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Determination of the absolute configuration of sugar residues using gas chromatography
Method with potential for elimination of references

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Abstract

The absolute configuration of a sugar can be determined by gas–liquid chromatography of the acetylated or trimethylsilylated dithioacetals from 1-phenylethanethiol. The isolation of both enantiomers of 1-phenylethanethiol is also described. Using the acetates and both thiol reagents the absolute configuration of C-2 can be determined, provided it is a hydroxyl group, with great certainty. A new way of determining the absolute configuration of sugars, without references, is thereby provided. The sugars analysed include aldoses, deoxyaldoses, 2-acetamido-2-deoxyaldoses and uronic acids. The analysis is made using columns with non-chiral stationary phase and the electron impact mass spectra of the acetylated and trimethylsilylated bis(1-phenylethyl)dithioacetals are described.

Keywords: Enantiomer separation; Sugars; Phenylethanethiol; Monosaccharides; Bis(1-phenylethyl) dithioacetals

1. Introduction

In structural studies of natural saccharides, full characterization should include determination of the absolute configurations of the constituent monosaccharides. In the last two decades, the use of gas–liquid chromatography for separation of enantiomers has been commonly used for that purpose. The developed methods has followed two directions; (1) separation of monosaccharide enantiomers using a chiral stationary phase [1–4] and (2) separation of the diastereomeric glycosides obtained by treatment of the sugars with a chiral reagent, usually an optically active alcohol, using non-chiral GLC columns [5–8]. However, in the approach with direct resolution on a chiral stationary phase, each enantiomer of a monosaccharide or monosaccharide glycoside, usually trifluoroacetylated, produces a chromatogram in which up to four forms may be present. One way to circumvent the multiplicity is to reduce the sugars to alditols but then some sugars lose their chirality, since a symmetrical polyol (a *meso* compound) is formed. Some sugars also give the same alditol as e.g. D-arabinose and D-lyxose. Also, separation of enantiomeric sugars as their diastereomeric glycosides gives rise to multiple-peak chromatograms. Another approach has been given by Little [8]. Using (+)-1-phenylethanethiol as a chiral re-

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agent each enantiomeric sugar produced a single acyclic diastereomer and the pairs of diastereomeric dithioacetals, subsequently acetylated or trimethylsilylated, were separated on common non-chiral capillary columns with good separation factors.

In this report we re-evaluate and extend the use of 1-phenylethanethiol as a chiral reagent. The method [8], was applied for neutral sugars but has not been commonly used in carbohydrate chemistry, most probably because the (+)-1-phenylethanethiol is not commercially available. Our results include the use of (−)-1-phenylethanethiol in combination with (+)-1-phenylethanethiol, which makes it possible to assign the D- or L-configuration of a monosaccharide with great certainty even if none of the two forms is available as standard. Furthermore, in addition to the more common neutral sugars, 2-acetamido-2-deoxyhexoses, dideoxyhexoses, L-*glycero*-D-*manno*-heptose as well as uronic acids have been included in this study. The mass spectra of the resulting derivatives are also analysed.

2. Experimental

2.1. General

Ethanethiol was obtained from Janssen (Geel, Belgium). (1-Bromoethyl)benzene, (−)-menthol and morpholine were purchased from Sigma–Aldrich (St. Louis, MO, USA). PTFE-lined screw-capped glass tubes (13×100 mm) were used for sample preparation, and for GLC analysis 1 μl of sample solution was injected, throughout.

A gas chromatograph (HP 5890) equipped with an autoinjector, an FI detector and a computer data system, was used. The GLC columns used were: fused-silica capillary columns coated with 0.25-μm DB-5 (28 m×0.25 mm) or, 0.40-μm DB-5 (13 m×0.25 mm, used for GLC–MS) which were obtained from J and W Scientific (Folsom, CA, USA). Helium carrier-gas velocity was maintained at 30 cm s^{-1} (50 cm s^{-1} in case of the longer DB-5 column) with a split ratio of about 20:1. Diastereomeric dithioacetals were analysed isothermally at 280°C, or using a temperature program (275°C for 1 min, 2°C min^{-1} to 300°C and hold for 4 min), using the DB-5 columns. A Nermag R 10-10 instrument was used for mass spectrometry.

