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TRIMETHYLSILYLATION AND G.L.C.–MASS SPECTROMETRY OF 3-KETOSES AND 2-HEPTULOSES*

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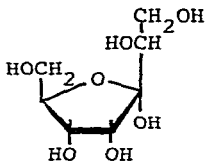
ABSTRACT

G.l.c.–mass spectrometry of trimethylsilylated DL-*gluco*-3-heptulose, D-*manno*-3-heptulose, D-*ido*-3-heptulose, D-*arabino*-3-hexulose, L-*xylo*-3-hexulose, D-*ribo*-3-hexulose, *erythro*-3-pentulose, L-*threo*-3-pentulose, D-*manno*-2-heptulose, D-*gluco*-2-heptulose, and sedoheptulose was performed. 3-Heptuloses and 3-hexuloses, upon trimethylsilylation with chlorotrimethylsilane–hexamethyldisilazane–pyridine, showed a g.l.c. peak for the open-chain form as the largest component. The open-chain form is presumed to have been produced mainly by opening of the hemiacetal ring during trimethylsilylation, and this transformation is regarded as one of the characteristic properties of 3-ketoses in both furanoid and pyranoid forms. The gas chromatograms of 3-heptuloses were analogous to one other, showing furanoid peaks of shorter retention-times than the other tautomers, a pyranoid peak, and a peak for the open-chain form having the longest retention time (OV-17). The main peak for sedoheptulose in g.l.c. was shown to be that of the furanoid form. Trimethylsilylation of coriose was also conducted with 1-(trimethylsilyl)imidazole and *N*,*O*-bis(trimethylsilyl)acetamide.

INTRODUCTION

Although a number of studies have been reported concerning g.l.c. and g.l.c.–mass spectrometry of aldoses and some 2-ketoses, g.l.c.–mass spectrometry of 3-ketoses has not been presented, except for data on α-coriofuranose[1,2] (**1**) and *erythro*-3-pentulose[3] (**2**). Among synthetic 3-heptuloses, only D-*manno*-3-heptulose (**3**) has been obtained crystalline[4]; DL- and D-*gluco*-3-heptulose[5] (**4**), and D-*ido*-3-heptulose[6] (**5**) have been obtained as syrups. The latter two syrupy 3-heptuloses showed four peaks in g.l.c. of their per(trimethylsilyl) derivatives. Among the synthetic 3-hexuloses, namely, D-*arabino*-3-hexulose[7] (**6**) (also produced by *Methylococcus capsulatus*[9]), L-*xylo*-3-hexulose[7] (**7**), L-*lyxo*-3-hexulose[7] (**8**), and D- and DL-*ribo*-3-hexulose[7,8] (**9**), only racemic **9** has been obtained crystalline. *erythro*-3-Pentulose[10,11] and L-*threo*-3-pentulose[11] (**10**) have also been obtained as syrups, as have sedoheptulose (**11**) and

*Part XII of the Series "Coriose and Related Compounds". For Part XI, see ref. 11.



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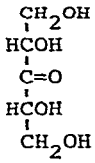

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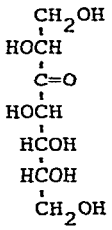

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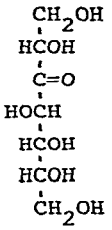

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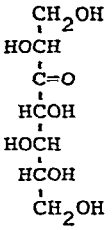

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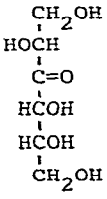

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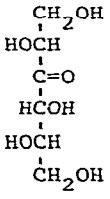

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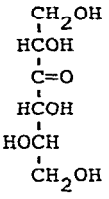

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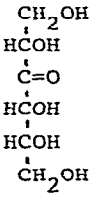

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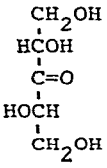

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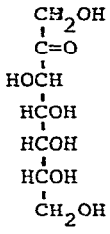

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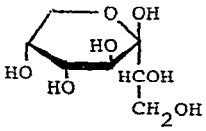

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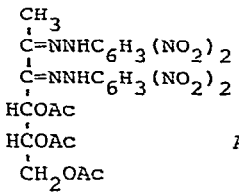

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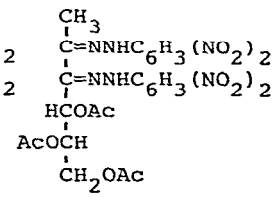

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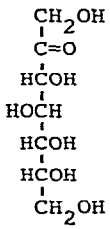

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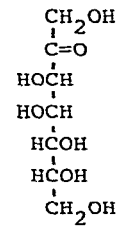

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several other 2-heptuloses. Gas chromatograms of these ketoses are often complicated by unknown equilibrium states in the syrups and in solution, and further by such possible transformations as ring opening upon trimethylsilylation (an example is α-coriofuranose[2]). It is not known whether ring opening upon trimethylsilylation of α-coriofuranose is due to formation of the α-furanose form of coriose, or is caused by the presence of the two-carbon side chain at C-3, although the transformations of the gas chromatogram of *β*-D-*manno*-3-heptulopyranose[12] (**12**) as trimethylsilylation[13] progresses is indicative of the latter. It has also not been determined whether the four peaks in the gas chromatogram of fully trimethylsilylated DL- and D-*gluco*-3-heptulose[5] and D-*ido*-3-heptulose[6] on an OV-17 column arise through equilibration between these syrupy sugars, or through their tautomerization during trimethylsilylation. G.l.c.–mass spectrometry of per(trimethylsilyl) derivatives of 2- and 3-ketoses, including sedoheptulose, is the subject of the present study.

