Electronic and Vibrational Spectral Investigation of the Molecular Association of the All-Trans Isomers of Retinal, Retinol, and Retinoic Acid

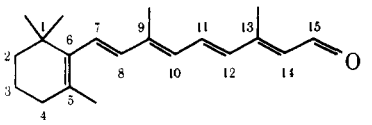
Kohji Chihara and Walter H. Waddell*

Contribution from the Department of Chemistry, Carnegie-Mellon University, Pittsburgh, Pennsylvania 15213. *Received November 26, 1979*

Abstract: Electronic (ultraviolet–visible) and vibrational (infrared) absorption spectra are recorded for the all-trans isomers of retinal, retinol, and retinoic acid in nonpolar solutions as a function of the polyenes concentration and of temperature. Although absorption spectral changes are not observed for *all-trans*-retinal, the vibrational spectra of *all-trans*-retinol and the electronic and vibrational spectra of *all-trans*-retinoic acid are sensitive to the polyenes concentration and to temperature. Their spectral changes are consistent with the formation of molecular aggregates as a result of intermolecular hydrogen bonding between the hydrogen and oxygen atoms of the alcoholic group for retinol and between the hydrogen and carbonyl oxygen atoms of the carboxylate group for retinoic acid. Values for the thermodynamic parameters of the molecular association, the equilibrium constant, enthalpy, and Gibbs free energy, are determined. The nature of the molecular aggregates formed by retinol and retinoic acid is discussed.

Introduction

Owing to the importance of the retinyl chromophore as the light-absorbing species of the visual protein rhodopsin,[1] and the proteins bacteriorhodopsin[2] and retinochrome,[3] the photochemical and spectroscopic properties of *all-trans*-retinal (structure **1**), related linear polyenes, and their configurational



all-trans-retinal, **1**

isomers have been the subject of extensive investigation. A number of interesting observations have been reported.[4-6] For example, the all-trans isomers of retinal (**1**),[7-9] retinol (**2**),[10,11] retinoic acid (**3**),[12] and retinal Schiff bases (**4**)[9] have absorption spectra that are characterized by a first band maximum that is broad and diffuse, even when recorded in a low-temperature matrix. The first band maximum of a nonpolar solution of *all-trans*-retinol undergoes a hypsochromic shift[11] (shift to higher energy; blue shift) upon lowering temperature, whereas the maxima of the other polyenes undergo a bathochromic shift (red shift). An unusually large "Stokes" shift exists between the absorption and fluorescence band maxima of these polyenes.[10] For *all-trans*-**1**[13-16] and *all-trans*-**2**,[13,17] a notable difference is found between the intrinsic fluorescence lifetime (τ_F^0) obtained by integration of the intensity of the first absorption band and that obtained by direct measurement of the radiative lifetime (τ_F) and fluorescence quantum yield (ϕ_F). One of the most interesting spectroscopic characteristics of *all-trans*-retinal is that ϕ_F is dependent upon the wavelength of the exciting light,[18-22] behavior not observed for polyenes **2–4.** In general, it is thought that many of these spectroscopic anomalies can be rationalized by the presence of a forbidden A_g-like excited singlet state of energy comparable to that of the allowed B_u excited singlet state, the state responsible for the large oscillator strength associated with the first band maximum in the absorption spectra of these retinyl polyenes[23] and related molecules.[24-28] The excited-state properties of *all-trans*-retinal are further complicated by the presence of an n,π^* excited singlet state of energy comparable to that of the two π,π^* excited singlet states.

Recently, it has been suggested that the wavelength dependence of ϕ_F of *all-trans*-retinal can be attributed to the presence of hydrogen-bonding species in solution or to the formation of retinal dimers.[14,15] In addition, the formation of dimers is also thought to account for the observed shifts in the position of the first band maxima of *all-trans*-retinal[29] with varying concentration and of *all-trans*-retinol[11] upon cooling a room temperature 3-methylpentane solution to 77 K. In order to obtain direct evidence for the existence of any molecular association for the retinyl polyenes, we have made a detailed investigation of the absorption spectral properties of the all-trans isomers of retinol, retinal, and retinoic acid. In particular, high-resolution infrared absorption spectra were recorded as a function of the polyenes concentration and of temperature, and ultraviolet–visible absorption spectra were recorded as a function of the polyenes concentration.

