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Note

Determination of the side-products formed during the nitroxide-mediated bleach oxidation of glucose to glucaric acid

Mathias Ibert,[a] Francis Marsais,[a,*] Nabyl Merbouh,[b] Christian Brückner[b,*]

[a] *UMR 1064-IRCOF-INSA de Rouen, BP08, F-76131 Mont Saint Aignan cedex, France*
[b] *Department of Chemistry, University of Connecticut, Storrs, CT 06269-3060, USA*

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Abstract

The side products formed in the TEMPO-mediated oxidation of glucose to glucaric acid were determined by GC. Next to glucaric acid, gluconic acid, the intermediate in the oxidation, the degradation products, oxalic acid, tartronic acid, meso- (erythraric) and DL-threaric (tartaric) acid were detected. Chiral GC determined the DL-tartaric acid to be non-racemic mixtures of L- and D-tartaric acids, with inverse D/L-ratios depending on the oxidation of D- or L-glucose. The origin of all degradation products is rationalized. This study details a fast screening method to optimize the reaction conditions toward minimal degradation. © 2002 Published by Elsevier Science Ltd.

Keywords: Glucaric acid; TEMPO-mediated oxidations; GC analysis of hydroxyacids

1. Introduction

Glucaric acid has potentially a number of industrially relevant applications in polymer, food, and medicinal chemistry.[1–3] Though naturally occurring,[3] it is most conveniently prepared by oxidation of D-glucose using a number of oxidation methodologies.[4–11] The most established method uses nitric acid as oxidant but produces, as its monopotassium salt, glucaric acid in only ~40% yield.[5,6] The remaining 60% of the starting material is degraded in due course of the reaction to a complex mixture consisting, in part, of carbonate, tartaric, oxalic, and '5-ketogluconic acid'.[5]

N-Oxide mediated oxidations in aqueous base using bleach (NaOCl) or other terminal oxidants have, in recent years, proven to be promising for the selective oxidation of alcohols in general,[12–15] and the production of aldaric acids from aldoses in particular.[10,11,16,17] 2,2,6,6-Tetramethyl-1-piperidinyloxy (TEMPO) and its 4-acetamido derivative are commonly used and commercially available nitroxide catalysts.[12,13,18] The system TEMPO–NaBr–NaOCl is utilized in the high yield oxidation of unprotected mono- and polysaccharides.[10,16,19,20] Thus, glucaric acid, isolated as its monopotassium or disodium salt, can be made under carefully controlled pH and temperature conditions from glucose in yields approaching 90%.[10] Deviations from the ideal protocol decreases the yield of glucaric acid and increases the formation of degradation (over-oxidation) products.

What are the over-oxidation products? Some older methods for the determination of the over-oxidation products oxalic and tartaric acid have been described.[5,21–23] However, these methods are not amenable to the convenient determination of a larger number of samples. ^{1}H NMR methods for the determination of the side products are, due to overlapping peaks or the absence of protons in the side products not ideal either.[24] ^{13}C NMR methods are time consuming and allow for the quantitative determination of the side products only under special conditions.[25] Can one devise a simple and reliable method to determine the amount and identity of the side products? Do the side products illuminate particular degradation pathways?

This contribution evaluates a fast method for the determination of the short-chain carboxylic acids side

* Corresponding authors. Tel.: +33-2-35522475 (F.M.); tel.: +1-860-4862743 (C.B.).
E-mail addresses: francis.marsais@insa-rouen.fr (F. Marsais), c.bruckner@uconn.edu (C. Brückner).

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products by gas chromatography. Using GC and NMR, we will describe the side products of the nitroxide-mediated oxidation of glucose to glucaric acid using bleach as terminal oxidant, and we propose a degradation pathway of glucose (and/or gluconic or glucaric acid) under these reaction conditions.

