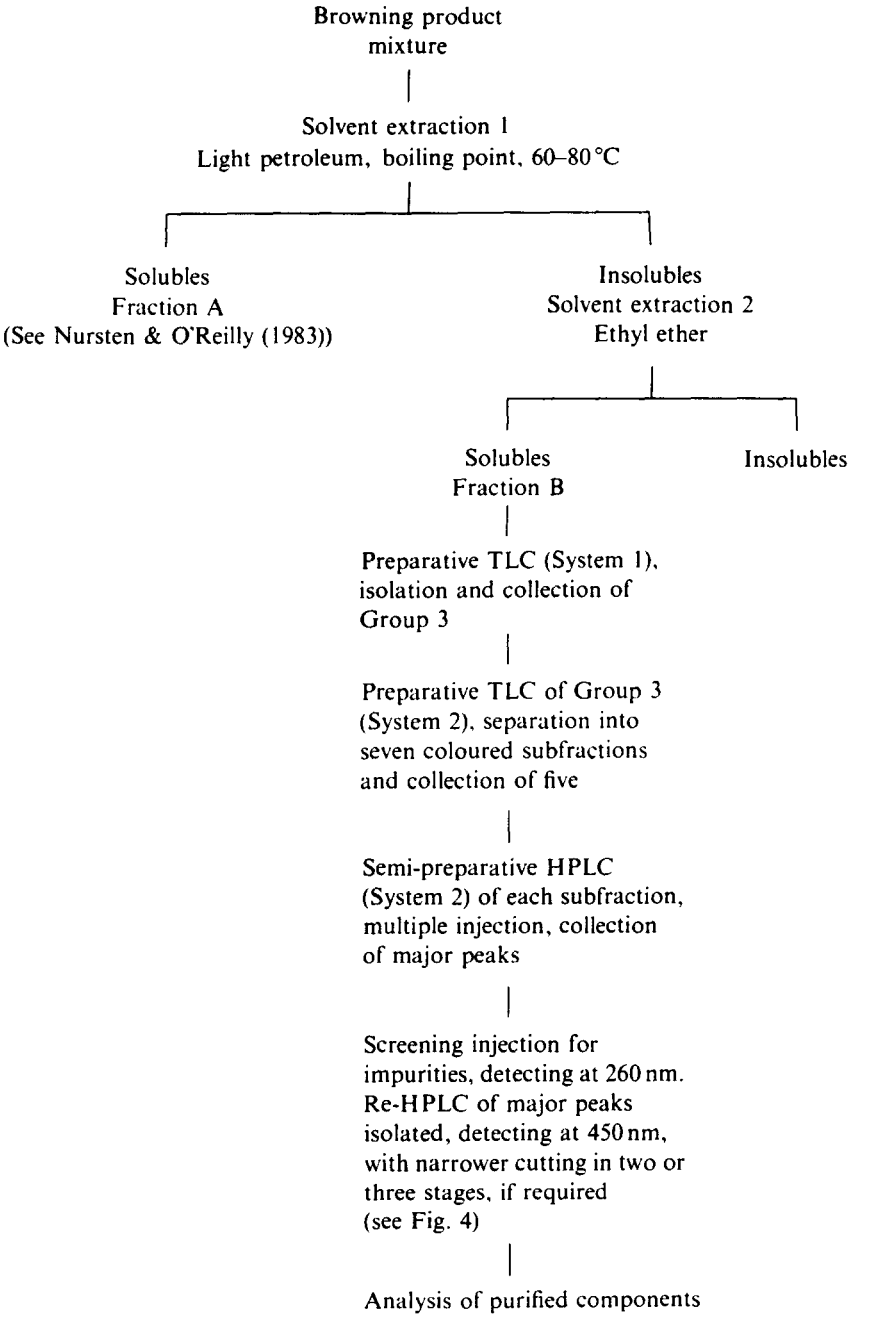


Fig. 1. Separation scheme for coloured Maillard products.

