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Formation of isomers of anionic hemiesters of sugars and carbonic acid in aqueous medium

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ABSTRACT

Hemiesters of carbonic acid can be freely formed in aqueous media containing HCO_3^-/CO_2 and mono- or poly-hydroxy compounds. Herein, ^{13}C NMR spectroscopy was used to identify isomers formed in aqueous solutions of glycerol (a prototype compound) and seven carbohydrates, as well as to estimate the equilibrium constant of formation (K_{eq}). Although both isomers are formed, glycerol 1-carbonate corresponds to 90% of the product. While fructose and ribose form an indistinct mixture of isomers, the anomers of D-glucopyranose 6-carbonate correspond to 74% of the eight isomers of glucose carbonate that were detected. The values of K_{eq} for the disaccharides sucrose (4.3) and maltose (4.2) are about twice the values for the monosaccharides glucose (2.0) and fructose (2.3). Ribose ($K_{eq} = 0.89$)—the only sugar without a significant concentration of a species containing a $-CH_2OH$ group in an aqueous solution—resulted in the smallest K_{eq}. On the basis of the K_{eq} value and the concentrations of HCO_3^- and glucose in blood, one can anticipate a concentration of 2–4 μmol L^{-1} for glucose 6-carbonate, which corresponds to ca. of 10% of its phosphate counterpart (glucose 6-phosphate).

1. Introduction

About a century ago, Hempel and Seidel[1] and Siegfried and Howwjanz[2] synthesized several hemiesters of carbonic acid (HECAs). They obtained calcium salts of hemiesters of different alcohols and sugars by passing CO_2 into an aqueous solution containing the hydroxy compound and excess of $Ca(OH)_2$. The general formula for these salts is $(ROCO_2)_2Ca$, which can be regarded as one of the carbonic acid hydroxyls that reacted with the alcohols while the other one is left as a free carboxylate.

Sir Haworth extended these studies about sugar carbonates, because he was motivated by the possibility of natural occurrence. He hypothesized the existence of sugar carbonates in plants, which would be formed during the absorption of CO_2 by the leaves.[3,4] He finally stated:[3]

> There can be no doubt that sugars possess the capacity of combining with carbon dioxide, and it appears singular that no evidence is available pointing to the isolation of sugar carbonates from plants.

An important issue in handling of HECA salts is that they easily hydrolyse in water regenerating the alcohol and releasing bicarbonate. The decomposition is an even bigger issue at low pH.[5–8] He realized that this behavior could affect the extraction techniques of sugar derivatives, which could explain the lack of evidence on HECAs in plants.

By the middle of the last century, however, these hypotheses seem to have vanished owing to the persistent lack of proofs about the formation of HECAs in biological medium.[9] Taking into account that (1) the concentration of inorganic carbon (HCO_3^-/CO_2) in living organisms is about 10^{-2} mol L^{-1}, (2) in prevailing aqueous media, the equilibrium

$$ROH + HCO_3^- \rightleftharpoons ROCO_2^- + H_2O \quad (1)$$

is shifted towards the formation of the free alcohol, and (3) the low kinetic barrier for this interconversion, one can understand the non-triviality of an isolation procedure, or even the detection of HECAs in living organisms.

Along the following decades, the HECAs—also known as monoalkyl carbonates (MACs)—were used only as models for other molecules of biochemical relevance[8] and in studies about enzymatic activity.[10,11] However, despite the presence of a great number of hydroxyl compounds and HCO_3^- in almost any compartment of the living cells, no possible role in biochemical processes has been identified for a HECA yet. Actually, this fact is more a consequence of the unawareness about the formation of such species than a proof

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of the intrinsic futility of HECAs for the metabolism of living organisms. There is a lack of analytical methods for detection of HECAs and fundamental studies about their properties that must be overcome before any attempt to establish biological roles.

In recent years, capillary electrophoresis with capacitively coupled contactless conductivity detection (CE-C^4D) allowed the detection of HECAs formed by the reaction of an alcohol and HCO_3^- in aqueous media.[12,13] This technique was used to detect monoethyl carbonate in beers and other carbonated drinks.[14] Subsequently, these findings were confirmed by electrospray ionization-mass spectrometry (ESI-MS),[15] and the limit of detection was improved by using CE–ESI-MS/MS.[16] This advancement in the analytical chemistry for the detection of HECAs was possible not only because of the modern instrumentation, but also because of a careful choice of the experimental conditions in order to preserve these evanescent species.

Not only simple alcohols, but also polyols and sugars have been investigated by CE, MS, and CE–MS. However, questions about the formation of isomers still remain. Taking into account a typical biological medium and the equilibrium constants, one can expect that only mono substituted species would be detected at significant levels.[13] Therefore, due to the different reactivity of the hydroxyls of a sugar, different amounts of the isomers should be formed. Herein, we report the detection and identification of isomers of anionic sugar carbonates by using ^{13}C NMR, which is, to the best of our knowledge, achieved for the first time.

2. Results and discussion

The presence of the carbonate group induces changes in the chemical shift of the backbone carbon atoms of the alcohol as demonstrated by Sauers et al.[8] At the same time, changes to the chemical shift of the carbonate group (when compared to free HCO_3^-) are observed as shown by Richardson et al.[17] However, due to the ^{13}C NMR low sensitivity, these peaks are not easily detected if the medium is primarily aqueous (see Eq. (1)). To access HECAs in aqueous medium by ^{13}C NMR, relatively high concentrations of $H^{13}CO_3^-$ and the alcohol as well as long acquisition time are needed. Therefore, the study was carried out using 0.2 mol L^{-1} $NaH^{13}CO_3$ solutions, and the spectra were accumulated for a long time (over a weekend) to improve the signal-to-noise ratio (SNR).

