IDENTIFICATION OF THE REACTION PRODUCTS OF PHENYL ISOCYANATE AND METHYL α-D-GLUCOPYRANOSIDE BY P.M.R. SPECTROSCOPY

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ABSTRACT

Reaction of methyl α-D-glucopyranoside with 1, 2, and 3.5 equivalents of phenyl isocyanate gave mixtures of 7, 9, and 4 carbanilated products, respectively. They were separated by column chromatography and identified by their p.m.r. spectra in Me_2SO-d_6 and pyridine-d_5. Hydroxyl-proton and low-field proton resonances were observed in Me_2SO-d_6. Substitution at a hydroxyl position causes a downfield shift of the corresponding ring-proton signal in both Me_2SO-d_6 and pyridine-d_5, and disappearance of the hydroxyl peak in Me_2SO-d_6. The 2-OH doublet has the largest splitting (7.0 Hz) and the 6-OH signal is the only triplet. The 3-OH and 4-OH doublets have nearly identical splitting (~5–6 Hz) but the 4-OH proton has the greater chemical shift. However, H-3 is deshielded to a greater extent than H-4 by substitution at the adjacent hydroxyl group. Spin-spin decoupling in pyridine-d_5 confirmed the ring-proton assignments. The 6-position is favored for substitution, and the substituent distribution is similar to that for equilibrium-controlled reactions of cellulose.

INTRODUCTION

P.m.r. spectroscopy has been used to determine the position of substitution in carbohydrate derivatives by examining the deshielding of protons *alpha* to the hydroxyl group at which substitution occurred. Forsen, *et al.*[1], concluded that xanthation occurs at O-6 of methyl α-D-glucopyranoside, because only the H-6 signals are shifted to lower field. Williams and Richardson[2] demonstrated, by observing low-field proton signals in pyridine solution, that dibenzoylation of methyl α-D-mannopyranoside and methyl α-D-glucopyranoside gives the 3,6-and 2,6-dibenzoates, respectively. Similar downfield shifts of the appropriate ring-proton signals were observed by Tulloch and Hill[3] in partially acylated derivatives of methyl β-D-glucopyranoside, and by Horton and Lauterbach[4] with derivatives of methyl α-D-glucopyranoside. The shift of the ring-proton resonances is affected by the type of the hydroxyl group (that is, primary or secondary)[5], by the nature of the solvent, and by the presence of substituents within the molecule[3,4].

The work described here was undertaken to determine whether the mono- and, in particular, the higher, substitution products of carbohydrates could be conveniently identified by observing the residual hydroxyl-proton and low-field, ring-proton signals in the p.m.r. spectra of the compounds isolated from the reaction mixture. The reaction of methyl α-D-glucopyranoside (**1**) and phenyl isocyanate was selected for study, as the resultant carbanilates are readily formed, are high melting, are readily crystallized, and are resistant to hydrolysis. The latter point is of importance in extending the study to such polysaccharides as cellulose and starch.

Hydroxyl-proton and low-field ring-proton resonances were observed in deuterated dimethyl sulfoxide (Me_2SO-d_6) solutions. The latter assignments were confirmed by using pyridine-d_5 as the solvent and by spin–spin decoupling experiments.

RESULTS AND DISCUSSION

Synthesis of carbanilated methyl α-D-glucopyranosides. — The 6-mono- (**2**) 2,3-di- (**11**), and 2,3,4,6-tetra- (**10**), carbanilates were prepared by known synthetic routes and were used as reference compounds. Reactions with 1, 2, and 3.5 equivalents of phenyl isocyanate were performed in pyridine solution at 25° and were monitored by thin-layer chromatography (t.l.c.). Mixtures of 7, 9, and 4 products, respectively, were obtained. They were separated by column chromatography on a silica gel support, with elution by various acetone–benzene mixtures. The results of the separations are summarized in Table I. The compounds are listed in the order of elution from the column.

