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NON-OXIDATIVE AND OXIDATIVE DEGRADATION OF D-GALACTURONIC ACID WITH ALKALI

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ABSTRACT

Degradation products from non-oxidative and oxidative treatment of D-galacturonic acid with alkali have been analysed by g.l.c.–m.s.; 13 hydroxy monocarboxylic acids and 26 dicarboxylic acids were identified in addition to several isomerisation products. In the absence of oxygen, the main degradation products were 3-deoxy-*lyxo*-hexaric, 3-deoxy-*xylo*-hexaric, malic, tartronic, *C*-methyltartronic, lactic, and 3-deoxytetronic acids. In the presence of oxygen, arabinaric, threaric, malic, tartronic, and glycolic acids were formed in large proportions. The routes of formation for most of the compounds are outlined.

INTRODUCTION

As part of our studies on the non-oxidative and oxidative degradation of wood polysaccharides with alkali, we have investigated the behaviour of pectic substances that are minor constituents of wood carbohydrates. In addition to other reaction routes, pectic acid is probably extensively degraded by alkali, and D-galacturonic acid residues are liberated. Indicative of this type of degradation is the presence of traces of 3-deoxyhexaric acids among the products after treatment of pectic acid with alkali[1]. We now report on the degradation of D-galacturonic acid by alkali in the absence and presence of oxygen. D-Galacturonic acid is known to isomerise under hot neutral[2,3] and mild alkaline[4,5] conditions, but little is known about its degradation under more drastic conditions.

EXPERIMENTAL

Degradation of D-*galacturonic acid.* — Samples (50 mg) of the acid were each treated with 0.5M sodium hydroxide (20 mL) under nitrogen or oxygen (0.4 MPa) at 80° for 3 h and at room temperature in the absence (5 days) or presence of oxygen (1 or 4 days). The resulting non-volatile carboxylic acids were trimethylsilylated as their ammonium salts and analysed by g.l.c.–m.s.[6]. An SE-54 fused-silica capillary column (0.32 mm i.d. × 25 m) was employed to improve the separa-

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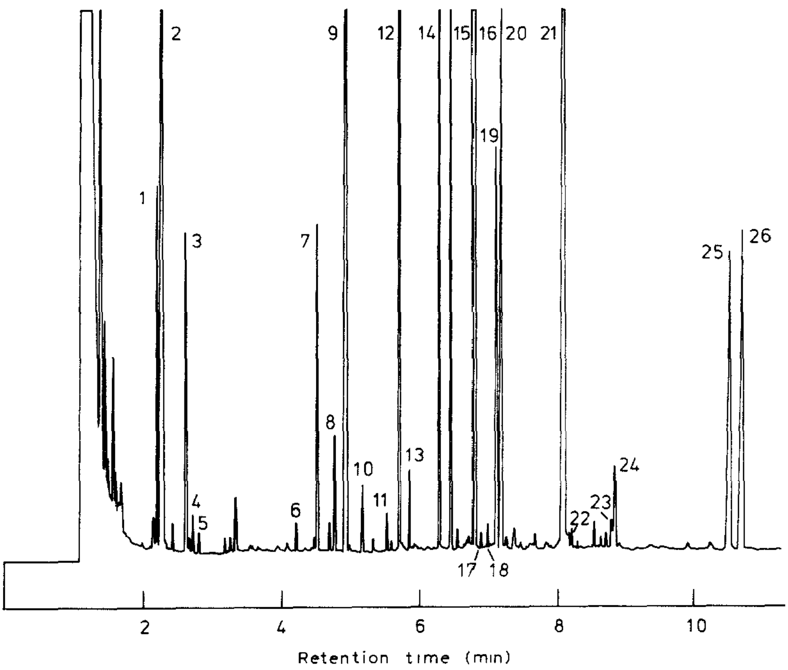


