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GAS CHROMATOGRAPHIC BEHAVIOUR OF CARBOHYDRATE TRIMETHYLSILYL ETHERS

II. ALDOHEXOSES

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SUMMARY

The tautomeric forms of the eight aldohexoses were separated as their O-trimethylsilyl ethers on several packed and capillary columns. Their chromatographic behaviour was similar to that previously found for aldopentoses, but different from that of other ethers. A mathematical approach developed for aldopentoses was applied to aldohexose retention indices on several stationary phases, in an attempt to relate these values to their structural characteristics.

INTRODUCTION

The study of carbohydrates by gas chromatography (GC) requires their derivatization in order to improve the volatility. Among the derivatives which do not cause changes in the initial configuration of the molecules, the trimethylsilyl (TMS) ethers are the most used[1,2].

Although many studies have dealt with the relationships between chemical structure and chromatographic behaviour, publications on sugar derivatives are scarce and incomplete. Several rules were deduced by Sweeley *et al.*[3] and have since been confirmed[4].

In the first part of this series[5] it was found that aldopentose TMS ethers show an unusual chromatographic behaviour on stationary phases of different polarities. Some structural features were correlated with retention, the highest positive contribution corresponding to TMS groups in equatorial positions. The retention indices of aldopentose TMS ethers decreased with increasing temperature. Now this study is extended to the TMS ethers of the eight aldohexoses.

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TABLE I

CHROMATOGRAPHIC COLUMNS USED IN THE GC ANALYSIS OF TMS ETHERS OF HEXOSES

Stationary phase	*Origin*	*Type*	*Material*	*Length (m)*	*I.D. (mm)*	*Support*	*Temperature (°C)*
SE-30	Teknokroma	Packed	Stainless steel	3	3	Supelcoport	150–210
Carbowax 20M	Teknokroma	Packed	Stainless steel	3	3	Supelcoport	160
DEGS	Teknokroma	Packed	Stainless steel	3	3	Supelcoport	160–180
SE-54	Laboratory-made	Open tubular	Glass	40	0.18	–	180–200
OV-17	Chrompack	Open tubular	Fused silica	25	0.22	–	160–190
Carbowax 20M	Hewlett-Packard	Open tubular	Fused silica	25	0.22	–	160
OV-215	Laboratory-made	Open tubular	Glass	25	0.18	–	170
OV-225	Laboratory-made	Open tubular	Glass	25	0.18	–	170

MATERIALS AND METHODS

Samples

β-D-Allose and D-altrose were obtained from Fluka (Buchs, Switzerland); D-mannose, D-gulose, D-idose, D-galactose and α-D-talose from Sigma (Eisenhofen, F.R.G.), and D-glucose from Ferosa (Spain).

A 1-mg amount of crystalline sample was dissolved in water or pyridine and left to stand for 48 h at room temperature, in order to attain the anomeric equilibrium. Syrup samples (idose and gulose) were equilibrated in water. Aqueous samples were lyophilized prior to silylation.

To silylate the samples, 0.1 ml of trimethylsilylimidazole was added and the mixture heated at 65°C for 30 min[6].

GC analysis

The chromatographic equipment, carrier gas and injection and detection conditions were as described previously[5]. Columns and conditions are summarized in Table I. Chromatograms were taken in the isothermal mode: oven temperatures were equal or slightly higher than those used previously[5].

Kováts retention indices were calculated from the retention times of TMS ethers and suitable *n*-alkanes. The dead time was determined by linear regression[7].

Calculations

Retention index calculations and normal and stepwise linear regressions were carried out by using several programs written by us in BASIC for a microcomputer Olivetti M-20.

RESULTS AND DISCUSSION

The eight aldohexose TMS ethers showed in capillary GC four peaks corresponding to the four cyclic tautomers (two pyranoses and two furanoses); only two peaks were observable for most sugars when using the packed columns. Retention indices are shown in Table II. They were similar on both capillary and packed columns, with the exception of Carbowax 20M columns where the retention indices were highly variable. The aldohexose identification was carried out by GC–mass spectrometry (MS) and by comparison with NMR data[8,9]. In two cases (mannose and glucose), it was impossible for us to assign some furanose forms present in the mixtures in very small amounts.