Experimental Section

Materials. *all-trans*-Retinol (**2**), *all-trans*-retinal (**1**), and *all-trans*-retinoic acid (**3**) were purchased from the Sigma Chemical Co. All compounds were stored under refrigeration in the dark. *all-trans*-Retinal and *all-trans*-retinoic acid were purified to >99% using high-pressure liquid chromatographic (LC) techniques. *all-trans*-Retinol was obtained in sealed vials as 96–98% pure and could not be further purified by LC methods. Thus, solutions of **2** were prepared using freshly opened vials and absorption spectra recorded immediately.

3-Methylpentane (3MP) was purchased from Phillips Petroleum Co. as 99+% pure and was refluxed and distilled from Dri-Na (Baker Chemical Co.) immediately prior to use. Carbon tetrachloride (CCl_4) was purchased from Fisher Scientific Co. and was stored over a Type 4AM-514 molecular sieve and filtered prior to use.

Methods. LC purifications were accomplished using the chromatographic conditions previously described,[30] namely, a Waters ALC/GPC 204 liquid chromatograph, a 12 × ¼ in. μ-Porasil column, 2–5% anhydrous ether in hexane, 2–3 mL/min, 1200–1500 psi, and 365-nm UV detection.

Electronic absorption spectra were recorded on a Perkin-Elmer Model 575 spectrophotometer using quartz cells of varying path length (Precision Cells).

Infrared absorption spectra were recorded on a Perkin-Elmer CDS/2 system that consists of a Perkin-Elmer 580 infrared spectrophotometer, Interdata 6/16 64 Kbyte computer, and Interdata Teletypewriter. Solution spectra were recorded in CCl_4 using a 0.1-mm NaCl solution cell. The infrared spectra of polyenes **1–3** were corrected by computer subtraction of the solvents spectrum which was recorded under identical conditions. Solvent cast films were recorded on newly polished sodium chloride plates. All spectra were recorded in the ratio-recording mode and are the average of at least two accumulated 16-min scans. All infrared spectral data were stored on cassette tapes, and data manipulations (accumulation, expansion, nor-

0002-7863/80/1502-2963$01.00/0

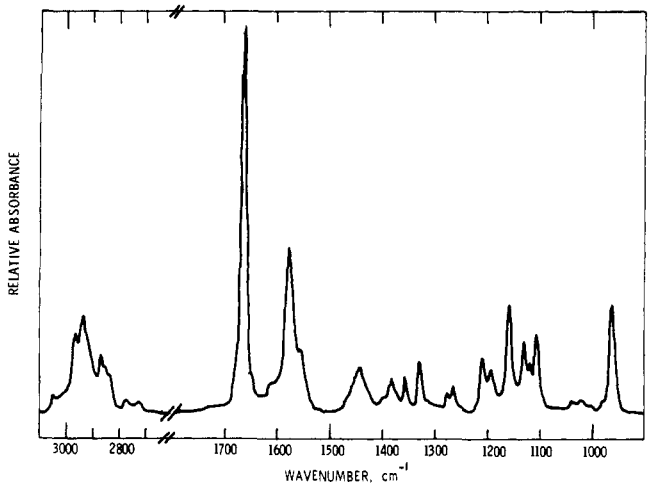


Figure 1. The room temperature infrared absorption spectrum of *all-trans*-retinal in carbon tetrachloride solution; average of two accumulated scans.

malization, smoothing, subtraction) were automatically performed using the Interdata 6/16 computer.

Temperatures of 0 and −20 °C (±1 °C) were obtained by heating nitrogen vapors that were delivered from a reservoir of liquid nitrogen into a double-jacketed metal Dewar equipped with sodium chloride windows. All chromatography and spectroscopy were performed under red room lighting.