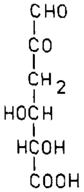
Fig. 1. Separation (OV-101 fused-silica capillary column) of the per(trimethylsilylated) carboxylic acids after treatment of D-galacturonic acid with oxygen–alkali at 80°: 1, lactic; 2, glycolic; 3, oxalic; 4, 2-hydroxybutanoic; 5, 3-hydroxypropanoic; 6, succinic; 7, glyceric; 8, *C*-methyltartronic; 9, tartronic; 10, 3-deoxytetronic; 11, dihydroxymalonic; 12, malic; 13, 3,4-dideoxypentonic; 14, *C*-(hydroxymethyl)tartronic; 15, erythraric; 16, threaric; 17, 3-deoxy-*erythro*-pentonic; 18, 3-deoxy-*threo*-pentonic; 19, 2-deoxy-*erythro*-pentaric; 20, 2-deoxy-*threo*-pentaric; 21, arabinaric; 22, xylaric; 23, 3-deoxy-*xylo*-hexaric; 24, 3-deoxy-*lyxo*-hexaric; 25, altraric; and 26, galactaric acids.

tion of some compounds, *e.g.*, the derivatives of *C*-(hydroxymethyl)tartronic acid and 2,3-dideoxypentaric acid.

Identification of the degradation products. — Most of the acids have been identified during the previous studies[1,6]. For the identification of diastereomeric forms of tetronic and aldaric acids, the corresponding retention-time data[7] were used. Identification of *C*-(hydroxymethyl)tartronic acid was based on the published mass spectrum[8], and the identity of dihydroxymalonic acid as the tetrakis(trimethylsilyl) derivative was confirmed by comparison with a commercial sample; *m/z* 409 (10%), 381 (8), 307 (M − 117, 100), 221 (12), 147 (30), 73 (65). The simplicity of its spectrum can be explained by the lack of carbon–hydrogen bonds[9]. The mass spectrum of *C*-(3-hydroxypropyl)tartronic acid will be discussed elsewhere. G.l.c. of the derivatised carboxylic acids on an OV-101 column is shown in Fig. 1.

RESULTS AND DISCUSSION

On the basis of the reaction products (Tables I and II), it can be assumed that the degradation routes of galacturonic acid are mainly analogous to those of hexoses[10]. The following intermediates are formed: 3-deoxy-*threo*-2-hexulosuronic acid (**1**) by β-hydroxy elimination at C-3, 5-hexulosonic acids (**2**) and 4-hexulosonic acids (**3**) by isomerisation, 3-deoxy-4,5-hexodiulosonic acid (**4**) by β-hydroxy elimination of **2**, and 4-deoxy-3-hexulosuronic acids (**5**) by isomerisation of **4**, tetruronic acids (**6**) and glycolaldehyde (**7**) by reverse aldol condensation of galacturonic acid, tartronaldehydic acid (**8**) and dihydroxyacetone (**9**) by reverse aldol condensation of **2**, 4-pentulosonic acids (**10**) and formaldehyde by reverse aldol condensation of **3**. The composition of the respective reaction mixtures varied, depending on whether oxygen was absent or present.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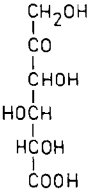
1


$$\begin{array}{c}\mathrm{CH_2OH}\\ |\\ \mathrm{CO}\\ |\\ \mathrm{CHOH}\\ |\\ \mathrm{HOCH}\\ |\\ \mathrm{HCOH}\\ |\\ \mathrm{COOH}\end{array}$$

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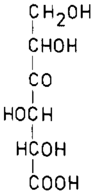
$$\begin{array}{c}\mathrm{CH_2OH}\\ |\\ \mathrm{CHOH}\\ |\\ \mathrm{CO}\\ |\\ \mathrm{HOCH}\\ |\\ \mathrm{HCOH}\\ |\\ \mathrm{COOH}\end{array}$$

