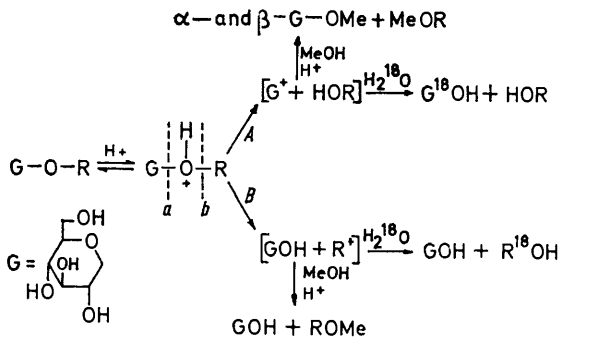
Reactions at Position 1 of Carbohydrates. Part IX.† A Reinvestigation of the Bond Cleavage in the Acid-catalysed Hydrolysis of Ferrocenylmethyl β-D-Glucopyranoside

By **P. M. Collins, W. G. Overend,* and B. A. Rayner,** Department of Chemistry, Birkbeck College (University of London), Malet Street, London WC1E 7HX

The acid-catalysed hydrolysis of ferrocenylmethyl β-D-glucopyranoside in $H_2{}^{18}O$ has been reinvestigated. Contrary to an earlier report, the glucose formed contained no ^{18}O isotopic enrichment. This shows that cleavage of the bond between the oxygen and aglycon–carbon occurred during the hydrolysis. Acid-catalysed methanolysis of ferrocenylmethyl β-D-glucopyranoside gave glucose and methoxymethylferrocene. Consequently bond cleavage occurred at the same position in both reactions.

THERE are two modes of bond cleavage commonly found to occur in the acid-catalysed hydrolysis of glycopyranosides,[1,2] namely glycosyl–oxygen fission and aglycon–oxygen fission (Scheme 1, *a* and *b* respectively). The mode of bond cleavage operating in the hydrolysis of several glycopyranosides has been studied using ^{18}O-enriched water. Thus, Bunton *et al.*[3] found that methyl and phenyl α- and β-D-glucopyranoside and maltose each gave either the appropriate alcohol or phenol derived from the aglycon portions, which was isotopically non-enriched, whereas t-butyl β-D-glucopyranoside gave the enriched butanol.[4] It was concluded that t-butyl β-D-glucopyranoside underwent reaction *via* bond cleavage (*b*) whereas in the other group of glycosides cleavage (*a*) occurred as depicted in Scheme 1. Furthermore, t-butyl β-D-glucopyranoside was found[4] to hydrolyse in 1M-perchloric acid at 25°, 10^3 times faster than the methyl analogue. Both this accelerated rate and the unusual mode of bond cleavage could be rationalised in terms of the stability of the tertiary butyl cation, $R^+ = Me_3C^+$, formed in path *B* (Scheme 1). It is noteworthy that other glycopyranosides with aglycons derived from tertiary alcohols have been found[5] to undergo hydrolysis very rapidly. This led to the conclusion[5] that glycosides having aglycons which can afford stable carbonium ions on hydrolysis, undergo reaction at an enhanced rate by route *B* in Scheme 1. The general validity of this conclusion was undermined by results obtained with ferrocenylmethyl β-D-glucopyranoside (1).[6] This glucoside was found[6] to hydrolyse 10^3 times faster than its t-butyl analogue, a result



SCHEME 1

compatible with the rapid hydrolysis of ferrocenylmethyl acetate. This acetate is hydrolysed[7] at a rate similar to that of triphenylmethyl acetate and indicates a high stability for the ferrocenylcarbinyl cation. However, the isolation of isotopically enriched glucose from a hydrolysis of the glucoside (1) carried out in $H_2{}^{18}O$ was unexpected since this suggested[6] that bond-cleavage occurred at position *a* in Scheme 1 to yield a glucosyl

† Part VIII, B. Capon, A. A. Levy, and W. G. Overend, *Carbohydrate Res.*, 1967, **5**, 93.

[1] J. N. BeMiller, *Adv. Carbohydrate Chem.*, 1967, **22**, 25; W. G. Overend, in 'The Carbohydrates,' ed. W. Pigman and D. Horton, Academic Press, New York, 1972, Vol. 1A, p. 279.

