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Partial Cyanoethylation of 2-Tetrahydropyranyl β-D-Glucopyranosides

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The rapid acid hydrolysis of 2-tetrahydropyranyl β-D-glucosides has made possible the hydrolysis of the partially cyanoethylated glucosides to glucose and glucose cyanoethyl ethers with the cyanoethyl groups intact. The glucosides were cyanoethylated in 2 % aqueous sodium hydroxide at 0° to low degrees of substitution and the relative proportion of each of the four monocyanoethyl ethers was after acid hydrolysis determined by quantitative paper electrophoresis in germanate buffer. The results indicate that the hydroxyl group at C-6 is more reactive than the three secondary hydroxyl groups together, followed by that at C-2.

Cyanoethyl cellulose which is of technical importance is generally made by the reaction of acrylonitrile with cellulose in aqueous sodium hydroxide, and *O*-2-cyanoethyl ethers are produced. The degree of substitution (D.S.) attained in the reaction varies with the reaction conditions. Little is known about the actual distribution of the *O*-2-cyanoethyl groups on the various positions of the cellulose. An attempt has been made by Carter [1] by using sodium-liquid ammonia treatment on cyanoethylcellulose, previously reported by Danilov and Lopatenok [2] to produce a deoxy cellulose. The distribution of the deoxy groups was examined by determining the 6-deoxyglucose content, by periodate oxidation and by enzymatic and controlled acid hydrolysis of the deoxy cellulose. It was concluded that the greater number of deoxy groups were located at C-2. Since the reductive cleavage of the *O*-2-cyanoethyl ethers mostly regenerated the original hydroxyl groups and cleavage to deoxy groups occurred only to a smaller extent, no reliable information about the original distribution of the *O*-2-cyanoethyl groups could be obtained by this method.

A study of the distribution of substituents on the partial cyanoethylation of cellulose by direct methods would seem difficult. *O*-2-Cyanoethyl groups should be liable to partial hydrolysis to amides and acids during acid hydrolysis

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of the cellulose derivatives, and in the previously reported preparations of *O*-2-cyanoethyl ethers of glucose substantial amounts of degradation products with low R_F-values on paper chromatography always accompanied the glucose *O*-2-cyanoethyl ethers on acid hydrolysis of the parent acetals and glycosides.[3] In the present study an attempt was therefore made to study the distribution of *O*-2-cyanoethyl groups obtained on the partial cyanoethylation of a β-D-glucopyranoside which could be hydrolysed to a mixture of glucose and *O*-2-cyanoethyl ethers without changing or removing the *O*-2-cyanoethyl groups. This requirement was met by the previously reported, isomeric 2-tetrahydropyranyl β-D-glucopyranosides.[4] A somewhat modified preparation of these, involving their separation as their tetraacetates by partial crystallisation instead of by chromatography is given in the experimental part.

Treatment of each of the previously reported 2-, 3-, and 6-*O*-(2-cyanoethyl)-D-glucose [3] with sulphuric acid under the conditions required for hydrolysis of the 2-tetrahydropyranyl glucopyranosides and examination of the products by paper chromatography indicated that no degradation of the *O*-2-cyanoethyl groups occurred. Hydrolysing conditions normally required to cleave glucopyranosides gave appreciable hydrolysis of the *O*-2-cyanoethyl groups.

O-2-Cyanoethyl groups are alkali labile,[5] undergoing hydrolysis to the parent alcohol as well as to amides and acids, and the cyanoethylations of the (−)-2-tetrahydropyranyl β-D-glucoside were therefore performed under mild conditions, in 2 % aqueous sodium hydroxide solution at 0°. The reaction times were 3 h or less. The degrees of substitution obtained were 0.27, 0.47, and 0.72, respectively. After mild acid hydrolysis [4] the resulting mixtures of glucose and the various 2-cyanoethyl ethers were separated into glucose, mono- and di-substituted glucoses on cellulose columns. The relative proportions of each of the 2-, 3-, 4-, and 6-*O*-(2-cyanoethyl)-D-glucose in the monosubstituted fraction were determined by quantitative paper electrophoresis in germanate buffer.[6] D-Glucose, 2-, 3-, and 6-*O*-(2-cyanoethyl)-D-glucose as well as a di-*O*-

Table 1. Composition of the hydrolysates from partially cyanoethylated 2-tetrahydropyranyl β-D-glucopyranosides.

D.S.	Mole %					
	D-Glucose	Mono-*O*-(2-cyanoethyl)-D-glucose				Di-*O*-(2-cyanoethyl)-D-glucose
		2-	3-	4-	6-	
0.14[a]	87.7	2.7	0.6	1.3	5.7	2.0
0.27[b]	77.4	4.1	1.0	1.7	11	4.6
0.47[b]	63.2	6.0	2.5	4.1	14	9.8
0.72[b]	48.0	7.2	2.7	4.7	17	20.3

[a] For the (+)-rotating isomer.
[b] For the (−)-rotating isomer.

