Carbohydrate Research, 34 (1974) 323–329

METHANOLYSIS OF GALACTURONIC ACID. DIMETHYL ACETALS.

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ABSTRACT

Dimethyl acetals of galacturono-6,3-lactone and methyl galacturonate are formed during methanolysis of galacturonic acid. The products of methanolysis were studied by ion exchange and gas chromatography. Trimethylsilyl (TMS) derivatives were used in gas chromatography. Structure determinations were made from mass spectra of the TMS derivatives. The course of methanolysis was investigated by means of a gas chromatographic study of the composition of the reaction mixture as a function of time.

INTRODUCTION

Esterification and subsequent formation of methyl pyranosides and furanosides on methanolysis of galacturonic acid have been studied previously by Schmidt and Neukom [1,2].

On preparation of the glycosides according to their methods, an additional product, a dimethyl acetal of galacturonic acid, was detected. Therefore, the products and the course of methanolysis were reinvestigated, applying analytical methods developed in this laboratory.

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

DIMETHYL ACETALS

Isolation and properties. - Ion exchange chromatography of the saponified products from methanolysis of galacturonic acid gave one band in addition to those corresponding to galacturonic acid and its methyl glycosides. With acetic acid as eluent on a resin in its acetate form, this band appeared first and was isolated. Acetic acid was removed from the eluate fraction by evaporation at reduced pressure. The rate of alkali consumption on titration with sodium hydroxide indicated a lactone. Rechromatography demonstrated that partial decomposition to galacturonic acid and its methyl furanosides had occurred during the preceding isolation. 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 results suggested a dimethyl acetal of galacturonic acid.

Mass spectrometric identification. - The sodium salt obtained on saponification was dried and silylated [6]. In Fig. 1, the mass spectrum of the resulting derivative of the new compound is reproduced. The base peak at m/e 75 is strong evidence for a dimethyl acetal structure. The TMS derivative of 3,6-anhydrogalactose 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 mass 292 is evidence for a