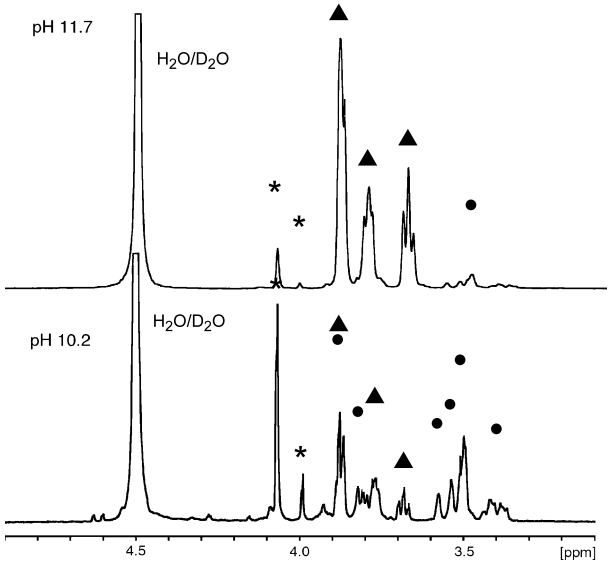

Fig. 1. ^1H NMR (300 MHz, D_2O) spectrum of crude reaction mixtures of nitroxide-mediated oxidations of glucose to glucaric acid using bleach as primary oxidant, performed at the pH indicated (for experimental details see Section 3). ● indicates signals characteristic of gluconate; ▲ indicates signals for glucarate; and * indicates the signals for the tartrates (meso- and DL-form).[24]

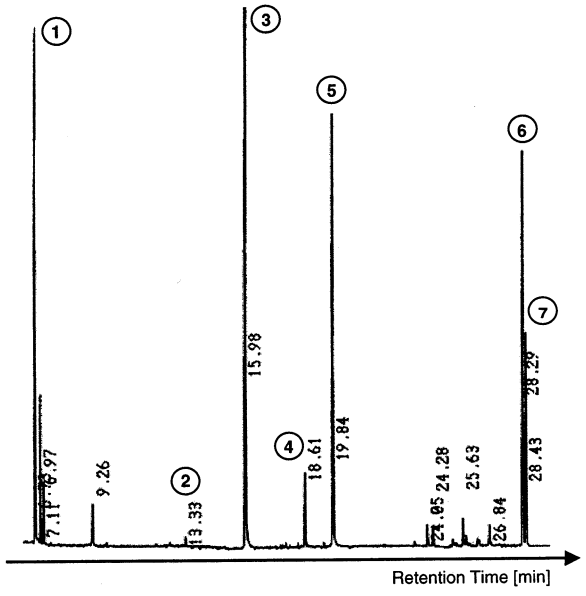

Fig. 2. GC trace of a silylated crude reaction mixture. Peak assignment, retention time, rel. amount (reference compound L-malic acid = 100%): (1) oxalic acid, 6.73 min, 30.8%; (2), tartronic acid, 13.33 min, 0.4%; (3), L-malic acid, 15.98 min; (4) meso-tartaric acid, 18.61 min, 3.7%; (5) DL-tartaric acid, 19.84 min, 23.9%; (6) gluconic acid, 28.29 min, 23.8%; (7) glucaric acid, 28.43 min, 17.4%. The unassigned peaks at low retention times can be attributed to reagent peaks.

2. Results and discussion

Glucose oxidation.—The oxidation protocol chosen for the oxidation of glucose is similar to that described by us in earlier contributions.[10,26] A bleach solution is, under tight pH and temperature control, added to an aqueous NaOH solution of glucose containing TEMPO and the co-catalyst, bromide. The oxidation products are isolated from the reaction mixture by precipitation with EtOH. At pH 11.7, ^1H NMR-spectroscopic yields close to 90% of sodium glucarate can be obtained.[26] At pH 10.5, the percentage of side-products increases on the expense of glucarate yields—they drop to as low as 25%. Analysis of the crude reaction mixtures by ^1H NMR, quantitative ^{13}C NMR, as well as a number of precipitation procedures allowed the determination of the over-oxidation products to be tartrate, oxalate, and carbonate.[10,24] Fig. 1 shows a comparison of the ^1H NMR spectra of a reaction mixture prepared at pH 10.5 and 11.7, clearly displaying the increased number of non-glucaric acid peaks. Overlapping peaks and the inherent difficulty in discerning minor components in mixtures using NMR led us to investigate an alternative analytical method of the identification of the side products.