(2-cyanoethyl)-D-glucose, which from its mobility on paper electrophoresis possibly is the 4,6-isomer, were obtained in a crystalline state. (+)-2-Tetrahydropyranyl β-D-glucoside was similarly reacted to a D.S. of 0.14 and the products were examined in the same way. The results are given in Table 1. From these it appears that the C-6 hydroxyl group is more reactive than those at the secondary hydroxyl groups taken together, followed by those at C-2, C-4, and C-3, respectively. An extrapolation of the ratio of substitution at each of these positions in the monosubstituted fraction to zero reaction time gives an approximate relative ratio of 3:1:2:8 for the rate of reaction of the hydroxyl groups at C-2, C-3, C-4, and C-6, respectively.

That the hydroxyl group at C-2 is the more reactive of the secondary hydroxyl groups is expected from previous alkylation studies on cellulose and model compounds in alkaline media, if it is presumed that the acrylonitrile reacts preferentially with alkoxide ions.[7,8] The rapid relative rate of reaction at the C-6 hydroxyl groups indicates rather more specific steric requirements of acrylonitrile than of alkyl halides. This would seem to be similar to the steric requirement of ethylene oxide.[9] In these previous studies however, higher alkali concentrations were used.

EXPERIMENTAL

All melting points are corrected. Evaporations were performed at reduced pressure at a bath temperature below 40°.

Paper chromatography. Solvents: A. Ethyl acetate-pyridine-water 8:2:1. B. Butanol-ethanol-water 10:3:5. C. Isopropyl ether-light petroleum (40–60°) 1:1 (on dimethyl sulphoxide impregnated paper).[10] Paper: Whatman No. 1.

Paper electrophoresis. Buffers: 0.05 M germanate buffer at pH 10.7;[11] for quantitative separations of the mono-2-cyanoethyl ethers 0.1 M germanate buffer at pH 10.0 at 1000 V an 40° for 3 h; 0.1 M borate buffer at pH 10.0. Paper: Whatman No. 3 MM.

Spray reagents: Anisidine hydrochloride for reducing sugars, silver nitrate and sodium hydroxide for the glucosides.

Materials. Each of the two isomeric 2-tetrahydropyranyl β-D-glucopyranosides were obtained by a somewhat different procedure to that previously described.[4] 2,3,4,6-Tetra-*O*-acetyl-β-D-glucose (118 g) was shaken overnight at room temperature with dihydropyran (distilled over sodium, b.p. 84–86°, 600 ml) containing 37 % hydrochloric acid (12 ml). The solution was neutralised with silver oxide, filtered and concentrated to near dryness. Crystallisation occurred on adding ethanol. The crystalline mixture obtained (117 g) had m.p. 100.5–121°, $[\alpha]_D^{20}$ −13° (*c*, 2 in chloroform) and was free from starting material on paper chromatography in solvent C.

The mixture (117 g) was fractionated by crystallisation from isopropyl ether, the (+) rotating, higher m.p. isomer tended to crystallise first, and the (−) rotating isomer was obtained from the mother liquors. Pure seed crystals obtained as previously described [4] were used. The (+)-2-tetrahydropyranyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside thus obtained (19 g) had m.p. 138–140°, $[\alpha]_D^{20}$ + 54° (*c*, 1 in chloroform) and the corresponding (−) isomer was obtained in two batches, one (10 g) with m.p. 109–111°, $[\alpha]_D^{20}$ −61° (*c*, 1 in chloroform) and the other (17 g) with m.p. 109–112°, $[\alpha]_D^{20}$ −56° (*c*, 1 in chloroform). The remainder of the original mixture was obtained as crystalline mixtures of varying proportions of the two isomers.

Each of the two 2-tetrahydropyranyl β-D-glucopyranosides was obtained as required, in quantitative yields, from the tetraacetates by deacetylation with Dowex 2–X8 (OH^- form) in 95 % ethanol.[4]

The acrylonitrile used in the partial cyanoethylations was freshly distilled, the fraction with b.p. 77–79° was collected.

The stability of glucose (2-cyanoethyl) ethers to acid. Each of 2-, 3-, and 6-*O*-(2-cyanoethyl)-D-glucose (3 mg) was treated with N sulphuric acid (0.2 ml) for 2 h at room temperature, neutralised with barium carbonate, filtered and concentrated. Paper chromatography in solvents A and B showed no change, each substance was chromatographically pure. Hydrolysis of each of the isomers (3 mg) in 8 % sulphuric acid (0.2 ml) for 3 h at 100° and working up as above gave products which on paper chromatography as above were contaminated with appreciable amounts of material with R_F values lower than those of glucose.

