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Note

Ion-exchange chromatography of 2-amino-2,6-dideoxyhexoses and of the methyl ethers of 2-amino-2,6-dideoxy-D-glucopyranose (quinovosamine) and 2-amino-2,6-dideoxy-D-galactopyranose (fucosamine)*

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The following 2-amino-2,6-dideoxyhexoses have been identified as components of bacterial polysaccharides: 2-amino-2,6-dideoxy-D-glucose (quinovosamine), 2-amino-2,6-dideoxy-L-mannose (rhamnosamine), 2-amino-2,6-dideoxy-D-galactose (fucosamine), 2-amino-2,6-dideoxy-L-galactose, and 2-amino-2,6-dideoxy-L-talose (pneumosamine)[1]. In order to assist in the identification of 2-amino-2,6-dideoxyhexoses present in the lipopolysaccharides of *Pseudomonas* species[2], analytical methods based on the gas–liquid partition chromatography of their fully acetylated 2-acetamido-2,6-dideoxyhexitol derivatives and on the paper-chromatographic separation of 2-amino-2,6-dideoxyhexose hydrochlorides and 2-acetamido-2,6-dideoxyhexoses were developed[3].

As it had been previously found that mixtures of 2-amino-2-deoxyhexoses could be conveniently analyzed on a micro scale by ion-exchange chromatography[4], the procedure was further investigated to see if it could be used for the rapid, routine analysis of 2-amino-2,6-dideoxyhexoses.

Fig. 1 is a chromatogram showing the separation of the four naturally occurring 2-amino-2,6-dideoxyhexoses: 2-amino-2,6-dideoxy-D-mannose (A), 2-amino-2,6-dideoxy-D-glucose (B), 2-amino-2,6-dideoxy-D-galactose (C), and 2-amino-2,6-dideoxy-D-talose (D). 2-Amino-2,6-dideoxy-D-gulose (E) and 2-amino-2,6-dideoxy-D-allose (F) had practically identical retention times, but were in a region distinguishable from the four naturally occurring 2-amino-2,6-dideoxyhexoses (A, B, C, and D). The elution times of the 2-amino-2,6-dideoxyhexoses, given relative to 2-amino-2-deoxy-D-glucose, are recorded in Table I. The 2-amino-2,6-dideoxyhexoses are eluted in a region free from amino acids and they are eluted after 2-amino-2-deoxy-D-glucose, -D-galactose, and -D-mannose. The analyses can be completed in 3 h, and samples containing 1–40 μg of the individual hexosamines are adequate for both quantitative and qualitative analysis.

*NRCC No. 14236.

