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Note

Evidence of benzilic rearrangement during the electrochemical oxidation of D-glucose to D-glucaric acid

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ABSTRACT

During the course of the 2,2,6,6-tetramethyl-1-piperidinyloxy free radical-catalyzed electrochemical oxidation of D-glucose to D-glucaric acid a new side-product was observed. This compound was isolated and identified as a tricarboxylic acid of unique structure, which was named maribersonic acid. Its structure was proven by different experiments coupled with several analytical methods, and its appearance during the electrochemical oxidation of D-glucose was rationalized through a thorough study.

In previous papers, we have described the preparative 2,2,6,6-tetramethyl-1-piperidinyloxy free radical (TEMPO) catalyzed chemical oxidation of D-glucose to D-glucaric acid. These oxidations, when performed with sodium hypochlorite or chlorine as an oxidant afforded high purity D-glucaric acid in yields up to 85%.[1–4] A thorough study of these reactions' side-products led us to a better understanding of the oxidations and degradation pathways occurring, along with the development of quick and efficient methodologies to analyze the complicated reaction mixtures often obtained during such oxidations.[5] Subsequently, we described the TEMPO-catalyzed electrochemical oxidation of D-glucose to D-glucaric acid. The optimized electrochemical oxidation of D-glucose had D-glucaric acid yields exceeding 90% with a small amount of degradation products.[6,7] However, although the outcome of TEMPO-mediated chemical oxidation of D-glucose has been well documented, we were rather puzzled by the appearance of a new side product during its TEMPO-mediated electrochemical oxidation.

This compound attracted our attention as it was unique in this oxidation and did not fit the 'profile' of the conventional side-products, which were mainly di-carboxylic acids, such as tartaric, tartronic, or oxalic acid.[5] Preliminary analyses led us to propose a tricarboxylic acid product resulting from a rearrangement rather than a degradation pathway. We present herein a study of this rearrangement and provide a rationale for the formation of this new tricarboxylic acid.

When D-glucose was electrochemically oxidized, and the reaction mixture analyzed by conventional methods, such as derivatization followed by gas chromatography (GC) (Fig. 1), ^{1}H NMR, or ^{13}C NMR spectroscopy, several results did not fit with those previously observed in the TEMPO-catalyzed chemical oxidation of D-glucose.[5] The appearance of new signals in the GC, and in the ^{1}H NMR and ^{13}C NMR spectra suggested the presence of a novel side-product. When the reaction mixture was persilylated (trimethylsilylated) and analyzed by GC–MS, a molecular mass of 656 g/mol was detected for this side-product, a difference of 14 mass units (mu) compared to persilylated D-glucaric acid. On the other hand, when the carboxylic acids were selectively methylated and the alcohol functions trimethylsilylated, the molecular mass observed dropped to 482 g/mol, a loss of 174 mu, consistent with the presence of an extra carboxylic acid in comparison with D-glucaric acid.

The ^{1}H NMR and COSY spectra in D_2O of the reaction mixture, showed the presence of two coupled doublets at 4.22 ppm and 4.71 ppm, with a coupling constant of 1.2 Hz, never observed in previous oxidation mixtures. ^{13}C NMR/DEPT experiments performed on the same mixture, showed the presence of six new carbons: three carbonyls from carboxylic acids (179.7, 176.9, and 176.8 ppm), a quaternary carbon at 84.7 ppm, and two tertiary carbons at 74.1 ppm and 73.2 ppm that an HMQC experiment showed to be correlated to the protons at 4.71 ppm and 4.22 ppm, respectively.

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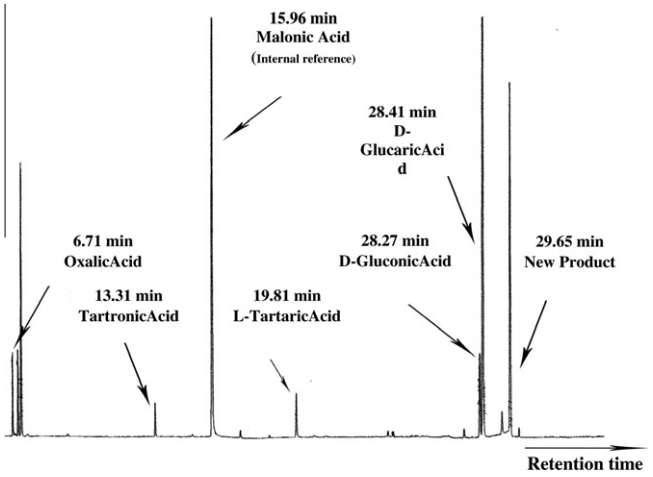
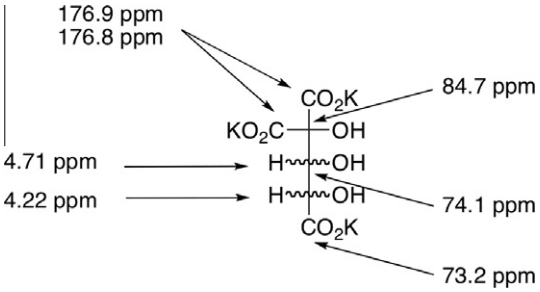


