Carbohydrate Research, 128 (1984) 73–78
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

HYDRAZINOLYSIS OF MONOSACCHARIDES: A TWO-STEP SYNTHESIS OF CHIRAL PENTANE-1,2,3-TRIOLS FROM PENTOSES AND OBSERVATIONS ON THE HYDRAZINOLYSIS OF GLYCOPROTEINS AND GLYCOPEPTIDES*

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(Received October 6th, 1983; accepted for publication, October 19th, 1983)

ABSTRACT

1,2-Dideoxyalditols, the corresponding 1-alkenes, and 1-deoxyalditols are formed in various proportions from D-glucose, D-mannose, L-arabinose, and D-xylose by the action of refluxing hydrazine. Sequential hydrazinolysis, catalytic hydrogenation, and chromatography afford a route to 1,2-dideoxyalditols. For example, 1,2-dideoxy-L-*erythro*-pentitol is formed from L-arabinose in 42% yield, and D-xylose is a source of 1,2-dideoxy-D-*threo*-pentitol (50%). Under the conditions (anhydrous hydrazine at 100° for 30 h in the absence of air) used by Montreuil for the hydrazinolysis of glycoproteins and glycopeptides, no 1,2-dideoxyalditol was formed; degradation was incomplete, there being some aldose hydrazone present. Under Kochetkov's hydrazinolysis conditions (105° for 10 h with hydrazinium sulphate), less degradation occurred and the product from D-galactose was identified as 1-deoxy-D-tagatose hydrazone.

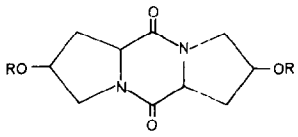
INTRODUCTION

Hydrazinolysis is used to isolate the carbohydrate moieties of certain glycopeptides and glycoproteins. For asparagine-bound oligosaccharides, the reaction cleaves the amide linkage between 2-acetamido-2-deoxy-D-glucose residues and asparagine[1]. For glycoproteins containing oligosaccharides linked to hydroxyproline, glycopeptides of type **1** can be formed as a result of cyclisation of neighbouring hydroxyproline residues[2].

Although Montreuil reported[3] in 1975 that oligosaccharides linked to serine and threonine were not released by hydrazinolysis, the lability to hydrazinolysis of the galactosylserine linkage in the glycoprotein extensin had been described in 1973 by Lamport *et al.*[4], who showed that the glycosylated serine residues were destroyed. Although hydrazine is not a strong base, it is possible, in principle, that β-

*Saccharide Hydrazones, Part 3. For Part 2, see ref. 9.

0008-6215/84/$ 03.00



1 R = oligosaccharide

elimination can occur under the conditions of hydrazinolysis. In a recent investigation of glycoproteins of plant cell-walls, aqueous hydrazine was preferred to aqueous alkali for the cleavage of glycosyl-serine (or -threonine) linkages[5]. Recent work in this laboratory has shown that hydrazinolysis can be used to release the oligosaccharide units of the glycopolypeptides derived from mucus[6]. The terminal reducing monosaccharides in the oligosaccharides released in such reactions would be converted into the hydrazone derivatives, and the fate of these hydrazones is of interest. Jones *et al.*[7] showed that treatment of D-galactose with boiling hydrazine for 48 h gave a mixture of 1-deoxy-D-galactitol, 1,2-dideoxy-D-*lyxo*-hexitol, and 1,2-dideoxy-D-*lyxo*-1-hexenitol. A further study of this reaction is now reported, its synthetic potential is examined, and its significance in the hydrazinolysis of glycopeptides and glycoproteins is discussed.

RESULTS

The results reported by Jones *et al.*[7] for the hydrazinolysis of D-galactose were confirmed and the reaction was applied to D-glucose, D-mannose, D-xylose, and L-arabinose The three major products, namely, the 1-deoxyalditol, 1,2-dideoxyalditol, and terminal alkene, were readily identified by ^{1}H- and ^{13}C-n.m.r. spectroscopy and their ratios were determined from the ^{1}H-n.m.r. spectra (see Table I). The proportion of alkene varied from ~0% for D-glucose to ~33% for D-galactose. Catalytic hydrogenation of the products over palladium gave mainly the 1-deoxyalditol and 1,2-dideoxyalditol, and the two-step sequence constitutes a useful short route to the latter compounds, the former being readily accessible by other routes.