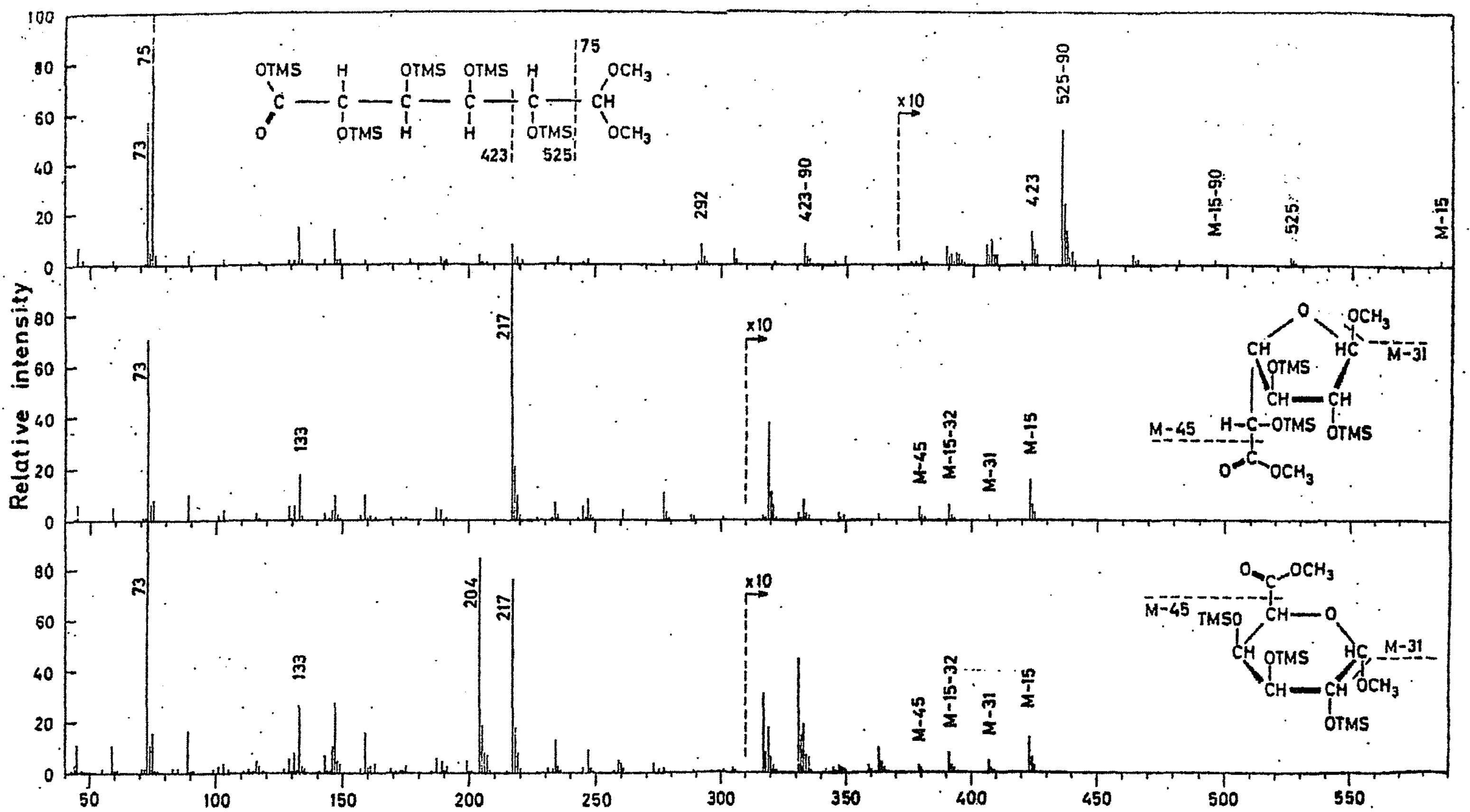

Fig. 1. Mass spectra at 70 eV of the trimethylsilyl derivatives of galacturonic acid dimethyl acetal (I), the methyl ester of methyl β-galactofuranosiduronic acid (II), and the methyl ester of methyl α-galactopyranosiduronic acid (III).

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 by analogy with the spectra of the aldonic acid derivatives [9]. The anticipated molecular weight (M = 600) is confirmed by the peaks at M-15 and M-15-90 [9].

Silylation of the isolated non-saponified compound produced a different derivative. The base peak of its mass spectrum is at m/e 75 indicating a dimethyl acetal. 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]. This lactone was expected since 1,4-lactones predominate for galactonic acid and most other aldonic acids.

The gas chromatographic study of methanolysis described below revealed the presence of one additional previously unknown component. Its mass spectrum is analogous with I in Fig. 1, but the masses of the ions containing the ester group are shifted 58 mass units towards lower values. Obviously, it is the TMS derivative of the methyl ester of the dimethyl acetal.

GLYCOSIDES

Preparation and ion exchange chromatography. - Methanolysis of galacturonic acid was carried out in two separate experiments; one 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. Ion exchange chromatography revealed the presence of four acid components in addition to galacturonic acid and its dimethyl acetal. These four acids were isolated by preparative ion exchange chromatography. All of them produced galacturonic acid on acid hydrolysis, thus confirming that they were the four anticipated methyl glycosides. The observed optical rotation data, $\alpha_D^{25} = 126^o$, c 0.5 (α-pyranoside), $\alpha_D^{25} = -37^o$, c 0.5 (β-pyranoside) and $\alpha_D^{25} = -110^o$, c 0.5 (β-furanoside), compare well with literature data.

Volume distribution coefficients (D_v) are given in Table I. The glycosides are eluted in the same order on all three columns. The α-furanoside is well separated from 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.

