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The formation of novel saccharinic acids, 2-*C*-(2-hydroxyethyl)tetronic acids, by alkaline degradation of leucrose

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Abstract

Two novel saccharinic acids, 2-*C*-(2-hydroxyethyl)erythronic and 2-*C*-(2-hydroxyethyl)threonic acids, were identified as their per(trimethylsilyl) derivatives by GLC–MS after alkaline degradation of leucrose (5-*O*-α-D-glucopyranosyl-D-fructopyranose). The identifications were supported by converting the acids into two different types of 1,4-lactones, that is, 3-(1,2-dihydroxyethyl)-3-hydroxydihydro-2(3*H*)-furanone and 3,4-dihydroxy-3-(2-hydroxyethyl)dihydro-2(3*H*)-furanone. The novel acids appear to be highly characteristic alkaline degradation products of (1 → 5)-linked hexose disaccharides, although several other hydroxy acids were formed and identified as well. © 1996 Elsevier Science Ltd.

Keywords: Leucrose; Alkaline degradation; Saccharinic acids; 2-*C*-(2-Hydroxyethyl)tetronic acids; Mass spectra

1. Introduction

Alkaline decomposition reactions of 1-*O*-, 4-*O*-, and 3-*O*-substituted hexoses or hexose polysaccharides to saccharinic (2-*C*-methylpentonic), isosaccharinic (3-deoxy-2-*C*-hydroxymethylpentonic), and metasaccharinic (3-deoxyhexonic) acids, respectively, have been thoroughly studied and are now well understood [1–4]. These reactions and the corresponding characteristic end-products have been applied for linkage or linkage-sequencing studies of carbohydrates [5–7].

The alkaline degradation reactions of 2-*O*-, 5-*O*-, and 6-*O*-substituted hexoses have received significantly less attention. 2-*C*-Methylglyceric acid has been identified [8,9] as a characteristic product derived from the 6-*O*-substituted hexoses, but no typical products have yet been established for the 2-*O*- and 5-*O*-substituted hexoses. These are

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generally believed to be relatively stable under alkaline conditions [2], even though some model experiments have shown [10] that at least at elevated temperatures the 2-*O*-substituted hexoses can give some acidic degradation products. By using a (1 → 5)-substituted hexose disaccharide, leucrose, as a model compound we have now demonstrated its limited alkali-stability, and we have identified novel branched-chain saccharinic acids, 2-*C*-(2-hydroxyethyl)tetronic acids, as highly characteristic degradation products. These new acids have the structure previously assigned by Kiliani to his galactose-derived "parasaccharinic" acid [1], but no evidence has now been found on the formation of these new saccharinic acids from simple hexoses.

2. Experimental

Alkaline treatments.—Samples (1 g) of leucrose (5-*O*-α-D-glucopyranosyl-β-D-fructopyranose; see ref. [11] for a recent review) were treated for 2 h in rotating autoclaves at 100 °C with 100-mL solutions of aq alkali (0.1 and 1 M NaOH). The treatments were carried out both under air and N_2 atmospheres. Additional treatments were carried out with 0.015 M $Ca(OH)_2$ as above, but with a reaction time of 6 h.

For comparison, samples (1 g) of D-galactose were treated for 2 h at 100°C with 100 mL of 0.1 and 1 M NaOH under N_2.

Gas chromatographic analyses.—The non-volatile carboxylic acids (2-mL liquor samples) from the alkaline treatments of leucrose and D-galactose were converted into the corresponding ammonium salts and per(trimethylsilylated) exactly as previously described [12]. Xylitol (0.5 mg) was added as the internal standard. The derivatised samples were analysed with a Hewlett–Packard 5880 A gas chromatograph equipped with a flame-ionisation detector and an SE-54 fused-silica capillary column (0.32 mm i.d. × 25 m). The temperature program was 2 min at 100 °C, 14 °C/min to 265 °C, and 15 min at 265 °C. The temperatures of the injection port and the detector were 270 °C. The carrier gas was hydrogen at 2 mL/min.

One of the leucrose-derived reaction mixtures (1 M NaOH, N_2 atmosphere) was also analysed after converting the hydroxy acids into the corresponding lactones. Thus, the liquor sample (2 mL) was subjected to a cation-exchange resin [Dowex 50W-X8 (H^+)], dissolved in 2 M HCl, and concentrated, and the residue was trimethylsilylated. The derivatised sample was analysed by GLC as above. In addition, retention indices of the trimethylsilylated lactones were determined on an isothermal run at 160 °C [13], using unbranched hydrocarbons as reference.

