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RESOLUTION OF THE ENANTIOMERS OF ALDOSES BY LIQUID CHROMATOGRAPHY OF DIASTEREOISOMERIC 1-(*N*-ACETYL-α-METHYLBENZYLAMINO)-1-DEOXYALDITOL ACETATES

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ABSTRACT

Acyclic diastereoisomers, namely, 1-(*N*-acetyl-α-methylbenzylamino)-1-deoxyalditol acetates are readily obtained by reductive amination of aldoses with chiral α-methylbenzylamine (MBA) in the presence of sodium cyanoborohydride, and may be separated by reversed-phase l.c. and, more effectively, by adsorption l.c. According to this procedure, enantiomers of the common, neutral aldoses are resolved. In adsorption l.c., L-L* [defined as an adduct of an L-aldose and L-(−)-MBA] is eluted before D-L* for erythrose, xylose, ribose, glucose, 4-*O*-methylglucose, galactose, and fucose, and the elution order is the reverse for arabinose, lyxose, mannose, rhamnose, and glyceraldehyde. This behavior is probably related to the configuration of C-2 of the aldoses.

INTRODUCTION

The configuration of a monosaccharide can only be determined by measurement of its specific rotation when a fairly large amount of pure sample is available. An enzymic method is specific to selected sugars. The method based on circular dichroism (c.d.) measurements of the derived alditol acetates[1] needs milligram amounts of pure sample, and is not applicable for configurational determination of sugars that give *meso*-alditols, or those which give alditol acetates with a weak c.d.

Chromatographic resolution of sugar enantiomers may be advantageous; configurations of sugars can be exactly determined on a microscopic amount of a sample, even in a mixture. For the chromatographic separation of enantiomers, an intramolecular, or intermolecular, asymmetric environment must be achieved; the former is introduced by conversion of sugars into diastereoisomeric derivatives, and the latter, by use of a chiral eluant or stationary phase.

Although diastereoisomeric glycosides of chiral alcohols have been separated by using capillary g.l.c.[2,3], intricate chromatograms are given, due to the presence of anomers of pyranose and furanose structures. Zablocki *et al.*[4] synthesized acyclic diastereoisomers of bis(ethyl L-lactate) acetals from diethyl dithioacetals of enantiomers of galactose and fucose, and separated the diastereoisomers derived from each pair

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by packed-column g.l.c. However, the derivatization is complicated, and the yield is low*.

Herein is developed a convenient method for the determination of the configuration of sugars by l.c. of acyclic diastereoisomers, 1-(*N*-acetyl-α-methylbenzylamino)-1-deoxyalditol acetates.

L-(−)-Methylbenzylamine was chosen as an asymmetric coupling agent, because (*1*) it is commercially available; (*2*) it can be readily coupled with sugars, to give diastereoisomers by reductive amination[6,7]; (*3*) the derived diastereoisomers do not assume pyranose or furanose structures that may result in complicated chromatograms; and (*4*) the isomers have an aromatic group which either facilitates sensitive detection in liquid-chromatographic operation, or enhances separation of the diastereomers as a result of multiple, intramolecular interactions[8].

EXPERIMENTAL

Apparatus. — A liquid chromatograph was constructed from a mini pump (Nihon Seimitsu Co., Tokyo), a damper (type DAM, Umetani Seiki, Osaka), and an injection valve (model 7025, Rheodyne Inc., CA) having a 20-μL loop. The effluent was monitored at 230 nm with a UVILOG-5III u.v.-detector (Oyo Bunko, Tokyo). Reversed-phase[8] (r.p.) l.c. was performed in a stainless-steel column (8 mm i.d. × 15 cm) packed with ODS-silica (Develosil ODS-3, 3 μm, Nomura Chemicals Co., Seto, Aichi), and adsorption l.c. in a column (4.6 mm i.d. × 15 cm) packed with spherical, porous silica gel (Develosil 60-3, 3 μm, or 60-5, 5 μm, mean pore-size 6.0 nm). The columns were packed by using 1 : 1 hexanol–CH_2Cl_2 as the gel-dispensing medium[9] at 400 kg/cm^2, and conditioned by pumping methanol for 1 h at 400 kg/cm^2.