Identification of reaction products by p.m.r. spectroscopy. — The 100-MHz, p.m.r. parameters for methyl α-D-glucopyranoside (**1**) and the known carbanilates (**2, 10, 11**) in Me_2SO-d_6 solution are summarized in Table II. The hydroxyl protons of the compounds resonate as doublets or triplets in the τ 6.0–6.3 region[6]. The assignments of hydroxyl-proton resonance for methyl α-D-glucopyranoside correspond to those of Perlin[7]. It may be noted that the vicinal coupling-constant, $J_{HOCH'}$, is about 6–7 Hz for the C-2 hydroxyl protons and about 5–6 Hz for those at C-3 and C-4. Substitution causes the remaining hydroxyl-proton resonance to shift downfield, relative to those of **1**. Substitution may also cause some change in the vicinal coupling-constants of the derivatives, possibly due to an alteration in ring conformation[6].

Substitution at the 2-OH group was easily recognized by the disappearance of the hydroxyl doublet resonance having the largest splitting. Similarly, substitution at the 6-OH group was easily identified by the disappearance of the triplet in the τ 5.5 region and the appearance of the typical H-6,6′ quartets in the same region; the latter are deshielded[1] by the 6-substituent. It is more difficult to distinguish between substituents at C-3 and C-4, and the assignments are based on the assumption that the 4-OH proton is deshielded to the greater extent, as reported by Perlin[7].

In each case the assignment was confirmed by the identification of the corresponding ring-proton signal, deshielded by substitution at the hydroxyl group. In

TABLE I

REACTION PRODUCTS OF PHENYL ISOCYANATE AND METHYL α-D-GLUCOPYRANOSIDE IN PYRIDINE SOLUTION AT 25°.

Compd. no.	*Compd. Identification*	*Phenyl isocyanate*[a]			*M.p.* °C	*Analysis, %*					
		1 eq.	*2 eq.*	*3 eq.*		*Found*			*Calc.*		
						C	*H*	*N*	*C*	*H*	*N*
1	Me α-D-glucopyranoside	2	trace		149–150						
2	6-monocarbanilate	1	2		146–147			4.25			4.47
3	2-monocarbanilate	4			209–211			4.63			4.47
4	4,6-dicarbanilate	5	6		209–210			6.37			6.48
5	2.6-dicarbanilate	3	1	3	97	58.12	5.37	6.22	58.20	5.60	6.48
6	3,6-dicarbanilate	6	7		207–208	57.88	5.69	6.37	58.20	5.60	6.48
7	Not identified	7	8								
8	2,4,6-tricarbanilate		3	2	214–216			7.33			7.62
9	2,3,6-tricarbanilate		4	2							
10	2,3,4,6-tetracarbanilate		5	1	229–232			8.35			8.36

[a]Numbers in columns below show relative intensity of products in each reaction mixture.

TABLE II

CHEMICAL SHIFTS[a], τ (P.P.M.) AND COUPLING CONSTANTS (Hz, IN PARENTHESES) OF METHYL α-D-GLUCOPYRANOSIDE AND KNOWN CARBANILATES

Compd.	*Solvent*	*Hydroxyl protons*				*Ring protons*				*H-6[c]*	*NH*
		2-OH (*d*)	*3-OH* (*d*)	*4-OH* (*d*)	*6-OH* (*t*)	*H-1* (*d*)	*H-2[b]* (*m*)	*H-3* (*t*)	*H-4* (*t*)	(*m*)	(*s*)
1	C_2D_6SO	5.35(7.0)	5.31(5.0)	5.17(5.0)	5.58(5.0)	5.47(3.5)					
2	C_2D_6SO	5.34(6.2)	5.23(4.9)	4.97(5.8)		5.51(3.4)				5.82	0.42
10	C_2D_6SO					5.02	5.14	4.54(9.7)	5.01(9.5)	5.79	0.12, 0.22, 0.35
	C_5D_5N					——4.8——		4.05(9.6)	4.60(9.6)	5.49	1.42
11	C_2D_6SO			4.68(5.0)	5.48(5.0)	5.16(3.5)	5.37[d]	4.82[d,e]			0.22, 0.45
	C_5D_5N					——5.3——		4.51[e]	——6.3——		1.90