Gas chromatography. - Raunhardt *et al.* [11] studied TMS derivatives of galacturonic acid, methyl galacturonate, and the corresponding methyl glycosides by gas chromatography, using SE-30 and SE-52 silicone phases. In the present investigation the fluorosilicone QF-1 was found to be well suited for the analysis of the silylated products from methanolysis and particularly for the dimethyl acetals. 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 with the methyl glycosides [11]. The dimethyl acetal of methyl galacturonate

TABLE I

Ion exchange chromatography. Volume distribution coefficients.

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

[a] Column: 4×900 mm Dowex 1-X8 (acetate form) 14-17 μm. [b] Column: 4×920 mm Dowex 1-X8 (acetate form) 14-17 μm. pH adjusted to 5.9 with acetic acid. [c] Column: 4.4×860 mm Dowex 1-X8 (borate form) 25-27 μm.

TABLE II

Gas chromatography of fully trimethylsilylated derivatives of galacturonic acid. Relative retentions [a] on QF-1 [b] at 160°.

		methyl ester	methyl glycoside	methyl ester, methyl glycoside
α-Galactofuranuronic acid	1.36	1.28	0.96	0.99
β-Galactofuranuronic acid	0.94	0.84	0.87	0.82
α-Galactopyranuronic acid	2.11	1.68	2.25	1.95
β-Galactopyranuronic acid	2.39	2.31	2.35	2.00
Galacturonic acid dimethyl acetal	1.78	1.50		
Galacturono-6,3-lactone dimethyl acetal	2.95			

[a] Adjusted retention times relative to the glucitol trimethylsilyl derivative (5.0 min).

[b] 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.

appeared between the furanoid and the pyranoid species, and that of galacturono-6,3-lactone after all other compounds.

Mass spectrometry. - Characteristic mass spectra, suitable for identification purposes, were obtained for all derivatives listed in Table II. Anomeric derivatives gave rise to similar spectra. Spectra of two representative isomeric furanoside and pyranoside derivatives are reproduced in Fig. 1. As with aldohexoses [12,13], characteristic features of 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 apparent difference. The m/e 133 ion for the methyl glycosides contains the substituent at C-1 and is replaced by a characteristic m/e 191 ion for the TMS glycosides [12,13]. The molecular weight is obtained from M-15 peaks 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 galacturonic acid is illustrated in Fig. 2.

Esterification proceeds faster than glycosidation [cf. 15]. The observed course of glycosidation is furanoside formation, furanoside anomerization, and ring expansion to pyranosides,

