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GAS CHROMATOGRAPHIC RETENTION OF CARBOHYDRATE TRIMETHYLSILYL ETHERS

III^a. KETOHEXOSES

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SUMMARY

Five tautomeric forms, four cyclic and one acyclic, of the four ketohexoses (fructose, sorbose, tagatose and psicose) were separated as their O-trimethylsilyl (TMS) ethers by gas chromatography on several capillary columns, and identified by gas chromatography – mass spectrometry. The retention indices changed slightly with temperature. As with aldoses, their chromatographic behaviour was different from that of other ethers. Principal component analysis showed the specific behaviour of some phases towards some compound groups. A mathematical approach developed for aldoses was applied to ketohexose retention indices in order to relate them to their structural characteristics. The results were similar to those found for aldoses, except for pyranose forms, where equatorial OTMS groups seemed to have a negative effect on the retention.

INTRODUCTION

Ketohexoses are carbohydrates of great interest in many fields of chemistry. Although the physical and chemical properties of fructose have been extensively described and its five equilibrium tautomeric forms have been quantified by NMR spectroscopy^{1–4} and gas chromatography (GC)^{5–7}, data on the tautomers of the other three ketoses are scarce. The retention times of their O-isopropylidene derivatives⁸ and O-*n*-butoxime pertrifluoroacetates⁹ on OV-225 have been reported. Nevertheless, these methods do not afford peaks corresponding to the present tautomeric forms. Hence, although complete NMR data exist for psicose⁴, sorbose^{3,10} and tagatose^{4,10}, only a few GC peaks have been identified for the trimethylsilyl derivatives of sorbose^{11,12}; eight unidentified peaks have been reported for those of tagatose¹³ and no data exist for psicose.

^a For Part II, see ref. 15.

TABLE I

CAPILLARY COLUMNS USED FOR GC ANALYSIS OF TMS ETHERS OF CARBOHYDRATES

<i>Stationary phase</i>	<i>Origin</i>	<i>Material</i>	<i>Dimensions (m × mm I.D.)</i>	<i>Temperature (°C)</i>
OV-1	Laboratory-made	Pyrex glass	25 × 0.22	170,180,190
OV-17	Chrompack	Fused silica	25 × 0.22	150,160,180
OV-215	Laboratory-made	Pyrex glass	25 × 0.18	180,190
OV-225	Laboratory-made	Pyrex glass	25 × 0.18	170,180,190
FFAP	Perkin-Elmer	Fused silica	24 × 0.22	150,160,170

In previous papers^{14,15}, chromatographic retention data for aldopentose and aldohexose O-trimethylsilyl ethers were presented. Some general features were found: an unusual chromatographic behaviour when these compounds and other ethers are compared; a decrease in their retention indices with increasing temperature; and a relationship between retention and structure, in which descriptors related to a planar conformation contribute positively to the retention.

In this work, GC retention data have been obtained for the TMS ethers of the five tautomeric forms of each ketohexose. Some aspects of their chromatographic behaviour are outlined, and their retention indices on several stationary phases are correlated with different structural parameters as was done with aldoses.

EXPERIMENTAL

Samples

D-Fructose and L-sorbose were obtained from Fluka (Buchs, Switzerland) and D-tagatose and D-psicose from Sigma (Eisenhofen, F.R.G.). Pure samples of sugars were dissolved in water, pyridine or dimethyl sulphoxide and left to stand until equilibrated. Aliquots containing about 1 mg of carbohydrate were silylated^{7,14,16}. Aqueous samples were first lyophilized.

GC analysis

Chromatographic analysis was carried out as described previously^{14,15}. The columns and conditions are summarized in Table I. Four of the stationary phases were silicones with different substituent groups (methyl, phenyl, trifluoropropyl, cyanopropyl) and the most polar was FFAP (polyethylene glycol 20M esterified with nitroterephthalic acid).

Kováts retention indices were calculated from the retention times of TMS ethers and suitable *n*-alkanes. The dead time was determined by linear regression¹⁷.