RESULTS AND DISCUSSION

Chlorotrimethylsilane–hexamethyldisilazane in pyridine effected complete trimethylsilylation of crystalline and lyophilized 3-ketoses in 48–120 h; 2-heptuloses required 2–6 h. As successive chromatograms of partially trimethylsilylated 3-ketoses changed in a complicated manner, the present paper is restricted to data obtained on the final products of trimethylsilylation.

The gas chromatogram and the mass spectra of the four components in trimethylsilylated DL-*gluco*-3-heptulose are shown in Fig. 1 and Fig. 2. The components, having R_{Glc} 1.50 (*a*), 1.56 (*b*), 1.91 (*c*), and 2.50 (*d*) on an OV-17 column at 180°, exhibit at highest mass only an $[M - 15]^+$ (*m/e* 627) ion peak, or both an M^+ (*m/e* 642) and an $[M - 15]^+$ ion peak. The mass spectra of *a*, *b*, and *c* show strong *m/e* 437 $[M - \text{-}CHOSiMe_3\text{-}CH_2OSiMe_3]^+$ peaks, indicating elimination of the C-1–C-2 portion from the hemiacetal ring. The mass spectra of *a* and *b* are practically identical, indicating a pair of anomers. Strong ion peaks at *m/e* 217 and weak ones at *m/e* 204 are exhibited by both *a* and *b*, whereas the relative strength of these ion peaks is reversed in *c*. The appearance of a strong $RO\text{-}CH{=}CH\text{-}CH{=}O^+R$ (R = $SiMe_3$: *m/e* 217) ion from furanoid, and $[RO\text{-}CH{=}CH\text{-}OR]^+$ (R = $SiMe_3$: *m/e* 204) ion from pyranoid hemiacetals have previously been recognized for aldoses[14–16] and for some 2-ketoses[3,17]. The fragmentations yielding these ions are presumed to occur also for furanoid and pyranoid forms of 3-ketoses, and occurrence of a strong *m/e* 217 ion accompanied by a weak one at *m/e* 204 in the spectrum of per(trimethylsilylated) α-coriofuranose has already been noted[2]. Therefore, *a* and *b* are considered to be furanoses, and *c* is considered to be a pyranose. Component *d* shows a strong ion at *m/e* 205, together with ions at *m/e* 409, 408, 319, 307, and 305, indicative of fragmentation of a trimethylsilylated, open-chain sugar[2,17]. Compound *d* is thus considered to be 1,2,4,5,6,7-hexakis(trimethylsilyl)-DL-*gluco*-3-heptulose.

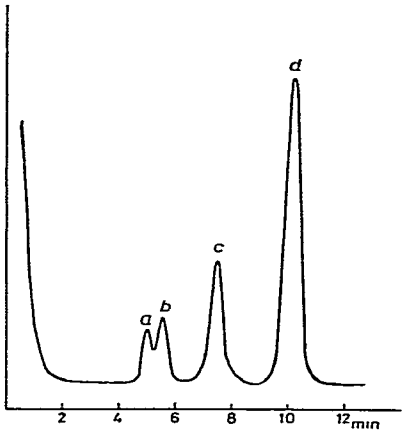

Fig. 1 Gas chromatogram of per(trimethylsilylated) DL-*gluco*-3-heptulose (**4**); 3% OV-17 on 80–100 mesh Chromosorb W in a glass column (2 m × 3 mm i d) at 180°; flow rate of nitrogen 40 ml/min; flame-ionization detector