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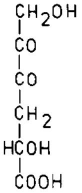
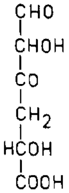

$$\begin{array}{c}\mathrm{CH_2OH}\\ |\\ \mathrm{CO}\\ |\\ \mathrm{CO}\\ |\\ \mathrm{CH_2}\\ |\\ \mathrm{HCOH}\\ |\\ \mathrm{COOH}\end{array}$$

4


$$\begin{array}{c}\mathrm{CHO}\\ |\\ \mathrm{CHOH}\\ |\\ \mathrm{CO}\\ |\\ \mathrm{CH_2}\\ |\\ \mathrm{HCOH}\\ |\\ \mathrm{COOH}\end{array}$$

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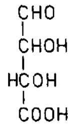
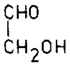
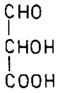

$$\begin{array}{c}\mathrm{CHO}\\ |\\ \mathrm{CHOH}\\ |\\ \mathrm{HCOH}\\ |\\ \mathrm{COOH}\end{array}$$

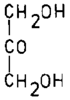
6


$$\begin{array}{c}\mathrm{CHO}\\ |\\ \mathrm{CH_2OH}\end{array}$$

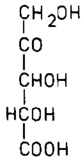
7


$$\begin{array}{c}\mathrm{CHO}\\ |\\ \mathrm{CHOH}\\ |\\ \mathrm{COOH}\end{array}$$

8


$$\begin{array}{c}\mathrm{CH_2OH}\\ |\\ \mathrm{CO}\\ |\\ \mathrm{CH_2OH}\end{array}$$

9


$$\begin{array}{c}\mathrm{CH_2OH}\\ |\\ \mathrm{CO}\\ |\\ \mathrm{CHOH}\\ |\\ \mathrm{HCOH}\\ |\\ \mathrm{COOH}\end{array}$$

10

In the absence of oxygen, about half of the compounds identified were dicarboxylic acids (Table I). The formation of 3-deoxy-*xylo*-hexaric and 3-deoxy-*lyxo*-hexaric ("galactometasaccharinaric") acids can be explained by the benzilic acid rearrangement of **1**. Surprisingly, the *lyxo* form preponderated, in contrast to the formation of 3-deoxy-*xylo*-hexonic acid from galactose[11].

3-Deoxy-2-*C*-hydroxymethylpentaric acid is formed mainly from **4** by benzilic acid rearrangement[12]. Although this acid (and especially its *threo* form) is the main alkaline degradation product of alginates[12], 4-*O*-methylglucuronic acid[13], and pectic acid[1], only small proportions (and only the *threo* form) were formed from galacturonic acid. The route to anhydroisosaccharinaric (1^1,4-anhydro-3-deoxy-2-*C*-(hydroxymethyl)pentaric acids from **4** has been described[1]. No acids of this type seem to have been detected after treatment of unsubstituted monosaccharides or their derivatives with alkali.

Hydrolysis of **5** during the formation of glycolaldehyde explains the formation of appreciable amounts of malic acid, but it is probable that some of this acid is

