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SPONTANEOUS REACTIONS OF THE IRREVERSIBLE β-D-GALACTOSIDASE INHIBITOR 2,6-ANHYDRO-1-DEOXY-1-DIAZO-D-*glycero*-L-*manno*-HEPTITOL*

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ABSTRACT

2,6-Anhydro-1-deoxy-1-diazo-D-*glycero*-L-*manno*-heptitol (**2**) decomposes in 0.01M methanolic sodium methoxide with a half-life of approx. 18 min. Decomposition in aqueous solution is too rapid for spectrophotometric measurement. Seven products could be identified in methanolic and aqueous reaction mixtures. 2,6-Anhydro-1-deoxy-D-*galacto*-hept-1-enitol (**6**), 2,7-anhydro-1-deoxy-β-D-*galacto*-hept-ulopyranose (**10**), and 4-*O*-vinyl-D-lyxose (**12**) are products of rapid intramolecular reactions. The major portion consists of the direct solvolysis products 2,6-anhydro-1-*O*-methyl-D-*glycero*-L-*manno*-heptitol (**3**) and 2,6-anhydro-D-*glycero*-L-*manno*-heptitol (**5**).

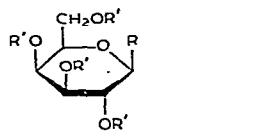
INTRODUCTION

2,6-Anhydro-1-deoxy-1-diazo-D-*glycero*-L-*manno*-heptitol (**2**) was shown[1] to be a "suicide substrate" for β-galactosidase from *Escherichia coli* (EC 3.2.1.23). It specifically blocks the active site of the enzyme. Diazo derivatives of carbohydrates have been prepared before[2–4], yet never used as highly reactive blocking agents for enzymes. For this purpose, the diazo compound has to be water soluble, which implies unsubstituted, free hydroxyl groups. The lability of diazo compounds is well known, and it is not surprising that **2** undergoes spontaneous decomposition and secondary reactions when generated in protic solvents. The present paper describes an investigation of the stability of **2** and of the secondary reactions, as deduced from the end-products of the spontaneous decomposition of **2** in methanol and water.

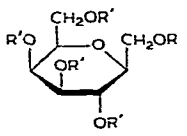
*Dedicated to Professor K. Heyns on the occasion of his 70th birthday.

RESULTS AND DISCUSSION

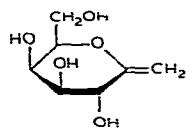
Compound **2** was formed almost instantaneously (<2 min)* when 3,4,5,7-tetra-*O*-acetyl-2,6-anhydro-1-deoxy-1-nitrosoacetamido-D-*glycero*-L-*manno*-heptitol (**1**) in methanol was treated with sodium methoxide. The transformation could be measured spectrophotometrically, as **2** in M sodium methoxide** and **1** in methanol differ significantly in their electron spectra (Fig. 1). The rate of decomposition of **2**



1 R = $CH_2N(NO)Ac$, R' = Ac
2 R = CHN_2, R' = H



3 R = Me, R' = H
4 R = Me, R' = Ac
5 R = R' = H



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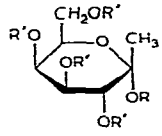
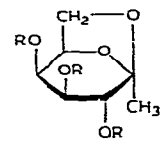
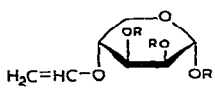


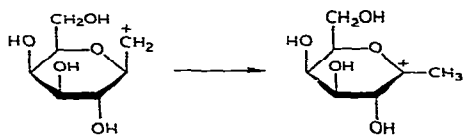
7 R = Me, R' = H
8 R = Me, R' = Ac
9 R = R' = H



10 R = H
11 R = Ac



12 R = H
13 R = Ac



14 → 3,5,6,12
15 → 7,9,10

could be best followed in a spectrophotometer at 435 nm, the maximum absorbance of **2**. In diluted methanolic sodium methoxide solution, the rate is clearly first-order and the half-life under these conditions is 18 min. Addition of water or acetic acid*** accelerated the decomposition to such an extent that its rate could no longer be measured spectrometrically. The decomposition was also indicated by a lively evolution of nitrogen and disappearance of the yellow color. In protic solvents, the initial step is protonation of C-1. Expulsion of nitrogen follows, and the carbenium ion **14** can undergo several secondary reactions. T.l.c. of the mixture after complete decomposition of **2** revealed at least six compounds. By g.l.c., up to 10 peaks could be

*A sample injected into an excess of aqueous acetic acid showed as a major product (t.l.c.) 2,6-anhydro-D-*glycero*-L-*manno*-heptitol (**5**). Alkaline treatment of the evaporated mixture did not change the t.l.c. pattern, indicating the absence of partially acetylated products in the original mixture.

