

GAS-LIQUID CHROMATOGRAPHY OF CARBOHYDRATE DERIVATIVES: THE SEPARATION AND QUANTITATIVE DETERMINATION OF SYNTHETIC AND NATURALLY OCCURRING GLYCOSYL DERIVATIVES OF AMINO ACIDS*

GEORGE W. JOURDIAN AND JACK J. DISTLER*

*The Rackham Arthritis Research Unit and the Department of Biological Chemistry,
The University of Michigan, Ann Arbor, Michigan 48104 (U. S. A.)*

(Received April 22nd, 1971; accepted in revised form, November 22nd, 1971)

ABSTRACT

A gas-liquid chromatographic separation of the trimethylsilyl derivatives of several synthetic and naturally occurring glycosyl derivatives of amino acids is described. This technique may be used for the qualitative and quantitative determination of several compounds of biological interest including 3-*O*- β -D-xylopyranosyl-L-serine, 3-*O*- α -L-arabinopyranosyl-L-serine, 5-*O*- β -D-galactopyranosyloxy-D,L-lysine, and 2-acetamido-*N*-(L-aspart-4-oyl)-2-deoxy- β -D-glucopyranosylamine. In addition, the mobilities of these glycosyl derivatives of amino acids in paper chromatographic and paper electrophoretic systems are reported.

INTRODUCTION

Substituted and unsubstituted glycosyl derivatives of amino acids are found in a number of biological fluids and tissues. In tissues, they comprise the branch point between protein and covalently bound carbohydrate residues in acid mucopolysaccharides, glycoproteins, and collagen. For purposes of classification, protein-carbohydrate compounds may be divided into two groups. In one group, the bond or branch point involves the amide nitrogen of L-asparagine, whereas in the second, the carbohydrate portion is linked through a glycosidic bond to the hydroxyl group of an amino acid contained in the polypeptide chain. Unsubstituted glycosyl derivatives of amino acids occur in urine. These are believed to originate from the catabolism of tissue protein-carbohydrate complexes¹.

During the course of studies concerned with the metabolism of vertebrate glycoproteins, a method was required that would allow separation and estimation of glycosyl derivatives of amino acids in microgram quantities. The efficacy of gas-liquid chromatography for the analysis of biological compounds, including carbohydrates^{2,3} and amino acids^{4,5} prompted development of the method described in

*This work was supported by research grants from the National Institutes of Health, United States Public Health Service (Grant AM-10531) and the Arthritis Foundation, Michigan Chapter.

*Recipient of an Arthritis Foundation Postdoctoral Fellowship.

this paper. We have selected the per(trimethylsilyl)ation procedure introduced by Sweeley *et al.*⁶ and the internal standard technique of Ray⁷.

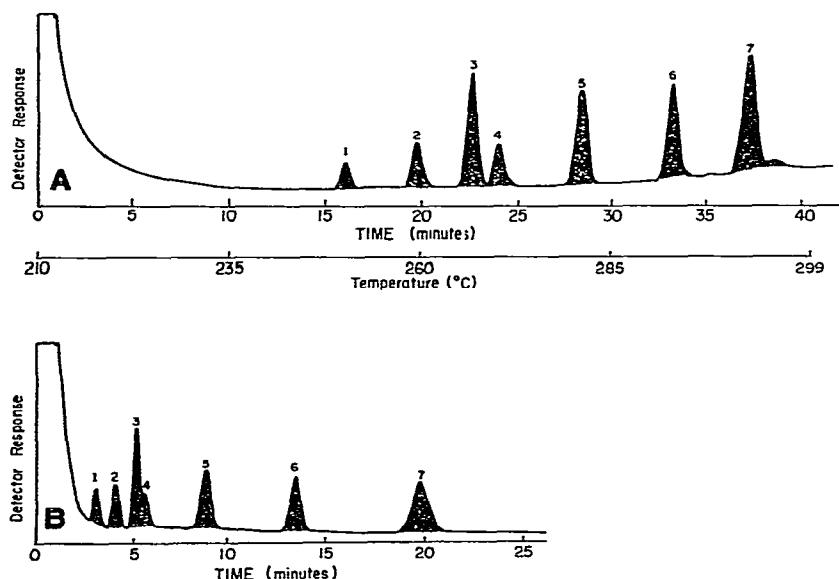


Fig. 1. A. Linear temperature-programmed separation of trimethylsilyl derivatives. The liquid phase and column parameters are given in the text. The instrument was programmed at 210–299° with a temperature increase of 2.5°/min, holding under isothermal conditions when a temperature of 299° was attained. B. Isothermal separation of trimethylsilyl derivatives. The components are: (1) 3-*O*- β -L-arabinopyranosyl-L-serine; (2) 3-*O*- β -D-xylopyranosyl-L-serine; (3) 3-*O*- β -D-galactopyranosyl-L-serine; (4) 3-*O*- β -D-glucopyranosyl-L-serine; (5) sucrose; (6) 5-*O*- β -D-galactopyranosyl-oxy-D,L-lysine; and (7) 2-acetamido-*N*-(aspart-4-oyl)-2-deoxy- β -D-glucopyranosylamine.

