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GAS CHROMATOGRAPHIC BEHAVIOUR OF CARBOHYDRATE TRIMETHYLSILYL ETHERS

I. ALDOPENTOSES

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SUMMARY

The four components of each aldopentose were separated as O-trimethylsilyl ethers on several packed and capillary columns. In order to establish which characteristics affect the retention and to achieve a better understanding of the chromatographic behaviour of these compounds, a mathematical approach was applied which tries to relate structural characteristics with retention indices on several stationary phases.

INTRODUCTION

The most widely used derivatives in the analysis of carbohydrates by gas chromatography (GC) and mass spectrometry (MS) are their trimethylsilyl (TMS) ethers[1–4], which have been applied to mono- and disaccharides[5–8]. Many other derivatives have been used: first, in order to decrease the number of possible peaks for each sugar (alditol derivatives[9–13], methoxime[14] and oxime TMS ethers[15–17]); secondly, in order to improve properties such as volatility [trifluoroacetates[18–20], dimethylsilyl (DMS) ethers[21]], or stereochemical differences (dithioacetals[22–24], boronates[25,26], etc.).

Although many analytical studies of carbohydrates have been carried out, few have concerned the relationships between their chemical structure and chromatographic retention[2,7]. Several factors which influence retention are well known, such

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TABLE I

COLUMNS USED FOR GC ANALYSIS OF TMS ETHERS OF CARBOHYDRATES

No.	*Stationary phase*	*Type*	*Material*	*Length (m)*	*I.D. (mm)*	*Support*	*Temperature (°C)*
1	SE-30	Packed	Stainless steel	3	3	Supelcoport	Several
2	DEGS	Packed	Stainless steel	3	3	Supelcoport	Several
3	SE-54	Capillary	Glass	40	0.18	–	180, 200
4	OV-17	Capillary	Fused silica	25	0.22	–	160, 190
5	Carbowax 20M	Capillary	Fused silica	25	0.22	–	160
6	OV-215	Capillary	Glass	25	0.18	–	150, 160
7	OV-225	Capillary	Glass	25	0.18	–	150, 160

as the size and form of molecules, position of substituents, etc. The members of a sugar family are isomers with identical substituents, which differ only in their relative orientation. This fact, and the lack of good resolution between tautomers of a simple sugar in packed columns, probably explain the scarcity of data on this topic.

The aim of this series is the study of TMS ethers of simple carbohydrates, pentoses, hexoses, disaccharides, in order to establish the main factors which affect their retention. In this paper, some general features of the GC behaviour of the TMS ethers of aldopentoses are outlined, and their retention indices are correlated with different structural parameters.

MATERIALS AND METHODS

Preparation of TMS derivatives

Pure samples of sugars were dissolved in water or pyridine and equilibrated for 24–48 h. Aliquots containing 0.5–1 mg of carbohydrate were silylated and analyzed by GC. Aqueous samples were first lyophilized. N-Trimethylsilylimidazole[27] and hexamethyldisilazane–trimethylchlorosilane in pyridine[2] were used as silylation agents.

GC analysis

Chromatographic data were obtained with a Perkin-Elmer Sigma 3B gas chromatograph connected to a Sigma 10 B data station. In the work with capillary columns we used Carlo Erba 4130, Perkin-Elmer 900 and Perkin-Elmer 3920 gas chromatographs, connected to Hitachi potentiometric recorders. The injection temperature was 300°C. The carrier gas (nitrogen) flow-rate was calculated to be near the Van Deemter optimum. A flame ionization detector was used in all cases. The columns are listed in Table I. Several Carbowax 20M columns were used in order to study the reproducibility of results.

Retention indices, I_x, were calculated from the retention times of TMS ethers and suitable *n*-alkanes[28].

Calculations

Calculations were carried out with an Olivetti M-20 microcomputer. Several programs were written in BASIC in order to perform normal and stepwise regressions, and principal component analysis.

RESULTS AND DISCUSSION

Identification

Silylation equilibrium mixtures of each of the four aldopentoses (arabinose, ribose, xylose and lyxose) were analyzed by GC–MS. Four components were found for each sugar. They were identified by GC–MS and by comparison with NMR data[29].