In M methanolic sodium methoxide, **2 is quite stable ($t_{0.5} > 1$ h).

***Attempts to use **2** as an esterifying reagent for carboxylic acids did not give the corresponding ester in reasonable yields.

detected (see Table I).* The four major products(**3**, **7**, **10**, and **12**) could be separated

TABLE I

PERCENTAGE OF DECOMPOSITION PRODUCTS OF **2** AS MEASURED BY G.L.C.[a]

Products	*Relative amount* (%) *in*		
	Sodium methoxide		M *Aqueous NaOH*
	0.1M	M	
2,6-Anhydro-1-*O*-methyl-D-*glycero*-L-*manno*-heptitol (**3**)	54	60	
2,6-Anhydro-D-*glycero*-L-*manno*-heptitol (**5**)	3	5	42
2,6-Anhydro-1-deoxy-D-*galacto*-hept-1-enitol (**6**)	2	3	1
Methyl 1-deoxy-α-D-*galacto*-heptulopyranoside (**7**)	14	13	
1-Deoxy-D-*galacto*-heptulose (**9**)			1
2,7-Anhydro-1-deoxy-β-D-*galacto*-heptulopyranose (**10**)	12	13	21
4-*O*-Vinyl-D-lyxose (**12**)	12	<1	19
Not identified	3	6	16

[a]Compounds **5** (ref. 5), **6** (ref. 6), and **9** (ref. 6) were identified by g.l.c. comparison with an authentic sample after trimethylsilylation.

on a silica gel column and were acetylated prior to n.m.r. analysis. Although the products, except the solvolysis products **3** and **7**, were the same for each solvent, they differed significantly in relative amounts. It may be expected that the intermediate primary carbenium ion **14** is stabilized by hydride shift to give the tertiary carboxonium ion **15**. This reacts by intramolecular attack to yield the anhydro compound **10** or by intermolecular reaction to give, in water, 1-deoxy-D-*galacto*-heptulose (**9**) or, in methanol, methyl 1-deoxy-α-D-*galacto*-heptulopyranoside (**7**). The latter reaction is probably stereospecific because of the anomeric effect. Evidence for a methyl group in the equatorial orientation in **7** was given by the proton-undecoupled ^{13}C-n.m.r. spectrum of **8**; in addition to a one-bond C–H coupling at 127(±1) Hz, the signal for C-1 only showed long-range couplings of less than 2 Hz. For a methyl group in the axial orientation, an axial–axial C–H coupling with H-3 of at least 4 Hz would be expected[8]. 4-*O*-Vinyl-D-lyxose (**12**) was formed by bond fission between C-2 and C-3 in the carbenium ion **14**. Comparable reactions of free carbenium ions are known in the chemistry of aliphatic hydrocarbons[9]. The shortest way of stabilizing the carbenium ion by loss of a proton was only favored to a minor extent as the very small yield of 2,6-anhydro-1-deoxy-D-*galacto*-hept-1-enitol (**6**) indicates. The high velocity

*There may be also oligomeric material formed which does not pass the g.l.c. column.

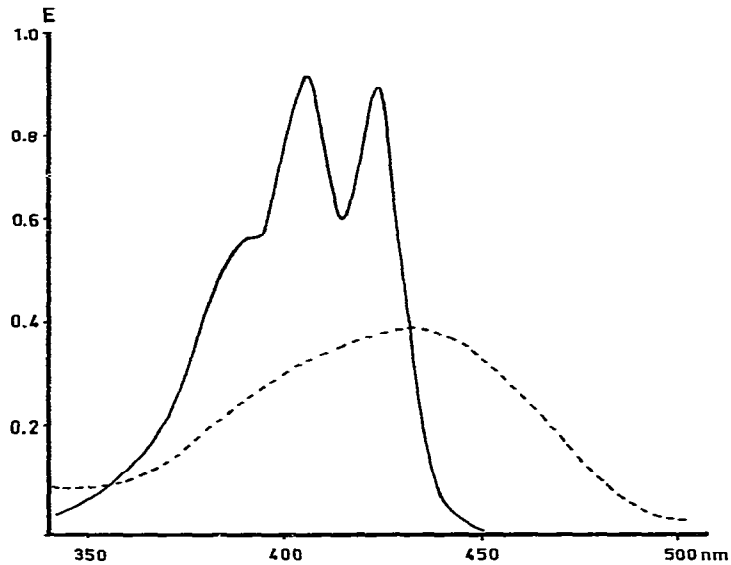


Fig. 1. Electron spectra of 0.01M 3,4,5,7-tetra-*O*-acetyl-2,6-anhydro-1-deoxy-1-nitrosoacetamido-D-*glycero*-L-*manno*-heptitol (**1**) in methanol (———) and 0.05M 2,6-anhydro-1-deoxy-1-diazo-D-*glycero*-L-*manno*-heptitol (**2**) in M methanolic sodium methoxide solution (- - -).

of the spontaneous reactions described so far is also underlined by the fact that the expected dianhydroheptitols, formed by intramolecular attack of the hydroxyl groups in the carbenium ion **14**, could not be isolated and identified. If present, they would amount to less than 3% of the total products formed in 0.1M methanolic sodium methoxide. Since only the unfavored $^{1}C_{4}$ or $^{2,5}B$ conformers could yield dianhydroheptitols, the generation of these conformers from the equilibrium mixture must have been slow in comparison with the rearrangement and solvolysis reactions of the carbenium ion **14**.

