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SEPARATION OF ALDONIC, DEOXYALDONIC, HEXURONIC AND HEXARIC LACTONES AND ACIDS USING THERMOSTABLE OPEN TUBULAR GLASS CAPILLARY COLUMNS

J. SZAFRANEK, C. D. PFAFFENBERGER and E. C. HORNING
Institute for Lipid Research, Baylor College of Medicine, Houston, Texas 77025 (U.S.A.)
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SUMMARY

Successful gas–liquid chromatographic separation of mixtures of aldonic, deoxyaldonic, hexuronic and hexaric lactones and acids has been achieved. The compounds were analyzed as mixtures of lactone trimethylsilyl ethers and linear trimethylsilyl ester trimethylsilyl ethers. Both kinetically defined and equilibrium product distributions were considered. Analyses were made using a 60-m SE-30 open tubular glass capillary column with temperature programming.

INTRODUCTION

The separation of complex mixtures of aldoses[1] and alditols[2] with high-resolution thermostable open tubular glass capillary columns has recently been reported. During the course of an investigation of methods for the study of alditols and aldoses[2] it appeared feasible to attempt a separation of related sugar acid lactones as trimethylsilyl (TMS) derivatives. Earlier studies of this problem were based on the use of packed columns containing either polar or nonpolar phases, *viz.* NGS[3], XF-1112[4], F-60[5], SE-30[6], SE-52[6], and QF-1[6].

Sweeley and co-workers[7] obtained two lactone forms from some aldonic acids prepared by bromine/water oxidation of the corresponding aldoses. Mixtures of aldoses led to mixtures of isomeric lactones which, when analyzed as TMS derivatives, were incompletely separated on packed columns. Morrison and Perry[8] and Sjöström *et al.*[4] also obtained incomplete separation of these derivatives.

Hexuronic acids have previously been separated as TMS derivatives by Imanari and Tamura[6] and as the corresponding 1→4 lactone TMS ethers by Perry and Hulyalkar[3]. Furthermore, Jaakonmaki *et al.*[5] separated the anomers of O-ethyl pyranosylglucuronic acids as TMS derivatives. Attempts to analyze aldonic or uronic acids as the corresponding alditol acetates were also successful, but terminal dissymmetry was forfeited as evidenced by four hexuronic acids yielding only two hexitols. Moreover, aldonic and uronic acids led to identical reaction products[9].

The separation of deoxyaldonic and hexaric lactones and acids as TMS derivatives has not been reported previously.

EXPERIMENTAL

Reagents

The aldonic acid lactones D-gulono-1→4-lactone and L-manno-1→4-lactone were purchased from Pfanstiehl (Waukegan, Ill., U.S.A.), D-ribono-1→4-lactone from Fluka (Buchs, Switzerland), D-galactono-1→4-lactone from K&K Labs. (Plainview, N.Y., U.S.A.), D-glucono-1→5-lactone from Matheson, Coleman and Bell (East Rutherford, N.J., U.S.A.), and D-*glycero*-D-*gulo*-heptono-1→4-lactone from Aldrich (Milwaukee, Wisc., U.S.A.). The hexuronic and hexaric acids D-galactaric acid and D-glucaric acid 1→4-lactone were purchased from Sigma (St. Louis, Mo., U.S.A.), D-glucuronic acid 6→3-lactone from Fluka. L-Threonolactone, L-xylonolactone and the barium salt of D-lyxonic acid were available from previous work. D-Erythronic, D-arabonic, D-talonic, and the deoxyaldonic acids were prepared from the corresponding aldoses and deoxyaldoses (Supelco, Bellefonte, Pa., U.S.A.) by bromine/water oxidation. Bis(trimethylsilyl)trifluoroacetamide (BSTFA) and trimethylchlorosilane (TMCS) were purchased from Pierce (Rockford, Ill., U.S.A.).

Oxidation of aldoses and deoxyaldoses[8]

Two milligrams of an aldose or a deoxyaldose was dissolved in 200 μl of water and treated with 4 μl of bromine. The mixture was shaken for 3 h; then 0.5 mg of ammonium carbonate was slowly added to the mixture in a PTFE-lined screw-cap vial. After remaining at room temperature for 24 h, 100 μl of 0.01 *N* hydrochloric acid was added and the water was evaporated at 40° using a stream of dry nitrogen. The product(s) was converted to the TMS derivative(s) as described below.