Effect of the stationary phase polarity

Retention indices changed with the polarity of the stationary phase, but no correlation was established. In a plot of the stationary phase polarity calculated according to McReynolds[10] against the first principal component, a positive correlation is expected[5]. However, Fig. 1 shows that the overall retention decreases in the order SE-54, OV-215, OV-17, OV-225 and Carbowax 20M. Similar chromatographic behaviour was observed for the packed columns, where Carbowax 20M showed the lowest *I* values. These results agree with those found[5] for aldopentoses, and confirm that the usual criteria of stationary phase polarity are not suitable for TMS ethers of sugars.

TABLE II

RETENTION INDICES, I_x, OF ALDOHEXOSES

Stationary phase, McReynolds polarity and temperature (°C).

Component		*SE-54* *334* *180*	*OV-17* *884* *180*	*OV-215* *1545* *170*	*OV-225* *1813* *170*	*C 20M* *2308* *160*
17	α-Allofuranose	1857	1829	1849	1772	1768
18	β-Allofuranose	1896	1886	1849	1831	1843
1	α-Allopyranose	1862	1814	1849	1789	1754
2	β-Allopyranose	1879	1829	1849	1789	1778
19	α-Altrofuranose	1837	1972	1774	1699	1739
20	β-Altrofuranose	1912	1871	1739	1699	1838
3	α-Altropyranose	1830	1765	1872	1826	1703
4	β-Altropyranose	1830	1758	1777	1710	1695
21	α-Glucofuranose	–	–	1824	1783	–
22	β-Glucofuranose	–	–	1824	1800	–
5	α-Glucopyranose	1924	1908	1905	1853	1793
6	β-Glucopyranose	2022	2002	2175	1984	1972
23	α-Mannofuranose	1944	–	1915	1870	–
24	β-Mannofuranose	–	–	2032	–	–
7	α-Mannopyranose	1835	1798	1794	1729	1716
8	β-Mannopyranose	1937	1886	1963	1882	1862
25	α-Gulofuranose	1908	1867	1824	1830	1832
26	β-Gulofuranose	1982	1883	1938	1858	–
9	α-Gulopyranose	1858	1803	1826	1762	1757
10	β-Gulopyranose	1825	1789	1765	1734	1729
27	α-Idofuranose	1896	1832	1853	1796	1815
28	β-Idofuranose	1858	1816	1793	1766	1771
11	α-Idopyranose	1858	1812	1837	1784	1764
12	β-Idopyranose	1909	1865	1893	1846	1841
29	α-Galactofuranose	1941	1909	1922	1878	1869
30	β-Galactofuranose	1852	1827	1779	1759	1763
13	α-Galactopyranose	1894	1859	1874	1817	1786
14	β-Galactopyranose	1941	1902	1946	1904	1869
31	α-Talofuranose	1882	1867	1918	1816	1823
32	β-Talofuranose	1863	1836	1833	1800	1794
15	α-Talopyranose	1882	1848	1840	1813	1890
16	β-Talopyranose	1943	1900	2021	1960	1896

Effect of temperature

The retention indices of aldohexose TMS ethers decreased with increasing temperature in both packed and capillary columns. Fig. 2 shows some examples for capillary (a) and packed (b) columns. Values of $\Delta I/10°C$ for capillary columns are shown in Table III. A similar chromatographic behaviour was found[5] for aldopentoses.

