# Gas Chromatographic Separation of Aldonic Acids as Trimethylsilyl Derivatives

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Separering av trimetylsilylderivat av olika aldono-1,4-laktoner har studerats gaskromatografiskt med ett stort antal stationära faser. Dessa laktoner kan framställas enkelt genom indunstning av lösningar som innehåller aldonsyror i närvaro av saltsyra och ger endast upphov till en kromatografisk topp för varje aldonsyra. Under andra betingelser t. ex. vid indunstning i närvaro av kalciumklorid registreras ofta tre toppar för varje aldonsyra. Dessa har identifierats genom masspektra och visats motsvara de fullständigt substituerade derivaten av 1,4-laktonen, 1,5-laktonen och av trimetylsilylestern.

The separation of trimethylsilyl derivatives of various aldono-1,4-lactones was studied by gas chromatography on a large number of stationary phases. These lactones, which are conveniently prepared by evaporating sample solutions containing aldonic acids in the presence of hydrochloric acid, give rise to single chromatographic peaks. Under other working conditions e.g. by evaporation in the presence of calcium chloride, three peaks can be recorded for each aldonic acid. These were identified by their mass spectra as the fully trimethylsilyl substituted derivatives of the 1,4-lactone, 1,5-lactone and the trimethylsilyl ester.

Die Trennung von Trimethylsilylderivaten verschiedener Aldono-1,4-laktone wurde chromatographisch mit einer grösseren Zahl stationärer Phasen studiert. Derartige Laktone, die einfach durch Eindampfen von Aldonsäurelösungen in Gegenwart von Salzsäure hergestellt werden können, geben für jede Aldonsäure nur einen chromatographischen peak. Unter anderen Bedingungen, z. B. beim Eindampfen in Anwesenheit von Kalciumchlorid, werden oft drei peaks für jede Aldonsäure beobachtet. Diese wurden massenspektroskopisch identifiziert. Es zeigte sich, dass sie den vollständig substituierten Derivaten des 1,4- und 1,5-Laktons sowie des Trimethylsilylesters entsprechen.

A partial depolymerization of cellulose and hemicellulose is unavoidable both in the pulping and bleaching procedures used in practice. Some of these reactions, or more often consecutive reactions which follow after the cleavage of the macromolecules, give rise to large losses of carbohydrate material. Depending upon the type of reaction various types of end groups are formed. With most wood pulps the reactions which give rise to aldonic acid end groups are less harmful than those which give rise to, for instance, saccharinic acid and aldehyde end groups. In addition the formation of aldonic acids by oxidation of sugars is a side reaction which largely contributes to the destruction both of hydrogen sulfite and monosaccharides during the sulfite cooking. The separation of aldonic acids from complex mixtures is therefore of great interest in research work on pulping and bleaching procedures.

The first successful separations of such mixtures were carried out on anion exchangers in the borate form (1, 2). Several species can be separated in acetate media as well (3, 4). A more recent method for separations of aldonic acids is gas-liquid chromatography of their trimethylsilyl (TMS) derivatives (5, 6, 7). This technique can be conveniently combined with identification of the separated species by mass spectrometry (8).

In this paper the separation of TMS-derivatives of aldono-1,4-lactones on various stationary phases is reported. In addition the pretreatment of the sample and the identity of the chromatographic peaks recorded after various types of pretreatment are discussed.

## Experimental

The aldonolactones were the same as described previously (8). These were used both for direct preparation of TMS-derivatives and for preparation of sample solutions containing 1,4- and 1,5-lactones as well as free aldonic acids.

The trimethylsilyl derivatives were prepared according to Sweeley et al. (5) with slight modifications. The sample (5—20 mg) was dissolved or suspended in 0.5—2 ml pyridine at room temperature and then about 0.5 ml hexamethyldisilazane and 0.3 ml trimethylchlorosilane were added. The reaction mixture was shaken for two minutes. If dissolution was difficult the mixture was treated by means of ultrasonic agitation (80 kHz). The solvent was removed in a rotating vacuum evaporator at 40°C. After dissolution in n-hexane to a concentration of about 10 mg/ml the sample (0.05—0.5 μl) was applied to the chromatographic column.