Figure 1. Gas chromatogram of the reaction mixture from the electrochemical oxidation of D-glucose (0.02 mol) and TEMPO (0.5 mmol) at an intensity of 600 mA and a pH of 11.8 (stopped after a transfer of 7.2 Faraday mol^{-1}: 20% excess).[7]

An HMBC experiment helped determine the position of the functional groups, showing a correlation between the carbons at 179.7 and 84.7 ppm and the two protons at 4.22 and 4.71 ppm, respectively. Finally, the unique correlation between the two remaining carbonyl carbons at 176.9 and 176.8 ppm and the only proton at 4.71 ppm led us to the tentative structure of this novel side-product shown below (Scheme 1).

While NMR and mass spectroscopic analyses enabled us to determine the overall structure of this triacid, the configuration at the stereogenic centers needed to be investigated. A thorough literature search revealed that no such compounds had been isolated or synthesized, with the closest related structures being D-hamamelose,[8,9] 2-(L-*threo*-1,2,3-trihydroxypropyl) tartronic acid,[10–13] D-isosaccharinic acid,[14] or 2,3-dihydroxypropyltartronic acid (Fig. 2).[15]

We focused our attention on structures resembling D-hamamelose and 2-(L-*threo*-1,2,3-trihydroxypropyl) tartronic acid for obvious reasons as the isosaccharinic derivatives lacked one secondary alcohol function. D-Hamamelose was first described by Freuderger et al. in 1924,[8] but was assigned the wrong structure. It was not until 1934 that Schmidt et al. described the correct structure,[9] which was later extensively studied, after it became available from tannin of witch hazel (*Hamamelis virginiana* L.).[16–19] To this day, very few syntheses of D-hamamelose have been described and most of them involved low yielding multistep syntheses.[20–22] 2-(L-*threo*-1,2,3-Trihydroxypropyl) tartronic acid, on the other hand, was prepared from the reaction of dehydroascorbic acid under alkaline conditions; however, no large scale analytical sample could be obtained for further experimentation.[10–13,23] We speculated that the observed triacid was a possible product of the D-hamamelose or 2-(L-*threo*-1,2,3-trihydroxypropyl) tartronic acid



Scheme 1. Tentative structure of the observed side-product.

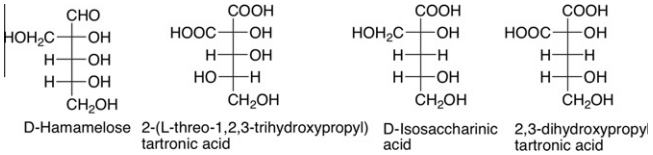


Figure 2. Related structures to the triacid present in the literature.

oxidation, and decided to investigate its isolation and mechanism of formation.

As the formation of such a triacid had never been observed during the chemical TEMPO-catalyzed oxidation of D-glucose, it was only logical to assume that such product was specific to the electrochemical oxidation process. We have observed that this triacid acid was formed only when at least 50% of D-glucaric acid had been formed, and after all the D-gluconic acid had been consumed,[7] while the electrochemical oxidation of the D-glucuronic acid yielded only a small amount of this triacid.

After a thorough literature search, the benzilic rearrangement seemed the plausible mechanism to explain the formation of this triacid, especially since geminal carboxylates were present in the final product.[10–13,23–27] D-Glucose, D-mannose, and D-galactose were oxidized, and GC analyses showed that D-glucose and D-galactose gave rise to the same triacid, while D-mannose, gave rise to a diastereoisomer (with a different GC retention time) showing that the C-2 and C-3 configurations of the starting hexoses were retained during the rearrangement. Oxidation of 3-methoxy-D-glucose gave rise to a methylated triacid (confirmed by NMR and mass spectrometry) further corroborating this hypothesis as the resulting C-3 configuration of the methylated triacid was retained. These results hinting at the fact that the postulated benzilic rearrangement is involving primarily the C-4, C-5, and C-6 centers of the oxidized hexose (Fig. 3). *It should be noted that one can also rationalize the same rearrangement involving the C-1, C-2, and C-3 centers, which will lead to a minor diastereoisomer.*

