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2-*C*-CARBOXYALDOSES AND ALDONIC ACIDS FROM CELLOBIOSE, MALTOSE, AND 4-*O*-METHYL-D-GLUCOSE WITH 2-ANTHRAQUINONE-SULFONIC ACID

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ABSTRACT

Cellobiose, maltose, and 4-*O*-methyl-D-glucose were treated with 0.1–20mM 2-anthraquinonesulfonic acid in 0.1M sodium hydroxide at 40°. The hydroxy carboxylic acids formed were separated by ion-exchange, and analyzed by g.l.c.–m.s. as their per(trimethylsilyl) derivatives. The acidic oxidation products of cellobiose were further fractionated into aldonic acids and carboxylaldoses by ion-exchange chromatography. The isolated carboxyaldoses were reduced with sodium borohydride, and then analyzed by g.l.c.–m.s. before and after hydrolysis. The *O*-D-glucosyl- and *O*-methyl-substituted products of the sugars consisted of erythronic, arabinonic, ribonic, gluconic, and mannonic acids, in addition to 2-*C*-carboxypentoses. The nonsubstituted products of the reducing D-glucose unit were formic, glycolic, 2-deoxytetronic, and 3-deoxypentonic acids, and 2-*C*-carboxy-3-deoxypentoses.

INTRODUCTION

It was earlier shown[1] that an aldose (D-glucose) is oxidized quantitatively to the corresponding aldos-2-ulose by a quinone (2-anthraquinonesulfonic acid). To obtain more knowledge about the further reactions of the aldos-2-uloses, some 4-*O*-substituted aldoses (cellobiose, maltose, and 4-*O*-methyl-D-glucose) have now been treated under similar conditions. The kinetic evidence for the different reaction paths still remained inadequate, but, on the other hand, a new type of product, namely, 2-*C*-carboxypentoses, was identified in the reaction mixture.

RESULTS AND DISCUSSION

Oxidation products. — The reducing D-glucose residue of all of the aldoses studied yielded erythronic, arabinonic, ribonic, gluconic, and mannonic acid residues (see Table I). The same aldonic acids had also been identified[2] after oxidation of cellulose with 2-anthraquinonesulfonic acid (AMS). Instead, only hexonic

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TABLE I

PRODUCTS FORMED AFTER NON-DEGRADATIVE OXIDATION OF CELLOBIOSE, MALTOSE, AND 4-*O*-METHYL-D-GLUCOSE WITH 10mM AMS IN 0.1M SODIUM HYDROXIDE AT 40° (THE YIELDS ARE GIVEN IN MOLE-% OF THE STARTING MATERIAL)

Product	*Cellobiose*[a]	*Maltose*[b]	*4-O-methyl-D-glucose*[c]
2-*O*-R-D-Erythronic acid	19	32	20
3-*O*-R-D-Arabinonic acid	2.0	5.5	17
3-*O*-R-D-Ribonic acid	0.5	1.7	
4-*O*-R-D-Gluconic acid	2.1	5.3	25
4-*O*-R-D-Mannonic acid	2.5	7.3	
2-*C*-Carboxy-3-*O*-R-D-pentose	8.1	7.8	32
Total	35	60	94

[a]R = β-D-glucopyranosyl. [b]R = α-D-glucopyranosyl. [c]R = Me.

acid end-groups were formed when cellobiose was oxidized with anthraquinone, possibly because of its lower oxidation power[3,4].

In addition to the aldonic acid residues, significant proportions of 2-*C*-carboxypentose derivatives were formed from all of the 4-*O*-substituted aldoses studied. In contrast, only traces of similar compounds were identified in the oxidation-product mixture from an unsubstituted aldose (D-glucose).

The unsubstituted oxidation products of the reducing D-glucose residue consisted of formic, glycolic, 2-deoxytetronic, and 3-deoxypentonic acids, in adition to 2-*C*-carboxy-3-deoxypentoses.

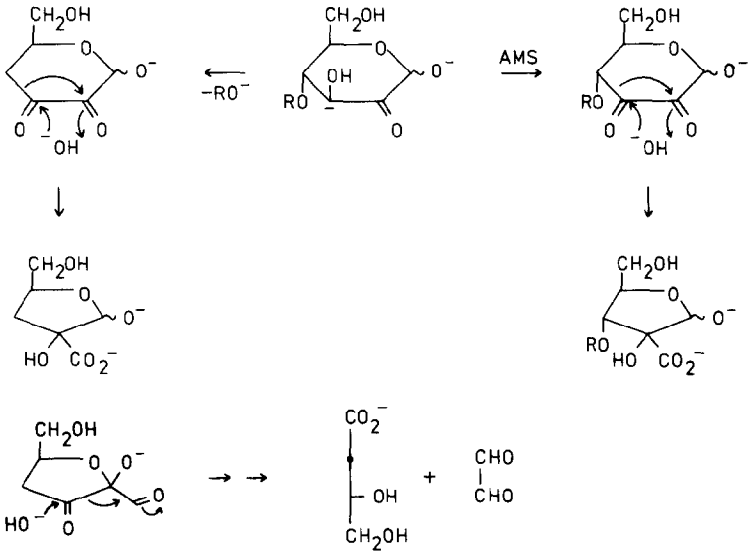
The substituted 2-*C*-carboxypentoses were quite stable towards alkali, whereas the 2-*C*-carboxy-3-deoxypentoses were degraded during prolonged reaction-times.