The conditions of the reaction were varied in an attempt to optimise the yield of 1,2-dideoxyalditol (after hydrogenation) from D-galactose. Hydrazinolysis with anhydrous hydrazine in the presence of hydrazinium sulphate, with hydrazine hydrate, and with aqueous 82% hydrazine gave no alkene. In most of the reactions in anhydrous hydrazine, a small proportion (<10% with respect to the major component) of what appeared to be a second 1-deoxyalditol was present. In experiments using the aforementioned conditions, this minor product was present in larger amount; thus, the products were less useful from a synthesis point of view. Addition of sodium hydroxide also increased the relative amount of the minor product and decreased the amount of alkene.

Decreasing the duration of the hydrazinolysis was also investigated. A 16-h reaction gave less alkene, and the product gave several signals in the hydrazone re-

TABLE I

N.M.R. DATA FOR PRODUCTS OBTAINED FROM ALDOSES IN REFLUXING HYDRAZINE

Aldose	*Partial n.m.r. data (δ)*						*Product ratios*
	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C	
	1-Deoxyalditol[a]		*1,2-Dideoxyalditol*[b]		*Alkene*[c]		
D-Galactose	1.24	21.4	0.96	11.8	6.0 5.3	120.7 139.6	3:1:2
D-Glucose	1.20	21.0	0.94	12.3			1:1:0
D-Mannose	1.26	21.7	0.94	12.3	5.9 5.3	119.2 140.3	3:1:0.3
D-Xylose	1.20	21.1	0.94	12.1	5.9 5.3	120.0 139.3	2:1:1.1
L-Arabinose	1.22	21.3	0.95	12.0	6.27[d] 5.52 5.19	120.6 138.9	3:1:1

[a]Data for CH_3; ^1H signal was a doublet ($J_{1,2}$ 6 Hz). [b]Data for CH_3; ^1H signal was a triplet (J 7 Hz); also δ_H 1.5–1.6 (m) and δ_C 27.6–28.4 for CH_2. [c]See text for description; H-1 signals were partially obscured by solvent (HOD); the H-2 signal, at low field, was an octet. [d]Data obtained from measurements at 70° for a solution in pyridine-d_5 after D_2O exchange. Similar results were obtained for the product from D-xylose.

gions of the n.m.r. spectra. The products from 8- and 4-h reactions gave stronger (complex) hydrazone signals. The optimum reaction time for deoxygenation at both C-1 and C-2 was 36–48 h.

Catalytic hydrogenation of the product of hydrazinolysis (48 h) of L-arabinose gave a mixture of the 1-deoxy- and 1,2-dideoxy-pentitols in a ratio of ~1.4:1. The minor component, tentatively identified as a 1-deoxypentitol, was present to the extent of 10% of the amount of the major product. The two major products were partially separated by fractional recrystallisation and sublimation. Pure, crystalline 1,2-dideoxy-L-*erythro*-pentitol*, isolated in 17% yield, gave a mass spectrum that was consistent with its structure, the base peak at *m/z* 59 being due to $[EtCHOH]^+$; ions at *m/z* 102, 91, and 89 were due to loss of H_2O, Et, and CH_2OH, respectively, from the molecular ion. Pure 1-deoxy-L-arabinitol was isolated in 38% yield. These compounds were well separated by liquid chromatography (l.c.), and preparative l.c. would clearly be the best method for their isolation.

Similar catalytic hydrogenation of the hydrazinolysis product from D-xylose gave equal amounts of the 1-deoxy- and 1,2-dideoxy-pentitols with ~5% of a minor product.