Effect of temperature

The chromatographic behaviour of aldopentose TMS derivatives was studied in both packed and capillary columns. It was found that I_x decreases with temperature

TABLE II

TEMPERATURE DEPENDENCE OF THE ALDOPENTOSE RETENTION INDICES ON SEVERAL STATIONARY PHASES, AS ΔI/10°C VALUES

Component	*Stationary phase and temperature range*			
	SE-54 180–200°C	*OV-215 150–160°C*	*OV-225 150–160°C*	*OV-17 160–190°C*
α-Ribofuranose	1.5	−26	−16	−4
β-Ribofuranose	2.0	−27	−8	−1
α-Ribopyranose	2.5	−14	−10	−6
β-Ribopyranose	1.5	−22	−14	−4
α-Arabinofuranose	−5.0	−18	−6	−9
β-Arabinofuranose	−10.5	−16	−14	−7
α-Arabinopyranose	−55.0	−21	−17	−9
β-Arabinopyranose	−6.5	−14	−16	−7
Xylofuranose	−6.5	−1	−7	−3
Xylofuranose	−5.5	−3		−1
α-Xylopyranose	−6.0	−2	−6	−1
β-Xylopyranose	−4.5	−2	8	−1
α-Lyxofuranose	2	−19	−10	−6
α-Lyxopyranose	4.5	−21	−4	0
β-Lyxopyranose	0.5	−14	−8	−1

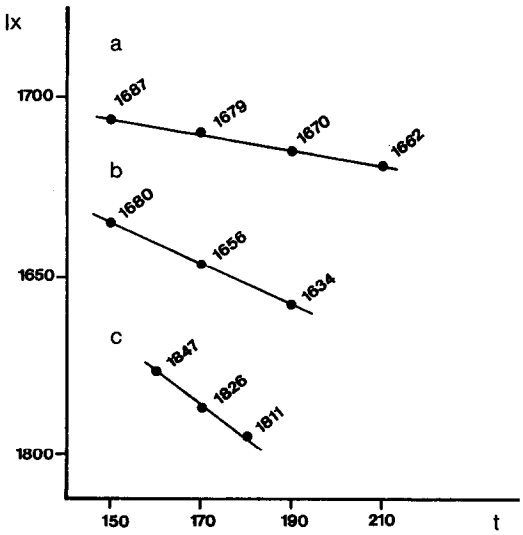
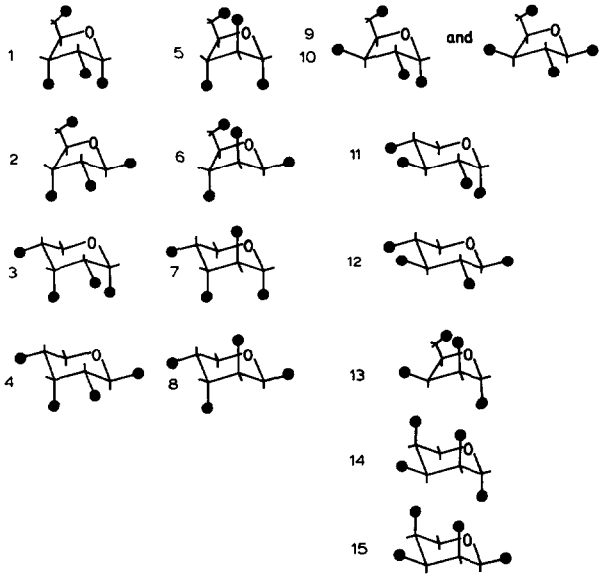


Fig. 1. Variation of I_x with the temperature for several aldopentoses on packed columns. (a) α-Ribose; (b) β-arabinose (both on SE-30); (c) β-xylose on DEGS.

in most cases (Table II), and that the variation is higher on polar phases. Fig. 1 shows the relationships I_x *vs.* t for several aldopentoses on packed columns. Carbowax 20M showed anomalous results.