RESULTS AND DISCUSSION

The resolution of a mixture of standard glycosyl derivatives of amino acids at programmed temperature and isothermal modes is shown in Fig. 1. Of the seven compounds tested, only 3-*O*- β -D-glucopyranosyl- and 3-*O*- β -D-galactopyranosyl-L-serine exhibited a significant peak overlap at an isothermal temperature (270°); when a temperature program was utilized, complete resolution was obtained. Some variation in the resolution of 3-*O*- β -D-glucopyranosyl- and 3-*O*- β -D-galactopyranosyl-L-serine was observed with column packings prepared at different times and with commercially prepared packing. These differences in relative retention time (<5%) are not attributable to variability in the manner in which the columns were packed, since those prepared from the same batches of packing material gave essentially identical results. In no instance was the overlap of 3-*O*- β -D-glucopyranosyl- and 3-*O*- β -D-galactopyranosyl-L-serine too extensive for identification or quantitation when the gas-liquid chromatograph was temperature programmed. In general, as

found with the trimethylsilyl derivatives of free sugars, there is an increase in retention time as the number of carbon atoms is increased⁶.

Each test compound could be reliably determined under the conditions described. This was indicated by (a) the appearance of a single peak with each test compound; (b) a linear detection response (Fig. 2); and (c) a variation of $\pm 1.2\%$ in

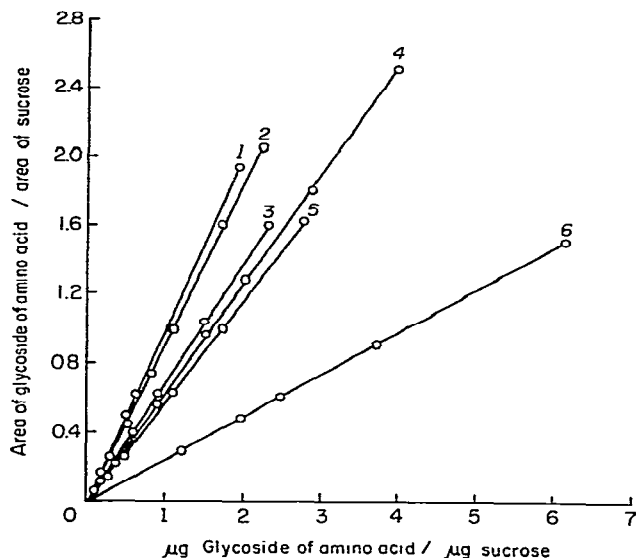


Fig. 2. Detector response obtained with glycosyl derivatives of amino acids at 270°. Varying amounts of compound were chromatographed with constant amounts of internal standard. The concentrations of the glycosyl derivatives of amino acids applied to the g.l.c. column varied between 1 and 25 nmoles. The compounds are: (1) 3-*O*- β -D-xylopyranosyl-L-serine; (2) 3-*O*- β -D-glucopyranosyl-L-serine; (3) 3-*O*- α -L-arabinopyranosyl-L-serine; (4) 3-*O*- β -D-galactopyranosyl-L-serine; (5) 5-*O*- β -D-galactopyranosyloxy-D,L-lysine; (6) 2-acetamido-*N*-(aspart-4-oyl)-2-deoxy- β -D-glucopyranosylamine.

relative retention time on duplicate derivatizations and injections. All trimethylsilyl derivatives except that of 2-acetamido-*N*-(L-aspart-4-oyl)-2-deoxy- β -D-glucopyranosylamine were stable over a 12-h period when maintained under anhydrous conditions. The latter derivative exhibited a second peak (relative retention time 1.69) after being kept 4–5 h at room temperature.

Although a linear detector response was obtained for each trimethylsilyl derivative, the slope of the detector response for the derivative of 2-acetamido-*N*-(L-aspart-4-oyl)-2-deoxy- β -D-glucopyranosylamine was less than that noted with the other glycosyl derivatives of amino acids tested.