TABLE I

DICARBOXYLIC ACIDS FORMED[a] ON TREATMENT OF D-GALACTURONIC ACID WITH ALKALI

Dicarboxylic acid	*N_2*		*O_2*		
	80°, 3 h	*20°, 5 days*	*80°, 3 h*	*20°, 1 day*	*20°, 4 days*
Oxalic	0.3	0.4	1.3	4.6	6.4
Malonic	0.1		+[b]		
Succinic	0.4	0.4	0.2		0.1
C-Methyltartronic	4.7	2.5	0.4	0.4	0.1
Tartronic	2.0	4.6	14.4	7.5	25.6
Dihydroxymalonic			0.2	0.1	
2-Deoxy-3-*C*-methyltetraric	1.0	0.6			
Malic (deoxytetraric)	7.8	3.8	6.7	5.8	7.2
2,3-Dideoxypentaric	0.7	0.5		0.3	0.3
C-(Hydroxymethyl)tartronic				1.8	3.5
Erythraric	1.0	0.5	2.4	3.2	3.5
Threaric	1.6	0.8	18.4	14.6	20.7
β-Anhydroisosaccharinaric	0.4				
α-Anhydroisosaccharinaric	0.3				
2-Deoxy-*erythro*-pentaric			1.6	0.9	3.3
2-Deoxy-*threo*-pentaric			3.7	1.9	8.4
3-Deoxy-*erythro*-pentaric	0.8	0.6	+	+	+
3-Deoxy-*threo*-pentaric	1.7	1.6	+	+	+
C-(3-Hydroxypropyl)tartronic	0.7	0.3	+		
Arabinaric	0.4	0.4	22.4	37.3	0.6
Xylaric			0.2		
3-Deoxy-2-*C*-hydroxymethyl-*threo*-pentaric	1.0	1.9			
3-Deoxy-*xylo*-hexaric	7.0	11.3	0.2	0.7	0.3
3-Deoxy-*lyxo*-hexaric	21.1	24.4	0.5	1.2	0.8
Altraric	0.1	0.2	2.6	4.2	2.2
Galactaric	0.1	+	3.0	2.9	0.7
Total	53.2	54.8	81.7	87.4	84.4

[a] Percentage of non-volatile carboxylic acids. [b] Trace.

formed also from **6** by β-hydroxy elimination and benzilic acid rearrangement. Loss of C-1 as formic acid from **5** gives rise to 3-deoxy-4-pentulosonic acid, from which 2,3-dideoxypentaric acid is formed by β-hydroxy elimination and benzilic acid rearrangement[14]. 2,3-Dideoxypentaric acid is a major product of the degradation of pectic acid[1] and alginates[15] with alkali.

3-Deoxypentaric acids are most likely formed from **10** after isomerisation by β-hydroxy elimination and benzilic acid rearrangement. The most probable route to *C*-methyltartronic acid is the isomerisation of **6** to 2-tetrulosonic acid followed by β-hydroxy elimination and benzilic acid rearrangement.

Two other branched-chain dicarboxylic acids were present in small proportions after the non-oxidative treatment. Of these, 2-deoxy-3-*C*-methyltetraric (citramalic) acid is formed in appreciable amounts during the treatment of pectic acid[1] and alginates[15] with alkali, whereas *C*-(3-hydroxypropyl)tartronic acid has

TABLE II

HYDROXY MONOCARBOXYLIC ACIDS FORMED[a] ON TREATMENT OF D-GALACTURONIC ACID WITH ALKALI

Hydroxy monocarboxylic acid	*N_2*		*O_2*		
	80°, 3 h	*20°, 5 days*	*80°, 3 h*	*20°, 1 day*	*20°, 4 days*
Lactic	24.1	16.0	2.5	2.0	2.3
Glycolic	2.5	2.2	12.6	6.7	10.1
2-Hydroxybutanoic	0.3	0.1	0.2		
3-Hydroxypropanoic	0.1	+[b]	0.1		
2-*C*-Methylglyceric	2.2	3.4	+	0.1	+
Glyceric	0.4	0.7	1.3	0.4	1.3
3-Deoxytetronic	7.8	9.4	0.2	0.2	0.3
2-Deoxytetronic	0.1	+			
3,4-Dideoxypentonic	0.1		0.3		
Erythronic	+	0.1			
Threonic	0.1	0.2			
3-Deoxy-*erythro*-pentonic	0.4	0.4	0.1		
3-Deoxy-*threo*-pentonic	0.6	0.5	0.2		
Total proportion (%)	39.0	33.0	17.5	9.4	14.0
Hexuronic and hexulosonic	2.0	8.7		2.7	
Unidentified	5.8	3.5	0.8	0.5	1.6

[a]Percentage of non-volatile carboxylic acids. [b]Trace.

been only tentatively identified after treatment of cellobiose[16] with oxygen–alkali and after treatment of hydrocellulose[17] with hot alkali. The final step in the formation of *C*-(3-hydroxypropyl)tartronic acid is a benzilic acid rearrangement of 4,5-dideoxy-2,3-hexodiulosonic acid, but the preceding sequence is obscure. The absence of 2-*C*-methylpentaric acids in the reaction mixtures indicates that no β-hydroxy elimination of **3** occurred.

