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Isotope studies of the transfer of the carbon atoms of carbohydrate derivatives into aromatic compounds (especially xanthene) under degradation conditions

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ABSTRACT

Treatment of various ^{13}C-carbohydrate-labelled phenyl β-D-glucopyranosides at 350°C in aqueous phenol in the presence of zinc chloride, with ^{13}C NMR analysis of the xanthene formed as the major neutral product, indicated that the methylene carbon atom (C-9) of this compound was derived from C-1 (30%), C-2 (20%), and C-6 (50%) of the glucosyl units. In addition, 4.5% of the carbon from the sugar was incorporated into the aromatic rings of the xanthene. Mass spectrometry of the phenol produced on heating methyl α-D-glucopyranoside (50% U-^{13}C) at 350°C for 1 h in aqueous zinc chloride showed the aromatic rings to be derived from the glucosyl moiety, partly without cleavage of the carbon chain and also after cleavage and recombination of the fragments.

INTRODUCTION

In the preceding paper[1], it was observed that reaction of cellulose at 350°C in aqueous phenol in the presence of zinc chloride gave a complex mixture of products, of which the major neutral component was xanthene (**1**). It was proposed that the xanthene was formed mainly from phenol in the solvent and formaldehyde produced from the cellulose. Some of the aromatic ring carbon had cellulose as source. The origins of the various carbon atoms in the xanthene are now addressed specifically.

RESULTS AND DISCUSSION

The main products of depolymerisation of cellulose at 350°C in aqueous phenol in the presence of zinc chloride were glucose, 1,6-anhydroglucose, and phenyl

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D-glucopyranoside[1], and each gave appreciable yields of xanthene on further treatment. In order to examine this process further, phenyl β-D-[U-^{13}C]glucopyranoside (6.4% ^{13}C per carbon atom) was subjected to the degradation reaction. The neutral product fraction gave a ^{13}C NMR spectrum consistent with that of xanthene, and with the resonance of C-9 enhanced by a factor of 6.1 which indicated that the methylene group (C-9) originated from the carbohydrate. Moreover, the aromatic carbons of the xanthene contained more than the natural abundance of ^{13}C, although the low degree of labelling did not permit an accurate determination of the enhancement factor.

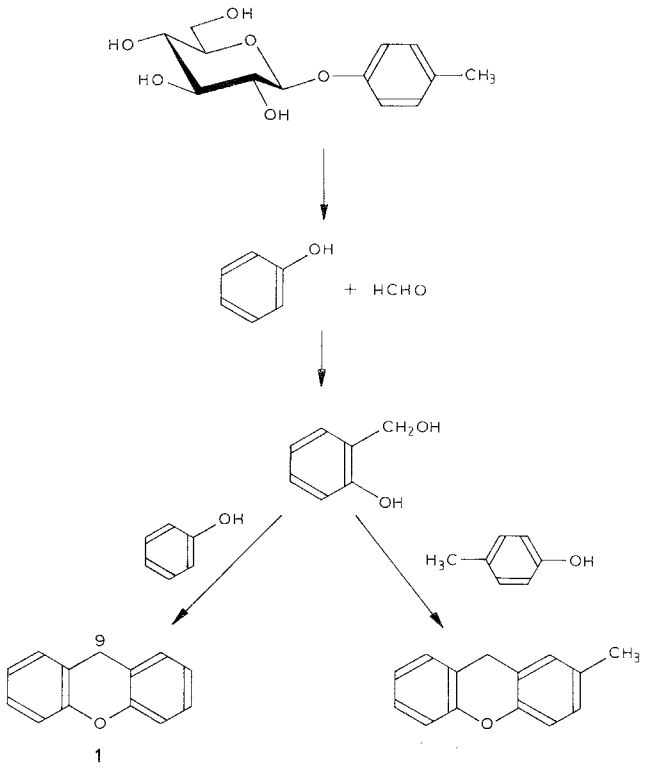
Experiments with phenyl β-D-glucopyranoside mono-labelled with ^{13}C at C-1, C-2, or C-6 then revealed that these carbon atoms contributed, respectively, 30, 20, and 50% (each ±5%) to C-9 of the xanthene; hence, C-3,4,5 were minor contributors. In contrast, the formaldehyde produced during the pyrolysis of ^{14}C-labelled D-glucose was shown to be derived[2] from C-1, C-2, and C-6 to the extents of 15, 5, and 65%, respectively, with C-3,4,5 again contributing little. Therefore, the liquefaction conditions appear to favour the conversion of C-1 and C-2 into formaldehyde rather than into carbon dioxide to which they make major contributions during pyrolysis[3].

