Carbohydrate Research, 217 (1991) 99–106
Elsevier Science Publishers B.V., Amsterdam

The stepwise degradation of a glycosylated aldose. A potential method for sequencing branched oligosaccharides*

Bernhard Jäger, Helga Lay, Jochen Lehmann†, and Lothar Ziser
Institut für Organische Chemie und Biochemie der Universität Freiburg i. Br., Albertstr. 21, D-7800 Freiburg i. Br. (Germany)

(Received October 30th, 1990; accepted for publication, December 24th, 1990)

ABSTRACT

2,3,6,2′,3′,4′,6′-Hepta-*O*-methylmaltose diethyl dithioacetal (**2**), obtained under mild conditions from *N*-methyl-hepta-O-methyl-*N*-*p*-nitrophenyl-β-maltosylamine (**1**), was converted into the disulfone **4**. Storage of a solution of **4** in aqueous tetrahydrofuran gave 1,2-dideoxy-1,1-bis(ethylsulfonyl)hepta-*O*-methylmalt-1-enitol (**5**). Fragmentation of **5** with aqueous ammonia released 2,3,4,6-tetra-*O*-methyl-D-glucose. This degradation procedure has potential for the sequencing of branched oligosaccharides.

INTRODUCTION

A satisfactory method for the stepwise chemical degradation of oligosaccharides, comparable to the well-established procedures for sequencing in peptide chemistry, is not yet known. Strictly selective reactions are possible only with the hemiacetal group of the reducing-end moiety of an oligosaccharide. Moreover, branching, which does not occur in peptides and oligonucleotides, presents problems in structural analyses of complex oligosaccharides. Although the structures of complex oligosaccharides can be determined by using g.l.c.–m.s. and high-resolution n.m.r. spectroscopy, a chemical method would be useful, especially for determining the positions of the side chains in branched oligosaccharides.

We have described[1] a procedure for the cleavage by an electrophilic neighbouring-group reaction of the glycosidic bond in an oligosaccharide adjacent to the reducing end. However, this degradation is not sufficiently selective to be generally applicable for the sequencing of complex carbohydrates[1]. The method now described, which has this potential, is based on the well-known MacDonald–Fischer sulfone degradation of monosaccharides[2].

Methylated, complex oligosaccharides can be isolated in pure form as their *N*-*p*-nitrophenylglycosylamines[3], which can be used in the determination of structure. Maltose has been used as a simple model compound in order to demonstrate the potential of the new technique.

* Dedicated to Professor Dr. Kurt Wallenfels on the occasion of his 80th birthday.
† Author for correspondence.

0008-6215/91/$03.50

RESULTS

The MacDonald–Fischer sulfone degradation has not been applied to oligosaccharides, because their dithioacetals, unlike the monosaccharide analogues, are not readily accessible. Wolfrom *et al.*[4] described the preparation of maltose diethyl dithioacetal and its crystalline octa-acetate and emphasised the difficulties involved. The standard procedure is not suitable for the preparation of the dithioacetals of higher oligosaccharides.

The fact that glycosylamines, unlike free pyranoses, undergo facile, proton-catalysed ring opening via immonium ions[5] led to their use for the easy preparation of oligosaccharide dithioacetals under mild conditions. The almost quantitative preparation of *N*-*p*-nitrophenylglycosylamines of complex oligosaccharides has been described[3]. These compounds, due to their light absorption and solubility properties, are amenable to h.p.l.c. as are the *O*- and *N*-methylated derivatives.

When crystalline *N*-methyl-hepta-*O*-methyl-*N*-*p*-nitrophenyl-β-maltosylamine (**1**), prepared from crystalline *N*-*p*-nitrophenyl-β-maltosylamine[6,7], was treated in dichloromethane at 0° with ethanethiol and a small amount of trifluoroacetic acid, the reaction proceeded smoothly as monitored by h.p.l.c. of the *N*-methyl-*p*-nitroaniline released (Fig. 1). Under these conditions, the glycosidic bond remained intact. 2,3,6,2′,3′,4′,6′-Hepta-*O*-methylmaltose diethyl dithioacetal (**2**) was isolated subsequently as a syrup and identified by ^{1}H-n.m.r.spectroscopy. The free hydroxyl group in **2** could be methylated under standard conditions to give octa-*O*-methylmaltose diethyl dithioacetal (**3**), which was oxidised readily by 3-chloroperoxybenzoic acid to yield crystalline 1-deoxy-1,1-bis(ethylsulfonyl)octa-*O*-methylmaltitol (**4**).