TABLE III

ALDOPENTOSE RETENTION INDICES, I_x



Component	*Stationary phase, McReynolds polarity, temperature (°C)*				
	SE-54	*OV-17*	*OV-215*	*OV-225*	*Carbowax 20M*
	334	*884*	*1545*	*1813*	*2308*
	180	*160*	*160*	*150*	*160*
(1) α-Ribofuranose	1626	1624	1675	1660	1528
(2) β-Ribofuranose	1636	1647	1688	1676	1550
(3) α-Ribopyranose	1663	1651	1775	1710	1550
(4) β-Ribopyranose	1642	1626	1709	1683	1531
(5) α-Arabinofuranose	1596	1597	1609	1600	1531
(6) β-Arabinofuranose	1666	1682	1738	1711	1603
(7) α-Arabinopyranose	1601	1597	1690	1633	1524
(8) β-Arabinopyranose	1635	1643	1717	1703	1586
(9) Xylofuranose	1610	1628	1670	1650	1530
(10) Xylofuranose	1622	1629	1680	1650	1538
(11) α-Xylopyranose	1762	1729	1804	1744	1630
(12) β-Xylopyranose	1777	1772	1844	1807	1736
(13) α-Lyxofuranose	1633	1627	1676	1676	1543
(14) α-Lyxopyranose	1593	1599	1667	1630	1486
(15) β-Lyxopyranose	1660	1646	1724	1703	1553

Effect of the stationary phase

The main advantage of the retention index is its independence of many chromatographic parameters: it depends on the chemical nature of the solute and stationary phase, and has a small variation with temperature.

Table III shows the I_x values of the four isomers from each pentose, on five stationary phases: α- and β-xylofuranose were not assigned. The values found on Carbowax 20M columns are lower than those on the other stationary phases, including SE-54 and SE-30. This is highly unusual. Moreover, there is no clear relationship between the silicone "polarity" and I_x values.

For comparison, we have selected from McReynolds data[30] a set of I_x values for thirteen ethers on five stationary phases having polarities similar to those used in the present work (Table IV). Both data matrices were submitted to a principal component analysis[31]. The first component vector can be interpreted as a measure of the relative polarity of the phases towards the compounds selected.

Fig. 2 shows the coefficients of the first component for both our data matrix (a) and the McReynolds ethers (b) plotted *versus* the phase polarity according to McReynolds. There is a clear correlation in case (b), but not in (a).

From these results it can be deduced that the differences in chromatographic behaviour between the TMS ethers of pentoses and of other compounds, including normal ethers, are highly significant. The similarity of the –OTMS groups of silylated sugars and the $-OSiCH_3$ groups of the stationary phase molecules may partly explain this anomalous behaviour, but it is clear that the usual polarity criteria are not suitable for TMS ethers of sugars.

Effect of carbohydrate structure

The elution order of TMS pentopyranoses varies on different phases as shown in Table III. The β-anomer is more strongly retained than the α for arabinose, xylose and lyxose; the opposite is true for ribose. These results are in accord with those

TABLE IV

RETENTION INDICES AT 120°C OF ETHERS ON FIVE STATIONARY PHASES

Data from McReynolds[30].

Ether	*SE-30*	*DC-550*	*QF-1*	*Carbowax 20M*	*XF-1150*
Dibutyl	691	776	726	794	809
Diallyl	593	631	656	771	773
Dipropyl	687	708	708	773	785
Diisopropyl	593	611	617	652	646
tert.-Butyl isopropyl	665	678	699	720	725
Dipentyl	1076	1104	1143	1165	1189
Diisopentyl	1002	1021	1067	1063	1094
Ethyl vinyl	499	531	560	677	659
Butyl vinyl	695	731	778	858	880
Isobutyl vinyl	655	684	736	800	806
2-Ethyl-1-hexyl vinyl	1036	1064	1126	1179	1198
Ethylene glycol dimetyl	646	706	814	918	970
Ethylene glycol dibutyl	1160	1213	1278	1359	1387