2.2. Synthesis of (+)- and (−)-1-phenylethanethiol

(+)-1-Phenylethanethiol was synthesized as described by Isola et al. [9]. Briefly, metallic sodium (16 g) was reacted with (−)-menthol (100 g) and following the addition of carbon disulfide sodium (−)-menthyl dithiocarbonate was formed [10]. The product is then reacted with racemic (1-bromoethyl)benzene, which yielded (−)-O-menthyl-(*S*)-1-phenylethyl dithiocarbonate and (−)-O-menthyl-(*R*)-1-phenylethyl dithiocarbonate. Both diastereomers were isolated after fractional crystallisation. The (−)-O-menthyl-(*S*)-1-phenylethyl dithiocarbonate was precipitated and recrystallized using absolute ethanol [9]. A second crop was obtained when the mother liquor was reduced by one third of its volume and kept at −20°C overnight, and some crystals from the first step were added as seeds. Concentration of the mother liquor in a rotary evaporator yielded an orange–yellow oil. Addition of ethanol (99%, ≈300 ml) to the oil and cooling to −20°C gave the next day a white crystalline precipitate of (−)-O-menthyl-(*R*)-1-phenylethyl dithiocarbonate which was filtered off and washed with ethanol (95%, ≈60 ml, −20°C). Both (−)-O-menthyl-(*R*)-1-phenylethyl dithiocarbonate and (−)-O-menthyl-(*S*)-1-phenylethyl dithiocarbonate were recrystallized once and then dried in vacuo. The (−)-O-menthyl-(*R*)-1-phenylethyl dithiocarbonate had $[\alpha]_{436}$ 460 (*c* 2.0, toluene, lit. 473.3 [9]) and (−)-O-menthyl-(*S*)-1-phenylethyl dithiocarbonate had $[\alpha]_{436}$ −452 (*c* 2.0, toluene). ^{13}C NMR data: signals at 20.9, 22.2, 31.5, 34.2, 47.3, 76.7, 77.2, 77.6, 84.3, 127.5, 128.7 142.2 and 212.8 ppm were observed for both diastereomers. The following six signals, associated with the environment of the chiral center, differed: 16.8, 22.2, 23.6, 26.3, 39.9 and 48.9 ppm for (−, *S*) compound and for the (−, *R*) compound 17.0, 21.9, 23.8, 26.7, 39.5 and 49.1 ppm.

Decomposition of (−)-O-menthyl-(*S*)-1-phenylethyl dithiocarbonate was accomplished with morpholine and precipitation with mercury(II) chloride to yield (+)-1-phenylethylthiomercury chloride. The latter compound was added to concentrated hydrochloric acid (under N_2) and stirred until the solid had

disappeared. The oily layer was collected and washed by partitioning between chloroform and water to yield (+)-1-phenylethanethiol. After removal of solvent the product was used without further purification.

By the same method, (−)-1-phenylethanethiol was prepared from (−)-O-menthyl-(*R*)-1-phenylethyl dithiocarbonate. About 3 g each of (+)- and (−)-1-phenylethanethiol were obtained.

2.3. Sample solutions

Stock solutions of each enantiomeric monosaccharide (2.0 mg ml^{-1}) in methanol–water (1:4, v/v), were kept at 5°C. Glucuronic acid and its 6,3-lactone react with thiols to afford the dithioacetal of the lactone [11], thus glucuronic acid 6,3-lactone was used as standard for glucuronic acid.

Samples of 3,6-dideoxyhexoses were prepared by hydrolysis of lipopolysaccharides (10 mg ml^{-1}), for 1 h at 100°C with aqueous trifluoroacetic acid (50 m*M*). Under these weak hydrolysis conditions, the 3,6-dideoxyhexoses, which occurred terminally in the polysaccharides, were obtained in an almost pure state. After cooling the reaction mixture it was quenched with methanol and centrifuged (20 000 *g* at 4°C for 4 min) to remove the precipitated polysaccharide. Hydrolysates were reconstituted to the original volume in methanol–water (1:4, v/v), and were kept at 5°C. The lipopolysaccharides used were prepared from *Salmonella enterica* serovar *typhimurium* (strain SH4809), *S. enterica* serovar *enteritidis* (strain SH1262), *S. enterica* serovar *paratyphi A* (strain IS2) and from *Escherichia coli* O111, and contained; abequose (3,6-dideoxy-D-galactose), tyvelose (3,6-dideoxy-D-mannose), paratose (3,6-dideoxy-D-glucose) and colitose (3,6-dideoxy-L-galactose), respectively.

