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Formation of Aromatic Compounds from Carbohydrates. V.* Reaction of D-Glucose and Methylamine in Slightly Acidic, Aqueous Solution

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The title reaction produced a humin-like precipitate. In the supernatant, D-fructose and compounds *1*–*18*, comprising furans, pyrroles, phenols, and enols, were identified. The bicyclic products, 1-methyl-5-[5-(hydroxymethyl)-2-furfuryl]pyrrole-2-carboxaldehyde (*13*), 5-(5-formyl-2-furfuryloxymethyl)-1-methylpyrrole-2-carboxaldehyde (*15*), 5,5′-(oxydimethylene)-bis-[1-methylpyrrole-2-carboxaldehyde] (*16*), 5-(hydroxymethyl)-1,1′-dimethyl-4,5′-methylenedipyrrole-2-carboxaldehyde (*17*), and 1-methyl-5-(3,4,5-trihydroxybenzyl)pyrrole-2-carboxaldehyde (*18*) seem to be new compounds. 2′,4′,5′-Trihydroxyacetophenone (*12*) has apparently not been identified previously as a degradation product of carbohydrates.

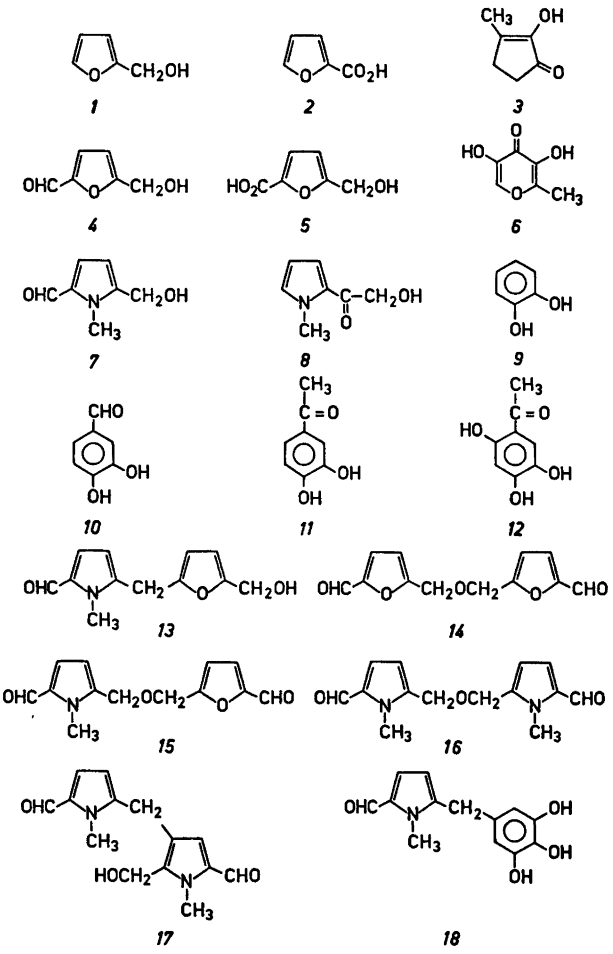
The previous papers [1–4] in this series focused on the degradation of aldoses or hexuronic acids to phenolic and enolic compounds, believed to be intermediates in the yellowing of cellulosic products during manufacture, storage and use. After accelerated ageing of bleached pulp, low-molecular carbohydrates and their degradation products may be extracted with ethanol.[5] Such extracts also contain amino acids,[6] which are known to accelerate and modify the degradation of carbohydrates under neutral or slightly acidic conditions.[7] In modern forestry, where the utilization of the whole tree increases the protein content of the pulp, a pronounced effect on the yellowing process may be expected. The so-called non-enzymic browning or Maillard reaction between carbohydrates and amino compounds is particularly important in food chemistry and may considerably affect the colour, aroma, and nutritional value of food.[7]

The present paper deals with the Maillard reaction in a simple model system, *viz.*, D-glucose and methylamine in aqueous acetic acid. The results were reported briefly at a recent meeting.[8] The same reaction has been studied previously,[9–11] and several products (particularly furans and pyrroles) were identified. These were separated mainly by gas chromatography or distillation. We preferred liquid chromatography for the separation to be able also to identify hydrophilic and less volatile products.

