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Formation of 3-hexuloses in aldol reactions, analysis of the products as their *O*-isopropylidene derivatives by GC–MS

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Abstract—A method for analysis of mixtures of 3-hexuloses by gas chromatography mass spectrometry of their di-*O*-isopropylidene derivatives has been elaborated. The origin of characteristic fragment ions in the mass spectra is suggested on the basis of the spectra of d_{12} analogues, obtained by acetonation with acetone-d_6 and on MS/MS investigations. The method has been applied to product mixtures from aldol reactions between *glycero*-tetrulose and glycolaldehyde and between 2-pentuloses and formaldehyde. An interesting result is the formation of *ribo*-3-hexulose with a high degree of stereoselectivity in alkali catalysed reaction between *erythro*-2-pentulose and formaldehyde.

Keywords: Aldol reactions; 2-Pentuloses; *glycero*-Tetrulose; 3-Hexuloses; Mass spectrometry; Isopropylidene derivatives; Glycolaldehyde; Formaldehyde

1. Introduction

3-Hexuloses are found in the product mixtures of reactions such as oxidation of hexitols,[1] aldol reactions[2,3] and the formose reaction.[4] Reported synthetic methods are based on CrO_3 oxidation of the tetraacetates of ethylidene, methylene or benzylidene derivatives of hexitols,[5,6] and Br_2 oxidation of 1,2:5,6-di-*O*-isopropyl-idene hexitols.[7] These hexuloses have not received much attention. An important exception is D-*arabino*-3-hexulose, which is formed as its 6-phosphate as primary product in formaldehyde fixation in some methylotrophic bacteria by aldol reaction with ribulose-5-phosphate in the ribulose monophosphate (RuMP) pathway.[8–10] This reaction is catalysed by 3-hexulose-phosphate synthase (HPS), and the free hexulose may be obtained on dephosphorylation.[11]

In continuation of our work with 3-hexuloses, we are interested in their formation by aldol reactions of small sugars. It was shown previously that D-*threo*-2-pentulose (xylulose) and formaldehyde in 0.1 M NaOH solution gave about equal amounts of the D-*xylo*-and D-*lyxo*-3-hexuloses, isolated as their di-*O*-isopropylidene derivatives.[2] When DL-*glycero*-tetrulose (erythrulose) and glycolaldehyde were used, and strongly basic anion exchange resin applied as catalyst, acetonation showed that the DL-*xylo*-and DL-*lyxo*-3-hexuloses were formed in a ratio of about 2:5, and this reaction was used for preparation of 1,2:3,4-di-*O*-isopropylidene-β-DL-*lyxo*-3-hexulofuranose.[3]

We have now extended the investigation of aldol reactions of small sugars to the reaction of formaldehyde with D-*erythro*-2-pentulose (ribulose). This seemed of particular interest in the light of the earlier mentioned formaldehyde fixation in methylotrophic bacteria.[8–11] We have also included in our investigation the alkali catalysed aldol reaction between DL-*glycero*-tetrulose and glycolaldehyde, with and without the presence of Sr^{2+} ions. It has been found previously that Ca^{2+} and Sr^{2+} ions strongly influence the product composition in alkali catalysed triose aldol reactions.[12]

A method for analysis of the products in these reactions was needed. Our experience with GC–MS of

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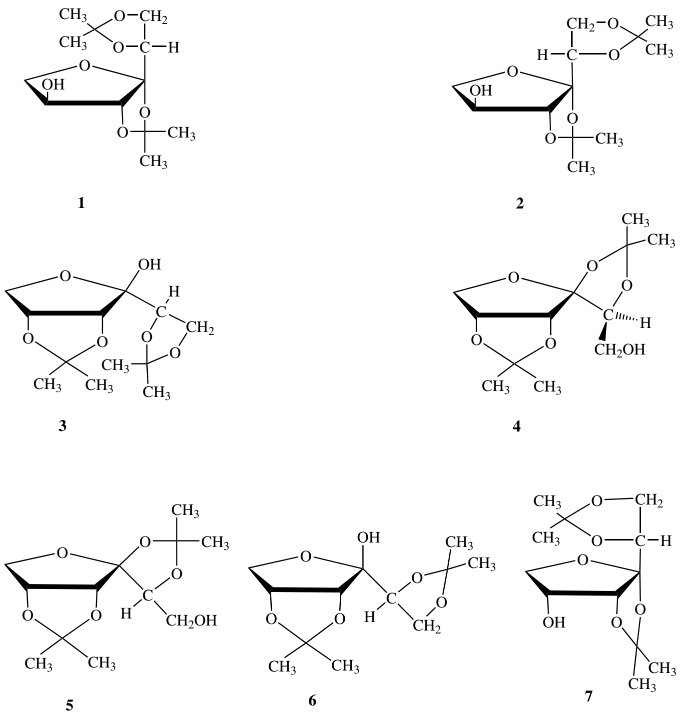