2.1. Glycerol carbonate

Glycerol was initially studied as a prototype for the sugars, because of the presence of only two distinct hydroxyls (primary and secondary). Fig. 1 shows the ^{13}C NMR spectra, in the vicinity of the bicarbonate peak, of solutions containing glycerol, $NaH^{13}CO_3$, and a mixture of them in D_2O. As previously observed,[13] the reaction between bicarbonate and glycerol takes place in a matter of minutes. Therefore, the spectra, which were obtained by accumulation during ca. 60 h, are representative of the dynamic equilibrium state.

The spectrum of the mixture shows two new peaks (162.18 and 161.73 ppm), which were attributed to the isomers of glycerol carbonate and clearly observed at upfield of the HCO_3^- peak (163.19 ppm). Although usually the intensities of the peaks for ^{13}C NMR cannot be directly related to the abundance, this approximation was acceptable in the present case, because of the similarity of the chemical environment and proximity of the chemical shifts. Therefore, one can estimate the equilibrium constant of formation (K_{eq}) according to Eq. 1 as follow. Starting with a bicarbonate concentration b and no HECA, when the equilibrium is reached, the HECA concentration is x, and bicarbonate concentration is $b–x$. Taking into account the proportionality of the peak area and concentration, the HECA concentration is now given by

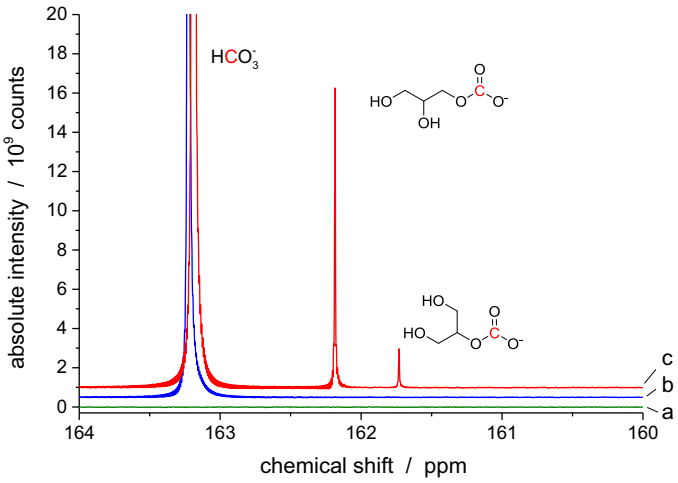

Fig. 1. ^{13}C NMR spectra of aqueous solutions of glycerol plus NaCl (a), $NaH^{13}CO_3$ (b), and glycerol plus $NaH^{13}CO_3$ (c).

$$x = \frac{A_H}{A_B + A_H} b \quad (2)$$

where A_H and A_B are the peak areas for the HECA and bicarbonate, respectively. Finally, the equilibrium constant of formation is given by

$$K_{eq} = \frac{x(w+x)}{(a-x)(b-x)} \quad (3)$$

where w and a are the initial concentrations of water and alcohol, respectively.

Taking the sum of the peak areas of the glycerol carbonate isomers and the one for HCO_3^-, the equilibrium constant of formation (K_{eq}) of glycerol carbonate was estimated at 2.1, which is in good agreement with 2.2 previously obtained by CE-C^4D.[13] The individual K_{eq} values for the isomers were 1.9 and 0.2, which show that one of them greatly prevails.

The simulation of the ^{13}C NMR spectra (ChemBioDraw v. 14, CambridgeSoft) indicated that the carbon atoms at positions 1 and 2 in the glycerol structure are shifted by +5.9 and –3.6 ppm for glycerol 1-carbonate and –2.5 and +3.6 ppm for glycerol 2-carbonate. Small peaks shifted by +4.0 and –1.9 ppm were detected in the spectrum of the mixture of glycerol and $NaHCO_3$ (Fig. S1), which suggests that glycerol 1-carbonate is the most abundant isomer. Unfortunately, contrary to the glycerol carbonate, the identification of the isomers based on the $\Delta\delta$ caused by the carbonate group on the backbone carbon atoms could not be successfully applied to the other sugars, because of the complexity of the spectra and the low SNR of the backbone peaks of the anionic sugar carbonates.

The tendency to form greater amount of the HECA at the primary carbon atom is in agreement with a previous observation about the correlation between K_{eq} and pK_a of the hydroxyl group.[8,13] On the basis of estimated pK_a values and experimental K_{eq} values, Sauers et al[8] estimated that $\log(K_{eq})$ varies according to 1.4 times the pK_a value of the hydroxy group of a monohydric alcohol. Although this finding could be used to predict the relative amount of HECAs in a mixture of alcohols, it cannot be straightly applied to a polyol, because the tendency to a new ionization diminishes after the ionization of a first hydroxyl. Therefore, the pK_a to be considered in the present case would be that one for the ionization of a specific hydroxyl keeping neutral all the other ones. This value has been obtained by simulation (Chemicalize, ChemAxon, Budapest, Hungary)