Carbohydrate Research, 80 (1980) 215–222

GAS CHROMATOGRAPHY–MASS SPECTROMETRY OF HEXULOSES AND PENTULOSES AS THEIR *O*-ISOPROPYLIDENE DERIVATIVES: ANALYSIS OF PRODUCT MIXTURES FROM TRIOSE ALDOL-CONDENSATIONS

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(Received May 28th, 1979; accepted for publication, July 23rd, 1979)

ABSTRACT

Identification and quantitation of hexuloses and pentuloses in mixtures has been achieved by gas chromatography–mass spectrometry of their *O*-isopropylidene derivatives. The method has been applied to product mixtures from triose aldol-condensations. The use of strongly basic anion-exchange resins as catalysts in the aldol condensation gives considerably higher proportions of fructose than when alkali or alkaline-earth hydroxides are applied.

INTRODUCTION

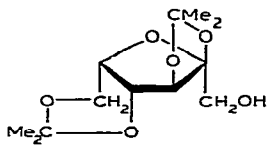
The formation of D-sorbose and D-fructose in the alkali-catalysed aldol condensation of D-glyceraldehyde and 1,3-dihydroxy-2-propanone was reported by Fischer and Baer[1]. It appears that the formation of compounds having the *threo* configuration at the new chiral centers is favoured in these aldol condensations[1,2]. The branched-chain ketohexose DL-dendroketose, arising from the combination of two molecules of 1,3-dihydroxy-2-propanone[3], is also formed in the triose aldol-condensation. The relative proportions of the various products depend on the catalyst employed. With glyceraldehyde as the starting material, a similar product mixture is obtained as that from an equimolar mixture of glyceraldehyde and 1,3-dihydroxy-2-propanone. This is due to a rapid isomerisation of the former to the latter under the alkaline conditions used.

There has been interest in the triose aldol-condensation as a possible secondary reaction in the formation of sugars by the formose reaction[4], and as a model reaction for the aldolase-catalysed, triose phosphate aldol-condensation. A g.l.c. method based on trimethylsilylation of the products has been used to determine the relative proportions of fructose, sorbose, and dendroketose formed in the triose aldol-condensation[5]. However, the ketohexoses psicose and tagatose have also been detected by paper chromatography as products of this reaction[6,7], although, in quantitative analyses, the amounts of these sugars were given as traces, or they were not mentioned at all. We now report on the g.l.c.–m.s. analysis of hexuloses and pentuloses as their *O*-isopropylidene derivatives, and the application of the method to the

hexulose mixture resulting from the triose aldol-condensation. G.l.c.–m.s. analysis of *O*-isopropylidene derivatives of aldoses has been described[8] and has advantages over the use of trimethylsilyl derivatives and acetates, in discriminating between configurational isomeric sugars by m.s.[9], as well as in giving less-complex gas chromatograms.

RESULTS AND DISCUSSION

2,3:4,6-Di-*O*-isopropylidene-α-L-sorbofuranose (**1**), 1,2:3,4-di-*O*-isopropylidene-β-D-psicofuranose (**2**), 1,2:3,4-di-*O*-isopropylidene-β-L-*erythro*-pentulofuranose (**3**), and 2,3-*O*-isopropylidene-β-D-*threo*-pentulofuranose (**4**) were, as expected[10], the only products detected by g.l.c. on OV-225 (Fig. 1) and Dexsil 300 when the parent ketoses were treated with acetone containing 2% of sulphuric acid (v/v) for 2 h at room temperature. 2,3:4,5-Di-*O*-isopropylidene- (**5**) and 1,2:4,5-di-*O*-isopropylidene--β-D-fructopyranose (**6**) were formed from D-fructose in a ratio of ~10:1. D-Tagatose gave traces of a di-*O*-isopropylidene derivative in addition to the known 1,2:3,4-di-*O*-isopropylidene-α-D-tagatofuranose (*7*). Fig. 1 shows the g.l.c. separation on OV-225 of the *O*-isopropylidene derivatives of the unbranched hexuloses and pentuloses, obtained from a mixture of the ketoses. The branched-chain ketohexose DL-dendroketose gave, in addition to the expected[11,12] 4-*C*-(hydroxymethyl)-1,2:3,4-di-*O*-isopropylidene-β-DL-*erythro*-pentulofuranose (**8**) and 4-*C*-(hydroxymethyl)-2,3:4,4¹-di-*O*-isopropylidene-β-DL-*threo*-pentulofuranose (**9**), a third di-*O*-isopropylidene derivative **10**, which had a mass spectrum very similar to that of **9** and is probably