When dissolved in aqueous tetrahydrofuran, **4** slowly lost 1 mol of methanol by β-elimination to give **5** which, apparently, underwent an equilibrium reaction with water to yield some 1-deoxy-1,1-bis(ethylsulfonyl)-3,5,6,2′,3′,4′,6′-hepta-*O*-methylmaltitol (**6**) and its 2-epimer **7**. Only **5** and one of the hydrated compounds were isolated by

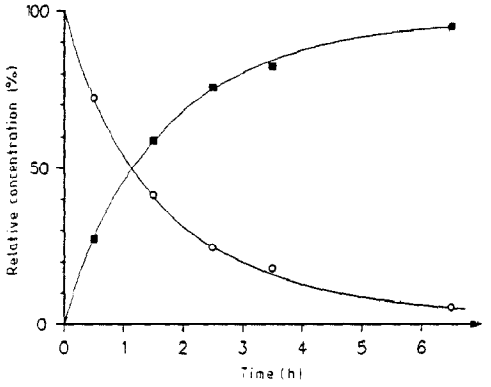
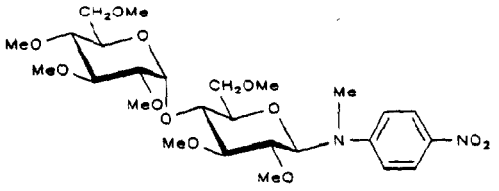
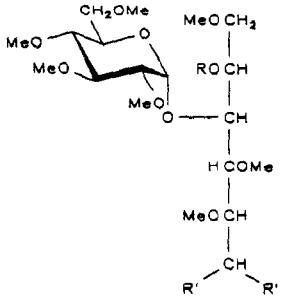


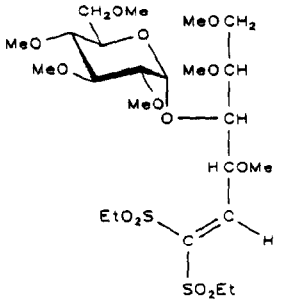
Fig. 1. Mercaptolysis of **1** (17.55 mg) in 70:15:6 CH_2Cl_2–ethanethiol–trifluoroacetic acid (273 μL). H.p.l.c. assay: column (250 × 8 mm) of ODS Hypersil (5 μm), MeCN–water (0–5 min, 0–10%; 5–11 min, 10–70% MeCN at 4.0 mL/min), 375 nm. Key: ○, **1** (*T* 14.4 min); ■, *N*-methyl-*p*-nitroaniline (*T* 13.6 min).

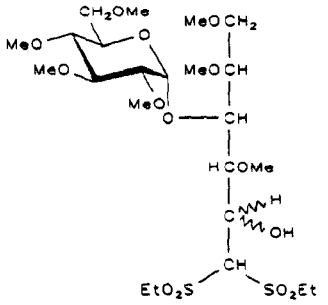
h.p.l.c. and identified. The formation (t.l.c.) of **5** proceeded slowly. The elimination of methanol may be assisted by simple protonation or acid–base catalysis in the aqueous solvent mixture. No elimination occurred when a solution of **4** in dry tetrahydrofuran was stored.



1







2 R = H R' = SEt

3 R = Me R' = SEt

4 R = Me R' = SO_2Et

5

6/7

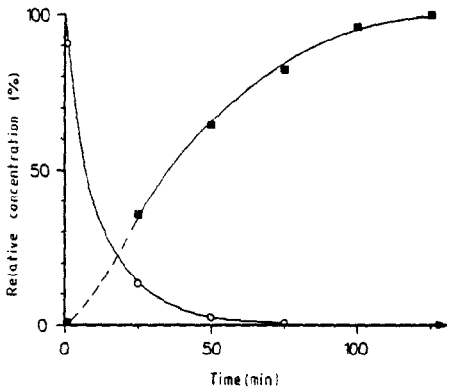
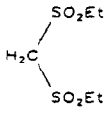


Fig. 2. Fragmentation of **4** (2.5 mg) in 2:2:1 tetrahydrofuran–water–aqueous 1% ammonia (2.5 mL). H.p.l.c. assay: column (250 × 4 mm) of Nucleosil C-18 (5 μm), MeCN–water (0–15 min, 50–65% MeCN at 0.75 mL/min), 220 nm. Key: ○, **5** (*T* 9.8 min); ■, **10** (*T* 5.1 min).