Calculations

Calculations of retention indices and normal and stepwise linear regressions were carried out on an Olivetti M-20 microcomputer.

RESULTS AND DISCUSSION

Identification

Silylated equilibrium mixtures of each of the four ketohexoses were analysed by GC-mass spectrometry (GC-MS). Chromatographic patterns on OV-17 are presented in Fig. 1. The five peaks of each sugar were well resolved under these conditions. The proportion of acyclic forms in fructose, tagatose and psicose is clearly excessive, and depends on the silylation method used¹⁸; however, it was useful in order to obtain good mass spectra.

The ring size of cyclic forms could be clearly assigned from the mass spectra. Furanoses were characterized by a very intense fragment at m/z 217 and pyranoses by a high value of the ratio m/z 204/217. The fragment at m/z 437, typical of ketoses¹⁹ was also an intense peak in both furanoses and pyranoses. Open forms were detected by the very intense fragment at m/z 306 (ref. 6).

Some traces of sorbose were detected in tagatose, as previously found by Angyal²⁰ in the NMR spectrum. Sorbose and tagatose also contained a component which was characterized from its mass spectrum as a 2,5-hexodiulose, with the base peak at m/z 305, as described by Novina¹¹. This can explain the eight peaks found by Tesařík¹³ for tagatose samples.

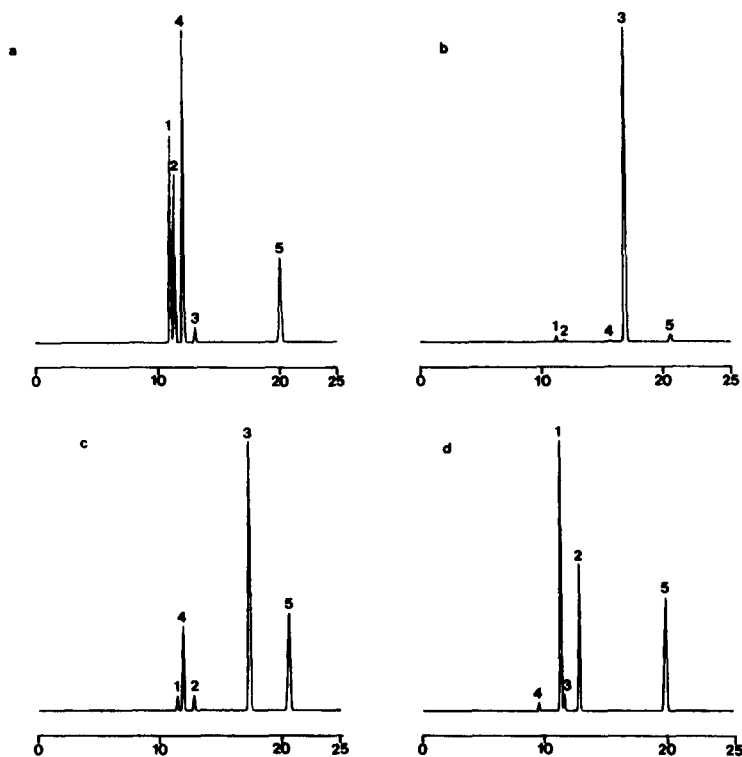


Fig. 1. Chromatographic patterns of ketohexose TMS ethers on OV-17 at 150°C. (a) Fructose; (b) sorbose; (c) tagatose; (d) psicose. Peaks: 1 = α -furanose; 2 = β -furanose; 3 = α -pyranose; 4 = β -pyranose; 5 = acyclic form.