The experiments with phenyl β-D-[U-^{13}C]glucopyranoside provided qualitative evidence that the aromatic rings of the derived xanthene contained carbon atoms of carbohydrate origin. Thus, in the ^{13}C NMR spectrum of the neutral products under peak-resolution-enhancement conditions[4], the signal (d, J 46 Hz) for C-9 of the xanthene was consistent with the presence of ^{13}C at position 9 and position 1a or 8a since $^{1}J_{C,C}$ for sp^2–sp^3 carbons atoms is 40–60 Hz (cf. 42 Hz for $J_{8a,9}$ in 9,10-dihydroanthracene[5]).

Attention was then turned to the quantitation of the aromatic carbon atoms of xanthene derived from solvent phenol and from the carbohydrates. Heyns et al.[6] reported that methylbenzenes in particular are formed during the pyrolysis of glucose at 300°C. At higher temperatures, a wide range of aromatic substances, including phenols, were produced from simple sugars[7] and cellulose[8]; similarly, complex mixtures of substituted benzenes and phenols were formed from carbohydrates under conditions of high-temperature liquefaction at low[9] and high[10] pH.

When a solution of *p*-tolyl β-D-glucopyranoside in aqueous *p*-cresol was heated at 350°C for 1 h, the neutral products contained, as expected from the work with phenol[1], several di-, tri-, and tetra-methylxanthenes and some xanthene, and significant proportions of methylxanthenes were detected by GLC–MS. The formation of ions with m/z 181 revealed methyldibenzofurans, xanthene ($[M - H]^+$), and methylxanthene ($[M - Me]^+$), and the ions with m/z 195 revealed methylxanthenes ($[M - H]^+$) and dimethylxanthenes ($[M - Me]^+$). The identity of these compounds was confirmed by comparing their retention times and mass spectra with those of authentic samples or with available mass-spectral data[11].

The possibility that the xanthene and methylxanthenes were products of demethylation of higher homologues was discounted since the reaction of



Scheme 1.

paraformaldehyde with cresol under similar conditions afforded only dimethylxanthenes. Therefore, it is concluded that the xanthene and methylxanthenes (0.5 and 4%, respectively) were produced from carbohydrate-derived phenol and formaldehyde with the solvent being incorporated in the latter products (Scheme 1). If it is assumed that both the benzenoid rings of the xanthene and one of those of the methylxanthene are derived from the sugar moiety, then it can be deduced from the yields that 4.1% of the carbohydrate carbon was converted into phenol. It has been concluded that the benzofurans formed during the liquefaction of cellulose in aqueous guaiacol arise from the carbohydrate[12].

A xanthene-producing aromatic compound was formed during the liquefaction of 1,6-anhydro-β-D-[U-^{13}C]glucopyranose (7%, ^{13}C) at 350°C for 1 h in aqueous [U-$^{12}C_6$]phenol (> 99.95% ^{12}C, prepared from [U-$^{12}C_6$]benzene by a thallation procedure[13]) in the presence of zinc chloride. The neutral products gave a ^{13}C NMR spectrum (Fig. 1) which indicated xanthene to be the sole component. The C-9 resonance was enhanced, as expected, but, since the only ^{13}C atoms in the system were carbohydrate in origin, the xanthene benzenoid rings, to some degree, were derived from this source. If it is assumed that C-9 of the xanthene originates exclusively from the carbohydrate (and no evidence to the contrary was obtained),

