Prebiotic Chemistry

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Diastereoselective Self-Condensation of Dihydroxyfumaric Acid in Water: Potential Route to Sugars**

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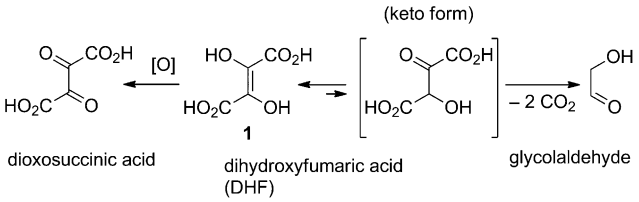
Dihydroxyfumaric acid (DHF, **1**, Scheme 1) has a long history since the days of its synthesis and extensive studies by Fenton in the 1890s.[1] The chemistry of DHF and that of its corresponding ester derivatives in organic solvents has been investigated.[2] However, studies of the (non-enzymatic) aqueous chemistry of DHF have been sparse, perhaps as result of a) its perceived "instability" in aqueous solutions owing to its oxidative transformation into dioxosuccinic acid[3] and its more widely known decarboxylative conversion into glycolaldehyde[4] (Scheme 1), and b) the sparing solubility not only of the parent acid, but also of its Na^+, K^+, and NH_4^+ salts, in water.[4b,c]



Scheme 1. The aqueous (non-enzymatic) chemistry of DHF.[3,4]

Our investigation into the aqueous chemistry of DHF was initiated in the context of the proposals of Eschenmoser that DHF is a molecule of interest[5] in the search for primordial metabolism, wherein DHF and glyoxylate could serve as source molecules for the formation of organic building blocks by reactions deemed to be compatible with the constraints of prebiotic chemistry ("glyoxylate scenario").[6]

We report herein the discovery of uncharted reactivity of water-soluble Li, Cs, and Mg salts of DHF. Our findings show that it is possible to expand the scope and spectrum of the chemical reactivity of DHF to include carbon–carbon bond-forming reactions, which exemplify its capacity to act both as a nucleophile and as an electrophile.

In our preliminary investigations we found that we could handle DHF as its lithium and cesium salts with ease. Our studies began with the monitoring of degassed aqueous solutions of the dilithium salt of DHF (0.45 M, pH 8–9) at room temperature and 4 °C by ^{13}C NMR spectroscopy (Figure 1), which initially showed signals that corresponded only to the enolic form; no signals corresponding to the keto form were seen.[7] Within 30 min (at room temperature), much to our surprise, we observed the appearance of eight new signals and a concomitant decrease in the intensity of the two DHF signals, which disappeared after 6 h. Continued monitoring showed that these eight signals were slowly replaced by six different signals over a period of 24–72 h; after this time the spectrum remained unchanged at room temperature.

The observations by ^{13}C NMR spectroscopy suggested the following reaction pathway (Scheme 2): DHF (**1**) condenses with itself (via its putative keto form) by intermolecular dimerization to yield a (presumed) linear dimer intermediate **2**, which immediately undergoes ring closure to form the cyclic dimer **3**. This cyclic dimer undergoes successive decarboxylation (perhaps via intermediate **2**) to form the final compound, pentulosonic acid (**4**). The ^{13}C NMR spectrum indicates that predominantly one diastereomer of **3** and essentially one diastereomer of **4** are formed. At the lower temperature of 4 °C, the reaction was slower, and the DHF signals persisted for up to 24 h. Reactions at 0.9 and 1.8 M concentrations of **1** at room temperature resulted in the formation of a 1:3–1:4 anomeric/diastereomeric mixture of **3**; nevertheless, essentially a single diastereomer of **4** was formed.[7]