The experiments were carried out using a Perkin-Elmer 800 Gas Chromatograph equipped with a dual differential flame ionization detector. Stainless steel columns (200 × 0.2 cm ID) were used. Suitable column temperatures were chosen for all column fillings investigated whereas the injection

*Table 1. Retention times on SE-30 for mass spectrometrically identified components relative to that of 2, 3, 5, 6-tetra-O-trimethylsilylglucono-1,4-lactone (18.9 min at 140° and 6.4 min at 170°)*

| | 1.4-lactone | 1.5-lactone | TMS-ester |
|---|---|---|---|
| Arabinonic acid (140°C) ......... | 0.25 | 0.29 | 0.63 |
| Xylonic acid (140°C) ............ | 0.26 | 0.31 | 0.57 |
| Gluconic acid (170°C) ........... | 1.00 | 0.93 | 1.72 |
| Mannonic acid (170°C) .......... | 1.37 | — | 1.53 |

Pretreatment of the acids: 30 mg 1,4-lactone dissolved in 5 ml 1 % CaCl₂ in 1 N HCl was kept on a boiling steam bath in a plastic stoppered glass vial for one hour. The solvent was removed in a rotating vacuum evaporator at 50°C and the residue subjected to trimethylsilylation.
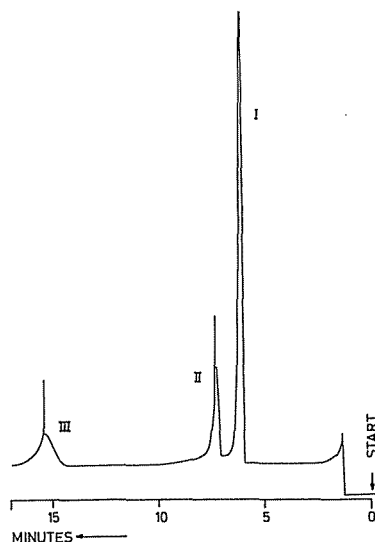


Fig. 1. Gas chromatographic separation of different TMS-derivatives from arabinonic acid (pretreated as described under Table 1).
Column: 300 × 0.3 cm ID glass. Column packing: 1 % SE-30 on Chromosorb P AW silan. 100/120 mesh. Column temperature: 140°C. Carrier gas: He. Flow rate: 35 ml/min.
I    2,3,5-tri-O-trimethylsilylarabinono-1,4-lactone
II   2,3,4-tri-O-trimethylsilylarabinono-1,5-lactone
III  2,3,4,5-tetra-O-trimethylsilylarabinonic acid trimethylsilyl ester

block temperature was 260°C and the detector temperature 120—150°C throughout. Purified nitrogen (max. limit of impurities 25 ppm) was used as carrier gas at a flow rate of 20—30 ml/min.

The investigations by gas chromatography-mass spectrometry were carried out as described previously (8) using the equipment installed at the Swedish Institute for Food Preservation Research. The G.L.C. data for SE-30 were obtained from this equipment.

### Influence of the pretreatment

As observed by Sweeley (5), the TMS-derivatives prepared from aldono-1,4-lactones give rise to a single gas chromatographic peak in each case. This is, of course, a great advantage when complex mixtures have to be separated. Chemical analyses of the derivatives from hexono-1,4-lactones isolated from a preparative column showed that these contained four trimethylsilyl groups (6). A gas chromatographic-mass spectrometric study of a great number of aldonolactones confirmed that all peaks corresponded to fully TMS-substituted lactones (8).

As reported by Perry and Hulyalkar (6), the 1,4-lactones are conveniently prepared from solutions of aldonic acid salts by removal of the metallic ions with a cation exchange resin and concentration of the solutions in the presence of hydrochloric acid. Finally the residue is heated at 100° under vacuum for a short period to complete the conversion into 1,4-lactones. These observations have been confirmed in the present work both by comparing the retention times of TMS-ethers from species pretreated in this way with those of TMS-ethers from authentic 1,4-lactones and by mass spectrometry. Perry and Hulyalkar obtained two peaks for each aldonic acid in the absence of hydrochloric acid. These were ascribed to 1,4- and 1,5-lactones. Sweeley found that if the calcium salts of aldonic acids were treated with hydrochloric acid before trimethylsilylation two peaks were obtained in each case except for xylonic acid, which gave three peaks. The identity of these peaks was not discussed.