In the presence of oxygen, the total amount of dicarboxylic acids was increased and marked changes in the pattern of composition occurred. The oxidative cleavage of dicarbonyl compounds is an important reaction in the treatment of carbohydrates[18,19] with oxygen–alkali, and such scissions are probably the main reason for the formation of dicarboxylic acids from galacturonic acid.

Arabinaric acid is formed by cleavage of D-*lyxo*-2-hexulosuronic acid in a manner analogous to the formation of arabinonic acid from glucose[20,21] and threonic acid from xylose[22]. The yield of arabinaric acid after treatment for 24 h was almost the same as that (40%) of D-lyxonic acid after treatment of D-galactose[23] with oxygen–alkali. After prolonged treatment, the arabinaric acid was almost completely degraded. The formation of altraric and galactaric acids can be explained by benzilic acid rearrangement of D-*lyxo*-2-hexulosuronic acid.

Large proportions of tartronic and tetraric acids were formed in all the oxygen–alkali experiments and it can be assumed that **6**, **8**, and **10** (after isomerisation) are important precursors in their formation. In the presence of oxygen, the cleavage of **1** to give 2-deoxypentaric acids was more rapid than benzilic acid rearrangement. Surprisingly, their proportions were highest after prolonged treatment.

Samuelson and co-workers[16,24–26] have shown that *C*-(hydroxymethyl)tartronic acid can be formed during treatment of various carbohydrates with oxygen–alkali, and it was also identified in the present work. It is most probably formed by benzilic acid rearrangement of 2,3-tetrodiulosonic acid, but the formation of this dicarbonyl compound cannot be explained. The detection of even small amounts of dihydroxymalonic acid (mesoxalic acid hydrate) was surprising, and no mechanism for its formation can be offered.

In the absence of oxygen, lactic and 3-deoxytetronic acids were the most abundant hydroxy monocarboxylic acids (Table II). By studying the decomposition of ^{14}C-labelled aldoses in alkali, Harris[27] concluded that recombination of C_2 fragments (**7**) contributed significantly to the formation of 3-deoxytetronic acid. Pyruvaldehyde, which is formed from **9** by isomerisation and β-hydroxy elimination, is an important precursor of lactic acid[11]. In the presence of oxygen, **7** was almost completely converted into glycolic acid, whereas the conversion of **9** into glyceric acid took place with lower yield. The formation of small proportions of 3-deoxypentonic acids can be explained by the recombination of **7** and glyceraldehyde.

Small proportions of several other acids were also detected, and their formation is also most probably the result of various recombinations of fragments of low molecular weight. According to de Wit[10], such reactions are responsible for the formation of 2-*C*-methylglyceric acid as a minor product of the treatment of hexoses with alkali and it was also formed from galacturonic acid. Of the other trace hydroxy monocarboxylic acids, 3-hydroxypropanoic, 2-deoxytetronic, and 3,4-dideoxypentonic acids have been identified after non-oxidative or oxidative treatment of glucose[20,21,28] or xylose[22] with alkali. The formation of 2-hydroxybutanoic acid is confusing, although very small proportions have been reported to be formed from pectic acid[1].

G.l.c.–m.s. revealed hexuronic and hexulosonic acids, especially after treatment with alkali under nitrogen, but they were not identified. However, the presence of 5-hexulosonic and 4-hexulosonic acids was confirmed.

ACKNOWLEDGMENTS

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