[2] B. Capon, *Chem. Rev.*, 1969, **69**, 407.

[3] C. A. Bunton, T. A. Lewis, D. R. Llewellyn, and C. A. Vernon, *J. Chem. Soc.*, 1955, 4419.

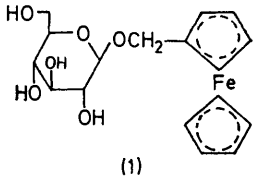
[4] C. Armour, C. A. Bunton, S. Patai, L. H. Selman, and C. A. Vernon, *J. Chem. Soc.*, 1961, 412.

[5] T. E. Timell, *Canad. J. Chem.*, 1964, **42**, 1456.

[6] A. N. de Belder, E. J. Bourne, and J. B. Pridham, *J. Chem. Soc.*, 1961, 4464.

[7] E. A. Hill and J. H. Richards, *J. Amer. Chem. Soc.*, 1959, **81**, 3484; R. E. Carter, M. S. Nugent, and J. H. Richards, *J. Amer. Chem. Soc.*, 1969, **91**, 6145. For reviews see M. Rosenblum, 'The Chemistry of the Iron Group Metallocenes; Ferrocene, Ruthenocene, Osmocene,' Interscience, New York, 1965, p. 129; M. Cais, *Organometallic Chem., Rev.* 1966, **1**, 435.

cation. The rate enhancement found with glucoside (1), which is derived from a primary alcohol, would not arise from steric acceleration of the kind which has been proposed to explain the rapid hydrolysis of triethylmethyl [5] and adamantyl glucosides.[8] Consequently it



(1)

seemed to us that the hydrolysis of compound (1) should be re-examined because it appeared to be anomalous and conflicted with the accepted rationale of bond cleavage in glycoside hydrolysis. While this work was in progress similar doubts about the published results concerning the hydrolysis of compound (1) were expressed by Capon.[2]

Two approaches to the problem have been employed in this work, namely the methanolysis and the hydrolysis in $H_2{}^{18}O$ of ferrocenylmethyl glucopyranoside.

RESULTS AND DISCUSSION

(a) *Methanolysis Studies.*—Recently one of us [9] showed that methanolysis studies provide a rapid method for obtaining information about bond cleavages in reactions at the anomeric centre of sugar derivatives. Consequently such a study of glucoside (1) was undertaken before embarking upon a more protracted investigation with isotopically enriched water.

The products which could arise by the two modes of bond scission during a methanolysis reaction of glucoside (1) are depicted in Scheme 1, where R = ferrocenylcarbinyl cation. (A cyclic glucosyl carbonium ion, which is generally accepted [10] as the intermediate involved in a reaction proceeding *via* path *a*, is assumed rather than an acyclic ion). Experiment has demonstrated that ferrocenylcarbinol is rapidly converted into methoxymethylferrocene in methanol under mild acid conditions of the kind which are insufficient for significant glycosidation of glucose. Therefore analysis of the reaction mixture for glucose and methyl glucosides is a satisfactory way to ascertain the mechanism by which glucoside (1) is solvolysed.

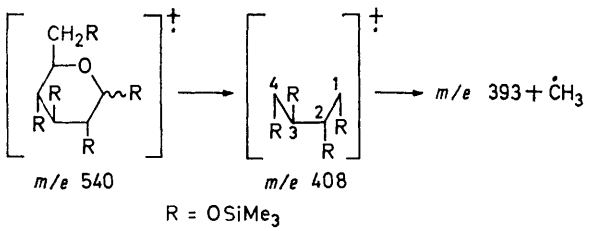
A solution containing glucoside (1) (prepared as described in the Experimental section) (0·022M) was treated at 22° with dry methanolic hydrogen chloride (0·044M) for 35 min. The products were trimethylsilylated and analysed by g.l.c. Methoxymethylferrocene and a peak comprising either penta-*O*-trimethylsilyl-α-D-glucopyranose or methyl 2,3,4,6-tetra-*O*-trimethylsilyl-β-D-glucoside appeared as major components, but a small amount of bisferrocenylmethyl ether and a trace of methyl tetra-*O*-trimethylsilyl-α-D-glucoside were also present.

