Carbohydrate Research, 34 (1974) 323–329

METHANOLYSIS OF D-GALACTURONIC ACID: DIMETHYL ACETALS

KENNETH LARSSON AND GÖRAN PETERSSON
Department of Engineering Chemistry, Chalmers University of Technology, Göteborg (Sweden)
(Received January 16th, 1974; accepted for publication, January 22nd, 1974)

ABSTRACT

Dimethyl acetals of D-galacturono-6,3-lactone and methyl D-galacturonate have been detected during methanolysis of D-galacturonic acid. The products of methanolysis were studied by ion-exchange chromatography and by g.l.c. of the trimethylsilyl (TMS) derivatives. Structural determinations were made from the mass spectra of the TMS derivatives. The course of methanolysis was monitored by g.l.c.

INTRODUCTION

Esterification and subsequent formation of methyl pyranosides and furanosides on methanolysis of D-galacturonic acid have been studied by Schmidt and Neukom[1,2]. On preparation of the glycosides according to their methods, an additional product, a dimethyl acetal of D-galacturonic acid, was detected. Therefore, the products and the course of methanolysis were reinvestigated, applying analytical methods developed in this laboratory.

The formation of dimethyl acetals during methanolysis has been demonstrated for aldoses[3,4].

RESULTS AND DISCUSSION

Dimethyl acetals. — Ion-exchange chromatography of the saponified products of the methanolysis of D-galacturonic acid gave one band in addition to those corresponding to D-galacturonic acid and its methyl glycosides. With acetic acid as eluent on a resin in its acetate form, this band appeared first and contained a lactone (*X*), as indicated by the rate of alkali consumption on titration with sodium hydroxide. Rechromatography demonstrated that partial decomposition of *X* to D-galacturonic acid and its methyl furanosides had occurred during the isolation procedure. Similar decomposition was observed during other treatments involving acidic conditions. Since dimethyl acetals of aldoses decompose to methyl furanosides and aldoses on acid hydrolysis[5], the above results suggested *X* to be a dimethyl acetal of D-galacturonic acid.