Scheme 1.

O-isopropylidene derivatives of monosaccharides[13,14] suggested this as an attractive method. The usefulness of *O*-isopropylidene derivatives for identification of monosaccharides by mass spectrometry was first shown by DeJongh and Biemann.[15] The advantage of these compounds over acetates and trimethylsilyl ethers is due to the fact that they frequently are structural, and not merely configurational, isomers. This method has previously been found useful for analysis of 2-hexuloses and 2-pentuloses.[14] In a previous paper, some EI-MS data were reported for di-*O*-isopropylidene derivatives of 3-hexuloses without discussion of the spectra.[7] In the present paper, the origin of some important fragments in the spectra is suggested, the possibility to discriminate between the 3-hexuloses is investigated, and the application of the GC–MS method to the product mixtures from aldol reactions is described.

2. Results and discussion

In accordance with a report by Angyal and Evans,[6] and confirmed in previous papers in this series,[2,3,7] the *xylo*- and *lyxo*-3-hexuloses gave the 1,2:3,4-di-*O*-isopropylidene-β-furanose derivatives **1** and **2**, respectively, as single products (see Scheme 1 for structures **1**–**7**).[†] These compounds are diastereomers, and their EI mass spectra are, as a consequence, almost identical (Table 1). The difference in the chromatographic mobilities of **1** and **2** (Table 2) eliminates this as a problem in analysis of the 3-hexuloses. An explanation of this difference, observed on Silica gel as well as in GC, was suggested in a previous paper in this series based on the ability of **1** to form intramolecular hydrogen bond in a favourable conformation.[2] The 3-hexuloses with *ribo* and *arabino* configurations give more than one di-*O*-isopropylidene derivative. From *ribo*-3-hexulose are obtained two derivatives **3** and **4** in relative proportions about 6:1. The major product **3** is known[6,7] to be the 1,2:4,5-di-*O*-isopropylidene-*ribo*-3-hexulofuranose, whereas **4** is the 2,3:4,5-di-*O*-isopropylidene-β-furanose derivative. From *arabino*-3-hexulose are obtained three derivatives, **5**, **6** and **7** in relative proportions 65%:30%:5%, respectively. They are known[6,7] to be 2,3:4,5-di-*O*-isopropylidene-β-*arabino*-3-hexulofuranose, 1,2:4,5-di-*O*-isopropylidene-*arabino*-3-hexulofuranose and the 1,2:3,4-di-*O*-isopropylidene-α-furanose derivative, respectively.

The EI mass spectra of di-*O*-isopropylidene derivatives of the common hexoses have been thoroughly described by DeJongh and Biemann.[15] The molecular ion is usually not observed, the highest mass peak is observed at *m*/*z* 245, due to the loss of a methyl radical from the molecular ion. The loss of acetone, acetic acid or both from this ion leads to fragments with *m*/*z* 187, 185 or 127, respectively. In addition, high intensity peaks at *m*/*z* 59 (protonated acetone) and 43 (acetylium ion) are observed. Suggested fragmentations leading to the more characteristic ions observed in the spectra of **1** and **2** are shown for compound **1** in Figure 1. The sug-

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[†] Only one enantiomer, D or L, of each hexulose and the derivatives is shown throughout this paper.

