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# Conformation analysis of D-glucaric acid in deuterium oxide by NMR based on its $J_{HH}$ and $J_{CH}$ coupling constants

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**D-Glucaric acid (GA) is an aldaric acid and consists of an asymmetric acyclic sugar backbone with a carboxyl group positioned at either end of its structure (i.e., the C1 and C6 positions). The purpose of this study was to conduct a conformation analysis of flexible GA as a solution in deuterium oxide by NMR spectroscopy, based on *J*-resolved conformation analysis using proton–proton ($^3J_{HH}$) and proton–carbon ($^2J_{CH}$ and $^3J_{CH}$) coupling constants, as well as nuclear overhauser effect spectroscopy (NOESY). The $^2J_{CH}$ and $^3J_{CH}$ coupling constants were measured using the *J*-resolved heteronuclear multiple bond correlation (HMBC) NMR technique. NOESY correlation experiments indicated that H2 and H5 were in close proximity, despite the fact that these protons were separated by too large distance in the fully extended form of the chain structure to provide a NOESY correlation. The validities of the three possible conformers along the three different bonds (i.e., C2–C3, C3–C4, and C4–C5) were evaluated sequentially based on the *J*-coupling values and the NOESY correlations. The results of these analyses suggested that there were three dominant conformers of GA, including conformer 1, which was H2H3:gauche, H3H4:anti, and H4H5:gauche; conformer 2, which was H2H3: gauche, H3H4:anti, and H4H5:anti; and conformer 3, which was H2H3:gauche, H3H4: gauche, and H4H5:anti. These results also suggested that all three of these conformers exist in equilibrium with each other. Lastly, the results of the current study suggested that the conformational structures of GA in solution were 'bent' rather than being fully extended. Copyright © 2016 John Wiley & Sons, Ltd.**

**Keywords:** glucaric acid; conformational analysis; carbon–proton coupling constant; NMR (*J*-resolved HMBC and NOESY)

## Introduction

D-Glucaric acid (D-GA) is an aldaric acid based on an acyclic sugar backbone with a carboxyl group at either end of its structure (Fig. 1). GA occurs naturally in low concentrations in a variety of different vegetables and fruits.[1] GA can be obtained by chemical synthesis as the corresponding monopotassium salt by the oxidation of D-glucose with nitric acid,[2] nitroxide derivatives,[3] or microorganisms.[4] GA has been reported to exhibit several interesting biological properties, including cholesterol-lowering,[1a] anticancer,[5] and metal chelating[6] activities. GA was recently listed as one of the top 12 value-added building blocks that can be produced from sugars via biological or chemical conversion processes in a report published by the US Department of Energy.[7] Furthermore, the central theme of this list was chemicals that can be subsequently converted to bio-based chemicals or materials. Aldaric acids such as GA have significant potential as building blocks for the construction of polymers, including polyesters[8] and polyamides.[9]

D-Glucaric acid [(2R,3S,4S,5S)-tetrahydroxyhexanedioic acid] is a flexible acyclic sugar with an asymmetric structure containing four chiral centers. It would be useful to develop a detailed understanding of the stereo-chemical structure of GA in solution and investigate the relationship between its structure and biological properties, as well as its chelating effect. The conformation of acyclic GA could have a significant impact on it reactivity for polymerization, as well as the structure of the resulting polymers, as mentioned by Styron *et al.*[10] However, previous studies pertaining to the conformational analysis of GA in solution have been limited to its cyclic lactone forms.[11] The results of a previous study by Denton *et al.*[12] revealed that the experimental $J_{HH}$-coupling values of GA in aqueous solution did not agree with the theoretical $J_{HH}$ values calculated for the crystalline and lowest energy models of GA. The theoretical values for this comparison were calculated based on the Karplus equation. Denton's groups also suggested that the conformation of GA in solution was different from that of the crystalline state, despite the fact that the conformation of GA in solution had not yet been determined. A unique method for studying the impact of solution effects on the conformational preference of acyclic compounds in solution by NMR spectroscopy was suggested by Gerken and co-workers in 2008.[13] However, it remains extremely difficult to determine the conformation or configuration of flexible and acyclic compounds in solution using only conventional $J_{HH}$-coupling constants or NOESY correlation

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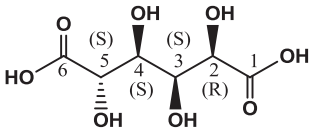


**Figure 1.** Chemical structure of D-glucaric acid.

experiments, because acyclic compounds can exist as multiple conformers with flexible rotation.