2.4. Preparation of diastereomeric sugars

The diastereomeric dithioacetals were prepared essentially as described, [8]. To the dry sample (0.01–0.2 mg sugar) trifluoroacetic acid (5 μl) was added and either (+)- or (−)-1-phenylethanethiol (20 μl). Following mixing by vortexing for 15 s the sample was incubated at room temperature (≈22°C) for 75 min and then, in order to stop the reaction, cold pyridine (≈4°C, 50 μl) was added. All equipment that had been in contact with the thiol reagents was rinsed with a saturated aqueous $KMnO_4$ solution before waste disposal or wash-up, in order to avoid the thiol smell.

The excess of thiol and pyridine was removed by placing the opened tube in a desiccator with containers of concentrated H_2SO_4, pellets of KOH, and activated charcoal impregnated with $CuSO_4$, and the vial was kept overnight in vacuo. To the dry sample acetic anhydride (100 μl) containing 4-(dimethylamino)pyridine (2.5 mg ml^{-1}) was added. Following mixing by vortexing for 15 s the sample was heated at 65°C for 2 h and then taken to dryness. The sample was reconstituted in dichloromethane (300 μl) and the collected clear solution was stored at 5°C or analysed directly.

Alternatively, for the preparation of trimethylsilylated derivatives, hexamethyldisilazane (HMDS, 50 μl) and trimethylchlorosilane (TMCS, 25 μl) were added to the reaction mixture, as obtained after the pyridine addition. The reaction mixture was heated for 2 h at 65°C, cooled, and hexane (150 μl) was added. The collected clear solution was used for analysis.

3. Results and discussion

3.1. GLC of diastereomeric dithioacetals

Numerous thiols have been found suitable for preparing dithioacetals of sugars but to date only (+)-1-phenylethanethiol leads to the formation of acyclic diastereomers that can be readily resolved by GLC [8]. The synthesis of (+)-1-phenylethanethiol has been described [9], but not that of the (−)-form. Through continued fractional crystallisation of a diastereomeric derivative it was, however, possible to obtain both forms. This is described in Section 2.2.

The formation of the sugar dithioacetals involves a dynamic equilibrium; therefore, a significant excess of the derivatizing agent is necessary to force the equilibrium towards sugar dithioacetal formation. Diastereomeric dithioacetals were prepared at room temperature using a 100-fold excess of the (+)- or

the (−)-1-phenylethanethiol and the product was acetylated or trimethylsilylated and analyzed on the non-chiral DB-5 column. The time course of the reaction for six different sugars in a mixture, is illustrated in Fig. 1. The maximum yield was obtained after different times. The maximum yield of arabinose and galactose dithioacetals occurred at 60 min, whereas the yield of products from fucose, 2-acetamido-2-deoxy-D-glucose, 2-acetamido-2-deoxy-D-mannose and glucuronic acid 6,3-lactone was maximal at 90 min. At longer reaction times the yields decreased. At higher temperatures (30–45°C) both product formation and product degradation were significantly faster than at room temperature. For example, after 60 min at 45°C the yields were only 10–25% of the maximum yield for most sugars. For (+)-1-phenylethanethiol the time course and yields were, as expected, similar to those obtained with (−)-1-phenylethanethiol. The yields of 2-acetamido-2-deoxy-D-hexose derivatives were significantly lower than those of aldoses, and the order of derivatising efficiency was 2-acetamido-2-deoxy-D-mannose>2-acetamido-2-deoxy-D-galactose>2-acetamido-2-deoxy-D-glucose. Based on these observations, a reaction period of 75 min at room temperature (≈22°C) was chosen as standard procedure. This reaction period is longer than that given by Little [8], which may be explained, at least in part, by the differences in equipment and relative amounts of reactants.