Results

Electronic (ultraviolet-visible) absorption spectra of the all-trans isomers of retinal (**1**), retinol (**2**), and retinoic acid (**3**) were recorded at wavelengths between 200 and 600 nm in the nonpolar solvents 3-methylpentane (3MP) and carbon tetrachloride (CCl_4) at room temperature as a function of polyene concentration and in 3MP at 77 K. Vibrational (infrared) absorption spectra of the all-trans isomers of retinyl polyenes **1–3** were recorded at frequencies between 4000 and 900 cm^{-1} in CCl_4 as a function of polyene concentration at room temperature (28 °C). Infrared spectra of **2** and **3** were also recorded at 0 and −20 °C. Solvent cast films on sodium chloride plates were recorded at room temperature for each polyene. Owing to the insolubility of polyenes **1–3** and the complexity of the infrared absorption spectrum of 3-methylpentane, useful infrared spectra were not obtained in that solvent.

***all-trans*-Retinal (1).** Electronic absorption spectra were recorded at room temperature for six solutions of **1** in 3MP with concentrations ranging from 5×10^{-6} to 1.2×10^{-3} M. For each solution, the first absorption band maximized (λ_{max}) at 369 nm. In addition, no variation is observed for the molar extinction coefficient (ϵ_{max} 50 000 M^{-1} cm^{-1}) or the bandwidth at half-height (h = 5610 cm^{-1}) beyond experimental reproducibility (±2%). At 77 K in 3MP λ_{max} = 385 nm (ϵ_{max} 54 000 M^{-1} cm^{-1}) and in room temperature solutions of carbon tetrachloride λ_{max} = 381 nm (ϵ_{max} 48 000 M^{-1} cm^{-1}) and h = 4420 cm^{-1}. All electronic absorption spectral data are essentially identical with that previously reported.[7,9,31,32]

Infrared absorption spectra of **1** in CCl_4 were recorded for five room temperature solutions ranging in retinal concentration from 5.9×10^{-3} to 9.5×10^{-2} M. The five spectra displayed no notable differences in the 4000–900-cm^{-1} energy region, Figure 1; hence, temperature-dependent infrared spectral studies were not performed. Table I is a list of the notable infrared bands observed for **1** and their vibrational assignments based upon characteristic infrared frequencies[33,34] and resonance Raman data of the retinyl polyenes.[35-39]

***all-trans*-Retinol (2).** Electronic absorption spectra of **2** were recorded at room temperature for 3MP solutions ranging in concentration from 1×10^{-6} to 2.6×10^{-3} M and for CCl_4 solutions ranging in concentration from 3×10^{-6} to 4.8×10^{-3} M. In addition, electronic absorption spectra were measured at 77 K for 3MP solutions ranging in concentration from 5.4×10^{-6} to 3.5×10^{-3} M. For these three sets of spectral measurements, there is no detectable change in either the maximum of the first absorption band, its molar extinction coefficient, or its width at half-height that results from a change in the polyenes concentration. At room temperature in a 3MP solution λ_{max} = 325 nm, ϵ_{max} = 48 500 M^{-1} cm^{-1}, and h = 5380 cm^{-1}. λ_{max} = 333 nm for a CCl_4 solution of **2**. At 77 K in a 3MP matrix, λ_{max} = 322 nm. These data are essentially identical with that previously reported.[10,11]

Infrared absorption spectra were recorded for four CCl_4 solutions of **2** ranging in concentration from 1.9×10^{-2} to 1.9×10^{-1} M. Notable infrared spectral changes are immediately evident, the most significant of which occurs in the 3700–3100-cm^{-1} region, Figure 2, whereby the relative absorbance of the sharp band at 3619 cm^{-1} observed in the 1.9×10^{-2} M solution decreases with increasing retinol concentration. A broad absorption that maximizes at approximately 3400 cm^{-1} becomes evident for a 4.7×10^{-2} M solution and is observed to both increase in relative area and undergo a slight bathochromic shift as the concentration of retinol is further increased.[40] Indeed the infrared absorption spectrum of a solvent cast film of **2** reveals the total absence of the 3619-cm^{-1} band. The relative absorbance of the infrared band at 961 cm^{-1} is also sensitive to variations in the retinol concentration.

