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The Structure of Dihydroxyacetone in Solution

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Nuclear magnetic resonance, infrared and ultraviolet spectral data for dihydroxyacetone are reported, together with a brief kinetic study of the dimer–monomer equilibrium. The results are interpreted in terms of a dimeric dioxan structure for solid dihydroxyacetone which dissociates into a mixture of free carbonyl and hydrated monomer upon dissolution. Lyophilization of aqueous solution results in isolation of solid monomer which converts to dimer in the solid state over several months on the shelf. A study of monohydroxyacetone revealed no such complexities.

INTRODUCTION

The observation has been made that galactose oxidase, a copper-requiring enzyme isolated from *Polyporus circinatus*, catalyzes the uptake of oxygen in the presence of dihydroxyacetone (*1*) and monohydroxyacetone (*2*). Because hydroxyaldehydes and hydroxyketones frequently exist as cyclic hemiacetals and hemiketals or dimers, we have studied the various forms of dihydroxyacetone and monohydroxyacetone in solution as an initial step to ascertaining the forms which are acted upon by galactose oxidase.

Dihydroxyacetone exists as a dimer in the solid state, but when it is dissolved in water dihydroxyacetone dissociates (*3–5*). Evidence for a dimer–monomer conversion upon dissolution was first obtained from the time-dependent change in molecular weight as determined from freezing-point depression (*3*, *4*). A study of monohydroxyacetone and dihydroxyacetone in solution is necessary to determine which species is acted upon by galactose oxidase. Such a study is also important from chemical and biological standpoints since these simple compounds, especially dihydroxyacetone, are the basis of ketose carbohydrate chemistry.

This paper reports on a study of the conformation and various species in solution of monohydroxyacetone and dihydroxyacetone using proton magnetic resonance, infrared and ultraviolet spectroscopy.

EXPERIMENTAL SECTION

Nuclear magnetic resonance spectra were obtained on a Varian A-60 spectrometer. *t*-Butanol was used as an external reference for samples dissolved in deuterium oxide and [$^2H_6$] dimethyl sulfoxide. Spectra were also obtained without the external reference and differed from the spectrum of the mixtures only in the absence of the *t*-butanol resonance. Infrared spectra were obtained on a Perkin-Elmer Model 521 grating spectrometer at 25°C. Samples were dissolved in deuterium oxide and placed in a 0.05-mm calcium fluoride cell previously balanced against a variable-thickness cell containing solvent so that a relatively straight base line was obtained in the region of 1600–1900 cm^{-1}. Ultraviolet spectra were obtained on a Durrum double-beam spectrometer using 1-cm cells at 25°C. Dihydroxyacetone–monomer was prepared by dissolving in water commercial dihydroxyacetone, which was 100% in the dimeric form, allowing this solution to stand for 24 h and lyophilizing. This procedure allowed us to isolate crystalline dihydroxyacetone–monomer, which was dried and stored over KOH. The monomer was observed to undergo a slow conversion to dimer in the solid state over a period of several months.

RESULTS AND DISCUSSION

Proton magnetic resonance spectroscopy. The proton magnetic resonance spectrum of freshly dissolved dihydroxyacetone–dimer in deuterium oxide revealed a marked time dependence, suggesting that a chemical change was occurring. The initial spectrum is composed of a pair of doublets at τ 5.90 and τ 6.42 (J_{gem} 12 Hz) which constitutes an AB system and a singlet at τ 6.46 as shown in Fig. 1. This initial pmr pattern is consistent with a dioxan structure for the dimer (Table 1).

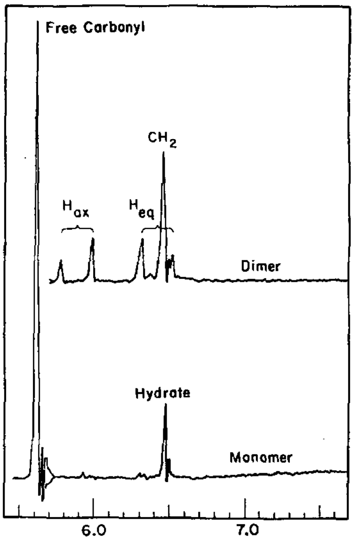
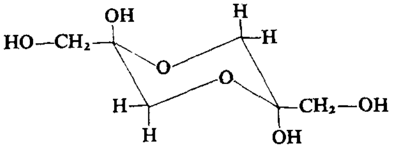
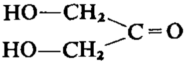
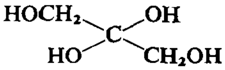
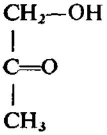


FIG. 1. Spectra of dihydroxyacetone dimer and monomer in deuterium oxide at 25°C 60-mHz pmr.

The fact that a singlet is observed for the hydroxymethyl protons argues for the existence of the *trans*-isomer assuming a chair conformation. The *cis*-isomer should have nonequivalent (ax–eq) CH$_2$-groups. However, the protons for the most symmetrical (*trans*) conformation are diastereotopic and magnetically nonequivalent.