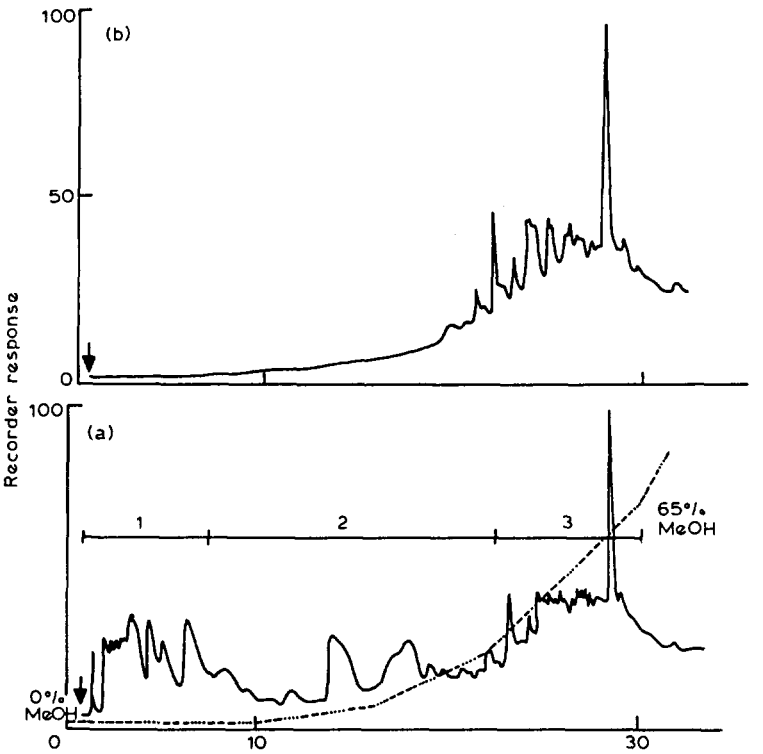


Fig. 2. HPLC separation. (a) Fraction B. (b) Group 3, obtained by HPLC of Fraction B and reinjection under the same conditions. Column: 0·8 inside diameter × 25 cm, Spherisorb ODS 5 μm average particle size, $N = 18\,000$–$20\,000\ m^{-1}$. Detection: 450 nm, sensitivity 10 mV FSD; 0·5 AUFS. Loading: 30 μl un methanol. Solvent: flow rate, 6 ml min^{-1}. Solvent programme 1: step 1, 0% min^{-1} (10 min); step 2, 1% min^{-1} (5 min); step 3, 2% min^{-1} (5 min); step 4, 5% min^{-1} (10 min); step 5, 10% min^{-1} (3 min).

two preparative TLC separations and semi-preparative HPLC in sequence. The solvent was removed at each stage (Fig. 1). For characterisation of compounds, Fraction B was repeatedly injected as a solution in methanol and the peaks were collected. Individual peaks were then subjected to HPLC again, sometimes twice more, checking peak symmetry. Each step causes losses of 30–50%, so, for most peaks, complete purification was not possible, because of insufficient material. The process for B319 is illustrated in Fig. 4. For all compounds, the number represents the molecular weight as determined by mass spectrometry and B denotes the parent fraction.

For B319 there was sufficient sample for high resolution MS, FT pmr

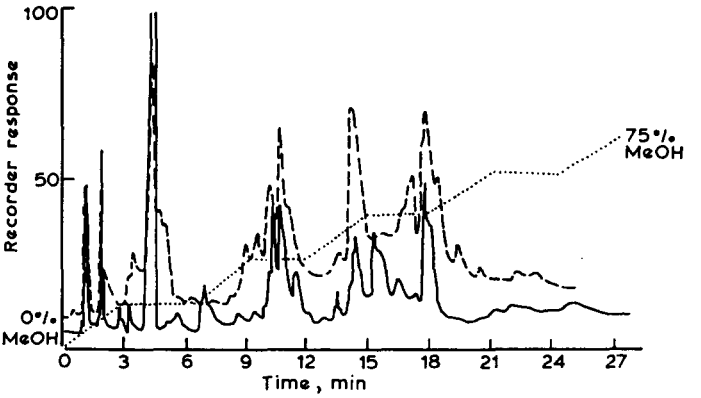


Fig. 3. HPLC separation of Group 3 obtained by TLC (System 1 of Fraction B). Column: See Fig. 2. Detection: —— 260 nm, 0·2 AUFS; 10 mV FSD; ----- 450 nm, 0·5 AUFS, 10 mV FSD. Loading: 5 μl, – – – 20 μl, sample in methanol. Solvent: Flow rate, 6 ml min^{-1}. Solvent programme 2: Initial, 0% methanol (100% water); final, 75% methanol (25% water). Solvent change, 5% min^{-1} for 3 min every 6 min.

and electronic absorption spectroscopy. For the remaining twenty-one compounds there was sufficient sample for HPLC and low resolution MS data only.