TABLE II
RETENTION INDICES OF KETOHEXOSE TMS ETHERS (*Lx*)

No.	Component	Stationary phase, McReynolds polarity, temperature (°C)				
		OV-1, 217, 180	OV-17, 884, 180	OV-215, 1545, 180	OV-225, 1813, 180	FFAP, 2546, 170
1	α -Psicofuranose	1875	1755	1752	1883	1709
2	β -Psicofuranose	1911	1783	1804	1938	1762
3	α -Psicopyranose	1897	1763	1781	1900	1709
4	β -Psicopyranose	1869	1719	1734	1868	1673
5	Acyclic form	1956	1896	1952	2124	1869
6	α -Fructofuranose	1849	1763	1730	1877	1693
7	β -Fructofuranose	1857	1763	1757	1877	1712
8	α -Fructopyranose	1873	1804	1800	1928	1731
9	β -Fructopyranose	1867	1775	1745	1877	1685
10	Acyclic form	1932	1887	1942	2099	1859
11	α -Sorbofuranose	1835	1746	1717	1858	1682
12	β -Sorbofuranose	1846	1762	1731	1858	1696
13	α -Sorbopyranose	1908	1844	1813	1949	1757
14	β -Sorbopyranose	1908	1833	1813	1949	1757
15	Acyclic form	1922	1885	1939	2092	1847
16	α -Tagatofuranose	1861	1764	1740	1896	1721
17	β -Tagatofuranose	1871	1788	1767	1926	1753
18	α -Tagatopyranose	1935	1860	1832	1983	1776
19	β -Tagatopyranose	1879	1780	1751	1913	1698
20	Acyclic form	1929	1891	1945	2119	1853

The α - and β -forms were assigned by comparison with NMR equilibrium data in water or pyridine, as in previous work²¹. For psicose, assignment of α - and β -pyranoses was made according to the equilibrium values in dimethyl sulphoxide²².

The retention indices of the different tautomeric forms are presented in Table II.

Effect of temperature

The retention indices of ketohexoses changed slightly with temperature, except in the FFAP column, where the change was more evident (see Table III).

Effect of stationary phase

As with other monosaccharides^{14,15}, no correlation was found between the polarity of the stationary phase and retention indices. The overall retention was higher on OV-225 and OV-1, decreased on OV-215 and OV-17 and attained a minimum on FFAP (it was not possible to obtain data at 180°C because the retention times became too short to be measured reliably). Comparative data for other monosaccharides are shown in Table IV.

A data matrix including the retention indices in Table II (180°C for the silicone

TABLE III

TEMPERATURE DEPENDENCE OF THE RETENTION INDICES OF KETOHEXOSE TMS ETHERS ON SEVERAL STATIONARY PHASES ($\Delta t/10^\circ\text{C}$ VALUES)

Component	Stationary phase and temperature range ($^\circ\text{C}$)				
	OV-1, 170-190	OV-17, 150-180	OV-215, 180-190	OV-225, 170-190	FFAP, 150-170
α -Fructofuranose	-4	-5	-13	-13	-28
β -Fructofuranose	-5	-8	-7	-13	-28
α -Fructopyranose	-14	-5	-5	-8	-24
β -Fructopyranose	-2	-9	-12	-13	-24
Acyclic form	-4.5	-9	2	-2	-23
α -Sorbofuranose	-2.5	-9	-12	-16	-30
β -Sorbofuranose	-2.5	-8	-12	-16	-30
α -Sorbopyranose	-0.5	-7	-5	-11	-23
β -Sorbopyranose	-0.5	-6	-5	-11	-23
Acyclic form	-2	-9	1	-4.5	-25
α -Tagatofuranose	-9	-7	-2	-11	-28
β -Tagatofuranose	-8	-8	-3	-9	-23
α -Tagatopyranose	-1.5	-7	-1	-5	-22
β -Tagatopyranose	-2	-5	0	-11	-24
Acyclic form	-5	-9	2	1	-23
α -Psicofuranose	-	-7	-14	-6	-27.5
β -Psicofuranose	-	-8	-16	-4	-30
α -Psicopyranose	-	-6	-12	-8	-27.5
β -Psicopyranose	-	-6	-12	-10	-27
Acyclic form	-	-3	-3	4	-26

phases, 170°C for FFAP) was submitted to principal component analysis²³. The first component values can be used as an estimate of the relative polarity of the stationary phases towards the selected compounds, and they are usually positively correlated with the McReynolds polarity values. The other components are related to the selectivity of the stationary phases towards some compounds in the data set; however, the physical significance of the lower components is less reliable, as they are more affected by experimental errors²³. Fig. 2 shows a plot of the first (a) and second (b) component loadings against the stationary phase polarity according to McReynolds. No correlation is found for the first component, and the second-component plot shows a correlation only for the silicone-based stationary phases. The third and fourth components are related to OV-17 and FFAP, respectively.