Reaction mechanism. — As for D-glucose[1], the oxidation of cellobiose and maltose to the corresponding aldos-2-uloses was quantitative at very low concentrations of AMS [the ratios of the rate-constants of oxidation and isomerization (k_{ox}/k_{is}, see ref. 1) were $1.4 \cdot 10^4$ (cellobiose) and $0.7 \cdot 10^4$ M^{-1} (maltose) at 25°]. Accordingly, under the present conditions (0.4–20mM AMS), no non-oxidative degradation-products were actually formed.

The consumption of AMS (20mM) was 1.6 and 1.7 mol per mol of the reducing D-glucose residue of cellobiose and maltose, respectively, which verified that some of the reaction paths consumed more than one equivalent of AMS. The calculated consumption of AMS is ~1.3 (cellobiose) and 1.5 mol/mol (maltose) if the formation of erythronic acid, pentonic acid, and 2-*C*-carboxypentose residues consumes 2 mol of AMS/mol. Because the concentration of AMS (0.4–20mM) had, however, no significant influence on the composition of the reaction-product mixture, it seems probable that the proportions of the products are exclusively controlled by the enolization, isomerization, rearrangement, and elimination steps in each route. Theoretically, it would also be possible that the proportions of the

conformers of the intermediates would determine the product composition, but mutarotation in alkaline solutions is not generally regarded as a rate-determining step[5].

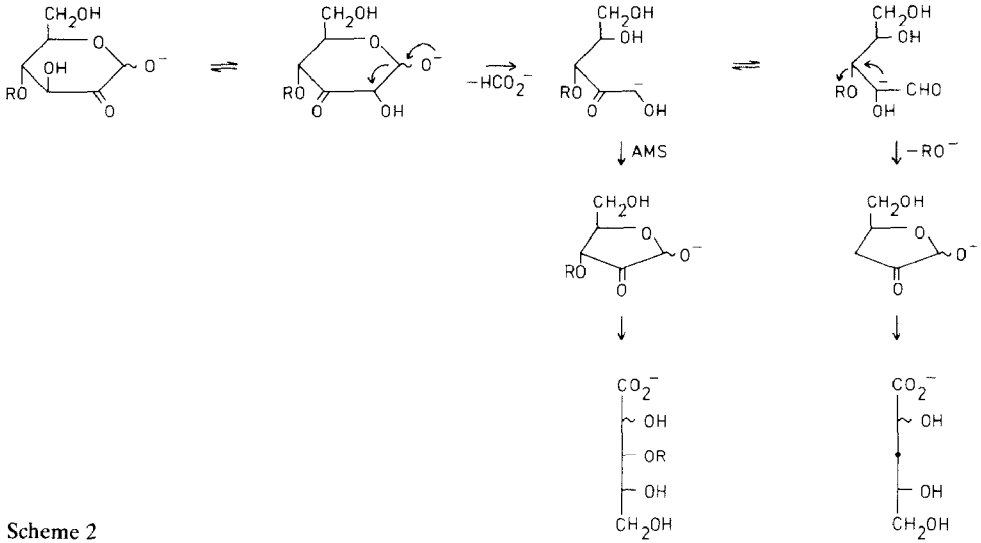
The 2-*C*-carboxypentose moieties are undoubtedly formed through oxidation of the aldos-2-ulose (or its isomers) to the corresponding aldos-2,3-diulose, which is followed by a benzilic acid type of rearrangement between the keto groups (see Scheme 1). The 2-*C*-carboxy-3-deoxypentoses are formed analcgously from the eliminated 4-deoxyhexos-2,3-diulose.



Scheme 1

The only possible source of 2-deoxytetronic acid is the 4-deoxyhexos-2,3-diulose (the other elimination product, 3-deoxypentos-2-ulose, does not yield 2-deoxytetronic acid[6]), from which glyoxal is eliminated[7,8] (see Scheme 1). Glyoxal is naturally rearranged to glycolic acid. Formation of an erythronic acid group from the aldos-2,3-diulose is an analogous reaction. Theoretically, the erythronic acid group could also be derived from the corresponding aldos-3-ulose, but, according to the literature[9,10], this route seems unimportant.