The fact that two or three peaks can be recorded from a single aldonic acid is of course of practical

interest for the identification of compounds isolated by ion exchange chromatography or by other methods. In the present investigation two or three distinct peaks were obtained on the silicon gum SE-30 for the aldonic acids studied when using conditions that differed somewhat from those used by Perry and Hulyalkar. Some relevant results are given in *Table 1*. A chromatogram obtained with arabinonic acid is given in *Fig. 1*. Mass spectra were recorded for all peaks. The first and the second peaks were identified as completely TMS-substituted 1,4- and 1,5-lactones by using the criteria described in a previous paper (8). The last peak gave a mass spectrum which was quite different from those recorded with the lactones. As will be shown below, this derivative is the TMS-ester of 2,3,4,5-tetra-O-trimethylsilylarabinonic acid. The results obtained with arabinonic, xylonic, gluconic and mannonic acids are summarized in Table 1. It is seen that on this column the peak corresponding to the TMS-ester appeared after the lactones. With both pentonic acids the 1,4-lactones appeared before the 1,5-lactones whereas with gluconic acid the order was reversed. With mannonic acid a significant lactone peak was recorded only for the 1,4-lactone.

In this connection it is worth mentioning that with some species more than one chromatographic peak was recorded for each acid also under other working conditions. As an example it may be mentioned that an eluate fraction containing xylonic acid obtained from an anion exchange column by elution with 0.5 M acetic acid gave rise to three peaks after evaporation under vacuum and trimethylsilylation. These were identified as the TMS-derivatives of the 1,4-lactone, the 1,5-lactone and the acid.

## Mass spectrometric evidence for the formation of fully trimethylsilylated TMS-esters of aldonic acids

The mass spectrum of the mannonic acid band which in Table 1 is denoted as the TMS-ester is reproduced in *Fig. 2*. This spectrum will serve as an example in discussing mass spectra of this type.

The peak at $m/e = 73$, characteristic of TMS-derivatives, was the base peak of the spectrum. The second most intense peak was obtained for $m/e = 147$, which is very often a prominent peak in spectra of compounds having more than one TMS-group (8).

The $m/e$-value of the molecule ion (M) for the TMS-ester of a hexonic acid would be 628. No distinct peak at this position could be detected, but a significant peak at $m/e = 613$ (M-15) was recorded. This peak had the highest $m/e$-value in the spectrum. This observation is in agreement with the previously observed dominance of M-15 compared with M for TMS-derivatives of open-chain hydroxy compounds (9, 10). The occurrence of expected peaks at $m/e = 538$ (M-90) and $m/e = 523$ (M-15-90) corresponding to ions formed by the splitting off of trimethylsilanol confirmed that the parent mass was 628.

With regard to the structure of a fully TMS-substituted aldonic acid, important fragment ions should result from splitting of carbon-carbon bonds with formation of resonance stabilized ions either from the alcohol portion or from the acid portion of the molecule. From Fig. 2 it is seen that most of the important peaks can be explained as arising from ions of this type ($m/e = 103, 205, 307, [409], 423, 525$) and from such ions after splitting off of trimethylsilanol ($m/e = 217, 319, 333, 435$). The intense peak at $m/e = 292$ which cannot be explained by this scheme probably originates from a rearrangement ion.

The spectrum recorded for the corresponding gas chromatographic band obtained with gluconic acid differed only slightly from that of mannonic acid. The spectra confirm that the bands were those of the fully TMS-substituted esters.