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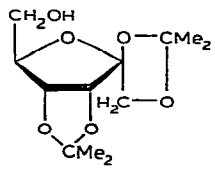

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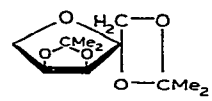

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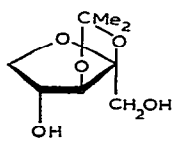

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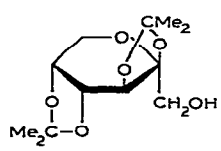

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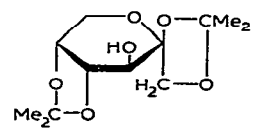

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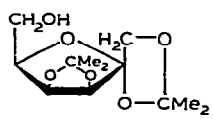

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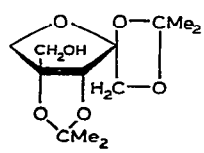

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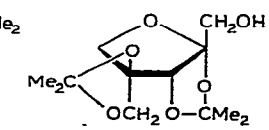

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For **8** and **9**, only the respective D- and L-enantiomers are shown.

TABLE I

CHROMATOGRAPHIC DATA FOR THE *O*-ISOPROPYLIDENE-KETOSES

Ketose	*Acetal*	T *values*[a]		*Molar responses*[b]	
		OV-225	*Dexsil 300*	*OV-225*	*Dexsil 300*
L-*erythro*-Pentulose	**3**	0.23	0.27	0.73	0.73
D-*threo*-Pentulose	**4**	0.85	0.45	0.55	—[c]
DL-Dendroketose	**8**	0.65	0.75	0.44	0.44
	9	0.75	0.83	0.27	0.32[d]
	10	0.89	0.83	0.04	
D-Psicose	**2**	0.65	0.81	0.80	0.79
D-Tagatose	**7**	0.75	0.87	0.90	0.89
D-Fructose	**5**	0.87	1.00	0.96	1.05
	6	0.80	0.90		
L-Sorbose	**1**	0.93	1.05	0.77	0.76

[a]Retention time relative to that of 2,3:5,6-di-*O*-isopropylidene-D-mannose. [b]Based on the parent ketose relative to that of D-mannose. [c]Depends on concentration when the ketose is present in low proportions. [d]Represents the sum of the responses from **9** and **10**.

TABLE II

MASS-SPECTRAL DATA FOR THE MAIN DI-*O*-ISOPROPYLIDENE-HEXULOSES

m/e[a]	*Relative intensity (%)*				
	1	**5**	**7**	**2**	**8**
245 (254)	40	47	23	19	45
229 (241)	7	14	4	12	
187 (190)	10	4	2	3	3
171 (177)	17	26	3	8	
169 (172)		8			
159 (165)	15				
145 (146)					16
144 (144)			7	13	19
142 (148)			21	4	
141 (147)					19
139 (142)	8				
130 (136)	14	7			
127 (127)	8	26	11	16	26
117 (123)			40	42	53
115 (118)	9	9			14
113 (113,119)	22	10	32	37	14
109 (109)			43	11	10
101 (101,107)	12				
97 (97,98)	15		16	21	18
86 (86)			18	17	37
85 (85,88,91)	11	16	22	28	43
84 (84)			30	10	
72 (78)			19	23	21
69 (69)	23	37	10	12	9
68 (68)		7	29	28	27
59 (65)	50	42	68	72	100
43 (46)	100	100	100	100	86

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

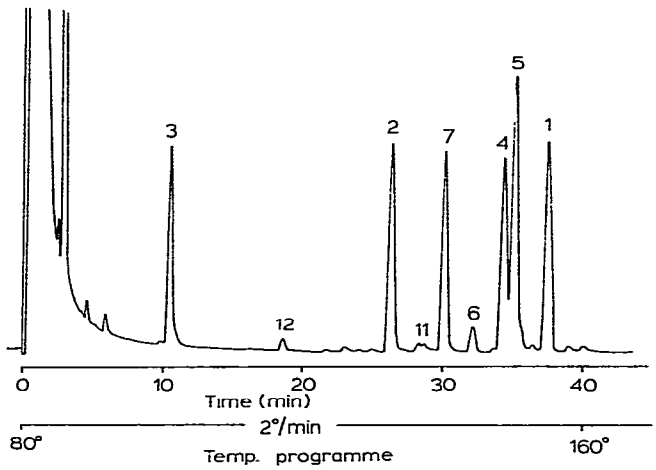

Fig. 1. Gas chromatogram on OV-225 of *O*-isopropylidene-ketoses prepared from a mixture of the parent sugars. The derivatives are those of 1, L-sorbose; 2, D-psicose; 3, L-*erythro*-pentulose; 4, D-*threo*-pentulose; 5, D-fructose; 6, D-fructose (minor); 7, D-tagatose; 11, D-tagatose (minor); and 12, L-arabinose (present in the L-*erythro*-pentulose sample as a contaminant).