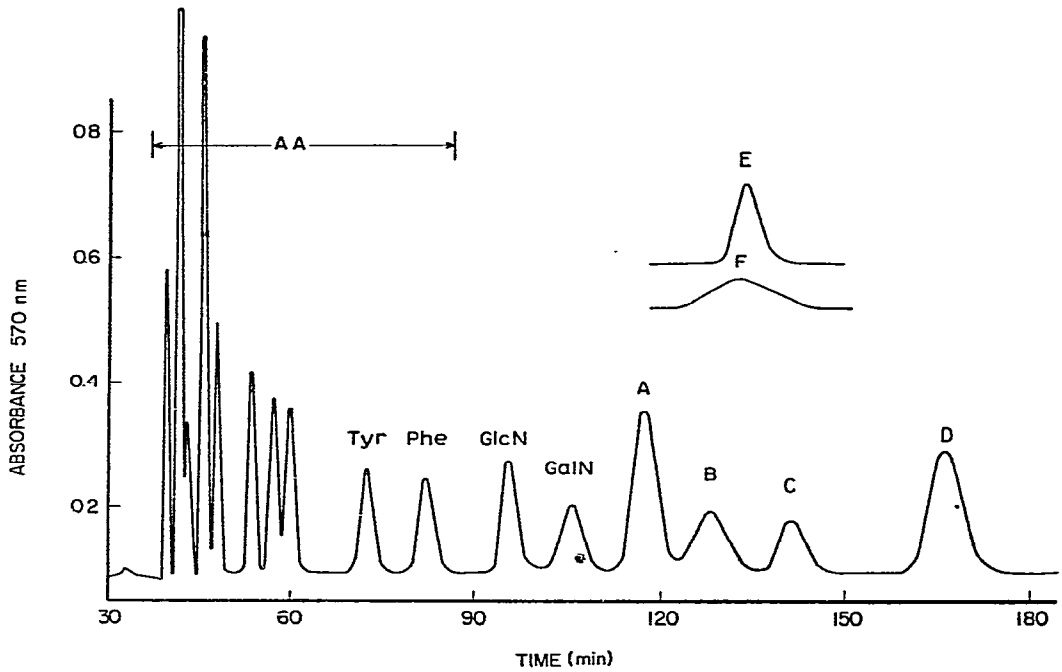

Fig. 1. Ion-exchange chromatographic separation of: AA, neutral and acidic amino acids (25 nanomoles each); GlcN, 2-amino-2-deoxy-D-glucose; GalN, 2-amino-2-deoxy-D-galactose; A, 2-amino-2,6-dideoxy-D-mannose; B, 2-amino-2,6-dideoxy-D-glucose; C, 2-amino-2,6-dideoxy-D-galactose; D, 2-amino-2,6-dideoxy-D-talose; E, 2-amino-2,6-dideoxy-D-gulose; and F, 2-amino-2,6-dideoxy-D-allose. Samples 5–10 μg of each glycose.

TABLE I

RELATIVE RETENTION-TIMES[a] OF 2-AMINO-2,6-DIDEOXYHEXOSES

Aminohexose	*Relative retention-time*
2-Amino-2-deoxyhexoses	
-D-glucose (glucosamine)	1.00
-D-mannose (mannosamine)	1.05
-D-galactose (galactosamine)	1.11
2-Amino-2,6-dideoxyhexoses	
-D-mannose (rhamnosamine)	1.24
-D-glucose (quinovosamine)	1.35
-D-allose	1.38
-D-gulose	1.42
-D-galactose (fucosamine)	1.48
-D-talose (pneumosamine)	1.75

[a]True elution-time plus delay by the analytical system. Determined with at least two buffer preparations. Retention time of 2-amino-2-deoxy-D-glucose was 92–96 min with three buffer preparations.

The identification of the methyl ethers of aminoglycoses obtained from the hydrolyzates of methylated glycosaminoglycans is usually a part of the procedure involved in determining their mode of substitution[5]. A variety of chromatographic methods can be used for the identification and determination of methylated amino-deoxyglycoses[6]; however, the finding that the separation of the methyl ethers of 2-amino-2-deoxy-D-glucose could be conveniently made by ion-exchange chromatography[7] prompted us to investigate the possibility of using this method for the analysis of the methyl ethers of 2-amino-2,6-dideoxy-D-glucose[8] and 2-amino-2,6-dideoxy-D-galactose[9]. Fig. 2A shows the complete separation obtained for the 3-, 4- and 3,4-di-methyl ethers of 2-amino-2,6-dideoxy-D-glucose. Fig. 2B shows a single,