Chromatography has indicated that one of the products diagnostic of the mechanism, *i.e.* methyl α-D-glucopyranoside, was not formed in significant amounts during the methanolysis, but was less definitive for the β-D-glucoside and glucose. The composition of the g.l.c. fraction containing these two compounds, as their trimethylsilyl derivatives, was determined by n.m.r. spectroscopic examination of a collected sample. This showed that it was the α-D-glucopyranose derivative contaminated with less than 4% of the derivative from methyl β-D-glucopyranoside. The formation of glucose in high yield and methyl glucosides in very low yield demonstrated that bond scission *b* (Scheme 1) had occurred during the methanolysis. A similar mode of bond cleavage was found in the methanolysis of the 2,3,4,6-tetra-*O*-acetyl derivative of the ferrocenylmethyl glucoside (1).

Hydrolysis Studies.—In view of the above results, the hydrolysis of the glucopyranoside (1) was reinvestigated by a procedure similar to that used by earlier workers,[3,4,6] except that volatile organic molecules could be studied directly with the MS902 mass-spectrometer employed in this investigation. This obviated the need to oxidise the hydrolysis products (glucose and hydroxymethylferrocene) to carbon dioxide in order to examine their oxygen isotope enrichment.

Hydroxymethylferrocene was sufficiently volatile to be used directly for mass spectral studies.[11] Its molecular ion, *m/e* 216, was intense and there was only a weak ion at *m/e* 218 (see Table 2), and so this region of its spectrum was suitable for estimating any ^{18}O enrichment in this compound.

The low volatility of glucose precluded direct mass spectral measurements and so the sugar was trimethylsilylated [12] prior to analysis. The choice of this derivative was fortunate because of the timely publication [13] of its mass spectrum. Although the molecular ion was very weak and unsuitable for this study, the ion *m/e* 393, which retained the oxygen atom at C-1 (see Scheme 2)



SCHEME 2

was suitable, especially since the *m/e* 395 ion was fairly weak. Consequently, examination of this region of its spectrum was satisfactory for the present study.

[8] D. Cocker and M. L. Sinnott, *Chem. Comm.*, 1972, 414.
[9] P. M. Collins, *Tetrahedron*, 1965, **21**, 1909.
[10] B. E. C. Banks, Y. Meinwald, A. J. Rhind-Tutt, I. Sheft, and C. A. Vernon, *J. Chem. Soc.*, 1961, 3240; W. G. Overend, C. W. Rees, and J. S. Sequiera, *J. Chem. Soc.*, 1962, 3429.
[11] H. Egger, *Monatsh. Chem.*, 1966, **97**, 602.
[12] E. J. Hedgley and W. G. Overend, *Chem. and Ind.*, 1960, 378; C. C. Sweeley, R. Bentley, M. Mabita, and W. W. Wells, *J. Amer. Chem. Soc.*, 1969, **91**, 1728.
[13] D. C. DeJongh, T. Radford, J. D. Hribar, S. Hanessian, M. Bieber, G. Dawson, and C. C. Sweeley, *J. Amer. Chem. Soc.*, 1969, **91**, 1728.

A solution of ferrocenylmethyl β-D-glucoside (1) (0·022M) was hydrolysed at 25° in sulphuric acid (0·051M) in isotopically-enriched water (^{18}O, 46·6%; ^{17}O, 4·48%). After 45 min (*ca.* five half-lives [6]) the acid was neutralised. Any isotopic enrichment in either the hydroxymethylferrocene or the per(trimethylsilyl) derivative of the glucose fraction was determined with the mass spectrometer, operating as described in the Experimental section. Errors in the estimations were minimised by making identical measurements upon the same two products derived from hydrolysis in non-enriched water.

From these two sets of readings the oxygen isotopic enrichment, recorded in Table 1, for each compound,

TABLE 1

Oxygen isotope distribution in glucose and hydroxymethylferrocene obtained either as a control after treatment with isotopically enriched water or as products from the hydrolysis of glucoside (1) in enriched water

		Percentage		
Source	Sample	^{16}O	^{17}O	^{18}O
Hydrolysis of compound (1)	Glucose fraction	99	0	1
	Ferrocenyl fraction	49	4	47
Control	Glucose fraction	84	2	14
	Ferrocenyl fraction	89	1	10
Yeda Research and Development Co. Ltd.	Isotopically enriched water	48·9	4·5	46·6

was calculated as described in the Experimental section. Significant ^{18}O exchange between glucose or hydroxymethylferrocene under hydrolysis conditions was ruled out by a control experiment.*

The oxygen isotopic abundances given in Table 1 clearly indicate that the glucose is formed with retention of the C-1 oxygen atom that was present in the glucoside (1). Such a result is consistent with cleavage at position *b* in Scheme 1.