Mass spectrometry.—Electron impact mass spectra (EIMS) were recorded at 70 eV with a JEOL JMS-DX303 instrument combined with a Hewlett–Packard 5790 A gas chromatograph and the above SE-54 column. The temperature program was similar to that used in GLC. The scanning range was 60 to 600 with a cycle time of 1 s, and the resolution was 500.

Most of the hydroxy carboxylic acids could be identified on the basis of our previous studies [14–17]. Mass spectrometric identification of the novel saccharinic acids and their lactones is described below.

3. Results and discussion

Mass spectrometric identifications.—The GLC investigations revealed the formation of several low molecular weight hydroxy acids from leucrose, most of which could readily be identified by mass spectrometry (Fig. 1). In addition to these well-established hydroxy acids, two new compounds (peaks 22 and 23 in Fig. 1) were found in moderate amounts, being eluted just before the glucoisosaccharinic acids. The identical mass spectra suggested the presence of diastereomeric compounds. Interpretation of these spectra was based on several known features of the mass spectra of trimethylsilylated polyhydroxy carboxylic acids [18].

The mass spectra of the trimethylsilyl derivatives of these acids (Fig. 2) show low-intensity ions at m/z 525 and 435. These obviously refer [18] to the $[M - 15]^+$ and $[M - 15 - 90]^+$ fragmentations, respectively, and suggest deoxyhexonic acids (mol wt of the trimethylsilyl derivative, 540). Elution of the new acids just before the glucoisosaccharinic acids is a clear indication [13] of a branched structure. Among the most structure-specific ions in the new mass spectra are those evidently derived from rearrangement fragmentations. The intense m/z 408 and 305 ions can refer [19,20] only

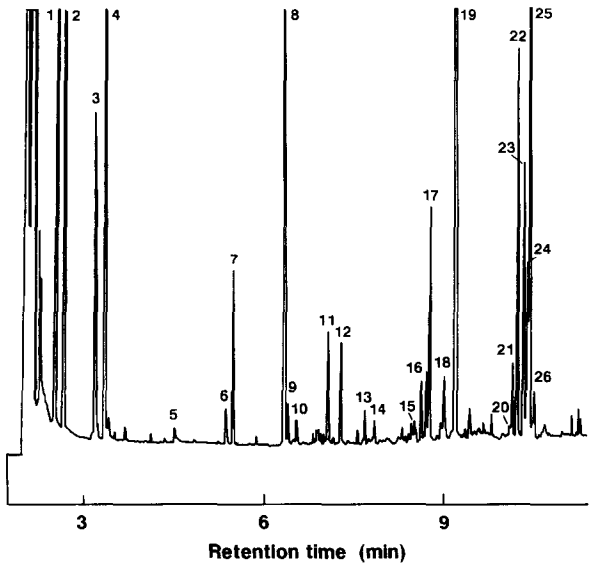

Fig. 1. Separation on an SE-54 fused-silica capillary column of the trimethylsilylated carboxylic acids obtained after treatment of leucrose with 1 M NaOH at 100 °C under air: 1, lactic; 2, glycolic; 3, 2-hydroxybutanoic; 4, 3-hydroxypropanoic; 5, 4-hydroxybutanoic; 6, 2-*C*-methylglyceric; 7, glyceric; 8, 3-deoxytetronic (2,4-dihydroxybutanoic); 9, 3-deoxy-2-*C*-methyltetronic (2,4-dihydroxy-2-methylbutanoic); 10, 2-deoxytetronic (3,4-dihydroxybutanoic) + 3,5-dideoxy-*threo*-pentonic; 11, unknown; 12, 3,4-dideoxypentonic; 13, erythronic; 14, threonic; 15, xyloisosaccharinic; 16, 3-deoxy-*erythro*-pentonic; 17, 3-deoxy-*threo*-pentonic; 18, a branched dideoxyhexonic; 19, xylitol (internal standard); 20, 2-*C*-methylarabinonic; 21, 2-*C*-methylribonic, 22, 2-*C*-(2-hydroxyethyl)threonic (β-parasaccharinic); 23, 2-*C*-(2-hydroxyethyl)erythronic (α-parasaccharinic); 24, 3-deoxy-*ribo*-hexonic (α-glucometasaccharinic); 25, β-glucoisosaccharinic + 3-deoxy-*arabino*-hexonic (β-glucometasaccharinic); and 26, α-glucoisosaccharinic acids.