The sugar derivatives were all, except for galacturonic acid, essentially eluted as a single peak at retention times which exceeded 4 min. After that time no significant amounts of byproducts were observed in the chromatograms when the sugars were analysed one by one. Galacturonic acid gave a secondary peak ≈10% of the main peak, possibly

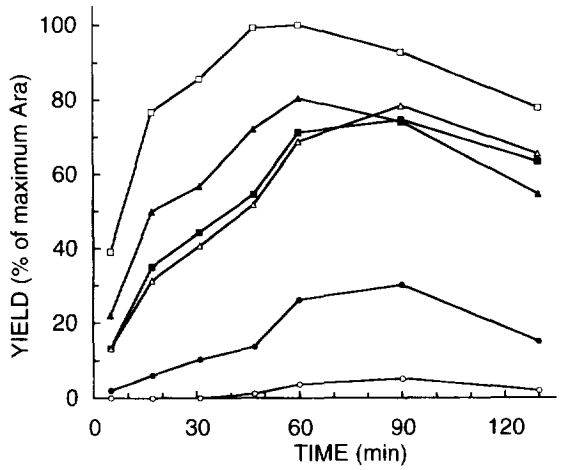

Fig. 1. Time course of the reaction between (−)-1-phenylethanethiol and a mixture of six compounds: D-arabinose (□), D-fucose (■), L-galactose (▲), D-glucuronic acid 6,3-lactone (△), 2-acetamido-2-deoxy-D-mannose (●), and 2-acetamido-2-deoxy-D-glucose (○). To the reaction mixture, containing 30 μg of each compound, 5 μl trifluoroacetic acid and 20 μl (−)-1-phenylethanethiol, 50 μl cold pyridine was added at the time indicated and then the sample was trimethylsilylated.

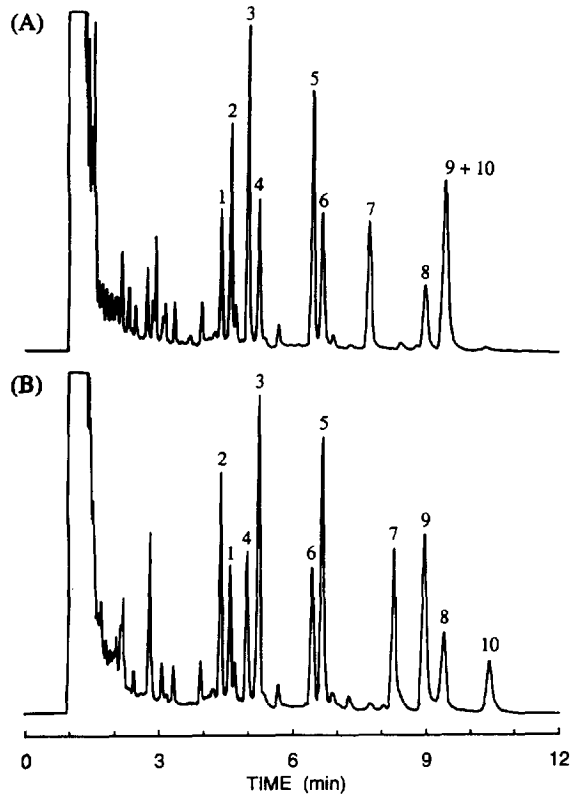

Fig. 2. Gas–liquid chromatograms of trimethylsilylated bis[(+)-1-phenylethyl] dithioacetals (a) and bis[(−)-1-phenylethyl] dithioacetals (b) of a mixture of colitose [3,6-dideoxy-L-galactose (1)], abequose [3,6-dideoxy-D-galactose (2)], D-arabinose (3), L-arabinose (4), D-rhamnose (5), L-rhamnose (6), D-glucuronic acid 6,3-lactone (7), L-galactose (8) D-galactose (9) and 2-acetamido-2-deoxy-D-galactose (10) on the DB-5 column (280°C). The reaction mixtures contained 20 μg of each D-compound (50 μg of 2-acetamido-2-deoxy-D-galactose) and 10 μg of each L-compound.