It has recently been recognized that proton–proton and carbon–proton *J*-coupling constants are dependent on their dihedral angles and that *J*-coupling constants can be used to determine the conformation or configuration of acyclic compounds bearing chiral centers.[14] *J*-coupling values ($^3J_{HH}$, $^3J_{CH}$, and $^2J_{CH}$) are generally categorized as small or large based on their magnitude in relation to their dihedral angles, as illustrated in Fig. 2, and represent an average of the possible conformers of a given structure.[14a] Despite the difficulties associated with these complex measurements for the accurate determination of small $^{2,3}J_{CH}$ values, recent progress in NMR techniques has allowed for the facile measurement of $^{2,3}J_{CH}$ values using two-dimensional NMR spectroscopy, such as *J*-resolved heteronuclear multiple bond correlation (HMBC) and heteronuclear long-range coupling (HETLOC) experiments.[14,15] Several successful studies on *J*-based conformation or configuration analysis have been reported.[16] However, this method has not yet been applied to acyclic sugar compounds. It would therefore be valuable to use this technique to develop a deeper understanding of the conformations of acyclic sugars in solution from the perspective of basic NMR analytical techniques.

In this study, we have applied *J*-based conformation analysis techniques to GA. In this way, we have evaluated the possible conformations of GA in solution and proposed three dominant conformers based on the $^3J_{HH}$, $^3J_{CH}$ and $^2J_{CH}$ values of GA, as well as its NOESY correlations.

## Experimental

### Materials

D-Glucaric acid monopotassium salt was kindly provided by the Ensuiko Sugar Refining Co., Ltd (Yokohama, Japan).

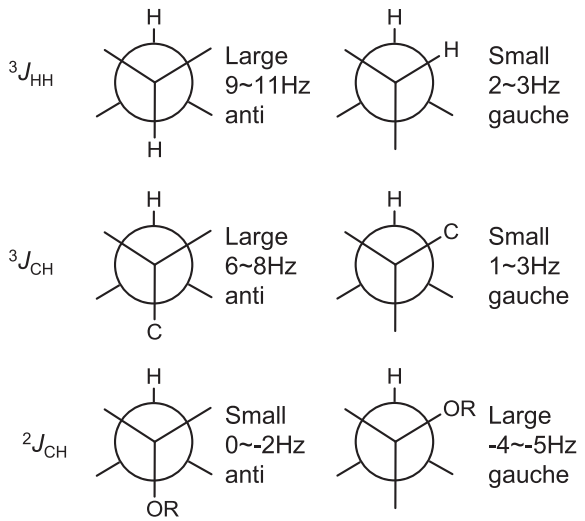


**Figure 2.** Relationships between the dihedral angles and *J*-coupling constants ($^3J_{HH}$, $^3J_{CH}$, and $^2J_{CH}$).

### D-glucaric acid

D-Glucaric acid monopotassium salt was converted to acyclic GA using an ion exchange resin. D-Glucaric acid monopotassium salt (50 mg) was dissolved in deuterium oxide ($D_2O$) (1 ml), and the solution was treated with an ion-exchange resin (Amberlyst $H^+$). The resulting slurry was stirred for about 5 min at room temperature and turned clear. The solution was then loaded into an NMR sample tube for NMR analysis.

### Nuclear magnetic resonance measurements

Nuclear magnetic resonance spectra, including $^1H$, $^{13}C$, double-quantum filtered correlation spectroscopy (DQF-COSY), heteronuclear single-quantum coherence (HSQC), heteronuclear multiple-bond correlation (HMBC), *J*-resolved HMBC1 and double-pulsed-field-gradient-spin-echo nuclear overhauser effect spectroscopy (DPFGSE-NOESY) (1D-selective NOESY), were recorded on a 500 MHz Varian INOVA500 NMR spectrometer (Varian, Palo Alto, CA, USA) at 25 °C, using deuterium oxide as a solvent. Chemical shifts ($\delta$) and coupling constants (*J*) have been reported in parts per million and Hertz, respectively. The *J*-resolved HMBC1 was measured to determine the $^{2,3}J_{CH}$-coupling constants with a scaling factor of 20. The *J*-coupling constants were calculated as follows: $^3J_{HH} = \Delta\delta_H \times 500$, $^{2,3}J_{CH} = \Delta\delta_H \times 500$, or $(\Delta\delta_C \times 125)/20$.