RESULTS

In previous investigations of the title reaction, a fairly dilute aqueous solution, of D-glucose, methylamine, and acetic acid in the approximate molar ratio 3:1:1 was refluxed for 2 h, cooled and then extracted with ethyl acetate.[9,10] Alternatively, a concentrated solution of equimolar amounts was refluxed for 1 h.[11] We followed essentially the former procedure, but exploratory, small-scale experiments using various reflux periods indicated that the maximum yield of extracted material was approached only after *ca.* 24 h. In subsequent large-scale experiments, the solution was accordingly refluxed for 24 h. During that time, the pH dropped from 5.1 to 4.2, and a dark-brown powder precipitated. The elemental composition of the dry material approximated

* Part IV. See Ref. 4.



to $C_{13.0}H_{14.2}NO_{4.6}$. On the basis of eqn. 1, the precipitate accounted for 3.7 % of the glucose and 5.7 % of the methylamine. Sugar analysis [12] of the supernatant showed glucose in 51 % of the original amount and a 7 % yield of D-fructose. The solution was extracted with ethyl acetate, followed by 2-butanone. The extracted material accounted for 2.5 – 3 % of the glucose and probably 4 – 5 % of the methylamine. Chromatography of the extracts on Sephadex LH-20 columns resulted in 9 main fractions, which were rechromatographed on silica gel columns (Table 1). In this way each of the compounds *1* – *18* was isolated, except for the mutual separation of the minor products *10* and *11*. No efforts were made to obtain optimum yields.

Compounds *1* – *6*, *9* – *12*, and *14* were identical (TLC, MS, and ^{1}H NMR) with authentic samples, while *7* and *8* were identified by means of previously published spectral data (MS, IR, and ^{1}H NMR).[10] The new compounds *13* and *15* – *18* may be regarded as anhydro-dimers of *7* or as anhydro-codimers of *7* with *1*, *4*, or pyrogallol (*19*). Their molecular formulas were established by high resolution mass spectrometry and their structures determined mainly by comparing their spectra (Table 2) with those of the respective monomers and the known anhydro-dimer *14*.[3,13]

Thus, the mass spectrum of each new compound showed the two arylcarbonium ions, expected from simple cleavage of the interannular chain, and/or closely related fragments.

Table 1. CC fractionation of extracts from the filtered title reaction mixture.

Fraction [a] No. (mg) [b]	Major (minor) components	Silica gel eluent (v/v)	Isolated compounds (mg)
Ethyl acetate extract (7.3 g)[b]			
1 (268)	*1*, *4* (*5*)	CH_2Cl_2–MeCN, 4:1	*4* (51)
2 (1257)	*4*, *7*, *8* (*1–3*, *6*)	CH_2Cl_2–MeCN, 4:1	*1* (9), *2* (8), *3* (5), *6* (6)
3 (876)	*7*, *8* (*15*)	CH_2Cl_2–Me_2CO, 8:1	*7* (183), *8* (162)
4 (141)	*14* (*15*)	CH_2Cl_2–MeCN, 4:1	*14* (3),[c] *15* (2)
5 (185)	*13* (*9*, *16*, *17*)	$(CH_2)_6$–Me_2CO, 2:1	*9* (2), *13* (18), *16* (7)
6 (135)	(*17*)	CH_2Cl_2–MeCN, 8:1	*17* (4)
7 (108)	(*10*, *11*)	$(CH_2)_6$–Me_2CO, 2:1	*10*+*11* (7)[d]
8 (462)	*18* (*12*)	CH_2Cl_2–MeCN, 4:1	*12* (9), *18* (14)
2-Butanone extract (2.5 g)[b]			
9 (242)	*4*, *7* (*5*, *6*, *16*)	CH_2Cl_2–EtOH, 9:1	*5* (11)

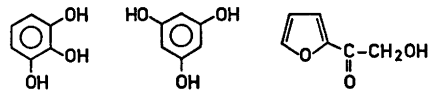
[a] Eluted from Sephadex LH-20. [b] After evaporation. [c] Identified only by TLC and MS. [d] Identical (TLC, MS, ^1H NMR) with an authentic 1:1 mixture.