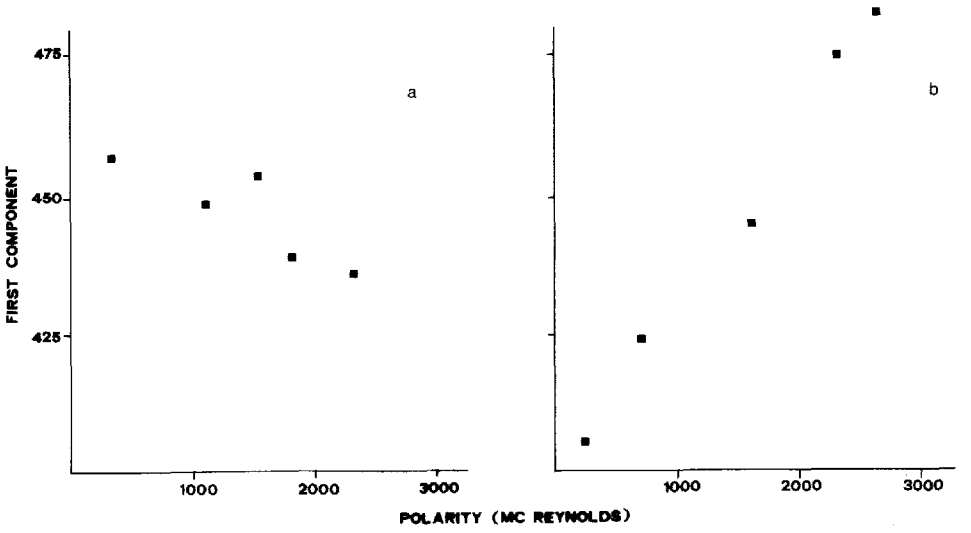


Fig. 1. (a) Values of the coefficients of the first principal component of the data matrix for aldohexose TMS ethers *versus* the polarity of the stationary phase. (b) Values obtained with thirteen ethers from McReynolds[5], given for comparison.

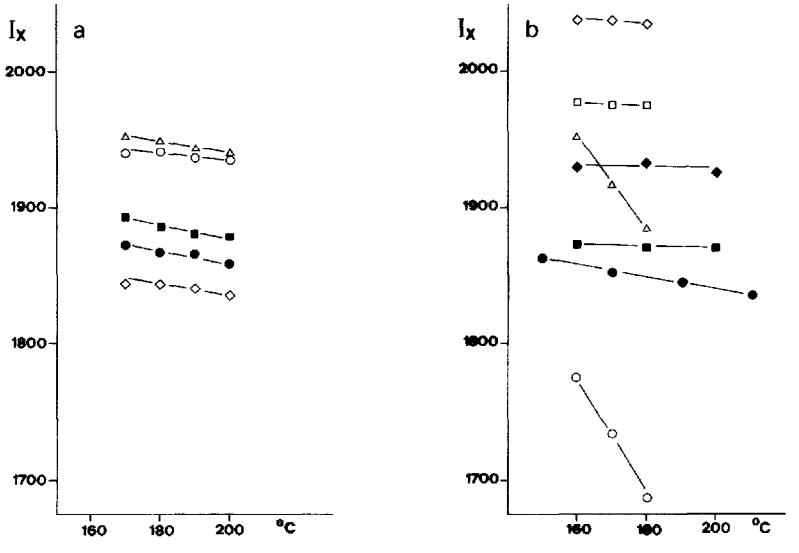


Fig. 2. Variation of I_x with temperature for several aldohexose TMS ethers. (a) Capillary column of SE-54: △, α-mannofuranose; ◇, α-mannopyranose; ○, β-mannopyranose; ■, α-talopyranose; ●, β-talofuranose. (b) Packed columns: □, α-talose (DEGS); ◇, β-talose (DEGS); ○, α-mannose (DEGS); △, β-mannose (DEGS); ■, α-talose (SE-30); ◆, β-talose (SE-30); ●, α-mannose (SE-30).

TABLE III

TEMPERATURE DEPENDENCE OF THE RETENTION INDICES, $\Delta I/10°C$, OF TMS ETHERS OF ALDOHEXOSES

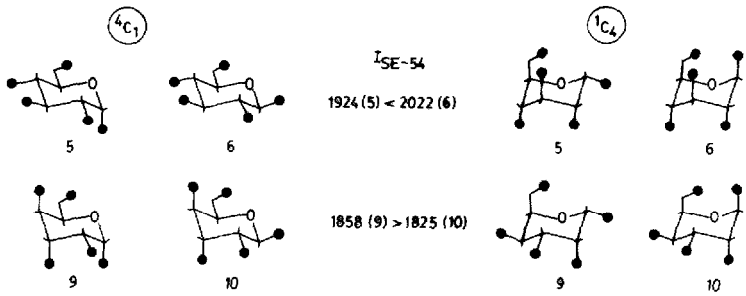
Component	*SE-54* *180–200°C*	*OV-17* *160–190°C*
α-Allofuranose	−4.0	−12.3
β-Allofuranose	−5.0	−12.3
α-Allopyranose	−1.5	−7.3
β-Allopyranose	−4.5	−8.0
α-Altrofuranose	−6.5	−9.7
β-Altrofuranose	−5.0	−8.7
α-Altropyranose	−3.0	−6.3
β-Altropyranose	−3.0	−9.3
α-Glucopyranose	−3.5	−10.0
β-Glucopyranose	−7.0	−9.3
α-Mannopyranose	−4.0	−9.7
β-Mannopyranose	−0.5	−8.0
α-Gulofuranose	−7.0	−7.0
β-Gulofuranose	−3.5	−5.7
α-Gulopyranose	−4.0	−6.3
β-Gulopyranose	−6.0	−2.0
α-Idofuranose	−2.0	−6.3
β-Idofuranose	−2.5	−7.3
α-Idopyranose	−2.5	−7.3
β-Idopyranose	−4.5	−9.0
α-Galactofuranose	−1.0	−11.7
β-Galactofuranose	−5.0	−10.7
α-Galactopyranose	−1.5	−8.5
β-Galactopyranose	−1.0	−7.7
α-Talofuranose	−4.5	−9.3
β-Talofuranose	−0.5	−9.3
α-Talopyranose	−2.0	−7.7
β-Talopyranose	−2.5	−5.7