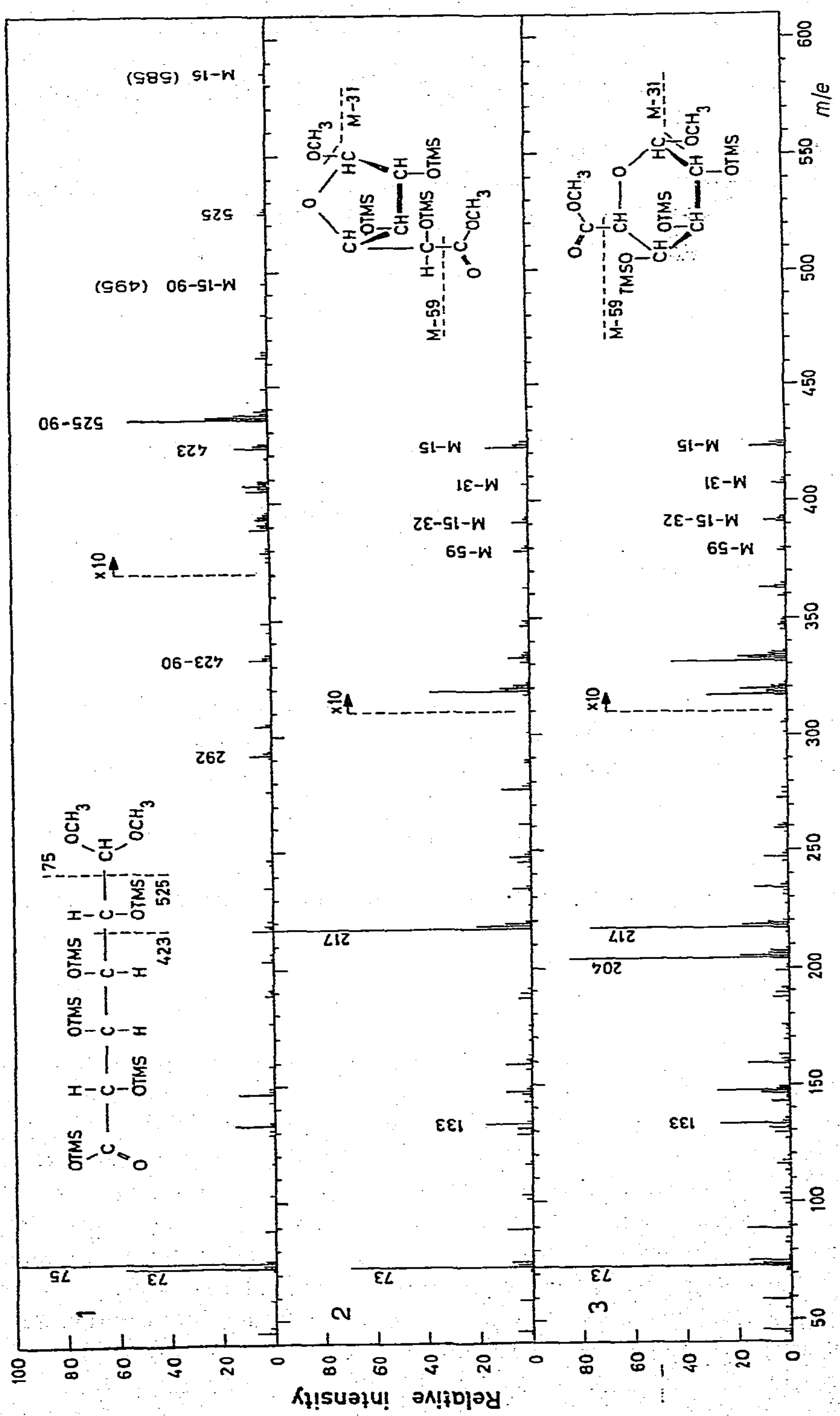

Fig. 1. Mass spectra at 70 eV of the TMS derivatives of D-galacturonic acid dimethyl acetal (1), the methyl ester of methyl β-D-galactofuranosiduronic acid (2), and the methyl ester of methyl α-D-galactopyranosiduronic acid (3).

The mass spectrum of the TMS derivative prepared[6] from the sodium salt obtained on saponification of *X* is shown in Fig. 1. The base peak at *m/e* 75 is strong evidence for a dimethyl acetal structure, since the TMS derivative of 3,6-anhydro-D-galactose dimethyl acetal gives rise to a *m/e* 75 ion of similar prominence[7]. Delocalization of the positive charge over two oxygen atoms promotes the formation of this ion. The rearrangement ion of *m/e* 292 is evidence for an α,β-dihydroxy acid[8]. The *m/e* 423 ion and the abundant *m/e* 333 daughter ion, formed by elimination of trimethylsilanol, confirm the structure **1** by analogy with the spectra of the aldonic acid derivatives[9]. The anticipated molecular weight (600) is confirmed[9] by the peaks at M—15 and M—15—90.

The mass spectrum of the TMS derivative of *X* also has a base peak at *m/e* 75, indicating a dimethyl acetal structure. Peaks at *m/e* 438 (M) and *m/e* 423 (M—15) are consistent with the anticipated lactone structure, and a prominent peak at *m/e* 217 indicates a γ-lactone rather than a δ-lactone[10]. A γ-lactone was expected since 1,4-lactones preponderate for galactonic acid and most other aldonic acids.

The g.l.c. study of methanolysis described below revealed the presence of one additional, previously unknown component. Its mass spectrum is analogous with that of **1** in Fig. 1, but the masses of the ions containing the ester group are shifted 58 mass units towards lower values. The component is therefore identified as the TMS derivative of the methyl ester of the dimethyl acetal.

Glycosides. — Methanolysis of D-galacturonic acid was carried out in two separate experiments; one of these was designed to give mainly the methyl esters of the methyl furanosides, and the other to give mainly the methyl esters of the methyl pyranosides[1]. The product mixtures were saponified at pH 10, and ion-exchange chromatography then revealed the presence of four acid components in addition to D-galacturonic acid and its dimethyl acetal. These four acids were isolated by preparative ion-exchange chromatography, and each produced D-galacturonic acid on acid hydrolysis, thus confirming that they were the four anticipated methyl glycosides. The observed optical rotation data $\{[\alpha]_D^{25}$ +126° (α-pyranoside), −37° (β-pyranoside), and −110° (β-furanoside)$\}$ compare well with literature data.