Preparation of browning product mixtures

Xylose (15 g) and glycine (7·5 g) were dissolved in Sørensen's phosphate buffer (Diem, 1968) (1/15M, pH 8·2, 100 ml) and heated under reflux for

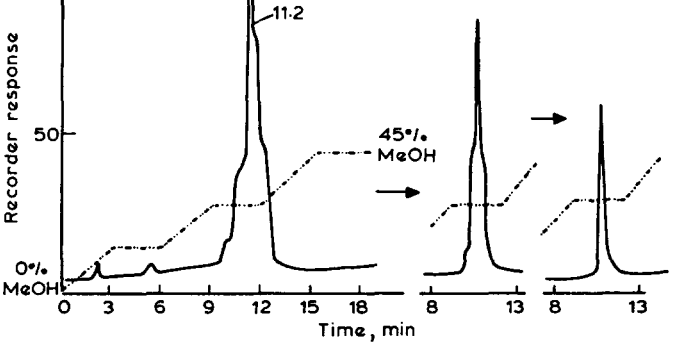


Fig. 4. Purification of B319 by HPLC. Loading: 20 μl in methanol. Detection: 450 nm; 0·2 AUFS. Other conditions as Fig. 3.

2 h. On 250-fold dilution with water, the absorbance at 450 nm was 0·75 ± 0·05. The preparation was also carried out in the absence of buffer, initial pH, 6·0. Larger amounts were prepared on five and ten times this scale.

Solvent extraction

Immediately after preparation, the browning product mixture was continuously extracted, first with light petroleum (boiling point, 60–80 °C) and then ethyl ether. About 2 h were required for each stage before no further colour was extracted. In each case the solvent was removed under vacuum <60 °C.

HPLC

The sample was injected on to an ACS Model LC750 chromatograph with dual pumps (Applied Chromatography Systems Ltd, Luton, Beds., Great Britain) by means of a loop injection valve, Model 7120 (Rheodyne Inc., Berkeley, CA94710, USA). A single channel, variable wavelength UV detector with a 1 cm pathlength quartz flowcell (8 μl volume) was used, Model CE212 (Cecil Instruments Ltd, Cambridge, Great Britain). The chart recorder, Model BS6000 (Bryans Southern Instruments Ltd, Surrey, Great Britain), was preset at 10 mV full scale deflection. For semi-preparative HPLC, a 50 μl loop was made from AISI Type 316 stainless steel tubing.

HPLC was carried out at room temperature on a 0·8 inside diameter × 25 cm stainless steel column, laboratory-packed with Spherisorb ODS (Phase Separation, Ltd, Queensferry, Clwyd, Wales, Great Britain), a reversed phase material. HPLC grade methanol (Rathburn Chemicals Ltd, Walkerburn, Peebleshire, Scotland, Great Britain) was used. Assessment of peak purity using 260 nm takes advantage of the increased sensitivity as well as detecting colourless, but UV-absorbing, compounds.

TLC

Preparative TLC was performed on silica layers 1 mm × 20 × 20 cm (BDH Chemicals Ltd, Poole, Dorset, Great Britain). Separated coloured components were detected visually.

Solvent system number 1, methyl acetate:water (8:1 v/v).
Solvent system number 2, ethyl acetate:toluene (1:1 v/v).

Electronic absorption spectroscopy

Samples were examined in methanol or ethanol on a double-beam spectrophotometer, Model SP800B (Pye Unicam Instruments Ltd, Cambridge, Great Britain).