Effect of carbohydrate structure

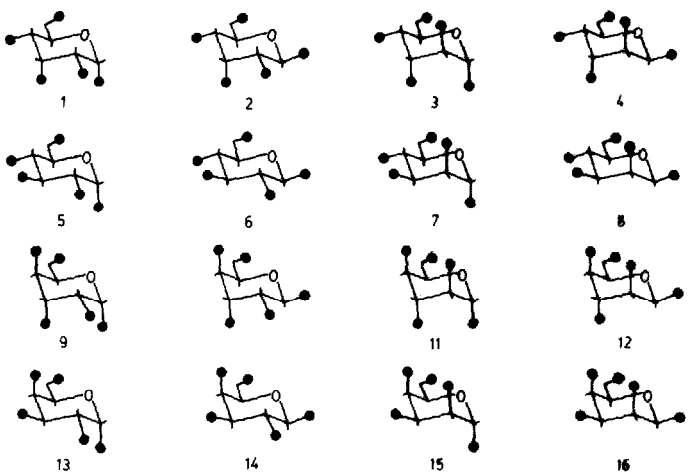
Table II shows the retention indices of aldohexoses on five stationary phases.

For pyranose forms, except altrose and gulose, the α-anomer was eluted before the β-anomer. Sweeley *et al.*[3] explained the gulose behaviour by supposing that while β-gulose is almost certainly in the conformation $^4C_1$, α-gulose may well be in the conformation $^1C_4$ with three axial OTMS groups including the anomeric one (Scheme 1). A similar explanation would be valid for altrose, although these authors did not assign the α- and β-forms.

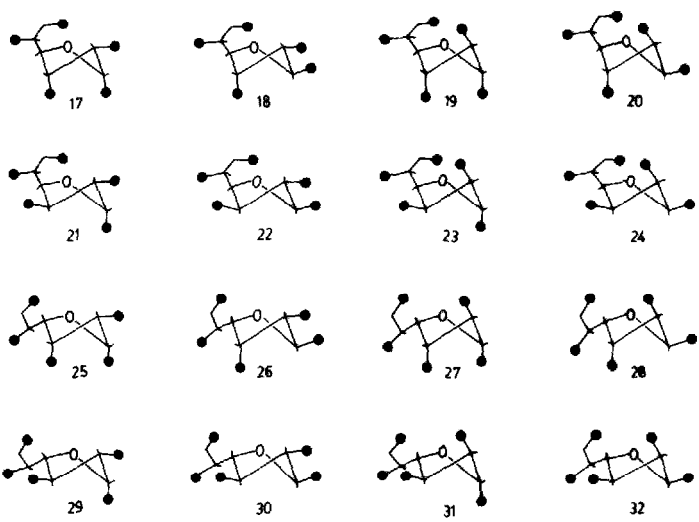
The planar structures are usually more strongly retained[3], as in the case of pentoses[5]. β-Glucopyranose, whose OTMS groups are all equatorial, always showed the highest retention. On the contrary, the different altrose tautomers, with many axial substituents, were the least strongly retained on most phases.



Scheme 1.



Scheme 2.



Scheme 3.