### *J*-based conformation analysis

The relationships between the dihedral angles and *J*-coupling constants ($^3J_{HH}$, $^3J_{CH}$, and $^2J_{CH}$) are shown together with a rough categorization in Fig. 2. These values were determined according to the reported method of *J*-based configuration analysis[14a,d] with the following characterizations: $^3J_{HH}$ = small (2–3 Hz) (gauche) or large (9 to 11 Hz) (anti); $^3J_{CH}$ = small (1 to 3 Hz) (gauche) or large (6 to 8 Hz) (anti); and $^2J_{CH}$ = small (0 to −2 Hz) (anti) or large (−4 to −5 Hz) (gauche). The $^2J_{CH}$ value was obtained as an absolute value in this study. The $^2J_{CH}$ value can be used to provide an indication of the angle between the H and O atoms bound to a C atom.[14d] Experimentally determined *J*-coupling values that were outside of these ranges were categorized as 'medium' coupling values, and the equilibrium state of the conformers was examined.

## Results and discussion

### Nuclear magnetic resonance analyses of glucaric acid

The $^1H$ and $^{13}C$ chemical shifts of GA are listed in Table 1 together with the *J*-coupling constants and categorizations of the different atoms. The peaks in the $^1H$ and $^{13}C$ NMR spectra were assigned in accordance with those reported in a previous study using isotopically labeled D-GA.[12] The C–H coupling constants ($^2J_{CH}$ and $^3J_{CH}$) were determined by *J*-resolved HMBC-NMR analysis, as shown in Fig. 3. A representative cross-peak for the $^2J_{H5C6}$ coupling is marked with a dotted line in Fig. 3. As shown in Fig. 4, the strong NOE correlations between H2–H3 and H4–H5 indicated that these protons were in a gauche orientation. Furthermore, the weak NOE correlation observed between H3–H4 indicated that these protons were in an anti orientation. Surprisingly, the NOE correlation between H2 and H5 was as strong as that between H2 and H3 (gauche conformation), as shown in Fig. 4 and d, despite the distance between these protons in the chain structure. This observation

**Table 1.** $^{1}H$ and $^{13}C$ chemical shifts of D-glucaric acid

| | Chemical shift | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| | | 4.42 | 4.10 | 3.93 | 4.31 | |
| | | d | dd | t | d | |
| $^{1}H$ | | $^{3}J_{H2H3}$ 3.1 small | $^{3}J_{H3H4}$ 5.7 medium | $^{3}J_{H4C5}$ 4.9 medium | | |
| | 176.75 | 72.41 | 72.27 | 73.99 | 72.21 | 176.44 |
| $^{13}C$ | $^{2}J_{H2C1}$ 5.0 large | $^{3}J_{H1C1}$ <3 small | $^{3}J_{H4C2}$ <3 small | $^{2}J_{H3C2}$ n.d. small | $^{3}J_{H5C3}$ <3 small | $^{3}J_{H5C3}$ <3 small | $^{2}J_{H4C3}$ n.d. small | $^{3}J_{H2C4}$ <3 small | $^{2}J_{H3C4}$ <3 small | $^{2}J_{H5C4}$ 3.8 medium | $^{3}J_{H3C5}$ <3 small | $^{2}J_{H4C5}$ 3.3 medium | $^{3}J_{H4C6}$ 3.6 medium | $^{2}J_{H5C6}$ 4.2 large |