Because the relative amounts of 2-deoxytetronic and 3-deoxypentonic acids varied (cellobiose gave more 3-deoxypentonic acids than did maltose and 4-*O*-methyl-D-glucose), it seems probable that the principal source of 3-deoxypentonic acids is the 3-deoxypentos-2-ulose that is eliminated from a 3-*O*-glucosylpentose (see Scheme 2). The latter, in turn, is formed from the aldos-3-ulose *via* elimination of formic acid[9,10], and is also the source of the pentonic acid groups. The pentose group is formed as the very reactive enolate, and, therefore, *O*-D-glucosylpentoses were not found in the reaction mixtures from cellobiose and maltose. Although the routes *via* the pentose group are regarded as the most probable, the formation of



Scheme 2

3-deoxypentonic acids and pentonic acid residues from the corresponding aldos-2,3-diuloses cannot be excluded[7,8].

Identification of the reaction products from cellobiose and maltose. — Among the dimeric reaction-products from both disaccharides, an *O*-D-glucosyltetronic, two *O*-D-glucosylpentonic, and two *O*-D-glucosylhexonic acids were identified by g.l.c.–m.s. (see Fig. 1). Characteristic for the mass spectra of the per(trimethylsilyl)ated *O*-D-glucosylaldonic acids, analogously to those of *O*-D-glucosylaldonolactones[11], were the peaks originating from ion M-349 (*m/z* 453, 555, and 657) in addition to the fragments of the glucosyl unit (*m/z* 361, 451, 466, and 539).

On hydrolysis, the dimeric reaction-products gave erythronic, arabinonic, ribonic, gluconic, and mannonic acids, corresponding to the aldonic acid moieties in the particular glucosylaldonic acids. To determine the order of elution of the isomeric glucosylaldonic acids in the gas–liquid chromatograms, oxidations were carried out in the presence of hydrogen peroxide[12] and calcium ion[13], and glucosylarabinonic and glucosylmannonic acid, respectively, were the main products.

In addition to the D-glucosylaldonic acids, three significant peaks appeared in the gas chromatograms of the dimeric reaction-products (peaks *12–14* in Fig. 1). In the mass spectra of these products, a prominent peak at *m/z* 583 was observed, in addition to the peaks originating from a D-glucosyl unit (*m/z* 361, 451, 466, and 539), which indicated that they could be either carboxy-*O*-D-glucosylpentoses or D-glucosylketohexoses, provided that the peak at *m/z* 583 resulted from ion M-349, in accordance with the fragmentation pattern of numerous related compounds[11,14,15] (see Fig. 2).

After separation by ion-exchange chromatography, and reduction with sodium borohydride, the compounds from peaks *12–14* gave only one peak in