A Hitachi model 063 gas chromatograph was used for g.l.c. analysis. ^{1}H-N.m.r. spectra were recorded with a JEOL JNM-MH-100 spectrometer at a resonance frequency of 100 MHz, for solutions in CCl_4 at room temperature. Specific rotations were measured with an automatic, digital polarimeter (model PM-101, Union Giken Co., Osaka).

Materials. — D-Glyceraldehyde and L-galactose were purchased from Sigma Chemicals Co. (St. Louis, MO), and L-glucose and D- and L-lyxose from Nakarai Kagaku Yakuhin Co. (Kyoto). 4-*O*-Methyl-D-glucose was a gift from Dr. A. Ishizu. Other sugars were obtained from Tokyo Kasei Kogyo Co. (Tokyo). L-(−)- and DL-(±)-α-Methylbenzylamine were obtained from Aldrich Chemical Co. (Milwaukee, WI) and Tokyo Kasei Kogyo Co., respectively. Sodium cyanoborohydride was purchased from Nakarai Kagaku Yakuhin Co. Reagent-grade solvents were used in chromatography without purification.

Preparation of diastereoisomers. — A solution of L-(−)-α-methylbenzylamine

*We have recently been informed of a paper presented at the 179th ACS National Meeting that described[5] l.c. resolution of D- and L-arabinose, D- and L-galactose, and D- and L-mannose as the diastereoisomeric dithioacetals formed from the sugars and chiral 2,3-dimercaptosuccinic acid.

TABLE I

CHARACTERISTICS OF THE DIASTEREOISOMERS

Aldose	*Configuration*		*Analysis*			$[\alpha]_{589}^{25}$ *(degrees)*	(c)[a]	*M.p. (degrees)*
	Sugar	*MBA*	*C*	*H*	*N*			
Glyceraldehyde	D	L	63.30	7.22	4.44	−53	(0.47)	
	D	DL	63.58	7.42	4.09	—		
			(calc. 63.54	7.21	4.36)			
Erythrose	D	L	61.14	7.05	3.48	−34	(0.42)	
	D	DL	—	—	3.28	—		
			(calc. 61.06	6.92	3.56)			
2-Deoxy-*erythro*-pentose	D	L	61.86	7.02	3.22	−36	(0.69)	
	D	DL	62.26	7.02	3.88	—		
			(calc. 61.90	7.17	3.44)			
Ribose	D	L	58.64	6.77	3.44	−29	(0.44)	
	D	DL	59.06	6.40	2.74	—		
Arabinose	D	L	59.37	6.69	3.31	+13	(0.42)	
	L	L	58.62	6.65	2.80	−63	(0.47)	106–107
Xylose	D	L	59.80	6.40	2.87	−41	(0.74)	87–89
	L	L	58.87	6.72	2.82	−18	(0.56)	
Lyxose	D	L	59.70	6.99	3.09	−2	(1.05)	
	L	L	58.97	6.84	3.11	−57	(0.92)	
			(calc. 59.34	6.71	3.01)			
Rhamnose	L	L	59.54	6.84	3.10	−42	(0.30)	
	L	DL	60.03	6.91	2.77	—		
Fucose	D	L	60.68	7.28	3.08	−49	(0.14)	164–165
	L	L	59.36	6.74	3.23	−8	(0.45)	114–115
2-Deoxy-*lyxo*-hexose	D	L	59.58	6.97	2.83	−25	(0.44)	
	D	DL	59.71	7.01	2.47	—		
			(calc. 60.11	6.94	2.92)			
Glucose	D	L	57.95	6.81	2.81	−22	(0.73)	
	L	L	58.24	6.56	2.62	−25	(0.60)	
Mannose	D	L	58.08	6.58	2.53	−10	(0.50)	69–70
	D	DL	57.43	6.31	2.39	—		
Galactose	D	L	57.81	6.81	3.02	−30	(0.78)	111–113
	D	DL	58.25	6.62	2.09	—		
	L	L	57.60	6.59	2.40	−21	(0.77)	134–136
			(calc. 58.09	6.56	2.61)			
4-*O*-Methylglucose	D	L	58.46	6.92	2.64	−23	(0.15)	
	D	DL	58.51	6.60	2.94	—		
			(calc. 58.93	6.92	2.75)			

[a]Determined for a solution in $CHCl_3$.

[L-(−)-MBA] (10 mg, 83 μmol) and $NaBH_3CN$ (3 mg, 32 μmol) in methanol (0.2 mL) was added to a solution of a sugar (10 mg) in water (0.2 mL). The mixture was allowed to stand overnight, acidified to pH 3–4 by addition of glacial acetic acid, and evaporated to dryness.