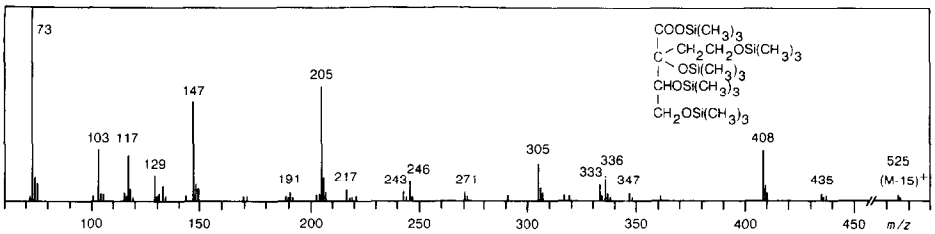

Fig. 2. The EIMS at 70 eV of the per(trimethylsilyl) derivative of 2-*C*-(2-hydroxyethyl)tetronic (parasaccharinic) acid.

to either 2-*C*-carboxy- or 2-*C*-(2-hydroxyethyl)-substituted α,β-dihydroxy acids. The former alternative, *C*-(1,2-dihydroxyethyl)tartronic acid, can readily be excluded as the spectrum [21] of this acid also exhibits an intense, characteristic [20,21] ion at m/z 393, and because it cannot be resolved into two diastereomeric peaks.

The above fragmentations thus strongly support the identification of the new hydroxy acids as isomeric 2-*C*-(2-hydroxyethyl)tetronic acids. Other intense ions which favour this conclusion include those at m/z 336, 246, and 205. The formation of the first two ions can be traced to a conventional McLafferty rearrangement, and they have m/z 220 and 130 ion counterparts in the mass spectra of the trimethylsilyl derivatives of non-substituted tetronic acids [18]. The intense m/z 205 ion, in turn, must be derived from the terminal dihydroxyethyl structure.

If this structure elucidation is correct, it should then be possible to convert the new hydroxy acids into a mixture of two diastereomeric forms of two different 1,4-lactones, 3-(1,2-dihydroxyethyl)-3-hydroxydihydro-2(3*H*)-furanone (lactone **1**) and 3,4-dihydroxy-3-(2-hydroxyethyl)dihydro-2(3*H*)-furanone (lactone **2**). After acidification, concentration, and trimethylsilylation, the four expected lactones were found and identified by GLC–MS as their tris(trimethylsilyl) ethers, confirming the above identification. The mass spectra of the trimethylsilyl ethers (Fig. 3) of the lactones **1** and **2** show a number of similarities, but also some highly structure-specific differences. The most striking feature of the MS of the trimethylsilylated lactone **1** is the intense m/z 246 ion. Its formation can be explained by a McLafferty-type rearrangement [22], resulting in the loss of the dihydroxyethyl side chain as $CHOCH_2OSi(CH_3)_3$. The similar fragmentation is not possible for the trimethylsilylated lactone **2**. In turn, this spectrum shows several intense ions also found in the MS of the bis(trimethylsilyl) ether of its parent compound, tetronic acid 1,4-lactone [23]. These ions especially include those at m/z 101, 103, and 116. For further comparisons, the mass spectra of the tris(trimethylsilyl) ethers of the 1,4-lactones of six-carbon saccharinic, metasaccharinic, and isosaccharinic acids can be consulted [24].