Derivatization of the oxidation product-mixture and GC analysis.—The oximation–trimethylsilylation and subsequent gas-chromatographic analysis of carbohydrates is a well established technique.[27–30] Thus, the precipitated, dried and powdered reaction mixture was converted into its volatile trimethylsilyl ethers and esters. Fig. 2 shows the GC trace of the silylated reaction mixture resulting from the oxidation of D-glucose. Next to glucaric acid and gluconic acid, the degradation products, oxalic acid (major product), tartronic acid (traces), meso- (erythraric) and DL-threaric (tartaric) acid (minor and major products, 1:6 ratio) can be detected. While not revealing unexpected degradation products, the trace demonstrates the power of the GC method for the convenient and rapid analysis of such reaction mixtures. It also confirms the absence of D-*xylo*-5-hexulosonic '5-ketogluconic' acid, a common oxidation product in the nitric acid oxidation of glucose.[5,23]

Interpretation of the oxidation products.—NMR investigations of the mechanism of the hypochlorite oxidation have shown that the oxidation of glucose to gluconic acid is fast and accompanied by the formation of relatively few side products.[11] In fact, this fast and selective oxidation has been utilized for the preparation of gluconate.[31,32] The *N*-oxide-mediated oxidation of

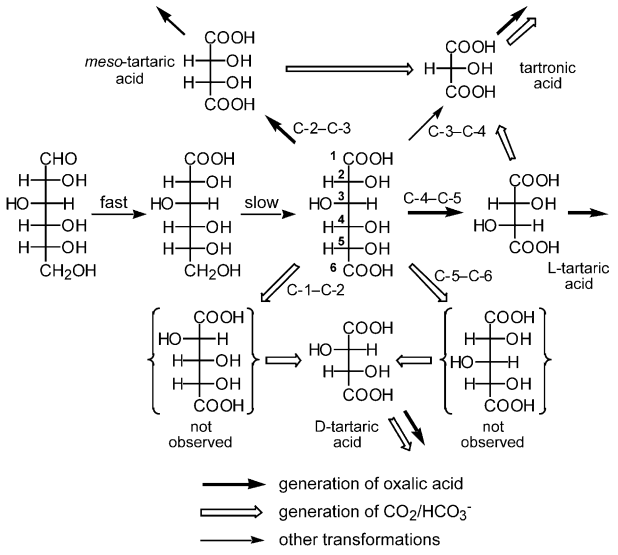

Fig. 3. Proposed genealogy of the degradation products observed in the nitroxide-mediated oxidation of D-glucose to D-glucaric acid.

the primary alcohol of the gluconate to a carboxylate is slow. In the absence of ideal reaction conditions, this conversion is accompanied by extensive over-oxidation. It is noteworthy, though, that no side products with primary alcohol termini other than gluconic acid were detected.

Fig. 3 depicts the proposed genealogy of the degradation products with glucaric acid at its origin. We realize that glucaric acid is not the only possible source of the degradation products detected. Pure gluconic or glucaric acid, exposed to the oxidation conditions, exhibit very similar degradation patterns as those shown in Fig. 2. Thus, the degradation products detected by GC (oxalic, tartronic, and meso- and DL-tartaric acid) can be rationalized by the degradations steps indicated: C-3–C-4 cleavage of glucaric acid forms tartronic acid; cleavage between C-2–C-3 and C-4–C-5 generates tartaric acids: meso-tartaric acid in the former and L-threaric (L-tartaric) acid in the latter case. Hypochlorite and hypobromite in the absence of a catalyst are generally not known to be able to cleave diols, although examples to the contrary are known.[33] However, control experiments in which glucaric acid was exposed to a several-fold molar excess bleach in the absence of the nitroxide catalyst but in the presence and absence of bromide at high pH indicated that, over 24 h, glucaric acid was entirely oxidized to the point where no CH signals were detectable in the ^{1}H NMR spectrum. Likewise, reaction of glucarate with a severalfold stoichiometric excess of independently prepared 4-acetamido–TEMPO-based oxammonium salt[18] under basic conditions resulted in complete decomposition. Thus, the oxammonium salt as well as the terminal oxidant are capable of over-oxidizing glucaric acid. Evidently, the observed high selectivity for the conversion of glucose to glucaric acid is the result of a fortuitous scaling of the rates of the various competing oxidation reactions and the low concentration of oxidants during the experiment.