Open forms can be clearly distinguished from the others from their high second-component scores, as their retention is relatively higher for the polar silicone stationary phases. The negative third-component scores for psicose forms are related to the low retention of these compounds on OV-17. Furanose and pyranose forms can be distinguished from their fourth-components scores, as FFAP retains the furanoses selectively.

A plot of second- and fourth-component scores (Fig. 3) shows that open forms, furanoses and pyranoses can be clearly distinguished.

TABLE IV

ELUTION RANGE (i.u.) OF MONOSACCHARIDES ON DIFFERENT STATIONARY PHASES

Temperatures (°C) are given in parentheses.

Stationary phase	Polarity	Aldopentoses	Aldohexoses	Ketohexoses
OV-1	217	—	—	1830–1955 (170–190)
SE-54	334	1610–1780 (180–200)	1810–2020 (180–200)	—
OV-17	884	1585–1760 (160–190)	1730–2000 (160–190)	1720–1920 (150–180)
OV-215	1545	1610–1845 (150–160)	1740–2175 (170)	1705–1950 (180–190)
OV-225	1813	1600–1720 (150–160)	1700–1985 (170)	1840–2110 (170–190)
Carbowax 20M	2308	1545–1860 (160)	1695–1972 (160)	—
FFAP	2546	—	—	1670–1920 (150–170)

Effect of the carbohydrate structure

The most retained peaks were usually the acyclic forms (see Table II). The elution order was sorbose, fructose, tagatose and psicose (except on FFAP, where tagatose eluted before fructose) (Scheme 1). The I_x values on each phase were very close, covering a maximum of 35 i.u. A similar behaviour has been reported for isomeric alditols²⁴.

The elution orders of pyranose forms were similar on the five stationary phases used. The β -anomers eluted before the α -anomers. When conformation 5C_2 is as-

TABLE V

STRUCTURAL DESCRIPTORS AND CODES

Code	Value	Structural significance
P/F	0–1	Pyranose/furanose ring
Σ Eq	0–4	Total number of equatorial OTMS groups
Σ 2c	0–3	Total number of OTMS pairs in a <i>cis</i> configuration
Σ 3c	0–2	Total number of OTMS triplets in a <i>cis</i> configuration
Eq2	0–1	OTMS equatorial at C-2 (α/β)
Eq3	0–1	OTMS equatorial at C-3
Eq4	0–1	OTMS equatorial at C-4
23c	0–1	OTMS/OTMS <i>cis</i> at C-2–3
23C	0–1	OTMS/CH ₂ OTMS <i>cis</i> at C-2–3
34c	0–1	OTMS/OTMS <i>cis</i> at C-3–4
45c	0–1	OTMS/OTMS <i>cis</i> at C-4–5
13c	0–2	OTMS/OTMS/OTMS <i>cis</i>
13C	0–2	OTMS/OTMS/CH ₂ OTMS <i>cis</i>
13a	0–1	OTMS axial at C-2 and C-4
13A	0–1	CH ₂ OTMS axial in C-2 and OTMS axial at C-4