At the turn of the century, it was believed [1] that 2-*C*-(2-hydroxyethyl)tetronic acid(s) could be formed by alkaline degradation of galactose, but this was never fully substantiated. However, Kiliani introduced the name parasaccharinic acid, and for historical reasons we suggest that this name should now be retained and re-adopted. Following the analogy with glucoisosaccharinic acids, the *erythro* and *threo* isomers of

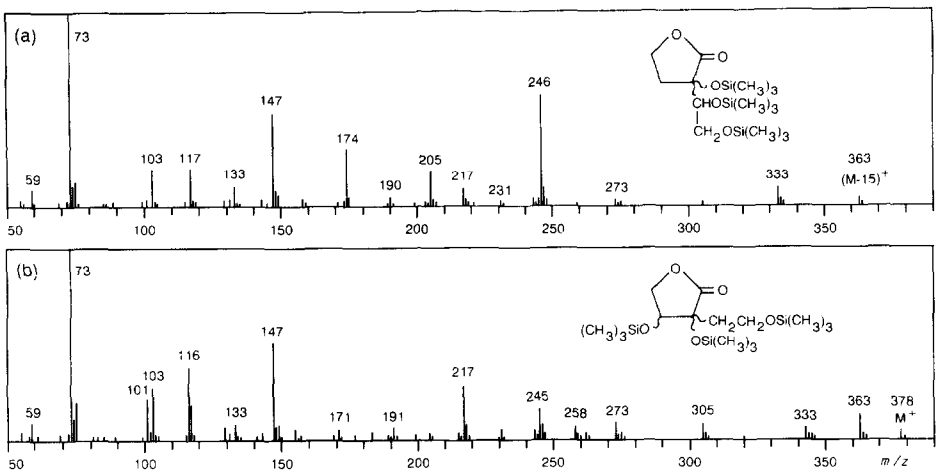

Fig. 3. The EIMS at 70 eV of the tris(trimethylsilyl) derivatives of (a) 3-(1,2-dihydroxyethyl)-3-hydroxydihydro-2(3*H*)-furanone (parasaccharinic acid lactone **1**) and (b) 3,4-dihydroxy-3-(2-hydroxyethyl)dihydro-2(3*H*)-furanone (parasaccharinic acid lactone **2**).

2-*C*-(2-hydroxyethyl)tetronic acids can be called α- and β-parasaccharinic acids, respectively.

Gas chromatography of the parasaccharinic acids.—The elution of the per(trimethylsilyl) derivatives of the parasaccharinic acids between 2-*C*-methylpentonic (saccharinic) and glucoisosaccharinic acids has already been noted. The elution order of the parasaccharinic acid isomers can be expected to be the same as that of isomeric glucoisosaccharinic acids [13] and 4-deoxy-2-*C*-methyltetronic (2,3-dihydroxy-2-methylbutanoic) acids [25], that is, the *threo* isomers appear earlier. This is further supported by the preferred formation of the *threo* isomer over the *erythro* isomer, characteristic of the formation of the *erythro–threo* pairs of hydroxy acids by alkaline degradation of carbohydrates [26].

The trimethylsilylated lactones of 2-*C*-substituted aldonic acids are eluted in the opposite order to the corresponding parent acids [13]. Taking this into account, the retention indices of the trimethylsilyl derivatives of all the six-carbon saccharinic acid lactones formed from leucrose could be compiled (Table 1, cf. ref. [13]). The retention indices show that the parasaccharinic acid lactones of type **1** were eluted before the glucoisosaccharinic acid lactones, and that the lactones of type **2** were clearly eluted later. The significant retention index differences between the *erythro* and *threo* forms of the parasaccharinic acid lactones are noteworthy.

Degradation of leucrose.—The formation of the parasaccharinic acids from leucrose can be envisaged as follows. Alkali-catalysed isomerisation of leucrose to the corresponding 5-*O*-α-D-glucopyranosyl-3-hexulose, followed by beta-alkoxy elimination, gives a 2-deoxy-3,4-hexodiulose intermediate. Finally, a benzilic acid-type rearrangement of this diketone produces the isomeric parasaccharinic acids. It is noteworthy that the diketonic intermediate is a DL isomer, and thus the final compounds also are racemic mixtures.

Table 1
Retention indices (RI) of the tris(trimethylsilyl) ethers of leucrose-derived saccharinic acid 1,4-lactones on SE-54 (isothermal run at 160 °C)