TABLE I

ION-EXCHANGE CHROMATOGRAPHY: VOLUME DISTRIBUTION COEFFICIENTS

	M *HOAc*[a] (*30°*)	*0.08*M *NaOAc*[b] (*30°*)	*0.15*M $K_2B_4O_7$[c] (*25°*)
D-Galacturonic acid	10.5	8.40	19.7
Methyl α-D-galactofuranosiduronic acid	9.82	8.39	3.32
Methyl β-D-galactofuranosiduronic acid	14.2	12.8	5.19
Methyl α-D-galactopyranosiduronic acid	5.24	4.09	2.71
Methyl β-D-galactopyranosiduronic acid	7.54	4.66	2.77
D-Galacturonic acid dimethyl acetal	3.71	5.17	14.9

[a]Column: 4×900 mm of Dowex-1 x8 (acetate form, 14–17 μm). [b]Column: 4×920 mm of Dowex-1 x8 (acetate form, 14–17 μm), pH adjusted to 5.9 with acetic acid. [c]Column: 4.4×8.60 mm of Dowex-1 x8 (borate form, 25–27 μm).

Volume distribution coefficients (D_v) are given in Table I. The glycosides were eluted in the same order on all three columns. The α-furanoside was well separated from D-galacturonic acid only in the borate medium. The two other ion-exchange systems are well suited to both analytical and preparative work with the remaining components.

Reference samples of the methyl esters of the glycosides were prepared by mild methanolysis of the isolated glycosides.

Raunhardt *et al.*[11] studied the TMS derivatives of D-galacturonic acid, methyl D-galacturonate, and the corresponding methyl glycosides by g.l.c., using SE-30 and SE-52 silicone phases. In the present investigation, the fluorosilicone QF-1 was suitable for the analysis of the silylated products of methanolysis, particularly for the dimethyl acetals, and the retention data are given in Table II. The retention order of the isomeric methyl glycosides is β-furanoside <α-furanoside <α-pyranoside <β-pyranoside, with QF-1 as well as with SE-30 and SE-52[11]. The ring sizes of the TMS glycosides were established by mass spectrometry, and the retention order of the anomeric TMS-glycosides was assumed to be the same as for the methyl glycosides[11]. The dimethyl acetal of methyl D-galacturonate appeared between the furanoid and the pyranoid species, and that of D-galacturono-6,3-lactone after all other compounds.

TABLE II

G.L.C. DATA[a] FOR TMS DERIVATIVES OF D-GALACTURONIC ACID

	Relative retentions[b] at 160°			
		Methyl ester	*Methyl glycoside*	*Methyl ester, methyl glycoside*
α-D-Galactofuranuronic acid	1.36	1.28	0.96	0.99
β-D-Galactofuranuronic acid	0.94	0.84	0.87	0.82
α-D-Galactopyranuronic acid	2.11	1.68	2.25	1.95
β-D-Galactopyranuronic acid	2.39	2.31	2.35	2.00
D-Galacturonic acid dimethyl acetal	1.78	1.50		
D-Galacturono-6,3-lactone dimethyl acetal	2.95			

[a] 3% DC QF-1 on 100–120 mesh Gas Chrom Q. Column: 300 × 0.2 cm i.d., stainless steel. Carrier gas: purified nitrogen, 30 ml/min. [b] Adjusted retention times relative to that of TMS-D-glucitol (5.0 min).

Characteristic mass spectra, suitable for identification purposes, were obtained for all the derivatives listed in Table II. Anomeric derivatives gave rise to similar spectra. Spectra of representative furanoside (2) and pyranoside (3) derivatives are reproduced in Fig. 1. As with aldohexoses[12,13], the spectra permit pyranosides to be distinguished from furanosides. The ratio between the intensities of the peaks at *m/e* 204 and 217 is the most significant difference. The *m/e* 133 ion for the methyl glycosides contains the substituent at C-1 and is analogous to the characteristic *m/e* 191 ion for the TMS glycosides[12,13]. The molecular weight is obtained from the

M—15 peaks which are of similar relative intensity for all the glycosidic derivatives. The other peaks in the upper-mass region are analogous to those of aldohexose derivatives[12,13]. The pyranoside spectra are also related to the spectra of fully methylated hexopyranuronic acids[14].