DL-tartaric acid forms over the meso-form in a 6:1 ratio, indicating a pronounced regioselectivity for the degradation reaction. Likely, this differentiation reflects the effects of coordination of glucarate to the sodium ions in solution. The oxygen atoms attached to C-1, C-2, and C-3 of glucaric, as well as gluconic acid, have been shown by NMR, UV–CD spectroscopy, and X-ray crystallography studies to bind preferentially over all other oxygen atoms to a number of metal ions.[34–39] Coordination to, in this case, the sodium cations in the alkaline reaction solution may serve as a steric and/or electronic protection towards C-2–C-3 cleavage, directing degradation toward C-4–C-5 cleavage. Alternatively, the cation may induce a conformationally restricted, cyclic chelate structure. This may set up sets of cis- and trans-diols exhibiting differing cleavage rates. These explanations suggest a counter-ion influence on the outcome of the oxidation reaction, a hypothesis as yet not tested. Oxalic acid and CO_2 (or CO_3^{2-}), the ultimate degradation products are generated by a number of degradation reactions.[40]

Remarkably, no direct evidence for any C-1–C-2 cleavage or C-5–C-6 cleavage are found, that is, no arabinose or xylose (or their corresponding -onic, -aric, or -uronic acids) were detected. While the preparation of arabinose from D-glucose using hypochlorite is known, this reaction requires acidic conditions and long reaction times in the absence of a catalyst.[31,32] However, there is indirect evidence that (fast) terminal carbon cleavages occur: Chiral GC analysis of the silylated oxidation mixture generated from oxidation of D-glucose indicated the DL-tartaric acid to be a 16:3 mixture of L- to D-tartaric acid (Fig. 4(B)). The L-form of tartaric acid is the expected degradation product resulting from C-4–C-5 cleavage of glucaric acid. The origin of the D-form is less obvious. It cannot originate from the L-form because the reaction conditions for an epimerization are too mild.[41] The only way we can perceive the formation of D-tartrate is by consecutive C-1–C-2 and C-5–C-6 cleavages of D-glucaric (or any of its precursors). The larger quantity of L-tartaric acid and the absence of any five-carbon acids in the oxidation mixture imply a very efficient cleavage reaction. We cannot offer an explanation for this circumstantial evidence. If this hypothesis for the origin of D-glucaric acid is in general true, oxidation of L-glucose under identical reaction conditions ought to produce an inverse amount of L- to D-tartaric acid. As Fig. 4(C) demonstrates, this is indeed observed.

In summary, the degradation products formed during the nitroxide-mediated oxidation of glucose to glucaric

acid under basic conditions using bleach as terminal oxidant were determined by GC analysis. A rationale for their formation is offered. We have thus provided the necessary analytical tools for the facile optimization of the reaction conditions for this or related reactions in industrial or laboratory settings.

3. Experimental

Materials.—All chemicals, including anhyd D- and L-glucose and TEMPO were analytical-grade commercial products (Aldrich) and were used without further purification.