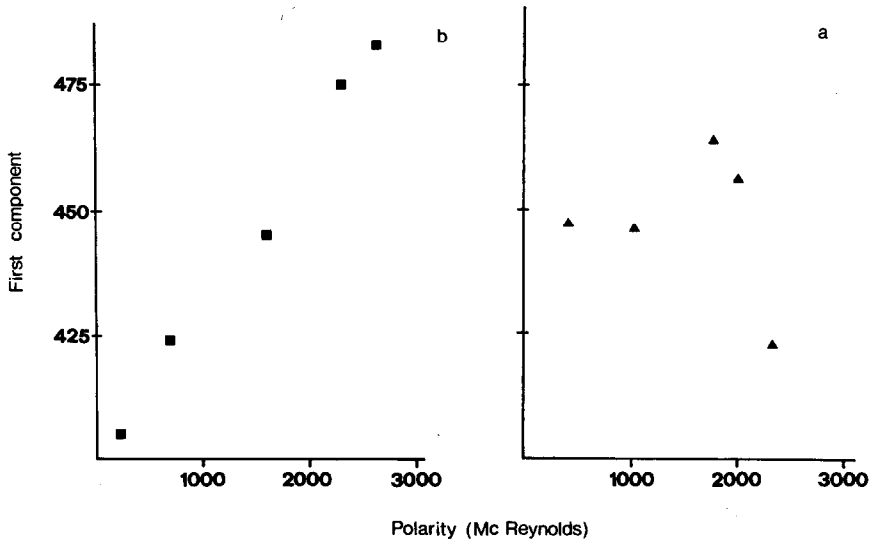
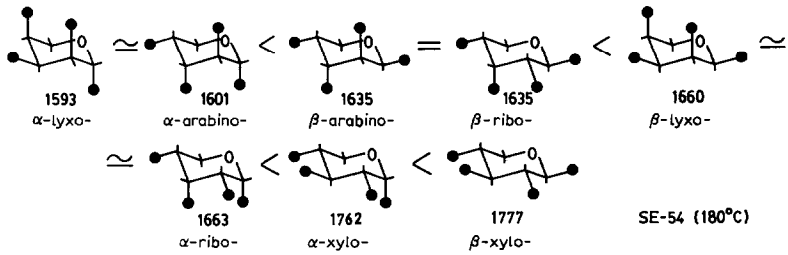


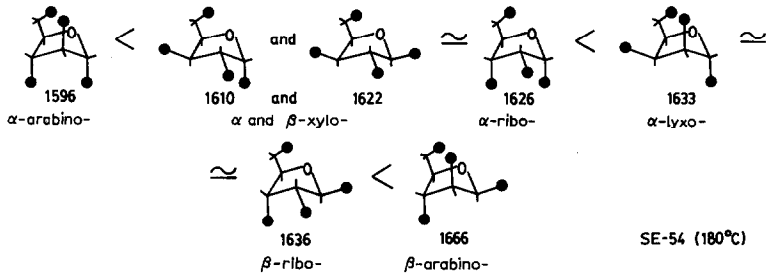
Fig. 2. Values of the coefficients of the first component for data matrices corresponding to retention indices for (a) TMS ethers of aldopentoses and (b) McReynolds ethers, *versus* the polarity of the stationary phase.

given by Sweeley *et al.*[2] for lyxose and xylose. The maximum difference between retention indices for a pair of anomers (106 I_x units) was found for xylose on Carbowax 20M. The smallest difference (19–65 I_x units) corresponds to ribose. In general, the compounds having planar structures are more strongly retained, as previously stated[2]. The first peaks eluted are always α-lyxo- and α-arabinopyranoses; they are also the less planar pyranose forms when the conformation $^4C_1$ is suposed. Maximum retention is always attained in the case of β-xylose, whose TMS groups are all equatorial (Scheme 1).



Scheme 1.

Retention data for pentofuranoses are scarce. Sweeley *et al.*[2] identified a γ-component in several sugars, which was assigned as a furanose form. The elution order of these compounds is shown in Table III; α-anomers are always eluted before β-anomers. The xylofuranoses are eluted close together, and overlap in two cases. The most strongly retained of these compounds is always the β-anomer of arabinose, and the least retained is its α-anomer. Thus, the anomeric pair of arabinose is the most resolved (70–190 units).



Scheme 2.

Correlation between structure and retention

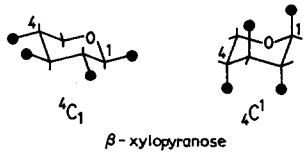
We have tried quantitatively to correlate the retention indices of pentoses and their chemical structures. It is convenient to study furanoses and pyranoses separately since it can be supposed that ring size plays an important rôle in retention. However, the number of furanose forms unambiguously assigned is too small to allow any data analysis.

Two different models were used in order to relate retention indices and structures of pyranoses.

(i) Prediction of retention data from structural descriptors. We suppose than the retention index, I_x, of a compound can be expressed as a sum of contributions, c_i, from their descriptors, d_i:

$$I_x = \Sigma c_i d_i \qquad (1)$$

The conformations of pentopyranose TMS ethers have not been reported. Free sugars in pyranose forms can appear in two conformations: $^4C_1$ and $_4C^1$ (ref. 32) (Scheme 3). As a first approximation, we have supposed a $^4C_1$ conformation for our compounds, which is the preferred one for most free sugars.