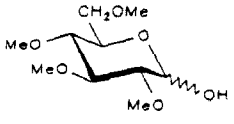
When **4**–**7** were each treated with dilute aqueous ammonia in tetrahydrofuran, a rapid reaction occurred and three new compounds were formed, isolated, and identified as bis(ethylsulfonyl)methane[2] (**8**), 2,3,4,6-tetra-*O*-methyl-D-glucose[8] (**9**), and 3-deoxy-2,4,5-tri-*O*-methyl-D-*glycero*-pent-2-enose (**10**). The formation of **10** could be monitored by t.l.c. or h.p.l.c. (Fig. 2).

Reduction of **10** with sodium borohydride gave syrupy **11** which was converted into the *p*-nitrobenzoate **12**. The enol ethers **11** and **12** were sensitive to hydrolysis when kept in the open or dissolved in aqueous solvents.

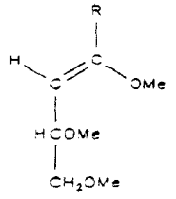
Compound **12**, as expected, was cleaved by ozonolysis[9] quantitatively into glycolic acid methyl ester[10], identified as the *p*-nitrobenzoate (**13**), and 2,3-di-*O*-methyl-D-glyceraldehyde[11] (**14**), identified as the crystalline condensation product with *N*,*N*-diphenylethylenediamine (Wanzlick's reagent)[12]. As described[11], **14** underwent β-elimination of methanol on treatment with base to form 2-methoxy-2-propenal (**15**).



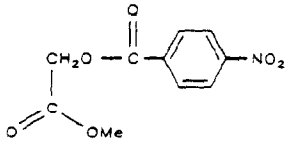
8



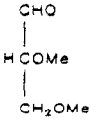
9



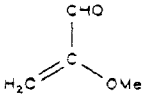
10 R = CHO
11 R = CH_2OH
12 R = CH_2O—C(=O)—C$_6H_4$—NO_2



13



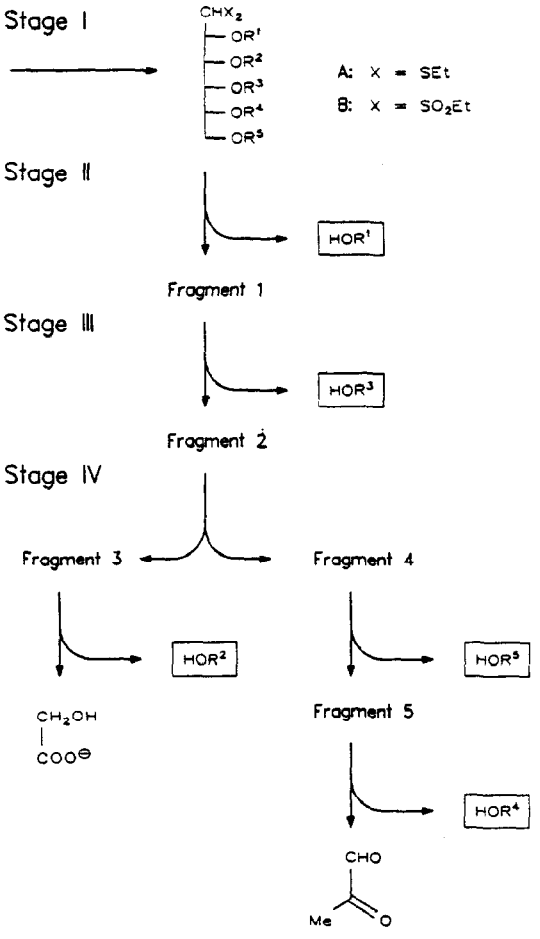
14



15

DISCUSSION

Methylated maltose diethyl dithioacetal **3**, which was used as a model glycosylated monosaccharide dithioacetal (A in Scheme 1), was obtained by a very mild method and converted (stage I in Scheme 1) into the highly reactive bis(ethylsulfonyl) derivative B. In the sequence of reactions II→IV, the substituents R^1–R^3 in B can be expelled separately as methanol, or as a reducing permethylated sugar derivative, until the C_3 fragment (C-4/6) is left as 2-oxopropanal. Thus, C-1 is lost as bis(ethylsulfonyl)methane