Subsequent oxidation of labeled D-glucose gave a better insight into the mechanism of formation of this triacid. When 1-^{13}C-D-glucose was oxidized, a major diastereoisomer of the triacid was formed, involving the C-4, C-5, and C-6 centers. The strong and characteristic signal at 179.4 ppm in the ^{13}C NMR spectrum is in accord with the carboxylic acid function at the C-1 position, while the signal at 177.1 ppm is attributed to the carboxylic acid of a minor diastereoisomer, involving the C-1, C-2, and C-3 centers (Fig. 4). The ^{1}H NMR spectrum afforded the heteronuclear coupling constants J_{1H-13C} between H-2 and C-1 (4.5 Hz), and H-3 and C-1 (1.5 Hz), along with the homonuclear coupling constant between H-1 and H-2 J_{H1-H2} (1.5 Hz) (Fig. 5).

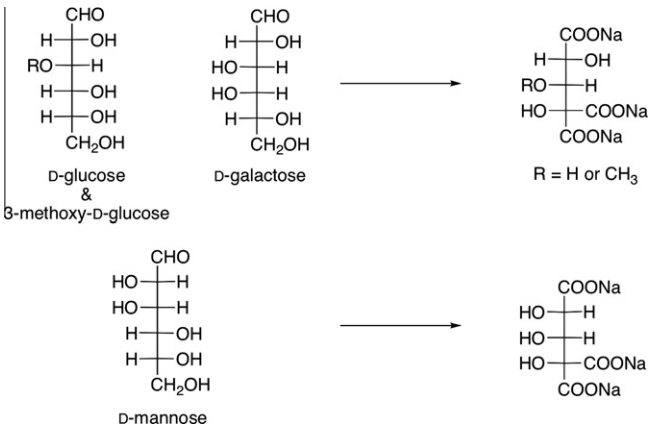


Figure 3. Outcome of the oxidation of D-glucose, D-mannose, and D-galactose.

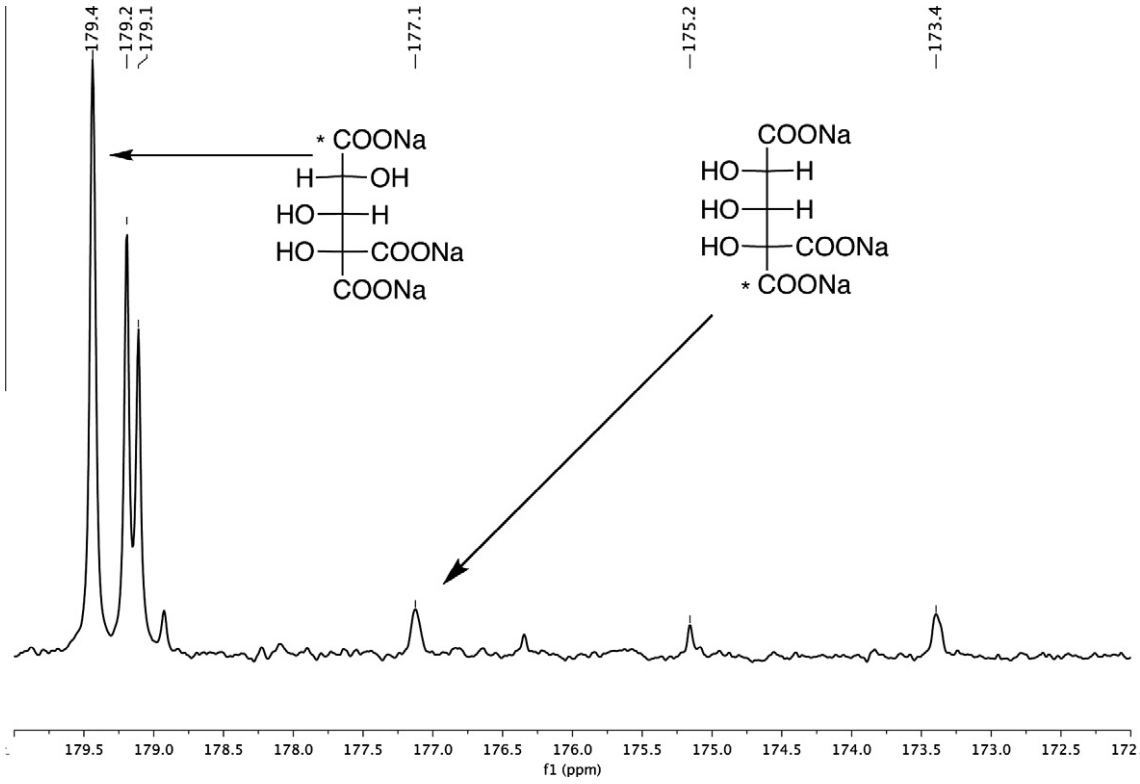


Figure 4. ^{13}C NMR spectrum of the 1-^{13}C-D-glucose oxidation mixture.