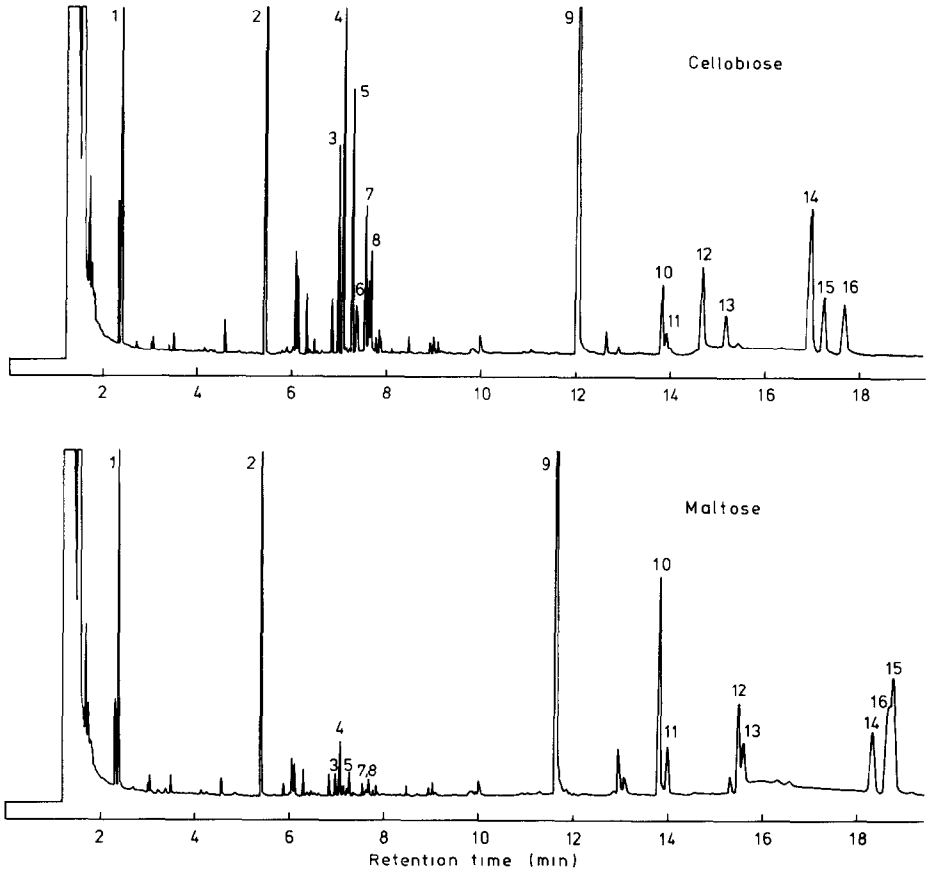


Fig. 1. Separation of the acidic reaction-products from cellobiose and maltose (20% conversion) as their per(trimethylsilyl) derivatives on an OV-101 fused-silica capillary column. [Peaks: *1*, glycolic acid; *2*, 2-deoxytetronic acid; *3*, 3-deoxy-*erythro*-pentonic acid; *4*, 3-deoxy-*threo*-pentonic acid; *5* and *7*, 2-*C*-carboxy-3-deoxypentofuranoses; *6* and *8*, 2-*C*-carboxy-3-deoxypentoses (acyclic); *9*, *O*-D-glucosyl-erythronic acid; *10*, *O*-D-glucosylarabinonic acid; *11*, *O*-D-glucosylribonic acid; *12*, *13*, and *14*, 2-*C*-carboxy-*O*-D-glucosylpentoses; *15*, *O*-D-glucosylmannonic acid; and *16*, *O*-D-glucosyl-D-gluconic acid.]

g.l.c. In the mass spectrum of the reduction product, a characteristic peak for an *O*-D-glucosylhexonic acid (*m/z* 657) appeared. The retention time of the reduction product in the gas–liquid chromatogram differed, however, from those of *O*-D-glucosylgluconic and *O*-D-glucosylmannonic acids.

After hydrolysis, the reduction product again gave in g.l.c. (in addition to D-glucose) only one peak, the mass spectrum of which was identical with the published spectrum of 2-*C*-(hydroxymethyl)pentonic acid[16]. The compounds causing peaks *12–14* must, therefore, be isomeric forms of a 2-*C*-carboxy-*O*-D-glucosylpentose.

Unlike the *O*-D-glucosylaldonic acids, the 2-*C*-carboxy-*O*-D-glucosylpentoses showed no tendency to lactonize, but, under hydrolytic conditions, their acid

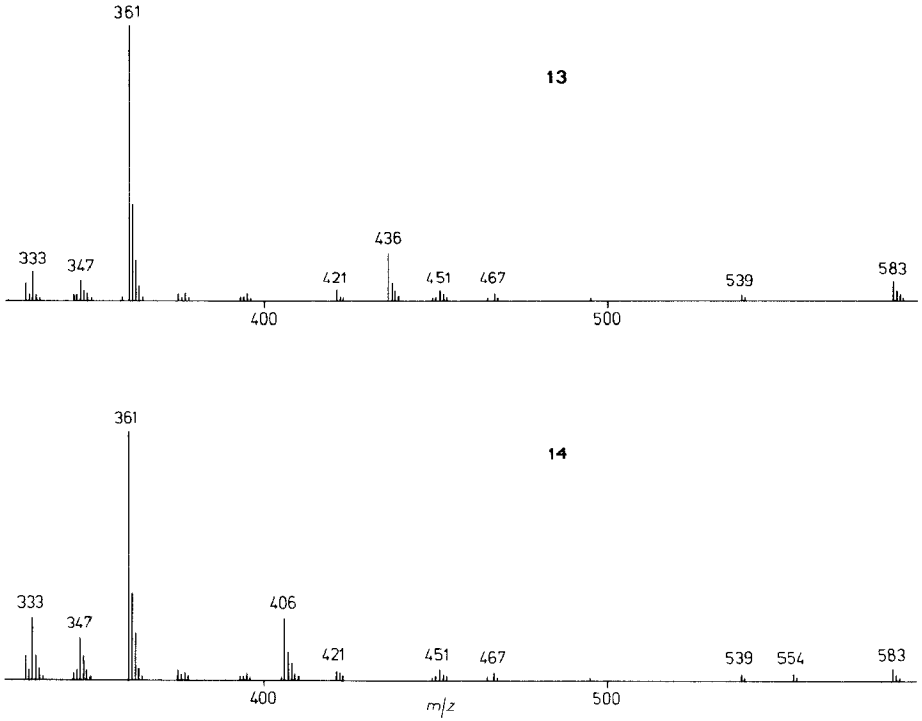


Fig. 2. Partial mass spectra of per(trimethylsilyl) derivatives of 2-*C*-carboxy-3-*O*-β-D-glucopyranosyl-D-pentoses (peaks *13* and *14* in Fig. 1). [The mass spectra of compounds *12* and *13* were analogous.]