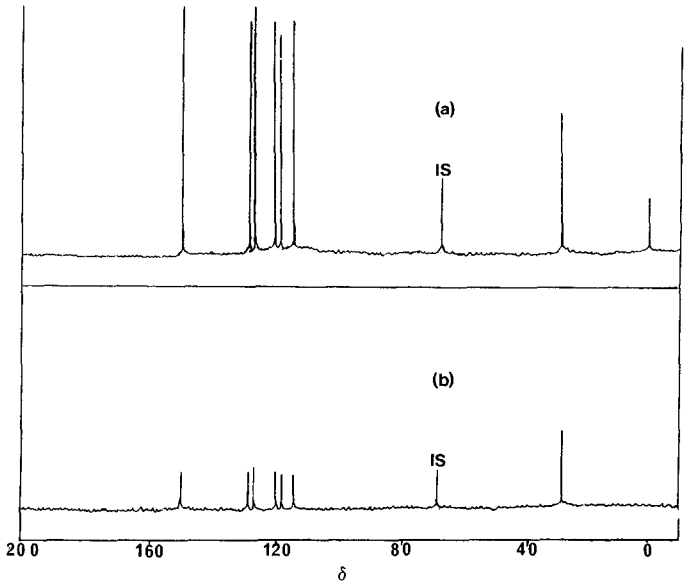


Fig. 1. ^{13}C NMR spectra of (a) normal xanthene and (b) the neutral products obtained from the treatment of 1,6-anhydro-β-D-[U-^{13}C]glucopyranose in aqueous [U-^{12}C]phenol under liquefaction conditions: IS, the internal standard dioxane (δ 67.5).

then the ratio of the intensities of resonances of C-9 and the aromatic carbons indicates that 12% of the latter originated from the sugar derivative. Since 100 mg of 1,6-anhydroglucose gave 21 mg of xanthene, the percentage conversion of carbohydrate carbon into the aromatic carbons of xanthene was 4.5% (cf. 41.% in the *p*-tolyl glycoside experiment). These data suggest that the labelled xanthene is produced other than via free phenol since there appears to be no isotope exchange with the solvent. This observation is not consistent with our other conclusions and remains to be explained.

MS of the xanthene produced in the above reaction of 1,6-anhydro-β-D-[U-^{13}C]glucose in [U-^{12}C]phenol gave mainly ions with *m/z* 181 and 182 (as did "natural" xanthene). However, unlike the unlabelled sample, the labelled compound also gave ions with *m/z* 186, 187, and 188 (Fig. 2a), which suggests that the uniformly labelled carbohydrate was converted into uniformly labelled phenol, that is, C_6 sugar chains became 6-membered aromatic rings without fragmentation. This possibility, as opposed to fragmentation and reassembly, was investigated further.

Thus, a 1:1 mixture of methyl α-D-[U-^{13}C]glucopyranoside and its unlabelled analogue was heated at 350°C for 1 h in aqueous zinc chloride. GLC–MS of the organic-solvent-soluble products revealed that phenol was a significant component, and cyclohexen-3-one, *o*-cresol, *p*-cresol, catechol, hydroquinone, and three methyldihydroxybenzenes were also identified.

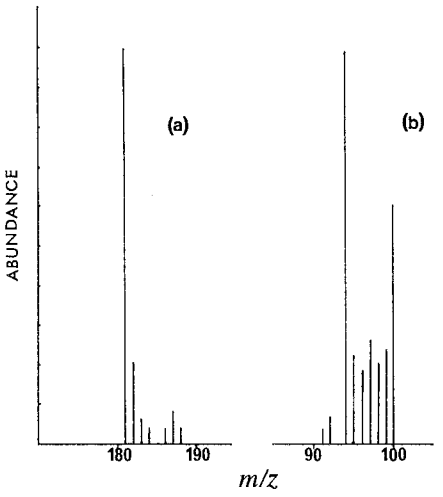


Fig. 2. Region for molecular ions in the mass spectrum of (a) xanthene obtained by treatment of 1,6-anhydro-β-D-[U-^{13}C]glucopyranose [7% ^{13}C] in [U-^{12}C]phenol under liquefaction conditions, and (b) phenol obtained from the hydrothermolysis of methyl α-D-[U-^{13}C]glucopyranoside (50% ^{13}C) at 350°C for 1 h.