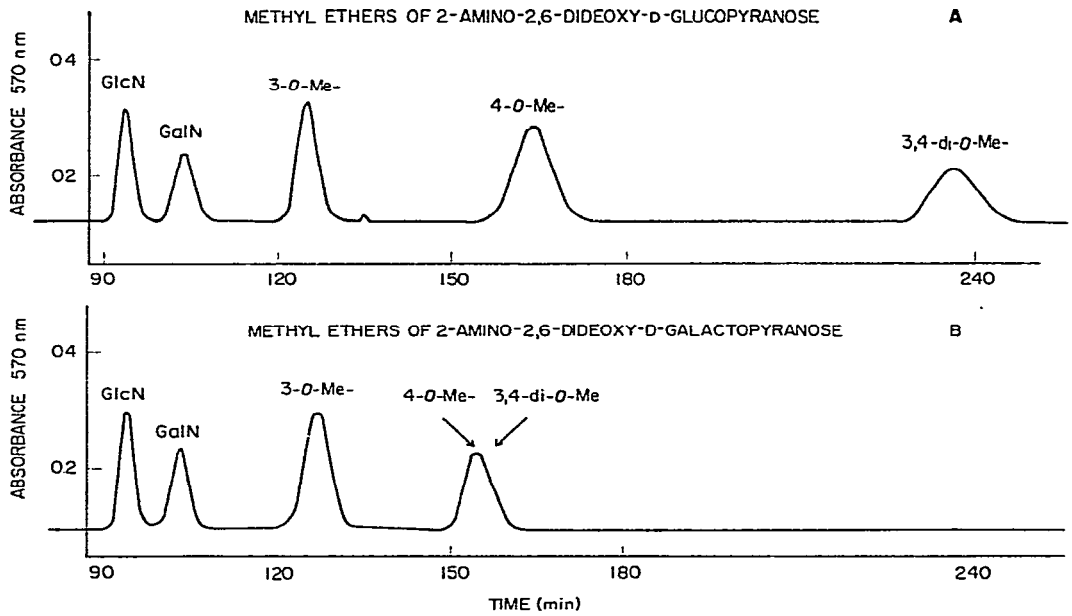

Fig. 2. (A) Ion-exchange separation of the 3-, 4-, and 3,4-di-methyl ethers of 2-amino-2,6-dideoxy-D-glucose. (B) Ion-exchange chromatographic separation of the 3-, 4-, and 3,4-di-methyl ethers of 2-amino-2,6-dideoxy-D-galactose.

separated peak for 2-amino-2,6-dideoxy-3-*O*-methyl-D-galactose, but the retention times for the 4- and 3,4-di-methyl ethers of 2-amino-2,6-dideoxy-D-galactose were too close to permit their separation, and the identification of these two glycoses should be established by paper or gas–liquid partition-chromatographic methods[9]. The retention times of the methyl ether derivatives of the 2-amino-2,6-dideoxyhexoses, given relative to 2-amino-2-deoxy-D-glucose, are recorded in Table II. The separations can be made on 1–50 μg of the individual glycoses, and the analyses can be completed within about 4 h.

TABLE II

RELATIVE RETENTION-TIMES OF THE 3-, 4-, AND 3,4-DI-METHYL ETHERS OF 2-AMINO-2,6-DIDEOXY-D-GLUCOSE AND 2-AMINO-2,6-DIDEOXY-D-GALACTOSE

2-Amino-2,6-dideoxyhexose methyl ether	*Relative retention time (2-amino-2-deoxy-D-glucose = 1.00)*
2-Amino-2,6-dideoxy-D-glucose	
3-*O*-methyl-	1.34
4-*O*-methyl-	1.76
3,4-di-*O*-methyl-	2.54
2-Amino-2,6-dideoxy-D-galactose	
3-*O*-methyl-	1.38
4-*O*-methyl-	1.71
3,4-di-*O*-methyl-	1.73

EXPERIMENTAL

Materials. — 2-Amino-2-deoxy-D-glucose, -D-galactose, and -D-mannose hydrochlorides were purchased from Pfanstiehl Laboratories Inc. The 2-amino-2,6-dideoxyhexose hydrochlorides and methyl ether derivatives of 2-amino-2,6-dideoxy-D-glucose and 2-amino-2,6-dideoxy-D-galactose were synthesized in the laboratory and were pure by paper and gas–liquid partition chromatography.

Chromatography. — A Technicon Sequential Multi-Sample Amino Acid Analyzer, Model TSM for physiologic sample chromatograms was used. The glass column, 5 mm inside diameter by 45 cm long, was packed with a slurry of Chromobeads type C (similar to Dowex 50 × 8) (Technicon Corp., Tarrytown, N.Y.) and was jacketed to maintain the column at 47°. Between runs, the column was automatically regenerated for 14 min with 0.3M lithium hydroxide and was then equilibrated for 16 min with a pH 2.85 lithium citrate buffer (see later).

The flow rate throughout the runs was 0.66 ml/min with a back pressure of 400–500 lb. $in.^{-2}$, and the effluent stream was analyzed (570 nm) automatically after reaction with ninhydrin–hydrazine sulfate reagent. Buffer solutions contained 10.51 g of citric acid monohydrate and 12.59 g of lithium hydroxide monohydrate per liter [0.3M Li^+]. A pH 2.85 lithium citrate buffer containing 4% of 2-methoxyethanol, and an elution buffer of pH 4.0 were made by adjustment of the lithium citrate solution to the required pH by the addition of ammonia-free 6M hydrochloric acid. After the automatic injection of the samples (*ca.* 10 μl = 10 μg of each aminoglycose), the pH 2.85 buffer was pumped for 4 min, and then the pH 4.0 buffer was pumped for 4.5 h.

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