To the resultant, oily material was added 1 : 1 acetic anhydride–pyridine (1 mL) and the mixture was heated in a sealed tube for 1 h at 100°. Water (1 mL) was added,

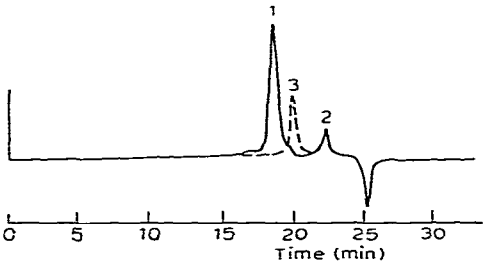

Fig. 1. Separation of acetylated products of reductive amination of D-xylose with L-(−)-α-methylbenzylamine by g.p.c. (TSK-gel G1000H_8, 7.6 × 600 mm × 2; eluant, THF; flow rate, 1 mL/min; detection, r.i.). Peak 1, 1-[*N*-acetyl-L-(−)-α-methylbenzylamino]-1-deoxy-D-xylitol acetate; 2, *N*-acetyl-L-(−)-α-methylbenzylamine. [The chromatogram of xylitol pentaacetate (peak 3) is superimposed in the Figure as a broken line.]

and the mixture was evaporated; to the oily residue was added water (1 mL), and the mixture was extracted with chloroform (1 mL). The extract was evaporated, to give an oily product which was purified by consecutive chromatography in a g.p.c. column (7.6 × 600 mm × 2) of TSK-gel G 1000H_8 with oxolane (THF) as the eluant at a flow rate of 1 mL/min and detection by r.i., and in a semi-preparative, reversed-phase column (8 mm i.d. × 25 cm) of Unisil Q C-18, Gasukuro Kogyo (5 μm) with 7:3 acetonitrile–water as the eluant at a flow rate of 2.5 mL/min and detection by r.i. Analytical data for the diastereoisomers prepared are listed in Table I.

RESULTS AND DISCUSSION

Derivatization. — A g.p.c. elution-curve for the acetylated reaction-products of D-xylose with L-α-methylbenzylamine (MBA) is shown in Fig. 1. Peak 2 was assigned to *N*-acetyl-L-MBA, judging from the ^{1}H-n.m.r. and i.r. data and the following physical constants: m.p. 102.5°, $[\alpha]_{589}^{19}$ −149.6° (*c* 2.01, ethanol) {lit.[10] m.p. 101–102°, $[\alpha]_D^{20}$ −150° (ethanol); lit.[11] m.p. 100–101.5°, $[\alpha]_D^{25}$ −152.8° (*c* 1.4, absolute ethanol)}. Peak 1 was identified as that of 1-(*N*-acetyl-α-methylbenzylamino)-1-deoxy-D-xylitol acetate from ^{1}H-n.m.r. and i.r. data and elementary analysis (see Table I and the next paragraph). Peak 3 (the dashed line) is due to xylitol acetate (prepared according to the literature[12]) and the amount of it remaining after derivatization was estimated to be ~10% from the chromatogram (the bold line). Other sugars were also derivatized to afford the corresponding diastereoisomers almost quantitatively.

The diastereoisomers obtained exhibited infrared absorption bands due to ester (1750, 1230, and 1050 cm^{-1}) and amide groups (1655 cm^{-1}). In their ^{1}H-n.m.r. spectra there appeared a phenyl resonance (δ 7.20), connected multiplets from methine protons in the MBA and sugar portions (δ 4.5–5.4), a doublet from -N-CH_2- (δ 3.0–3.4), and singlets from the acetyl methyl protons (δ 1.95–2.10). A doublet of doublets from -CH_2-OAc was recognized at δ 4.0, except for the diastereoisomers from the 6-deoxyhexoses (fucose and rhamnose), for which the C-5 methyl signal

appeared at δ 1.05. The position of the resonance of the methyl protons in the MBA portion (δ 1.45–1.6) was found to vary, depending on the configuration of the sugar.