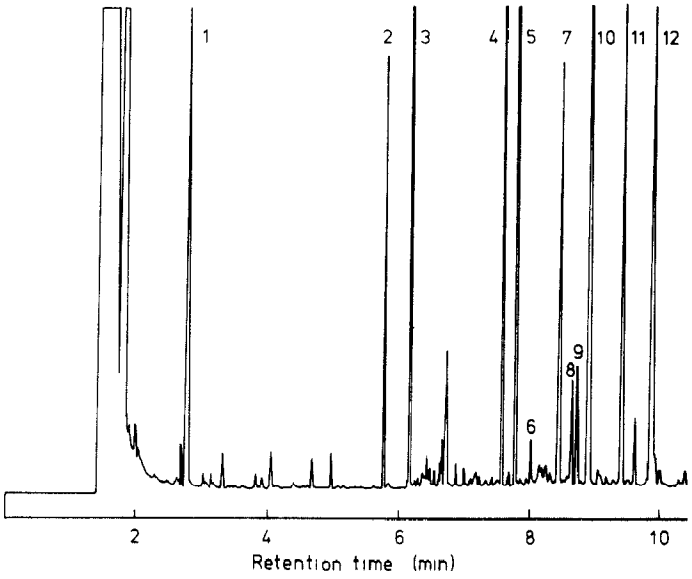


Fig. 3. Separation of the acidic reaction-products from 4-*O*-methyl-D-glucose (2.5 h at 40°) as their per(trimethylsilyl) derivatives on an OV-101 fused-silica capillary column. [Peaks: *1*, glycolic acid; *2*, 2-deoxytetronic acid; *3*, 2-*O*-methyltetronic acid; *4* and *5*, 3-*O*-methylpentonic acids; *6*, 2-*C*-carboxy-3-*O*-methylpentose (acyclic); *7* and *9*, 2-*C*-carboxy-3-*O*-methylpentofuranoses; *8* and *10*, 2-*C*-carboxy-3-*O*-methylpentopyranoses; and *11* and *12*, 4-*O*-methylhexonic acids.]