If the phenol was formed from the hexose moiety without carbon-chain cleavage, two types of molecular ions would be present with *m/z* 94 (from unlabelled carbohydrate) and 100 (from the labelled carbohydrate), and, because of the 50% labelling, two molecular ions of equal intensity would be expected (assuming that no isotope effects occurred). Alternatively, had the hexose chains fragmented and recombined, then the isotope scrambling would have resulted in a cluster of molecular ions in the range *m/z* 94–100 symmetrically distributed about *m/z* 97. The pattern observed (*m/z* 94–100 of 100:24:16:26:17:25:64) indicated that direct conversion of glucose into phenol had not occurred exclusively, but the higher intensities of the ions at *m/z* 94 and 100 showed that some direct conversion had occurred. The differences in intensities of the ions with *m/z* 94 and 100 may reflect the occurrence of an isotope effect.

No evidence was obtained to indicate how either a direct hexose-into-phenol conversion could occur or how a fragmentation/recombination process could be involved, but chemical[14] and biochemical[15] precedents for the former can be considered, and reactions such as the Michael addition of acetone to acrolein could account for the latter process[16]. Miller and Saunders[17] showed that many of the aromatic products of the liquefaction of cellulose can be formed from possible fragments such as acetaldehyde, crotonaldehyde, acrolein, and hydroxypropanone.

EXPERIMENTAL

General methods. — The ^{13}C NMR spectra were measured with a Varian FT 80A instrument on solutions in $CDCl_3$ (internal Me_4Si). Quantitative studies were

carried out using ^{1}H-decoupled ^{13}C NMR spectra obtained with full suppression of NOE effects (inverse gated) on 0.25 M solutions (internal dioxane) with 0.1 M tris(2,4-pentanedionate)chromium(III) added as a paramagnetic relaxation agent[18]; 1000 pulses and the pulse ($5 \times$ largest measured T_1 value[19]) ensured complete nuclear relaxation[20]. The accuracy of the results, based on peak-height measurements, were assessed using phenyl tetra-*O*-acetyl-β-D-[6-^{13}C]glucopyranoside of known ^{13}C content. EI (70 eV)-mass spectra were obtained with a Hewlett–Packard HP 5997OB instrument.

D-[U-^{13}C]-, D-[1-^{13}C]-, and D-[6-^{13}C]-glucose, D-[1-^{13}C]mannose and [U-^{12}C]benzene were obtained from Merck, Sharp and Dohme (Quebec, Canada). D-[2-^{13}C]Glucose was prepared (53.5%, identified by ^{13}C NMR characteristics[21]) from D-[1-^{13}C]mannose by application of the Bilik reaction[22] and was isolated on an ion-exchange column[23].

^{13}C-Labelled phenyl β-D-glucopyranosides. — Each ^{13}C-labelled D-glucose (0.035 g) was added separately to D-glucose (0.465 g) to give a mixture containing 7% of ^{13}C. The β anomers were prepared by an adaptation of the method of Pfeffer and Hicks[24]. Water (50 μL) was added to each mixture (0.50 g) of glucoses which was heated at 100°C for 1 h then at 160°C to give melts. Acetic acid (0.5 mL) was added at 100°C, the solution was cooled at 10°C/h, and seed crystals of β-D-glucose were added at 70°C. The crude products were washed with EtOH at 2°C (2×10 mL) to give β-D-glucopyranose (0.37 g, 74%), mp 149–150°C, $[\alpha]_D + 20° \rightarrow +53°$; lit[25] mp 148–150°C, $[\alpha]_D + 19° \rightarrow +53°$.

Acetylations were carried out using β-D-glucopyranose (0.36 g), pyridine (2 mL), and acetic anhydride (2 mL) added dropwise at 0°C. Standard work-up procedures gave the β-penta-acetate (0.76 g, 97%), mp 134–135°C, $[\alpha]_D + 3.8°$ ($CHCl_3$); lit.[26] mp 132°C, $[\alpha]_D + 4°$.

Penta-*O*-acetyl-β-D-glucopyranose (0.1 g) and tributylphenoxystannane [0.11 g, 1.1 mol equiv; prepared by heating phenol and bis(tributyltin)oxide in refluxing toluene] were added to a stirred suspension of anhyd zirconium tetrachloride (0.6 g, 1.0 mol equiv) in $CHCl_3$ (5 mL). The mixture was heated at 70°C for 16 h, $CHCl_3$ (10 mL) was added, and the usual processing then gave phenyl β-D-glucopyranoside tetra-acetate (0.102 g, 92%), mp 124–125°C, $[\alpha]_D -22°$ ($CHCl_3$); lit.[26] mp 124–125°C, $[\alpha]_D -22°$.