For sugars for which both enantiomers were available, D-L* and L-L* were prepared therefrom† with L-(−)-MBA. If only one member of a pair of aldoses was at hand, a mixture of D-L* (L-D*) and D-D* (L-L*) derived from D(or L)aldoses and DL-(±)-MBA was prepared (see Table I). The D-L* (D-D*) form is the enantiomer of the L-D* (L-L*) form, and so each pair behaves completely identically in an achiral environment such as the present chromatographic system composed of an achiral eluant and a stationary phase. In order to confirm these criteria, we examined, for galactose and fucose, the chromatographic behavior of pure D-L* and L-L*, and of a mixture of D-L* and D-D* prepared from the D-sugar and (±)-MBA. Then, it was feasible to identify peaks as those given either by D-L* or L-L* by comparing chromatograms of the mixture and of a pure diastereoisomers prepared from the D(or L)-aldose and L-(−)-MBA.

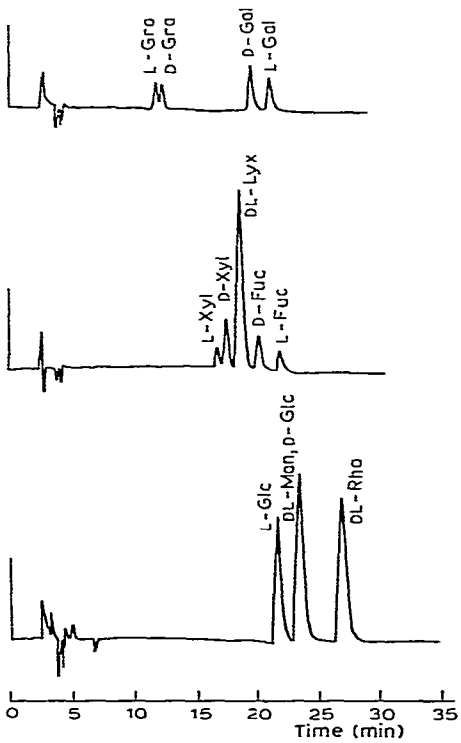

Fig. 2. Resolution of monosaccharides by reversed-phase l.c. as diastereoisomeric 1-[*N*-acetyl-L-(−)-α-methylbenzylamino]-1-deoxyalditol acetates. [Conditions: column, 8 × 150 mm, Develosil ODS-3, 3 μm; eluant, 2:3 acetonitrile–water; flow rate, 2.5 mL/min; detection, 230 nm, 0.08 aufs. Glyceraldehyde is abbreviated as Gra.]

†An adduct of a D-aldose and L-MBA is abbreviated as D-L*; the letter with the asterisk designates the configuration of the MBA added.

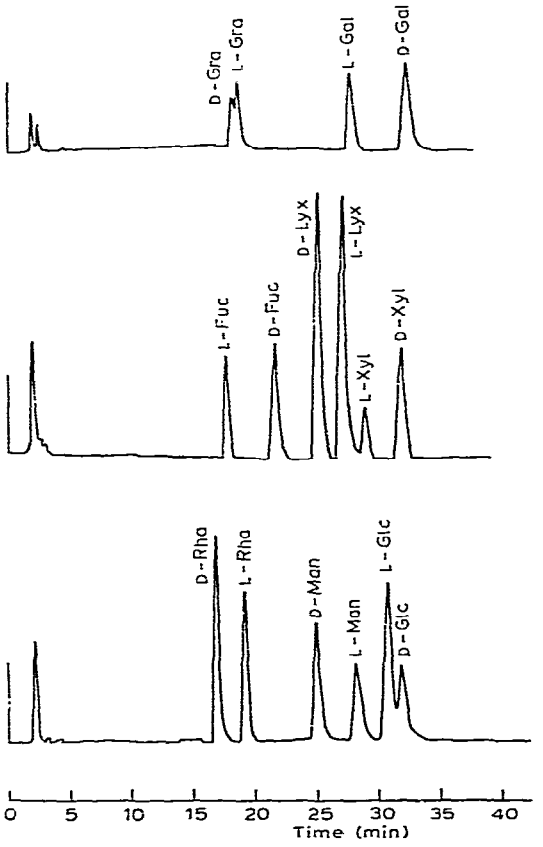

Fig. 3. Resolution by adsorption l.c., of aldoses as diastereoisomeric 1-[*N*-acetyl-L-(—)-α-methylbenzylamino]-1-deoxyalditol acetates. [Conditions: column, Develosil 60-3, 3 μm, 4.6 × 150 mm; eluant, 19:1 hexane–ethanol; flow rate, 1.25 mL/min; detection, 230 nm, 0.04 aufs.]