Table 1. Mass spectral data for the main di-*O*-isopropylidene-3-hexuloses

m/*z*[a]	Relative intensity (%)			
	1	2	3	5
245 (254)	100	79	40	100
243 (255)			1	
229 (241)				4
227 (236)	3	3		88
217 (229)			7	
202 (208)			6	5
200 (212)	59	35		
187 (190)	18	14	6	3
185 (191)	27	21	8	8
171 (177)				20
169 (172)	10	8		4
159 (165)	76	89	68	12
147 (153)			13	30
143 (146)			6	10
142 (148)	8	5		4
131 (134)			11	12
127 (127,128)	37	39	18	35
115	9	8	10	
114 (120)			14	25
113 (116)	6	7		20
101 (107)	64	61	28	7
99	13	12	7	12
97	6	8	5	24
85 (88,91)	16	14	15	52
83	14	12		10
73	22	21	7	11
71	24	23	13	34
69	12	10		27
59 (65)	73	78	100	93
57	16	19	17	38
55	37	31	19	34
43 (46)	90	100	75	88

[a] The figures in parentheses refer to *m*/*z* for the d_{12}-analogues.

gestions are based on the known fragmentation of 2-hexulose derivatives[14,15] and is supported by the spectra of d_{12} analogues, obtained by derivatisation with acetone-d_6. For some of the fragments, product/precursor ion techniques have also been applied. Suggested fragmentation in the mass spectrum of **3**, which is almost identical to that of its diastereomer **6**, is shown in Figure 2, and the possible origin of the most characteristic fragments in the spectrum of **5** is shown in Figure 3, the spectrum is for the same reason similar to that of **4**.

The mass spectra of the *xylo* (**1**) and *lyxo* (**2**) derivatives are readily differentiated from those of the main derivatives of the *ribo*-and *arabino*-3-hexuloses (**3** and **5**) (Table 1). The high intensity *m*/*z* 200 fragment, due to a C-3–O-6 and a C-4–C-5 cleavage, and the fragment ion at *m*/*z* 142, resulting from loss of acetone from the *m*/*z* 200 fragment, are not observed in the spectra of the *ribo* and *arabino* derivatives. In addition, peaks at *m*/*z* 101 from C-2–C-3 cleavage of high abundance are observed in the spectra of **1** and **2**. A fragment ion with *m*/*z* 159, also resulting from C-2–C-3 cleavage, is observed in the spectrum of **3** as well as in those of **1** and **2**. However, in the spectrum of **3**, the *m*/*z* 101 fragment ion is much less abundant than in the spectra of **1** and **2**, whereas the *m*/*z* 159 fragment is of high abundance. An explanation could be that the 159 fragment ion from **3** is more easily stabilised than those from **1** and **2** due to the possibility to achieve planarity within the group of atoms involved in the resonance stabilisation of the fragment ion (Fig. 2). It is, however, observed an other route, which may be more important, resulting in a *m*/*z* 159 fragment with the same elemental composition. This route is confirmed by a product ion mass spectrum of a *m*/*z* 217 fragment ion as precursor. This fragment is observed in the spectrum in relatively low abundance, and could be represented as shown in Figure 2. Loss of acetone from the *m*/*z* 217 fragment leads to a *m*/*z* 159 fragment. A very small, but significant peak is observed at *m*/*z* 243 in the spectrum of **3** due to loss of the OH-3 as a radical from the molecular ion. This is characteristic in spectra of derivatives with the anomeric hydroxyl group free.[15] In the spectrum of compound **5**, a characteristic peak of high abundance, not observed in the spectrum of **3**, and of very low intensity in the spectra of **1** and **2,** is seen at *m*/*z* 227, due to the loss of water from $M^+{-}CH_3$, whereas a unique fragment for **5** is observed at *m*/*z* 171, arising from loss of acetone from the fragment ion *m*/*z* 229, which is observed in surprisingly low abundance in the spectrum (Fig. 3). A peak at *m*/*z* 147, seen in the spectra of **3** and **5**, is obviously an analogue of the *m*/*z* 117 ion, which is characteristic of a 1,2-*O*-isopropylidene group in derivatives of 2-hexuloses[15,16] and 2-pentuloses[14] containing the C-1 to C-3 part of the molecule, as well as protonated O-6 (Figs. 2 and 3). Finally, a fragment ion with *m*/*z* 114 is observed in the spectra of **3** and **5**, not observed in the spectra of **1** and **2**, containing the C-4 to C-6 part of the molecule, being characteristic of 3-hexulose derivatives with a 4,5-*O*-isopropylidene

Table 2. Chromatographic data for the main di-*O*-isopropylidene derivatives of the hexuloses

Hexulose	di-*O*-Isopropylidene-acetal	Retention time/min	Molar responses[a]
xylo-3-Hexulose	1,2:3,4	10.3	1.00
ribo-3-Hexulose	1,2:4,5	11.0	0.85
arabino-3-Hexulose	2,3:4,5	11.6	0.65
lyxo-3-Hexulose	1,2:3,4	13.5	1.00
arabino-2-Hexulose	2,3:4,5	13.3	0.95
xylo-2-Hexulose	2,3:4,6	14.1	0.80

[a] Relative peak areas of the *main* acetal of each hexulose.