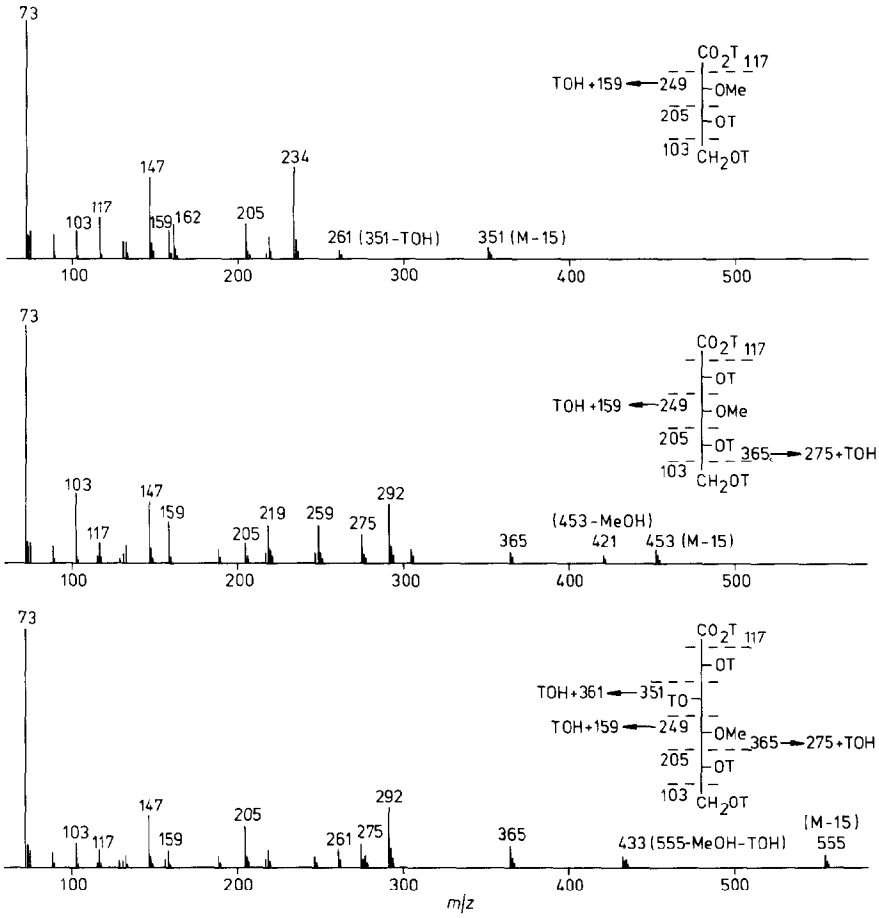


Fig. 4. Mass spectra of the per(trimethylsilyl) derivatives of 2-*O*-methyltetronic, 3-*O*-methylpentonic (peak *4* in Fig. 3), and 4-*O*-methylhexonic acids (*11*).

residue was degraded, probably to 2-furaldehyde (no peak appeared in the gas chromatogram in addition to D-glucose).

The monomeric reaction-products of the reducing D-glucose residue of the disaccharides consisted of formic, glycolic, 2-deoxytetronic, and 3-deoxypentonic acids. At relatively short reaction-times (*e.g.*, after 20% conversion of the disaccharides), four additional peaks were detected by g.l.c. (peaks *5–8* in Fig. 1). These compounds were, on ion-exchange, eluted in the same fraction as the carboxyglucosylpentoses. After reduction with sodium borohydride, they gave the isomeric 3-deoxy-2-*C*-(hydroxymethyl)pentonic acids (~60 and 40% of the *threo* and *erythro* forms, respectively), as determined by g.l.c.–m.s.[17,18]. Compounds in peaks *5–8* were, consequently, regarded as isomers of the corresponding 2-*C*-carboxy-3-deoxypentoses. The nature of the compounds causing peaks *5–8* is

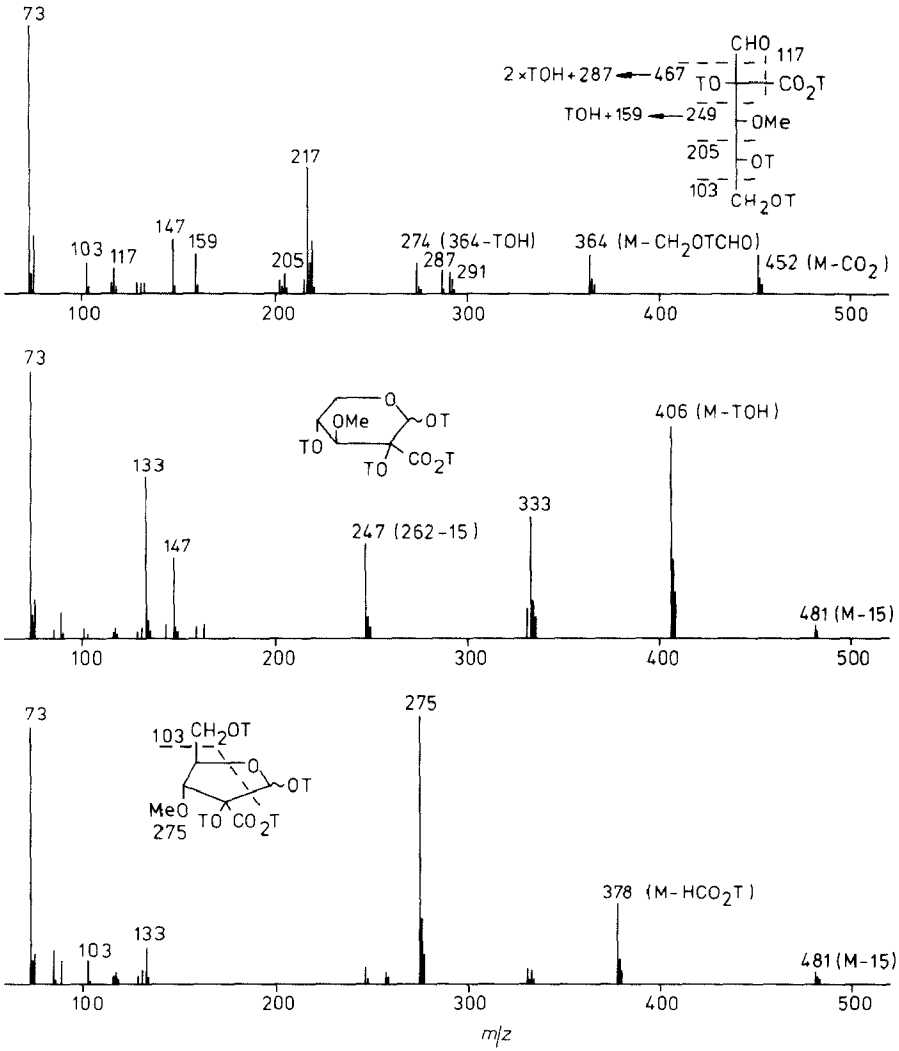


Fig. 5. Mass spectra of the per(trimethylsilyl) derivatives of acyclic, pyranoid (peak *8* in Fig. 3), and furanoid (*9*) forms of 2-*C*-carboxy-3-*O*-methylpentose.

discussed, on the basis of their mass spectra, in a later paragraph.