The hydrazinolysis of D-galactose in a sealed tube in the absence of air at 100° for 30 h gave 1-deoxy-D-galactitol (40%), D-galactose hydrazone (36%), and 1,2-

*The enantiomer of this compound having a lower m.p. was previously prepared from 2-deoxy-D-*erythro*-pentose[8].

dideoxy-D-*lyxo*-1-hexenitol (24%). A 15-h reaction gave the same products in the proportions 24, 64, and 12%, respectively. Hydrazinolysis in the presence of hydrazinium sulphate at 105° for 10 h in a sealed tube in the absence of air resulted in less degradation; only a trace of 1-deoxy-D-galactitol was formed, 1,2-dideoxy-D-*lyxo*-1-hexenitol was absent, and ~24% of a compound identified as 1-deoxy-D-tagatose hydrazone by n.m.r. data [CH_3 at δ_H 1.84(s), δ_C 12.5; C=N at δ_C 157.8] was present. This compound was formed[6] in the hydrazinolysis of 2-amino-2-deoxy-D-galactose hydrochloride, in a reaction that parallels the formation of 1-deoxy-D-fructose hydrazone from 2-amino-2-deoxy-D-glucose hydrochloride[9]. Traces of 1-deoxy-D-tagatose hydrazone could be detected by n.m.r. spectroscopy in the products of the hydrazinolysis of D-galactose in refluxing hydrazine; similarly, traces of 1-deoxy-D-fructose hydrazone were detected in the products from D-glucose and D-mannose.

DISCUSSION

The 1-deoxyalditols formed in the hydrazinolysis reactions result from a Wolff–Kishner reduction of the aldose hydrazones. It has been proposed that the donation of a proton from a hydroxylic component to the hydrazone anion is involved in the rate-determining step[10]. For aldose hydrazones, there is the possibility of an intramolecular proton transfer from HO-3 *via* a five-membered ring transition state. This may account for the occurrence of the reaction under relatively mild conditions in the absence of strong base.

Elimination of a substituent adjacent to the hydrazone group to form an alkene is a known side-reaction of the Wolff–Kishner reduction[11]. Such elimination is not favoured when the adjacent substituent is an amino group, and the thermal decomposition of 2-amino-2-deoxy-D-glucose hydrazone follows a different pathway to give 1-deoxy-D-fructose hydrazone[9]. The 1,2-dideoxyalditols result from the reduction of the alkene by diimide, which is produced by atmospheric oxidation of hydrazine. In the absence of air, no reduction of the alkene occurs. Sequential hydrazinolysis and hydrogenation thus affords a short synthetic route to 1,2-dideoxyalditols when preparative l.c. is used to separate the two major products.

The thermal decomposition of aldose hydrazones in hydrazine is particularly relevant to the hydrazinolysis of glycoproteins and glycopeptides because degradation of any released reducing saccharides is undesirable. The shortest reaction time and lowest temperature should be used to minimise the Wolff–Kishner reduction and elimination. Hydrazinolysis in the presence of hydrazinium sulphate at 105° for 10 h, the conditions of Yosizawa *et al.*[1] as modified by Kochetkov *et al.*[12], caused much less degradation than reaction at 100° for 30 h in the absence of hydrazinium sulphate[13] and yielded a different degradation product, namely, 1-deoxy-D-tagatose hydrazone. Presumably, the formation of the anion of D-galactose hydrazone is inhibited in the presence of hydrazinium sulphate, and the Wolff–Kishner reaction is thus prevented.

Hydrazinolysis is most commonly applied to glycoproteins or glycopeptides containing asparagine-bound carbohydrate[14]. Although 2-acetamido-2-deoxy-D-glucose is usually the monosaccharide attached to asparagine, a glycopeptide isolated from rat glomerular-basement membrane has been reported to contain D-glucose linked to asparagine (or glutamine)[15,16]. Hydrazinolysis of such compounds would cause some degradation of the D-glucose hydrazone derivative formed unless sufficiently mild conditions were used. A knowledge of the side reactions that can occur in the hydrazinolysis of glycoproteins and glycopeptides is necessary in order to interpret the results of experiments carried out on the hydrazinolysis products.

EXPERIMENTAL*

Hydrazinolysis (reflux conditions). — A 5% solution of the aldose in anhydrous hydrazine was refluxed with the exclusion of moisture for 48 h (or under other conditions as specified in the Discussion). The hydrazine was removed *in vacuo* over conc. H_2SO_4, and the residue was analysed by ^1H- and ^{13}C-n.m.r. spectroscopy. Signals for the alkene products were absent after catalytic hydrogenation, which was carried out at atmospheric pressure with methanolic or aqueous methanolic solutions over 10% Pd/C. The 1,2-dideoxyalditol formed from D-glucose and D-mannose corresponded (n.m.r. data) to that formed in the hydrazinolysis of 2-deoxy-D-*arabino*-hexose.