Thus both the methanolysis and hydrolysis experiments support the conclusion that aglycon–oxygen bond cleavage occurs to give the stable ferrocenylcarbinyl cation in both these reactions. Consequently, the earlier reported reverse mode of bond cleavage [6] must be erroneous.

EXPERIMENTAL

Unless stated otherwise optical rotations were measured with a Bellingham and Stanley polarimeter; n.m.r. spectra were measured with a Varian Associates A-60D instrument and mass spectra were measured as described in detail below.

T.l.c. was carried out on thin layers of Silica Gel G (Merck 7731) with benzene–ethyl acetate (3 : 1) as solvent system. G.l.c. was undertaken on a Varian-Aerograph instrument (Model 202C) fitted with a thermal conductivity detector. A column (10 ft × 0·25 in) packed with Chromosorb W, 60–80 mesh, impregnated with SE30, 10%, was used with hydrogen carrier gas.

* This agrees with an earlier result.[6] However, low solubility of the hydroxymethylferrocene in water might be responsible for the surprisingly small amount of exchange that occurred with this compound; *cf.* the methanolysis experiment where hydroxymethylferrocene is converted into its methyl ether.

Ferrocenylmethyl 2,3,4,6-Tetra-O-acetyl-β-D-glucopyranoside.—2,3,4,6-Tetra-*O*-acetyl-α-D-glucosyl bromide (6·15 g) in methylene chloride (50 ml) was added, during 12 h, to a stirred mixture of hydroxymethylferrocene (4·95 g), silver oxide (13·8 g), and anhydrous calcium sulphate (10 g) in methylene chloride (50 ml). After a further 6 h of stirring, the mixture was filtered, the solvent evaporated, and the residue crystallised from methanol to give a solid (3·4 g) which t.l.c. showed to be composed of four components. They were, in order of decreasing mobility: bisferrocenylmethyl ether, hydroxymethylferrocene, the title compound, and an unidentified spot which was probably 2,3,4,6-tetra-*O*-acetylglucose. The non-carbohydrate ferrocene derivatives were removed from the product by five successive extractions with hot, light petroleum (b.p. 60—80°). The remaining solid was then recrystallised from benzene–hexane to give a pure sample of the title compound (1·0 g, 12%), m.p. 178—180°, $[\alpha]_D^{22}$ −11·0° (*c* 1·0 in $CHCl_3$), which was homogeneous on t.l.c. de Belder *et al.* reported [6] m.p. 183—185°, $[\alpha]_D^{21}$ −11·7°. The n.m.r. spectrum ($CDCl_3$) showed τ 4·7—5·25 (m, H-1, -2, -3, -4), 5·3—5·6 (m, H-6 and -6′), 5·8—6·0 (m, H-5 and $C_{10}H_9Fe$), 6·1—6·5 (m, CH_2), 7·93 (s), 8·02 (s), 8·06 (s), and 8·07 (s, 4 OAc).

Ferrocenylmethyl β-D-Glucopyranoside (1).—The tetra-acetate of compound (1) (0·4 g) was deacetylated in chloroform (10 ml) with sodium methoxide in methanol (30 ml) during 1 h. The glycoside (1) was isolated in the usual way (0·27 g, 85%). Recrystallisation from water gave yellow plates, m.p. 133—135°, $[\alpha]_D^{22}$ −37° (*c* 1·0 in H_2O) [reported values,[6] m.p. 135—136°, $[\alpha]_D^{22}$ −37·7°].

Methoxymethylferrocene.—This compound was prepared according to the method of Nesmeyanov *et al.*[14] It had b.p. 101—102° (0·6 × 133 Nm^{-2}) and $n_D^{22.5}$ 1·59803; the reported [14] b.p. is 106—107·5° (1·5 × 133 Nm^{-2}) and n_D^{20} 1·6003.