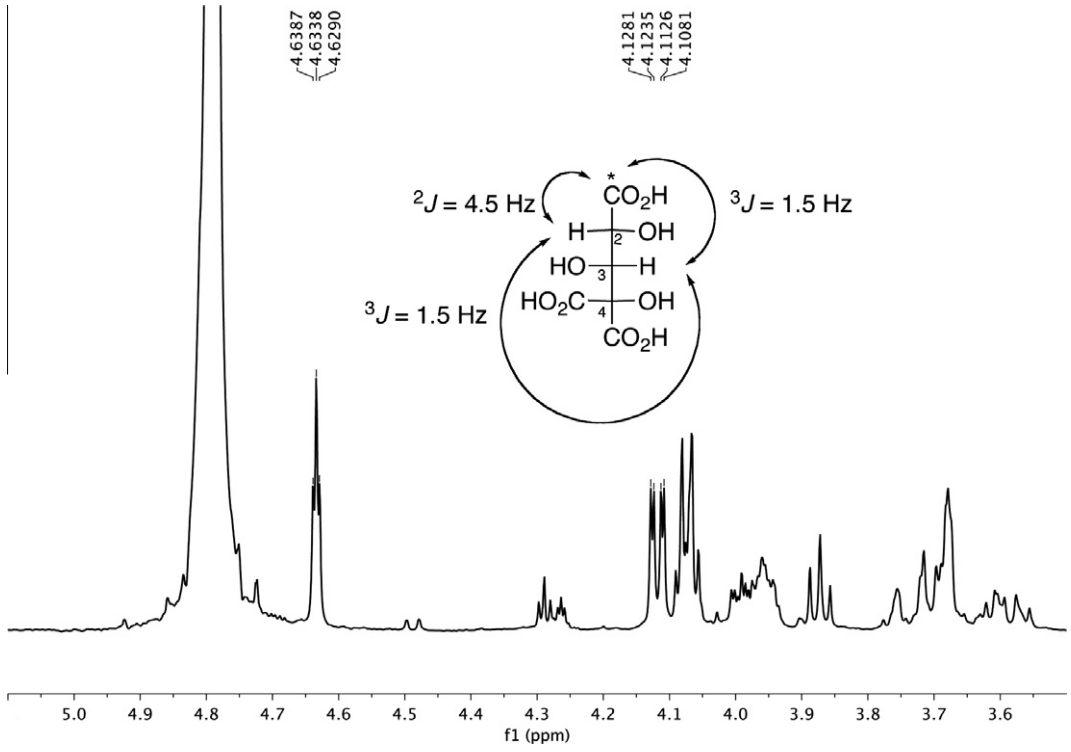


Figure 5. ^{1}H NMR spectrum of the 1-^{13}C-D-glucose oxidation mixture.

The electrochemical oxidation of 4-^{13}C-D-glucose, followed by detailed NMR analysis, showed the appearance of the strong signal at 84.7 ppm (quaternary carbon), along with the minor signal at 75.8 ppm, provided definitive evidence that the quaternary carbon of triacid acid was derived from the C-4 position of D-glucose (Fig. 6). This quaternary carbon chemical shift is in accordance with the observed chemical shift for the 2-(L-*threo*-1,2,3-trihydroxypropyl) tartronic acid around 86.7 and 85.36 ppm.[24]