In addition, the infrared absorption spectrum of a 1.9×10^{-1} M CCl_4 solution of **2** was recorded at frequencies between 4000 and 3000 cm^{-1} at +28 (room temperature), 0, and −20 °C. These spectra indicate, that upon cooling, the 3619-cm^{-1} band decreases in relative intensity as the broad band at 3600–3200 cm^{-1} both increases in relative intensity and undergoes a bathochromic shift,[40] Figure 3. Table I also lists the infrared vibrations observed for a 1.9×10^{-2} M solution of **2** in CCl_4.

***all-trans*-Retinoic Acid (3).** Room temperature electronic absorption spectra were recorded for nine solutions of **3** in CCl_4

Table I. Infrared Vibrations of *all-trans*-Retinal, *all-trans*-Retinol, and *all-trans*-Retinoic Acid[a]

assignment[b]	frequency, cm^{-1} [c] *all-trans*-retinal	*all-trans*-retinol	*all-trans*-retinoic acid
O—H stretch, nonassociated		3619	3538
O—H stretch, associated		3600–3200	3400–2400
C—H stretch, olefinic	3040	3036	3038
C—H stretch, aliphatic[d]	2960–2820	2960–2820	2960–2820
C—H stretch, aldehydic	2718–2762		
C=O stretch, nonassociated	1660		1722
C=O stretch, associated			1680
C=C stretch	1575[e]	1570[e]	1603, 1575
C—H bend, methyl group	1443	1442	1439
C—O stretch, associated			1343
C—O stretch, nonassociated		961	1102

[a] Carbon tetrachloride solutions, room temperature. [b] See ref 33–39. [c] ±1 cm^{-1}. [d] Several peaks; see Figures 1 and 4. [e] A number of weak bands appear in the 1620–1550-cm^{-1} region.

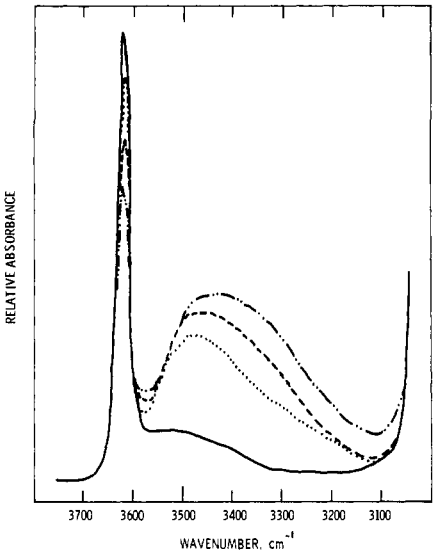


Figure 2. Room temperature infrared absorption spectra of *all-trans*-retinol in carbon tetrachloride solution: 1.9×10^{-2} M (—), eight scans; 4.7×10^{-2} M (·····), eight scans; 1.1×10^{-1} M (- - - - -), four scans; 1.9×10^{-1} M (- · - · - · -), four scans. The intensities of these spectra were normalized to the 3036-cm^{-1} band (not shown).

Table II. Electronic Absorption Spectral Data for *all-trans*-Retinoic Acid

concn, M[a]	λ_{max}, nm[b]	ϵ_{398nm}, M^{-1} cm^{-1} [c]
5.5×10^{-6}	368	30 000
1.1×10^{-5}	368.5	29 200
2.8×10^{-5}	369	26 500
5.5×10^{-5}	370	24 300
2.8×10^{-4}	372	23 800
5.5×10^{-4}	373	21 300
1.1×10^{-3}	374	20 200
1.8×10^{-3}	374.5	19 700
5.5×10^{-3}	376	19 200