*Identification of the reaction products from 4-O-methyl-*D*-glucose.* — As determined by g.l.c., the aldonic acids in the products of oxidation of 4-*O*-methyl-D-glucose consisted of a 2-*O*-methyltetronic acid, a pair of 3-*O*-methylpentonic acids, and a pair of 4-*O*-methylhexonic acids, in addition to a smaller proportion of a 2-deoxytetronic acid (see Fig. 3). Although the absolute configurations of the *O*-methyl-substituted acids were not determined, they undoubtedly consisted of the *erythro*, *arabino*, *ribo*, *gluco*, and *manno* forms. In the mass spectra of the per(trimethylsilyl)ated, *O*-methyl-substituted aldonic acids, the most characteristic

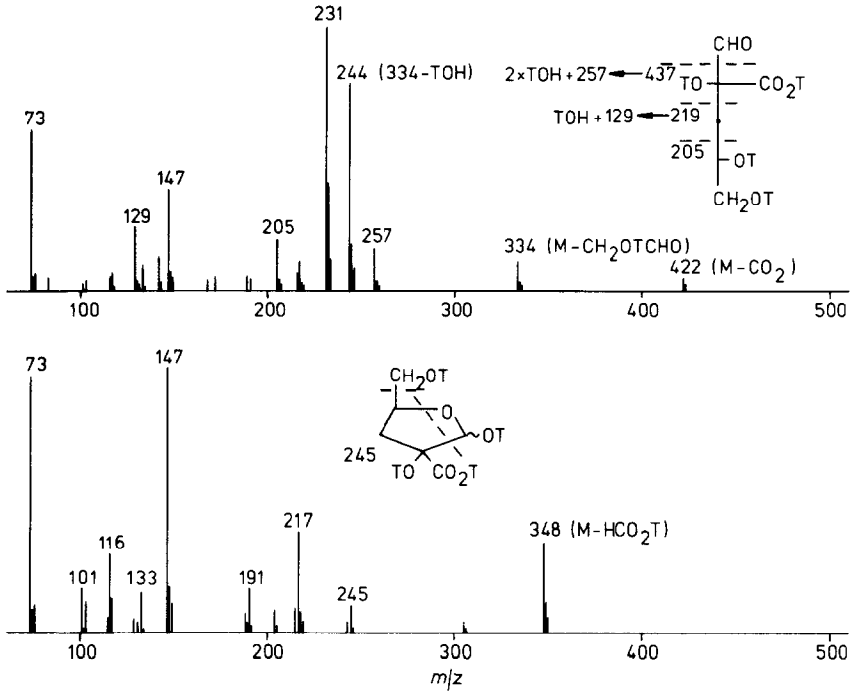


Fig. 6. Mass spectra of the per(trimethylsilyl) derivatives of acyclic (peak *8* in Fig. 1) and furanoid (*5*) forms of 2-*C*-carboxy-3-deoxypentose.

fragments were formed either *via* a direct chain-cleavage, which in some cases was accompanied by elimination of trimethylsilanol, or *via* a McLafferty (*m/z* 162) or a related rearrangement (*m/z* 234 and 292)[19,20] (see Fig. 4).

In addition to the aldonic acids, an acyclic (<1% of the total), two furanoid (24%), and two pyranoid forms (75%) of 2-*C*-carboxy-3-*O*-methylpentoses were identified, among the oxidation products, by g.l.c.–m.s. Typical of the mass spectrum of the acyclic form was elimination of carbon dioxide *via* a McLafferty type of rearrangement (*m/z* 452), in addition to the general fragmentation *via* chain cleavage and elimination of trimethylsilanol and methanol (see Fig. 5). The peaks at *m/z* 364 and 274 were obviously formed after successive eliminations of *O*-(trimethylsilyl)glycolaldehyde and trimethylsilanol *via* a rearrangement similar to that observed in the m.s. of per(trimethylsilyl)ated 2-deoxyaldonic acids[19].