another lactone than the prevalent one. Fig. 2 shows chromatograms obtained from a mixture of four enantiomeric pairs in a D–L-ratio of 2:1, and two single enantiomers, as trimethylsilylated bis[(+)-, (Fig. 2a) and as bis[(−)-1-phenylethyl]-dithioacetals (Fig. 2b). As expected, the relative peak intensities in each pair are changed when the other thiol is used, since the (+)-thiol-D-sugar derivative is enantiomeric to the (−)-thiol-L-sugar derivative, and (+)-thiol-L-sugar derivative is enantiomeric to the (−)-thiol-D-sugar derivative, and hence they are not resolved on a non-chiral column. The retention data for acetylated and trimethylsilylated derivatives are given in Tables 1 and 2, respectively. For previously reported [8] sugars given in Tables 1 and 2, the retention data were close to those described. The resolution of different sugars of a complex mixture increased by using both (+)- and (−)-1-phenylethanethiol in the analyses. For example, acetylated bis[(+)-1-phenylethyl] dithioacetals of D-glucose and L-mannose have the same retention time but, as acetylated bis[(−)-1-phenylethyl] dithioacet-

Table 1
GLC data for acetylated diastereomeric dithioacetals formed with (+)- or (−)-1-phenylethanethiol on a DB-5 column

Parent sugar	Retention time (min)		α[a]
	(+)-1-Phenylethanethiol	(−)-1-Phenyl ethanethiol	
2-Deoxy-D-rib	5.13	5.15	(1.00)
2-Deoxy-L-rib	5.13	5.15	
D-Ara	6.00	6.31	1.068
L-Ara	6.32	5.98	
D-Lyx	6.03	6.36	1.065
L-Lyx	6.34	6.02	
D-Rib	6.01	5.63	1.081
L-Rib	5.62	6.00	
D-Xyl	6.49	5.94	1.110
L-Xyl	5.94	6.48	
D-Fuc	6.30	6.03	1.051
L-Fuc	6.03	6.29	
D-Rha	5.83	6.12	1.058
L-Rha	6.10	5.82	
Abe[b]	4.95	4.81	1.035
Col[b]	4.81	4.95	
Tyv[b]	4.67	4.78	1.030
Par[b]	4.90	4.64	1.069
D-Gal	9.99	9.53	1.056
L-Gal	9.51	10.01	
D-Glc	9.70	9.05	1.081
L-Glc	9.05	9.70	
D-Man	9.23	9.70	1.058
L-Man	9.70	9.21	
D-GlcNAc	10.10	10.12	1.002
D-GalNAc	10.29	10.37	1.009
D-ManNAc	10.11	10.33	1.024
L-D-Hep[c]	12.11	12.49	1.034

[a] α, Separation factor, t'_{R2}/t'_{R1}, where $t'_{R2} > t'_{R1}$ and are the corrected retention times for the slower and the faster component, respectively. Corrected retention time (t'_R) is the retention time for a component minus the retention time for the solvent. When both D- and L-sugar enantiomers were analysed, the separation factor was the mean value of the four different values which could be calculated. Otherwise the separation factor was calculated from the retention times obtained for the two diastereomeric dithioacetals formed from a sugar enantiomer with (+)-1-phenylethanethiol and with (−)-1-phenylethanethiol.

[b] 3,6-Dideoxysugars, (see Section 2.3).

[c] L-*Glycero*-D-*manno*-heptose.

Temperature gradient program, 275°C (1 min, 2°C min^{-1})→300°C (4 min).

Table 2
GLC data for trimethylsilylated diastereomeric dithioacetals formed with (+)- or (−)-1-phenylethanethiol, on a DB-5 column at 280°C

Parent sugar	Retention time (min)		α[a]
	(+)-1-Phenylethanethiol	(−)-1-Phenylethanethiol	
2-Deoxy-D-rib	4.59	4.63	1.013
2-Deoxy-L-rib	4.63	4.58	
D-Ara	5.10	5.37	1.064
L-Ara	5.37	5.11	
D-Fuc	6.98	6.64	1.060
L-Fuc	6.64	6.96	
D-Rha	6.35	6.71	1.065
L-Rha	6.70	6.37	
Abe[b]	4.83	4.62	1.066
Col[b]	4.60	4.85	
D-Gal	9.65	9.19	1.057
L-Gal	9.17	9.64	
D-GlcNAc	9.72	10.58	1.099
D-GalNAc	9.56	10.66	1.128
D-ManNAc	10.99	10.88	1.011
D-Galacturonic acid[c]	7.03[d]	7.12[d]	1.015
D-Glucuronic acid[c]	7.90	8.48	1.084