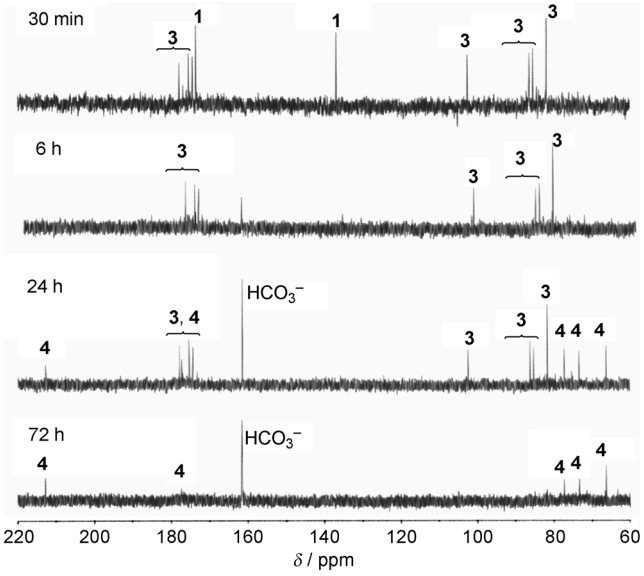


Figure 1. Monitoring of the reaction of aqueous Li_2DHF (0.45 M) by ^{13}C NMR spectroscopy (H_2O/D_2O).

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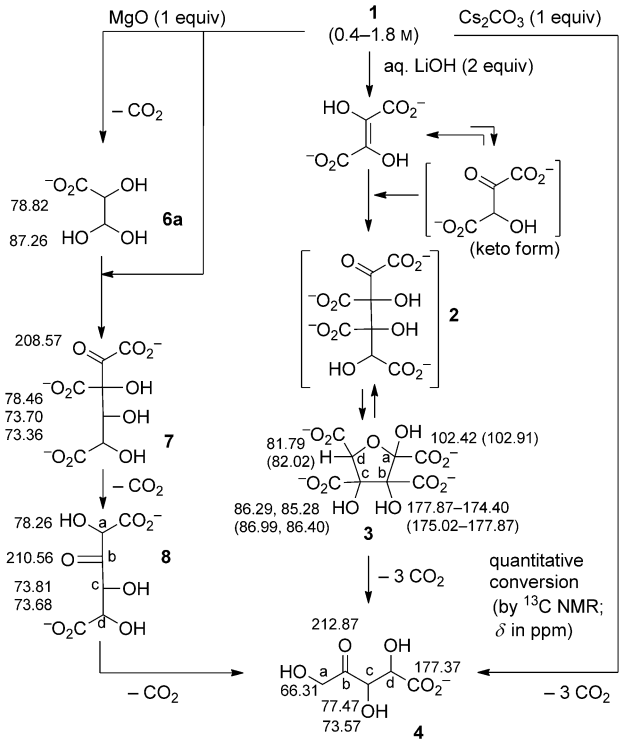


Scheme 2. Pathways for the formation of **4** from DHF on the basis of ^{13}C NMR spectra measured at 4 °C and room temperature (in H_2O containing D_2O; chemical shifts, δ, are given in ppm). Intermediates shown in square brackets are inferred; ^{13}C NMR chemical shifts in parentheses refer to the other diastereomer.

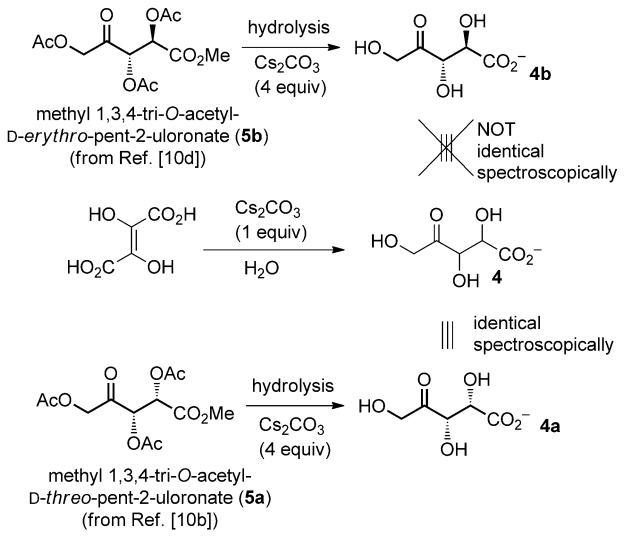
The constitutional assignment of **3** is based on the ^{13}C NMR chemical shifts and the multiplicity of the carbon signals, as well as comparison with structurally closely related compounds (see Table S1 in the Supporting Information).[8] Concentration of the reaction mixture at the stage at which **3** is formed furnished a gray-white powder. The ^{13}C NMR spectrum of this powder, when redissolved in water, was unchanged, which shows that this cyclic dimeric structure is stable. Solid **3** was found to be stable at −20 °C for 1 month and at room temperature for 10 days. An aqueous solution of compound **3** was converted into **4** over a period of hours at room temperature. Additional evidence for the DHF dimer **2**/**3** was obtained by benzylation of the reaction mixture; mass spectral data of the resulting mixture confirmed the presence of the tetrabenzyl ester of the dimer along with the corresponding tribenzyl ester derivative.[7] Attempts thus far to convert **3** into suitable derivatives for further characterization have met with failure.