Scheme 3.

Several structural features related to the absolute and relative positions of OTMS groups were selected in order to describe the molecules in a very complete form. The values of some of these descriptors are correlated with the rest, and we need to eliminate them in order to get significant results. After the elimination step, the following descriptors were selected: A, OTMS group equatorial on C-1; B, OTMS group equatorial on C-2; C, OTMS group equatorial on C-3; D, two OTMS groups *cis* on C-1 and C-2; E, two OTMS groups *cis* on C-2 and C-3; F, two axial OTMS groups on alternate carbon atoms. Each descriptor takes a value $d_i = 1$ when the corresponding structural feature is present in the molecule.

TABLE V

MULTIPLE LINEAR REGRESSION LEASTS-SQUARES FIT FOR PENTOPYRANOSES

Correlation coefficient, $r = 0.99998$.

Compound	*O* *Ring size (pyranose)*	*A*	*B*	*C*	*D*	*E*	*F*	I_x	
								Found	*Predicted*
α-Ribopyranose	1	0	1	0	1	1	1	1663	1662.6
β-Ribopyranose	1	1	1	0	0	1	0	1642	1642.4
α-Arabinopyranose	1	0	0	0	0	0	1	1601	1601.4
β-Arabinopyranose	1	1	0	0	1	0	0	1635	1634.6
α-Xylopyranose	1	0	1	1	1	0	0	1762	1762.4
β-Xylopyranose	1	1	1	1	0	0	0	1777	1776.6
α-Lyxopyranose	1	0	0	1	0	1	0	1593	1592.6
β-Lyxopyranose	1	1	0	1	1	1	0	1660	1660.4
Contribution (I_x units)	1566.9	41.0	88.8	80.0	26.8	−54.3	34.5		

The values of the descriptors and the results of the multiple regression fit using the I_x values from the SE-54 column (Table III) are shown in Table V. When the I_x values from other stationary phases are used, the results are similar although the quality of fit is lower.

Although the fit is good, the relative number of variables is too high. It is possible to reduce this number when the contributions from similar descriptors have similar values. If we suppose that the contribution from an OTMS group does not depend on its position, the first three variables in Table V can be replaced by the total number of equatorial OTMS groups. In Table VI the results of this multiple regression fit are shown for the five stationary phases studied. It is impossible to omit more variables from the fit while keeping a high correlation coefficient.

It is worth noting the positive contribution from equatorial OTMS groups on the five stationary phases. Apparently, high retention indices are related to planar

TABLE VI

MULTIPLE LINEAR REGRESSION LEASTS-SQUARES FIT FOR PENTOPYRANOSES ON FIVE STATIONARY PHASES

Contribution of descriptor (I_x units) and correlation coeficient.

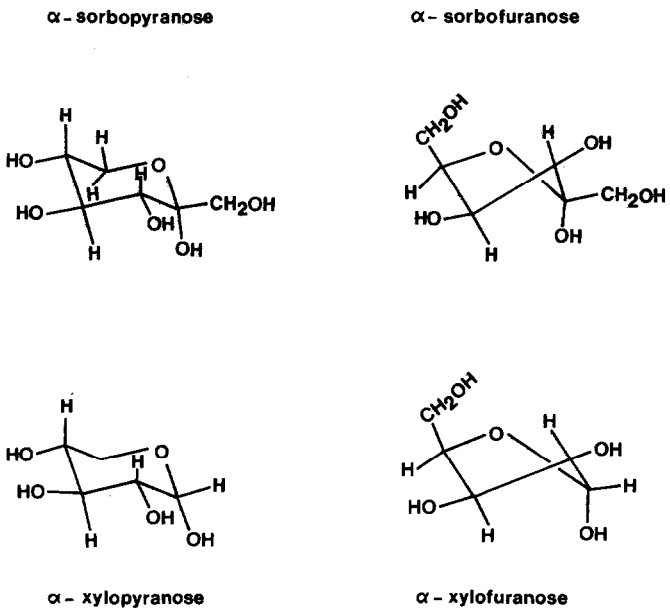
Descriptors (see text)	*Phase*				
	SE-54	*OV-17*	*OV-215*	*OV-225*	*Carbowax 20M*
A+B+C	77.5	67.9	74.0	67.3	79.2
D	26.8	18.8	27.5	26.8	10.5
E	−54.3	−54.8	−45.0	−40.3	−80.0
F	57.1	45.3	87.0	49.5	55.6
Pyranose ring	1549.9	1562.8	1617.3	1595.1	1481.1
Correlation coefficient	0.966	0.970	0.967	0.978	0.977

structures like the xylopyranoses. The first two peaks eluted, α-lyxo- and α-arabinopyranose are the less planar pyranose forms when the conformation $^4C_1$ is supposed.