Trimethylsilyl derivatives of lactones

One milligram of a lactone or the oxidation product(s) described above was dissolved in 100 μl of dry pyridine. After addition of 85 μl of BSTFA and 15 μl of TMCS, the mixture was heated at 60° for 1 h. The resulting solution was immediately used for gas–liquid chromatographic analysis.

Gas chromatography

Separations of TMS derivatives were carried out with an F&M Model 400 gas chromatograph equipped with a flame ionization detector modified to accept a 60-m ×0.3 mm I.D. glass capillary column coated with SE-30 containing Silanox 101 (Cabot, Boston, Mass., U.S.A.). A Keithley Model 417 picoammeter and a Texas Instrument recorder (Texas Instruments, Houston, Texas, U.S.A.) were employed. The precolumn helium and flame hydrogen flow-rates[10,11] were controlled by Brooks Model 8744 flow controllers (Brooks, Hatfield, Pa., U.S.A.) and measured with Model 8110-0121 mass flowmeters from Matheson.

Methylene unit (*MU*) values were measured through use of *n*-alkanes co-injected with the samples using an initial temperature of either 120° or 150° with temperature programming at 1°/min. Other gas chromatographic (GC) conditions were: sample volume, 0.5 μl; split ratio, 5:1; precolumn-inlet splitter, 230°; detector bath, 300°; column inlet pressure, 10 p.s.i., resulting in a carrier gas (helium) linear velocity of 18–20 cm/sec (200°); hydrogen flow-rate, 10 ml/min; air flow-rate, 30 ml/min. An HETP of 0.44 mm was measured at 250° for *n*-tetracosane.

RESULTS AND DISCUSSION

The four chemical families included in this study may be conveniently represented by the linear structures for aldonic acids (I), 2-deoxyaldonic acids (II), hexuronic acids (III) and hexaric acids (IV). Members of families I and II may exist as linear

ALDONIC	2-DEOXYALDONIC	HEXURONIC	HEXARIC
COOH (CHOH)$_4$ CH$_2$OH	COOH CH$_2$ (CHOH)$_3$ CH$_2$OH	CHO (CHOH)$_4$ COOH	COOH (CHOH)$_4$ COOH
I	II	III	IV

acids or as the 1→4 or 1→5-lactones. Most members are found as 1→4-structures, gluconic acid being the notable exception in which the 1→5-form predominates[12]. Hexuronic acids may form pyrano or furano hemiacetals, 6→2 or 6→3-lactones and various bicyclic combinations[13–15]. Hexaric acids[16,17] may form 1→4, 1→5, 6→2, or 6→3-lactones as well as several dilactones. Mixtures containing members from each of families I, III and IV cannot be analyzed as the corresponding alditols[9,18,19] because the reduction step destroys family identity. We thus chose a method, trimethylsilylation, which does not involve chemical modification of the sample prior to the derivatizing step.

The first analysis was of a mixture of twelve aldonic acid lactones (Fig. 1). Complete separation of every component was obtained except for the D-gulonic/D-talonic pair. Even for this case the partial separation obtained allowed the determination of *MU* values for both TMS derivatives (Table I). Only four of the compounds included in this mixture were prepared under conditions which assured equilibrium

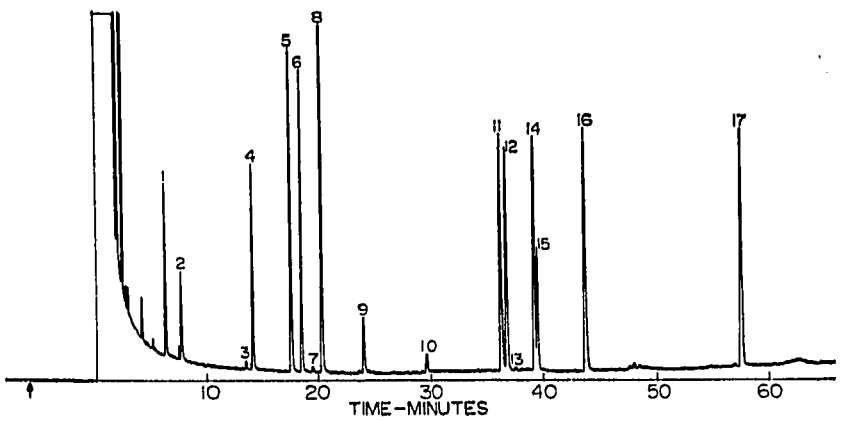

Fig. 1. Separation of a mixture of TMS derivatives of aldonic lactones and acids on a 60-m × 0.3 mm I.D. SE-30 capillary column (temperature programmed 1°/min from 165°). 1,4= L-Threonic; 2,3= D-erythronic; 5,7= D-arabonic; 6= L-xylonic; 8= D-ribonic; 9,10= D-lyxonic; 11,13= D-gluconic; 12= D-galactonic; 14= D-gulonic; 15= D-talonic; 16= L-mannonic; 17= D-*glycero*-D-*gulo*-heptonic.