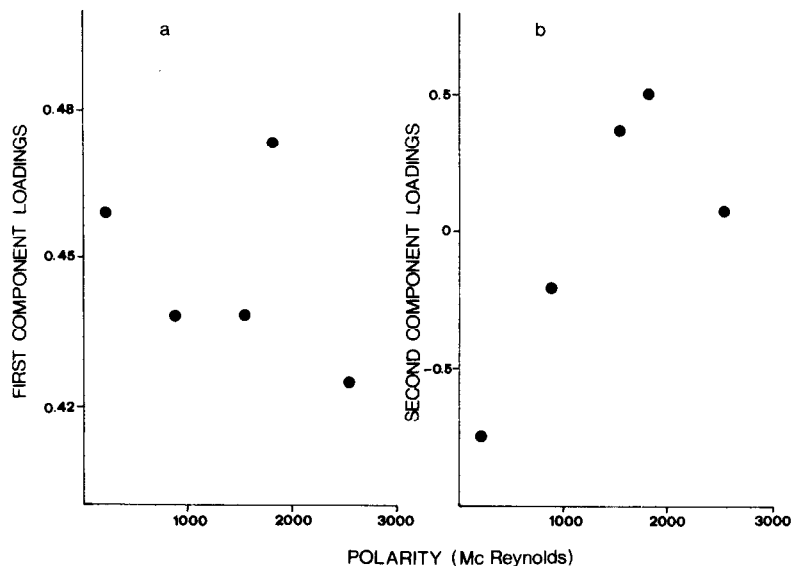


Fig. 2. (a) First- and (b) second- component loadings, calculated from data in Table II, versus McReynolds polarity.

sumed, the bulky CH_2OTMS group is axial in β -anomers (Scheme 2) and equatorial in α -anomers. Hence these are more planar molecules and their retention would be greater, as was found for similar compounds¹⁴⁻¹⁶.

The most retained compound on the five stationary phases was the TMS deriv-

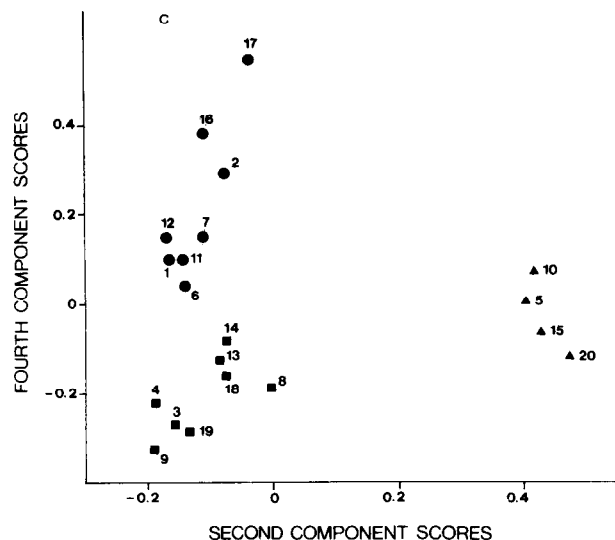
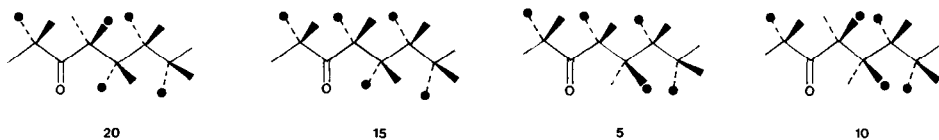


Fig. 3. Plot of second- and fourth-component scores calculated from data in Table II: (●) furanoses; (■) pyranoses; (▲) acyclic forms.



Scheme 1.

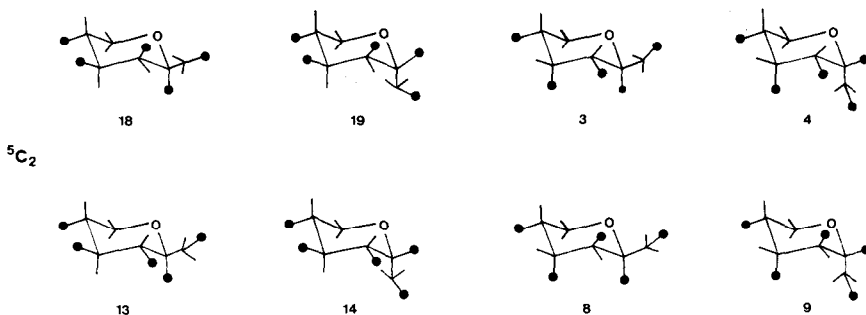
ative of α -tagatopyranose, followed by those of α - and β -sorbitose. β -Psicopyranose and β -fructopyranose were the least retained compounds. The better resolved anomeric pair corresponded to tagatose (covering a range from 56 i.u. on OV-1 to 82 i.u. on OV-17) and the least resolved corresponded to sorbitose, whose α - and β -anomers coeluted on most phases.