The water-soluble Cs salt of DHF was also found to react, albeit slowly, to yield essentially a single diastereomer of pentulosonic acid (**4**); however, the reaction pathway could not be defined by ^{13}C NMR spectroscopy, unlike that of the Li salt.[7] The treatment of a suspension of DHF (0.5–0.9 M) in water with solid Cs_2CO_3 (1 equiv) at 4 °C (or room temperature) resulted in a clear dark solution within 2–6 h (pH 7.2). Monitoring by ^{13}C NMR spectroscopy at room temperature revealed that the dicesium salt of DHF remained unreacted longer (18 h at 4 °C) than the corresponding dilithium salt. With time, the formation of a single diastereomer of **4** (plus HCO_3^-) with simultaneous disappearance of starting material was observed (ca. 434 h at 4 °C and 24–28 h at room temperature).[7] At no stage in the monitoring process could the signals of any other intermediate be observed—only signals for **1** and **4** were detected. It is possible that the lower solubility of the Cs salt of DHF results in concentrations of intermediates that are below the detection limits of the instruments.

The structure of pentulosonic acid (**4**) was deduced, again, on the basis of ^{13}C NMR chemical shifts and multiplicities.[7,9] We were able to verify its constitution and configuration by preparing **4** independently from known compounds, methyl 1,3,4-tri-*O*-acetyl-D-*threo*-2-pentuloronate (**5a**) and the corresponding *erythro* isomer **5b** (Scheme 3).[10] Thus, when compound **5a** (0.14 M) was treated with aqueous Cs_2CO_3 (0.55 M) at room temperature, complete and clean hydrolysis of **5a** within 2 h was observed by ^{13}C NMR spectroscopy with the generation of a ^{13}C NMR spectrum identical to that of **4** (along with MeOH and AcO^-). When the NMR sample of a fresh reaction mixture for the production of **4** from **1** was spiked with the material produced from the hydrolytic reaction of **5a**, an increase in the intensity of only the peaks corresponding to the pentulosonic acid **4** was observed, in support of its *threo* configuration.[7] In an equivalent spiking experiment with the hydrolytic product from D-*erythro* isomer **5b**, peaks were observed in the ^{13}C NMR spectrum that did not match the peaks of **4** in the reaction mixture. This result confirmed the *threo* configuration of pentulosonic acid (**4**). No epimerization to produce the other diastereomer was observed in either case.

While investigating other reaction conditions (see Table S2 in the Supporting Information), we observed that a suspension of $MgCO_3$ and DHF (pH ≈ 7–8) produced **4** much faster (74 h, 4 °C) than the reaction in the presence of Cs_2CO_3; however, the reaction mixture remained heterogeneous, which complicated monitoring by ^{13}C NMR spectroscopy.

Additional screening revealed that MgO provides an excellent "solution" to this problem. An aqueous suspension



Scheme 3. Confirmation of the constitution and configuration of **4**.

