Ultraviolet and Infrared Absorption Spectra of Malonaldehyde in Organic Solvents

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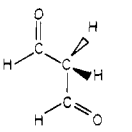
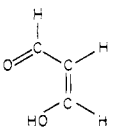
The molar absorptivity of the weak ultraviolet absorption band ($n \rightarrow \pi^*$) of malonaldehyde in aqueous solution is 7.8. Previously, much higher values were reported and are due apparently to polymerization of the aldehyde. In organic solvents, the intense absorption bands ($\pi = \pi^*$) are at 234 and/or 271 mμ and are attributable to the open *sym-cis* and *sym-trans* enols, respectively. With increasing cyclohexane content in diethyl ether–cyclohexane mixtures, the 234-mμ band diminishes, the 271-mμ band increases, and the sum of the two decreases. The decreased sum may be due to the transformation of the enols to the diketo form. The infrared spectra and bromine titration of the compound in dichloromethane support this concept. Only one ultraviolet absorption band at 271 mμ is observed in dichloromethane, and the calculated enol content from the spectrum surprisingly is less than 2% of the total aldehyde.

The 2-thiobarbituric acid (TBA) reaction has been used widely as a measure of lipid oxidation in foods and biological tissues. It has been assumed that malonaldehyde (MA) is responsible for the reaction because the TBA chromogen obtained with oxidized fat has the same absorption spectrum (max. 532 mμ) as that with MA (Patton *et al.*, 1951; Sinnhuber *et al.*, 1958). Although the validity of the assumption was questioned recently by several reports (Saslaw *et al.*, 1963, 1966; Saslaw and Waravdekar, 1965), the original assumption has been confirmed by a nondestructive method (Kwon and Olcott, 1966a, 1966b). Fractionation of the water extract from autoxidized lipids by Sephadex G-10 column clearly demonstrated that MA is indeed the most important TBA reactive substance from the oxidation of polyunsaturated fatty esters (Kwon and Olcott, 1966b) and squalene (Kwon and Olcott, 1966a). Malonaldehyde reacts with amino acids (Crawford *et al.*, 1966), proteins (Buttkus, 1967; Crawford *et al.*, 1967; Kwon and Brown, 1965; Kwon and Olcott 1966c), and other food constituents (Kwon *et al.*, 1965; Kwon and Norgaard, 1966). Furthermore, MA inhibits lipase activity (Landsberg and Sinnhuber, 1965) and is toxic to rats (Crawford *et al.*, 1965).

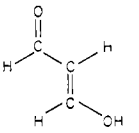
Malonaldehyde exists predominantly as the thermodynamically stable enolic tautomer (Hüttel, 1941; Mashio and Kumura, 1960). The main ultraviolet (UV) absorption band ($\pi \rightarrow \pi^*$) of MA in an aqueous solution is pH dependent (Kwon and Watts, 1963; Kwon *et al.*, 1965; Mashio and Kimura, 1960; Saunders and May, 1963), shifting progressively from 245 to 267 mμ, while pH is changing from 2.8 to 6.5. No further changes occur in the spectra outside of this pH range. The band at 245 mμ below pH 2.8 must be that of the nondissociated molecular species, the *sym-cis* chelated form (IV) (Kwon and Watts, 1963; Saunders and May, 1963). This interpretation is supported by the volatility during distillation (Kwon and Watts, 1964), disappearance of the $n \rightarrow \pi^*$ band at 350 mμ (Saunders and May, 1963), and a K_d value greater



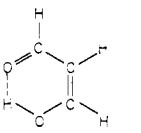
diketo form (I)



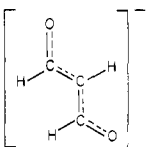
open s-cis enol (II)



s-trans enol (III)



chelated form (IV)



enolate anion (V)

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than unity during Sephadex gel filtration (Kwon, 1966) of MA in acidic aqueous solution. Saunders and May 1963) arrived at the same conclusion from their infrared (IR) data of MA in dichloromethane apparently by assuming that MA has the same conformation in water as in dichloromethane. Although their conclusion coincided with the above interpretation, such an assumption may not be true. Actually, MA does not occur as the chelated form in such organic solvents. The band at 267 mμ above pH 6.5 is attributed to completely dissociated MA, the *sym-trans* enolate anion (V) (Kwon and Watts, 1963; Mashio and Kimura, 1960). Recently, Bacon *et al.* (1965) arrived at the same conclusion from the nuclear magnetic resonance spectral data of the anion. Such conformational assignments for the observed absorption bands were originally predicted from the calculated transition moment for the allowed $\pi \rightarrow \pi^*$ transition of the chelated form and anion (Kwon, 1963).