Scheme 1. Schematic representation of the stepwise expulsion of all substituents (OR^1/OR^5) attached to a fully alkylated or glycosylated 1,1-bis(ethylsulfonyl)aldohexose during fragmentation of the original carbon skeleton into bis(ethylsulfonyl)methane (C-1), glycolic acid (C-2,3), and pyruvic aldehyde (C-4/6).

(stage III) from fragment 1, and C-2,3 as a glycolic acid derivative (stage IV) from fragment 3. Should the expelled hydroxy component (R^1OH/R^5OH) be a reducing sugar derivative, then a new cycle of degradations can be started.

All of the products of the degradation, except methanol, could be identified by t.l.c. and/or h.p.l.c., and also recovered by the latter method if necessary. If [^{14}C]-methylated oligosaccharides were used, the degradations could be carried out on a micro-scale and applied to structural analyses of complex oligosaccharides.

Although, during the degradation sequence, the configurational identity of the reducing-end monosaccharide is lost, this is not a severe drawback since the identity and number of sugar residues in an oligosaccharide can be determined on minute quantities of material[14]. Thus, in combination with the known methods, the above procedure may be a useful, additional tool in the investigation of the structures of oligosaccharides, and a micro-method is being developed.

EXPERIMENTAL

General methods. — All reactions were monitored by t.l.c. on Silica Gel 60 F_{254} (Merck) and column chromatography was carried out with Silica 32-63, 60 A (ICN), using EtOAc–cyclohexane mixtures *A*, 3:1; *B*, 2:1; *C*, 1:1; and *D*, 1:3. H.p.l.c. was performed with an LKB h.p.l.c. controller, two pumps, a variable wavelength monitor, and a Shimadzu C-R2Ax integrator. I.r. spectra were measured with a Perkin–Elmer 1320 spectrophotometer, and optical rotations with a Schmidt & Haensch Polartronic I. ^{1}H-N.m.r. spectra were obtained with a Bruker WM 250 spectrometer at 250 MHz for solutions in $CDCl_3$ (internal Me_4Si). Melting points are uncorrected.

*2,3,6,2′,3′,4′,6′-Hepta-*O*-methylmaltose diethyl dithioacetal* (**2**). — To a stirred solution of **1** (30 g, 52.2 mmol) in dry CH_2Cl_2 (500 mL) and ethanethiol (240 mL) was added trifluoroacetic acid (80 mL) slowly at 0–5°. After 4 h, half of the solvent was evaporated *in vacuo*, the residue was diluted with water (1 L) and then extracted with CH_2Cl_2 (3 × 200 mL), and the combined extracts were washed with water (500 mL), neutralised with saturated aqueous $NaHCO_3$ (2 × 250 mL), washed with water (500 mL), dried ($CaSO_4$), and concentrated. Column (10 × 15 cm) chromatography (solvent *B*) of the residue gave syrupy **2** (21.0 g, 74%), $[\alpha]_D^{22}$ +149° (*c* 1.4, chloroform), R_F 0.13 (solvent *C*). ^{1}H-N.m.r. data: δ 4.11 (d, 1 H, $J_{1,2}$ 5.7 Hz, H-1), 4.00 (q, 1 H, $J_{5,6}$ 5.3 Hz, H-5), 5.11 (d, 1 H, $J_{1',2'}$ 3.6 Hz, H-1′), 3.18 (dd, 1 H, $J_{2',3'}$ 9.8 Hz, H-2′), 3.51 (t, 1 H, $J_{3',4'}$ 9.0 Hz, H-3′), 3.23 (dd, 1 H, $J_{4',5'}$ 9.8 Hz, H-4′), 3.83 (dt, 1 H, $J_{4',5'}$ 2.9 Hz, H-5′), 3.67 (d, 1 H, OH), 2.59–2.86 (m, 4 H, 2 SCH_2), 1.28 and 1.29 (2 t, 6 H, *J* 7.5 Hz, 2 CH_3), 3.38–3.64 (7 s, 21 H, 7 OMe).