the *erythro* analogue of **9**. Compounds **9** and **10** were separated on OV 225, but not on Dexsil 300 which separated the derivatives of the other hexuloses from those of dendroketose (Table I). The electron-impact mass spectra (Table II) of **1**, **5**, and **6** have been discussed by DeJongh and Biemann[9]. Differences between the spectra of the L-sorbose derivative **1** and the main acetal **5** derived from D-fructose are readily observed. For example, "h rupture" affords[13] relatively prominent peaks at *m/e* 159 and 101 in the spectrum of **1**, whereas such a fragmentation is impossible for **5**.

The mass spectra of the di-*O*-isopropylidene derivatives **2** and **7** of D-psicose and D-tagatose show characteristic differences from those of **1** and **5**; fragments having *m/e* 117 and 72 are known to be due to the 1,2-*O*-isopropylidene-grouping[9,10], and the lack of prominent peaks at *m/e* 130 and 115 is indicative of the absence of a 2,3-*O*-isopropylidene group. The spectra of **2** and **7** are qualitatively similar, but the relative abundances of fragment ions having *m/e* 229 and 171, resulting from cleavage of the C-5–C-6 bond and subsequent loss of acetone, are considerably higher in the spectrum of **2**. On the other hand, a peak at *m/e* 109 ($C_6H_5O_2$, unchanged in the spectra of d_{12}-analogues, obtained with acetone-d_6) is more prominent in the spectrum of **7**, and this peak could result from the *m/e* 127 fragment (M^+ — Me — Me_2CO — AcOH) by the loss of water, as observed with 1,2:5,6-di-*O*-isopropylidene derivatives of aldohexoses[14]. The appearance of a peak with relatively high abundance at *m/e* 142 ($C_7H_{10}O_3$, *m/e* 148 in the d_{12}-analogue) in the spectrum of the tagatose derivative **7** is remarkable. Since this fragment is also formed from the L-*erythro*-pentulose derivative **3** and D-psicose acetal **2** in small proportions, it possibly results from the C-1/C-4 part of the molecules after C-4–C-5/C-2–O-5 cleavage and loss of one molecule of acetone. This suggestion is supported by the formation of a characteristic *m/e* 84 fragment-ion ($C_4H_4O_2$, unchanged in the d_{12}-analogues) in high

(from **7**) and intermediate (from **2** and **3**) proportions, which could result from the *m/e* 142 fragment-ion by the loss of a further molecule of acetone. The difference between **2** and **7**, in their tendency to form the *m/e* 142 (and the presumed, subsequent *m/e* 84) fragment, obviously reflects steric differences. The preference of this fragmentation in **7** may explain the low intensities of the *m/e* 229 and 171 peaks in its mass spectrum.

The mass spectra of the dendroketose derivatives **8** (Table II) and **9** (Table III) allow a ready differentiation of the two compounds on the basis of the presence or absence of the characteristic *m/e* 117/72 and *m/e* 130/115 pairs of peaks. The fragments with *m/e* 229 and 171, found in the spectra of all of the other di-*O*-isopropylidene-hexuloses, are not formed from the main acetal **8** of dendroketose, indicating that C-4–C-4′ bond-cleavage is unfavourable.

TABLE III

MASS-SPECTRAL DATA[a] FOR ACETALS **3**, **4**, AND **9**

1,2:3,4-Di-*O*-isopropylidene-β-L-*erythro*-pentulofuranose (**3**): *m/e* 215 (224,28), 172 (178,4), 157 160,2), 155 (161,5), 142 (148,3), 117 (123,27), 114 (114,29), 97 (97,42), 85 (85+91,25), 84 (84,13), 72 (78,20), 59 (65,56), and 43 (46,100).

2,3-*O*-Isopropylidene-β-D-*threo*-pentulofuranose (**4**): *m/e* 175 (178,29), 159 (165,27), 157 (160,13), 130 (136,4), 115 (115+118,15), 101 (101,6), 97 (97+98,19), 71 (71,35), 59 (65,100), and 43 (46,60).