The present paper reports the accurate molar absorptivity for the $n \rightarrow \pi^*$ transition of MA in water, and discusses the ultraviolet and infrared spectra of MA in organic solvents.

EXPERIMENTAL

Malonaldehyde bis(diethyl acetal) was obtained from Kay-Fries, and purified by distillation. A 500-ml. portion of $2 \times 10^{-3}M$ MA acetal in $10^{-2}N$ HCl was hydrolyzed by heating for 60 minutes at 50°. The MA concentration in the hydrolyzate determined by both the ultraviolet spectrophotometry (Kwon and Watts, 1963) and the TBA test (Sinnhuber *et al.*, 1958) was the same, indicating that the acetal was completely hydrolyzed into MA. The MA was the only homogeneous TBA positive component of the hydrolyzate during Sephadex G-10 column chromatography (Kwon, 1966). The hydrolyzate was diluted with water, and dilute HCl or NaOH was added to obtain the pH dependent ultraviolet absorption spectra. For solvent-dependent spectra, the hydrolyzate was extracted three times with 100-ml. portions of diethyl ether, and the extracts were combined, concentrated, dried with anhydrous sodium sulfate, and diluted with diethyl ether or its cyclohexane mixtures. Similar extracts were also made with chloroform or dichloromethane. All organic solvents were distilled in the presence of TBA to remove any TBA reactive substances (Ho and Brown, 1966). Ultraviolet (Cary, Model 15) and infrared (Perkin-Elmer, Model 137) spectra were obtained at room temperature. Total MA concentrations in the extracts were determined by TBA test.

RESULTS AND DISCUSSION

Molar Absorptivity of the $n \rightarrow \pi^*$ Band. The transition moment for the $n \rightarrow \pi^*$ band of carbonyl oxygen in MA is zero by local symmetry. However, this forbidden transition appears as a weak absorption band at 350 mμ due to weak electronic interactions; the molar absorptivity calculated from Figure 1 was 7.8. The absorption spectra were recorded with 5-cm. cells and a carefully purified MA solution ($2.4 \times 10^{-3}M$). The absorbance is linear only with MA concentration up to $2.4 \times 10^{-3}M$, but sharply increased above this concentration. In contrast, the previously reported values ranged from about 300 to 1000, according to Mashio and Kimura (1960) and Saunders and May (1963). Such values are too high for the $n \rightarrow \pi^*$ transition of carbonyl groups. Heating of MA solution also increases the absorbance. Thus, the previously reported high molar absorptivity may be mainly attributable to the aggregation of MA (Kwon and Olcott, 1966b) caused by the high concentration and high temperature employed during hydrolysis of MA acetal. When this aggregated MA solution was diluted, the extrapolated absorbance at zero concentration was always higher than zero, suggesting some irreversible modifications.