*2,3,5,6,2′,3′,4′,6′-Octa-*O*-methylmaltose diethyl dithioacetal* (**3**). — To a stirred solution of **2** (5.0 g, 9.2 mmol) in dry methyl sulfoxide (40 mL) was added powdered sodium hydroxide (2.0 g, 50 mmol) followed by methyl iodide (3.3 g, 23.2 mmol). After 2 h, the mixture was diluted with water (500 mL) and extracted with Et_2O (3 × 100 mL), and the combined extracts were washed with water (5 × 100 mL), dried ($MgSO_4$), and concentrated. Column (5.5 × 15 cm) chromatography (solvent *C*) of the residue gave syrupy **3** (4.2 g, 81.7%), $[\alpha]_D^{22}$ +104° (*c* 1.2, chloroform), R_F 0.18 (solvent *C*). ^{1}H-N.m.r. data: δ 4.14 (d, 1 H, $J_{1,2}$ 6.8 Hz, H-1), 5.23 (d, 1 H, $J_{1',2'}$ 3.6 Hz, H-1′), 3.16 (dd, 1 H, $J_{2',3'}$ 9.8 Hz, H-2′), 3.46 (t, 1 H, $J_{3',4'}$ 9.8 Hz, H-3′), 3.27 (dd, 1 H, $J_{4',5'}$ 9.0 Hz, H-4′), 3.89 (dt, 1 H, $J_{5',6'}$ 3.3 Hz, H-5′), 2.59–2.85 (m, 4 H, 2 SCH_2), 1.27 and 1.28 (2 t, 6 H, *J* 7.5 Hz, 2 CH_3), 3.36–3.66 (8 s, 24 H, 8 OMe).

*1-Deoxy-1,1-bis(ethylsulfonyl)-2,3,5,6,2′,3′,4′,6′-octa-*O*-methylmaltitol* (**4**). — A solution of 3-chloroperoxybenzoic acid (17.3 g, 100 mmol) in CH_2Cl_2 (150 mL) was added dropwise to a stirred solution of **3** (10.0 g, 17.9 mmol) in CH_2Cl_2 (30 mL). After 4 h, the mixture was neutralised with saturated aqueous $NaHCO_3$ (3 × 100 mL), washed with water (100 mL), dried ($CaSO_4$), and concentrated. Column (8 × 12 cm) chromatography (solvent *C*) of the residue and crystallisation from EtOAc–light petroleum yielded **4** (8.0 g, 72%), m.p. 74°, $[\alpha]_D^{22}$ +93° (*c* 0.75, chloroform), R_F 0.35 (solvent *A*). ^{1}H-N.m.r. data δ 5.38 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 4.69 (dd, 1 H, $J_{2,3}$ 9.3 Hz, H-2), 4.08 (dd, 1 H, $J_{3,4}$ 3.5 Hz, H-3), 4.20 (dd, 1 H, $J_{4,5}$ 2.1 Hz, H-4), 5.26 (d, 1 H, $J_{1',2'}$ 3.5 Hz, H-1′), 3.16

(dd, 1 H, $J_{2',3'}$ 9.8 Hz, H-2'), 3.24 (dd, 1 H, H-4'), 1.42 and 1.45 (2 t, 6 H, *J* 6.5 Hz, 2 CH_2CH_3), 3.39–3.68 (8 s, 24 H, 8 OMe).

Anal. Calc. for $C_{24}H_{48}O_{14}S_2$: C, 46.14; H, 7.74; S, 10.26. Found: C, 46.33; H, 7.73; S, 10.37.