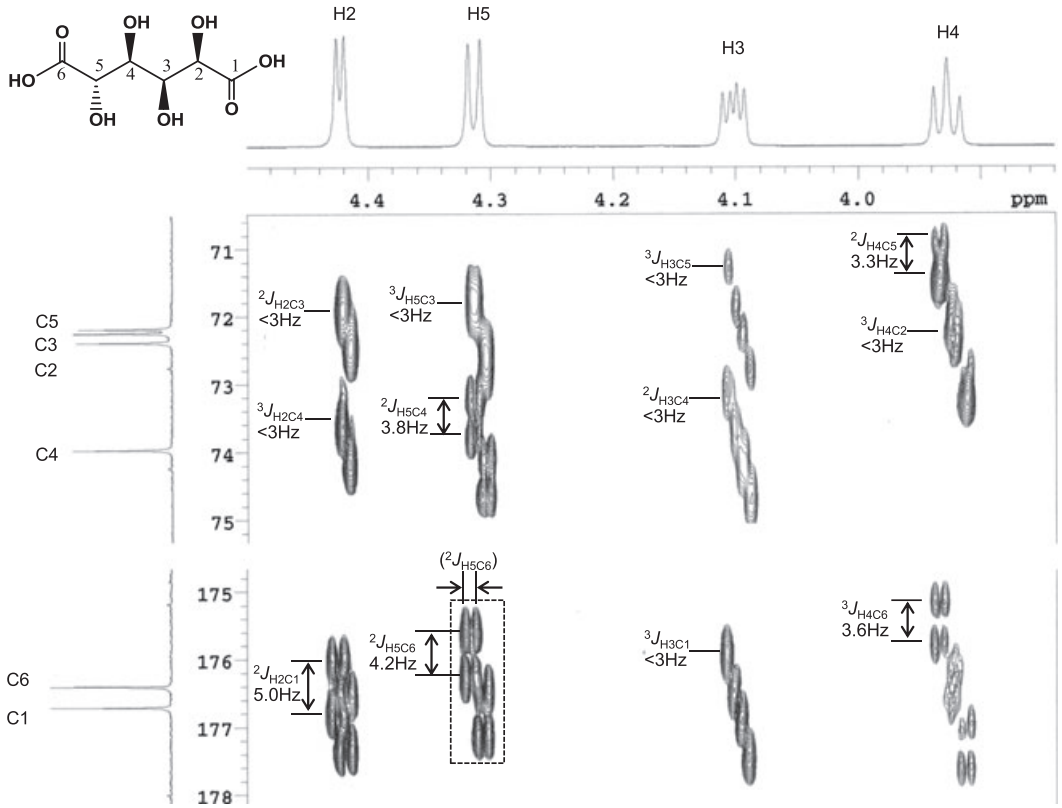


**Figure 3.** *J*-resolved HMBC-NMR spectrum of D-glucaric acid.

strongly suggested that H2 and H5 were in close proximity and that the distance between these two protons was very similar to that of the distance between H2 and H3, which were positioned vicinal to each other.

### Conformation analysis of GA

The conformation of GA was investigated based on its *J*-coupling constants. The NOESY data collected for this compound were examined with high priority to reduce the number of possible conformations. The configurations of the four stereocenters were fixed to C2(R), C3(S), C4(S), and C5(R), according to the original stereochemical structure of D-GA.

### Conformation along the C2–C3 bond

We initially examined the three possible staggered conformers of the C2–C3 bond shown in Fig. 5A. The results revealed a $^{3}J_{H2H3}$ value of 3.1 (small), which was indicative of a gauche orientation between H2 and H3, which was in agreement with the NOESY result. We also obtained $^{3}J_{H3C1}$ and $^{3}J_{H2C4}$ values of less than 3 (small), which were indicative of gauche orientations between H3 and C1, and H2 and C4, respectively. The $^{2}J_{H3C2}$ and $^{2}J_{H2C3}$ values were both less than 3 (small) and, therefore, indicative of anti orientations between H3 and O2, and H2 and O3, respectively. It is noteworthy that the measured *J*-coupling values were in agreement with all of the theoretical values for conformer **5a**. With regard to conformers **5b** and **5c**, we observed several differences between the theoretical



  