The course of methanolysis. — The change in the composition of the reaction mixture during methanolysis of D-galacturonic acid is illustrated in Fig. 2:

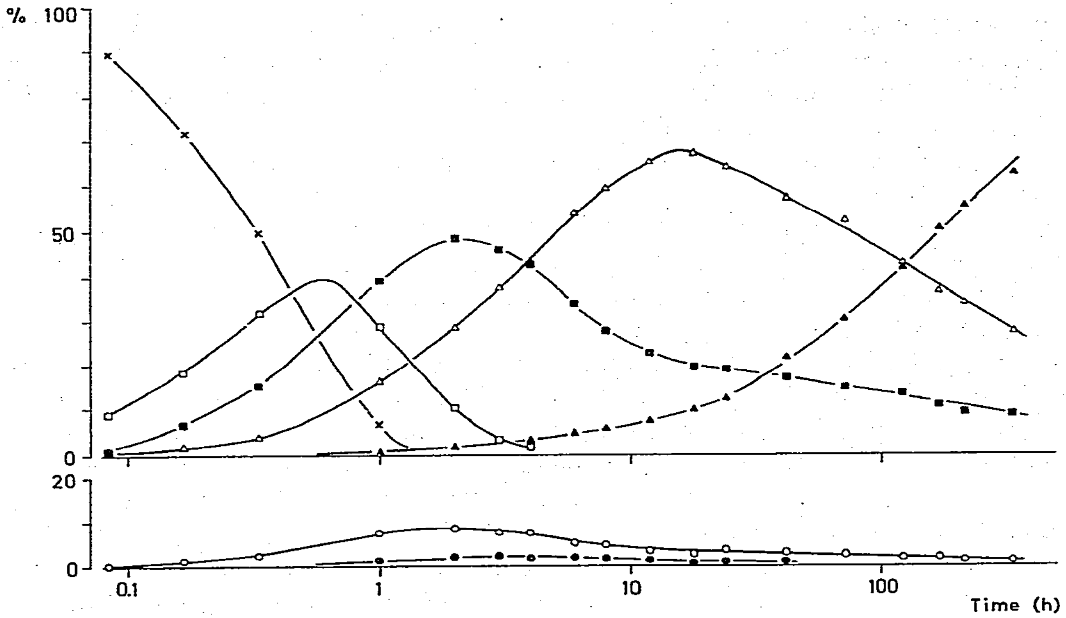

Fig. 2. Methanolysis of D-galacturonic acid at 23°. Molar distribution (%) of components in the reaction mixture as a function of time: ○ D-galacturono-6,3-lactone dimethyl acetal; ● methyl D-galacturonate dimethyl acetal; × D-galacturonic acid; □ methyl D-galacturonate; ■ methyl ester of methyl α-D-galactofuranosiduronic acid; △ methyl ester of methyl β-D-galactofuranosiduronic acid; ▲ methyl esters of methyl D-galactopyranosiduronic acids.

Esterification proceeds faster than glycosidation (*cf.* Ref. 15). The observed course of glycosidation is furanoside formation, furanoside anomerization, and ring expansion to pyranosides, in accord with the normal reaction sequence for methanolysis of aldoses[16]. Initially, the α-furanoside is formed in preference to the β-furanoside. All these results are in agreement with those of Schmidt and Neukom[1].

The lactone dimethyl acetal reached its maximum content ($\sim$8%) when all the D-galacturonic acid was consumed. Formation *via* a hemiacetal of D-galacturonic acid or its lactone appears likely, and supports the hypothesis[3,4] that acyclic hemiacetal intermediates are involved in the glycosidation of aldoses. The lactone of the dimethyl acetal exhibited a stability similar to that observed for galactono-1,4-lactone during the applied conditions of methanolysis.