Hydrazinolysis of L-*arabinose (preparative experiment).* — A solution of L-arabinose (1 g) in anhydrous hydrazine (10 mL) was boiled under reflux for 48 h. After removal of hydrazine, the product (0.958 g) solidified on standing. An aliquot was removed for n.m.r. analysis, and the remainder (0.752 g) was hydrogenated in methanol–water (5:1, 30 mL) over 10% Pd/C (0.1 g). Removal of catalyst and solvent gave a syrupy residue (0.703 g) which solidified on standing. A one-seventh aliquot was removed for n.m.r. and l.c. analysis. The latter was effected by using a Whatman 10 PAC column (25 cm), elution with aqueous 95% acetonitrile at 0.8 mL/min, and a refractive index detector. The dideoxy- and monodeoxy-pentitols had retention times of 7.2 and 11.0 min, respectively. Recrystallisation of the crude product from methanol–ether gave large prisms of 1-deoxy-L-arabinitol (0.135 g), m.p. 132–134°; lit.[17] m.p. 129–131°. ^1H-N.m.r. data (CD_3OD): δ 3.98 (dq, 1 H, J 2.5 and 6.5 Hz, H-2), 3.67 (m, 3 H), 3.30 (m, 1 H), and 1.21 (d, 3 H, J 6.5 Hz, H-1). Sublimation at 60–70°/0.1 mmHg of the residue (0.488 g) obtained from the mother liquor gave 1,2-dideoxy-L-*erythro*-pentitol (91 mg, 17%), m.p. 64.5–65.5°, $[\alpha]_D^{20}$ +6.2° (*c* 0.6, water); lit.[8] m.p. 39° for the enantiomer. ^1H-N.m.r. data (CD_3OD): δ 3.2–3.8 (m, 4 H), 1.50 (m, 2 H), 0.97 (t, 3 H, J 7 Hz). Mass spectrum: *m/z* 59 (100%), 44 (97), 31 (55), 28 (33.8), 43 (17.0), 61 (25.8), 41 (25.2), 45 (24.6), 89 (9.4), 91 (3.3), and 102 (0.4).

*See ref. 9 for general methods.

Anal. Calc. for $C_5H_{12}O_3$: C, 50.0; H, 10.1. Found: C, 50.1; H, 10.25.

A second sublimate (123 mg) was obtained which was shown to be impure dideoxypentitol. The sublimation residue, on recrystallisation from methanol, gave more (98 mg) 1-deoxy-L-arabinitol (total yield, 38%).

Hydrazinolysis of D-*xylose.* — D-Xylose (1 g) was subjected to hydrazinolysis and hydrogenation as in the preceding experiment. L.c. analysis gave two peaks at 7.4 and 11.3 min under the conditions described above. Fractional distillation of the oily product in a Kugelrohr apparatus at 0.01 mmHg did not give the main products in pure form.

Hydrazinolysis in the absence of air. — Hydrazinolyses according to Bayard and Montreuil[13] and to Kochetkov *et al.*[12] were carried out as described in ref. 9. The proportions of the products were calculated from the ^{1}H-n.m.r. spectra, with the assumption that the ratio of acyclic–cyclic tautomers of D-galactose hydrazone was the same (5:1) as that found for a hydrazine solution[18] because the signals for the cyclic tautomer could not be readily integrated. The hydrazone tautomerises slowly in D_2O solution[17]. The product from the Kochetkov hydrazinolysis experiment contained, in addition to D-galactose hydrazone [δ_H 7.41 (d, J 5 Hz); δ_C 151.7, 94.4], a new compound, namely, 1-deoxy-D-tagatose hydrazone [δ_H 1.85 (s), δ_C 12.5, 157.8], in the ratio of 3.15:1, together with traces of 1-deoxy-D-galactitol and other compounds. The above chemical shifts are identical to those of 1-deoxy-D-tagatose hydrazone, which is the major degradation product formed in the hydrazinolysis of 2-amino-2-deoxy-D-galactose hydrochloride[6].

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