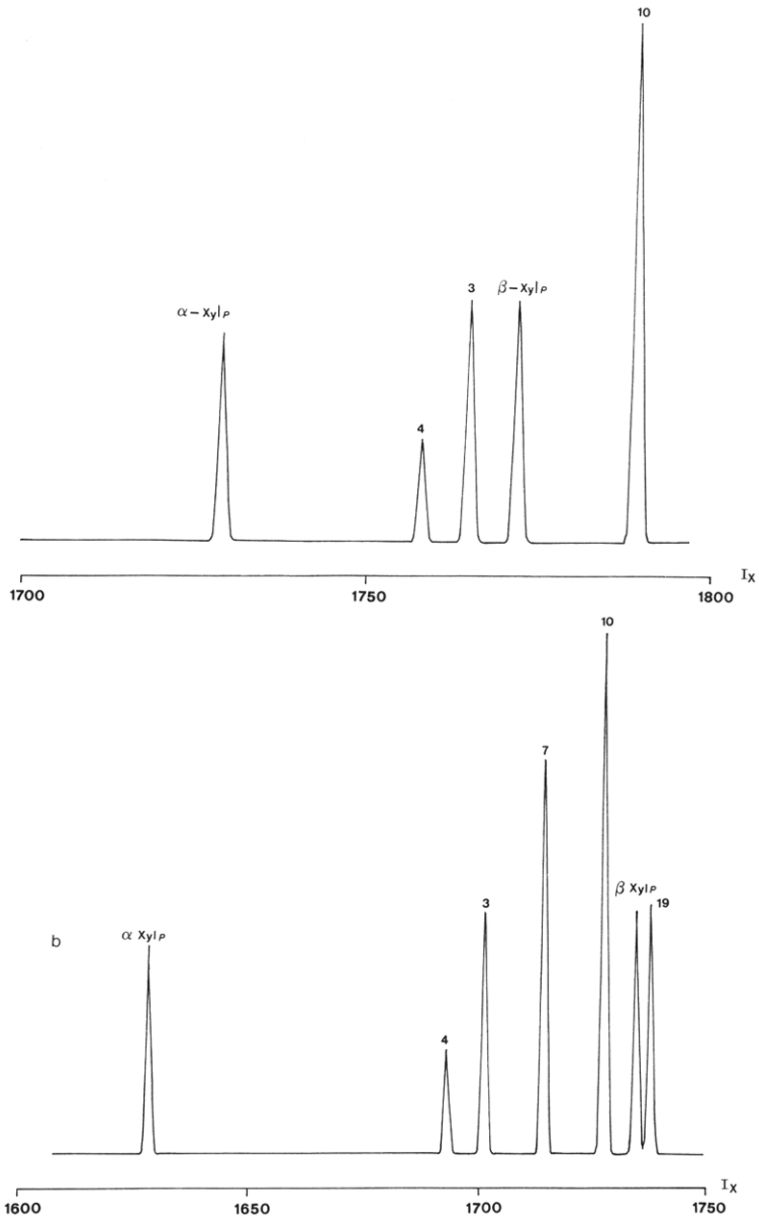


Fig. 3. Chromatographic patterns for some hexose TMS ethers eluted before β-xylopyranose on (a) OV-17 and (b) Carbowax 20M (both at 160°C). Numbers of peaks correspond to structures in Schemes 2 and 3.

The highest separation between anomeric pairs was found in glucopyranoses (179 I.U. on Carbowax 20M). The least separated were those corresponding to allose and altrose, which overlapped on some phases.

The behaviour of furanoses was opposite to that of pyranoses: the β-anomers were eluted before the α-anomers, with the exception of gulose and allose. In general,

TABLE IV

STRUCTURAL DESCRIPTORS OF HEXOSE TMS ETHERS

Code	*Range*	*Structural significance*
Eq1	0–1	OTMS group equatorial on C-1 (anomeric)
Eq2	0–1	OTMS group equatorial on C-2
Eq3	0–1	OTMS group equatorial on C-3
Eq4	0–1	OTMS group equatorial on C-4
ΣEq	0–4	Total number of equatorial OTMS groups
2c	0–4	Two OTMS groups in a *cis* disposition
2t	0–4	Two OTMS groups in a *trans* disposition
3c	0–3	Three OTMS groups in a *cis* disposition
A13	0–2	Two OTMS groups in alternate diaxial disposition
AA	0–3	Two OTMS groups in adjacent diaxial disposition
AE	0–4	Two OTMS groups in adjacent axial–equatorial disposition
EE	0–4	Two OTMS groups in adjacent diequatorial disposition

the separation range was smaller than for pyranoses. The most strongly retained compound (on three phases) was α-galactofuranose, whereas α- and β-altrose were the least strongly retained. The most easily resolved anomeric pair was α- and β-galactose (except on Carbowas 20M).