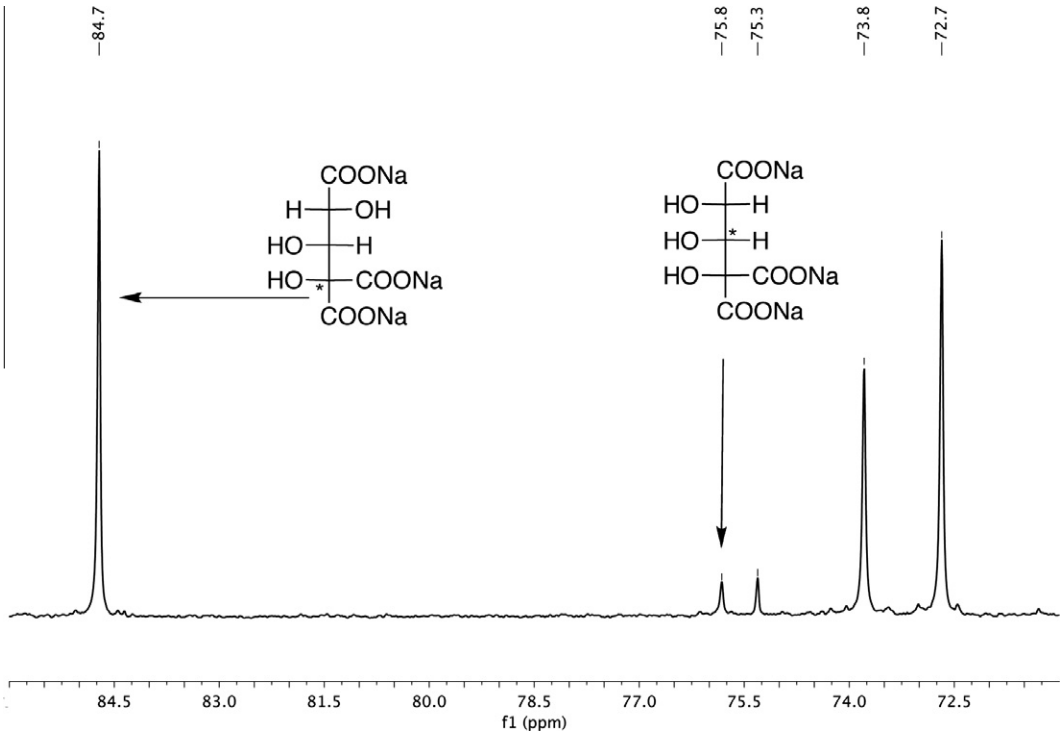


Figure 6. ^{13}C NMR of the 4-^{13}C-D-glucose oxidation mixture.

Finally, the spectrum of the product from the electrochemical oxidation of 6-^{13}C-D-glucose helped confirm the formation of the major triacid acid diastereoisomer, as a strong signal at 176.6 ppm, characteristic of the geminal carboxylic acids appeared (Fig. 7). The ^{1}H NMR spectrum provided further support for the structure of the major stereoisomer as a unique heteronuclear coupling constant J_{1H-13C} between the labeled ^{13}C-5 and H-3 was observed (2.2 Hz), and a 'longer range coupling constant' between ^{13}C-5 and H-2 was absent (Fig. 8). The homonuclear coupling constant (1.5 Hz) between H-2 and H-3 was the same as observed in Figure 5.