[a] α, Separation factor, for explanation see Table 1.
[b] 3,6-Dideoxy sugars, (see Section 2.3)
[c] The dithioacetal of galacturonic acid lactonizes upon warming. Glucuronic acid and its 6,3-lactone react with thiols to form the dithioacetal of the lactone [11].
[d] Main peak, an additional secondary peak formed with the (+)-thiol occured at 8.43 min and formed with the (−)-thiol at 8.37 min.

als these sugars were resolved (Table 1). Also, trimethylsilylated bis[(+)-1-phenylethyl] dithioacetals of 2-acetamido-2-deoxy-D-galactose and D-galactose have the same retention time but were resolved as trimethylsilylated bis[(−)-1-phenylethyl] dithioacetals (Fig. 2). Furthermore, sugars which have the same or similar retention time as acetylated derivatives, may be separated as trimethylsilylated derivatives and vice versa. For example, acetylated derivatives of D-arabinose and L-fucose are eluted at the same time (Table 1) but the trimethylsilylated derivatives of same sugars are resolved (Table 2).

Good separation factors, usually 5–10%, were obtained for all pairs of the sugars tested, with one significant exception. The acetylated derivatives of 2-deoxyribose could not be resolved (Table 1). The trimethylsilylated derivatives of 2-deoxyribose were resolved but the separation factor was only 1.01. This discrepancy from the other sugars may be explained by the finding that the order in which the acetylated diastereomers were eluted depended primarily upon the stereochemistry at C-2, of the parent sugar. Thus, for the acetylated bis[(+)-1-phenylethyl] dithioacetals, sugars having the (*S*)-configuration at C-2 were eluted prior to those having the (*R*)-configuration. This characteristic feature was found applicable to all the pairs of sugar enantiomers which had a hydroxy group at C-2 (Table 1). Consequently, the absolute configuration of a monosaccharide can be assigned with a fairly large degree of certainty without the use of any standard, since the stereochemistry at C-2 can be determined. In other words, if a new sugar of unknown absolute configuration is isolated, it is possible to make a very likely guess about its absolute configuration. The relative configurations at the other chiral centers in the molecule must however be known, something which often can be determined with NMR spectroscopy. This requires that both (+)- and (−)-1-phenylethanethiol are used as reagents for the analysis. If an unknown monosaccharide gives a shorter retention time with the (+)-1-phenylethyl derivative, C-2 has the (*S*)-configuration and vice versa. However, for maximum security, at least one pure enantiomer of known configuration should be used as reference and this should also be

analysed with the use of both (+)- and (−)-1-phenylethanethiol.

In addition to GLC on a 28 m DB-5 column with 0.25 μm phase, a shorter DB-5 column with a 0.40 μm phase was used when running GLC–MS. The separation factor obtained from the shorter column was in most cases some percent higher, probably attributable to the phase thickness.

3.2. Mass spectra of diastereomeric dithioacetals

It is important that sugars to be analysed can be identified in a gas–liquid chromatogram. This is best achieved using detection by mass spectrometry and we have therefore analysed the different types of sugars, hexoses, deoxyhexoses etc. with GLC–MS.

3.2.1. Mass spectra of acetylated diastereomeric dithioacetals

The mass spectra of acetylated bis(1-phenylethyl)dithioacetals contain several fragments which are analogous to those of acetylated diethyl dithioacetals [12], as the fragmentation should be similar. However, a molecular ion was found for deoxyhexoses and pentoses only. Elimination of acetic acid from the molecular ion forms a fragment which was found for all sugars tested except 2-deoxypentose. As for diethyl dithioacetals [12], the fission of a carbon–sulfur bond in the molecular ion, led to fragment 1a (Fig. 3 and Table 3,). However, in contrast to diethyl dithioacetals, a fragment (labeled 3a) 32 atomic mass units (u) higher than that of 1a was found in all spectra, and corresponded to elimination of a phenylethyl radical (105 u). The phenylethyl ion was the most intense in the spectra. Other significant fragments derived from the upper part of the molecule had *m/z* 77, 79, 91, 121, 136, 137 and 287 (fragment 2). Loss of acetic acid, ketone, and/or acetic anhydride from fragments 1a and 3a, as well as, loss of the second phenylethyl entity yields other significant fragments (Fig. 3 and Table 3). The fragments which are most useful for an easy interpretation are given in italic characters in Table 3.