TABLE I

MU VALUES OF TMS DERIVATIVES OF ALDONIC LACTONES AND ACIDS

Aldonic acid lactone	*Peak No.*	*% Isomer**	*MU value***
L-Threonic	1		13.71
	4		15.85
D-Erythronic	2	91	14.13
	3	9	15.66
D-Arabonic	5	98	16.36
	7	2	16.73
L-Xylonic	6		16.56
D-Ribonic	8		16.87
D-Lyxonic	9	76	17.41
	10	24	18.20
D-Gluconic	11		19.08
	13		19.23
D-Galactonic	12		19.14
D-Gulonic	14		19.45
D-Talonic	15	100	19.46
L-Mannonic	16		20.03
D-*Glycero*-D-*gulo*-heptonic	17		21.71

* Only equilibrium product distributions reported.

** For L-threonic through D-lyxonic acid lactones an initial temperature of 120° was employed. For D-gluconic through D-*glycero*-D-*gulo*-heptonic acid lactones the initial temperature was 150°.

product distributions: D-erythronic, D-arabonic, D-lyxonic and D-talonic acid lactones. Inasmuch as a kinetically defined product distribution can differ considerably from an equilibrium product distribution, a mixture of the eight aldonic acid lactones analyzed as kinetic products in Fig. 1 was further analyzed as an equilibrium mixture (Fig. 2; Table II).

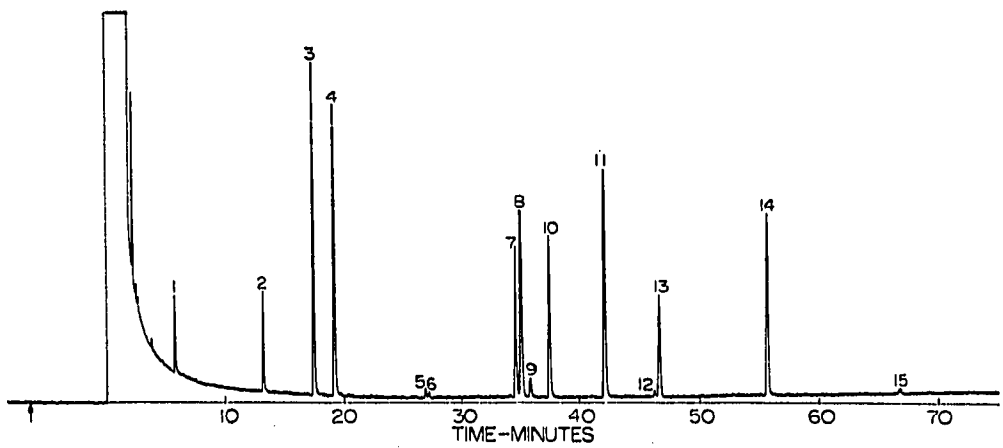

Fig. 2. Separation of an equilibrium mixture of TMS derivatives of eight of the aldonic lactones and acids included in Fig. 1 under identical analysis conditions. 1,2= L-Threonic; 3,5= L-xylonic; 4,6= D-ribonic; 7,9,13= D-gluconic; 8,12= D-galactonic; 10= D-gulonic; 11= L-mannonic; 14, 15= D-*glycero*-D-*gulo*-heptonic.

TABLE II

MU VALUES AND ISOMER PERCENTAGES OF TMS DERIVATIVES OF ALDONIC LACTONES AND ACIDS AT EQUILIBRIUM

Aldonic acid lactone	*Peak No.*	*% Isomer*	*MU value*
L-Threonic	1	57	13.71
	2	43	15.85
L-Xylonic	3	97	16.56
	5	3	18.07*
D-Ribonic	4	99	16.87
	6	1	18.10*
D-Gluconic	7	55	19.08
	9	7	19.23
	13	38	20.59*
D-Galactonic	8	96	19.14
	12	4	20.54*
D-Gulonic	10	100	19.45
L-Mannonic	11	100	20.03*
D-*Glycero*-D-*gulo*-heptonic	14	97	21.71
	15	3	23.03

* This compound was not in the kinetically defined product distribution shown in Fig. 1. An initial temperature of 150° was used to determine the *MU* values.