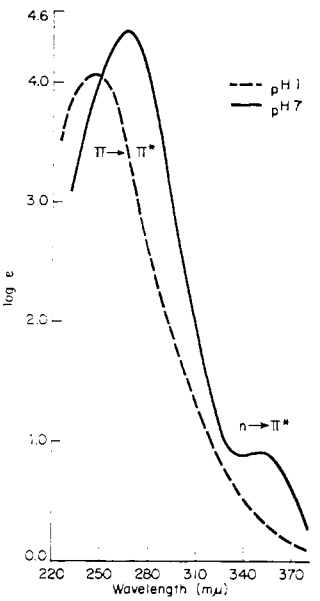

Figure 1. Ultraviolet absorption spectra of MA in aqueous solution at pH 1 and 7

Ultraviolet Absorption Spectra of MA in Organic Solvents. In diethyl ether, two bands at 234 and 271 mμ are observed, and the absorbance at 234 mμ is about three times higher than 271 mμ (Figure 2). Furthermore, with increasing cyclohexane content in diethyl ether–cyclohexane mixtures, the absorbance at 234 mμ gradually diminishes while that at 271 mμ increases, and at 99% cyclohexane essentially only the latter band remains. The spectra in such organic solvents are very different from those in aqueous solution, indicating that the solvent has very striking effects on position and intensity of the band. The possibility that these effects are due to molecular aggregation was eliminated by dilution studies. Such changes are greater than what might be expected from simple perturbation by solvents; thus, the only feasible interpretation is the conformational changes of MA in different solvents.

Recently, Bothner-By and Harris (1965) concluded from their nuclear magnetic resonance studies that the enol form of MA predominantly occurs as the *sym-trans* form (III) in chloroform with very similar coupling constants for the anion (Bacon *et al.*, 1965). The absorption spectrum of MA in chloroform has a band at 271 mμ, as in dichloromethane and diethyl ether–cyclohexane mixtures. Therefore, this band is reasonably attributable to the *sym-trans*

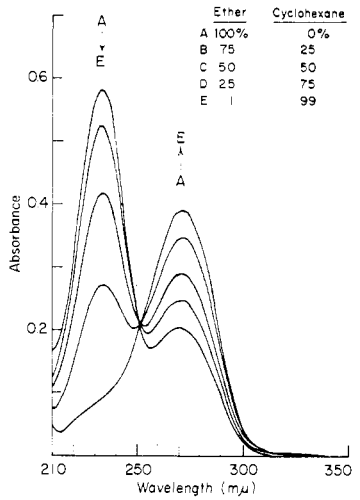

Figure 2. Ultraviolet absorption spectra of MA in diethyl ether–cyclohexane mixtures

Concentration of MA 4.5×10^{-5} *M*

form. This band cannot be attributed to the enolate anion, since the anion in alkaline aqueous solution is not extractable in such organic solvents. The band at 234 mμ in diethyl ether or its cyclohexane mixtures is attributed to the open *sym-cis* form (II) for the following reasons: Diethyl ether is a dechelating agent (Eistert *et al.*, 1951); breakage of the intramolecular hydrogen bond would be expected to give a blue shift of the 245 mμ $\pi \rightarrow \pi^*$ band observed in aqueous solution; and this is the only other reasonable conformation which would give absorption in this wavelength region.

The apparent molar absorptivities of β-dicarbonyl compounds are linear functions of the enol content in solvents, and the absorption characteristics are apparently almost independent of solvent (Hammond *et al.*, 1959). If one assumes the molar absorptivities of the *cis* and *trans* enols are similar to those of the chelated enol and the enolate anion, respectively, from the geometry considerations, then the calculated MA concentration from the spectra (Figure 2) gradually decreases with increasing cyclohexane content in the solvent mixtures, while the total MA concentration ($4.5 \times 10^{-5}M$) determined by the TBA reaction is constant. Furthermore, the solvent-dependent spectral changes are reversible, so there is no irreversible loss of MA by changing proportions in the solvent mixtures. These observations suggest that in cyclohexane the enols are converted to the diketo form (I), which is not expected to absorb in this ultraviolet region. Surprisingly, the calculated enolic content in dichloromethane was less than 2% of the total MA, and thus more than 98% was present as the diketo form. This observation agreed with bromine titration of the enolic form of MA in dichloromethane (Kwon, 1967).

Infrared Spectra of MA in Dichloromethane. A dichloromethane extract was made from a low concentration of MA ($2 \times 10^{-3}M$) and its infrared spectrum was examined. There were two main bands, at 5.80 and 3.56 microns (Figure 3). The former is attributable to free C=O and the latter to C—H (Rassmussen *et al.*, 1949), indicating that the diketo form is the predominant molecular species and supporting the above findings.