Aldopyranoses and ketopyranoses show a different chromatographic behaviour: the aldopyranoses having an all-equatorial substitution (β -xylose among pentoses¹⁴ and β -glucose among hexoses¹⁵) were the most retained, whereas the most retained ketopyranose (α -tagatose) has an axial substituent at C-3.

The elution order of ketofuranose TMS ethers on the five columns was also regular. The α -anomers eluted before the β -anomers. The least retained compound was always α -sorbofuranose and the most retained was β -psicopyranose. The least separated anomeric pairs were those corresponding to sorbitose and fructose, and the best resolved corresponded to psicose (which ranged from 40 i.u. on OV-1 to 55 i.u. on OV-255).

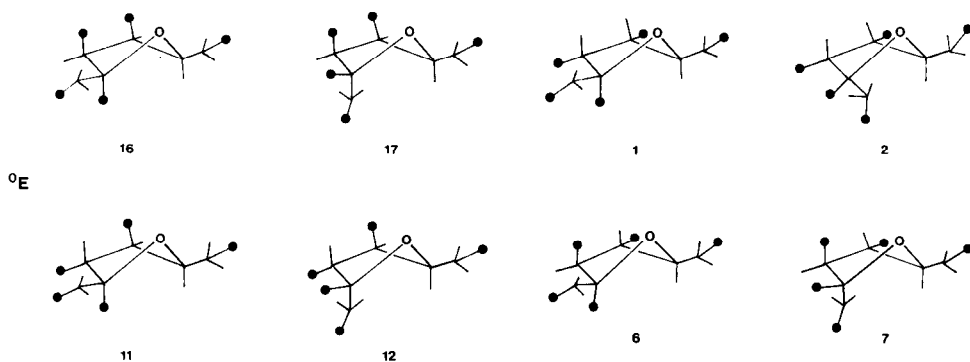
Correlation between structure and retention

The preferred conformations of ketohexose TMS ethers have not been reported. For ketopyranoses we used a 5C_2 conformation (see Scheme 2) in which the OTMS group at C-5 is always equatorial. The ketofuranose substituents were considered as pseudo-axial or pseudo-equatorial in order to determine their descriptor values, from the envelope 0E conformations shown in Scheme 3.



Scheme 2.

Table V lists the structural descriptors used in this work and their identification codes. The number of descriptors is too high, and many of them are redundant as they are related to others in the set; for instance, the total number of equatorial TMS groups is equal to the sum of equatorial TMS groups at C-2, -3, -4 and -5. When the



Scheme 3.

ketoses are divided into furanose and pyranose groups, more descriptors become redundant. Only six descriptors are really independent in the furanose and pyranose sets.

As in previous papers^{14,15}, we used two different approaches in order to relate the retention indices and structures of cyclic ketoses. The small number of acyclic forms precludes their use in the calculation of these models.

Prediction of retention data from structural descriptors. We assume that the retention index I_{xp} of a compound x on a stationary phase p can be expressed as a sum of contributions c_{jp} from its descriptors d_{xj} :

$$I_{xp} = \sum d_{xj} c_{jp}$$

I_{xp} values are listed in Table II, descriptor values (d_{xj}) were obtained from Schemes 2 and 3 and c_{jp} values can be calculated by multiple linear regression.

When ketofuranoses and ketopyranoses were considered as a single data set, the quality of fit was not good ($r = 0.45$ – 0.70 using seven variables). Only a few descriptor values (the positive contribution of pyranose rings P/F and the negative contribution of 1,3-diaxial substituents, 13a and 13A) seem to have significance. In order to improve the fit, it seemed to be necessary to distinguish furanose and pyranose descriptors.