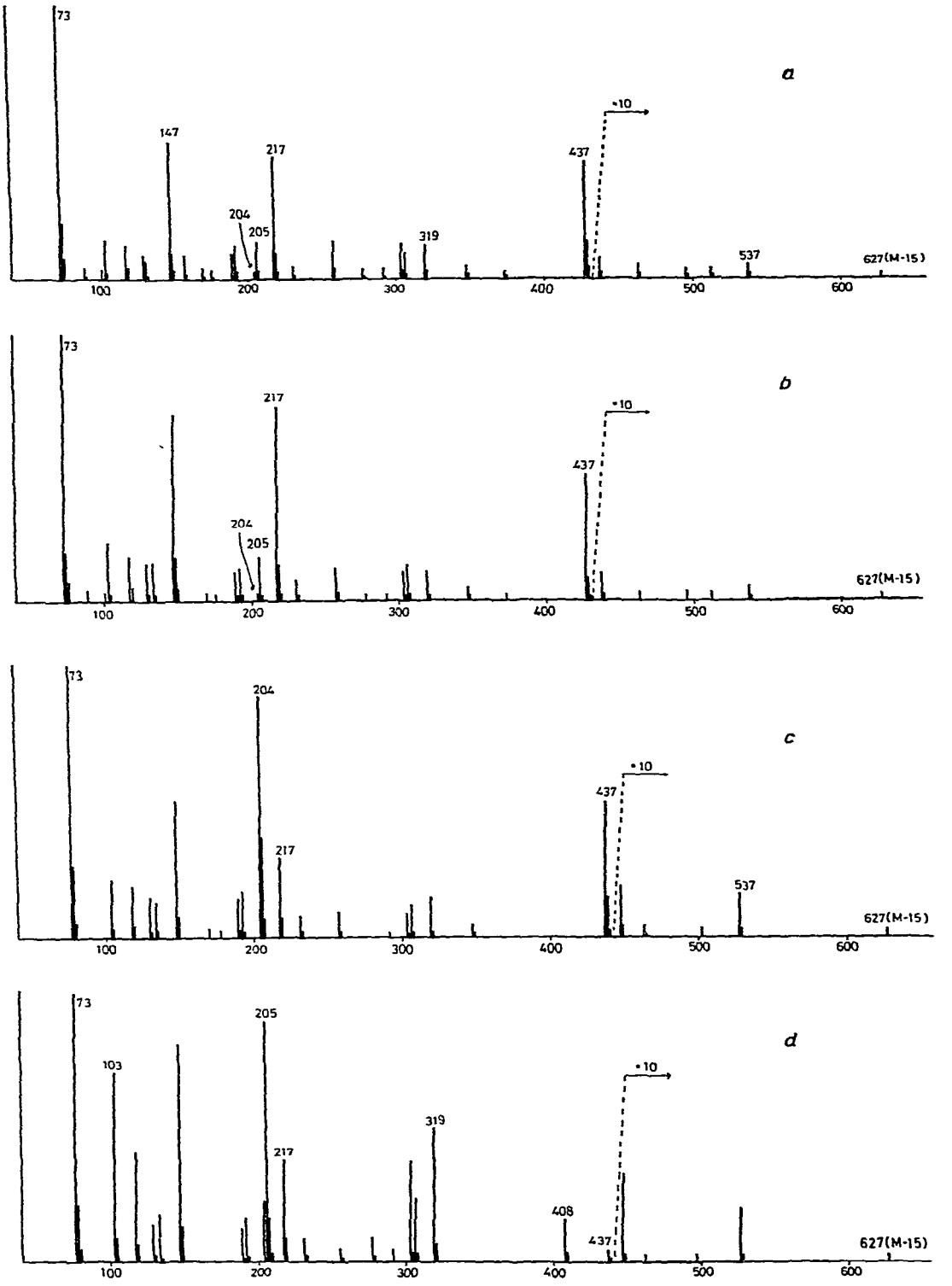

Fig. 2. Mass spectra obtained by g.l c.–mass spectrometry of components *a*, *b*, *c*, and *d* in Fig. 1 of per(trimethylsilylated) DL-*gluco*-3-heptulose.

When g.l.c. of trimethylsilylated DL-*gluco*-3-heptulose was performed on OV-1 and NPGS columns, only three peaks were observed in each gas chromatogram.

The per(trimethylsilylated) DL-*gluco*-3-heptulose was distilled *in vacuo*, and the distillate, whose gas chromatogram was identical to that of the solution in the trimethylsilylating reagent, exhibited four spots in t.l.c. Separation by preparative t.l.c. and then g.l.c. of the isolated components revealed that the slowest-moving spot in t.l.c. is that of open-chain form, and two spots, of moderate mobility and close to each other, are furanoses. The fastest-moving spot in t.l.c. was found by g.l.c. to be a pyranose.

D-*ido*-3-Heptulose also showed four g.l.c. peaks for trimethylsilylated derivatives, R_{Glc} 1.47, 1.58, 2.04, and 2.44, and its gas chromatogram showed marked similarity to that of **4** (Table I). G.l.c.–mass spectrometry showed, as for **4**, that the g.l.c. peak having the shortest retention-time and the second peak are those of furanoses, the third peak is that of a pyranose, and that the fourth (largest) peak is that of the open-chain form.

Crystalline β-D-*manno*-3-heptulopyranose, after complete trimethylsilylation, also showed four peaks, R_{Glc} 1.40, 1.50, 1.79, and 2.30 in g.l.c., and the pattern was analogous to those of **4** and **5** (Table I). Upon g.l.c.–mass spectrometry, the first and second peak in g.l.c. were found identical to each other in the mass spectra, and were shown to be furanoses by strong ion peaks at *m/e* 217 and weak ones at *m/e* 204. The third g l.c. peak exhibited a fairly strong ion peak at *m/e* 204, although the ion peak at *m/e* 217 was also of comparable intensity. This third component is expected to be a pyranose, as the fourth peak was shown to be that of the open-chain form by the strong ion peak at *m/e* 205. Syrupy D-*manno*-3-heptulose was also trimethylsilylated, and no marked difference was observed (g.l.c.) between the crystals and the syrup.

TABLE I

G.L C. DATA FOR PER(TRIMETHYLSILYL) DERIVATIVES OF 3-HEPTULOSES, 3-HEXULOSES, AND 2-HEPTULOSES (3% OV-17)

Ketoses	*Retention time[a] relative to per(trimethylsilyl)ated α-D-glucopyranose*
DL-*gluco*-3-Heptulose (**4**)[b]	1 50 (5 1)[a], 1.56 (10 4), 1.91 (22.8), 2.50 (61.7)
D-*manno*-3-Heptulose (**3**)[b]	1 40 (5.3), 1.50 (9 1), 1.79 (26 3), 2 30 (59 3)
D-*ido*-3-Heptulose (**5**)[b]	1 47 (5 2), 1.58 (8 3), 2 04 (29 8), 2 44 (56 7)
D-*arabino*-3-Hexulose (**6**)[c]	0 82 (31 3), 1 01 (68.7)
L-*xylo*-3-Hexulose (**7**)[c]	0 51 (13 8), 0 65 (11.5), 0 99 (74.7)
D-*ribo*-3-Hexulose (**9**)[c]	0 74 (10 2), 0.84 (14 1), 0 94 (75 7)
D-*gluco*-2-Heptulose (**15**)[b]	1 88 (100)
D-*manno*-2-Heptulose (**16**)[b]	1.67 (100)

[a]Calculated from the ratio of individual peak areas to the total area for the peaks listed [b]Column temperature 180°. [c]Column temperature 170°.