Consistent with the general fragmentation-pattern of furanoses and pyranoses[21], the per(trimethylsilyl)ated 2-*C*-carboxy-3-*O*-methylpento-furanoses and -pyranoses gave significant peaks at *m/z* 275 and at *m/z* 333, 247 (262 − 15), and 133, respectively. Characteristic for the furanoses and pyranoses were also the fragments formed from the molecular ion *via* elimination of trimethylsilyl formate (*m/z* 378) and trimethylsilanol (*m/z* 406), respectively.

The peaks of the 2-*C*-carboxy-3-deoxypentoses in the gas–liquid chromato-

gram (*cf.*, Fig. 1) were assigned to the acyclic and furanoid forms, because of the features of their mass spectra common to those of the 2-*C*-carboxy-3-*O*-methylpentoses (see Fig. 6). Very indicative in this respect were considered the fragments formed after elimination of carbon dioxide (*m/z* 422, acyclic form), *O*-(trimethylsilyl)glycoaldehyde and trimethylsilanol (*m/z* 334 and 244, acyclic form), and trimethylsilyl formate (*m/z* 348, furanoid form). The proportion of the acyclic forms was ~30% of the total.

EXPERIMENTAL

Materials. — 4-*O*-Methyl-D-glucose was obtained from methyl 4-*O*-methyl-β-D-glucopyranoside (kindly supplied by Mr. Matti Stén, Enso-Gutzeit Osakeyhtiö, Research Centre) after hydrolysis with M hydrochloric acid at 100°. The purity of the product was >95%, as determined by g.l.c.

Performance of the oxidations. — The de-aerated solution of AMS (0.1–20mM) in sodium hydroxide (0.1M) was heated to the reaction temperature (40°), and the reaction was started by adding the sugar, in a small volume of water, under a nitrogen atmosphere. The initial concentration of the sugar ranged from 0.1 to 20mM, but the molar ratio of AMS and the converted sugar was always 4–12. After a selected degree of conversion of the sugar (usually 0.2; the rate constants for the disappearance of cellobiose and maltose were $2.6 \cdot 10^{-4}$ and $3.1 \cdot 10^{-4}$ s^{-1}, respectively; the reaction times ranged from 10–240 min), the solution was cooled, and the acidic reaction-products were separated by ion-exchange[1]. The consumption[12] of AMS, and the ratio of oxidation and isomerization rates[1], were determined as described earlier.

Fractionation of the acidic reaction-products from cellobiose. — A mixture of the acidic reaction-products (50 mg in 0.5 mL) was eluted with 0.5M formic acid through a column (10 mm i.d. × 130 cm) of Dowex-1 X1 (50–100 mesh) resin in formate form. The flow rate of the eluant was 80 mL/h. The acids in the eluate were detected with a refractometer having a flow-through cell, and analyzed by g.l.c. The elution volumes of the aldonic and deoxyaldonic acids and carboxy sugars were ~70–200 and 200–300 mL, respectively.

Reduction and hydrolysis of the carboxyaldoses. — A fraction of pure carboxyaldoses (~10 mg) was treated overnight with an excess of sodium borohydride (20 mg) in 0.01M sodium hydroxide (1 mL). The treatment was repeated, and then the sodium ions were removed by means of Dowex 50 W (H^+), a cation exchanger. To remove the boric acid liberated, methanol was several times added to, and evaporated from the residue. The 2-*C*-carboxy-3-deoxypentoses were entirely reduced to the 3-deoxy-2-*C*-(hydroxymethyl)pentonic acids, whereas the conversion of the 2-*C*-carboxy-*O*-D-glucosylpentoses remained incomplete. The mixture of the reduction products was further treated with M hydrochloric acid for 4 h at 100°, in order to hydrolyze the dimeric reduction-products. The hydrochloric acid was removed by evaporation under diminished pressure.

Gas–liquid chromatography and mass spectrometry. — After their conversion into ammonium salts[22], the acidic reaction-products were per(trimethylsilyl)ated, and the products analyzed by g.l.c. in an OV-101 fused-silica capillary column (0.35 mm i.d. × 25 m). The oven-temperature program was 2 min at 100°, 20°/min to 200°, and 4 min at 200° (4-*O*-methyl-D-glucose) or 2 min at 100°, 20°/min to 250°, and 10 min at 250° (cellobiose and maltose). The injection port and manifold were kept at 280°. The flow rate of the carrier (hydrogen) was 2 mL/min. Due to the incomplete separation of maltobionic and 4-*O*-α-D-glucopyranosyl-D-mannonic acids, these were analyzed as their per(trimethylsilyl)ated lactones on[13] OV-1701.

The mass spectra were recorded with a Hewlett–Packard quadrupole mass spectrometer (70 eV), equipped with an OV-101 fused-silica capillary column.

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