PMR

PMR spectra were recorded at 100 MHz, using a Varian HA-100 instrument (Varian Associates Ltd, Walton-on-Thames, Surrey, Great Britain), chloroform-*d* as solvent and tetramethylsilane as internal standard (NMR Ltd, High Wycombe, Bucks, Great Britain), with a normal sweep width of 1000 Hz and sweep time of 500 s. Fourier Transform pmr spectra were recorded using a Bruker WH300 instrument (Bruker Instruments Inc., Manning Park, Billerica, MA01821, USA) and methanol-*d* as solvent (NMR Ltd).

Mass spectrometry

Low resolution EI spectra were determined with an MS9 (AEI Ltd, Urmston, Manchester, Great Britain) at 70 eV. A probe was used, normally at 100 °C. For B319, the molecular ion was also examined under high resolution on the MS9. More extensive high resolution data were obtained using the ARC/FRI Mass Spectrometry Service (Food Research Institute, Norwich, Norfolk, Great Britain) and perfluorokerosene as reference.

RESULTS

TLC, HPLC and MS data for all components isolated are summarised in Table 1. Molecular ions and up to ten prominent fragment ions in decreasing order of relative abundance are listed. Mass spectra were examined alongside relevant available data. Data for B319 are given in Table 2 and Fig. 5. Both pmr and MS data are complicated by impurities. Peaks between *m/z* 219 and 181 were excluded from the high resolution data, as the reference peaks used in this region were wrongly chosen. The base peak *m/z* 193 is therefore missing, having been mistaken by the data system as a reference ion. The low resolution spectrum was used as a check for the high resolution data. The impurities did not correspond to

TABLE 1
TLC, HPLC and Mass Spectral Data for Compounds Isolated from Group 3

TLC[a] *(Rf)*	*HPLC retention (cm)*[b]	*Molecular ion, m/z (% RA)*[c]	*Mass spectrum prominent peaks m/z (% RA)*[c]
	15·4	178 (100)	29(64), 60(54), 150(49), 57(46), 77(38), 107(36), 55(35), 31(31)
0·49	19·7	192 (81)	43(100), 44(86), 121(67), 31(47), 45(40), 29(35), 40(28), 114(25), 77(21), 57(21)
	20·5	448 (4)	43(100), 29(100), 168(47), 55(37), 346(37), 41(30), 60(28), 45(28), 81(27)
0·41–0·47	4·4	114 (40)	43(100), 44(38), 176(17), 29(11), 40(10), 55(9)
	11·3	281 (2)	43(100), 114(45), 44(39), 59(32), 29(32), 222(23), 107(23), 60(22), 45(20), 176(17)
	4·45–4·7	114 (72)	43(100), 55(8), 29(6), 42(6), 44(5), 115(5), 71(4), 58(4)
0·42[e]	9·15–9·65	192 (2)	43(100), 44(95), 29(22), 114(21), 40(17), 45(10), 60(10), 31(10), 95(9·5)
	11·0–11·3	222 (18)	43(100), 162(36), 114(27), 29(24), 107(18), 95(14), 192(14), 193(11), 176(10)
	14·9	385 (1)	193(100), 43(39), 194(21), 162(17), 107(16), 192(13), 81(12), 44(11), 121(10), 176(9)
0·38	[d]	114 (100)	43(100), 31(45), 29(29), 55(19), 44(10), 115(8)
	[d]	222 (0·2)	43(100), 114(46), 29(10), 55(8), 31(5), 192(4)
	2.0	368 (1)	192(100), 121(87), 43(74), 81(27), 31(24), 193(20), 32(18), 114(18), 65(13)
0·35–0·37	4·4	114 (40)	43(100), 44(38), 29(11), 55(9), 102(2), 115(2)
	14·9	364 (1)	162(100), 43(52), 193(44), 44(25), 163(19), 29(15), 114(13), 134(8), 107(8), 45(8)
	21·9	192 (14)	29(100), 43(73), 114(27), 120(16), 192(14), 60(10)
	25·1	385 (1)	193(100), 43(100), 192(72), 114(72), 44(47), 81(44), 107(30), 194(22), 121(18), 162(15)
	10·2	165 (4)	31(100), 29(48), 44(19), 69(16), 57(11), 81(10), 43(10), 41(10), 55(9), 149(9)
0·30	11·2	319 (23)	see Table 2
	14·3	149 (20)	31(100), 29(55), 57(39), 55(31), 43(31), 69(30), 41(26), 83(23), 71(20), 149(20)
	1·85	279 (6)	31(100), 44(88·5), 29(61·5), 149(50), 57(38·5), 69(29), 41(27), 43(23), 71(19)
0·07–0·16	10·85	191 (100)	43(44), 60(39), 106(26), 57(26), 41(26), 55(24), 45(22), 77(22), 69(21)
	11·55	60 (27)	31(100), 29(50), 44(30), 60(27), 40(12), 45(5), 57(4)