[a]At 100 MHz. Peak multiplicity noted as: s, singlet; d, doublet; t, triplet; m, multiplet. [b]Doublet of a doublet; J values, 3.5 and 9.5 Hz. [c]AB portion of an ABX system; τ value at center of multiplet. [d]From spectrum after addition of deuterium oxide. [e]Doublet of a doublet.

the absence of a substituent at the hydroxyl group, the ring-proton signals, with the exception of H-1, are located well up-field and overlap so extensively in Me_2SO-d_6 solution that they cannot be differentiated. However, for the most part, they are well separated in pyridine-d_5 solution and therefore spin decoupling is more conveniently performed in this solvent.

Table III summarizes the p.m.r. parameters for the carbanilates isolated from the reactions of phenyl isocyanate and **1**. The degree of substitution is readily observed from the number of NH peaks in the Me_2SO-d_6 spectra, or, when these peaks overlap, from the integration curve. In each case, elemental analysis provided supporting data. Analysis of the individual spectra provided the distinction between the possible isomers. Hydroxyl-proton resonances were verified by the addition of deuterium oxide.

Considering first the products from the reaction with one equivalent of phenyl isocyanate, compound **1** was characterized as the starting material. The spectrum of **2** in Me_2SO-d_6 is identical with that of the known 6-monocarbanilate (Tables II and III), and the elemental analysis and m.p. substantiated the identification.

Compound **3** could not be isolated in sufficient purity because of its relatively low concentration in the mixture and the similarity of its R_F value to those of **2** and **4**. To identify **3**, compound **1** was treated with 0.34 equivalent of phenyl isocyanate. T.l.c. showed four products having R_F values corresponding to those of compounds **1**, **2**, **3**, and **5**, but not of **4**. This experiment permitted separation of sufficient, pure **3** to obtain its spectrum; the single NH signal indicated that it is a monosubstituted product. There were two overlapping doublets, at τ 4.94 and 4.98, having J values of 5.5 and 5.0 Hz, respectively, and a triplet at τ 5.52 having a coupling constant of 5.0 Hz that overlapped another signal. These signals disappeared on addition of (deuterium oxide, leaving the H-1 doublet at τ 5.25, $J = 3.6$ Hz, and revealing a doublet of a doublet centered at τ 5.52. As the hydroxyl-proton doublet having the largest coupling constant (2-OH) was absent, but a doublet of a doublet appears downfield showing the expected coupling constants namely, 3.5 Hz (axial–equatorial) and 9.5 Hz (axial-axial), it was apparent that **3** is the 2-monocarbanilate. Confirmation was provided by the p.m.r. data for the known 2,3-dicarbanilate, which also has a similar resonance at τ 5.37.

Compounds **4**, **5**, and **6** were similarly identified. Each is a dicarbanilate having C-6 substituted. The ring-proton signals (Table III) were identified by spin-decoupling and favor the following assignments: **4**, the 4,6-dicarbanilate; **5**, the 2,6-dicarbanilate; and **6**, the 3,6-dicarbanilate. In each instance, the proton *alpha* to the substituent resonates at low field relative to the corresponding proton of the parent compound **1** (Tables II & III). Consistent also with these assignments were the chemical shifts and spacings of the hydroxyl-proton resonances in Me_2SO-d_6 solution (Table III). In these instances, the absence of an OH signal corresponding to one produced by the parent glycoside, indicates that a carbanilate has been formed at that position.

The spectrum of **6** in pyridine-d_5 showed a somewhat poorly resolved doublet of a doublet instead of the triplet expected for the axial H-3 coupled with the axial