Partial cyanoethylations and hydrolyses. (−)-2-Tetrahydropyranyl β-D-glucopyranoside (1.35 g) in 2 % aqueous sodium hydroxide (100 ml) was cooled to 0°. Acrylonitrile (5.0 ml) was added and the mixture was stirred for 3 h at 0°. The solution was neutralised with Dowex 50 W−X 8 (H^+ form) and filtered. The resin was washed with water and 50 % aqueous ethanol. The combined filtrate and washings were concentrated to a syrup. The syrup was dissolved in N sulphuric acid (80 ml) and allowed to stand at room temperature for 1 h, the solution was neutralised with Dowex 3 (free base), filtered and the resin was washed with water and with 50 % aqueous ethanol. The combined filtrate and washings on concentration and drying in a vacuum over phosphorus pentoxide gave a syrup (979 mg). Paper chromatography in solvent A indicated glucose (major spot), 6-*O*-(2-cyanoethyl)-glucose,[3] a somewhat weaker, elongated spot corresponding to the other mono-(2-cyanoethyl)-glucose ethers,[3] and still weaker spots of higher R_F values. The other two partial cyanoethylations of (−)-2-tetrahydropyranyl β-D-glucopyranoside and of the (+)-isomer were performed as above, but with reaction times of 2 h, 1 h, and 15 min, respectively.

Fractionations. Of the above 979 mg syrup 871 mg was added to the top of a cellulose column (Whatman standard grade, 3.5 × 40 cm) and eluted with solvent A. Fractions of 10 ml were collected, examined by paper chromatography in solvent A and B, similar fractions were combined, concentrated and dried in a vacuum over phosphorus pentoxide. Fractions 16−33 (178.3 mg) gave three spots with R_F 0.81, 0.70 and 0.63 in solvent A, the R_F values in solvent B were 0.68, 0.56 and 0.49. The intermediate spots predominated in both solvents. The M_G values on paper electrophoresis in germanate buffer at pH 10.7 were 0.0 (major spot, probably representing disubstituted glucose substituted at C-2), 0.4 and 1.3. The M_G values in borate buffer were 0.00, 0.08 (major spot), 0.13, 0.17, and 0.48. Fractions 34−41 (42.1 mg) on paper chromatography in solvent A had R_F 0.63 and in solvent B 0.49, the M_G value on paper electrophoresis in germanate buffer at pH 10.7 was 0.4 and that in borate buffer was 0.17. This fraction crystallised; its further characterisation is described below. Fractions 42−51 (23.6 mg) consisted of 3-*O*-(2-cyanoethyl)-D-glucose [3] with traces only of the other mono-*O*-(2-cyanoethyl) ethers. (Paper electrophoresis in germanate). Fractions 52−62 (131.1 mg) consisted of a mixture of 2-, 4-, and 6-*O*-(2-cyanoethyl)-D-glucose.[3] Only a trace of the 3-isomer was present, detectable on paper electrophoresis, but present in too small an amount to be detected on the subsequent quantitative paper electrophoresis. Fractions 63−86 (125.7 mg) contained 6-*O*-(2-cyanoethyl)-D-glucose [3] only. After elution with a further 2 l of solvent A, D-glucose (330.3 mg) was obtained. The mixtures obtained from the other cyanoethylations were similarly resolved, 3-*O*-(2-cyanoethyl)-D-glucose was however not quantitatively separated and was therefore combined with the mixed mono-*O*-(2-cyanoethyl)-D-glucose fraction. The overall yields for the cyanoethylations, hydrolyses and fractionations were of the same order as that described above (82−90 %).

The amounts of aldose in each of the combined fractions were in the case of the completely water soluble fractions (mono-*O*-(2-cyanoethyl) ethers and glucose) determined by hypoiodite oxidation [12] and although the absolute values were up to 5 % lower than the weighed amounts the relative values were unchanged. Hypoiodite oxidation [12] of weighed amount of 3-*O*- and 6-*O*-(2-cyanoethyl)-D-glucose gave iodine consumptions corresponding to yields of 103 and 99 % aldose, respectively.