The results with D-*manno*-3-heptulose show that the pyranoid form of this 3-ketose also undergoes ring-opening tautomerization, thus indicating that such tautomerization may occur, regardless of the size of the hemiacetal ring in the 3-ketoses. It is also noteworthy that transformation of the pyranose into the furanose form occurred for D-*manno*-3-heptulose, whereas transformation of the furanose into the pyranose form did not take place for coriose[2] upon trimethylsilylation with Me_3SiCl–hexamethyldisilazane–pyridine.

It is observed that the order of furanose, pyranose, and open-chain form in the gas chromatogram is identical for these three 3-heptuloses. Coriose shows shorter retention-times than these three 3-heptuloses for all of the tautomers. Among these four 3-heptuloses, D-*manno*-3-heptulose shows the second shortest retention-time of the tautomer peaks, except for one of the furanoid peaks. Comparatively large differences of retention time are observed for the pyranoid forms of 3-heptuloses, that of D-*ido*-3-heptulose showing the longest retention-time.

G.l.c.–mass spectrometry of per(trimethylsilylated) 3-hexuloses (which can not form pyranose forms) may be expected to permit confirmation of the g.l.c. peak-assignments for 3-heptuloses. D-*arabino*-3-hexulose, L-*xylo*-3-hexulose, and D-*ribo*-3-hexulose were prepared by acid hydrolysis of 1,2,4,5,6-penta-*O*-acetyl-D-*arabino*-3-hexulose[7], 1,2:3,4-*O*-isopropylidene-L-*xylo*-3-hexulose[7], and 1,2,4,5,6-penta-*O*-acetyl-D-*ribo*-3-hexulose[18], respectively, and their structures were confirmed by formation of the triacetates, **13** and **14**, of the 1-deoxy-(2,4-dinitrophenyl)osazones[19], and by borohydride reduction[19].

Per(trimethylsilylated) D-*arabino*-3-hexulose showed two peaks, R_{Glc} 0.82 and 1.01, in the gas chromatogram (Table I). The peak of longer retention-time showed a strong ion-peak at *m/e* 205, and the peak of shorter retention-time exhibited a strong ion peak at *m/e* 217 and weak one at *m/e* 204; these are assigned, therefore, to open-chain and furanoid form, respectively. The ion peak of *m/e* 218 is exhibited in strength comparable to that of the ion peak at *m/e* 217 (Fig. 3). L-*xylo*-3-Hexulose exhibited three g.l.c. peaks, R_{Glc} 0.51, 0.65, and 0.99 (Table I). The components of the first and second g.l.c. peak showed mass spectra that were practically identical with those of the shorter retention-time g l.c. peak of D-*arabino*-3-hexulose, and the spectra of the third g.l.c. peak showed fragmentation of the characteristic open-chain form. D-*ribo*-3-Hexulose also exhibited three g.l.c. peaks, R_{Glc} 0.74, 0.84 and 0.94. The two faster-moving peaks are regarded as arising from a pair of furanoid anomers, and the third one is assigned to the open-chain form, on the basis of their mass spectra, which are analogous to those of the three components of L-*xylo*-3-hexulose. The common occurrence of a strong ion peak at *m/e* 218, together with an ion peak at *m/e* 217 in the mass spectra of the furanoid form of these three 3-hexuloses is attributed to participation of the C-6 methylene group in formation of the *m/e* 218 ion, which occurred in a way analogous to the fragmentation yielding an *m/e* 217 ion from the furanoid form of trimethylsilylated aldoses and 2-ketoses. 3-Hexuloses resemble 3-heptuloses in that cyclic forms show a shorter retention-time in the gas chromatogram than the open-chain forms. G.l.c.–mass spectrometry of *erythro*-3-