In the initial phases of this work, each of the chemically synthesized glycosyl derivatives of amino acids (including 3-*O*- α -L-arabinopyranosyl-, 3-*O*- β -D-xylopyranosyl-, 3-*O*- β -D-glucopyranosyl-, and 3-*O*- β -D-galactopyranosyl-L-serine) exhibited double peaks (Table I). In all cases, the second minor peak disappeared after recrystal-

TABLE I

GAS CHROMATOGRAPHY OF TRIMETHYLSILYL DERIVATIVES OF MONOSACCHARIDES AND GLYCOSYL DERIVATIVES OF AMINO ACIDS^a

Derivatives	Temperature Conditions	
	Isothermal (270°)	Programmed (210–299°)
3- <i>O</i> - α -L-Arabinopyranosyl-L-serine	0.29	0.52 (0.49) ^b
3- <i>O</i> - β -D-Xylopyranosyl-L-serine	0.41	0.66 (0.59) ^b
3- <i>O</i> - β -D-Galactopyranosyl-L-serine	0.55	0.78 (0.67) ^b
3- <i>O</i> - β -D-Glucopyranosyl-L-serine	0.62	0.83 (0.72) ^b
Sucrose	1.00	1.00
5- <i>O</i> - β -D-Galactopyranosyloxy-D,L-lysine	1.52	1.17
2-Acetamido- <i>N</i> -(aspart-4-oyl)-2-deoxy- β -D-glucopyranosylamine	2.48	1.30
L-Arabinose		0.26,0.33
D-Xylose		0.22,0.25
D-Galactose		0.16,0.19
D-Glucose		0.12,0.14

^aThe values given in this Table are retention times relative to per(trimethylsilyl)ated sucrose at an isothermal and at a programmed temperature mode. Each value represents an average of 4 runs; a maximum deviation in retention time of ± 0.02 was found. The retention time for the internal standard, per(trimethylsilyl)ated sucrose, was 8.9 min in the isothermal mode and 28.3 min in the programmed temperature mode. Trimethylsilyl derivatives of L-hydroxylysine, L-aspartic acid, and L-serine had retention times of less than 0.25 when columns were programmed at 200–299°. ^bRetention time for a minor component detected in each of the crude preparations of chemically synthesized glycosyl derivatives of amino acids.

lization. This second peak cannot be attributed to the presence of free L-serine or carbohydrate, since in all cases their trimethylsilyl derivatives have distinctly shorter retention times than the parent amino acid–sugar compound (Table I). It is probable that these peaks were due to the presence of a smaller proportion of a different anomer, but sufficient amounts of the minor constituents were not available for further study. Resolution of the anomers would be expected in view of the results of Sweeley *et al.*⁶

In addition, Sweeley *et al.*⁶ found that the trimethylsilyl derivatives of di- and tri-saccharides could be separated on silicone columns at elevated temperatures. In the present study, an attempt was made to establish the retention time for per(trimethylsilyl)ated *O*- α -D-glucopyranosyl-(1 \rightarrow 2)- β -D-galactopyranosyloxy-(1 \rightarrow 5)-D,L-lysine. No peak was obtained under the isothermal or programmed gas-chromatographic conditions described in this paper or under a variety of other experimental conditions including (a) incubation in the presence of Regisil for prolonged-time periods, (b) heating with Regisil at 75° for periods up to 2 h, and (c) maintaining an isothermal mode of 300° for periods up to 4 h. These results suggest that the upper limit for detection of per(trimethylsilyl)ated glycosyl derivatives of amino acids in this system approximates 27 carbon atoms.

The present study of urine specimens fractionated on Dowex 2 ion-exchange resin suggests that several pentoses and hexoses occur in urine covalently bound to

compounds containing negatively charged groups. The trimethylsilyl derivative of 3-*O*- β -D-xylopyranosyl-L-serine isolated from urine or from chondromucoprotein exhibited the same relative retention time as the trimethylsilyl derivative of the chemically synthesized compound. Estimation by this procedure of 3-*O*- β -D-xylopyranosyl-L-serine in four 24-h urine specimens from a normal individual gave an average of 14.7 μ moles of 3-*O*- β -D-xylopyranosyl-L-serine/m²/24 h*. Subsequent