Table 2. Spectral data.

Assignment [a]	*7* [b]	*13* [c]	*15*	*16*	*17*	*18*
MS [IP 70 eV; *m/e* (% rel. int.)]						
M, obs.		219.089	247.083	260.115	260.114	247.083
M, calc.		219.090	247.084	260.116	260.116	247.084
Strongest peaks above *m/e* 65	139 (100)	219 (100)	122 (100)	122 (100)	260 (100)	110 (100)
	122 (53)	94 (73)	94 (83)	94 (76)	151 (79)	247 (60)
	82 (30)	190 (51)	123 (48)	123 (71)	110 (61)	138 (54)
	67 (26)	160 (40)	67 (24)	67 (25)	123 (59)	81 (33)
	94 (22)	111 (33)	109 (22)	93 (20)	82 (58)	82 (32)
	138 (22)	117 (33)	81 (21)	260 (15)	94 (36)	108 (19)
	110 (20)	132 (33)	93 (18)	108 (13)	122 (31)	109 (17)
	124 (17)	93 (28)	82 (15)	82 (12)	150 (30)	218 (15)
	68 (17)	77 (22)	95 (15)		185 (27)	77 (14)
	80 (11)	108 (21)	247 (12)		93 (24)	122 (12)
IR [$CHCl_3$; $\tilde{\nu}_{max}$ (cm^{-1})]						
2-CHO	1660 (s)	1660 (s)	1660 (s)	1660 (s)	1660 (s)	1660 (s)
2′-CHO	–	–	1680 (s)	1660 (s)	1660 (s)	–
OH	3380 (s)	3420 (s)	–	–	3420 (m)	3380 (s)
^1H NMR [100.0 MHz, $(CD_3)_2CO$; δ]						
1-Me	3.92 (s)	3.89 (s)	3.92 (s)	3.91 (s)	3.88 (s)	3.86 (s)
2-CHO	9.48 (s)	9.46 (s)	9.55 (s)	9.55 (s)	9.45 (s)	9.43 (s)
3-H	6.84 (d)	6.87 (d)	6.90 (d)	6.90 (d)	6.88 (d)	6.85 (d)
4-H	6.16 (d)	6.06 (d)	6.28 (d)	6.26 (d)	5.98 (d)	5.97 (d)
5-CH_2	4.62 (s)	4.03 (s)	4.66 (s)	4.61 (s)	3.93 (s)	3.90 (s)
1′-Me	–	–	–	3.91 (s)	4.00 (s)	–
2′-CHO	–	–	9.62 (s)	9.55 (s)	9.48 (s)	–
3′-H	–	6.02 (d)	7.37 (d)	6.90 (d)	6.67 (s)	–
4′-H	–	6.18 (d)	6.66 (d)	6.26 (d)	–	6.22 (s)[d]
5′-CH_2	–	4.46 (s)	4.62 (s)	4.61 (s)	4.69 (s)	–
^1H NMR [100.0 MHz, $(CD_3)_2CO$; $\|J\|$ (Hz)]						
3-H, 4-H	4.1	4.0	4.1	3.9	4.1	4.0
3′-H, 4′-H	–	3.1	3.6	3.9	–	–

[a] Primed numbers refer to the right-hand ring in the formula. [b] *Cf.* Ref. 10. [c] *m/e* 122 (20 %). [d] 4′- and 6′-H.

Such ions derived from the *7* residue(s) appeared at *m/e* 122 and 94 (loss of CO). As expected,[14] this mode of cleavage was most pronounced for the ethers *15* and *16*. Each formyl group attached to a pyrrole or furan ring gave rise to a carbonyl band in the IR spectrum at 1660 or 1680 cm^{-1}, respectively. Broadening of certain ^1H NMR signals revealed weak but unambiguous allylic couplings to all methylene protons, except for those of the hydroxymethyl group in *17*. Although disregarded in Table 2, these couplings permitted complete analysis of the spectra by successive spin decoupling. The resulting assignments are listed in Table 2 and establish the proposed substitution patterns.