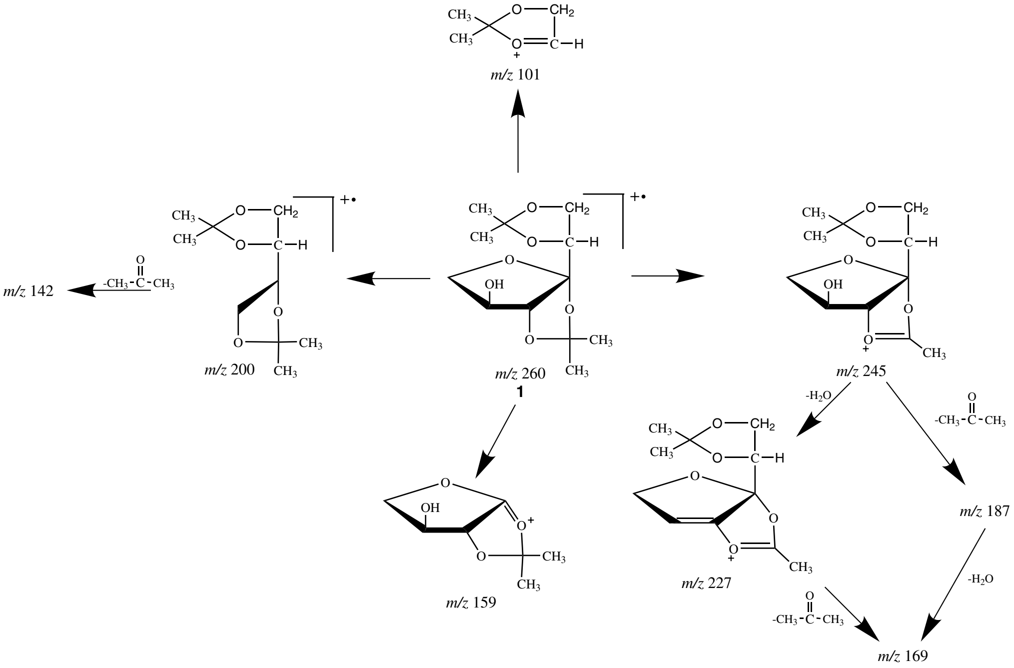

Figure 1. Suggested origin of characteristic fragment ions in the mass spectrum of **1**.

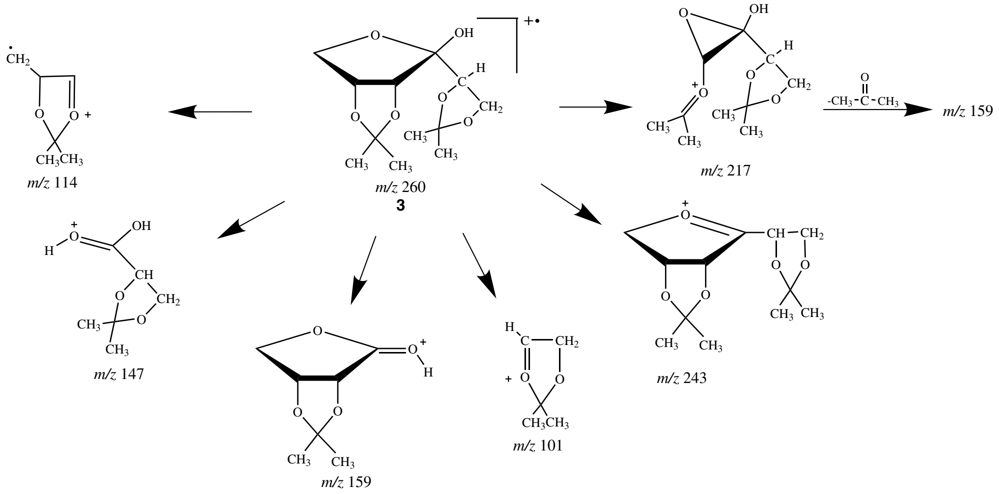

Figure 2. Suggested origin of characteristic fragment ions in the mass spectrum of **3**.

group. The MS data are shown in Table 1. The main di-*O*-isopropylidene derivatives of the 3-hexuloses are well separated under the applied GC conditions (Table 2). This, in combination with the MS data, allows ready discrimination between the 3-hexuloses.