Standard *O*-deacetylation of the tetra-acetates (0.2 g) involved MeOH, triethylamine, and water (8:2:1). Removal of the solvents and recrystallisation of the residues from water at 2°C gave the phenyl β-D-glucopyranosides (0.095 g, 91%), mp 174–175°C, $[\alpha]_D -72°$ (H_2O); lit.[27] mp 174–175°C, $[\alpha]_D -72°$.

1,6-Anhydro-β-D-[U-^{13}C]glucopyranose (cf. *Coleman*[28]). — Phenyl tetra-*O*-acetyl-β-D-[UL-$^{13}C_6$]glucopyranoside (0.5 g) was heated under reflux with 3.5 M NaOH (5 mL) for 20 h. The solution was cooled at 0°C, neutralised with H_2SO_4, and concentrated, and the residue was treated with pyridine (1 mL) and acetic anhydride (1 mL). Standard work-up procedures and column chromatography gave 2,3,4-tri-*O*-acetyl-1,6-anhydro-β-D-[U-^{13}C]glucopyranose (0.38 g, 63%), mp 109–

110°C, $[\alpha]_D$ −61.5° ($CHCl_3$); lit.[29] mp 109–110°C, $[\alpha]_D$ −62°. Standard *O*-deacetylation (MeOH, triethylamine, water) of the ester (0.38 g) and crystallisation of the product from EtOH gave the title compound (0.15 g, 97%), mp 183–184°C, $[\alpha]_D$ −67° (H_2O); lit.[29] mp 178–180°C, $[\alpha]_D$ −66°.

Methyl α-D-[U-^{13}C]glucopyranoside. — The glycoside was made from the labelled free sugar (0.1 g) by heating under reflux for 29 h in methanolic 0.6 M HCl (7 mL). The solution was neutralised with anion-exchange (HO^-) resin, then concentrated, and the residue was recrystallised from MeOH to give the title compound (0.08 g, 61%), mp 166–168°C, $[\alpha]_D$ +159° (H_2O); lit.[27] mp 165–166°C, $[\alpha]_D$ +158°.

[U-^{12}C]Phenol. — [U-^{12}C]Benzene (1 g) was added to a solution of thallium tris(trifluoroacetate) (6.75 g, 1.1 mol equiv) in trifluoroacetic acid (15 mL), the mixture was stirred at 20°C for 48 h, and crystalline phenylthallium bis(trifluoroacetate)[13] (6.2 g, 95%) was collected and washed with CH_2Cl_2. A solution of this product (6.8 g) in trifluoroacetic acid (10 mL) at 0°C was stirred with a solution of lead tetra-acetate (6.6 g, 1.1 mol equiv) in trifluoroacetic acid (10 mL) for 30 min, then concentrated, 6 M HCl (20 mL) was added, and the mixture was filtered and concentrated. The resulting oily phenyl trifluoroacetate was stirred with 2 M NaOH (20 mL) for 30 min, the solution was neutralised and extracted with CH_2Cl_2 (3 × 50 mL), and the combined extracts were washed with aq $NaHCO_3$ and water, dried, and concentrated to give [U-$^{13}C_6$]phenol (0.31 g, 30%), mp 41°C; lit.[30] mp 40.9°C.

Degradation procedures. — Each aryl glycoside and 1,6-anhydro-β-D-glucopyranose (each, 100 mg) was heated[1] for 1 h at 350°C in a thick-walled tube (8 × 100 mm, i.d. 4 mm) with the appropriate phenol (300 mg), zinc chloride (25 mg), and water (100 μL). Prior to sealing the tubes, dissolved gases were removed by freezing and thawing the solutions under vacuum. On completion of the reactions, the neutral products were extracted into hexane from water as described[1].

In the degradation experiment involving labelled methyl α-D-glucopyranoside, the sample (50 mg) was heated in water (0.8 mL) containing zinc chloride (2.5 mg) for 1 h at 350°C. The CH_2Cl_2-soluble products were examined by GLC–MS.