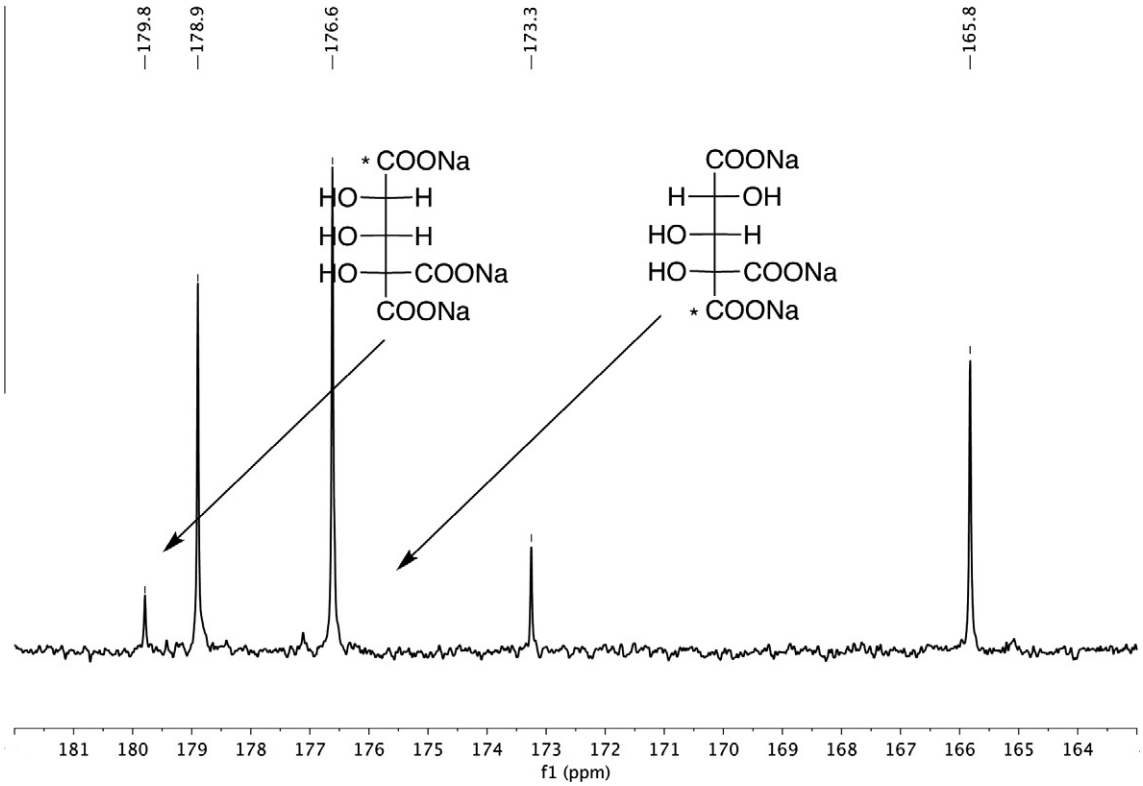


Figure 7. ^{13}C NMR spectrum of the 6-^{13}C-D-glucose oxidation mixture.

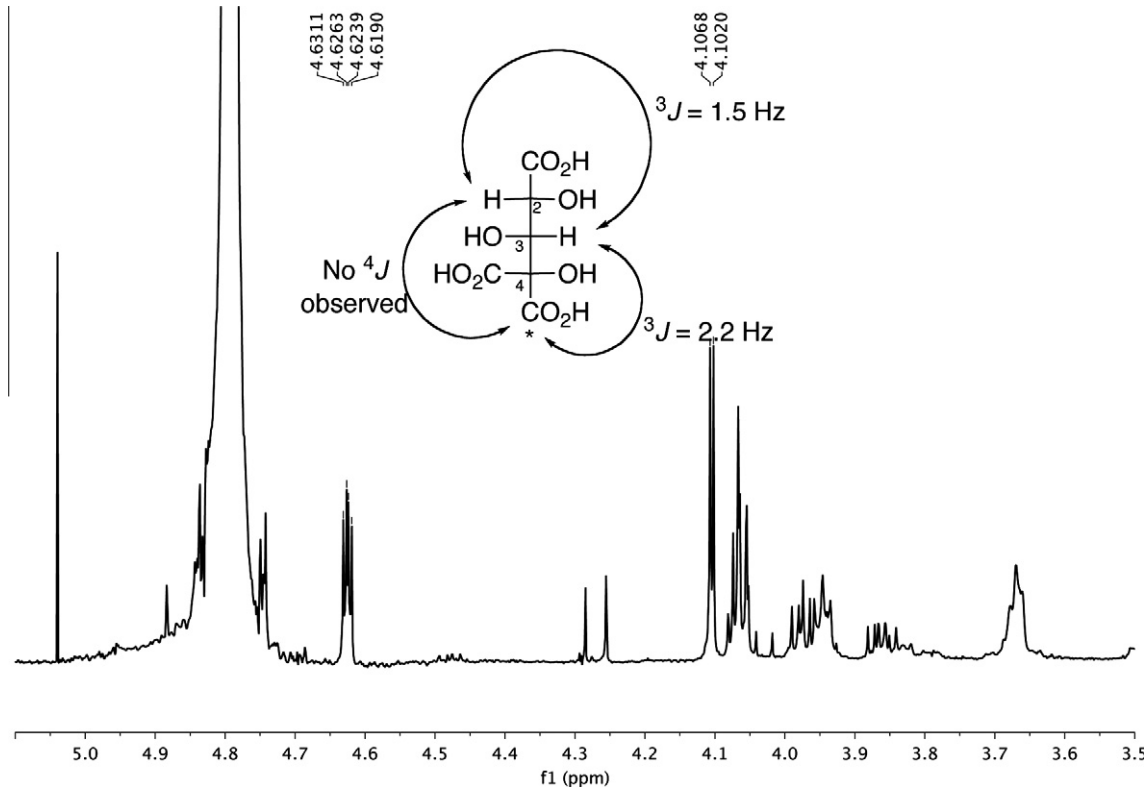
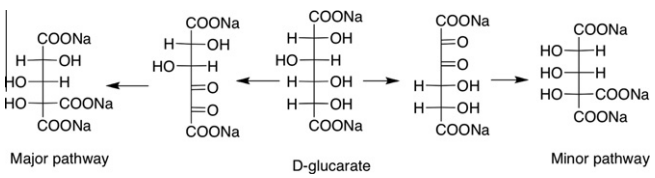


Figure 8. ^{1}H NMR spectrum of the 6-^{13}C-D-glucose oxidation mixture.