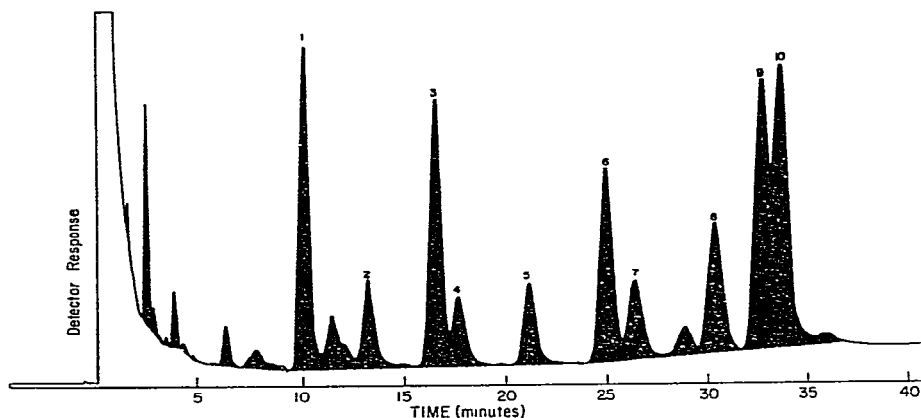


Fig. 3. G.L.C. of carbohydrate (as alditol acetate derivatives) found in human urine. The anionic fraction was hydrolyzed with Dowex 50 (H^+) for 24 h, desalted with Dowex 1 (HCO_3^-), and acetylated according to the conditions of Lehnhardt and Winzler⁸. The components cochromatographed with acetate esters of: (1) 2-deoxy-D-erythro-pentitol; (2) 6-deoxy-L-galactohexitol; (3) ribitol; (4) L-arabinitol; (5) xylitol; (7) 2-deoxy-D-xylo-hexitol or 2-deoxy-D-lyxo-hexitol or both; (8) D-mannitol; (9) galactitol; and (10) D-glucitol. Methyl β -D-glucopyranoside (6) was used as internal standard.

studies with a less specific, but somewhat simpler, procedure (Fig. 3) have shown a range of 18.0 to 42.7 (mean value 24.0) μ moles of 3-*O*- β -D-xylopyranosyl-L-serine/m²/24 h for urine from normal individuals and concentrations as high as 146 μ moles of 3-*O*- β -D-xylopyranosyl-L-serine/m²/24 h in patients suffering from connective tissue diseases⁹.

The presence of covalently bound arabinose in human urine⁹ and brain tissue¹⁰, and a report that L-arabinose occurs as a linkage point between peptide and hyaluronic acid¹¹, perhaps in a manner analogous to that found for D-xylose in chondroitin 4-sulfate, prompted the chemical synthesis of 3-*O*- α -L-arabinopyranosyl-L-serine**. Free 3-*O*- α -L-arabinopyranosyl-L-serine has not been demonstrated in human urine, but the synthetic compound serves as an acceptor for D-glucose residues when incubated in the presence of uridine-5-(α -D-glucopyranosyl pyrophosphate) and a novel glucosyltransferase obtained from embryonic-chicken brain¹³. These results suggest a possible biological role for this compound.

*Excretion relative to body surface.

**In a recent report, Katzman¹² concluded that arabinose is not a component of bovine-cerebral hyaluronic acid nor a component of crude mucopolysaccharide from bovine brain.

EXPERIMENTAL

Methods. — Descending paper chromatography was conducted on Whatman No. 40 and Schleicher and Schuell No. 589 Green papers with the following solvent systems (v/v): ethyl acetate–pyridine–water (50:2:7, upper layer, solvent A); 2-methyl-2-propyl alcohol–30% formic acid–water (5:1:1, solvent B); 2-butanone–propionic acid–water (3:1:12, solvent C); butyl alcohol–acetic acid–water (4:1:5, upper layer, solvent D); and ethyl acetate–pyridine–water (8:2:1, solvent E). Paper electrophoresis was conducted in a Gilson High Voltage Electrophorator (Model D) with Whatman 3MM paper and Isopar H (Humble Oil Co., Houston, Texas) as the coolant. The following conditions were used: 0.15M formic acid–1.6M acetic acid, pH 2.1, for 45 min at 58v/cm (buffer A); 50mM pyridine acetate, pH 6.5, for 30 min at 47 v/cm (buffer B). The chromatograms and electrophoretograms were air dried, sprayed with 0.2% ninhydrin in 95% acetone containing 2% pyridine, and heated at 100° to locate the compounds.

Gas-liquid chromatography. — Two Packard gas chromatographs were used in this study, a Series 7508 and a Model 7401. Each instrument was equipped with a temperature programmer and a dual-flame ionization detector. Coiled glass columns (4 mm i.d. and 244 cm in length) were packed with 3% OV-17 on 80/100 mesh Gas Chrom Q (Applied Sciences Laboratories, State College, Pa., Lot 767/60). The packed columns were conditioned with nitrogen at 270° for one week prior to use. This treatment reduced bleeding of the stationary phase to a level at which an electronic differential operation to compensate for baseline drift was not required. A number of column septa were employed. Of these, silicone rubber septa (Applied Science Laboratories, Type W-10) were found to withstand the high detector-cell temperature (370°) with minimal contribution to baseline response. The use of stainless steel in place of polypropylene tubing for detector leads, or stainless steel columns in place of glass columns resulted in degradation of the trimethylsilyl derivatives and the formation of extraneous peaks. Unless otherwise specified, the g.l.c. conditions were: column temperature 210–299°, at a program rate of 2.5°/min; detector cell temperature, 370°; injection temperature, 330°; hydrogen flow to detector, 30 ml/min; air to detector burner, 300 ml/min; carrier nitrogen, 45 ml/min; and chart speed 76 cm/h. Satisfactory resolution was obtained at program rates as high as 5°/min.