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TABLE III

CHEMICAL SHIFTS[a], τ (P.P.M.) AND COUPLING CONSTANTS (Hz, IN PARENTHESES) OF REACTION PRODUCTS

Compd.	*Solvent*	*Hydroxyl protons*				*Ring protons*					*H-6*[c]	*NH*
		2-OH (*d*)	*3-OH* (*d*)	*4-OH* (*d*)	*6-OH* (*t*)	*H-1* (*d*)	*H-2*[b] (*m*)	*H-3* (*t*)	*H-4* (*t*)	*H-5* (*m*)	(*m*)	(*s*)
1	C_2D_6SO	5.35(7.0)	5.31(5.0)	5.17(5.0)	5.58(5.0)	5.47(3.5)						0.42
2	C_2D_6SO	5.36(6.2)	5.25(4.5)	5.00(5.5)		5.52(3.5)				5.02		0.24
3	C_2D_6SO		4.98(5.0)	4.94(5.5)	5.52(5.0)	5.25(3.6)	5.52[d]				5.85	0.34, 0.36
4	C_2D_6SO	5.02(7.0)	4.88(5.5)			5.38(3.2)			5.41(9.6)		5.90	1.95
	C_5D_5N					5.66(3.5)	6.66	6.19(9.5)	5.25(9.5)		5.71	0.22, 0.36
5	C_2D_6SO		4.80(5.5)	4.67(5.3)		5.19(3.6)	5.43				5.82	2.0
	C_5D_5N					5.46(3.1)	5.40	6.20(9.5)	——6.6——		5.69	0.35, 0.48
6	C_2D_6SO	5.01(7.0)		4.64(6.3)		5.35(3.5)		5.05(9.0)[d]			5.80	
	C_5D_5N					5.63(3.5)	6.70	4.45[e]	6.65(9.5)	6.53	5.78	0.12, 0.25, 0.28
8	C_2D_6SO		4.45(6.0)			5.07(3.5)	5.4		5.3(9.5)	6.06	5.75	0.14, 0.29, 0.35
10	C_2D_6SO					5.01	5.08	4.50(9.5)	4.99(10.0)		5.49	1.42
	C_5D_5N					——4.8——		4.04(9.6)	4.60(9.6)	5.92		

[a]At 100 Hz. Peak multiplicity noted as: s, singlet; d, doublet; t, triplet; m, multiplet. [b]Doublet of doublet, *J* values, 3.5 and 9.5 Hz. [c]AB portion of an ABX system; τ value at center of multiplet. [d]From spectrum after addition of deuterium oxide. [e]Doublet of doublet.

H-4 and H-2. Likewise, the H-3 signal of **11** showed a poorly resolved doublet of a doublet in both Me_2SO-d_6 (τ 4.82) and pyridine-d_5 (τ 4.18). Substitution at C-3 may change the ring conformation slightly so that H-3 approaches an axial–equatorial disposition with one of the vicinal ring protons. There may also be a solvent effect, since the signal was a well-resolved triplet in the case of **6** in Me_2SO-d_6.

The reaction of 2 equivalents of phenyl isocyanate gave a mixture of 9 products (Table I). The first five compounds (**3**, is absent) were separated by column chromatography and identified by comparison of their R_F values and p.m.r. spectra with the products isolated from the reaction with one equivalent of phenyl isocyanate.

Compounds **8**, **9**, and **10** were not present in sufficient amount to be readily separated by column chromatography. Their R_F values suggested that they were tri- and tetra-substituted derivatives. To obtain adequate amounts for identification, compound **1** was treated with 3.5 equivalents of phenyl isocyanate, to give a mixture of four products (Table I). Compound **5**, present in the smallest amount, was identified from its R_F value and p.m.r. spectrum.

Compound **8** was the 2,4,6-tricarbanilate, as indicated by its p.m.r. data (Table III). There were three NH peaks and only one hydroxyl-proton signal, a doublet, at τ 4.45, whose its coupling constant is relatively large (6.0 Hz) but it is the 3-OH signal as shown by the doublet of a doublet at $\tau \sim 5.4$, (H-2) overlapping a triplet at $\tau \sim 5.3$ (H-4). Substitution at C-3 would displace the H-3 signal well below τ 5. Substitution at C-6 was shown by the two AB quartets centered at τ 5.78, and, of course, by the absence of a hydroxyl-proton triplet. Due to the similarity of the R_F values of compounds number **8**–**10**, it was not possible to obtain complete separation of **9**. The R_F value of **9** showed it to be a trisubstituted glucopyranoside. Based on the reactivity of the hydroxyl groups and the fact that compound **8** is the 2,4,6-triester, compound **9** was very probably the 2,3,6-tricarbanilate.