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REFERENCES

1 R.J. Ferrier, W.B. Severn, R.H. Furneaux, and I.J. Miller, *Carbohydr. Res.*, 237 (1992) 79–86.
2 Y. Houminer and S. Hoz, *Isr. J. Chem.*, 8 (1970) 97–98.
3 Y. Houminer and S. Patai, *Tetrahedron Lett.*, (1967) 1297–1300.

4 D. Shaw, *Fourier Transform N.M.R. Spectroscopy*, Elsevier, Amsterdam, 1976, p 191.
5 V. Wray and P.E. Hansen, *Annu. Rep.* NMR *Spectrosc.*, 11A (1981) 99–182.
6 K. Heyns, R. Stute, and H. Paulsen, *Carbohydr. Res.*, 2 (1966) 132–149.
7 R.H. Walter and I.S. Fagerson, *J. Food Sci.*, 33 (1968) 294–297.
8 E.B. Higman, I. Schmeltz, and W.S. Schlotzhauer, *J. Agric. Food. Chem.*, 18 (1970) 636–639.
9 T. Popoff and O. Theander, *Acta Chem. Scand., Ser. B*, 30 (1976) 397–402.
10 P.M. Molton, R.K. Miller, J.M. Donovan, and T.F. Demmitt, *Carbohydr. Res.*, 75 (1979) 199–206.
11 National Bureau of Standards, I10, Revision 3.1, NBS/NIH/EPA/MSD Database, Hewlett–Packard Company.
12 I.J. Miller and S.K. Fellows, *Fuel.*, 64 (1985) 1246–1250.
13 E.C. Taylor, H.W. Altland, R.H. Danforth, G. McGillivray, and A. McKillop, *J. Am. Chem. Soc.*, 92 (1970) 3520–3522.
14 R. Blattner, R.J. Ferrier, and S.R. Haines, *J. Chem. Soc., Perkin Trans. I*, (1985) 2413–2416.
15 D.E. Kiely and W.R. Sherman, *J. Am. Chem. Soc.*, 97 (1975) 6810–6814.
16 J.A. Russell, R.K. Miller, and P.M. Molton, *Biomass*, 3 (1983) 43–57.
17 I.J. Miller and E.R. Saunders, *Fuel*, 66 (1987) 130–135.
18 J.N. Shoolery, *Prog. Nucl. Magn. Reson. Spectrosc.*, 11 (1977) 79–93.
19 D. Shaw, *Fourier Transform NMR Spectroscopy*, Studies in Physical and Theoretical Chemistry 30, 2nd ed., Elsevier, Amsterdam, 1984, pp 249–294.
20 R.K. Harris and R.H. Newman, *J. Magn. Reson.*, 24 (1976) 449–456.
21 K. Bock and C. Pederson, *Adv. Carbohydr. Chem. Biochem.*, 41 (1983) 27–66.
22 M.L. Hayes, N.J. Pennings, A.S. Serianni, and R. Barker, *J. Am. Chem. Soc.*, 104 (1982) 6764–6769.
23 S.J. Angyal, G.S. Bethell, and R.J. Beveridge, *Carbohydr. Res.*, 73 (1979) 9–18.
24 P.E. Pfeffer and K.B. Hicks, *J. Carbohydr. Chem.*, 3 (1984) 197–217.
25 R.L. Whistler and J.N. BeMiller, *Methods Carbohydr. Chem.* 1 (1962) 130–131.
26 M.L. Wolfrom and A. Thompson, *Methods Carbohydr. Chem.*, 2 (1963) 211–215.
27 J. Conchie, G.A. Levvy, and C.A. Marsh, *Adv. Carbohydr. Chem.*, 12 (1957) 157–187.
28 C.H. Coleman, *Methods Carbohydr. Chem.*, 2 (1963) 397–399.
29 M. Cerný and J. Stanek, *Adv. Carbohydr. Chem. Biochem.*, 34 (1977) 23–177.
30 S. Coffey (Ed.), *Rodd's Chemistry of Carbon Compounds*, Vol. IIIA, Elsevier, Amsterdam, 1971, p 289.