Nine aldonic acid lactones give at least two TMS derivatives at equilibrium. L-Threonic, D-erythronic, L-xylonic, D-ribonic, D-lyxonic, D-galactonic and D-*glycero*-D-*gulo*-heptonic acid lactones exist in one cyclic structure, the 1→4-lactone, and one linear form. Their respective cyclic to linear structural ratios (equilibrium conditions) are 57:43, 91:9, 97:3, 99:1, 76:24, 96:4, and 97:3. D-Arabonic acid lactone exists as two cyclic structures, the 1→4 and 1→5-lactones, in the ratio 98:2. D-Gluconic acid

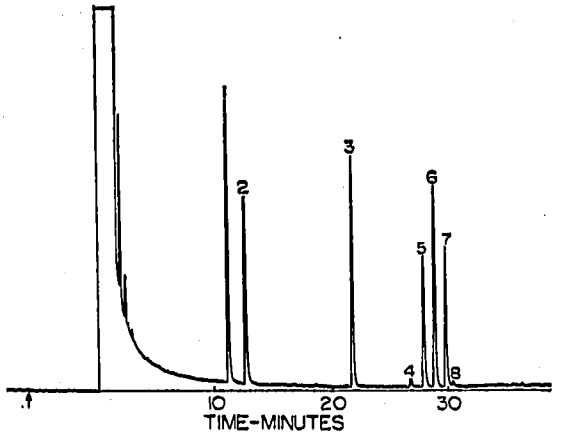

Fig. 3. Separation of TMS derivatives of deoxyaldonic lactones and acids on a 60-m × 0.3 mm I.D. SE-30 capillary column (temperature programmed 1°/min from 165°). 1= 2-Deoxy-D-ribonic; 2= 2,6-dideoxy-D-allonic; 3,4= 6-deoxy-L-galactonic; 5= 3-deoxy-D-gluconic; 6= 2-deoxy-D-galactonic; 7, 8= 2-deoxy-D-gluconic.

TABLE III

MU VALUES OF TMS DERIVATIVES OF DEOXYALDONIC LACTONES AND ACIDS

Deoxyaldonic acid lactone	*Peak No.*	*MU value**
2-Deoxy-D-ribonic	1	14.92
2,6-Dideoxy-D-allonic	2	15.25
6-Deoxy-L-galactonic	3	16.91
	4	17.63
3-Deoxy-D-gluconic	5	17.82
2-Deoxy-D-galactonic	6	17.95
2-Deoxy-D-gluconic	7	18.07
	8	18.11

* For 2-deoxy-D-ribonic through 3-deoxy-D-gluconic acid lactones an initial temperature of 120° was employed. For 2-deoxy-D-galactonic and 2-deoxy-D-gluconic acid lactones, the initial temperature was 150°.

TABLE IV

MU VALUES OF TMS DERIVATES OF HEXURONIC AND HEXARIC ACIDS AND ACID LACTONES

Parent compound	*Peak No.*	*MU value**
D-Glucuronic acid 6→3-lactone	1	18.18
	2	18.32
D-Glucaric acid 1→4-lactone	3	19.72
D-Galacturonic acid	4	19.91
D-Galactaric acid	5	20.94

* An initial temperature of 150° was employed.

actone yields three structures, the 1→5 and 1→4-lactones and the linear form, in the respective ratio 55:7:38. Under equilibrium conditions D-gulonic, D-talonic and L-mannonic acid lactones give only one derivative, the 1→4-lactone TMS ether.

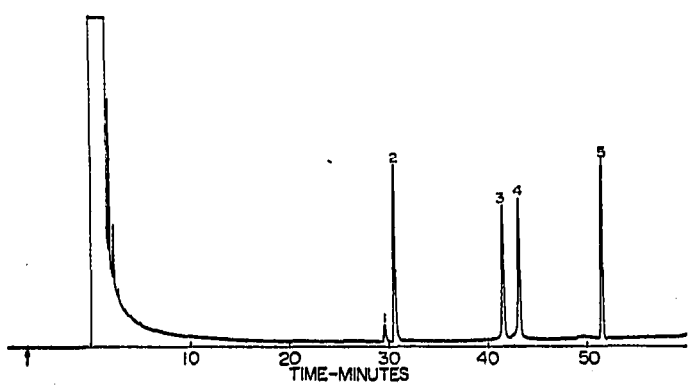

Fig. 4. Separation of TMS derivatives of hexuronic and hexaric lactones and acids on a 60-m × 0.3 mm I.D. SE-30 capillary column (temperature programmed 1°/min from 165°). 1,2= D-Glucuronic; 3= D-glucaric; 4= D-galacturonic; 5= D-galactaric.