[a] Carbon tetrachloride solutions. [b] Room temperature, ±0.5 nm. [c] ϵ_{max} = 40 500 ± 500 M^{-1} cm^{-1} for solutions up to 1.1×10^{-3} M.

ranging in concentration from 5.5×10^{-6} to 5.5×10^{-3} M. Both λ_{max} and ϵ_{398nm} (calculated assuming no association) are dependent upon the varying retinoic acid concentration, whereby λ_{max} undergoes a bathochromic shift and ϵ_{398nm} decreases in intensity with increasing retinoic acid concentration. The measured band width at half-height (h) decreases continually from 5390 cm^{-1} for the dilute solution to 4980 cm^{-1} for the concentrated retinoic acid solution. Table II is a summary. It is also observed that upon addition of ca. 10% (by volume) anhydrous ether to the CCl_4 solution a hypsochromic shift occurs for the first band maximum, λ_{max} = 360 nm. Similar electronic absorption spectral results are observed for 3MP solutions of **3** at room temperature, namely, the variation in λ_{max}, ϵ, and h with retinoic acid concentration.[41]

Infrared absorption spectra were recorded at room temperature for four solutions of **3** in CCl_4 ranging in concentration from 3.8×10^{-3} to 3×10^{-2} M. Infrared spectral differences are observed for the four retinoic acid solutions, the most notable of which are for bands at 3538, 3400-2400, 1722, 1680, and 1102 cm^{-1} that have relative intensity changes with varying concentration of **3**, Figure 4. Similar spectral changes are observed for a solution whose infrared spectra are recorded at room temperature (28), 0, and −20 °C. The infrared spectrum recorded as an evaporated film on a NaCl plate does not exhibit bands at 3538, 1722, and 1102 cm^{-1}. Table I lists the infrared vibrations observed for a 3.8×10^{-3} M solution of **3** in CCl_4.

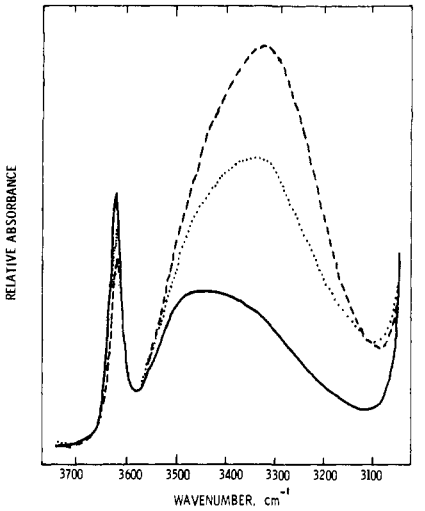


Figure 3. Infrared absorption spectra of a 1.9×10^{-1} M carbon tetrachloride solution of *all-trans*-retinol at 28 (—), 0 (·····), and −20 °C (- - - - -). Each spectrum is the average of four accumulated scans. The intensities of these spectra were normalized to the 3036-cm^{-1} band (not shown).

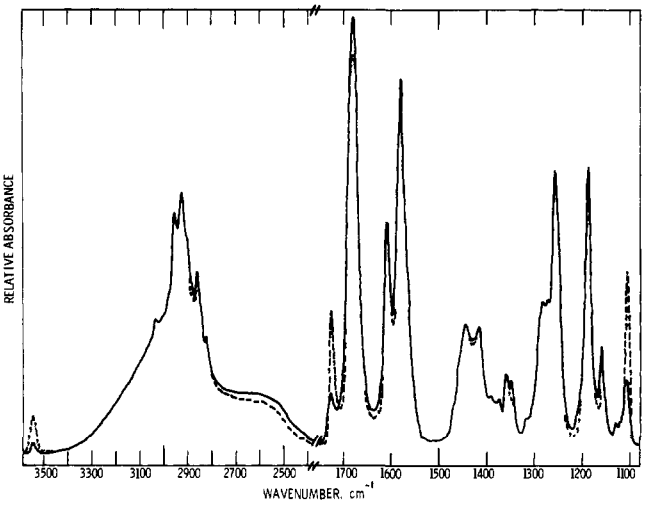


Figure 4. Infrared absorption spectra of *all-trans*-retinoic acid in carbon tetrachloride solution: 3.8×10^{-3} M (- - - - -), 48 scans; 3.0×10^{-2} M (—), 6 scans. Intensities were normalized to the 1575-cm^{-1} band.