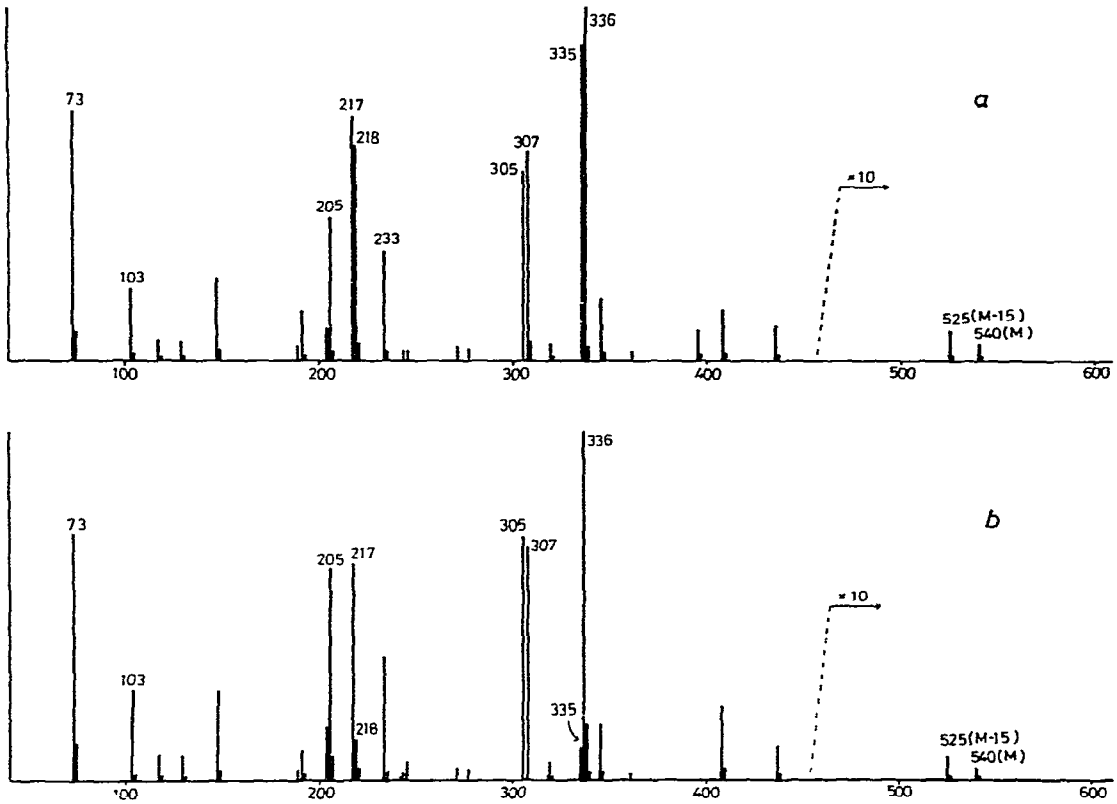

Fig 3. Mass spectra of components of shorter retention-time (*a*) and of longer retention-time (*b*) in g l c. of per(trimethylsilylated) D-*arabino*-3-hexulose

pentulose and L-*threo*-3-pentulose, in accord with that reported for the former[3], showed the characteristic mass spectra of the open-chain form by a single component.

Per(trimethylsilylated) D-*gluco*-2-heptulose (**15**) and D-*manno*-2-heptulose (**16**), which are presumed to form mainly α-pyranoses, based on conformational analysis, exhibited single g.l.c. peaks, R_{Glc} 1.88 and 1.67, respectively. Their mass spectra were almost identical with each other and also with *b* in Fig. 5, exhibiting strong ion peaks at *m/e* 204 and 539 $[M - -CH_2OSiMe_3]^+$. These results are in accord with the ^{13}C-n.m.r. spectral data[21]. Such tautomerization as found upon the trimethylsilylation of 3-ketoses was not observed.

Per(trimethylsilylated) sedoheptulose showed a small peak of shorter retention-time, and the main peak of longer retention time, on an OV-17 column (R_{Glc} 1.30 and 1.48), and also on an OV-225 column, whereas a small peak of longer retention-time was exhibited at the foot of the main peak in the gas chromatogram obtained on OV-210 (Fig. 4). G.l.c.–mass spectrometry showed, by a strong ion peak at *m/e* 217 and a weak one at *m/e* 204, that the main g.l.c. peak is that of the furanoid form, and that the small g.l.c. peak arises from the pyranoid form, by reversed correlation of the strength of these ion peaks (Fig. 5). Syrupy sedoheptulose, therefore, is pre-

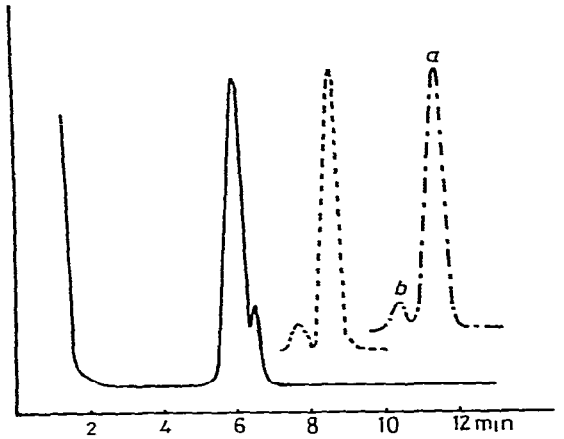

Fig. 4. Gas chromatograms of per(trimethylsilylated) sedoheptulose: 2% OV-210 (solid line); 3% OV-225 (dashed line); 3% OV-17 (dot-dash line); on 80–100 mesh Chromosorb W in a glass column (2 m × 3 mm i.d) at 160°; flow rate of nitrogen 40 ml/min; flame-ionization detector.