The relative amounts of each of 2-, 3-, 4-, and 6-*O*-(2-cyanoethyl)-D-glucose obtained in the mixed monocyanoethyl ether fraction from the various cyanoethylations were determined by quantitative paper electrophoresis in germanate buffer. The papers were previously eluted with water, and the hypoiodite consumed by a water eluted 6 × 9 cm paper strip corresponded to less than 0.1 ml 0.02 M sodium thiosulphate. The papers were cut while still wet and the amounts of aldose were after elution by water from the paper strips determined by hypoiodite oxidation. The eluate from a part of the paper containing

no carbohydrate, of a similar area to the other paper strips, was used as a blank. The total recovery of aldose from the papers was generally of the order of 85—90 % when corrected for the amounts removed in the guide strips. The procedure was checked with known amounts of 3-*O*- and 6-*O*-(2-cyanoethyl)-D-glucose and was found to give complete separation (paper chromatography on the eluates) and the relative proportions were within ± 2 % of those expected. The figures in Table 1 are calculated from the average of four determinations by paper electrophoresis, the variation of the relative amounts of each of the four isomers being ± 3 % when calculating the percentage of each of the isomers in the monocyanoethyl fraction. The order of the amount of each of the isomers was always the same. The M_G and R_F values of the various monocyanoethyl ethers obtained in the fraction ation are given in Table 2.

Table 2. M_G and R_F values of mono-2-cyanoethyl ethers of glucose.

Compound	R_F, solvent A	R_F, solvent B	M_G, germanate buffer pH 10.0
2-*O*-(2-cyanoethyl)-D-glucose	0.42	0.41	0.0
3-*O*-(2-cyanoethyl)-D-glucose	0.45	0.43	1.7
4-*O*-(2-cyanoethyl)-D-glucose	approx. 0.4	approx. 0.4	0.3
6-*O*-(2-cyanoethyl)-D-glucose	0.33	0.34	0.8

Characterisation of the components

D-*Glucose*. The sugar had m.p. 144—146° undepressed on admixture with authentic α-D-glucose, $[\alpha]_D^{20}$ + 52° (*c*, 0.6, in water, equilibrium value).

2-O-*(2-Cyanoethyl)*-D-*glucose*. The ether was obtained in 42 % yield (based on the amount originally present) by fractional crystallisation from the mixed fraction of mono-*O*-(2-cyanoethyl) ethers after samples had been withdrawn for quantitative paper electrophoresis, m.p. 177—180° undepressed on admixture with an authentic specimen,[3] $[\alpha]_D^{20}$ + 55° (*c*, 0.3, in water, equilibrium value).

3-O-*(2-Cyanoethyl)*-D-*glucose*. The ether had m.p. 140—143° undepressed on admixture with an authentic specimen,[3] $[\alpha]_D^{20}$ + 47° (*c*, 0.1 in water, equilibrium value).

4-O-*(2-Cyanoethyl)*-D-*glucose*. The ether was not characterised beyond paper electrophoresis and paper chromatography.

6-O-*(2-Cyanoethyl)*-D-*glucose*. The ether had m.p. 127—129° (transition at 121—123°) undepressed on admixture with an authentic specimen,[3] $[\alpha]_D^{20}$ + 47° (*c*, 0.4 in water, equilibrium value).

A di-O-*(2-cyanoethyl)*-D-*glucose*. The above mentioned crystalline di-*O*-(2-cyanoethyl) ether after recrystallisations from methyl ethyl ketone had m.p. 129—131.5°, $[\alpha]_D^{20}$ + 81 (6 min) → + 58° (72 h, *c*, 0.2, in 95 % aqueous ethanol, equilibrium value). (Found: C 50.2; H 6.58; N 9.88; O 33.5. Calc. for $C_{12}H_{18}N_2O_6$: C 50.3; H 6.34; N 9.79; O 33.5). The mobility of the ether on paper electrophoresis (see above) suggests that the ether is the 4,6-isomer.

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REFERENCES

1. Carter, M. E. *J. Polymer Sci.* **51** (1961) 199.
2. Danilov, S. N. and Lopatenok, A. A. *Zh. Obshch. Khim.* **28** (1958) 3184.
3. Garegg, P. J., Kubo, J. and Lindberg, B. *Acta Chem. Scand.* **17** (1963) 1761.
4. de Belder, A. N., Garegg, P. J., Lindberg, B., Petropavlovskii, G. and Theander, O. *Acta Chem. Scand.* **16** (1962) 623.
5. Bruson, H. A. *Org. Reactions* **5** (1949) 79.
6. de Belder, A. N., Lindberg, B. and Theander, O. *Acta Chem. Scand.* **16** (1962) 2005.
7. Croon, I. *Svensk Papperstid.* **63** (1960) 247.
8. Compton, J. in Whistler, R. L., Green, J. W., BeMiller, J. N. and Wolfrom, M. L. *Methods in Carbohydrate Chemistry, III, Cellulose.* Academic Press, New York and London 1963, p. 317.
9. Croon, I. and Lindberg, B. *Svensk Papperstid.* **59** (1956) 794.
10. Wickberg, B. *Acta Chem. Scand.* **12** (1958) 615.
11. Lindberg, B. and Swan, B. *Acta Chem. Scand.* **14** (1960) 1043.
12. Hirst, E. L., Hough, L. and Jones, J. K. N. *J. Chem. Soc.* **1949** 928.

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