19 *20* *21*

When sprayed with *p*-anisaldehyde in sulfuric acid, all the furan derivatives displayed a blue colour, whereas all pyrroles turned yellow to brown. Accordingly, *13* and *15* showed a blue-green colour. Unlike phloroglucinol (*20*), compounds *18* and *19* produced a characteristic dark-grey colour when sprayed with aqueous iron(III) chloride or ammonium molydate,[15] and formed borate complexes, ruling out the isomer of *18* derived from *7* and *20*. The complex formation was demonstrated by electrophoresis experiments at pH 9.0 with and without borate.[16] The migration rates (mm/h) were as follows.

Compound	*7*	*18*	*19*	*20*
Without borate	0	0	5	0
With borate	0	44	68	0

DISCUSSION

The total yields of *1–18* were not determined but in each of fractions 1–9 (Table 1), the approximate proportions of the components were estimated by TLC. From the results and from the weights of the evaporated fractions, it was clear that *4*, *7*, and *8* were the main products, followed by *1*, *13*, *14*, and *18*. Compounds *1*, *4*, and *6–8* have previously been identified as title reaction products.[10] Compounds *9*, *10*, and *14* have been obtained from glucose or fructose,[3] and compound *2* from D-glucuronic acid,[1] under similar conditions but in the absence of methylamine. Compounds *3* and *11* are formed on degradation of glucose in alkali,[2] and compound *5* from solutions of *4* by autoxidation.[17] However, *12*, *13*, and *15–18* have apparently not been identified previously as degradation products of carbohydrates.

The dehydration of hexoses to *4*, 2-furyl hydroxymethyl ketone (*21*), and other products is well-known and its mechanism is fairly well understood.[18] The reaction is catalyzed by amino compounds, owing to the transient formation of enamines and/or immonium ions.[7] Cyclization of such intermediates is no doubt responsible for products *7* and *8*.[19] The ready conversion of *4* to *14*[13] indicates that the bicyclic products *13–17* are formed by acid-catalyzed S_N1 reactions between the relatively abundant compounds *1*, *4*, and *7*, the aryl-carbonium ion corresponding to *4* or *7* alkylating *1*, *4*, or *7* at oxygen or in the most reactive free ring position (Friedel-Crafts alkylation). It is not clear whether *18* is formed by similar alkylation of *19* or one of its precursors, since *19* should react in position 4 rather than 5. Although no free *19* was detected in the title reaction mixture, traces are formed in the absence of methylamine.[3]

Products *3*, *5*, *6* and *9* may have been formed by dehydration, accompanied by oxidation or reduction. Since all these products are minor, air oxidation and disproportionation reactions – of the Cannizzaro type, for example – are perhaps not ruled out. However, a fragmentation-recombination sequence seems more probable for *3* and *9*, because these are formed on degradation of either xylose or glucose in alkali.[2] In any case, as pointed out previously for *11* and related compounds,[2] such reactions are necessary to explain the formation of products *1*, *2*, and *10–12*. Experiments with pure *9* or hydroxyhydroquinone in acetate or formate buffer indicated that *10–12* are not formed by Friedel-Crafts acylation.

The elemental composition of the precipitate is close to that of soil humin [20] and indicates considerable aromatization. It is also in good agreement with the net reaction scheme (eqn. 1)

$$2.0\ C_6H_{12}O_6 + CH_3NH_2 \rightarrow C_{13.0}H_{14.2}NO_{4.6} + 7.4\ H_2O \qquad (1)$$

Disregarding the modest amounts of *1*, its derivative *13*, and some minor products, the title reaction is therefore essentially composed of isomerization and dehydration steps.

The above results indicate that the formation of pyrroles is partly responsible for the loss of lysine and other essential amino acids in food through the Maillard reaction. However, a comparable part of the lost amino nitrogen is found in the insoluble fraction. Being fairly stable, the isolated pyrroles are probably not intermediates in the formation of this fraction.[21] An exception is *18*, which is easily converted to coloured products. Minor products like *9–12*, *19*, or other derivatives of *9* [3] may also be reactive and important in colour formation.