The increment in molecular weight with respect to aldopentoses produces an increase in the overall retention which averaged 200 I.U. (231 ± 31 on SE-54, 194 ± 35 on OV-17). All the aldohexoses were eluted after the aldopentose (β-xylopyranose) on SE-54, but there were several exceptions on OV-17 and Carbowax 20M (Fig. 3).

TABLE V

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

Contribution of hexopyranose descriptors (I_x units) and correlation coefficient.

Descriptor (see Table IV)	*Phase*				
	OV-17	*Carbowax 20M*	*OV-215*	*OV-225*	*SE-54*
ΣEq	78.6	75.7	249.7	221.4	98.4
Eq1	35.4	82.3	10.7	8.9	24.5
Eq2	30.9	37.9	18.1	30.3	13.1
Eq3	111.1	127.9	149.8	145.7	87.6
2c	35.6	22.8	−8.6	−14.5	35.2
3c	−61.9	−45.5	−73.9	−62.3	−62.4
A13	142.9	160.6	241.9	194.0	137.7
EE	−3.4	−13.2	−114.8	−131.4	−17.8
Ring	1507.9	1443.9	1388.9	1424.9	1558.7
Correlation coefficient	0.972	0.962	0.952	0.986	0.970
α-Allopyranose Exptl.	1814	1754	1849	1789	1862
Calc.	1818.7	1757.9	1860.3	1791.7	1869.1
β-Allopyranose Exptl.	1829	1778	1849	1789	1879
Calc.	1812.7	1765.0	1846.3	1774.3	1863.8

Correlation between structure and retention

The correlation between the retention indices and the chemical structure of aldohexose TMS ethers has been examined using the approach described[5] for pentoses. Two different models have been used.

Prediction of retention data from structural descriptors. In this model, we suppose that the retention index, I_x, of a compound x on the stationary phase p can be expressed as a sum of the contributions, c_{jp}, of their descriptors, d_{xj}:

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

The c_{jp} values can be calculated from experimental I_{xp} retention indices and descriptor values by multiple linear regression.

We have selected as molecular descriptors several structural features related to the absolute and relative positions of the OTMS groups on the furanose and pyranose rings. Unfortunately, the conformations of many hexose TMS ethers have not been reported. Scheme 2 presents the $^4C_1$ (D) conformation of hexopyranoses, that has been shown to be the preferred one for the TMS ethers of β-allo- and β-altropyranose, and of gluco-, manno-, galacto- and talopyranose α- and β-anomers at 25°C[11,12]. There are no data for the other aldohexoses (idose, gulose and α-anomers from allose and altrose), although it may be supposed that α-gulose, α-altrose and α-idose are in a $^1C_4$ (D) conformation.

The actual conformations of hexofuranose TMS ethers have not been reported. They are presented in Scheme 3 in a twist $^2T_3$ conformation, where some extracyclic carbon atoms are neither truly axial nor equatorial.

Descriptor values were calculated from the conformations shown in Schemes 2 and 3. The furanose OTMS substituents were considered as pseudoaxial or pseudoequatorial according to the conformation in Scheme 3. The significance and range of values of the hexose descriptors is shown in Table IV.

In a first step, both furanose and pyranose forms were included in the calculations. Although up to twelve descriptors were used, the quality of fit, measured from the correlation coefficient, r, was not good (r values between 0.76 and 0.78 for the five stationary phases). For this reason, we decided to consider as different the descriptor sets for furanose and pyranose forms.

For pyranoses, sixteen experimental I_x values were available for each phase (Table II). Eight descriptors provide a reasonably good fit, the r values being between 0.95 and 0.99 (Table V). The quality of fit decreased when the descriptors of α-gulose and α-altrose were calculated from their $^1C_4$ conformation; in the case of α-idose it increased slightly. The descriptor contributions were positive, with two exceptions: three OTMS groups in a *cis*-arrangement (3c) and two equatorial OTMS groups on connected carbon atoms (EE), which take negative values for the five stationary phases used. The highest contributions corresponded, in the five phases, to the number of pairs of OTMS groups on alternate carbon atoms (A13), and to the descriptors related to the number of equatorial OTMS groups (Eq1-4). Table V shows also, as an example, the experimental and calculated retention indices of α-allopyranose and β-allopyranose on the five stationary phases. Although the quality of fit was good, the method does not allow the identification of compounds having similar retentions; nevertheless, when the problem is an equilibrated mixture (as in biological samples)

this can be accomplished by considering the chromatographic pattern of the different tautomers.