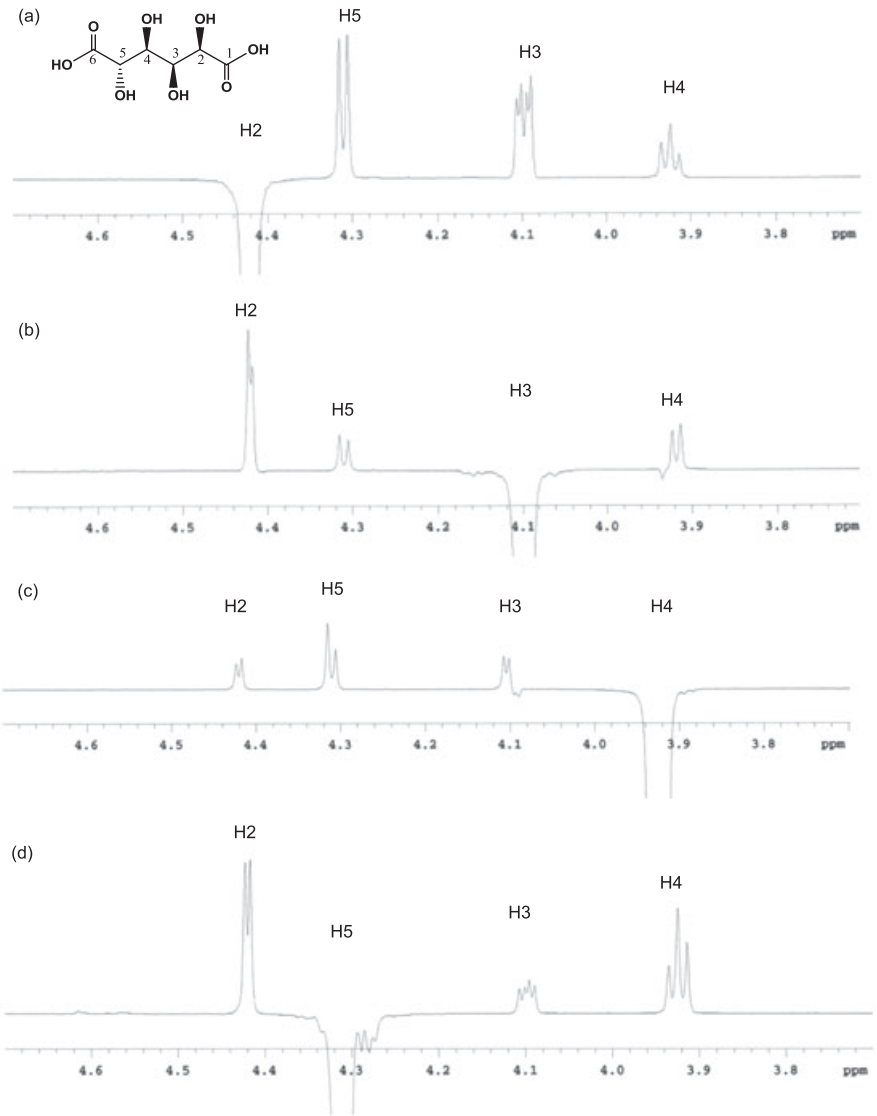


**Figure 4.** Nuclear overhauser effect spectroscopy NMR spectra of D-glucaric acid. Excited at (a) H2, (b) H3, (c) H4, and (d) H5.

and experimental *J*-coupling and NOE values. Based on these results, conformer **5a** was determined to be the dominant conformer for the C2–C3 bond.

### Conformation along the C3–C4 bond

The conformation of the C3–C4 bond was also investigated based on the three possible conformers shown in Fig. 5B. However, the measured *J*-coupling values did not completely satisfy any of these three conformers. This result suggested that there was an equilibrium between the three different conformers and that the measured coupling constant therefore represents an average value for the three possible conformers.[14a] The eclipsed conformation for this bond was considered to be impossible.

For conformer **5d**, H5 extended away from C5 in the opposite direction to H2, which was in agreement with the molecular model. The distance between H2–H5 was not small enough to allow for a strong NOE correlation between these protons. Furthermore, the weak NOE correlation observed experimentally between H3 and H4 was inconsistent a gauche conformation for these two protons. Based on these results, conformer **5d** was considered to be impossible.

We then proceeded to investigate conformer **5e**, where H3–H4 existed in the anti conformation. Notably, this conformer also allowed for H2 and H5 to be placed close enough to each other to give a strong NOE, as shown in Fig. 6, whilst the H4–H5 conformations were gauche and anti. The H5 proton could rotate along the C4–C5 axis within the same range; maintaining its close proximity to H2. This conformation agreed well with the NOESY results. Taken together, these results suggested that conformer **5e** was possible.

Lastly, we investigated conformer **5f**. None of the *J*-coupling or NOE values for this conformer completely satisfied the measured values. However, this conformer did allow for the H2 and H5 protons to be held in close proximity to each other, as shown in Fig. 6c, although the H5 position had to be strongly restricted to maintain its close proximity with H2. Conformer **5f** was therefore considered to be less possible than conformer **5e**.

