CHEMISTRY LETTERS, pp. 943-946, 1981.

DETERMINATION OF CONFIGURATION OF MONOSACCHARIDES BY HPLC ON DIASTEREOISOMERIC 1-DEOXY-1-(N-ACETYL-α-METHYLBENZYLAMINO)ALDITOL ACETATES

Ryuichi OSHIMA and Ju KUMANOTANI
Institute of Industrial Science, University of Tokyo
7-22-1, Roppongi, Minatoku, Tokyo 106

Acyclic diastereoisomers, 1-deoxy-1-(N-acetyl-α-methylbenzylamino)alditol acetates, are readily obtained by reductive amination of sugars with chiral α-methylbenzylamine in the presence of sodium cyanoborohydride. Ten pairs of enantiomers of common monosaccharides are resolved by an adsorption HPLC on the diastereoisomers.

Configuration of monosaccharides can be assigned by specific-rotation measurement only when considerably great amounts of pure sample are available. An enzymic method, for which microquantities are sufficient, is specific to selected sugars. The method based on circular dichroism (CD) measurement of alditol acetates needs milligram amounts of pure sample and is not applicable to sugars which give meso-alditols or alditols with weak CD.[1]

Chromatographic resolution of sugar enantiomers is likely to be advantageous, viz., configuration of sugars can exactly be determined on microscopic amounts of sample even in a mixture. Although diastereoisomeric glycosides with chiral alcohols have been separated using capillary g.l.c. by Gerwig et al.[2] and Leontein et al.,[3] intricate chromatograms were given due to the presence of anomers. Zabolski et al.[4] synthesized acyclic diastereoisomers of bis(ethyl L-lactate)acetals from diethyl dithioacetals of galactose and fucose, and separated a pair of diastereoisomers derived from each sugar by g.l.c. on a packed column. However, the derivatization procedure was complicated and the yield was unsatisfactory.

Here is presented a convenient method for determination of the configuration of sugars by HPLC on acyclic diastereoisomers, 1-deoxy-1-(N-acetyl-α-metylbenzylamino)-

alditol acetates, which are derived from sugars and chiral α-methylbenzylamine (MBA) in the presence of sodium cyanoborohydride.

A solution of L-(-)-MBA (Aldrich, Wis.) (10 mg) and $NaBH_3CN$[5,6] (Nakarai Chemicals, Kyoto) (2 mg) in 0.2 ml of methanol was added to a solution of a sugar (10 mg) in 0.2 ml of water. The mixture was allowed to stand overnight, acidified to pH 3 to 4 by addition of glacial acetic acid and evaporated to dryness. The resultant oily material was acetylated by acetic anhydride-dry pyridine (1:1) (1 ml) at 100 °C for one hour in a sealed tube. After codistillation of the acetic anhydride with toluene, water (1 ml) was added to the residue and the mixture was extracted with chloroform (1 ml). A chloroform layer was evaporated to dryness to give an oily product as residue, which was purified by consecutive chromatography on a g.p.c. column (TSK-gel $G1000H_8$, 7.6x600mmx2; eluant, THF) and on a semi-preparative reversed-phase column (Unisil Q C-18, 5 μm, 8x250mm; eluant, acetonitrile-water (7:3)). The g.p.c yield was almost quantitative. Most of the prepared diastereoisomers were oily, but some of them crystallized; D-L*[7] of xylose (mp 87-89 °C), L-L* of arabinose (mp 106-107 °C), D-L* (mp 164-165 °C) and L-L* (mp 114-115 °C) of fucose, D-L* (mp 109-113 °C) and L-L* (mp 132-136 °C) of galactose.

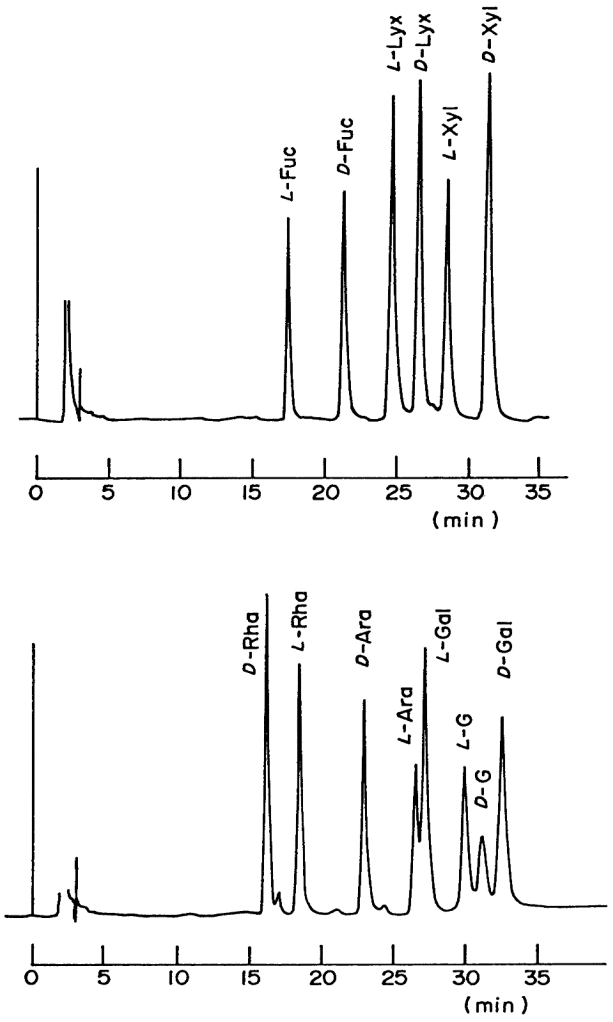

Fig. 1. Separation of diastereoisomeric alditol acetates derived from sugars and L-(-)-α-methylbenzylamine by HPLC. Conditions: column, Develosil 60-3 (3 μm, 4.6x150mm); eluant, n-hexane-ethanol (95:5); flow rate, 1.2 ml/min; detection at 230 nm, 0.04 aufs. Conventions for carbohydrate nomenclature (J.Org.Chem., 28, 281 (1963)) are followed for abbreviations.