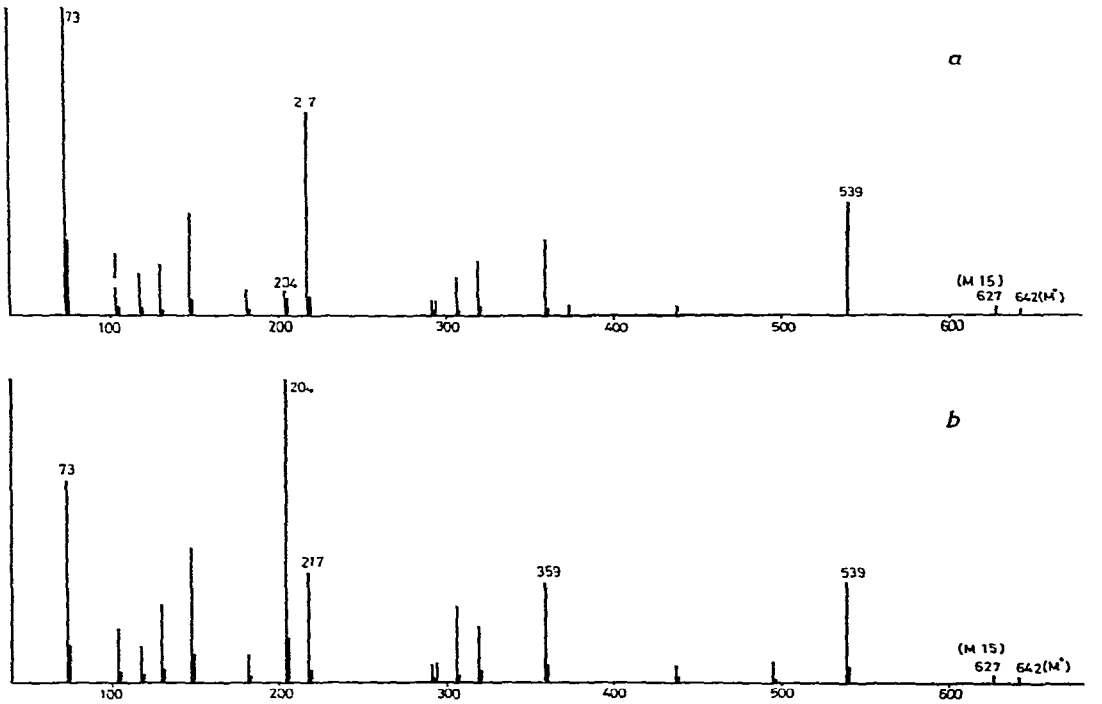

Fig. 5 Mass spectra of components *a* and *b* (Fig. 4) of per(trimethylsilylated) sedoheptulose.

sumed to be mainly furanose, accompanied by a small proportion of pyranose, if tautomerization did not take place upon trimethylsilylation. This presumption is in accord with the ^{13}C-n.m.r. spectral data[21].

The present mass spectra of both furanoid and pyranoid 3-ketoses generally feature a strong $M^+ - 205$ peak (*m/e* 437 for 3-heptuloses and *m/e* 335 for 3-hexu-

loses), which may be produced by elimination of -$CHOSiMe_3$-CH_2OSiMe_3, whereas an $M^+ - 103$ peak is shown by 2-ketoses. These ion peaks are smaller in the open-chain forms of these 3-ketoses, and a fairly strong *m/e* 408 ion-peak is exhibited, as well as *m/e* 103 and 205 ion-peaks, by the open-chain forms of 3-heptuloses.

It must be noted that the equilibrium states of free 3-ketoses could be markedly different from those of their per(trimethylsilyl) derivatives. All 3-ketoses in the present study showed, in the gas chromatograms of their per(trimethylsilyl) derivatives, the open-chain forms as the strongest tautomers. However, these open-chain forms are presumed to have been produced mainly by ring-opening tautomerization during the trimethylsilylation, as in the trimethylsilylation of α-coriofuranose. This presumption is based on the u.v.[21], o.r.d., c.d., and ^{13}C-n.m.r.[21,22] spectra of 3-ketoses, which do not exhibit such large proportions of the open-chain forms, although the proportions of open-chain forms in equilibrated solutions of 3-ketoses are generally found higher than those of the 2-ketoses. Such ring-opening tautomerization upon trimethylsilylation of 3-ketoses is considered to be due to the presence of the two-carbon side chain at the hemiacetal carbon. 2-Ketoses, and aldoheptoses that have the two-carbon side chain on the other carbon atom, did not show such transformations upon trimethylsilylation under analogous reaction conditions.

The furanose–pyranose ratio, and α,β anomeric ratio, in the syrupy free 3-ketoses could also be different from those of the trimethylsilyl derivatives, as there could be differences of velocity of the ring-opening tautomerization among the tautomers of each 3-ketose.

As 1,2,4,5,7-pentakis(trimethylsilyl)-α-coriofuranose has been found as the initial product of the trimethylsilylation of α-coriofuranose[2], and the trimethylsilylation of 2-ketoses has also been found to proceed *via* initial products having a free hemiacetal hydroxyl group[23], analogous initial products having a free hydroxyl group at C-3 are presumed to have been formed upon the trimethylsilylation of 3-ketoses in the present study.

Trimethylsilylation with other reagents, namely, 1-(trimethylsilyl)imidazole and *N,O*-bis(trimethylsilyl)acetamide, was performed for α-coriofuranose. Trimethylsilylation with 1-(trimethylsilyl)imidazole was complete within 20 min to give a gas chromatogram in which two peaks were identified with those of 1,2,3,4,5,7-hexakis(trimethylsilyl)-α-coriofuranose, and 1,2,4,5,6,7-hexakis(trimethylsilyl)coriose. The second peak (*b*), upon g.l.c.–mass spectrometry, showed a mass spectrum almost identical to that of the first peak, and it was assigned as 1,2,3,4,5,7-hexakis(trimethylsilyl)-β-coriofuranose (Fig. 6). Therefore, transformation of α-coriofuranose into pyranoses did not take place with these two reagents. Trimethylsilylation of coriose equilibrated in pyridine and dimethyl sulfoxide, with 1:2 chlorotrimethylsilane–hexamethyldisilazane, gave products whose gas chromatograms were almost identical and identical with that of the product obtained by trimethylsilylation of α-coriofuranose with *N,O*-bis(trimethylsilyl)acetamide. However, the mass spectrum of the component exhibiting the second g.l.c. peak showed an ion peak at *m/e* 204 of about one half the height of the ion peak at *m/e* 217. As the *m/e* 204 ion-peaks of per(trimethyl-

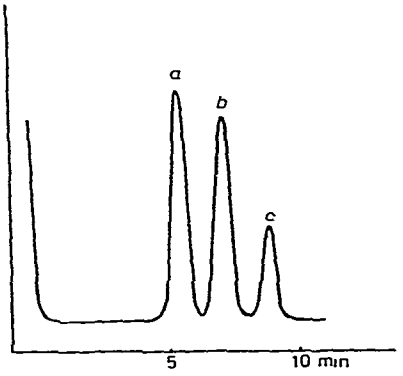

Fig 6. Gas chromatogram of coriose, per(trimethylsilylated) with *N,O*-bis(trimethylsilyl)acetamide; 3% OV-17 on 80–100 mesh Chromosorb W in a glass column (2 m × 3 mm i d.) at 180°; flow rate of nitrogen 40 ml/min; flame-ionization detector.

silylated)coriofuranoses are less than 7% of the height of the *m/e* 217 ion-peak, the second g.l.c. peak of the per(trimethylsilyl) derivative of equilibrated coriose, having an identical retention time as that of *b*, is regarded as arising from a mixture of furanoid and pyranoid forms.