With regard to the $^{2}J_{CH}$ values, the measured values (small) were contrary to the theoretical values (large) for conformers **5e** and **5f**. However, there have been very few studies concerning acyclic

(A)

| C2-C3 | | (a) | (b) | (c) |
|---|---|---|---|---|
| Possibility of the presence | | (O) | (×) | (×) |
| | | Measured | Theoretical | Theoretical | Theoretical |
| NOE | $NOE_{H2H3}$ | strong | strong | weak | strong |
| $^3J_{HH}$ | $J_{H2H3}$ | small (3.1) | small | large | small |
| $^3J_{CH}$ | $J_{H2C4}$ | small (<3) | small | small | large |
| | $J_{H3C1}$ | small (<3) | small | small | large |
| $^2J_{CH}$ | $J_{H2C3}$ | small (<3) | small | large | large |
| | $J_{H3C2}$ | small (<3) (n.d.) | small | large | large |

(B)

| C3-C4 | | (d) | (e) | (f) |
|---|---|---|---|---|
| Possibility of the presence | | (×) | (O) | (△) |
| | | Measured | Theoretical | Theoretical | Theoretical |
| NOE | $NOE_{H3H4}$ | weak | strong | weak | strong |
| | $NOE_{H2H5}$ | strong | 0 (impossible) | strong (possible) | weak (less possible) |
| $^3J_{HH}$ | $J_{H3H4}$ | medium (5.7) | small | large | small |
| $^3J_{CH}$ | $J_{H3C5}$ | small (<3) | small | small | large |
| | $J_{H4C2}$ | small (<3) | small | small | large |
| $^2J_{CH}$ | $J_{H3C4}$ | small (<3) | small | large | large |
| | $J_{H4C3}$ | small (<3) (n.d.) | small | large | large |

(C)

| C4-C5 | | (g) | (h) | (i) |
|---|---|---|---|---|
| Possibility of the presence | | (×) | (O) | (O) |
| | | Measured | Theoretical | Theoretical | Theoretical |
| NOE | $NOE_{H4H5}$ | strong | strong | weak | strong |
| | $NOE_{H2H5}$ | strong | 0 (impossible) | strong (possible) | strong (possible) |
| $^3J_{HH}$ | $J_{H4H5}$ | medium (4.9) | small | large | small |
| $^3J_{CH}$ | $J_{H4C6}$ | medium (3.6) | small | small | large |
| | $J_{H5C3}$ | small (<3) | large | small | small |
| $^2J_{CH}$ | $J_{H4C5}$ | medium (3.3) | small | large | large |
| | $J_{H5C4}$ | medium (3.8) | large | large | small |

**Figure 5.** Conformation analysis of D-glucaric acid based on its NOESY and *J*-coupling constants. Bold (blue): reasonable, plain (red): unreasonable.



  

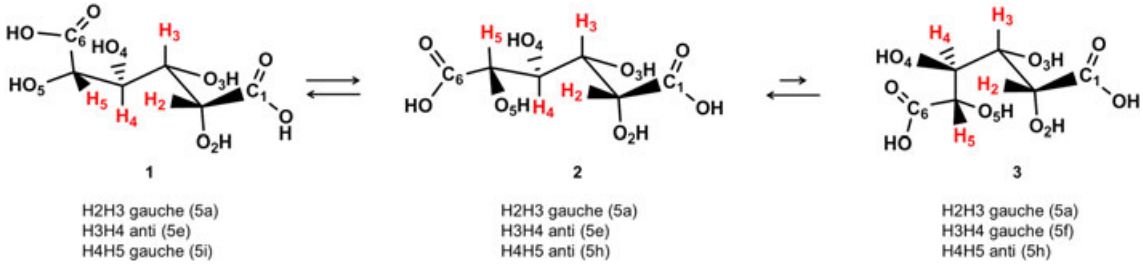


**Figure 6.** Dominant conformers of D-glucaric acid.

sugars or polyols, and it is therefore difficult to interpret these results in a meaningful way. For example, it is not clear from previous results whether conformational analysis based on $^{2}J_{CH}$ can be applied to GA or any other sugar, such as glucose, galactose, or mannose.[16c] Furthermore, the effects of neighboring O atoms or NOE effects on the *J*-coupling values of O–H moieties have not been thoroughly investigated. For this reason, we did not investigate the differences observed in the $^{2}J_{CH}$ values in this study any further.

Having conducted a comprehensive evaluation of the *J*-value and NOE results, it was concluded that conformer **5e** was the dominant conformer and that **5f** was only present as a small component of the mixture. Conformer **5f** may have contributed to the medium $^{3}J_{HH}$ value of 5.7, but would not have made a significant enough contribution to effect the $^{3}J_{CH}$ value.