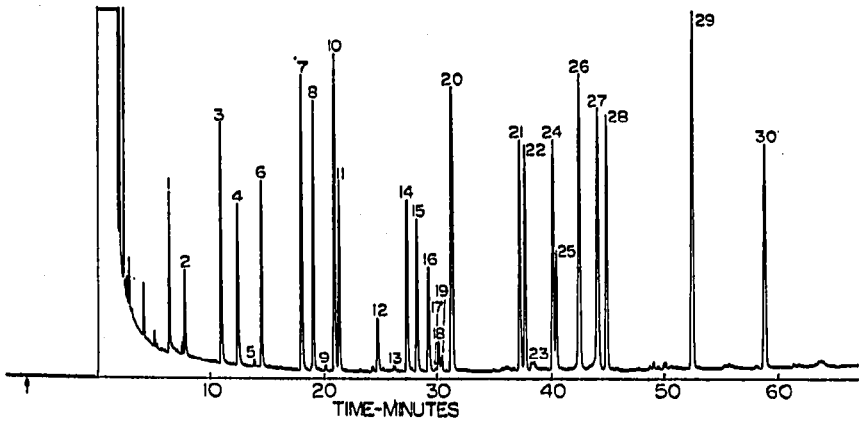

Fig. 5. Separation of TMS derivatives of aldonic, deoxyaldonic, hexuronic and hexaric lactones and acids on a 60-m × 3 mm I.D. SE-30 capillary column (temperature programmed 1°/min from 165°). 1,6= L-Threonic; 2,5= D-erythronic; 3= 2-deoxy-D-ribonic; 4= 2,6-dideoxy-D-allonic; 7,9= D-arabonic; 8= L-xylonic; 10= D-ribonic; 11,13= 6-deoxy-L-galactonic; 12,19= D-lyxonic; 14= 3-deoxy-D-gluconic; 15= 2-deoxy-D-galatonic; 16,17= 2-deoxy-D-gluconic; 18,20= D-glucuronic; 21,23= D-gluconic; 22= D-galactonic; 24= D-gulonic; 25= D-talonic; 26= D-glucaric; 27= D-galacturonic; 28= L-mannonic; 29= D-galactaric; 30= D-*glycero*-D-*gulo*-heptonic.

Most aldonic acid lactones yield only 4% or less of the linear derivative at equilibrium. The four exceptions include L-threonic, D-erythronic, D-lyxonic, and D-gluconic acid lactones. L-Threonic acid lactone exhibits a much higher amount of linear derivative (43%) than does its diastereomer (9%). Within the family of five-carbon aldonic acid lactones only D-lyxonic acid lactone yields a high amount of linear derivative (24%). And of the six-carbon aldonic lactones studied, only D-gluconic acid lactone gives more than 4% of linear derivative (38%). Furthermore, this compound is the only one which gives at equilibrium a large amount (38%) of an isomer not present in the kinetically defined product distribution.

Most deoxyaldonic acid lactones (Fig. 3, Table III) yield only one derivative at equilibrium, a lactone TMS ether. 6-Deoxy-L-galactonic acid lactone gives 3% of a second derivative, presumably the linear TMS esther TMS ether (based on retention behaviour). 2-Deoxy-D-gluconic acid lactone also yields a second derivative (4%), presumably another lactone TMS ether.

The few hexuronic and hexaric acids and acid lactones included in this study offer no separation problems. An analysis of a kinetically defined product distribution of the TMS derivatives (Table IV) of these compounds appears in Fig. 4. An equilibrium product distribution of the same compounds is very complicated. Although it offers no separation problems, identification of its many components has been reserved for a thorough structural analysis study using GC–MS-computer techniques.

A chromatogram of a composite mixture of all the families included in this study is given in Fig. 5. The analysis was made on a 60-m SE-30 open tubular glass capillary column temperature programmed 1°/min from 165°.

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