For each pure diastereoisomer, no peak corresponding to the antipode was detected in the adsorption chromatogram, the detailed conditions for which are described in the next section. Consequently, epimerization is negligible during the derivatization procedures.

Separation. — Attempted separation of the diastereoisomers by g.l.c. failed on capillary columns of fused silica: FFAP, PEG-20M (0.25 mm i.d. × 25 m, Gasukuro Kogyo, Tokyo) and SP-2100 (0.2 mm i.d. × 25 m, Hewlett–Packard).

Enantiomers of six aldoses (glyceraldehyde, arabinose, xylose, fucose, glucose, and galactose) could be resolved in the form of the diastereoisomers by r.p.-l.c., using 2:3 acetonitrile–water as the eluant (see Fig. 2). An eluant having an acetonitrile content of <30% may be needed in order to separate the diastereoisomers of other sugars, but it could not elute the solutes, presumably due to adsorption onto the gel.

Successful separation was achieved for almost all of the pairs of sugars studied, except the 2-deoxy sugars, by adsorption l.c. on 5- or 3-μm silica gel using 1:19

ethanol–hexane as the mobile phase (see Fig. 3). The separation factor (the ratio of the capacity factor of the second peak to that of the first peak) was found to be larger for the adsorption l.c. than for the reversed-phase l.c., except with glyceraldehyde and glucose (see Table II).

Sugars may be classified into three groups, based on their elution behavior in the adsorption l.c.: (*1*) erythrose, xylose, ribose, glucose, 4-*O*-methylglucose, galactose, and fucose, for which L-L* is eluted before D-L*; (*2*) arabinose, lyxose, mannose, rhamnose, and glyceraldehyde, for which the elution order is the reverse; and (*3*) 2-deoxy-*erythro*-pentose and 2-deoxy-*lyxo*-hexose, for which the diastereoisomers have the same retention time.

This classification must be related to the configuration of C-2 of the aldoses. The D enantiomers of the aldoses belonging to group *1* have the (R) configuration of C-2, those of group *2* have the (*S*) configuration, and C-2 of the 2-deoxy sugars (group 3) is, of course, nonasymmetric. Only glyceraldehyde is an exception.

The present analytical procedure may prove to be an effective tool for determination of the configuration of sugars, and for analysis of the constituent sugars of polysaccharides.

ACKNOWLEDGMENT

The kind gift of 4-*O*-methyl-D-glucose by Dr. A. Ishizu is appreciated.

TABLE II

RETENTION TIME (min) AND SEPARATION FACTORS OF THE DIASTEREOISOMERS

Aldose	*Adsorption l.c.*[a]			*R.p. l.c.*[b]		
	D-L*	L-L*	*r*[c]	D-L*	L-L*	*r*[c]
Glyceraldehyde	18.3	18.7	1.02	12.3	11.8	1.04
Erythrose	23.6	23.1	1.02	15.2		1.00
2-Deoxy-*erythro*-pentose	27.9		1.00	16.8		1.00
Ribose	28.0	25.8	1.09	19.6		1.00
Arabinose	23.8	27.9	1.17	17.5	16.8	1.05
Xylose	32.1	29.1	1.10	17.6	16.9	1.04
Lyxose	25.3	27.2	1.08	18.7		1.00
Rhamnose	17.0	19.3	1.14	27.2		1.00
Fucose	21.8	17.8	1.22	20.2	22.0	1.09
2-Deoxy-*lyxo*-hexose	30.1		1.00	20.4		1.00
Glucose	32.1	31.0	1.04	23.8	22.0	1.08
Mannose	24.8	28.4	1.15	23.8		1.00
Galactose	32.5	27.9	1.16	19.6	21.1	1.08
4-*O*-Methylglucose	40.2	35.6	1.13	19.6		1.00

[a]Column, Develosil 60-3, 4.6 × 150 mm; eluant, 19:1 hexane–ethanol; flow rate, 1.25 mL/min; pressure, 50 kg/cm^2. [b]Column, Develosil ODS-3, 8 × 150 mm; eluant, 2:3 acetonitrile–water; flow rate, 2.5 mL/min; pressure, 150 kg/cm^2. [c]Separation factor, defined as the ratio of the capacity factor of the second peak to that of the first peak.

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