EXPERIMENTAL

General

Melting points are corrected. Evaporations were performed at reduced pressure below 50 °C. Electrophoresis was carried out on Whatman No. 1 paper in 0.05 M phosphate or phosphate-borate buffer (0.05 M in each) at pH 9, 15 °C, and 1500 V. The spots were visualized with aq. *p*-anisidine hydrochloride and iron(III) chloride. TLC was performed on silica gel (Merck, HF_{254}). After inspection of the plates in UV light, ethanolic *p*-anisaldehyde-sulfuric acid, aq. iron(III) chloride, and aq. ammonium molybdate–acetic acid [15] were used as spray reagents. CC was carried out on Sephadex LH-20 or silica gel (Mallinckrodt, 100 mesh). Sugars were analyzed as their per(trimethylsilyl) ethers by GLC [12] on a Varian 1840 instrument, equipped with flame-ionization detectors and 1.8 m × 2 mm i.d. glass columns. These contained 3 % OV-1/Varaport 30 (100–120 mesh). The nitrogen flow rate was 30 ml/min and the temperature programmed from 100 to 250 °C at 6 °C/min. Low and high resolution mass spectra were recorded at 70 eV on Varian MAT CH 7 and AEI MS 902 instruments, respectively, the latter spectra at the Institute of Medical Biochemistry, University of Gothenburg. The samples were introduced directly into the ion source or through a Varian 1740 gas chromatograph, operated at 150 °C and 25 ml He/min and fitted with a 1.8 m × 2 mm i.d. glass column. This contained 3 % OV-225/Gas-Chrom Q (100-120 mesh). IR spectra were recorded on a Perkin-Elmer 337 instrument, using chloroform as solvent. ^1H NMR spectra were recorded at 100.0 MHz and *ca.* 30 °C on a Varian HA-100 D instrument, equipped with a Varian VFT-100 Fourier Transform System. Acetone-d_6 was used as solvent.

Materials

Solvents, reagents, and reference compounds other than those listed below were commercial samples of good grade. All solvents were freshly distilled before use.

5-(Hydroxymethyl)-2-furoic acid (5) was prepared by oxidation of *4* (94 mg) with freshly prepared silver(I) oxide in aq. alkali, *cf.* Ref. 22. The ethyl acetate extract of the acidified reaction mixture yielded *5* (55 mg, 52 %), which was recrystallized from chloroform-ethanol, m.p. 159–162 °C (lit.[23] 160–162 °C, decomp.).

3,5-Dihydroxy-2-methyl-4H-pyran-4-one (6) was a gift from Dr. P. E. Shaw, Winter Haven, Florida, U.S.A.

2′,4′,5′-Trihydroxyacetophenone (12), *cf.* Ref. 24. Perchloric acid (5 ml 70 %) was carefully added to a stirred solution of hydroxyhydroquinone (4.2 g, 33 mmol) in acetic anhydride (16 g, 160 mmol). The stirring was continued for 2 h on a steam bath. The mixture was cooled, neutralized with aq. sodium carbonate, and extracted with ethyl acetate. The concentrated extract was put on a silica gel column, which was eluted with dichloromethane-ethanol, 20:1 (v/v). Sublimation of the evaporation residue from the first fraction at *ca.* 0.5 mmHg yielded *12* (0.62 g, 11 %) as yellow crystals, m.p. 204–206 °C (lit.[25] 204–206 °C).

5,5′-(Oxydimethylene)di-2-furaldehyde (14) was obtained from D-fructose.[3]

Title reaction procedure

A solution of anhydrous D-glucose (520 g, 2.9 mol), aq. methylamine (72 g 40 %, 0.93 mol), and glacial acetic acid (59 g, 0.98 mol) in water (3.7 l) was refluxed for 24 h and then cooled to 20 °C. The dark-brown precipitate was collected, washed with water, and then dried to constant weight at 60 °C and 0.1 mmHg, yielding a hygroscopic amorphous powder (13.7 g). Found: C 60.5; H 5.5; N 5.1; O 28.6. Calc. for $C_{13.0}H_{14.2}NO_{4.6}$: C 60.5; H 5.6; N 5.4; O 28.5. Apart from a small portion used for sugar analysis,[12] the combined filtrate and washings were extracted with ethyl acetate (4 × 600 ml), followed by 2-butanone (4 × 600 ml). The extracts were fractionated as follows (Table 1).