Penta-O-trimethylsilyl-α-D-glucopyranose.—Glucose (0·2 g) was dissolved in anhydrous pyridine (1·0 ml) in a sample tube sealed with a serum cap. A mixture of hexamethyldisilazane (1 ml) and trimethylsilyl chloride (0·5 ml) was injected into the tube and the mixture was heated to 60° for 15 min. This gave a solution of the title compound suitable for g.l.c. studies. If required, the derivative could be isolated as follows: the excess of reagent was destroyed with methanol (2 ml) at 0°, diethyl ether (10 ml) was added and any insoluble material removed by filtration. The solution was evaporated to give the title compound as a colourless syrup, R_T 20 min, at 205°, and an n.m.r. spectrum (C_6D_6) which showed τ 4·84 (d, $J_{1,2}$ 3 Hz), 6·46 (q, $J_{2,3}$ 8·5 Hz), 5·7—6·3 (m, H-3, -4, -5, -6, -6′), 9·69 (s), 9·7 (s), 9·8 (s), 9·82 (s), and 9·89 (s, 5 $OSiMe_3$).

Methyl 2,3,4,6-Tetra-O-trimethylsilyl-β-D-glucopyranoside.—Methyl β-D-glucopyranoside (0·2 g) was trimethylsilylated as described above. The product was isolated as a colourless syrup which on g.l.c. examination had R_T 20 min. at 205°. The n.m.r. spectrum (C_6D_6) showed τ 6·01 (d, $J_{1,2}$ 7 Hz), 6·10—6·60 (m, H-2, -3, -4, -6, -6′), 6·80—7·10 (m, H-5), 6·75 (s, OMe), 9·71 (s), 9·73 (s), 9·78 (s), and 9·85 (s, 4 $OSiMe_3$).

Methanolysis of Ferrocenylmethyl β-D-Glucopyranoside (1).—Ferrocenylmethyl β-D-glucopyranoside (1) (0·150 g; 0·022M) was dissolved in anhydrous methanolic hydrogen chloride (0·044M; 16·85 ml) at 22°. After 35 min the acid

[14] A. N. Nesmeyanov, E. G. Perevalova, and Yu A. Vstynyuk, *Doklady Akad. Nauk S.S.S.R.*, 1960, **133**, 1105 (*Chem. Abs.*, 1960, **54**, 24,616*f*).

was neutralised with lead carbonate and the solution was filtered and evaporated. Hexamethyldisilazane (0·6 ml) and trimethylsilyl chloride (0·4 ml) in pyridine (0·5 ml) were added to the mixture and after 12 h at 22° the solution was analysed by g.l.c. (205°). This showed methoxymethylferrocene (R_T 6·4 min), bisferrocenylmethyl ether (R_T 8·8 min), the α-anomer of methyl tetra-*O*-trimethylsilyl-D-glucopyranoside (R_T 18·6 min) and possibly its β-anomer (R_T 20·0 min), penta-*O*-trimethylsilyl-α-D-glucopyranose (R_T 20·0 min) and the tetra-*O*-trimethylsilyl derivative of the starting material (R_T 26·8 min). All material with R_T 20·0 min was collected as a colourless syrup and its n.m.r. spectrum measured in C_6D_6. It was identical with that obtained with penta-*O*-trimethylsilyl-α-D-glucopyranose.

Methanolysis of Ferrocenylmethyl 2,3,4,6-Tetra-O-acetyl-β-D-glucopyranoside.—The acetylated glucoside (0·022M) was methanolysed as described for compound (1). The neutralised solution was deacetylated during 1 h at 22° with sodium methoxide. Carbon dioxide was then added, and after evaporation the residue was trimethylsilylated as described above. G.l.c. analysis at 205° showed that the product was identical with that from the non-acetylated glycoside.

Methanolysis of Hydroxymethylferrocene.—As a control experiment, hydroxymethylferrocene (0·022M) was methanolysed by the method described above. The product was extracted into diethyl ether (4 × 500 μl). The residue obtained after solvent evaporation was treated with the trimethylsilylating reagent and then analysed by g.l.c. (205°). This showed one peak with R_T 6·4 min identical with methoxymethylferrocene. Trimethylsilyloxymethylferrocene was not detected.