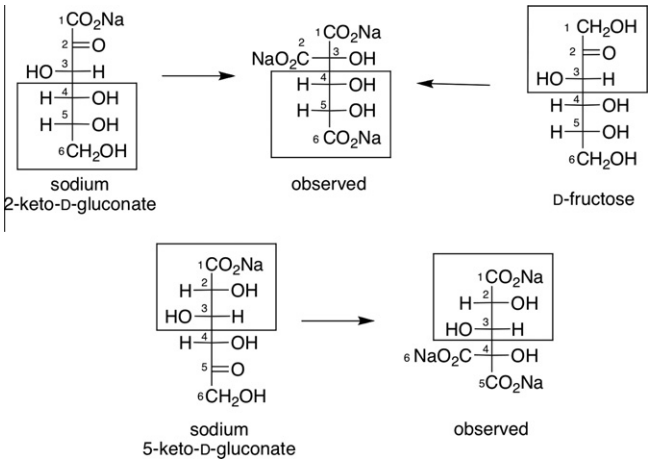
With these results in hands we could safely assume that the observed triacid was the result of a benzilic rearrangement at the C-4, C-5, and C-6 centers, occurring through the formation of a diketone by oxidation of the C-4 and C-5 (or C-2 and C-3 as minor pathway) secondary alcohols of D-glucaric acid (Scheme 2).

In order to test this hypothesis, we performed the electrochemical oxidation of 2-keto-D-gluconic acid and 5-keto-D-gluconic acid (intermediates in the proposed mechanism of over-oxidation of D-glucose), which should yield diastereoisomers of the triacid obtained from D-glucose. In the same manner we performed the electrochemical oxidation of D-fructose which should yield the same stereoisomer of the triacid obtained from 2-keto-D-gluconic acid or D-mannose, but the opposite diastereoisomer of the triacid was obtained from D-glucose. As shown in Scheme 3 the outcomes of these experiments are in accord with the retention of the C-2 and C-3 configurations when the over-oxidation occurs at the C-4–C-5 centers, and with the retention of the C-4 and C-5 configurations over-oxidation occurs at the C-2–C-3 centers.

Interestingly this result is in accordance with our previous findings that the over-oxidation of D-glucose occurs primarily at its C-4–C-5–C-6 end, as shown when we analyzed the outcome of the TEMPO-mediated chemical oxidation. The major cleavage observed was primarily at the C-4–C-5 bond due to over-oxidation.[5] Careful analysis of the diastereoisomers, revealed a ratio of 20:4



Scheme 2. Proposed over-oxidation pathways leading to the formation of the two diastereoisomers of the triacid.



Scheme 3. Outcome of the electrochemical oxidation of 2-, 5-keto-D-gluconic acid, and D-fructose.

in favor of the diastereoisomer resulting from the rearrangement at the C-4 and C-5 centers, in accord with the ratio of degradation side-products (85:15; L-tartaric acid/D-tartaric acid) observed during the TEMPO-catalyzed chemical oxidation of D-glucose.[3,5]

These observations led us to fully understand both chemical and electrochemical TEMPO-mediated oxidations of D-glucose. The electrochemical oxidation involved milder conditions leading to a rearrangement and much less degradation/cleavage products, while the chemical oxidation under harsher condition led to more degradation and no observed rearrangement. *The harsher conditions are due to the presence of oxidants other than the oxoammonium salts, such as sodium hypochlorite or hypobromite.*

In summary we have provided the evidence for the formation of a triacid in the electrochemical oxidation of D-glucose and other

monosaccharides, through benzilic rearrangement. The proposed mechanism accounts for the formation of all diastereoisomers in different monosaccharides series, along with the stereochemistry of the resulting compounds. GC analyses combined with NMR studies of the reaction mixtures established unambiguously the configuration of the stereogenic centers of the generated triacids.