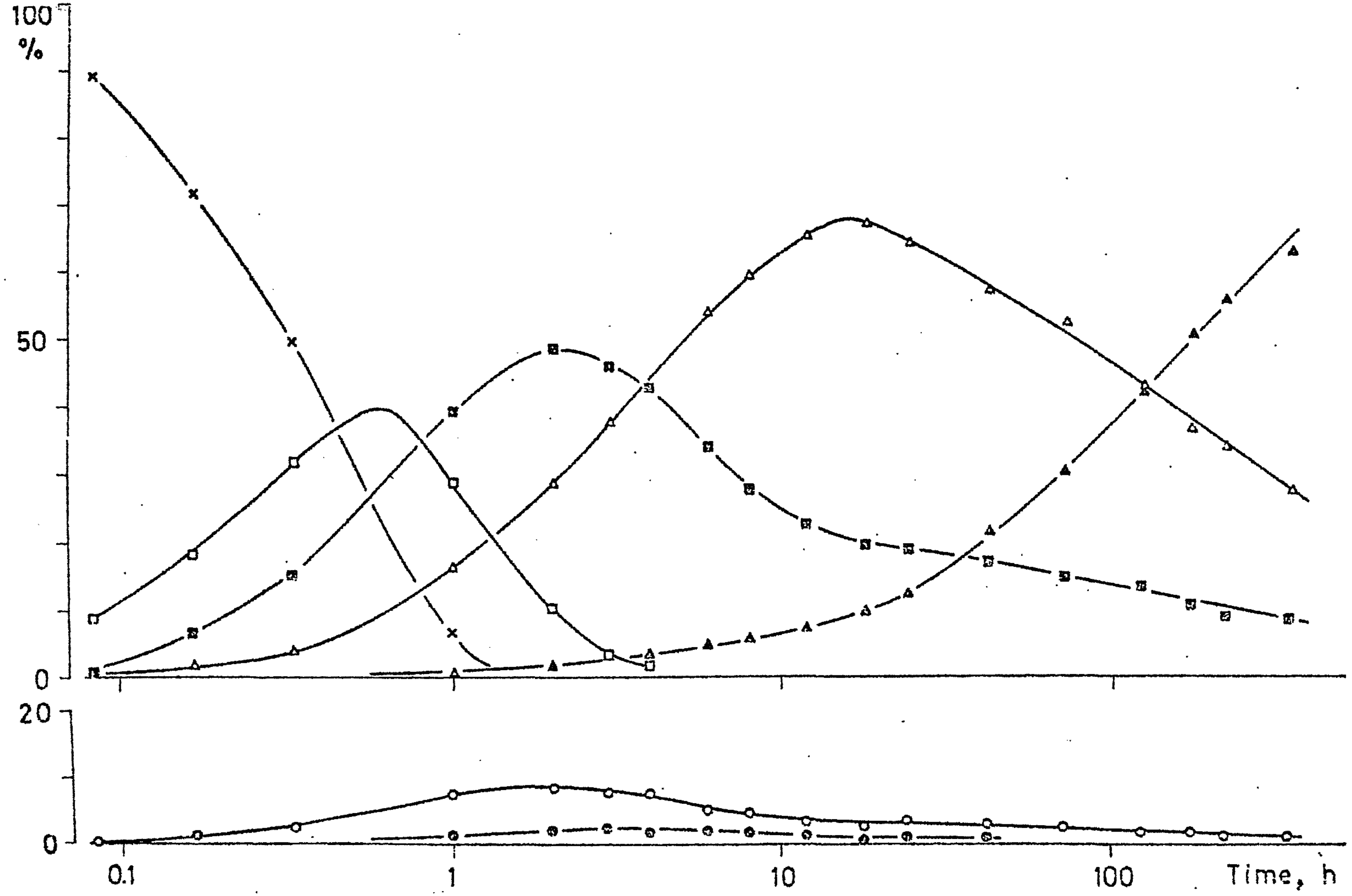

Fig. 2. Methanolysis of galacturonic acid at 23°. Molar distribution (%) of components in the reaction mixture as a function of time.

○ Galacturono-6,3-lactone dimethyl acetal

⊛ Methyl galacturonate dimethyl acetal

× Galacturonic acid

□ Methyl galacturonate

▨ Methyl ester of methyl α-galactofuranosiduronic acid

△ Methyl ester of methyl β-galactofuranosiduronic acid

▲ Methyl esters of methyl galactopyranosiduronic acids 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 (about 8%) when all galacturonic acid was consumed. Formation *via* a hemiacetal of galacturonic acid or its lactone appears likely. This 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 methanolysis conditions.

The dimethyl acetal of methyl galacturonate reached its maximum (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 the course of methanolysis, D-galacturonic acid (500 mg) was added to a glass tube containing 25 ml methanol and 20 ml methanol-washed ion exchange resin (Dowex 1-X8, 20-50 mesh, H^+-form). The tube was sealed and stored at 23±0.5° under rotation. Samples (0.3 ml) were withdrawn during the reaction, and methanol was removed from them in a rotating vacuum evaporator at 30°.

TMS derivatives were prepared in pyridine with bis(trimethylsilyl)trifluoroacetamide (BSTFA) and trimethylchlorosilane (TMCS) as reagents [6].

A Perkin-Elmer Model 900 with FID detector was used for gas chromatography. 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 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 reduced 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 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 an anion exchange column (20×810 mm, Dowex 1-X8, 25-32 μm) coupled to a fraction collector. Acetic acid (1,0 M) was used as eluent.

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