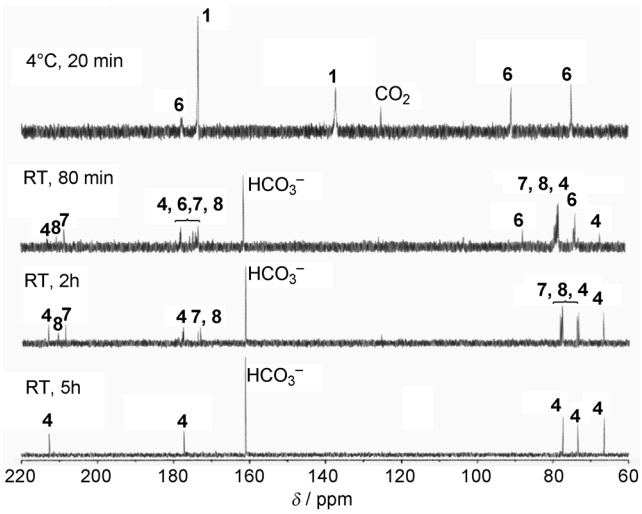


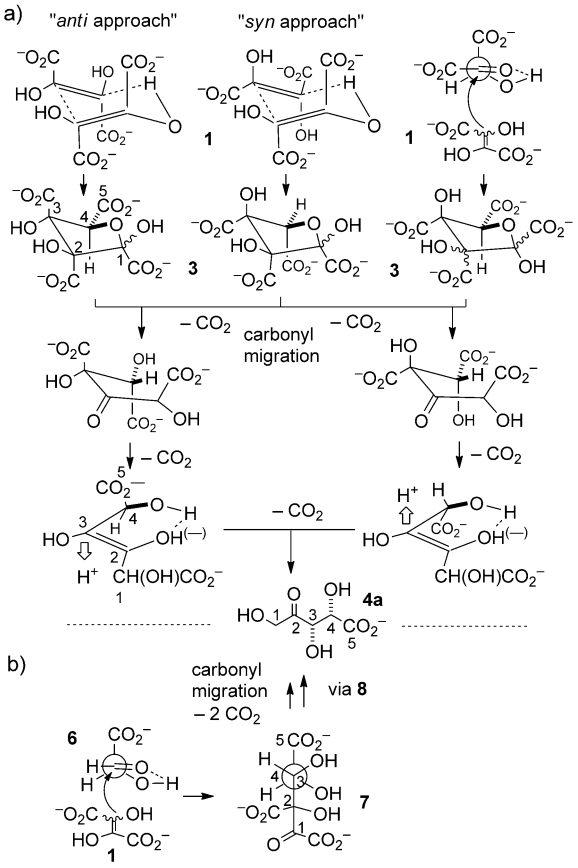
Figure 2. Monitoring of the reaction of aqueous DHF (0.4 M) with MgO (1 equiv) by ^{13}C NMR spectroscopy (H_2O/10% D_2O).

of DHF (0.4 M) in the presence of magnesium oxide (1 equiv)[11] became a clear solution within minutes at room temperature. The pH value of the solution increased from about 3.5 to about 7.1 over a period of 60 min and stabilized close to neutral (pH ≈ 8.0). At 4 °C, a clear solution was formed in 2 h with an initial pH value of 3.9, which rose to approximately 8.0 over a period of 20 h. ^{13}C NMR spectroscopy (at room temperature and at 4 °C) revealed the formation, once again, of a single diastereomer of **4** as the final product (Figure 2), but through a totally different pathway from that observed for the dilithium DHF salt. In the presence of MgO, a portion of the DHF undergoes decarboxylation as a result of the initial acidity of the medium to give the hydrated form, **6a** (Scheme 2), of α-carboxyglycolaldehyde (**6**); this aldehyde reacts with DHF to give intermediate **7**, which undergoes decarboxylation to give another intermediate **8**, which loses another molecule of carbon dioxide to provide **4** (final pH 8.1). The dimerization pathway may be concurrently operative, but is not discernible by ^{13}C NMR spectroscopy.

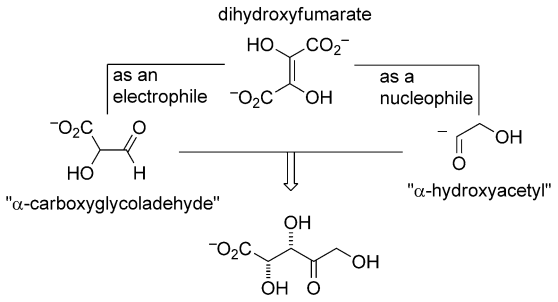
In all three cases, with the Li, Cs, and Mg salts of DHF, (*rac*)-*threo*-pentulosonic acid (**4a**) was formed quantitatively and essentially as a single diastereomer, as indicated by the ^{13}C NMR spectra of the corresponding reaction mixtures.[12] In one case, isolation of the product after ion-exchange workup (to remove the metal ions) afforded the crude acid (*rac*)-**4** in about 96% yield.[7]