Discussion

***all-trans*-Retinal (1).** The most notable result to be drawn from the electronic and vibrational spectral studies of *all-trans*-retinal in carbon tetrachloride solutions is that no positive evidence exists for any molecular association in the 5×10^{-6} to 10^{-1} M concentration range at room temperature. Based solely upon electronic absorption spectral studies, it may be possible to extend this result to the hydrocarbon solvent 3-methylpentane since the spectral behavior of **1** in the two nonpolar solvents parallels. This conclusion is contrary to that reached by Moore and Song[29] based upon electronic absorption and fluorescence spectral measurements; however, fluorescence measurements were made at 77 K, a temperature at which Takemura and co-workers[15] also provide fluorescence spectral evidence for the existence of dimers of *all-trans*-retinal. Finally, Fugate and Song[16] report fluorescence lifetime data measured at 77 K in which ϕ_F, but not τ_F, was found to vary with excitation wavelength. The invariance of τ_F with excitation wavelength suggests that one emitting species is present. A one-species emission is consistent with the results of Takemura and co-workers,[15] who conclude that the monomer of *all-trans*-retinal does not fluoresce (in non-hydrogen-bonding

Table III. Thermodynamic Data for the Molecular Association of *all-trans*-Retinol and *all-trans*-Retinoic Acid[a]

molecule	t, °C[b]	K, M^{-1}	$-\Delta G°$, kcal/mol	$-\Delta H°$, kcal/mol
all-trans-retinol	28	2.2 ± 1.0	0.48	2.1 ± 0.7
	0	3.2 ± 1.2	0.63	
	−20	7.0 ± 1.5	0.98	
all-trans-retinoic acid	28	1100[c]	4.2	7.7
	0	3900	4.5	
	−20	11 000	4.7	

[a] Carbon tetrachloride solutions. [b] ±1 °C. [c] $K = 1000 \pm 300$ M^{-1} from the electronic absorption spectral data; $K \sim 10^4$ in 3MP based upon electronic absorption spectral data (ref 12).

solvents), but that the dimer is an emitting species. Our result, that *all-trans*-retinal does not associate in nonpolar solvents at room temperature, is not inconsistent with conclusions based upon fluorescence studies since these latter studies were made at 77 K.

***all-trans*-Retinol (2).** Electronic absorption spectral data failed to substantiate the occurrence of a concentration-dependent phenomenon for nonpolar solutions of *all-trans*-retinol at concentrations less than approximately 5×10^{-3} M. Alternatively, room temperature infrared spectral data indicate that three bands are particularly sensitive to varying retinol concentration ([**2**]). They are the 3619-, ca. 3400-, and 961-cm^{-1} bands, which are readily assigned as arising from an O-H stretching vibration of the nonassociated (monomeric) and associated forms of the alcohol and its C-O stretching vibration,[33,34] respectively. The relative intensity of the 3619-cm^{-1} band decreases with increasing [**2**] while that of the broad 3400-cm^{-1} band increases with increasing [**2**], Figure 2. The relative intensities of the remaining infrared bands are essentially unaffected by changes in the retinol concentration.

Similar spectroscopic behavior is also observed for a solution of *all-trans*-retinol in which the infrared spectra are recorded at various temperatures. However, one difference is that the broad band that maximizes at 3440 cm^{-1} in the room temperature spectrum of **2** undergoes a notable bathochromic shift as the temperature is decreased. At 0 and −20 °C, the band maximizes at 3330 and 3300 cm^{-1}, respectively, Figure 3.