TABLE 1

PROTON MAGNETIC RESONANCE PARAMETERS FOR HYDROXY KETONES

Compound	Solvent	Protons	τ	J
Dihydroxyacetone dimer	D_2O	Hax	6.00, 5.80	12 Hz
		Heq	6.53, 6.32	
		—CH_2—	6.46	
	DMSO(d_6)	Heq	5.85, 6.05	12 Hz
		HO—	5.35, 5.25, 5.16	6 Hz
		HO—ax	4.10	
Dihydroxyacetone monomer	D_2O	—CH_2—	5.65	
 $(HO{-}CH_2)_2C{=}O$	DMSO(d_6)	—CH_2—	5.79, 5.69	6 Hz
		HO—	5.00, 4.90, 4.80	
Dihydroxyacetone monomer (hydrate)	D_2O		6.49	
 $HOCH_2C(OH)_2CH_2OH$				
Monohydroxyacetone neat		—CH_2—	5.76	
		—CH_3—	7.90	
		HO—	5.60	
 $CH_2OH{-}C{=}O{-}CH_3$				

Therefore, that a singlet was observed argues for "accidental equivalence" or degeneracy.

The resonances of the dimer are replaced in time by resonances which are assigned to a mixture of free carbonyl and hydrated monomer in a ratio of 4 to 1, respectively, as shown in Fig. 1. The hydrate has a resonance at τ 6.49 and the free carbonyl of a resonance at τ 5.65. This assignment is made because of the deshielding of the hydrogen nuclei adjacent to the carbonyl carbon; this deshielding is significantly reduced when the hydrate or gem diol is formed. Thus, as a result of this reduced deshielding, the resonance observed for the protons of dihydroxyacetone should occur at a lower field in the keto form than in the gem diol form. The observed shift of 0.9 ppm is very close to that of 0.8 ppm reported by Lombardi and Sogo (*6*) for the free carbonyl and hydrated forms of acetaldehyde.

The spectra of monomeric and dimeric dihydroxyacetone were also investigated in deuterated dimethyl sulfoxide. The spectrum of the dimer shows a very slow time dependence in this solvent, and the conversion to monomer is only 50% complete after 64 h. This slow conversion of dihydroxyacetone dimer to monomer in dimethyl sulfoxide is to be contrasted with the observation by Collins and George (*7*) of a 1 to 3-h period for a sizable fraction of the glycolaldehyde dimer to convert to monomer in the same solvent.

When the pmr spectrum of pure monomer was recorded in dimethyl sulfoxide (Table 1), coupling between the hydroxyl proton and the methylene protons was observed (J 6 Hz). This coupling observed in dimethyl sulfoxide is consistent with a relatively slow hydroxyl proton exchange in comparison with the pmr time scale. This spin–spin interaction disappears in D_2O as expected, due to a rapid proton–deuterium exchange.

The dimer of dihydroxyacetone, when dissolved in dimethyl sulfoxide, shows the same (J 6 Hz) coupling (Table 1); in addition, a second hydroxy proton is observed as a singlet at τ 4.10. This hydroxyl group is formed as a result of hemiketal formation. The ring protons, two axial and two equatorial, should exist as a pair of doublets. The equatorial protons exist as a doublet centered around τ 5.95 (J 12 Hz) but the axial protons are not resolved due to overlapping peaks in the expected region. The additional peaks are due to solvent and to the methylene protons at the 2 and 5 positions. Casu *et al.* (8), suggested a range of τ 3.7–4.05 for axial hydroxyl protons in molecules similar to dihydroxyacetone dimer. Therefore, the resonance at τ 4.1, in the spectrum of dihydroxyacetone–dimer in dimethyl sulfoxide, is assigned to a hydroxyl proton of this group in an axial position.

This evidence also suggests that in solution dihydroxyacetone dimer exists in the *trans*-isomer of a chair conformation with the hydroxymethyl groups in an equatorial position and the hemiketal hydroxy groups axial.

The pmr spectrum of monohydroxyacetone shows three resonances as indicated in Table 1. In spite of the presence of the same functional groups in both mono- and dihydroxyacetone, we could find no evidence for dimeric species of monohydroxyacetone either in solution or neat. At room temperature monohydroxyacetone exists as a liquid where dihydroxyacetone exists as a solid and neither time nor lyophilizing a water solution of this compound results in formation of a solid.

Ultraviolet and infrared spectroscopy. The ultraviolet spectrum of pure dihydroxyacetone–dimer, when dissolved in water, undergoes a marked time dependence. With time a very symmetrical ultraviolet band appears at 275 nm. This band has an ε of 24 and has been assigned to the n-π^* transition of the carbonyl group of dihydroxyacetone–monomer.

Infrared spectra were obtained for dihydroxyacetone–monomer and dimer in deuterium oxide. As expected from the pmr and ultraviolet studies the dimer showed no carbonyl absorption. The spectrum of the dimer shows a time-dependence with the appearance of a strong absorption band at 1730 cm^{-1}. Dihydroxyacetone–monomer reveals a strong band at 1730 cm^{-1} initially when its infrared spectrum is recorded.

Kinetic studies. We have used ultraviolet spectroscopy to measure the rate of dimer to monomer conversion in water for dihydroxyacetone using the appearance of the carbonyl frequency at 275 nm. The dimer-to-monomer conversion of dihydroxyacetone takes place with an apparent first-order rate constant of 0.034 min^{-1} which corresponds to a half-life of 20.4 min.

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