The results of the aldol reactions are shown in Table 3. It is seen that the application of strongly basic ion exchange resins as catalysts in the reaction between *glycero*-tetrulose and glycolaldehyde leads to a higher stereoselectivity than observed in the alkali catalysed reaction. This is in accordance with previous observations during triose aldol reactions[14,17,18] and the aldol reaction between dihydroxyacetone and glycolaldehyde.[19] The presence of Sr^{2+} ions during the alkali catalysed reaction between *glycero*-tetrulose and glycolaldehyde has a marked influence on the product composition. The relative amount of *lyxo*-3-hexulose is reduced, whereas that of the *arabino* isomer is more than doubled. In addition, substantial amounts of *arabino*-2-hexulose (fructose) and, in particular, *xylo*-2-hexulose (sorbose) are observed in the product mixture. The increase in the amounts of 2-hexuloses formed in the presence of Sr^{2+} ions must be a result of stabilisation as alkaline earth ion complexes. The 2-hexuloses are secondary products, they are not observed during the initial phase of the reaction. The change in product composition in the presence of Sr^{2+} ions is not surprising, the same phenomenon was observed during alkali catalysed

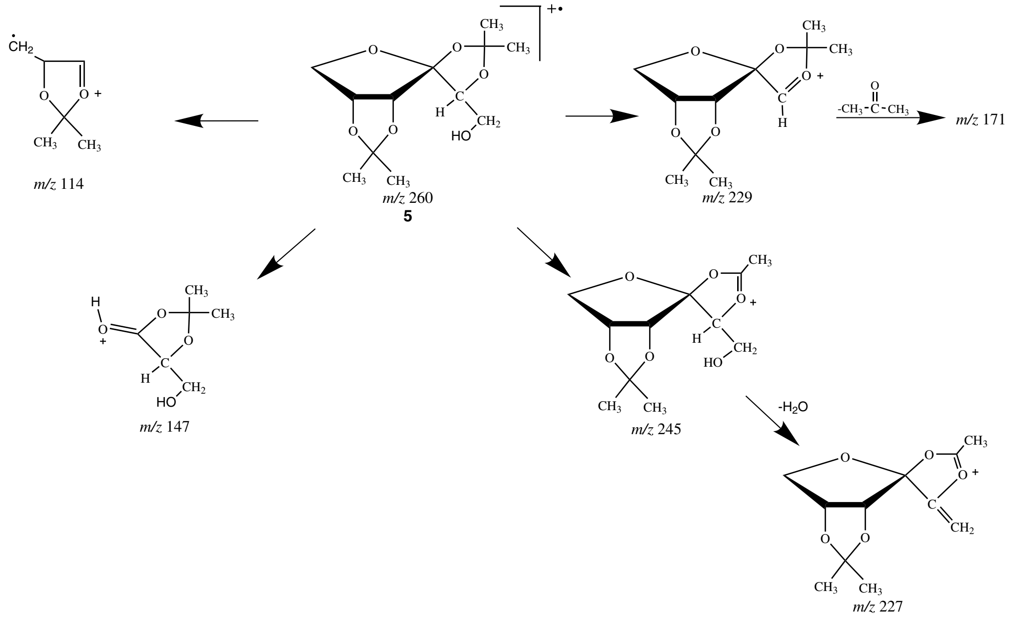

Figure 3. Suggested origin of characteristic fragment ions in the mass spectrum of **5**.