[a] TLC subfraction of Group 3 using solvent system 2.
[b] HPLC retention in centimetres, using solvent programme 2 (see Fig. 3).
[c] RA = Relative abundance (to base peak).
[d] Direct MS without HPLC.
[e] Band 0·41–0·47 taken with a narrower cut.

TABLE 2
Data for B319

HPLC retention = 11·2 cm (see Fig. 4).
λ_{max}^{MeOH} 235, 292, 375 nm, tailing into the visible region and accounting for the yellow colour.

FT pmr—Aromatic region

6·35–6·375	(J = 1·5 Hz)	quadruplet	1 H (3)
6·45–6·46	(J = 3·0)	doublet	1 H (4)
7·385–7·40	(J = 1·7)	quadruplet	1 H (2)

The aliphatic region was complicated by impurities.

MS (low resolution)
M^+ 319 (23), *m/z* 193 (100), 28 (100), 162 (46), 110 (29), 107 (18·5), 43 (18), 194 (11), 210 (11), 29 daltons (9% RA).

MS (high resolution)
Accurate molecular mass: 319.10592 daltons.[a] Calculated for $C_{16}H_{17}NO_6$: 319.10558 daltons.
Selected fragment data:

Observed ion mass	*Fragment ion formula*	*Observed ion mass*	*Fragment ion formula*
319·1026[a]	$C_{16}H_{17}NO_6$	134·0548	$C_5H_{10}O_4$
301·0984	$C_{16}H_{15}NO_5$		or: C_8H_8NO
291·1058	$C_{15}H_{17}NO_5$	126·0294	$C_6H_6O_3$
288·0898	$C_{15}H_{14}NO_5$		or: $C_4H_4N_3O_2$
276·0919	$C_{14}H_{14}NO_5$	107·0405	C_6H_5NO
260·0936	$C_{14}H_{14}NO_4$	95·0852	C_7H_{11}
210·0756	$C_{10}H_{12}NO_4$	95·0480	C_6H_7O
162·0558	$C_9H_8NO_2$	95·0347	C_5H_5NO

[a] The discrepancy between the exact masses for the molecular ions obtained with different instruments was expected. That obtained on the AEI MS9 had the higher accuracy.

perfluorokerosene reference fragments and are attributed to contamination of the very small amount of B319 isolated.

DISCUSSION

The discussion falls into two parts—B319 and the other compounds.