Compound **10** had the highest R_F value. Its spectrum in Me_2SO-d_6 did not show any hydroxyl-proton resonances and showed four NH protons (Table III), two of them overlapping. The downfield position of the H-2, H-3, H-4, and H-6 signals in both pyridine-d_5 and Me_2SO-d_6 also demonstrated this compound is the 2,3,4,6-tetracarbanilate, as did comparison of its p.m.r. data with that of a known sample of the tetracarbanilate (Table II).

In turn, the last three compounds, resulting from the reaction of 2 equivalents of phenyl isocyanate, were presumed by their R_F values to be the 2,4,6-tri-, 2,3,6-tri-, and 2,3,4,6-tetracarbanilates.

Distribution of substituents. — Calibration curves of t.l.c. spot-intensity *vs.* concentration were made by using **1** and the known 6-mono-, 2,3-di-, 2,3,4-tri-, and 2,3,4,6-tetracarbanilate. The assumption was made that, regardless of the isomer involved, the same number of carbon atoms should give the same spot intensity in t.l.c.

The relative amounts of substituted α-D-glucopyranosides in the reaction mixtures formed with one and two equivalents of phenyl isocyanate were determined with a microdensitometer. The results from three t.l.c. plates were averaged. The

sums of the estimated weights of the reaction products were in good agreement w the initial weight of the reaction mixtures employed.

For the reaction of one equivalent of phenyl isocyanate, the distribution substitutents among the C-6, C-2, C-3, and C-4 hydroxyl groups of methyl α-glucopyranoside was 1.0:0.30:0.02:0.03. This ratio is very similar to those found i equilibrium-controlled reactions of cellulose[8,9], that is, reaction at O-6 is favored.

EXPERIMENTAL

General methods. — Microanalyses were made by Schwarzkopf Microanalyti Laboratory. Melting points were determined with a Kofler micro hot stage (Arth H. Thomas Co., Philadelphia, Pennsylvania) and are uncorrected. P.m.r. spec were measured with a Varian HA-100 n.m.r. spectrometer operating at 100 MHz the field-sweep mode. Spin-decoupling experiments were performed with the HA-1 instrument operating in the frequency-sweep mode. Spectra were measured at concentration of 10% (w/v). Dimethyl sulfoxide-d_6 solutions also contained 2 (w/v) of tetramethylsilane ($\tau = 10.000$) as an internal standard and to provide a lo signal. For pyridine-d_5 solutions, a capillary containing sulfuric acid was placed the n.m.r. tube to provide a lock signal. A few drops of tetramethylsilane were add and its signal was set at 0 p.p.m. Chemical shifts are on the τ scale and were tak from the chart recording. The temperature in the probe was approximately 25°. T recorded first-order coupling constants are the measured peak spacings.

T.l.c. of the reaction products was made with Silica Gel G (E. Merck, Dar stadt, W. Germany), activated at 105°, as the absorbent. A 3:7 (v/v) acetone–benze mixture was used as developing solvent for the higher substitution-products and 7:3 mixture for the lower ones. Detection was effected with chromic acid. Intensities the products separated by t.l.c. were measured with an automatic recording mic densitometer MK IIIC (Joyce, Loebl and Co., Ltd., England). Activated silica (Davison Chemical), 100–200 mesh, was employed for column chromatograp after extraction for 4 h with 7:3 acetone–benzene. The column dimensions w 40 × 750 cm. The solvent flow-rate was maintained at 30–40 ml/h and 10-ml fractic were collected. The fractionations were monitored by t.l.c.

Methyl α-D-glucopyranoside 6-monocarbanilate (**2**). * — Prepared by the meth of Hearon, Hiatt, and Fordyce[10], overall yield was 15%. Recrystallized from et acetate until t.l.c. showed one spot, it had m.p. 143–144° (lit.[10] m.p. 132–133 R_F 0.10 (t.l.c.); p.m.r. data in Me_2SO-d_6 at 100 MHz (see Table II).