EXPERIMENTAL

General methods. — T.l.c. was performed on Silica gel F_{254} (Merck, Germany) with ethyl acetate–propane-2-ol–water (25:14:7, v/v). Detection was effected by charring with sulphuric acid. G.l.c. was performed with a Pye Unicam GCD chromatograph, operated with a temperature-gradient program of 170–250°, glass columns containing 3% of SE 52 on Chromosorb G AW-DMCS, and an FID detector. Distillation was carried out in half-micro scale in a "Kugelrohr" apparatus (Büchi, Switzerland). ^{1}H-N.m.r. data at 60 and 100 MHz (with tetramethylsilane as the internal standard) were recorded with Varian spectrometers A-60 D and HA-100 D. ^{13}C-N.m.r. spectra at 25.2 MHz were recorded in the Fourier mode with a Varian spectrometer XL 100/15. Electron spectra were obtained with a Cary Recording Spectrophotometer model 14 M-50.

Decomposition of 2,6-anhydro-1-deoxy-1-diazo-D-glycero-L-manno-*heptitol* (**2**). — 3,4,5,7-Tetra-*O*-acetyl-2,6-anhydro-1-deoxy-1-nitrosoacetamido-D-*glycero*-L-*manno*-heptitol[1] (**1**, 5 g) was disssolved in 0.1M methanolic sodium methoxide (100 ml). Formation of **2** was immediate, and decomposition of **2** was complete when the yellow colour had disappeared. Samples (10 μl) were taken, dried, and trimethylsilylated[7] for g.l.c. analysis. The bulk reaction-mixture was evaporated and subjected to chromatography on silica gel (column dimension, 7 × 100 cm) with ethyl acetate–propane-2-ol–water (25:14:7, v/v) as eluent. The eluate was analyzed by t.l.c. and divided into four fractions. Each fraction was, after evaporation, acetylated with 1:1 (v/v) acetic anhydride–pyridine (5 ml) and after 12 h evaporated under reduced pressure.

1,2,3-Tri-O-*acetyl-4*-O-*vinyl*-α-D-*lyxopyranose* (**13**). — The acetylated first fraction was distilled at 160°(0.001 torr) to give 360 mg (10.3%) of an anomer mixture, which could be separated by p.l.c. with 4:1 (v:v) ether–light petroleum as solvent. The faster-moving anomer was redistilled to give 230 mg (6.6%) of syrupy **13**, $[\alpha]_{578}^{25}$ +20.7° (*c* 1.4, chloroform); ν_{max}^{KBr} 1750 (CO) and 1630 cm^{-1} (C=C). The structure of **13** was established by ^{13}C- and ^{1}H-n.m.r. spectroscopy: ^{13}C-n.m.r. (25.2 MHz, $CDCl_3$, multiplicity in off-resonance spectrum in parentheses): δ 20.72 and 20.85 (2 q, 3 CH_3), 62.26 (t, C-5), 68.54 and 69.36 (2 d, C-2 and C-3), 71.95 (d, C-4), 89.89 (t, C-7), 90.74 (d, C-1), 150.62 (d, C-6), 168.66, 169.60, and 169.80 (3 s, 3 C=O); ^{1}H-n.m.r. (100 MHz, $CDCl_3$): δ 2.08 (s, 3 H, OAc), 2.18 (s, 6 H, 2 OAc), 3.72 ($J_{4,5ax}$ 9 Hz, H-5_{ax}), 4.0 ($J_{4,5eq}$ 5 Hz, H-5_{eq}), 4.08 ($J_{6,7trans}$ 7 Hz, H-7_{trans}), 4.2 (H-4), 4.40 ($J_{6,7}$ 14 Hz, H-7_{cis}), ($J_{7,7}$ 2 Hz), 5.22 ($J_{2,3}$ 2.5 Hz, H-2), 5.35 ($J_{3,4}$ 8.5 Hz, H-3), 5.96 ($J_{1,2}$ 2.5 Hz, H-1), 6.30 (H-6). On deacetylation and acid hydrolysis, **13** yielded a free sugar which, after trimethylsilylation, had the same chromatographic properties (g.l.c.) as an authentic sample of D-lyxose.