The descriptor values for ketopyranoses are highly related. Only ΣEq , $\Sigma 2c$, $\Sigma 3c$, $Eq2$, $23c$ and $13c$ were chosen for the regression, as the other descriptors can be calculated from these values (Table VI). The quality of fit was fair ($r = 0.90$ – 0.98 and mean error = 6–12 i.u. when using six descriptors). Although the ratio between variables in the fit and experimental data is too high and the high degree of redundancy avoids the assignment of an absolute significance to the descriptor values, some conclusions can be drawn from them. The highest positive contribution corresponded to $\Sigma 3c$ (number of triplets in a *cis* configuration, including both OTMS and CH_2OTMS groups), while $\Sigma 2c$ (number of OTMS pairs in a *cis* configuration) had the highest negative value. This difference must be related to the interaction between the bulky CH_2OTMS group and the *cis* OTMS groups in C-3 and C-4. Also it is worth noting that, in contrast to aldopyranoses^{14–16}, the total number of equatorial substituents (ΣEq) had a low negative contribution.

TABLE VI

MULTIPLE LINEAR REGRESSION LEAST-SQUARES FIT FOR KETOPYRANOSSES ON FIVE STATIONARY PHASES

Contributions of the six ketopyranose descriptors (I_x units) and correlation coefficients.

Descriptor ^a	Stationary phase				
	OV-1	OV-17	OV-215	OV-225	FFAP
Σ Eq	-17.5	-56.0	-17.7	-66.2	-27.0
Σ 2c	-73.0	-161.0	-76.0	-179.0	-103.0
Σ 3c	60.0	91.0	31.5	108.5	55.0
Eq2	-5.0	15.0	-28.0	28.0	-13.0
23c	-3.0	-9.0	-27.5	-25.5	-23.0
13c	-11.0	-9.0	10.5	6.5	2.0
Ring	2046.2	2192.5	1978.9	2358.6	1972.5
Correlation coefficient	0.902	0.971	0.963	0.985	0.970
α -Fructopyranose Exp.	1873	1804	1800	1928	1731
Calc.	1882.7	1814.5	1809.1	1934.4	1739.5
β -Fructopyranose Exp.	1867	1775	1745	1877	1685
Calc.	1857.2	1764.5	1735.8	1870.6	1676.5

^a See Table V.

The same six descriptors (Σ Eq, Σ 2c, Σ 3c, Eq2, 23c and 13c) were chosen for ketofuranoses (Table VII). The quality of fit was better than for pyranoses; when using six descriptors the r values were higher than 0.97 and the mean error was lower

TABLE VII

MULTIPLE LINEAR REGRESSION LEAST-SQUARES FIT FOR KETOFURANOSSES ON FIVE STATIONARY PHASES

Contributions of the six ketofuranose descriptors (I_x units) and correlation coefficients.

Descriptor ^a	Stationary phase				
	OV-1	OV-17	OV-215	OV-225	FFAP
Σ Eq	24.6	2.7	22.8	18.1	14.8
Σ 2c	-12.5	-9.0	-19.5	-19.0	-13.5
Σ 3c	34.7	21.3	40.7	55.7	44.7
Eq2	-15.1	5.3	-2.3	-18.1	1.7
23c	-7.3	-5.0	-3.0	-6.3	-4.0
13c	-13.5	-18.0	-19.0	-42.5	-26.0
Ring	1815.2	1765.2	1708.6	1863.0	1680.2
Correlation coefficient	0.991	0.993	0.983	0.984	0.993
α -Fructofuranose Exp.	1849	1763	1730	1877	1693
Calc.	1851.9	1761.5	1734.8	1880.1	1696.3
β -Fructofuranose Exp.	1857	1763	1757	1877	1712
Calc.	1854.1	1764.6	1752.3	1873.9	1708.8

^a See Table V.

than 5 i.u. The descriptor values seemed also to be more significant. Σ Eq and Σ 3c showed positive contributions, whereas Σ 2c, Eq2, 23c and 13c had negative values. The main difference between furanose and pyranose descriptor values corresponded to Σ Eq, which is clearly related to the overall structure of the molecule; some conformations in Scheme 2 are probably not the preferred ones. The 13c descriptor (OTMS groups *cis* in C-1,C-3) also has a negative value in aldoses¹⁴⁻¹⁵. The presence of a CH₂OTMS substituent on the anomeric carbon could be the reason, as for some ketopyranose descriptor values, for the negative contribution of Eq2 in ketofuranoses on OV-1 and OV-225.