From Fraction B, Group 3, twenty-two components were isolated and

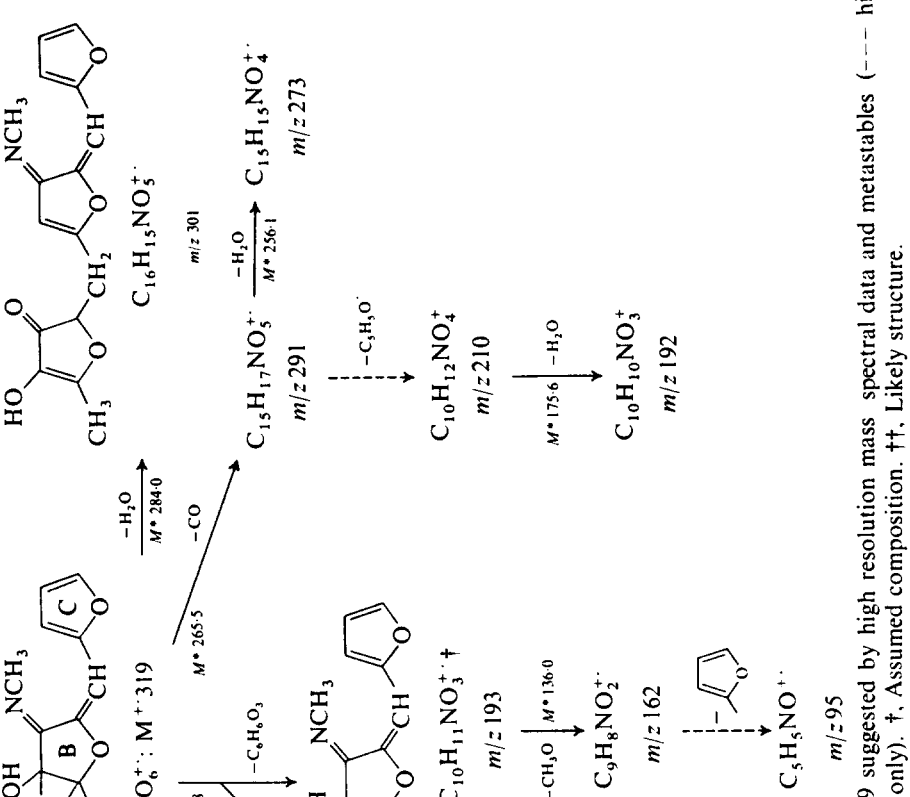


Fig. 5. A partial fragmentation scheme for B319 suggested by high resolution mass spectral data and metastables (--- high resolution mass spectral data only). †, Assumed composition. ††, Likely structure.

characterised by TLC, HPLC and MS, seventeen of these having molecular weights between 149 and 448 daltons and giving yellow or orange solutions in methanol.

B319

B319, $C_{16}H_{17}NO_6$, is a new compound (yield, 600 μg), giving a yellow solution in methanol. The molecular formula was derived by high resolution mass spectrometry (Table 2). (1) The molecular ion was 319·10592 daltons, with two possible formulae, $C_{17}H_{13}N_5O_3$ (319·10692) and $C_{16}H_{17}NO_6$ (319·10552). The high nitrogen content of the former makes it unlikely; the latter was closer to the measured mass, but the M + 1/M RA ratio favoured seventeen carbons (4·3/23 × 100 = 18·70/1·1 = 17·0). (2) Formulae found for ion fragments can only come from $C_{16}H_{17}NO_6$. The relatively stable molecular ion, 23% of the intensity of the base peak, and the high mass of the base peak, *m/z* 193, point to a cyclic structure. The formula implies nine rings and/or double bonds and doubly charged peaks at *m/z* 93·5 and 144·5 support a highly conjugated structure and/or linked heterocycles.

The composition of the base peak ion is not known from high resolution measurement, but $C_{10}H_{11}NO_3^{+\cdot}$ seems reasonable. Important losses from M^+ include M-18, M-28 and M-31. B319 is quite polar, as shown by the HPLC retention and the loss of water and $-CH_3O$ given by the high resolution data. There are numerous structures for $C_{16}H_{17}NO_6$ and many possible assignments to the high mass fragments. High resolution MS data on the fragments lessened these possibilities. There are seven metastable ions visible (Table 3) and the formulae for the neutral fragments lost in metastable transitions were confirmed by high resolution MS data, enabling a fragmentation pattern to be assigned, with suggested structures for M^+ and fragments *m/z* 301, 193 and 126 (see Fig. 5).

It is reasonable to assume that the C_{16} structure is built from three five-carbon units, which themselves originate from three xylose molecules. The sixteenth carbon is probably attached to the nitrogen and originates from a glycine molecule. The formula $C_{16}H_{17}NO_6$ represents $3 \times C_5H_{10}O_5 + C_2H_5NO_2 - CH_{18}O_{11}$ (i.e. a loss of $9H_2O + CO_2$). For simplicity, B319 is divided into three subunits as in Fig. 5. The presence of a furan monosubstituted at C-2 (Ring C) is evident from the aromatic region of the pmr spectrum. It could originate from 2-furaldehyde. This

TABLE 3
The Metastable Peaks of B319 (daltons)