Column calibration and calculations. — The peak area was determined by multiplying the height of the peak by the width at half height. A detection-response curve was obtained for each glycosyl derivative of an amino acid by chromatographing varying amounts of the corresponding trimethylsilyl derivative in the presence of a constant amount of per(trimethylsilyl)ated sucrose. The ratio of the total area of the derivative peak to that of the standard was plotted against the ratio of the weight of the amino acid derivative to that of sucrose, the internal standard¹⁴.

Preparation of trimethylsilyl derivatives. — Aliquots containing 5 nmoles of sucrose as internal standard and suitable amounts of amino acid derivatives (1–25 nmoles) were pipetted in Kontes Microflex tubes, 0.3-ml capacity (Kontes Glass

Co., Vineland, New Jersey) and evaporated to dryness on a rotary desiccator *in vacuo*. *N,N*-Dimethylformamide (5 μ l), and Regisil [*N,N*-bis(trimethylsilyl)trifluoroacetamide and 1% trimethylchlorosilane (Regisil Chemical Co., Chicago, Illinois)] (25 μ l) were added under anhydrous conditions. The tubes were sealed with teflon caps, vigorously shaken, and kept for 90 min at room temperature. Suitable aliquots (less than 10 μ l of the reaction mixture) were injected into the gas-liquid chromatograph.

The quantity of *N,N*-dimethylformamide used was not critical and could be varied for quantitative studies. The time required for maximal yields of the trimethylsilyl derivatives was studied using 3-*O*- β -D-xylopyranosyl-L-serine as a model compound. A series of tubes containing 3-*O*- β -D-xylopyranosyl-L-serine (5 nmoles), *N,N*-dimethylformamide (5 μ l), and Regisil (25 μ l) were incubated at room temperature. During the first hour, tubes containing reaction mixtures were removed at 15-min intervals, and thereafter, at 30-min intervals for a period of 5 h. At each time period, each tube was immediately opened, per(trimethylsilyl)ated sucrose (5 nmoles) added, the solution mixed, and a suitable aliquot injected into the gas-liquid chromatograph; the maximum yields were obtained in 30 min. Subsequent studies with 2-acetamido-*N*-(L-aspart-4-oyl)-2-deoxy- β -D-glucopyranosylamine gave variable results at this time interval, but consistent qualitative per(trimethylsilyl)ation was obtained at 90 min for each of the test compounds, and all subsequent studies were conducted at this longer reaction time.

Materials. — D-Glucose, D-galactose, D-xylose, L-arabinose, and sucrose were obtained from Pfanstiehl Laboratories, Inc. (Waukegan, Illinois); and 2-acetamido-*N*-(L-aspart-4-oyl)-2-deoxy- β -D-glucopyranosylamine, L-aspartic acid, and L-serine from Cyclo Chemical Co. (Los Angeles, California). 3-*O*- β -D-Xylopyranosyl-L-serine-*t* (104 mCi/ml) was prepared by the New England Nuclear Corporation (Boston, Massachusetts). The method of Kum and Roseman¹⁵ was used to synthesize 3-*O*- β -D-xylopyranosyl-L-serine, 3-*O*- β -D-glucopyranosyl-L-serine, and 3-*O*- β -D-galactopyranosyl-L-serine. 5-*O*- β -D-Galactopyranosyloxy-D,L-lysine and *O*- α -D-glucopyranosyl-(1 \rightarrow 2)- β -D-galactopyranosyloxy-(1 \rightarrow 5)-D,L-lysine were generous gifts of Dr. R. G. Spiro and were obtained by alkaline degradation of glomerular-basement membranes, prepared from bovine-renal cortex. In addition, 5-*O*- β -D-galactopyranosyloxy-L-lysine was isolated from human urine by the method of Cunningham *et al.*¹⁶. The isolated compound gave a single ninhydrin-positive spot when cochromatographed on paper with authentic 5-*O*- β -D-galactopyranosyloxy-D,L-lysine (Table II). After acid hydrolysis, the neutral fraction was shown to contain galactose by g.l.c.⁸. Paper chromatography (solvent D) of the hydrolyzate gave a material that migrated at the same speed as that of authentic 5-hydroxy-L-lysine.