Hydrolysis of Ferrocenylmethyl β-D-Glucopyranoside (1) *in* $H_2{}^{18}O$.—The glucoside (1) (1·76 mg, 0·022M) was weighed in a screw-capped sample tube (35 × 10 mm diam.) and then isotopically enriched water (^{18}O, 46·6%, ^{17}O, 4·48%) (supplied by Yeda Research & Development Co. Ltd.) (200 μl) was added. The solution was acidified with 10·3M-sulphuric acid (1 μl) and after a reaction period of 0·75 h it was neutralised with lead carbonate (8 mg). The non-carbohydrate ferrocene derivatives, produced in the reaction, were extracted into diethyl ether (4 × 500 μl). These extracts were combined and evaporated to give a solid residue which was dried at 25° (0·1 × 133 Nm^{-2}). This sample was freed from any glucose contaminant by dissolution in dry diethyl ether and filtration. The residue (*ca.* 0·6 mg) obtained after solvent evaporation was analysed by mass spectrometry (see Table 2).

The aqueous fraction was evaporated in the frozen state and the isotopically enriched water was recovered. The residual carbohydrate material was dried and then treated with a 2 : 1 mixture of hexamethyldisilazane and trimethylsilyl chloride (50 μl) in pyridine (50 μl) for 17 h at 20°. After removal of excess of reagent with methanol (50 μl) the solution was diluted with diethyl ether (1 ml) and any precipitated by-product was allowed to settle. The supernatant liquor, containing the trimethylsilylated carbohydrate fraction, was removed using a 250 μl syringe. The residue was washed with diethyl ether and the washings were withdrawn, combined with the solution, and then evaporated. The residue, so obtained, was purified by redissolving it in ether, separating the solution from any insoluble material, and then evaporating the ether. The sample was then analysed by mass spectrometry (see Table 2).

Hydrolysis of Ferrocenylmethyl β-D-Glucopyranoside (1) *in* $H_2{}^{16}O$.—The hydrolysis of the glucoside (1) in normal water and the isolation of the two products were carried out as described above, except that the water was not recovered. The results are recorded in Table 2.

TABLE 2

Voltage readings and calculated percentage voltages for various ions produced in the mass spectra of $H_2{}^{16}O$ and $H_2{}^{18}O$ hydrolysis products from glucoside (1)

m/e	Mean peak heights * (mm)		Corresponding ion voltages 10^2V		Percentage volts	
	Penta-*O*-trimethylsilyl-α-D-glucopyranose					
	$H_2{}^{16}O$	$H_2{}^{18}O$	$H_2{}^{16}O$	$H_2{}^{18}O$	$H_2{}^{16}O$	$H_2{}^{18}O$
393	81·0 [c]	126·8 [c]	154·0	242·0	100·0	100·0
394	151·5 [b]	47·78 [c]	58·0	91·1	37·68	37·65
395	84·48 [b]	136·1 [b]	32·4	52·2	21·04	21·57
	Hydroxymethylferrocene					
216	129·9 [c]	98·23 [c]	248·0	187·2	100·0	100·0
217	94·90 [b]	112·0 [b]	36·4	42·9	14·7	22·9
218	102·6 [a]	98·29 [c]	3·83	187·3	1·55	100·1

* Measured on the Rikadenki recorder chart and corrected for total ion-current decay.

[a] $3·73 \times 10^{-4}$ V mm^{-1} on the 100 mV attenuator scale. [b] $3·83 \times 10^{-3}$ V mm^{-1} on the 1·0 V attenuator scale. [c] $1·906 \times 10^{-2}$ V mm^{-1} on the 5·0 V attenuator scale.

Treatment of Glucose and Hydroxymethylferrocene with Acidified $H_2{}^{18}O$.—Hydroxymethylferrocene (0·022M) and D-glucose (0·022M) were treated separately with water (^{18}O, 46·6%; ^{17}O, 4·48%) (200 μl) containing 10·3M-sulphuric acid (1 μl) for 0·75 h at 25°. Each solution was worked up by the method employed in the hydrolysis experiments and the materials isolated were examined by mass spectrometry (see Table 3).