Observed m/z	*Calculated*	*Mass loss and fragment type*	*Transition m/z*
284·0	$\frac{(301)^2}{319} = 284{\cdot}0$	18 H_2O	319 → 301
265·5	$\frac{(291)^2}{319} = 265{\cdot}5$	28 CO or C_2H_4	319 → 291
256·1	$\frac{(273)^2}{291} = 256{\cdot}1$	18 H_2O	291 → 273
238·8	$\frac{(276)^2}{319} = 238{\cdot}8$	43 C_2H_3O	319 → 276
175·6	$\frac{(192)^2}{210} = 175{\cdot}6$	18 H_2O	210 → 192
136·0	$\frac{(162)^2}{193} = 136{\cdot}0$	31 CH_3O	193 → 162
116·8	$\frac{(193)^2}{319} = 116{\cdot}8$	126 $C_6H_6O_3$	319 → 193

uses three of the nine rings and/or double bonds. There are no other aromatic protons. No —CHO is present, there being no resonance at δ 9·7–10·5. The high resolution MS data place the nitrogen-bearing moiety in the centre of a trinuclear structure. The furan ring is directly attached to the nitrogen-bearing moiety and is part of the *m/z* 162 fragment, which is formed from *m/z* 193 by loss of CH_3O (see Fig. 5). In Table 2, *m/z* 126·0294 (6%) has two formulae listed, $C_4H_4N_3O_2$ and $C_6H_6O_3$. The former is ruled out by the formula for M^+ and the difference between the accurate masses for *m/z* 288 ($C_{15}H_{14}NO_5$) and *m/z* 162 ($C_9H_8NO_2$) also leaves $C_6H_6O_3$. The important alternative with one nitrogen in *m/z* 126, $C_6H_8O_2N$ 126·063 324, was ruled out, its mass falling outside the tolerance set for the high resolution data. $C_6H_6O_3$ could originate from 4-hydroxy-5-methyl-3(2*H*)-furanone and a bridge methine group derived from attack by an aldehyde on the activated ring methylene group, with loss of water. The λ_{max}^{MeOH} 292 nm fits in with such a five-membered heterocyclic α-substituted enone (Ring A) (Scott, 1965).

This group is not responsible for the colour. Ring B has three double bond equivalents ($C_{16}H_{17}NO_6 = 9$; Ring A + C = 6). A ring structure for B is favoured, leaving two double bonds, as well as bonds to both A and C.

Hodge (1967) described two routes by which pentoses can cyclise. Nitrogen can be incorporated, as suggested by Kato & Fujimaki (1968). The most plausible way of linking the three five-carbon units is by aldol condensation (Ledl & Severin, 1978).

$C_{16}H_{17}NO_6$ (319 daltons) is a new compound. The structure can be rationalised on the basis of a monosubstituted furan and 4-hydroxy-5-methyl-3(2*H*)-furanone linked by a nitrogen bearing moiety, $C_5H_5NO_2$, as suggested in Fig. 5.

The other compounds

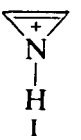
For the remaining twenty-one compounds there was sufficient sample for TLC, HPLC and low resolution MS data only. Seventeen of them gave yellow or orange solutions in methanol, their molecular weights being in the range 149–448 daltons. Some generalisations can usefully be made about the mass spectra.

(1) Several of the observed fragment ions are common to several spectra and thus may originate from similar structural units. The following fragments occur as one of the ten most prominent peaks in the mass spectra of five or more compounds: *m/z* 29, 31, 40, 41, 43, 44, 55, 57, 60, 69, 71, 81, 107, 114, 121, 192, 193 daltons. The more polar nature of this fraction is shown by some compounds giving fragments of *m/z* 31, 45 and 60 daltons.

(2) *m/z* 40, 41, 55, 57, 60 and 81 daltons are common low mass fragments for pyrrole and furan (Porter & Baldas, 1971). *m/z* 41 daltons is likely to be the aziridinium fragment I or $C_3H_5^+$.

(3) *m/z* 29, 43 and 81 daltons are likely to be formylium, acetylium and furfurylium radicals, respectively.

(4) *m/z* 121 and 192 daltons may have particular structural significance, the former probably having the structure II, and the latter possibly being derived from a 2-furfurylidene-4-hydroxy-5-methyl-3(2*H*)-furanone.



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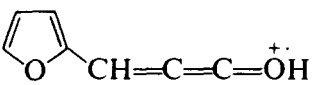

II

(5) The spectrum of B191 is noteworthy in having a molecular ion as base peak. B368, B364, B319 and B385 have base peaks of high mass, m/z 192, 162, 193 and 193 daltons, respectively.