Glucose oxidation procedure.—D-Glucose (5 g, 27.8 mmol), TEMPO (45 mg, 0.01 equiv), and NaBr (0.40 g, 0.15 equiv), were dissolved in water (60 mL) in a 200 mL beaker equipped with a thermometer and a pH electrode connected to a Logilap™ system. The reaction vessel was cooled in a salt-ice mixture to 0 °C. In a separate vessel, a 1.8 M NaClO solution (51 mL, equal to 2.2 equiv ClO^-/mmol primary OH plus 1.1 equiv ClO^-/mmol of hemiacetal OH) was adjusted to the desired pH with 4 M HCl and the solution was cooled to 0 °C. Controlled by the Logilap™ system, the chilled oxidant was added to the glucose solution at a rate not allowing the reaction temperature to exceed 5 °C. The pH was held constant at the desired pH by automated titration with an 1.5 M NaOH solution. When the oxidation was completed (all oxidant added and no further pH shift detectable), the reaction was quenched with EtOH, and the pH was adjusted to 8.5 with 1.0 M HCl. The solution was concentrated to 50 mL, and the oxidation products were precipitated by addition of EtOH (150 mL). The precipitate was dissolved in water (50 mL), and the precipitation operation was repeated. The resulting solid was washed with 4:1 EtOH–water, and dried at 50 °C under diminished pressure. An NMR of the mother liquors proved complete precipitation of the hydroxyacids.

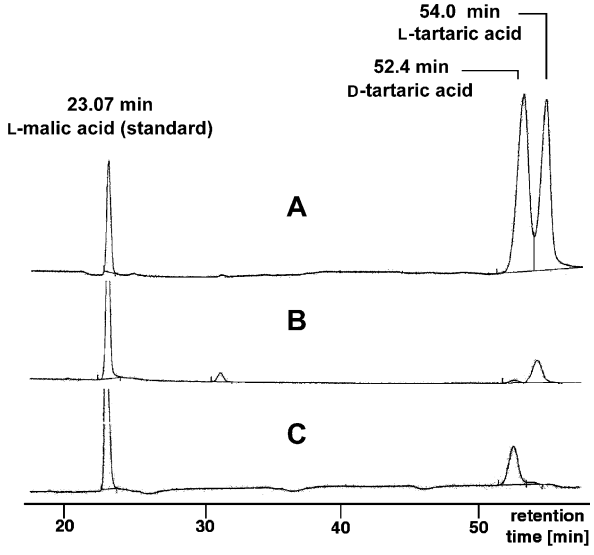

Fig. 4. Truncated chiral GC traces of (A) DL-tartaric acid (D-tartaric acid, 52.4 min, L-tartaric acid, 54 min), internal standard L-malic acid, 23.07 min. (B) Oxidation product mixture resulting from the oxidation of D-glucose (15% D-, 85% L-tartaric acid). (C) Oxidation product mixture resulting from the oxidation of L-glucose (90% D-, 10% L-tartaric acid).

GC analysis of the oxidation products.—The powdered reaction mixture (1–5 mg) was dissolved in 1 mL of an anhyd pyridine stock solution (100 mL) containing the internal reference compounds glutaric acid (50 mg) and L-malic acid (50 mg) (it was confirmed that neither compound was an over-oxidation product of glucose). Methoxylamine hydrochloride (30–40 mg) and 500 μL of [bis(trimethylsilyl)trifluoroacetamide] (BSTFA, containing 1% Me_3SiCl) are added. The mixture is sonicated for 5 min, and the resulting solution is heated to 60–70 °C for 30 min. About 1 μL of the resulting solution is injected into the gas chromatograph for analysis. All peak assignments were confirmed with genuine materials.

Instrumentation.—NMR spectra were recorded on Bruker AC300F or DRX400 spectrometers, operating at ^1H NMR frequencies of 300 and 400 MHz, respectively. The non-chiral GC spectra were recorded on a Varian 3400 using a J&W Scientific DB1 column (30 m, 0.32 mm diameter, 0.25 μm film). Injector temperature 300 °C, column temperature ramped from 80 to 250 °C at a rate of 5 °C/min and from 250 to 320 °C at a rate of 10 °C/min. Carrier gas rate 78 mL/min. FID detector at 300 °C. Under these conditions, all compounds were eluted between 4 and 34 min. The chiral GC was performed using a Hewlett–Packard 5890 instrument equipped with a Supelco Beta-Dex 120 column (15 m, 0.25 mm diameter, 0.25 μm film). Injector temperature 250 °C. The spectrum was recorded isothermally at 100 °C, carrier gas rate 50 cm/s and a split ratio of 1:20. FID detector at 250 °C.

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