4-*C*-(Hydroxymethyl)-2,3:4,4¹-di-*O*-isopropylidene-β-DL-*threo*-pentulofuranose (**9**): *m/e* 24 5 (254,100), 229 (241,20), 187 (190,22), 171 (177,57), 169 (172,59), 141 (147,32), 131 (137,37), 130 (136,76), 127 (127,26), 115 (118,48), 114 (120,45), 113 (113+119,48), 99 (102,33), 85 (85,22), 83 (83,19), 71 (74,68), 59 (65,70), and 43 (46,100).

[a]The figures in parentheses represent *m/e* of the analogues prepared from hexadeuterioacetone, and relative intensities (%), respectively.

The mass spectrum (Table III) of 1,2:3,4-di-*O*-isopropylidene-β-L-*erythro*-pentulofuranose (**3**) contains peaks corresponding to fragments analogous to those from the 1,2:3,4-di-*O*-isopropylidene-hexuloses **2** and **7**, with the expected modifications due to the lack of an exocyclic hydroxymethyl group. Peaks at *m/e* 114 and 97 (both are unchanged on deuterioacetonation) might be analogues of the *m/e* 144 ($M^+ - 2\ Me_2CO$)[9] and the *m/e* 127 ($M^+ - Me - Me_2CO - AcOH$)[9] peaks in the spectra of the hexulose derivatives. 2,3-*O*-Isopropylidene-β-D-*threo*-pentulofuranose (**4**) also gives a mass spectrum (Table III) in accordance with the known fragmentation-patterns of *O*-isopropylidene derivatives. Most of the peaks observed in the spectrum of 2,3-*O*-isopropylidene-D-ribose[9] are seen, and, in addition, the characteristic *m/e* 130 and 115 fragment-ions are formed.

Since the di-*O*-isopropylidene derivatives of the four unbranched hexuloses and dendroketose were separated on Dexsil 300, g.l.c.–m.s. could be applied in the identification of the triose aldol-condensation products. Peak areas in g.l.c. of the *O*-isopropylidene derivatives were found to be proportional to the concentration of

TABLE IV

PRODUCT COMPOSITIONS AFTER ALDOL CONDENSATION OF DL-GLYCERALDEHYDE AND 1,3-DIHYDROXY-2-PROPANONE WITH DIFFERENT CATALYSTS

Catalyst	*Temperature (degrees)*	*Reaction time (min)*	*Products (%)*[a]				
			DL-*Dendroketose*	DL-*Psicose*	DL-*Tagatose*	DL-*Fructose*	DL-*Sorbose*
$Ca(OH)_2$ (saturated)	60	30	15	12	5	43	25
$Ba(OH)_2$ (saturated)	25	90	10	12	3	45	30
NaOH (0.01M)	25	60	18	8	2	40	32
Dowex-1 (HO^-) resin	25	10	7	11	<1	67	14
Amberlite IRA-400 (HO^-) resin	25	10	<2	6	<1	78	13

[a]Determined from peak areas of gas chromatograms.