When the number of descriptors used in the regression was reduced, the quality of fit decreased, but the remaining descriptor contributions were roughly similar to those shown in Tables VI and VII. The most important descriptors for furanoses are Σ Eq and Σ 3c, whereas for pyranoses they are Σ 3c, Eq2 and 23c.

Tables VI and VII also show the calculated and experimental retention indices of cyclic forms of fructose on OV-17, using five descriptors that take different values for ketofuranoses and ketopyranoses.

Prediction of structural descriptor values from retention data. In this approach, we assume that the value of the descriptor y in a compound x (d_{xy}) can be calculated from the retention indices I_{xp} of compound x on the five stationary phases p , using the expression:

$$d_{xy} = \Sigma I_{xp} q_{py}$$

The coefficients q for each descriptor y and stationary phase p can be obtained by multiple linear regression. A good fit between real and calculated d_{xy} descriptor

TABLE VIII
TRUE AND CALCULATED VALUES OF FRUCTOSE DESCRIPTORS

Descriptor ^a	α -Fructofuranose		β -Fructofuranose		α -Fructopyranose		β -Fructopyranose	
	True	Calc. ^b	True	Calc.	True	Calc.	True	Calc.
Σ Eq	2	2.2(2)	3	3.3(3)	1	1.2(1)	2	2.2(2)
Σ 2c	1	1.1(1)	1	1.2(1)	2	1.9(2)	2	1.9(2)
Σ 3c	0	-0.1(0)	0	-0.2(0)	0	0.0(0)	0	0.0(0)
13a	0	-	0	-	1	1.0(1)	0	0.1(0)
13A	0	-	0	-	0	-0.1(0)	1	0.8(1)
Eq2	0	0.1(0)	1	1.1(1)	0	0.0(0)	1	0.9(1)
Eq3	0	0.2(0)	0	0.4(0)	0	0.1(0)	0	0.2(0)
Eq4	1	0.9(1)	1	0.8(1)	0	0.1(0)	0	0.1(0)
23c	0	0.1(0)	1	1.1(1)	0	0.1(0)	1	1.3(1)
23C	1	0.9(1)	0	-0.1(0)	1	0.9(1)	0	-0.3(0)
34c	0	0.0(0)	0	-0.1(0)	0	0.0(0)	0	0.0(0)
45c	0	0.1(0)	0	0.2(0)	1	0.9(1)	1	0.9(1)
13c	0	-0.1(0)	0	-0.3(0)	0	0.1(0)	0	0.3(0)
13C	0	0.0(0)	0	0.1(0)	0	-0.1(0)	0	-0.2(0)

^a See Table V

^b Numbers in parentheses are the calculated values rounded of.

values indicates that the descriptor y could be related to the differences in retention between stationary phases.

When using the full data matrix, the best results were found for acyclic/cyclic forms ($r=0.984$) and for pyranose/furanose rings (P/F, $r=0.903$). In order to find a better correlation for the other descriptors, it was necessary to study furanoses and pyranoses separately. The furanose retention indices were related to twelve different descriptors; the multiple correlation coefficients ranged from 0.68 to 0.99, eight being higher than 0.90. After rounding up (descriptor values are always integral numbers), nine descriptors were correctly predicted for the eight compounds. A similar fit was found for pyranoses, with $r = 0.66-0.99$; twelve of fourteen descriptors were correctly predicted. As an example, Table VIII shows real and calculated descriptor values for α - and β -fructopyranose.

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