3-*O*- α -L-Arabinopyranosyl-L-serine. — 2,3,4-Tri-*O*-acetyl-L-arabinopyranosyl bromide¹⁷, $[\alpha]_D^{25} + 282^\circ$ (*c* 2, chloroform) (1.65 g), was condensed with *N*-carbobenzoyloxy-L-serine benzyl ester as described by Brendel and Davidson¹⁸ for the corresponding D-xylose derivative. After catalytic hydrogenation and separation by column chromatography on microcrystalline cellulose, the white crystalline 3-*O*-(2,3,4-tri-*O*-acetyl-L-arabinopyranosyl)-L-serine was recrystallized from ethanol, 1.1 g (62%),

TABLE II

PAPER CHROMATOGRAPHIC AND ELECTROPHORETIC BEHAVIOR OF GLYCOSYL DERIVATIVES OF AMINO ACIDS

Compounds	Solvents ^a R _F				Electrophoretic mobility (cm) ^b	
	A	B	C	D	A	B
3-O-β-D-Xylopyranosyl-L-serine	0.47	0.59	0.52	0.48	13.1	3.3
3-O-α-L-Arabinopyranosyl-L-serine	0.42	0.53	0.45	0.48	13.3	3.3
3-O-β-D-Glucopyranosyl-L-serine	0.41	0.46	0.38	0.42	11.7	3.3
3-O-β-D-Galactopyranosyl-L-serine	0.34	0.44	0.33	0.40	11.7	3.3
2-Acetamido-N-(aspart-4-oyl)-2-deoxy-β-D-glucopyranosylamine	0.27	0.44	0.28	0.32	13.2	3.2
5-O-β-D-Galactopyranosyloxy-D,L-lysine	0.30	0.39	0.14	0.16	28.6	11.0
α-D-Glucopyranosyl-(1→2)-β-D-galactopyranosyloxy(1→5)-D,L-lysine	0.02	0.21	0.07	0.08	23.9	9.4
L-Serine	1.00	1.00	1.00	1.00	22.4	3.4

^aSolvent A, ethyl acetate-pyridine-water (50:2:7, upper layer); solvent B, 2-methyl-2-propyl alcohol-30 per cent formic acid-water (5:1:1); solvent C, 2-butanone-propionic acid-water (3:1:12); solvent D, butyl alcohol-acetic acid-water (4:1:5, upper layer). Whatman No. 40 paper was used for descending paper chromatography except for solvent A, where Schleicher and Schuell No. 589 Green paper was used. The glycosyl derivatives of amino acids were detected by spraying with 0.2% ninhydrin in 95% acetone containing 2% pyridine. ^bPaper electrophoresis was conducted in a Gilson High Voltage Electrophorator (Model D) with Whatman 3MM paper and Isopar H as the coolant. The following conditions were used: Buffer A, 0.15M formic acid-1.6M acetic acid, pH 2.1 (45 min at 58 v/cm); buffer B, 50mm pyridine acetate, pH 6.5 (30 min at 47 v/cm). The compounds were detected as described above.

m.p. 212–215° (dec), $[\alpha]_D^{25} + 18.1^\circ$ (c 1, water). This compound (1 g) was deacetylated with ammonia in methanol. The solvent was removed *in vacuo*, and on being kept overnight the syrupy residue crystallized. The crystals were dissolved in water (15 ml) and methanol (30 ml), and acetone was added until the mixture became turbid. On being kept at 5°, crystals (750 mg) were formed. After two additional recrystallizations, the product showed a constant $[\alpha]_D^{25} + 0.3^\circ$ (c 4.4, water) and m.p. 141–144° (dec). Analysis of this product by g.l.c. showed a single homogeneous peak.

Anal. Calc. for C₈H₁₅NO₇: C, 40.50; H, 6.37; N, 5.91. Found C 40.65; H, 6.47; N, 5.90.

The product was homogeneous on paper chromatograms and electrophoretograms (Table II), and gave only serine and arabinose after acid hydrolysis. The molar rotation ($[\text{M}]_D + 76^\circ$) corresponded more closely to that of methyl α-L-arabinopyranoside¹⁹ ($[\text{M}]_D + 2,840^\circ$) than to that of the β-D anomer ($[\text{M}]_D + 40,300^\circ$), suggesting that the compound has an α-L configuration.