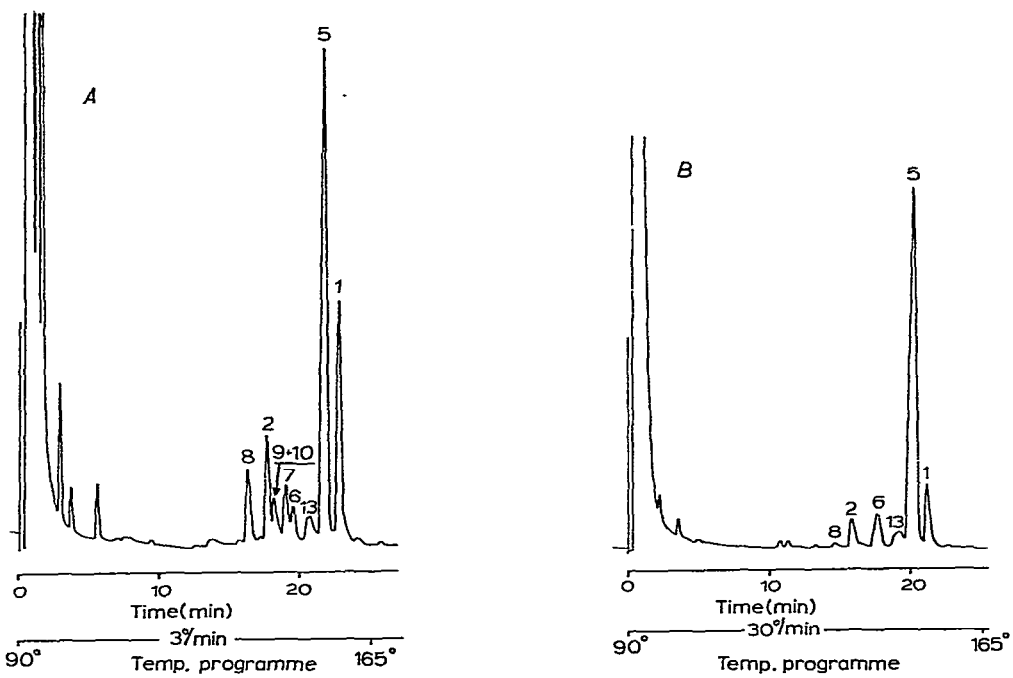

Fig. 2. Gas chromatogram on Dexsil 300 of *O*-isopropylidene derivatives of the products formed in triose aldol-condensations catalysed by *A*, $Ca(OH)_2$; and *B*, Amberlite IRA-400(HO^-) resin. The derivatives are those of 1, DL-sorbose; 2, DL-psicose; 5, DL-fructose; 6, DL-fructose (minor); 7, DL-tagatose; 8, DL-dendroketose; 9 + 10, DL-dendroketose (minor derivatives); and 13, unknown + glucose (formed in trace amounts from fructose under the alkaline conditions).

the parent ketose, and thus determination of the relative proportions of the products formed with different catalysts was also possible (Table IV). Gas chromatograms of the product mixtures from DL-glyceraldehyde–1,3-dihydroxy-2-propanone aldol-condensations, catalysed by calcium hydroxide and a strongly basic anion-exchange resin, respectively, are shown in Figs. 2*A* and 2*B*.

The results strongly support the observation[6] that the relative amount of fructose is enhanced when catalysis is effected with a strongly basic ion-exchange resin. The yield of dendroketose in the resin-catalysed reaction is low, and the amounts of psicose in the product mixtures are considerably higher than expected on the basis of previous reports. Since the proportions of the unbranched hexuloses formed decrease in the order fructose > sorbose > psicose > tagatose, with all of the catalysts, there is evidently a preference, of secondary importance, for formation of compounds having the *erythro* configuration at C-4–C-5, in addition to the primary preference for the formation of products having the *threo* configuration at C-3–C-4.

EXPERIMENTAL

DL-Dendroketose[3], D-psicose[15], and L-*erythro*-pentulose[16] were prepared according to literature methods; the other ketoses were obtained commercially. Thin-layer chromatography (t.l.c.) was performed on silica gel plates with 5:1 chloroform–methanol, and detection with diphenylamine–aniline–phosphoric acid[17]. G.l.c. was performed on a Perkin–Elmer F 11 gas chromatograph, equipped with a flame-ionisation detector and glass columns (6 ft. × 1.5 mm i.d.) filled with 3% of OV-225 and 3% of Dexsil 300 on 100/120 Supelcoport. For the g.l.c.–m.s. analyses, a Varian Aerograph 2400 gas chromatograph was applied in combination with a Micromass 12 F mass spectrometer, operating at 70 eV. High-resolution mass spectra were recorded with an AEI MS-902 mass spectrometer.

Aldol condensation. — DL-Glyceraldehyde (5 mg) and 1,3-dihydroxy-2-propanone (5 mg) were treated with the catalyst (Table IV) in water (2 ml) until t.l.c. showed complete disappearance of the starting materials. The solutions containing sodium, calcium, or barium hydroxide were then neutralised with Dowex 50W(H^+) resin and, after filtration, the solvent was removed under reduced pressure. For the resin-catalysed reactions, the resin was removed by filtration and washed with 50% aqueous acetic acid, and the filtrate and washings were combined before removal of the solvents.

Preparation of the O-isopropylidene derivatives. — The products of the above reactions, or from the concentration of aqueous solutions of reference sugars, were treated with acetone, or acetone-d_6, containing 2% (v/v) of conc. sulphuric acid (2 ml) for 2 h at room temperature. After neutralisation with solid sodium hydrogencarbonate, the solutions were immediately ready for g.l.c.

ACKNOWLEDGMENT

The author thanks Pasteurlegatet for financial support.

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