(6) B368, B385(a) and B385(b) have some fragments similar to those from 2-furfurylidene-4-hydroxy-5-methyl-3(2*H*)-furanone; namely, at $m/z = 43$, 121 and 192 daltons.

(7) B385(b) is composed of two stable units, m/z 192 and m/z 193 daltons, linked by a single bond, which is readily cleaved in the mass spectrometer. The nature of the linkage is uncertain, but high resolution MS data on these ions would be most helpful.

The occurrence of fragments in B385(b) which are common to 2-furfurylidene-4-hydroxy-5-methyl-3(2*H*)-furanone suggests formation by linking heterocycles, such as these or nitrogen-bearing analogues. This is particularly likely from observing abundant fragments at m/z 193 (100) and m/z 192 daltons (72%). This compound constitutes the first evidence for the build up of a tetranuclear structure during Maillard reactions.

(8) B222, B319, B364, B385(a) and B385(b) have abundant fragments at m/z 193, 162 and 107 daltons. High resolution MS data are available for these fragments for B319: m/z 193 $C_{10}H_{11}NO_3$ (via the metastable); m/z 162 $C_9H_8NO_2$; m/z 107 C_6H_5NO. The fact that the three fragments occur together strongly suggests their having the same composition in each case. The fragments are related in Fig. 6, and confirmed by metastables for B319 and B364.

Volatile low-mass N- and O-bearing heterocyclic compounds are well known products from Maillard systems. Colour requires conjugated unsaturation and appropriate linkage of such heterocyclic compounds could provide it. Aldol condensation and reaction of amines with carbonyl compounds are two mechanisms for linking two heterocycles.

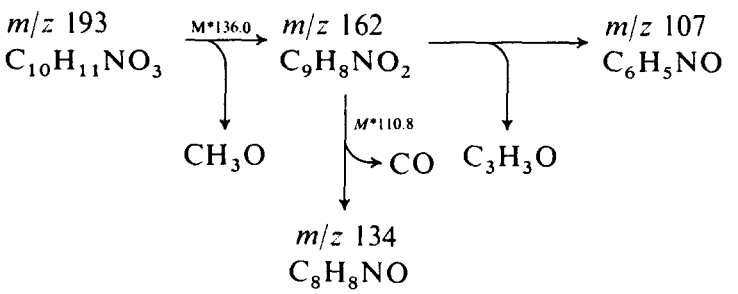

Fig. 6. A scheme relating fragments m/z 193, 162, 134, 107 daltons for B319 and B364.

Ledl and Severin's (1978) route to coloured compounds is of the aldol type, but it cannot proceed beyond three rings joined and so cannot alone explain the build up of the tetranuclear structures isolated here.

CONCLUSION

A new coloured compound, $C_{16}H_{17}NO_6$, molecular weight 319 daltons, has been isolated and a tentative structure proposed for it. Five of the seventeen remaining yellow or orange compounds, B364, B368, B385(a), B385(b) and B448, have provided evidence of the presence of 3 or 4 linked heterocyclic rings.

Determination of structure was made difficult by the small quantities available. Some fractions, even after being submitted to several separation techniques, remain impure. Reactivity has complicated matters and B448, in particular, may have been modified by reactions subsequent to HPLC. Further difficulties were due to the high molecular weights of the compounds examined, the limited high resolution MS data obtained and the few compounds of relevant structure known.

It is difficult to assemble this information into a coherent picture at this stage. Of particular interest are B191, B319, B364 and B385(b). High resolution MS data, Fourier Transform pmr and Fourier Transform ir are likely to lead to the data necessary for structure elucidation, but it will need considerable effort to obtain them because of poor yields. 2 D-nuclear magnetic resonance spectroscopy (2D-nmr) is a particularly promising technique for structural studies of complicated molecules (Coglan, 1983). For those compounds without a distinct molecular ion, chemical ionisation may lead to the molecular weight. Group 1 and 2 compounds remain to be studied, Peak 14 (see Fig. 2) being of special interest since it has $\lambda_{max}^{H_2O}$ 414 nm; they can be obtained directly from Fraction B by HPLC and this is currently being studied.

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