In a control experiment, when DHF alone was suspended in water (0.9 M when fully dissolved, without any adjustment of the pH value or metal ions, pH 2.2), it slowly underwent decarboxylation and was converted predominantly into the hydrated form of glycolaldehyde in 91 h at room temperature (Scheme 1). The oxidized form of DHF, dioxosuccinic acid (hydrated form), was also observed (after 170 h at room temperature). On the other hand, at pH > 10 (attained by the addition of LiOH (3.5 equiv)), an aqueous solution of DHF (0.5 M) at room temperature (or 4 °C) was found to undergo a fragmentation reaction to give predominantly oxalate and glycolate; the presence of formate was suggested by a CH signal at $\delta = 171.45$ ppm.[7] The aforementioned two control reactions imply that the generation of **4** from DHF occurs within a certain pH-value range in the neighborhood of neutrality in an aqueous medium;[13] the pathway for its formation is influenced by the nature of the counterion.

The formation of essentially a single diastereomer of a five-carbon-atom ketoaldonic acid **4** through an initial dimerization of four-carbon-atom DHF is striking.[14] This preference can be rationalized by the following mechanism (Scheme 4a): The enediol of DHF can approach the keto form of DHF in two possible ways (or by dimerization via a chairlike transition state) to give a (putative) linear dimer, which closes to form the corresponding anomers/diastereomers of the cyclic dimer **3**. The dimer undergoes decarboxylation at C2 with concomitant protonation and migration of the carbonyl group to the C2 position to yield another presumed β-carboxy intermediate. Loss of another molecule of CO_2 (at C3) produces a lower-energy *E* enolate (further stabilized by internal hydrogen bonding), which undergoes diastereoselective protonation at C3, wherein the proton approaches from the less hindered side. This step seems to be critical in determining the final *threo* configuration of the pentulosonic acid. In the case of the MgO-mediated reaction, face-selective attack on the intramolecularly hydrogen-



Scheme 4. Possible mechanistic rationale for the formation of **4a** a) through the dimerization of Li_2DHF and b) from DHF in the presence of MgO. Atom numbering is shown for the purpose of correlation (and not nomenclature).



Scheme 5. Latent reactivity of DHF as a nucleophile (umpolung equivalent of α-hydroxyacetyl anion) and as an electrophile (α-carboxyglycolaldehyde) under aqueous conditions.

bonded conformation of α-carboxyglycolaldehyde accounts for the configuration of **4a** (Scheme 4b). These observations are reminiscent of the stereoselectivity observed in the aldolization of glycolaldehyde phosphate.[15]

The results presented herein demonstrate that the scope of the chemistry of DHF in an aqueous medium can be diversified to encompass stereoselective carbon–carbon bond-forming reactions, which increase the potential of DHF to become a versatile building block in organic synthesis:[16] when DHF has the opportunity to act as the nucleophile, it is the umpolung equivalent of α-hydroxyacetyl anion, whereas as an electrophile, it is equivalent to α-carboxyglycolaldehyde (Scheme 5). Both of these synthons are not readily accessible in a straightforward manner by other means, especially under aqueous conditions.

Investigations of reactions of DHF with other small molecules in the context of the "glyoxylate scenario"[6] with the primary goal of discovering an alternative to the formose pathway for the formation of sugars are under way.

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[11] We undertook further experiments with Mg salts, as they can be considered to be prebiotically relevant in the context of the "glyoxylate scenario";[6] magnesium oxide (magnesia) is known to occur naturally in a mineral form called periclase.

[12] Peaks corresponding to the *erythro* isomer were not observed in the ^{13}C NMR spectra of the reaction mixtures. To confirm this observation, we spiked the NMR sample of *threo* isomer **4a** with the *erythro* isomer **4b** (5%); the ^{13}C NMR spectrum of this mixture clearly showed the presence of both isomers (see the Supporting Information).

[13] DHF is highly soluble in MeOH and THF. The resulting solutions were found to be stable with no further change at 4°C or room temperature over a 24 h period. The addition of *t*BuLi (2 equiv) to DHF dissolved in THF resulted in the quantitative formation of the dilithium salt of DHF as a precipitate; no further reactions were observed.

[14] Theoretical (computational) studies may aid in judging and elucidating the factors involved in this *threo*-selective product formation.

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