1,2-Dideoxy-1,1-bis(ethylsulfonyl)hepta-O-methylmalt-1-enitol (**5**). — A solution of **4** (1.5 g, 2.4 mmol) in 4:1 tetrahydrofuran–water (25 mL) was stored at room temperature for 10 h, then diluted with water (200 mL), and extracted with CH_2Cl_2 (3 × 50 mL), and the combined extracts were dried ($CaSO_4$) and concentrated. Column (3 × 30 cm) chromatography (solvent *A*) of the residue gave syrupy **5** (1.4 g, 98%), $[\alpha]_D^{22}$ +69° (*c* 1.1, chloroform), R_F 0.31 (solvent *A*). ^{1}H-N.m.r. data: δ 7.54 (d, 1 H, $J_{2,3}$ 9.3 Hz, H-2), 5.14 (dd, 1 H, $J_{3,4}$ 5.0 Hz, H-3), 5.08 (d, 1 H, $J_{1',2'}$ 3.8 Hz, H-1'), 3.11 (dd, 1 H, $J_{2',3'}$ 9.6 Hz, H-2'), 3.19 (dd, 1 H, $J_{4',5'}$ 9.9 Hz, H-4'), 3.74 (dt, 1 H, $J_{5',6'}$ 2.7 Hz, H-5'), 1.33 and 1.39 (2 t, 6 H, *J* 7.5 Hz, 2 CH_2CH_3), 3.31–3.61 (7 s, 21 H, 7 OMe).

Anal. Calc. for $C_{23}H_{44}O_{13}S_2$: C, 46.41; H, 7.48. Found: C, 46.49; H, 7.35.

Fragmentation of **5**. — A solution of **5** (1.05 g, 1.69 mmol) in tetrahydrofuran (20 mL) was added to aqueous ammonia (30 mL, 2.5%). After 2 h at room temperature, **5** had disappeared and three new compounds were detectable in t.l.c. (solvent *A*). The mixture was diluted with water (200 mL) and extracted with CH_2Cl_2 (3 × 100 mL), the combined extracts were dried ($CaSO_4$) and concentrated, and the residue was subjected to chromatography (column, 3 × 50 cm, and solvent *A* for **9**; column, 3 × 15 cm, and solvent *D* for **8** and **10**).

Part of **9**, which remained in the aqueous phase, was combined with **9** obtained from column chromatography, to give material identical with authentic 2,3,4,6-tetra-*O*-methyl-D-glucose[8], R_F 0.18 and 0.24 (solvent *A*).

Compound **8** (strong staining with iodine in t.l.c.) was identical with authentic bis(ethylsulfonyl)methane[2], R_F 0.54 (solvent *A*).

3-Deoxy-2,4,5-tri-*O*-methyl-D-*glycero*-pent-2-enose (**10**) (strong u.v. absorbance in t.l.c.) was obtained as an unstable syrup (250 mg, 85%), R_F 0.50 (solvent *A*). ^{1}H-N.m.r. data: δ 9.23 (s, 1 H, H-1), 5.79 (d, 1 H, $J_{3,4}$ 8.7 Hz, H-3), 3.43 (dt, 1 H, $J_{4,5}$ 4.8 Hz, H-4), 3.45 (d, 2 H, H-5), 3.31, 3.34, and 3.79 (3 s, 9 H, 3 OMe).

*3-Deoxy-2,4,5-tri-*O*-methyl-1-*O*-(p-nitrobenzoyl)-*D*-glycero-pent-2-enitol* (**12**). — A solution of **10** (400 mg, 2.3 mmol) in 1:1 tetrahydrofuran–water (20 mL) was treated with sodium borohydride (~ 100 mg). After 2 h at room temperature, the mixture was diluted with water (100 mL) and extracted with CH_2Cl_2 (8 × 50 mL), and the combined extracts were dried ($CaSO_4$) and concentrated, to yield crude **11** (380 mg) as an oily liquid, R_F 0.20 (solvent *A*). ^{1}H-N.m.r. data: δ 4.14 (d, 1 H, $J_{1a,1b}$ 13.5 Hz, H-1a), 4.18 (d, 1 H, H-1b), 4.64 (d, 1 H, $J_{3,4}$ 8.9 Hz, H-3), 4.35 (ddd, 1 H, $J_{4,5a}$ 4.4, $J_{4,5b}$ 6.2 Hz, H-4), 3.30, 3.36, and 3.69 (3 s, 9 H, 3 OMe).