EXPERIMENTAL

General methods. — Trimethylsilylation was effected with 1:2:10 chlorotrimethylsilane–hexamethyldisilazane–pyridine, 1:4 1-trimethylsilylimidazole–pyridine, or 1:4 *N,O*-bis(trimethylsilyl)acetamide–pyridine. G.l.c. was performed with a Shimadzu 5A gas chromatograph equipped with a flame-ionization detector and a glass column (2 m × 3 mm i.d.) packed with methyl silicone (1% OV-1 or 1.5% SE-30), methyl phenyl silicone (3% OV-17 or 1.5% SE-52), fluoropropyl silicone (2% OV-210), nitrile silicone (3% OV-225), ethylene glycol succinate (3.5% EGS), or neopentyl glycol succinate (3.5% NPGS) on 80–100 mesh Chromosorb W treated with hexamethyldisilazane. R_{Glc} is the retention time relative to that of per-*O*-(trimethylsilyl)-α-D-glucose. G.l.c.–mass spectrometry was conducted with Shimadzu-*LKB 9000 gas chromatograph*–mass spectrometer. The columns used for g.l.c. were applied as the introductory columns. The temperature of the ion source was 250°, ion-accelerating voltage 3.5 kV, ionizing potential 70 eV, trap current 60 μA. Background peaks were subtracted from the mass spectra. ^{1}H-N.m.r. spectra were determined at 90 MHz with a Hitachi R-22 spectrometer, and chemical shifts (δ) are given in p.p.m. relative to tetramethylsilane as the internal standard. T.l.c. was performed on Silica Gel G (Merck), developing with 1:1 petroleum ether (b.p. 60–80°)–benzene, and detection was effected with conc. sulfuric acid. Extraction of preparative t.l.c. zones was performed with chloroform. Paper chromatography (p.c.)

employed as developers solvent *A* (6:4:3 1-butanol–pyridine–water), solvent *B* (4:1:5, upper phase, 1-butanol–acetic acid–water), or solvent *C* (4:1.2:1 1-butanol–ethanol–water), and detection was effected with 1:30:300 orcinol–trichloroacetic acid–1-butanol. Concentration of solvents was carried out *in vacuo* below 40°.

D-ribo-*3-Hexulose*. — To a solution of 1,2,4,5,6-penta-*O*-acetyl-D-*ribo*-3-hexulose[18] (350 mg) in acetone (1 mL), 1.5% sulfuric acid in methanol was added. The mixture was kept overnight, diluted with water (30 mL), and extracted 3 times with dichloromethane. The aqueous layer was neutralized with barium hydroxide, and centrifuged. The supernatant liquor was evaporated to a syrup (110 mg) that was purified by preparative p.c., developing with solvent *A*, to give a syrup (43 mg) that showed a greyish-brown, single spot on p.c. by the orcinol reagent; $[\alpha]_D^{13}$ −32° (*c* 0.78, water); p.c.: R_F 0.38 (solvent *A*) and 0.12 (solvent *B*); g l.c. (OV-17, 170°)· R_{Glc} 0.74, 0.84, and 0.94.

Anal. Calc. for $C_6H_{12}O_6 \cdot H_2O$: C, 36.36; H, 7.12. Found: C, 36.29; H, 7.33.

A solution of sodium borohydride (1.2 mg) in water (1 mL) was added to an ice-cooled aqueous solution (1 mL) of D-*ribo*-3-hexulose (5 mg) obtained as just described. The mixture was refrigerated overnight, passed through a column of IR-120 (H^+) resin, and the aqueous eluate was evaporated. Methanol (2 mL) was added to the residue, and evaporated off. This procedure was repeated five times to give a syrup that was trimethylsilylated (chlorotrimethylsilane–hexamethyldisilazane–pyridine), and was identified by gas chromatography as a mixture of allitol and glucitol; R_{Glc} 1.41 (OV-17), 1.22 (SE-52).

1-Deoxy-(2,4-dinitrophenyl)osazone triacetate from D-ribo-*3-hexulose*. — A solution of D-*ribo*-3-hexulose (30 mg) in 2M hydrochloric acid (2 mL) was kept for 30 min in a boiling-water bath, and a warm solution of (2,4-dinitrophenyl)-hydrazine (50 mg) in a mixture of 2M hydrochloric acid (10 ml) and ethanol (1 mL) was added. The mixture was kept for an additional 1 h in a boiling-water bath, and the crystals (63 mg) deposited were filtered from the warm solution. Acetic anhydride (0.5 mL) was added to a solution of this product (43 mg) in pyridine (0.5 mL). The mixture was kept overnight, poured into ice–water, and extracted with dichloromethane. The dichloromethane layer was washed with 5% hydrochloric acid, aqueous sodium hydrogencarbonate, and water, and dried (magnesium sulfate). Evaporation of the solvent yielded a syrup (59 mg) that was purified by column chromatography (silica gel, Wakogel C-200, chloroform) to yield a syrup that was crystallized and recrystallized from ethanol to give orange needles (23 mg), m.p. 177–179° (dec.); n.m.r. (chloroform-*d*): δ 1.96 (s, Ac), 1.98 (s, Ac), 2.22 (s, Ac), 2.44 (s, Me-C=N-), 4.13 (dd, $J_{6,6'}$ 12 Hz, $J_{5,6}$ 6 Hz, H-6), 4.42 (dd, $J_{5,6'}$ 4 Hz, H-6'), 5.69 (m, H-5), 7.03 (d, $J_{4,5}$ 5.4 Hz, H-4), 7.90–10.40 (6 H, arom. H), 12.27 (s, H-bonded NH), and 14.04 (s, H-bonded NH).