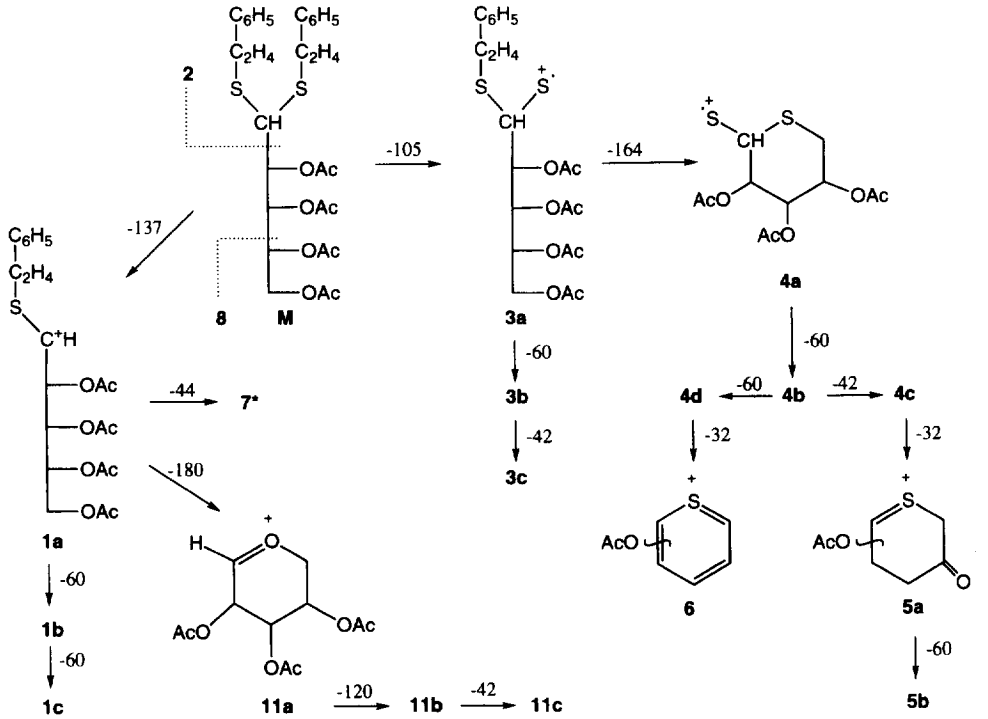

Fig. 3. Proposed structures of the major fragments from EI–MS of acetylated bis(1-phenylethyl) dithioacetals; exemplified with the ribose derivative. * There are no fragments 7 detected from derivatives of pentoses.

Table 3
Significant EI-MS fragments of acetylated bis(1-phenylethyl) dithioacetals of various sugars

Sugar	Fragment[a]																	
	11c	5b	*11b*	8	6	5a	4d	4c	4b	*11a*	4a	1c	3c	*1b*	7	3b	*1a*	3a
	m/z[b]																	
Heptose	241	257	283	289	299	317	331	*349*	391	403	*451*	–	513	*523*	539	555	583	*615*
Hexose	169	185	211	217	227	245	259	*277*	319	331	*379*	–	441	*451*	467	*483*	511	543
6-Deoxyhexose	111	127	153	159	169	187	201	*219*	261	273	*321*	333	383	393	409	*425*	453	*485*
3,6-Dideoxyhexose	–	–	95	159	111	129	143	*161*	203	215	*263*	275	325	335	*351*	*367*	395	427
Pentose	97	113	139	145	155	173	187	*205*	247	259	*307*	319	369	379	–	*411*	439	*471*
2-Deoxypentose	–	–	81	145	97	115	129	*147*	*189*	201	249	261	311	321	–	*353*	381	*413*

[a] The different fragments are numbered as the corresponding fragments of diethyl dithioacetals [12] (italic characters). Other designations as shown in Fig. 3.

[b] The four *m/z* values in each group of sugars, given in italic characters, are the most useful for identification of a sugar.

3.2.2. Mass spectra of trimethylsilylated diastereomeric dithioacetals

In contrast to the mass spectra of acetylated bis(1-phenylethyl) dithioacetals the mass spectra of the corresponding trimethylsilylated derivatives showed quite simple electron impact (EI) spectra, with abundant fragments derived from the carbohydrate chain.