Methyl α-D-glucopyranoside 2,3-dicarbanilate (**11**)*. — Prepared in three ste by the method of Hearon *et al.*[10], the overall yield was 20%. Purified by colu chromatography with 3:7 acetone–benzene as developer and recrystallization fr

*Compounds **2** and **11** were purified by recrystallization and column chromotography, respectiv Purification was continued until t.l.c. showed only one spot. This purification is believed to account the m.p. of **2** and **11** being higher than those recorded in the literature.

a mixture of petroleum ether and ethyl acetate gave **11**, m.p. 160–162° (lit.[10] m.p. 151–153°); p.m.r. data in Me_2SO-d_6 and pyridine-d_5 at 100 MHz (see Table II).

Methyl α-D-*glucopyranoside* **2,3,4,6-***tetracarbanilate* (**10**). — Prepared by the method of Wolfrom and Pletcher[11], it was obtained in 46% yield, m.p. 230–231° (lit.[11] m.p. 227° dec.); R_F 0.80 (t.l.c.); p.m.r. data in Me_2SO-d_6 and pyridine-d_5 at 100 MHz (see Table II).

Reactions of methyl α-D-*glucopyranoside and phenyl isocyanate.* — In each case, compound **1** (5.25 g, 2.7 mmole) was dissolved in dry pyridine (30 ml) and phenyl isocyanate was added with stirring. The temperature was maintained at 25° with a cooling bath. After 0.5 h the pyridine was removed under diminished pressure. Water was added to the residue and evaporated off under diminished pressure. This sequence was repeated several times to remove the last traces of pyridine. Phenyl isocyanate [0.91, 2.73, 5.46, and 9.56 mmoles, (0.34, 1.0, 2.0, and 3.5 equivalents)] respectively, were employed.

A portion of the reaction mixture (1.0–1.5 g) was dissolved in acetone (15 ml), loaded on the column, and eluted with an acetone–benzene mixture. The first 300–400 ml of effluent was discarded and then 10 ml fractions were collected. A 7:3 (v/v acetone–benzene mixture was used to separate the 2-monocarbanilate from the other three compounds in the reaction with 0.34 equivalents of phenyl isocyanate. For separation of the reaction products formed with 1 and 3.5 equivalents, a 3:7 acetone–benzene mixture was employed. This same mixture was used in the case of the reaction of 2 equivs. for the first 150 fractions. Then the ratio was changed to 5:5, and, at fraction 400, to 7:3. The elemental analyses, and m.p.* values are given in Table I, and the p.m.r. data in Me_2SO-d_6 and pyridine-d_5, where noted, are given in Table III.

REFERENCES

1 S. FORSEN, P. J. GAREGG, B. LINDBERG, AND E. PETTERSON, *Acta. Chem. Scand.*, 20 (1966) 2763.
2 J. M. WILLIAMS AND A. C. RICHARDSON, *Tetrahedron*, 23 (1964) 1369.
3 A. P. TULLOCH AND H. HILL, *Can. J. Chem.*, 46 (1968) 2487.
4 D. HORTON AND J. H. LAUTERBACH, *J. Org. Chem.*, 34 (1969) 86.
5 V. W. GOODLETT, *Anal. Chem.*, 37 (1965) 432.
6 R. D. GILBERT, C. G. MORELAND, AND R. KAMMERECK, *Text. Res. J.*, 40 (1970) 193.
7 A. S. PERLIN, *Can. J. Chem.*, 44 (1966) 539.
8 S. P. ROWLAND, V. O. CIVINO, AND A. L. BULLOCK, *Can. J. Chem.*, 44 (1966) 1051.
9 G. F. TOUZINSKY, *J. Org. Chem.*, 30 (1965) 426.
10 W. M. HEARON, G. D. HIATT, AND C. R. FORDYCE, *J. Amer. Chem. Soc.*, 66 (1944) 995.
11 M. L. WOLFROM AND D. E. PLETCHER, *J. Amer. Chem. Soc.*, 62 (1940) 1151.

*The m.p. of **2** (146–147°), as obtained from the column, was higher than (132–133°) reported by Hearon *et al.*[10].