Isolation of 3-O-β-D-xylopyranosyl-L-serine from human urine. — A 24-h specimen of normal human urine (100 ml), stored at 4° under toluene, was deproteinized with picric acid²⁰. Excess picric acid was removed by three extractions with 3 vol of water-saturated ethyl acetate. The deproteinized sample was transferred to a column of Dowex 2 (X-8, OH⁻, 200–400 mesh, 4 × 30 cm). The column was washed

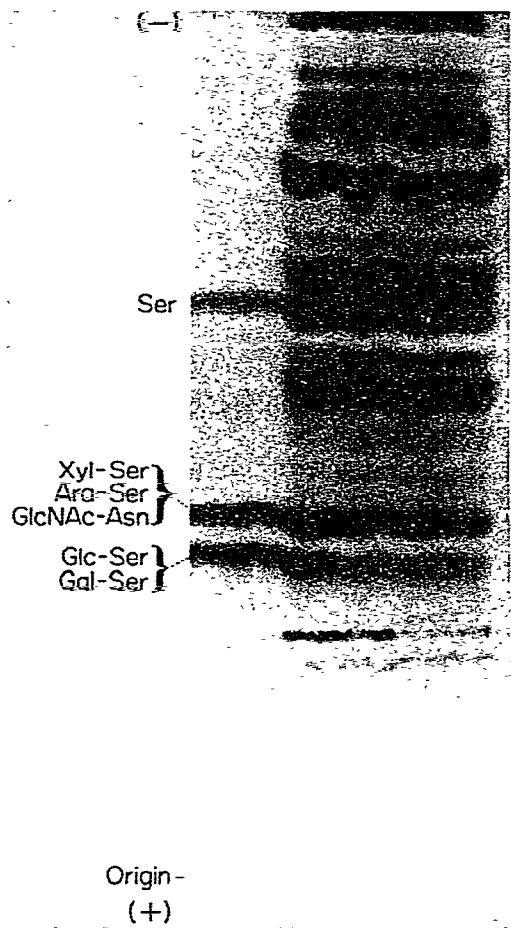


Fig. 4. Paper electrophoresis of chemically prepared glycosyl derivatives of amino acids with an anionic fraction obtained from normal human urine. To compensate for the effect of salt on the migration of the compounds, a mixture containing $0.25 \mu\text{mole}$ of each of the amino acid glycosides was bandstreaked to overlap the urine fraction. The conditions for paper electrophoresis and urine fractionation are described in the text. The amino acids and glycosyl derivatives of amino acids were detected with 0.2% ninhydrin in 95% acetone containing 2% pyridine. Ser, L-serine; Xyl-Ser, 3-*O*- β -D-xylopyranosyl-L-serine; Ara-Ser, 3-*O*- α -L-arabinopyranosyl-L-serine; GlcNAc-Asn, 2-acetamido-*N*-(aspart-4-oyl)-2-deoxy- β -D-glucopyranosylamine; Glc-Ser, 3-*O*- β -D-glucopyranosyl-L-serine; Gal-Ser, 3-*O*- β -D-galactopyranosyl-L-serine.

with 10 vol of distilled water, and the amino acid fraction was eluted with M acetic acid²¹. In separate studies where 3-*O*- β -D-xylopyranosyl-L-serine-*t* was added to urine, $93 \pm 3\%$ of the tritium was recovered in the acetic acid eluate. Acetic acid was removed *in vacuo*, and the residue was purified by preparative paper electrophoresis on Whatman 3MM in buffer A (35 min at 58 v/cm). The ninhydrin-positive band corresponding to 3-*O*- β -D-xylopyranosyl-L-serine (Fig. 4) was eluted and chromatographed on Whatman No. 40 with solvent C. The material that migrated at the same speed

as 3-O- β -D-xylopyranosyl-L-serine was extracted with water and reabsorbed on a column of Dowex 2 (X-8, OH⁻, 1 \times 18 cm). The column was washed with water and the xylosyl derivative of L-serine eluted in the manner just described. Use of the latter column of ion-exchange resin was necessary to remove the neutral materials that were solubilized from the paper matrix and found to interfere with the silylation reaction.

The yield of product was 0.35 mg. It migrated at the same rate as chemically synthesized 3-O- β -D-xylopyranosyl-L-serine in solvents A, B, C, and D and in buffers A and B (Table II). The sample was hydrolyzed in 1M sulfuric acid for 1 h at 100°; products of hydrolysis were found to migrate, on paper chromatograms in solvents C and E, at the same speed as those of L-serine and D-xylose, respectively. Xylose was also identified by g.l.c.⁸. The pentose (orcinol)^{2,2} to serine (ninhydrin)^{2,3} ratio was found to be 0.94:1.00.