The dimethyl acetal of methyl D-galacturonate reached its maximum content (2–3%) later than the lactone. By analogy with the aldose dimethyl acetals, it might be formed from the glycosides[3,4,16], and it should decompose primarily to furanosides[5].

EXPERIMENTAL

In the study of methanolysis, D-galacturonic acid (500 mg) was added to a glass tube containing 25 ml of methanol and 20 ml of methanol-washed Dowex-1 x8(H^+) resin (20–50 mesh). The tube was sealed and stored at 23 ±0.5° under rotation. Samples (0.3 ml) were withdrawn at intervals, and methanol was removed from them by use of a rotary evaporator at 30°.

TMS derivatives were prepared in pyridine with bis(trimethylsilyl)trifluoroacetamide and chlorotrimethylsilane[6].

A Perkin–Elmer Model 900 instrument with flame-ionisation detector (FID) was used for g.l.c. Quantitative analysis was performed on QF-1 at 160°. Peak height × retention time was used to calculate peak areas on the gas chromatograms. The peak areas of the TMS glycosides were summed to give one value for D-galacturonic acid and one for its methyl ester. The peaks from the dimethyl acetals were well separated from all other peaks. The derivatives of the two anomeric methyl esters of the methyl pyranosides were not resolved. Overlapping peaks may have decreased the accuracy for some components in the samples with TMS glycosides present. Most of these compounds were better resolved on OV-1 at 160°. The molar distribution (Fig. 2) was calculated by assuming the same FID weight-response for all derivatives.

Mass spectra were recorded on a LKB 9000 gas chromatograph–mass spectrometer, using a QF-1 column[6]. The temperature of the molecule separator was 210° and that of the ion source 270°.

D_v values were determined on ion-exchange columns (Table I) coupled to three-channel analyzers[17]. Preparative separations were made on columns (20 × 810 mm) of Dowex-1 x8 resin (25–32 μm) coupled to a fraction collector. M Acetic acid was used as eluent.

REFERENCES

1 H. W. H. SCHMIDT AND H. NEUKOM, *Helv. Chim. Acta*, 49 (1966) 510.
2 H. W. H. SCHMIDT AND H. NEUKOM, *Helv. Chim. Acta*, 47 (1964) 865.
3 D. D. HEARD AND R. BARKER, *J. Org. Chem.*, 33 (1968) 740.
4 R. J. FERRIER AND L. R. HATTON, *Carbohyd. Res.*, 6 (1968) 75.
5 B. CAPON AND D. THACKER, *J. Chem. Soc., B*, (1967) 1322.
6 G. PETERSSON, *Carbohyd. Res.*, 33 (1974) 47.
7 W. SCHMÖLCK AND E. MERGENTHALER, *Z. Lebensm. Untersuch. Forsch.*, 152 (1973) 263.
8 G. PETERSSON, *Org. Mass Spectrom.*, 6 (1972) 577.
9 G. PETERSSON, *Tetrahedron*, 26 (1970) 3413.
10 G. PETERSSON, O. SAMUELSON, K. ANJOU, AND E. VON SYDOW, *Acta Chem. Scand.*, 21 (1967) 1251.
11 O. RAUNHARDT, H. W. H. SCHMIDT, AND H. NEUKOM, *Helv. Chim. Acta*, 50 (1967) 1267.
12 G. PETERSSON AND O. SAMUELSON, *Sv. Papperstidn.*, 71 (1968) 731.

13 D. C. De Jongh, T. Radford, J. D. Hribar, S. Hanessian, M. Bieber, G. Dawson, and C. C. Sweeley, *J. Amer. Chem. Soc.*, 91 (1969) 1728.
14 V. Kováčik, Š. Bauer, J. Rosík, and P. Kováč, *Carbohyd. Res.*, 8 (1968) 282.
15 E. F. Jansen and R. Jang, *J. Amer. Chem. Soc.*, 68 (1946) 1475.
16 B. Capon, *Chem. Rev.*, 69 (1969) 440.
17 B. Carlsson, T. Isaksson, and O. Samuelson, *Anal. Chim. Acta*, 43 (1968) 47.