The ethyl acetate extracts were combined, dried with sodium sulfate and evaporated to constant weight. The residue was put on a 95 × 5 cm Sephadex LH-20 column. This was eluted with water, yielding fractions 1–7. Subsequent elution with 95 % ethanol yielded fraction 8. The separation was monitored by TLC, using chloroform-glacial acetic acid, 9:1 (v/v), as eluent. The 2-butanone extracts were treated in the same way, but an 80 × 2 cm column was used, and one aq. fraction (9) was

collected. Fractions 1–7 were extracted with ethyl acetate and fraction 9 with 2-butanone. The extracts and fraction 8 were dried and evaporated as above. Each residue was put on a silica gel column. This was eluted by the solvent system listed in Table 1. The separation was monitored by TLC, using the same eluent. Evaporation of the purest fractions yielded *1–9*, *12–18*, and a mixture of *10* and *11*, generally as brownish syrups and each in sufficient amount and purity for proper characterization and identification.

Characterization of new compounds

Compound *15* was crystallized from hexane-acetone, m.p. 63–65 °C; compound *16* from hexane, m.p. 108–109 °C; and compound *17* from hexane-acetone, m.p. 121–122 °C. For spectral data, the reader is referred to Table 2.

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REFERENCES

1. Popoff, T. and Theander, O. *Carbohydr. Res. 22* (1972) 135.
2. Forsskåhl, I., Popoff, T. and Theander, O. *Carbohydr. Res. 48* (1976) 13.
3. Popoff, T. and Theander, O. *Acta Chem. Scand. B 30* (1976) 397.
4. Popoff, T. and Theander, O. *Acta Chem. Scand. B 30* (1976) 705.
5. Dillén, S., Popoff, T. and Theander, O. *Sven. Papperstidn. 71* (1968) 377.
6. Popoff, T. and Theander, O. *Unpublished results.*
7. Feeney, R. E., Blankenhorn, G. and Dixon, H. B. F. *Adv. Protein Chem. 29* (1975) 135, and references therein.
8. Pernemalm, P.-Å., Popoff, T. and Theander, O. In *Eighth International Symposium on Carbohydrate Chemistry. Abstracts*, Kyoto 1976, p. 22.
9. Severin, T. and Seilmeier, W. *Z. Lebensm. Unters. Forsch. 137* (1968) 4.
10. Jurch, G. R., Jr. and Tatum, J. H. *Carbohydr. Res. 15* (1970) 233.
11. Kato, H. and Fujimaki, M. *Agric. Biol. Chem. 34* (1970) 1071.
12. Sweeley, C. C., Bentley, R., Makita, M. and Wells, W. W. *J. Am. Chem. Soc. 85* (1963) 2497.
13. Dunlop, A. P. and Peters, F. N. *The Furans*, Reinhold, New York 1953, p. 411.
14. Ferretti, A., Flanagan, V. P. and Ruth, J. M. *J. Agric. Food Chem. 22* (1974) 729.
15. Quastel, J. H. *Analyst (London) 56* (1931) 311.
16. Theander, O. *Sven. Kem. Tidskr. 70* (1958) 393.
17. Turner, J. H., Rebers, P. A., Barrick, P. L. and Cotton, R. H. *Anal. Chem. 26* (1954) 898.
18. Feather, M. S. and Harris, J. F. *Adv. Carbohydr. Chem. Biochem. 28* (1973) 161.
19. Kato, H. *Agric. Biol. Chem. 31* (1967) 1086.
20. Schnitzer, M. and Khan, S. U. *Humic Substances in the Environment*, Dekker, New York 1972, p. 31.
21. Kato, H. and Fujimaki, M. *J. Food Sci. 33* (1968) 445.
22. Campaigne, E. and LeSuer, W. M. In Rabjohn, N., Ed., *Org. Synth. Coll. Vol. 4* (1963) 919.
23. Curtis, R. F., Hassall, C. H. and Nazar, M. *J. Chem. Soc. C* (1968) 85.
24. Leonte, M., Beschia, M., Pascaru, E. and Stoica, M. *Stud. Univ. Babes-Bolyai, Ser. Chem. 8* (1963) 291.
25. Daly, J. W., Benigni, J., Minnis, R., Kanaoka, Y. and Witkop, B. *Biochemistry 4* (1965) 2513.

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