TABLE 3

Percentage voltages from various ions obtained from the mass spectra of hydroxymethylferrocene and D-glucose after treatment with $H_2{}^{16}O$ and $H_2{}^{18}O$ under hydrolysis conditions

	m/e	393	394	395
Penta-*O*-trimethylsilyl-α-D-glucose	$H_2{}^{16}O$	100·0	36·6	20·7
	$H_2{}^{18}O$	100·0	39·0	38·3
	m/e	216	217	218
Hydroxymethylferrocene	$H_2{}^{16}O$	100·0	14·9	1·8
	$H_2{}^{18}O$	100·0	15·4	13·4

Mass Spectral Measurements.—(i) *Instrumentation and methodology.* Mass spectra were measured on an AEI MS 902 instrument. For the low-resolution, full-scan spectra, the ionising voltage was 70 eV. For isotopic abundance studies the ionising voltage was reduced to 16 eV which decreased errors arising from $M + 1$ and $M + 2$ ions and also minimised the formation of instrumental background peaks (*e.g.* from grease). The trap current was 100 μA and the ion source temperatures were 130° for the hydroxymethylferrocene samples and 150° for the pertrimethylsilylglucopyranose samples. The source slit width was reduced to about one third of the collector slit width. The narrower ion beam so produced gave rise to flat topped peaks which were easier to measure accurately.

Peak heights were determined throughout the *m/e* ion range of interest on a Rikadenki two-pen chart recorder Model B241. This was adjusted so that weaker signals gave full scale deflection (25 cm) and the signals from the abundant ions were attenuated. The serious errors which

arise from the decreasing total ion-current reaching the collector region of the spectrometer were minimised by delaying measurements for 15 min after sample injection. The remaining small decay in ion current was recorded with the second pen simultaneously with the ion scan and in this way a correction could be made. The recorder response was calibrated by measuring, with a digital voltmeter, the voltage difference between the mass spectrometer collector and earth, while the peak was scanned at a selected attenuation. The complication, in the calculation of the heavy oxygen isotopic enrichment, caused by the contributions from the ^{13}C isotope and the iron isotopes (^{54}Fe 5·9%, ^{57}Fe 2·3%, and ^{58}Fe 0·33%) present in the ferrocenyl derivatives and the ^{13}C isotope and the silicon isotopes (^{29}Si 4·7% and ^{30}Si 3·1%) present in the trimethylsilyl derivatives, were minimised by making accurate measurements of peak heights throughout the appropriate ion ranges on both enriched and non-enriched samples. This procedure also minimised errors arising from the instrumentation and sample preparation.

(ii) *Analysis of mass spectra.* The ^{18}O isotopic enrichment, in the $H_2{}^{18}O$ hydrolysis products, was determined as follows. The spectrum of the hydroxymethylferrocene was measured in the region of its molecular ion (*i.e.* *m/e* 214 to 219) and for the pertrimethylsilylglucose in the region of its fragment ion *m/e* 393 (*i.e.* 392 to 397). Such measurements were made on samples from the hydrolysis products of glucoside (1) in $H_2{}^{18}O$ and $H_2{}^{16}O$. The relevant results are recorded in Table 2. Measurements were also made on samples of hydroxymethylferrocene and glucose, which had been recovered from an acidified $H_2{}^{18}O$ solution (H_2SO_4, 0·051M). The results are given in Table 3.

(iii) *Calculations.* The detailed calculation of ^{17}O and ^{18}O enrichment in the hydroxymethylferrocene component isolated from the hydrolysis of glucoside (1) in isotopically enriched water is given as an illustrative example.

The results in Table 2 show that hydroxymethylferrocene has, at *m/e* 217, a ratio: $^{17}O : {}^{16}O :: 8·2 : 100$. Thus the contribution at *m/e* 218 from ions containing ^{17}O is $(8·2 \times 14·7)/100 = 1·21$. Consequently the contribution at *m/e* 218 from ions containing ^{18}O is: $100·1 - 1·6 - 1·2$, therefore $^{18}O : {}^{16}O :: 97·3 : 100$. The ratio of oxygen isotopes in the hydrolysis sample of hydroxymethylferrocene is: $^{16}O : {}^{17}O : {}^{18}O :: 49 : 4 : 47$. Table 1 contains the oxygen isotopic abundances in various compounds, which were calculated by a similar procedure.

We thank Dr. D. Payling of the P.C.M.U. for helpful discussions concerning the mass spectral data. The Governors of Birkbeck College are thanked for support of this work.

[2/1819 *Received*, 1*st August*, 1972]