Anal. Calc. for $C_{24}H_{24}N_8O_{14}$: C, 44.45; H, 3.73; N, 17.28. Found: C, 44.28; H, 3.89; N, 17.01.

D-arabino-3-*Hexulose*. — A solution of 1,2,4,5,6-penta-*O*-acetyl-D-*arabino*-3-

hexulose[7] (1.0 g) in methanol (20 mL) containing 1.5% of sulfuric acid was kept for 36 h, and then evaporated *in vacuo* to a syrup that was dissolved in water (20 mL). The solution was extracted twice with dichloromethane, the aqueous layer was neutralized with barium hydroxide, and the supernatant liquor was evaporated to a syrup that was purified by preparative p.c., developing with solvent *A*, and extracting with water the area giving a greyish-brown spot with the orcinol reagent. A syrup (230 mg) was obtained; $[\alpha]_D^{20}$ −32.8° (*c* 5.15, water); p.c. R_F 0.47 (solvent *A*), 0.25 (solvent *B*) and 0.34 (solvent *C*); g.l.c. of trimethylsilyl derivative R_{Glc} 0.82 and 1.01 (OV-17, 170°), 0.99 and 1.02 (SE-30, 180°), 1.00 and 1.09 (SE-52, 180°).

Anal. Calc. for $C_6H_{12}O_6 \cdot H_2O$: C, 36.36; H, 7.12. Found: C, 36.09; H, 7.41.

Reduction of this 3-hexulose (5 mg) with sodium borohydride as for D-*ribo*-3-hexulose gave a syrup that was trimethylsilylated (chlorotrimethylsilane–hexamethyldisilazane–pyridine) for g.l.c., whereupon two peaks corresponding to mannitol (R_{Glc} 1.25, OV-17, 180°; 1.21, SE-52, 160°) and altritol (R_{Glc} 1.39, OV-17, 180°; 1.31, SE-52, 160°) were detected.

1-Deoxy-(2,4-dinitrophenyl) osazone triacetate from D-arabino-*3-hexulose.* — This product was prepared as for that from D-*ribo*-3-hexulose, and the product was found identical to that from D-*ribo*-3-hexulose, by i.r. and n m.r. spectra.

L-xylo-*3-Hexulose.* — A suspension of 1,2:3,4-di-*O*-isopropylidene-L-*xylo*-3-hexulose (1.0 g) in 1% sulfuric acid was kept for 5 h at 55°, cooled, and extracted with dichloromethane. The aqueous layer was neutralized with saturated aqueous barium hydroxide and centrifuged. The supernatant liquor was evaporated to a syrup (0.6 g) that was purified by preparative p.c., developing with solvent *A*, and extracting with water the area that showed a greyish-brown color with the orcinol reagent. The extract was evaporated to a syrup (390 mg); $[\alpha]_D^{20}$ +22° (*c* 4.8, water); p.c.: R_F 0.50 (solvent *A*), 0.26 (solvent *B*), and 0.33 (solvent *C*); g.l.c. of the trimethylsilyl derivative: R_{Glc} 0.51, 0.65, and 0.99 (OV-17, 170°); 0.65, 0.84, and 1.01 (SE-30, 180°); and 0.77, 0.92, and 1.15 (SE-52, 180°).

Anal. Calc. for $C_6H_{12}O_6 \cdot H_2O$: C, 36.36; H, 7.12. Found: C, 36.49; H, 7.25.

Reduction of the aforementioned L-*xylo*-3-hexulose (7 mg) was performed with sodium borohydride as for other 3-hexuloses, and the syrupy product was trimethylsilylated. The retention time of the trimethylsilylated product was identical with both corresponding derivatives of galactitol [R_{Glc} 1.38 (OV-17, 180°) and 1.26 (SE-52, 160°)], and glucitol [R_{Glc} 1.38 (OV-17, 180°) and 1.25 (SE-52, 160°)].

1-Deoxy-(2,4-dinitrophenyl)osazone triacetate from L-xylo-*3-hexulose.* — This product was prepared as for that from the other 3-hexuloses; m.p. 224–226° (dec.); $[\alpha]_D^{23}$ −164° (*c* 0.42, chloroform); n.m.r. (dimethyl sulfoxide-d_6): δ 1.78 (s, OAc), 2.01 (s, OAc), 2.19 (s, OAc), 2.39 (s, Me-C=N-), 4.18 (dd, $J_{6,6'}$ 11.5 Hz, $J_{5,6}$ 7.2 Hz, H-6), 4.51 (dd, $J_{5,6'}$ 5 Hz, H-6'), 5.58 (m, H-5), 6.76 (d, $J_{4,5}$ 3 Hz, H-4), 7.9–9.0 (6 H, aromatic), 11.25 (s, H-bonded NH), and 12.53 (s, H-bonded NH).

Anal. Calc. for $C_{24}H_{24}N_8O_{14}$: C, 44.45; H, 3.73; N, 17.28. Found: C, 44.09; H, 3.67; N, 17.05.

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