The contributions from descriptors D and F are also always positive. The descriptor E takes negative values on the five stationary phases. The ring contribution is very important in the absolute values of the retention indices.

The quality of fit drops if we choose for each compound the preferred conformation according to Angyal[33]; however, high positive values are again obtained for the contribution of equatorial OTMS groups and for the contribution of two axial groups on alternate carbons.

As the number of compounds used for the calculation of the contributions is too small (because no more simple aldopentoses exist), we have tried to apply our results to a similar family of compounds. Ketohexoses seemed to be the most adequate, because their structure is similar to that of pentoses, with a $-CH_2OH$ group at the anomeric carbon (Scheme 4).



Scheme 4.

Retention data for ketohexoses on SE-54 are shown in Table VII. The resolution between different tautomers is worse than that for aldopentoses; the open forms are more retained.

A comparison of Tables V and VII shows significant differences between the chromatographic behaviour of these compounds. The elution order of fructose and tagatose pairs is the reverse of that of their pentose analogues. The structure of aldopentoses and ketoses can be described using the same variables; it is clear, however, that the influence of the substituent at the anomeric carbon must change the

TABLE VII

RETENTION INDICES, I_x OF KETOSES ON SE-54 AT 180°C

Ketose	I_x	*Ketose*	I_x
α-Psicofuranose	1758	α-Sorbofuranose	1731
β-Psicopyranose	1791	β-Sorbofuranose	1742
α-Psicopyranose	1772	α-Sorbopyranose	1804
β-Psicopyranose	1749	β-Sorbopyranose	1804
Open form	1838	Open form	1866
α-Fructofuranose	1750	α-Tagatofuranose	1756
β-Fructofuranose	1757	β-Tagatofuranose	1765
α-Fructopyranose	1832	α-Tagatopyranose	1823
β-Fructopyranose	1767	β-Tagatopyranose	1773
Open form	1875	Open form	1830

values of their contributions to the retention. For instance, the bulkier substituent at C-5 is now CH_2OTMS, and the contribution of descriptor A becomes negative.

When aldohexoses are considered, a new substituent is introduced, and the importance of the other groups is altered. Thus the conclusions of the present paper must be contrasted with the results of further experiments. The GC retention of ketoses and aldohexoses will be the subject of subsequent work.

(ii) Prediction of molecular descriptor values from retention data. The contribution of a structural descriptor to the I_x values depends on the stationary phase considered. The different I_x values for each compound in Table III can then be used to predict when a given descriptor is present in a molecule. The expression used was

$$d_i = \Sigma I_{xp} c_{pi} \tag{2}$$

where d_i is the descriptor to be calculated, I_{xp} the retention index of the compound on phase p and c_{pi} the contribution of the phase p to the descriptor value. The latter

TABLE VIII

TRUE AND CALCULATED VALUES OF DESCRIPTORS A AND F IN PENTOPYRANOSES

Component	*A*		*F*	
	True	*Calc.*	*True*	*Calc.*
α-Ribopyranose	0	0.02	1	1.00
β-Ribopyranose	1	0.88	0	0.09
α-Arabinopyranose	0	−0.02	1	0.97
β-Arabinopyranose	1	1.15	0	0.04
α-Xylopyranose	0	0.09	0	0.02
β-Xylopyranose	1	0.91	0	−0.01
α-Lyxopyranose	0	−0.04	0	0.00
β-Lyxopyranose	1	1.01	0	−0.11
Correlation coefficient, *r*	0.986		0.992	

parameter can be calculated for each stationary phase and descriptor by multiple linear regression. We have fitted eqn. 2 to twelve different structural descriptors, obtaining correlation coeficients from 0.576 to 0.991. The prediction of descriptor values from eqn. 2 is 100% correct in six cases. In Table VIII are shown the two best results, predicting the position of an OTMS anomeric group (descriptor A) and the presence or absence of two axial OTMS groups on alternate carbon atoms (descriptor F). As the true values are integer numbers, the approach can be considered satisfactory.

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