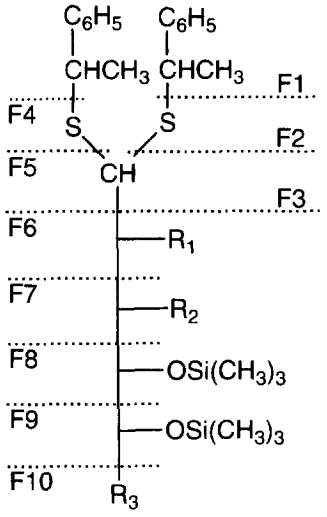

Fig. 4. General structure of trimethylsilylated dithioacetals and structure of primary fragments F1–F10. Fragments F4–F10 (Table 4) can lose $HOSi(CH_3)_3$ to give a series of secondary ions, 90 u lower. Key: hexose $R_1=R_2=OSi(CH_3)_3$, $R_3=CH_2OSi(CH_3)_3$; 6-deoxyhexose $R_1=R_2=OSi(CH_3)_3$, $R_3=CH_3$; 3,6-dideoxyhexose $R_1=OSi(CH_3)_3$, $R_2=H$, $R_3=CH_3$; hexuronic acid 6,3-lactone $R_1=OSi(CH_3)_3$, $R_2=6,3\text{-}O$, $R_3=CO$; 2-acetamido-2-deoxyhexose $R_1=NHCOCH_3$, $R_2=OSi(CH_3)_3$ $R_3=CH_2OSi(CH_3)_3$; pentose $R_1=R_2=OSi(CH_3)_3$, $R_3=H$; 2-deoxypentose $R_1=R_3=H$, $R_2=OSi(CH_3)_3$.

As for acetylated dithioacetals, the fission of a carbon–sulfur bond in the molecular ion, led to fragment F5 (corresponding to fragment 1a for the acetylated dithioacetals, Fig. 4) and a fragment F4, 32 u larger than the mass of F5, was found in all spectra, and corresponds to elimination of a phenylethyl radical (105 u). Here also, the phenylethyl ion was the most intense in the spectra. Another fragment significantly present in all spectra of trimethylsilylated bis(1-phenylethyl) dithioacetals is F3, 287 u. The primary fragments F4–F10, (Fig. 4 and Table 4) can lose $(CH_3)_3SiOH$ to give ions, 90 u lower.

4. Conclusion

The 1-phenylethanethiol method has several advantages over other methods used for determination of the absolute configurations of monosaccharides by GLC; good separation factors, only one derivative is formed which is easily prepared and stable and standard columns can be used. By analysing both the acetylated bis[(+)- and bis[(−)-1-phenylethyl] dithioacetals the absolute configuration at C-2, provided it carries a hydroxy group, can be determined with great certainty and thereby, if the relative configuration of the sugar is known, the absolute configuration can be deduced. In no case has more than one enantiomer of a pair to be available as reference, as the product of a D-sugar and (+)-1-phenylethanethiol is the enantiomer of the product of the L-form of same sugar and (−)-1-phenylethanethiol, and vice versa.

Table 4
Primary EI-MS fragments of trimethylsilylated dithioacetals

Parent sugar etc.	Primary fragments						
	F4	F5	F6	F7	F8	F9	F10
	m/z						
Hexose	(693)	(661)	511	409	307	205	103
6-Deoxyhexose	605	573	423	321	219	117	N.d.[a]
3,6-Dideoxyhexose	517	485	335	233	219	117	N.d.
Hexuronic acid	545	513	363	261[b]	N.d.	N.d.	N.d.
2-Acetamido-2-deoxyhexose	662	N.d.	480	409	307	205	103
Pentose	591	(559)	409	307	205	103	N.a.[c]
2-Deoxypentose	503	471	N.d.	307	205	103	N.a.

[a] N.d., not detected.
[b] The fragment F7 of hexuronic acids also lost CO_2 (44 u) in addition to the serial loss of $HOSi(CH_3)_3$, yielding a significant fragment at *m/z* 217.
[c] N.a., not applicable.
Loss of $HOSi(CH_3)_3$ gives a series of secondary ions, 90 u. lower. For explanation of F4–F10 see Fig. 4. Numbers given in parenthesis indicate that the primary ion is absent or very weak.

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