3,4,5-Tri-O-*acetyl-2,6-anhydro-1-deoxy*-β-D-galacto-*heptulopyranose* (**11**). — The acetylated second fraction was treated with 5% acetic acid in methanol (20°, 30 min) to hydrolyze contaminating **13**. After evaporation, the product was distilled at 170° (0.001 torr) to yield 220 mg (6.3%), $[\alpha]_{578}^{25}$ −16° (*c* 1.0, chloroform); n.m.r. (60 MHz, $CDCl_3$): δ 1.40 (s, 3 H, CH_3), 2.00 (s, 3 H, OAc), 2.12 (s, 6 H, 2 OAc), 3.6–4.5 (m, 3 H, H-6 and H-7), and 4.8–5.3 (m, 3 H, H-3, H-4, and H-5).

Anal. Calc. for $C_{13}H_{18}O_8$: C, 51.65; H, 6.00. Found: C, 51.43; H, 6.25.

Methyl 3,4,5,7-tetra-O-*acetyl-2,6-anhydro-1-deoxy*-α-D-galacto-*heptuloside* (**8**). — The acetylated third fraction was dissolved in chloroform (50 ml), washed with water (3 × 30 ml), and dried ($MgSO_4$). After evaporation, the residue was crystallized from water to give 305 mg (7.0%), m.p. 160–161°, $[\alpha]_{578}^{25}$ +103° (*c* 1.0, chloroform); ^{1}H-n.m.r. (60 MHz, $CDCl_3$): δ 1.35 (s, 3 H, CH_3), 1.93, 2.02, 2.07 and 2.12 (4 s, each 3 H, 4 OAc), 3.27 (s, 3 H, OCH_3), 4.1 (s, 3 H, H-6 and H-7), and 5.2–5.4 (m, 3 H, H-3, H-4, H-5); ^{13}C-n.m.r. (25.2 MHz, $CDCl_3$): δ 170.44, 170.04 (C=O), 99.98 (C-2), 71.21 (C-3), 68.72, 68.33, and 67.43 (C-4 C-5, and C-6), 61.87 (C-7), 20.82, 20.66 (C-methyl), and 19.71 (C-1).

Anal. Calc. for $C_{16}H_{24}O_{10}$: C, 51.06; H, 6.43. Found: C, 50.97; H, 6.61.

3,4,5,7-Tetra-O-acetyl-2,6-anhydro-1-O-methyl-D-glycero-L-manno-heptitol (**4**). — The acetylated fourth fraction was dissolved in chloroform (100 ml), washed with water (3 × 50 ml), and dried ($MgSO_4$). After evaporation, the residue (1.9 g, 43%) crystallized, m.p. 67° (from light petroleum), $[\alpha]_{578}^{25}$ +13.0° (*c* 1.0, chloroform); ^{1}H-n.m.r. (60 MHz, $CDCl_3$): δ 1.98 (s, 3 H, OAc), 2.05 (s, 6 H, 2 OAc), 2.15 (s, 3 H, OAc), 3.35 (s, 3 H, OCH_3), 3.50 (s, 2 H, H-1), 3.75–4.25 (m, 4 H, H-2, H-6 and H-7), and 5.05–5.4 (m, 3 H, H-3, H-4, and H-5).

Anal. Calc. for $C_{16}H_{24}O_{10}$: C, 51.06; H, 6.43. Found: C, 51.21; H, 6.67.

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REFERENCES

1 M. BROCKHAUS AND J. LEHMANN, *FEBS Lett.*, 62 (1976) 154–156.
2 J. FARKAŠ AND F. ŠORM, *Collect. Czech. Chem. Commun.*, 37 (1972) 2798–2803.
3 E. M. ACTON, A. N. FUJIWARA, L. GOODMAN, AND D. W. HENRY, *Carbohydr. Res.*, 33 (1974) 135–151.
4 M. S. ALEXANDER AND D. HORTON, *Abstr. Pap. Am. Chem. Soc. Meet.*, 174 (1977) CARB-012.
5 B. COXON AND H. G. FLETCHER, JR., *J. Am. Chem. Soc.*, 86 (1963) 922–926.
6 M. BROCKHAUS AND J. LEHMANN, *Carbohydr. Res.*, 53 (1977) 21–31.
7 C. C. SWEELEY, R. BENTLEY, M. MAKITA, AND W. W. WELLS, *J. Am. Chem. Soc.*, 85 (1963) 2497–2507.
8 K. BOCK AND C. PEDERSEN, *Acta Chem. Scand. Ser. B*, 31 (1977) 354–358.
9 J. T. KEATING AND P. S. SKELL, in G. A. OLAH AND P. v. R. SCHLEYER (Eds.), *Carbonium Ions*, Vol. 2, Wiley–Interscience, New York, 1970, pp. 599–601.