In the spectra of the bands denoted as TMS-esters of arabinonic and xylonic acids (M = 526), the base peak was the same as for mannonic acid. Again, the peak with the highest $m/e$-value (511) corresponded to M-15. Likewise a significant peak was recorded at $m/e = 421$ (M-15-90). Peaks at $m/e = 147$ and 292 of about the same relative intensity as for the mannonic acid derivative were recorded as well as expected peaks at $m/e$-values corresponding to fragment ions formed by single cleavages in the carbon chain ($m/e = 103, 205, 307, 321, 423$) and by elimination of trimethylsilanol ($m/e = 217$ and 333). No other intense peaks were recorded. The spectra confirm that the chromatographic bands corresponded to esters of pentonic acids. The spectra of arabinonic and xylonic acids were very similar. The relative intensity differences for peaks in the spectra of the diastereomeric TMS-esters investigated were smaller than in the spectra of the corresponding cyclic lactone derivatives (8). For identification of diastereomeric aldonic acids by mass spectrometry the TMS-derivatives of the lactones are, therefore, preferred to those of the free acids.
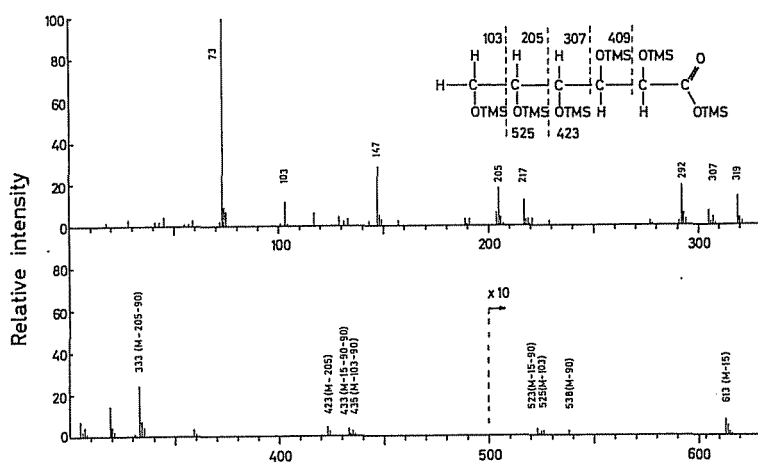


Fig. 2. Mass spectrum of a fully trimethylsilylated mannonic acid. Mass spectrometer: LKB 9000. Ionizing voltage: 70 eV. Inlet temperature: 210°C.

## Separations of aldono-1,4-lactones as their TMS-ethers

As already mentioned the 1,4-lactones can be conveniently prepared by concentrating aldonic acid solutions in the presence of hydrochloric acid. In this way only one peak is obtained for each aldonic acid, thus facilitating the separation of complex mixtures. In this study a few aldonic acids which were not studied by earlier investigators have been included. Moreover, a large number of stationary phases were investigated. The results are summarized in *Table 2* in which the retention times relative to that of glucono-1,4-lactone are given. The retention times were determined both with single species and with mixtures and the reported figures are average values. From the table it is seen that with a few exceptions the retention time increases with increasing molecular weight. It is noteworthy that on all stationary phases lactones with their TMS-groups on $C_2$ and $C_3$ in the trans position have shorter retention times than the corresponding cis isomers. Within each of these categories most of the derivatives with the trans configuration at $C_3$ and $C_4$ have shorter retention times than those having the cis configuration. The latter rule holds true for the pentonolactones on all stationary phases whereas there exist several exceptions among the hexonolactones.

The most satisfactory separations were achieved with the fluorinated silicone fluid QF-1. A chromatogram from a temperature programmed run with a mixture of 11 aldonolactones is reproduced in *Fig. 3*. Serious overlapping was observed only with mannono- and talonolactones but these can be easily separated on a number of other stationary phases. Some overlapping occurred also between the bands corresponding to galactono- and glucono-lactones when this temperature program was used. In a run at constant temperature (170°) no overlapping between these two species was observed whereas the overlapping between mannono- and talonolactones became worse. These experiments show that the choice of temperature is critical and that the temperature has to be adapted to the species present in the sample to be analyzed.