Preparation of 3-O- β -D-xylopyranosyl-L-serine from chondromucoprotein
Chondromucoprotein and chondromucoprotein glycopeptides were prepared from bovine-nasal septa in the manner described by Lindahl and Rodén²⁴. The purified glycopeptide fraction (50 mg) was dissolved in 30mM hydrochloric acid (25 ml) and pH was adjusted to 1.5, and the solution heated for 4 h at 100° in a sealed-glass tube. The hydrolyzate was neutralized with 4M ammonium hydrogen carbonate and the solvent evaporated to dryness; water was added and evaporated repeatedly, and the residue dried until all excess of ammonium hydrogen carbonate was removed. The residue was dissolved in water (25 ml) and fractionated on Dowex 2 in the same manner as described for the deproteinized urine specimens. The resulting compound migrated at the same speed as that of 3-O- β -D-xylopyranosyl-L-serine on paper chromatograms (Table II). After acid hydrolysis, colorimetric analysis showed a xylose to serine ratio of 0.91:1.0.

ACKNOWLEDGEMENTS

We thank Dr. Donald Kay for providing the results of the g.l.c. of 3-O- β -D-xylopyranosyl-L-serine found in urine for normal individuals and patients with connective tissue disease. In addition, the valuable technical assistance of Mrs. Czuma and Mrs. Wanda Torzewski is acknowledged.

REFERENCES

- 1 R. D. MARSHALL AND A. NEUBERGER, *Advan. Carbohydr. Chem.*, 25 (1970) 407.
- 2 C. T. BISHOP, *Advan. Carbohydr. Chem.*, 19 (1964) 95.
- 3 W. W. WELLS, C. C. SWEELEY, AND R. BENTLEY, *Biomedical Applications of Gas Chromatography*, H. A. SZYMANSKI (Ed.), Plenum Press, New York, 1964, p. 169.
- 4 B. WEINSTEIN, *Methods Biochem. Anal.*, 14 (1966) 253.
- 5 E. D. SMITH AND H. SHEPPARD, JR., *Nature*, 208 (1965) 878.
- 6 C. C. SWEELEY, R. BENTLEY, M. MAKITA, AND W. W. WELLS, *J. Amer. Chem. Soc.*, 85 (1963) 21.
- 7 N. H. RAY, *J. Appl. Chem.*, 4 (1954) 21.
- 8 W. F. LEHNHARDT AND R. J. WINZLER, *J. Chromatogr.*, 34 (1968) 471.
- 9 D. R. KAY, G. W. JOURDIAN, AND G. G. BOLE, *Arthritis Rheum.*, 14 (1971) 393.

- 10 Z. STARY, A. H. WARDI, D. L. TURNER, AND W. S. ALLEN, *Arch. Biochem. Biophys.*, 110 (1965) 388.
- 11 A. H. WARDI, W. S. ALLEN, D. L. TURNER, AND Z. STARY, *Arch. Biochem. Biophys.*, 117 (1966) 44.
- 12 R. L. KATZMAN, *J. Neurochem.*, 18 (1971) 1187.
- 13 J. DISTLER AND G. W. JOURDIAN, *Fed. Proc.*, 26 (1967) 345.
- 14 J. S. SAWARDEKER AND J. H. SLONEKER, *Anal. Chem.*, 37 (1965) 945.
- 15 K. KUM AND S. ROSEMAN, *Biochemistry*, 5 (1966) 3061.
- 16 L. W. CUNNINGHAM, J. D. FORD, AND J. P. SEGREST, *J. Biol. Chem.*, 242 (1967) 2570.
- 17 R. E. DERIAZ, W. G. OVEREND, M. STACEY, E. G. TEECE AND L. F. WIGGINS, *J. Chem. Soc.*, 1949, 1879.
- 18 K. BRENDDEL AND E. A. DAVIDSON, *Carbohyd. Res.*, 2 (1966) 42.
- 19 F. J. BATES *et al.*, *Polarimetry, Saccharimetry, and the Sugars*, National Bureau of Standards Circular C 440, Washington, D.C., 1942, p. 708.
- 20 C. W. GEHRKE, D. ROACH, R. W. ZUMWALT, D. L. STALLING, AND L. L. WALL, *Quantitative Gas-Liquid Chromatography of Amino Acids in Proteins and Biological Substances*, Analytical Biochemistry Laboratories, Inc., Columbia, Missouri, 1963, p. 68.
- 21 A. DRÈZE, S. MOORE, AND E. J. BIGWOOD, *Anal. Chim. Acta*, 11 (1954) 544.
- 22 A. H. BROWN, *Arch. Biochem.*, 11 (1946) 269.
- 23 J. R. SPIES, *Methods Enzymol.*, 3 (1957) 468.
- 24 U. LINDAHL AND L. RODÉN, *J. Biol. Chem.*, 241 (1966) 2113.

Carbohyd. Res., 22 (1972) 369-379