From the relative intensity changes observed for the O-H stretching vibration due to the associated and nonassociated forms of *all-trans*-retinol with varying concentration and temperature, it is possible to calculate thermodynamic values of the molecular association.[42-44] The equilibrium constant (K), enthalpy change ($\Delta H°$), and Gibbs free energy change ($\Delta G°$) were estimated using eq 1-4.

$$K = x(1 - 2x)^{-2}C^{-1} \quad (1)$$

where x denotes the molar fraction of the associated form of the alcohol and is equal to or less than $0.5C$, C is the concentration of the sample (nonassociated) in moles/liter (M), and x is determined from

$$(I_A/I_N)(\Delta I_N/\Delta I_A) = x(1 - 2x)^{-1} \quad (2)$$

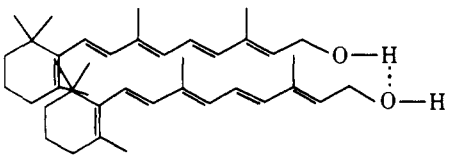
at a constant temperature and specified concentration. I_A and I_N denote the integrated intensities of the associated and nonassociated O-H bands,[40] respectively. $\Delta I_N/\Delta I_A$ was estimated by measuring the increase in I_A and decrease in I_N upon lowering the temperature. $\Delta H°$ and $\Delta G°$ were evaluated using the following equations:

$$(\delta \ln K/\delta T)_p = \Delta H°/RT^2 \quad (3)$$

$$\Delta G° = -RT \ln K \quad (4)$$

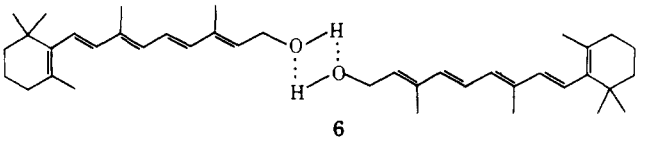
The concentration change of the sample with varying temperature was taken into account. Table III is a summary.

Although the infrared spectral measurements do not yield exact information on either the number of hydrogen bonds formed by each retinol molecule or the number of retinol molecules per aggregate, it is possible to gain insight into the structure of the molecular aggregate. For example, the absence of electronic absorption and certain vibrational absorption (C=C, C—C, or C—H) spectral changes at room temperature with varying retinol concentration eliminates a molecular association for which a complete or partial "card-stacked" structure is present (for example, structure **5**), since such

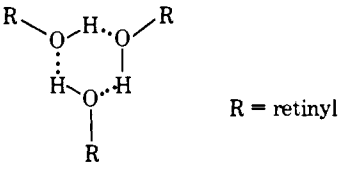


5

structures would probably have an exciton interaction between the two π systems of sufficient magnitude to produce electronic absorption spectral changes, which are not observed. Alternatively, the vibrational data require that the hydrogen and oxygen atoms of the alcoholic functional group participate in hydrogen bonding. Owing to the small value of the equilibrium constant for association (K), the molecular aggregate is probably composed of only a limited number of retinol molecules, for example, a dimer (**6**) or trimer (**7**). For a molecular



6



R = retinyl

7

aggregate having a small equilibrium constant it is still possible to form trimers or tetramers.[45] For example, methanol is thought to exist as a tetramer, ethanol as a trimer or tetramer, and *tert*-butyl alcohol as a trimer.[46] Also, it is thought that stronger hydrogen bonds are formed for a cyclic structure.[47] The thermodynamic data indicates that dimer formation is reasonable; however, if trimers are the only aggregates, K is calculated to decrease with increasing retinol concentration.

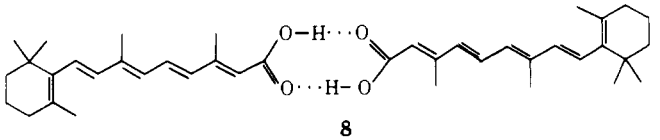
The spectral broadness and shift in the position of the O-H stretching frequency due to the associated species observed for the room temperature vibrational spectra at varying [**2**] clearly indicate the complex nature of the molecular aggregate. Indeed, it is probable that dimers and trimers exist in an equilibrium mixture at room temperature,[48] and/or dimers of varying conformations exist. The variable-temperature vibrational data reveal a more pronounced bathochromic shift with lowering temperature. These spectral shifts may result from a population redistribution between dimers and trimers. Alternatively, it is possible that at the lower temperatures a conformational change results. For example, a "card-stacking" arrangement may occur in addition to hydrogen bonding. Such a geometric arrangement is thought to exist for *all-trans*-retinol in low-temperature 3MP matrices.[11]