*Table 2. Retention times of the TMS-derivatives of aldono-1,4-lactones on various stationary phases.*
Stationary phase, column temperature and retention time of the reference substance (2,3,5,6-tetra-O-trimethylsilylglucono-1,4-lactone)

| | SE-30 | SE-52 | QF-1 | DC-560 EGSP-Z | NPGS | XE-60 | CW-1540 | ECNSS-M | EGSS-Y | HI-EFF-8BP | EGS |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 140° | 150° | 170° | 185° | 165° | 150° | 160° | 140° | 150° | 150° | 180° |
| | 18.9 | 6.0 | 6.4 | 13.6 | 13.3 | 10.9 | 7.0 | 10.3 | 5.8 | 6.1 | 3.3 min. |
| Threonolactone ......... | 0.08 | 0.09 | 0.23 | 0.13 | 0.29 | 0.20 | | 0.37 | 0.40 | 0.30 | |
| Erythronolactone ........ | 0.10 | 0.11 | 0.35 | 0.16 | 0.54 | 0.41 | 0.28 | 0.93 | 0.77 | 0.64 | 0.46 |
| Arabinonolactone ........ | 0.25 | 0.27 | 0.41 | 0.33 | 0.48 | 0.38 | 0.28 | 0.50 | 0.55 | 0.44 | 0.34 |
| Xylonolactone .......... | 0.27 | 0.30 | 0.54 | 0.36 | 0.58 | 0.51 | 0.34 | 0.69 | 0.66 | 0.57 | 0.42 |
| Ribonolactone ... ....... | 0.31 | 0.35 | 0.64 | 0.42 | 0.75 | 0.73 | 0.42 | 1.05 | 0.86 | 0.79 | 0.53 |
| Lyxonolactone .......... | 0.40 | 0.45 | 0.81 | 0.51 | 1.17 | 1.08 | | 1.66 | 1.27 | 1.17 | |
| Galactonolactone ........ | 0.92 | 0.98 | 0.88 | 0.95 | 0.95 | 0.95 | 0.84 | 0.98 | 0.96 | 0.90 | 0.80 |
| Gluconolactone ......... | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Talonolactone .......... | | 1.19 | 1.49 | 1.13 | 1.35 | 1.73 | | 1.84 | 1.40 | 1.48 | |
| Gulonolactone .......... | 1.03 | 1.15 | 1.12 | 1.13 | 1.51 | 1.69 | 1.17 | 1.80 | 1.44 | 1.52 | 1.16 |
| Mannonolactone ......... | 1.29[1] | 1.50 | 1.40 | 1.40 | 1.91 | 2.06 | | 2.20 | 1.80 | 1.78 | |
| D-glycero-D-guloheptonolactone | | 3.10 | | | | | | | | | |
| D-glycero-L-mannoheptonolactone | | 3.10 | | | | | | | | | |

[1] Column temperature 160°C

| | |
|---|---|
| SE-30: | 1 % SE-30 Silicone Gum on Chromosorb P AW silan. 100/120 mesh (LKB-Produkter). |
| SE-52: | 1 % SE-52 Silicone Gum on Chromosorb W silan. 80/100 mesh (Perkin-Elmer). |
| QF-1: | 3 % DC QF-1 Silicone Fluid on Gas Chrom Q 100/120 mesh (Appl. Science Lab.). |
| DC-560 EGSP-Z: | 7 % DC-560 Silicone Fluid plus 1 % EGSP-Z Organosilicon Polymer on Gas Chrom Q 100/120 mesh (Appl. Science Lab.). |
| NPGS: | 1 % Neopentylglycolsuccinate Polyester on Chromosorb W silan. 80/100 mesh (Perkin-Elmer). |
| XE-60: | 1 % GE XE-60 Silicone Gum on Gas Chrom Q 100/120 mesh (Appl. Science Lab.). |
| CW-1540: | 10 % Carbowax 1540 on Teflon 35/60 mesh (Perkin-Elmer). |
| ECNSS-M: | 3 % ECNSS-M Organosilicon Polymer on Gas Chrom Q 100/120 mesh (Appl. Science Lab.). |
| EGSS-Y: | 3 % EGSS-Y Organosilicon Polymer on Gas Chrom Q 100/120 mesh (Appl. Science Lab.). |
| HI-EFF-8BP: | 1 % HI-EFF-8BP Polyester on Gas Chrom Q 100/120 mesh (Appl. Science Lab.). |
| EGS: | 10 % EGS Polyester on Celite 545 60/100 mesh (Perkin-Elmer). |
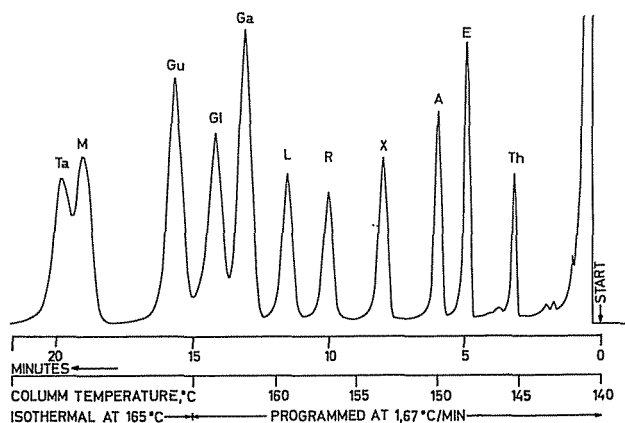