Table 3. Relative proportions of hexuloses in the reaction mixtures after the aldol reactions between DL-*glycero*-tetrulose and glycolaldehyde (Panel A), and between formaldehyde and 2-pentuloses in 0.05 M NaOH solution (Panel B)

Catalyst	Reaction time (min)	Products (% of total hexuloses)					
		xylo-3-Hexulose	*ribo*-3-Hexulose	*arabino*-3-Hexulose	*lyxo*-3-Hexulose	*arabino*-2-Hexulose	*xylo*-2-Hexulose
Panel A							
Amberlite, IRA-400 (OH^-) resin	12	21	6	7	52	5	9
Dowex, 1 × 16 (OH^-) resin	12	26	6	2	58	2	6
NaOH, 0.02 M	50	29	11	8	44	2	6
NaOH, 0.02 M, $SrCl_2$ 2M	50	25	11	25	12	5	22
Panel B							
erythro-2-Pentulose	30	—	84	16	—		
threo-2-Pentulose	30	46	—	—	54		

triose aldol reactions.[12] Attempts to use anion exchange resin as a catalyst in reactions between 2-pentuloses and formaldehyde were unsuccessful. An unexpected result was, on the other hand, observed on alkali catalysed aldol reaction between *erythro*-2-pentulose and formaldehyde. Whereas the hexulose phosphate synthase catalysed formaldehyde fixation gives the *arabino*-3-hexulose-6-phosphate, the *ribo* isomer is strongly favoured in the alkali catalysed reaction. The stereoselectivity here is high, unlike the situation in the analogous reaction with *threo*-2-pentulose. In addition to hexuloses, the product mixtures from these reactions also contain aldoses. Erythrose and threose are, as expected, formed by self aldolisation of glycolaldehyde, and small amounts of aldopentoses are occasionally produced by isomerisation of the 2-pentuloses. Based on some of the presented results, work is in progress to develop alternative procedures for preparation of 3-hexuloses.

3. Experimental

3.1. General methods

GC was performed with a Shimadzu GC-14B gas chromatograph, equipped with an open tubular fused silica column, 25 m × 0.32 mm ID, wall coated with CP-SIL 43 CB, programmed at 6°/min from 90 to 225 °C. For mass spectrometry, a JEOL JMX-DX 303 double focusing mass spectrometer with EB geometry was used, equipped with a 10 kV post acceleration conversion dynode detector and an electron ionisation—chemical ionisation (EI–CI) ion source, operated in EI mode at 70 eV and an ion source temperature at 180 °C. MS/MS investigations were performed with an AutoSpec Ultima 2000 (Micromass ltd) mass spectrometer with EBE geometry. A MIKE scan technique was used, the sample was introduced into the ion source by direct insertion probe (DIP) and ionised by electron ionisation at 70 eV. The ion source temperature was 200 °C. A Carlo Erba high resolution gas chromatograph (HRGC) was applied for the GC–MS combination.

3.2. Materials

D and L-*eythro*-2-Pentulose,[20] D-*threo*-2-pentulose,[21] DL-glycero-tetrulose[3] DL-*lyxo*-,[3] D-*arabino*-,[7] L-*xylo*-,[7] and D-*ribo*-3-hexulose[7] were prepared according to reported methods.

3.3. GC–MS analysis

The sugars were identified by GC–MS of their isopropylidene derivatives on the basis of previous work.[7,13,14,19] The relative proportions of the hexuloses in the product mixtures was calculated from the peak areas of the derivatives. For the hexuloses giving more than one derivative, the molar response of the main acetal was stipulated from the proportion formed of this acetal on acetonation of the hexulose.

3.4. Reaction of 2-pentuloses with formaldehyde

To the 2-pentulose (20 mg) in water (4.5 mL) were added 35% formaldehyde solution (0.5 mL) and 0.1 M NaOH (5 mL). The solution was flushed with nitrogen and kept at room temperature for 30 min under nitrogen. After neutralisation with Dowex 50 W (H^+) ion exchange resin, filtering of the solution and evaporation of the solvent under reduced pressure, the residue was dissolved in MeOH. Dowex 50 W (H^+) was added to the solution and the mixture was heated at reflux temperature under stirring for 2 h to remove excess formaldehyde as its dimethyl acetal, which was continuously distilled off during the reaction period together with about one third of the MeOH. After cooling, the solution was filtered and the solvent evaporated. The residue was heated in 0.1 M H_2SO_4 (10 mL) at 65 °C for 2 h to hydrolyse methyl glycosides. The solution was then cooled, neutralised with Amberlite IRA 400 (HCO_3^-) ion exchange resin, filtered and the solvent was evaporated. The residue was stirred for 90 min with 2% H_2SO_4 in acetone or acetone-d_6 (5 mL). After neutralisation with solid $NaHCO_3$ and filtration of the solution, it was subjected to GC and GC–MS.