1. Experimental

1.1. Classical electrolysis reactor

A control and an electrolysis device composed the reactor, which allowed a tight pH and temperature control during the electrolysis. A Micro-pump N21 (from Ismatec) allowed the circulation between the two parts and was calibrated to a flow rate of 80 mL min^{-1}. The temperature was controlled using a jacketed reactor equipped with a cryothermostat Ecoline RE107 from Lauda. The power generator was a SDL/PA-R from Sodilec. The pH was controlled by an Inlab 413 glass electrode connected to an automated ORSO Electronic System from Logilap™. The electrolysis time was programmed using a OTIO 93000-001 timer.[28]

1.2. Large scale Priam electrolysis reactor

Larger scale oxidations were achieved on a PRIAM 1-2C reactor of 3300 mL capacity, fitted with a graphite felt anode and two stainless steel cathodes, both anode and cathodes having a 190 cm^2 area and 5 mm thickness. A centrifugal pump MD-10-230GS01 (from Iwaki) allowed the circulation of the electrolyte between electrolysis and control devices with a calibrated flow rate of 11 L min^{-1}. Temperature, pH, and reaction time were controlled as for the classical electrolysis reactor.[28]

1.3. Oxidation general procedure[7]

D-Glucose (0.02 mol), TEMPO (0.5 mmol), and NaBr (0.02 mol, when needed) in water (MiliQ, 330 mL, 2 Mohm·cm) were electrolyzed at the desired scan rate with 20% faradic excess. Two electrodes composed the electrolysis system: a graphite felt anode (90 cm^2) and a stainless steel cathode (50 cm^2). The amount of current needed was calculated as the following: two electrons were required for the oxidation of the hemiacetal function, and four electrons for the primary alcohol function, topped with 20% excess of the oxidant to assure completion. At the end of the oxidation, the reaction was concentrated to 100 mL, under vacuum. The pH was adjusted to 8 by addition of Amberlyst resin (CA200, H^+). After removal of the resin by filtration, the remaining solvent was evaporated under vacuum, and the resulting product was dried under vacuum at 60 °C for 24 h.

1.3.1. Preparative oxidation of D-glucose to triacid under optimized conditions

Oxidative formation of triacid acid could be achieved by oxidation of D-glucose at a pH lower than 12, but better results were obtained using either a mixture of D-glucose and sodium D-gluconate or sodium glucarate itself due to better electrolyte conductivity.

1.3.2. Triacid synthesis from the oxidation of a mixture of D-glucose and sodium D-gluconate

D-Glucose (15 g, 75.8 mmol), sodium D-gluconate (8.5 g, 39 mmol) and TEMPO (0.3 g, 2 mmol) in water (3.3 L, 2 Mohm cm) were electrolyzed at pH 12.2 (adjustment with aqueous KOH 4 mol L^{-1}) at 5 °C with a 1000 mA intensity (190 cm^2 graphite felt anode—20% Faradic excess). A mixture containing D-gluconic acid (4%), glucaric acid (70%), and triacid acid (22%) as their potassium salts was obtained together with 4% degradation side-products.

1.3.3. Triacid synthesis from the oxidation of D-glucaric acid

Sodium D-glucarate (23.2 g, 81.2 mmol) and TEMPO (0.3 g, 2 mmol) in water (3.3 L, 2 Mohm cm) were electrolyzed at pH 12.2 (adjustment with aqueous KOH 4 mol L^{-1}) at 5 °C with a 1000 mA intensity (190 cm^2 graphite felt anode without faradic excess). A mixture containing D-glucaric acid (47%) and triacid acid (22%) as their potassium salts was obtained together with 27% of degradation side-products.

1.3.4. Oxidation of ^{13}C enriched D-glucose

^{13}C enriched glucose (100 mg), TEMPO (50 mg), and Na_2CO_3 (500 mg) in water (80 mL) were electrolyzed at a temperature of 5 °C with a 200 mA intensity (20% Faradic excess), to yield a mixture containing at least 90% of D-glucaric acid after work-up.

1.4. Purification of the triacid fraction

Purification of triacid was achieved in three steps: Concentration of the oxidation mixture and acidification to pH 8 afforded a mixture of the crude potassium salts of the different acids. Most of the D-glucaric acid was further removed by precipitation of its mono potassium salt by adjusting the pH to 3.8 by careful addition of a strong acid exchange H resin CA200. The mother-liquor (65% enriched in triacid) was subsequently passed over a cationic exchange column (PCR532H^+) eluted with water. The aqueous phase was dried in vacuo, to afford a diastereoisomeric mixture of triacid acid in a 8:2 ratio with a purity of 96% by GC.

1.5. Analytical analyses

All the GC analyses that were performed on the reaction mixtures were performed according to the procedure previously described by Ibert et al.[5] All ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker 300 MHz spectrometer in D_2O and the proton spectra referenced to the residual H_2O peak at 4.79 ppm.

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