Fig. 3. Analysis of aldono-1,4-lactones as TMS-ethers. Column: 200 × 0.2 cm ID stainless steel. Column packing: 3 % DC QF-1 on Gas Chrom Q 100/120 mesh. Column temperature: Programmed at 1.67°C/min from 140° to 170°C. Carrier gas: N2. Flow rate: 28 ml/min.
Th = Threono-1,4-lactone, E = Erythrono-1,4-lactone, A = Arabinono-1,4-lactone, X = Xylono-1,4-lactone, R = Ribono-1,4-lactone, L = Lyxono-1,4-lactone, Ga = Galactono-1,4-lactone, Gl = Glucono-1,4-lactone, Gu = Gulono-1,4-lactone, M = Mannono-1,4-lactone, Ta = Talono-1,4-lactone.
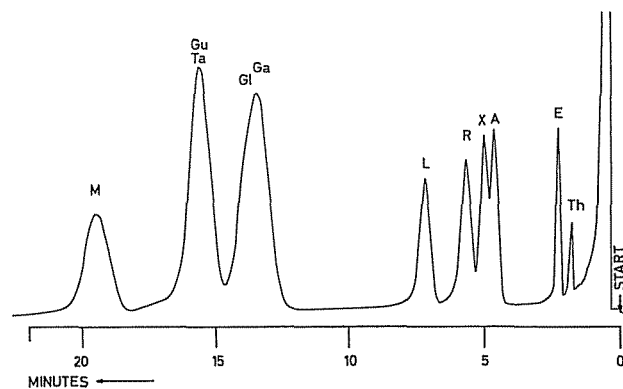


Fig. 4. Analysis of aldono-1,4-lactones as TMS-ethers. Column: 200 × 0.2 cm ID stainless steel. Column packing: 7 % DC-560 plus 1 % EGSP-Z on Gas Chrom Q 100/120 mesh. Column temperature: 185°C. Carrier gas: N2. Flow rate: 28 ml/min.
Th = Threono-1,4-lactone, E = Erythrono-1,4-lactone, A = Arabinono-1,4-lactone, X = Xylono-1,4-lactone, R = Ribono-1,4-lactone, L = Lyxono-1,4-lactone, Ga = Galactono-1,4-lactone, Gl = Glucono-1,4-lactone, Gu = Gulono-1,4-lactone, Ta = Talono-1,4-lactone, M = Mannono-1,4-lactone.

An excellent group separation of lactones differing in the number of carbon atoms was achieved with DC-560/EGSP-Z (*Fig. 4*). The separation of diastereomeric lactones was less satisfactory with this stationary phase, however. The strongly polar stationary phases investigated (EGS, EGSS-Y) were less satisfactory mainly because of a pronounced tailing.

The selectivity of the non-polar silicon elastomers (SE-52 and SE-30) is inferior to that of most other phases. On the other hand these materials exhibit a very high stability at high temperatures and are therefore suitable when identifications are made by means of mass spectrometry. Since mass spectra can be recorded for the first and the last part of two overlapping bands complete separation is not necessary for obtaining mass spectra for identification purposes.

In practical applications studied in this laboratory, gas chromatographic separations of aldono-1,4-lactones as TMS-ethers proved to be a valuable complement to separations by ion exchange chromatography. Examples are control of purity of fractions isolated by ion exchange chromatography and separation of mixtures obtained after group separations on anion exchange resins.

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