In the case of furanoses, the low number of experimental I_x values limits the number of descriptors which can be used. Even when using eight descriptors the quality of fit is not good (r values between 0.71 and 0.97). The descriptors showing higher positive contributions to the retention indices are those related to the existence of equatorial OTMS groups and also the number of pairs of axial OTMS groups on alternate carbon atoms, while the β-substitution and the existence of three OTMS groups in a *cis*-position have a negative contribution.

Although it was necessary to fit the pyranose and furanose data in separate ways, there are some relationships among the descriptor values calculated for these hexose forms. In both sets, equatorial OTMS groups (Eq1-Eq4) and pairs of axial OTMS groups on alternate carbon atoms (A13) had high positive contributions to the retention indices, and the presence of three *cis*-OTMS groups (3c) had a negative value.

The descriptor values for hexose (Table V) and pentose (Table VI from ref. 5) were also similar. Equatorial OTMS groups and pairs of axial OTMS groups had also the highest positive values in pentoses, and the only pentose descriptor showing a negative contribution was that for a pair of *cis*-OTMS groups at C-2 and C-3[5].

Although the descriptor values from different sets of compounds cannot be related in a quantitative way, these similiarities confirm their physical significance, which seems to be related[5] to the overall structure of the molecule.

Prediction of structural descriptor values from retention data. In the second model we suppose that the descriptor values for a compound can be approximated by the expression

$$d_{ix} = \sum I_{xp} c_{pi} \tag{2}$$

where d_{ix} is the value of the descriptor i in the compound x, I_{xp} is the retention index of the compound x on phase p and c_{pi} the contribution of phase p to the descriptor i. From both d_{ix} values and experimental I_{xp}, c_{pi} can be calculated by a least squares fit.

As in the first model, we grouped both furanose and pyranose forms in a first least squares fit. However, the quality of fit was poor for most of the fourteen descriptors considered; r values were lower than 0.6 except for the ring size (pyranose/furanose descriptor) which had a r value of 0.71.

For this reason, we applied eqn. 2 in a separate way to furanose and pyranose forms, as in the first model. Although the quality of fit was better (r values up to 0.92 for furanoses and 0.88 for pyranoses), it was not enough to allow the accurate prediction of descriptor values in most cases. In order to achieve a useful prediction, it was necessary to carry out different calculations for α- and β-compounds. The quality of fit improved and most descriptors provided r values higher than 0.9 for pyranoses. Calculated descriptor values were correct in 95.5% of cases. The r values were even higher for furanoses, but the number of experimental data available was lower and the results were less reliable.

Table VI shows the experimental and calculated values for the descriptors of α- and β-anomers from allo- and glucopyranose.

TABLE VI

TRUE AND CALCULATED VALUES OF ALLOSE AND GLUCOSE DESCRIPTORS

Descriptor	*α-Allopyranose*		*β-Allopyranose*		*α-Glucopyranose*		*β-Glucopyranose*	
	True	*Calc.*	*True*	*Calc.*	*True*	*Calc.*	*True*	*Calc.*
2c	3	3 (2.93)	2	2 (1.68)	1	1 (1.18)	0	0 (0.02)
2t	1	1 (1.06)	2	2 (2.31)	3	3 (2.81)	4	4 (3.97)
3c	2	1 (1.41)	1	0 (0.22)	0	0 (0.03)	0	0 (0.09)
ΣEq	2	2 (1.83)	3	3 (2.57)	3	3 (3.02)	4	4 (4.13)
Eq2	1	1 (0.85)	1	1 (0.67)	1	1 (1.01)	1	1 (1.02)
Eq3	0	0 (0.40)	0	0 (0.26)	1	1 (1.13)	1	1 (1.03)
Eq4	1	1 (0.58)	1	1 (0.64)	1	1 (0.87)	1	1 (1.07)
A13	1	1 (0.51)	0	0 (−0.03)	0	0 (−0.16)	0	0 (−0.06)
AA	0	0 (0.19)	0	1 (0.58)	0	0 (−0.11)	0	0 (−0.14)
AE	3	3 (2.93)	2	2 (1.68)	1	1 (1.18)	0	0 (0.03)
EE	1	1 (0.74)	2	2 (1.73)	3	3 (2.92)	4	4 (4.12)

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