Systematic name for parent acid of lactone	Saccharinic acid name	RI
2-*C*-Methylarabinonic	β-Glucosaccharinic	1653
2-*C*-Methylribonic	α-Glucosaccharinic	1660
2-*C*-(2-Hydroxyethyl)erythronic (lactone **1**)	α-Parasaccharinic	1647
2-*C*-(2-Hydroxyethyl)threonic (lactone **1**)	β-Parasaccharinic	1702
2-*C*-(2-Hydroxyethyl)erythronic (lactone **2**)	α-Parasaccharinic	1711
2-*C*-(2-Hydroxyethyl)threonic (lactone **2**)	β-Parasaccharinic	1740
3-Deoxy-2-*C*-(hydroxymethyl)-*erythro*-pentonic	α-Glucoisosaccharinic	1706
3-Deoxy-2-*C*-(hydroxymethyl)-*threo*-pentonic	β-Glucoisosaccharinic	1718
3-Deoxy-*ribo*-hexonic	α-Glucometasaccharinic	1816
3-Deoxy-*arabino*-hexonic	β-Glucometasaccharinic	1801

Under the conditions used, several competing reactions also occur and the formation of the parasaccharinic acids is not very selective (Table 2). Especially in the presence of oxygen (air), oxidative cleavage of the 2-deoxy-3,4-hexodiulose intermediate to glyceric and 3-hydroxypropanoic acids (Fig. 1) can take place, but other reactions may also be possible. The eliminated glucose, in turn, is probably mainly responsible for the formation of 3-deoxytetronic, 3-deoxypentonic, glucosaccharinic, glucoisosaccharinic, and glucometasaccharinic acids (Fig. 1). There are probably several competing degradation reactions which can produce glycolic, lactic, and 2-hydroxybutanoic acids.

The ratio of the formation of the *threo-erythro* isomers seemed to be fairly constant (~ 1.3) during all the NaOH treatments.

As summarised by Sowden [1], at the turn of the century some evidence was given on the formation of "parasaccharinic" acid(s) by alkali-catalysed degradation of galactose. At that time, however, structure distinction between the isomeric saccharinic acids was usually based on their reduction products and was occasionally unreliable. Therefore, the real nature of Kiliani's parasaccharinic acid(s) remained questionable. Taking the mechanism outlined above into account, the formation of 2-*C*-(2-hydroxyethyl)tetronic acids from simple hexoses looks most unlikely, and has never been supported by modern investigations. Nevertheless, we now subjected D-galactose to alkaline treatments, but not even traces of the parasaccharinic acids could be detected in the resulting mixture of acids. It may thus be concluded that only 5-*O*-substituted hexoses can produce parasaccharinic acids.

Table 2
Yields (mg/g leucrose) of parasaccharinic acid isomers from alkaline treatments of leucrose

Isomer	0.1 M NaOH		1 M NaOH		0.015 M $Ca(OH)_2$	
	Air	N_2	Air	N_2	Air	N_2
α-Parasaccharinic acid	20	28	41	49	–	4
β-Parasaccharinic acid	27	36	53	62	–	6

All the treatments carried out in NaOH solutions also produced relatively high amounts of late-eluting dimeric hydroxy acids, *O*-glucopyranosylsaccharinic acids, or related compounds (elution not shown in Fig. 1). The structures of these acids have not yet been established, but most probably they include isomeric 3-deoxy-5-*O*-α-D-glucopyranosyl-D-hexonic and 3-deoxy-4-*O*-α-D-glucopyranosyl-2-*C*-(hydroxymethyl)-D-pentonic acids. In addition, the formation [27] of some 4-*O*-α-D-glucopyranosyl-D-arabinonic acid is likely during the treatments carried out under air atmosphere.

Conclusions.—To the best of our knowledge, the currently identified parasaccharinic acids represent the first hydroxyethyl-substituted aldonic acids (or corresponding lactones) found after alkaline degradation of any carbohydrates. However, an isomeric compound — a 2-*C*-(1-hydroxyethyl)tetronic acid — has been obtained [28] as a lactone by reduction of a rare naturally occurring monosaccharide, 3-*C*-carboxy-5-deoxy-L-xylose. Another type of carboxylic acid with a hydroxyethyl branch, *C*-(2-hydroxyethyl)tartronic acid, is a known product [29] of oxidative alkaline degradation of xylan.

In general, the 5-*O*-substituted hexose disaccharides or polysaccharides are quite uncommon in Nature, and apparently for that reason little attention has so far been paid to their alkaline degradation reactions. Even though it has now been shown that this type of disaccharide can produce highly characteristic degradation products, it remains to be established whether or not more selective conditions for their formation can be found. It also remains to be established how selectively (1 → 5)-linked hexose polysaccharides, such as galactocarolose, could be degraded to any saccharinic acids.

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