The infrared spectrum shown in Figure 3 differs completely from that reported by Saunders and May (1963). However, when a dichloromethane extract was prepared from 0.5*M* MA solution, following the procedures described by Saunders and May (1963), an infrared spectrum similar to theirs was obtained (Figure 4), except for the absence of a broad band at 3.32 microns which they had interpreted as an intramolecular hydrogen band. Upon dilution, the bands at 6.20 and 5.98 microns (Figure 4) diminished, and a strong band at 5.80 microns for free C=O became dominant, suggesting that there was an increasing content of the diketo form with increasing dilution. The two bands (Figure 4) at 6.20 and 5.98 microns,

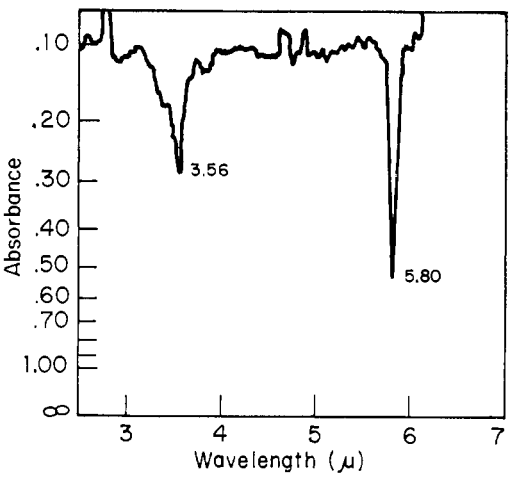

Figure 3. Infrared spectrum of malonaldehyde in dichloromethane

$2 \times 10^{-3}M$ MA acetal hydrolyzate was extracted with dichloromethane

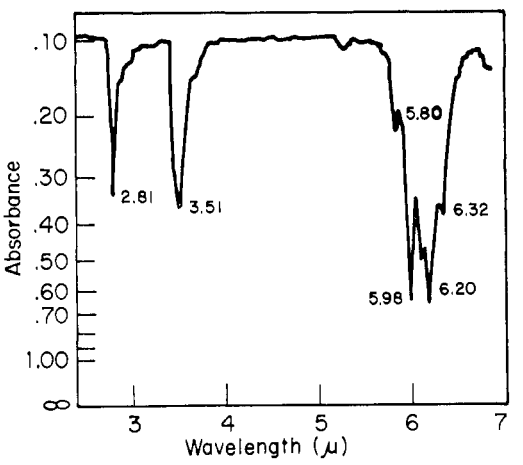

Figure 4. Infrared spectrum of malonaldehyde in dichloromethane

$5 \times 10^{-1}M$ MA acetal hydrolyzate was extracted with dichloromethane

therefore, can be assigned to C=C—C=O conjunction which is presumably stabilized by intermolecular hydrogen bonding (Rassmussen *et al.*, 1949), but not by intramolecular bonding. The 0.5*M* MA solution was light brown in color and there was a precipitate, suggesting the presence of polymeric MA. As pointed out earlier, however, Saunders and May (1963) attributed their infrared spectrum, obtained with a dichloromethane extract from 0.5*M* MA acetal hydrolyzate, to the chelated form. These combined observations suggest that their MA preparation might have been somewhat aggregated, and thus confused their interpretation of the spectrum.

Malonaldehyde occurs mainly as the enol in water and predominantly as the diketo form in nonpolar solvents with only a minute amount as its enolic tautomer. Thus, MA behaves more as the *trans*-fixed β-dicarbonyls (Eistert and Reiss, 1954a,b) rather than as the open chain β-dicarbonyl compounds (Kabachnik *et al.*, 1961; Sidgwick, 1925). Furthermore, the occurrence of the enolic tautomer as the *sym-trans* form, but not as the chelated form in such solvents is indeed a special feature of the MA molecule. When both H atoms at carbon numbers 1 and 3 diketo form of MA are substituted by either methyl and ethoxy groups (as in ethyl acetoacetate), or two methyl groups (as in acetylacetone), or where only one H atom is replaced by a methyl group (as in acetyl acetaldehyde (Bothner-By and Harris, 1965)), the substituted compounds occur predominantly in the chelated form in nonpolar solvents.

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