3.5. Anion exchange resin catalysed reaction of DL-*glycero*-tetrulose with glycolaldehyde

To DL-*glycero*-tetrulose (10 mg) in water (2 mL) was added a solution of glycolaldehyde (8 mg) in water (2 mL). Enough freshly regenerated anion exchange resin was added to fill the solution volume. The mixture was kept at room temperature for 12 min under nitrogen, 20% aqueous acetic acid was then added. After filtration, the resin was washed with more acetic acid solution and finally with water. The combined filtrates and washings were evaporated, and the residue was treated with acetone or acetone-d_6–H_2SO_4 as described above and the solution subjected to GC or GC–MS.

3.6. Alkali catalysed reaction of DL-*glycero*-tetrulose with glycolaldehyde

To DL-*glycero*-tetrulose (15 mg) in water (5 mL) were added a solution of glycolaldehyde (12 mg) in water (3 mL) and then 0.04 M NaOH solution (8 mL) with or without $SrCl_2 \cdot 6H_2O$ (8.5 g). After 50 min under nitrogen at room temperature, the solution without $SrCl_2$ was neutralised with Dowex 50 W (H^+) resin, filtered and evaporated under reduced pressure. The solution with $SrCl_2$ was deionised with IRA 400 (HCO_3^-) and Dowex 50 W (H^+) resins, filtered and evaporated. The residues were treated with acetone—H_2SO_4 as described above and subjected to GC and GC–MS.

References

1. Decker, P.; Schweer, H. *Carbohydr. Res.* **1982**, *107*, 1–6.
2. Morgenlie, S. *Acta. Chem. Scand. B* **1982**, *36*, 725–727.
3. Morgenlie, S. *Acta. Chem. Scand. B* **1987**, *41*, 745–748.
4. Mizuno, T.; Weiss, A. H. *Adv. Carbohydr. Chem. Biochem.* **1974**, *29*, 173–227.
5. Angyal, S. J.; James, K. *Chem. Commun.* **1970**, 320–321.
6. Angyal, S. J.; Evans, M. E. *Aust. J. Chem.* **1972**, *25*, 1495–1512.
7. Ekeberg, D.; Morgenlie, S.; Stenstrøm, Y. *Carbohydr. Res.* **2001**, *335*, 141–146.
8. Kemp, M. B. *Biochem. J.* **1974**, *139*, 129–134.
9. Strøm, T.; Ferenci, T.; Quayle, J. R. *Biochem. J.* **1974**, *144*, 465–476.
10. Colby, J.; Zatman, L. J. *Biochem. J.* **1975**, *148*, 513–520.
11. Brockamp, H.-P.; Steigel, A.; Kula, M.-R. *Liebigs. Ann. Chem.*, **1993**, 621–624.
12. Morgenlie, S. *Acta. Chem. Scand. B* **1988**, *42*, 546–549.
13. Morgenlie, S. *Carbohydr. Res.* **1975**, *41*, 285–289.
14. Morgenlie, S. *Carbohydr. Res.* **1980**, *80*, 215–222.
15. DeJongh, D. C.; Biemann, K. *J. Am. Chem. Soc.* **1964**, *86*, 67–74.
16. Brady, R. F., Jr. *Adv. Carbohydr. Chem. Biochem.* **1971**, *26*, 197–278.
17. Gutsche, C. D.; Redmore, D.; Buriks, R. S.; Nowotny, K.; Grassner, H.; Armbruster, C. W. *J. Am. Chem. Soc.* **1967**, *89*, 1235–1245.
18. Morgenlie, S. *Carbohydr. Res.* **1982**, *107*, 137–141.
19. Morgenlie, S. *Carbohydr. Res.* **1984**, *132*, 330–334.
20. Ekeberg, D.; Morgenlie, S.; Stenstrøm, Y. *Carbohydr. Res.* **2002**, *337*, 779–786.
21. Hough, L.; Theobald, R. S. *Methods Carbohydr. Chem.* **1962**, *1*, 94–98.