A solution of **11** in C_5H_5N (10 mL) was treated with *p*-nitrobenzoyl chloride (400 mg). After 20 h at room temperature, the excess of reagent was hydrolysed by adding ice (~ 1 g), the mixture was diluted with water (200 mL) and extracted with CH_2Cl_2 (3 × 50 mL), and the combined extracts were neutralised with saturated aqueous $NaHCO_3$, washed with water (100 mL), dried ($CaSO_4$), and concentrated. Column (2 × 30 cm)

chromatography (solvent *C*) of the residue gave syrupy **12** (490 mg, 65.5%), $[\alpha]_D^{22}$ +45° (*c* 2.1, chloroform), R_F 0.60 (solvent *C*). ^{1}H-N.m.r. data: δ 4.94 (s, 2 H, H-1), 4.93 (d, 1 H, $J_{3,4}$ 9.0 Hz, H-3), 4.42 (ddd, 1 H, $J_{4,5a}$ 4.2, $J_{4,5b}$ 5.9 Hz, H-4), 3.42 (dd, 1 H, $J_{5a,5b}$ 10.5 Hz, H-5a), 3.45 (dd, 1 H, H-5b), 3.35, 3.40, and 3.73 (3 s, 9 H, 3 OMe), 8.20–8.35 (m, 4 H, Ar-H).

Ozonolysis of **12**. — Ozonolysis[9] of **12** gave **13** and **14** in almost quantitative yield. Compound **13** was identical with an authentic sample prepared by *p*-nitrobenzoylation of commercial methyl glycolate. 2,3-Dimethoxypropanal (**14**) gave a condensation product with *N*,*N*-diphenylethylenediamine[12], which was identical with the product, m.p. 100.5° (methanol), obtained by condensation of the authentic aldehyde[13] with Wanzlick's reagent.

Anal. Calc. for $C_{19}H_{24}N_2O_2$: C, 73.05; H, 7.74; N, 8.97. Found: C, 72.88; H, 7.78; N, 9.21.

ACKNOWLEDGMENTS

Professors J. G. Buchanan and S. Stirm are thanked for encouraging discussion, and the Fonds der Chemischen Industrie for financial support.

REFERENCES

1 H. Kurth and J. Lehmann, *Carbohydr. Res.*, 113 (1983) c22–c26.
2 D. L. MacDonald and H. O. L. Fischer, *Biochim. Biophys. Acta*, 12 (1953) 203–206.
3 H. Kurth and J. Lehmann, *Biomed. Chromatogr.*, 1 (1986) 58–63.
4 M. L. Wolfrom, M. R. Newlin, and E. E. Stahly, *J. Am. Chem. Soc.*, 53 (1931) 4379–4383.
5 H. S. Isbell and H. L. Frush, *J. Org. Chem.*, 23 (1958) 1309–1319.
6 J. Honeyman, *Methods Carbohydr. Chem.*, 2 (1963) 95–99.
7 H. Kurth, Dissertation, University of Freiburg, 1986.
8 J. C. Irvine and J. W. H. Oldham, *J. Chem. Soc.*, 119 (1921) 1744–1759; W. N. Haworth, C. W. Long, and J. H. G. Plant, *ibid.*, (1927) 2809–2814.
9 M. A. Bernstein and L. D. Hall, *Carbohydr. Res.*, 78 (1980) c1–c3.
10 J. G. Deshmukh, R. B. Mane, M. H. Jagdale, and M. M. Salunkhe, *Chem. Ind.*, (1986) 179–180.
11 M. Fedoroňko, M. Petrušová, and T. Sticzay, *Carbohydr. Res.*, 115 (1983) 75–83.
12 H.-W. Wanzlick and W. Löchel, *Chem. Ber.*, 86 (1953) 1463–1467.
13 E. Baer and H. O. L. Fischer, *J. Biol. Chem.*, 145 (1942) 61–68; M. Fedoroňko, P. Temcovic, V. Milhálov, and I. Tvaroška, *Carbohydr. Res.*, 87 (1980) 51–62.
14 L. Kenne and S. Strömberg, *Carbohydr. Res.*, 198 (1990) 173–179.