### Conformation along the C4–C5 bond

Lastly, we investigated the conformation along the C4–C5 bond based on the three possible conformers shown in Fig. 5C. The measured *J*-coupling values for the C4–C5 bond were $^{3}J_{H5C3} < 3$ (small), $^{3}J_{H4C6} = 3.6$ (medium), $^{3}J_{H4H5} = 4.9$ (medium), $^{2}J_{H4C5} < 3.3$ (medium), and $^{2}J_{H5C4} = 3.8$ (medium), which suggested that there was an equilibrium between the three possible conformers. The eclipses conformations were all considered to be impossible.

We initially investigated conformer **5g**. A molecular model of this conformer suggested that it would not allow for the experimentally observed NOE between H2 and H5 based on the distance between these two protons in the model. This conformer was therefore considered to be impossible. We subsequently evaluated conformers **5h** and **5i**. The molecular models of these two conformers allowed for H2 and H5 to be placed close enough to other to exhibit a strong NOE correlation. Taken together with the medium *J*-coupling values observed along the C4–C5 bonds of **5h** and **5i**, these results indicated that the medium *J*-values represented the averaged theoretical values of the different conformers, with **5h** and **5i** existing as the dominant species.

### Dominant conformers of glucaric acid

Following our analysis of the C2–C3, C3–C4, and C4–C5 bonds, we proposed three dominant conformers, including **1** (H2H3:gauche, H3H4:anti, H4H5:gauche), **2** (H2H3:gauche, H3H4:anti, H4H5:anti) and **3** (H2H3:gauche, H3H4:gauche, H4H5:anti), which most likely existed in equilibrium with each other, as shown in Fig. 6. The dominant conformers **1** and **2** appeared to be preferred to conformer **3**, which was less dominant. The results of this study have successfully shown that there are three dominant conformers of GA (i.e., **1**, **2**, and **3**) and that these are preferred over one statistical conformation. Furthermore, the results have shown that the measured $J_{HH}$-coupling constants for GA in solution represent the averaged values of these three different conformers based on an equilibrium between the three states. The conformational structures of GA in solution were 'bent' rather than being fully extended. Similar conformational structures have been reported for GA following crystal analysis and computer modeling studies.[10b,12]

All four of the hydroxyl groups extending away from the main carbon chain in conformers **1**, **2**, and **3**. Interestingly, conformer **1** had the same conformational structure as crystalline GA and the lowest energy conformer determined by computer modeling.[12] This result suggested that conformer **1** was also the most stable structure in solution. However, in solution, the free rotation of the C2–C3 or C4–C5 axis in conformer **1** would provide access to several other conformations. However, the rotation around this bond would be restricted because of electronic repulsion and steric hindrance, meaning that the GA would most likely adopt conformers **2** and **3**. These results suggest that intermolecular electronic repulsion and steric hindrance effects between the hydroxyl and carboxyl groups could control the changes in the conformations of GA. Although it has been reported that the nature of the solvent can have a significant impact on the conformational preferences of a molecule,[13] water molecules had no significant effect on the conformation of GA in this study compared with these other factors. The C4–C6 bond appeared to be more flexible than the C1–C3 bond, most likely because the configuration of the OH group at C5 was different from that of the OH group at C2, resulting in the asymmetric conformation.

The conformation of GA disclosed in this study could have a significant impact on its biological activity, chelating ability, structure and reactivity toward polymerization, and the structure of the resulting polymers.

## Conclusion

We have conducted a conformational analysis of GA in $D_2O$ by NOESY and *J*-resolved HMBC-NMR. We have evaluated the conformation of GA based on its $^{3}J_{HH}$-coupling and $^{2,3}J_{CH}$-coupling constants, which were measured by $^{1}H$ NMR and *J*-resolved HMBC-NMR, respectively, as well as its NOESY correlations. We identified three dominant conformers of GA, including **1** (H2H3:gauche, H3H4:anti, and H4H5:gauche), **2** (H2H3:gauche, H3H4:anti, and H4H5:anti), and **3** (H2H3:gauche, H3H4: gauche, and H4H5:anti), which existed in an equilibrium state. The results of this study also suggested that GA exists in a 'bent' form in solution rather than its fully extended form. The results of the *J*-based conformational analysis method proved to be effective for determining the conformation of GA and could be used to determine the conformations of other acyclic sugars and their derivatives. The results of this study could also be used as a platform to investigate the biological

activity, chelating ability, structure and reactivity toward polymerization of these compounds, and the structure of the resulting polymers.

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