The obtained diastereoisomers show infrared absorption bands of amide (1655 cm^{-1}) and ester groups (1750,

1230 and 1050 cm^{-1}). In their ^1H NMR spectra in CCl_4 appeared a singlet of phenyl resonance (δ 7.20), connected multiplets from methine protons in MBA and sugar portions (δ 4.5 to 5.4), a multiplet from $-N-CH_2-$ (δ 3.0 to 3.4) and singlets from acetyl methyl protons (δ 1.95 to 2.10). Two double doublets from $-CH_2-OAc$ were observed around δ 4 except for derivatives of 6-deoxyhexoses, for which a doublet from C-6 methyl protons appeared at δ 1.05. The position of the resonance of methyl protons in the MBA portion (δ 1.45 to 1.6) varies due to configuration of sugars.[8]

For sugars, both enantiomers of which were available, D-L* and L-L* were made with L-(-)-MBA. When only one of enantiomers was at hand, a mixture of D-L* (or L-L*) and D-D* (or L-D*) was made from D- (or L-) sugar and DL-(±)-MBA. D-L* (or D-D*) is an enantiomer of L-D* (or L-L*), or they behave identically in an achiral environment as the present chromatographic system which is composed of achiral eluant and stationary phase. Therefore, retention times can be determined for the both diastereoisomers of D- and L-sugars.[9]

Table 1. Retention times t_R and separation factors r of 1-deoxy-1-(L-N-acetyl-α-methylbenzylamino)alditol acetates by adsorption HPLC. Chromatographic conditions are the same as in Fig. 1.

Sugars	t_R (min) D-form	t_R (min) L-form	r
glyceraldehyde	18.2	19.0	1.04
2-deoxyribose	26.0	26.0	1.00
ribose	27.0	25.5	1.06
arabinose	22.9	26.4	1.16
xylose	31.1	28.3	1.10
lyxose	26.6	24.4	1.09
rhamnose	16.1	18.4	1.15
fucose	21.1	17.3	1.22
glucose	31.1	29.8	1.04
mannose	25.1	28.5	1.14
galactose	32.4	27.1	1.20

Among several chromatographic systems examined to separate the diastereoisomers, including capillary g.l.c., reversed-phase and adsorption HPLC, the best result was given by adsorption HPLC on a 4.6x150-mm column packed with 3-μm silica (Develosil 60-3, Nomura Kagaku, Nagoya)[10] using n-hexane-ethanol (95:5) as eluant (see Fig. 1). Ten pairs of enantiomers of common neutral monosaccharides can be resolved on the diastereoisomers by the adsorption HPLC; retention times and separation factors r (r is the ratio of capacity factor of the second peak over that of the first peak) are summarized in Table 1.

The present analytical procedure will extend to an effective tool for determination of configuration of sugars and analysis of constituent sugars of oligo- and polysaccharides and glycoconjugates of biological importance.

The authors would like to acknowledge Dr. N. Takai of this institute for permission to use a reversed-phase column. Thanks are also due to Mr. Y. Yamauchi of this laboratory for packing of efficient columns and valuable comment about HPLC conditions.

References and Notes

1) G.M.Bebault, J.M.Berry. Y.M.Choy, G.G.S.Dutton, N.Funnell, L.D.Hayward, and A. M.Stephen, Can. J. Chem., 51, 324 (1973).

2) G.J.Gerwig, J.F.Kammerling, and J.F.G.Vliegenthart, Carbohydr. Res., 62, 349 (1978).

3) K.Leontein, B.Lindberg, and J.Lonngren, Carbohydr. Res., 62, 359 (1978).

4) W.Zabolski, E.Behrman, J.Edwards, and G.A.Barber, J. Biochem. Biophys. Methods, 1, 253 (1979).

5) R.F.Borch, M.D.Bernstein, and H.D.Durst, J. Am. Chem. Soc., 93, 2897 (1971).

6) S.Hase, T.Ikenaka, and Y.Matsushima, Biochem. Biophys. Res. Commun., 85, 257 (1978).

7) An adduct of D-sugar and L-MBA is abbreviated as D-L*; a letter with asterisk designates the configuration of MBA.

8) The details of the analyses of ^{1}H NMR spectra will be described in a subsequent paper in connection with the mechanism of separation in the present HPLC.

9) B.Halpern, "Handbook of Derivatives for Chromatography", K.Blau and G.S.King Ed., Heydon and Son Ltd., London, 1978, p.457.

10) Column packing was performed using n-hexanol-chloroform (1:1) as gel-dispersing medium; Y.Yamauchi and J.Kumanotani, Seisan Kenkyu, 32, 255 (1980).

(Received April 27, 1981)