***all-trans*-Retinoic Acid (3).** From the electronic absorption spectral data (Table II), it is clear that some form of molecular phenomenon is occurring for *all-trans*-retinoic acid as its concentration ([**3**]) is varied in the carbon tetrachloride solu-

tions. Room temperature infrared spectral measurements (Table I and Figure 4) reveal that in particular the bands at 3538, 1722, and 1102 cm^{-1} are quite sensitive to the variation in [3], whereby their relative intensities decrease with increasing retinoic acid concentration. These bands may be readily assigned as the O—H, C=O, and C—O stretching vibrations of the nonassociated (monomeric) form of the carboxylic acid,[33,34] respectively. Additional stretching frequencies of retinoic acid in the more concentrated solutions are observed at 3400-2400, 1680, and 1343 cm^{-1}, respectively. These bands increase in relative intensity with increasing [3]. Thus, the molecular phenomenon is a molecular association, probably dimer or trimer formation. The vibrational data then implies that the molecular phenomenon that causes the electronic absorption spectral changes for the more dilute CCl_4 solutions is also a molecular association. In addition, the relative intensities of the other infrared vibrations are essentially unaffected by the variation in retinoic acid concentration. Similar spectral results are observed when a room temperature solution is cooled to 0 °C and then to −20 °C, with infrared spectra recorded at all three temperatures.

From the infrared intensity changes observed for the O—H and C=O stretching vibrations of the associated and monomer bands with temperature, K, $\Delta H°$, and $\Delta G°$ for aggregation of *all-trans*-retinoic acid were estimated using eq 1-4. K, the equilibrium constant, was also evaluated from the electronic absorption spectral data. Table III is a summary. These thermodynamic parameters are similar to those estimated for *all-trans*-retinoic acid based upon absorption and fluorescence spectral measurements in 3MP[12] and to those of benzoic acid in nonpolar solvents as determined by infrared spectroscopy.[49]

Utilization of the experimental data to construct a geometry for the retinoic acid molecular aggregate must include the fact that relative intensity shifts are observed for the O—H, C=O, and C—O stretching vibrations with changes in [3] and temperature, but that the relative intensities of the C=C, C—C, C—H stretching vibrations do not change appreciably with [3]. Structure **8**, a cyclic dimer in which an eight-membered



8

ring is formed, is proposed to account for the observed spectral characteristics, including the hypsochromic shift in the C-O stretching vibration upon dimer formation.[33] Recall that a loss in relative intensity of the 1102-cm^{-1} band is observed with increasing *all-trans*-retinoic acid concentration. Carboxylic acids are generally assumed to form cyclic dimers in nonpolar solvents,[50] although it has been suggested[51] that they may exist as an equilibrium mixture of open-chain and cyclic structures.

Summary

The absence of electronic and vibrational absorption spectral changes with varying *all-trans*-retinal concentration implies that a molecular association phenomenon is not occurring at room temperature in nonpolar solvents. Although the electronic absorption spectral characteristics of *all-trans*-retinol do not vary with concentration in the 10^{-6}-10^{-3} M range, vibrational spectra recorded in carbon tetrachloride solution at polyene concentrations of up to 10^{-1} M provide positive evidence for molecular association. The nature of the molecular aggregates, which result from intermolecular hydrogen bonding, depends upon the polyenes concentration and the temperature. At room temperature, it is thought that an equilibrium mixture of dimers and trimers exists, and at lower temperatures exciton interactions may also occur between these aggregate components. *all-trans*-Retinoic acid is thought to form a tail-to-tail, cyclic dimer involving two hydrogen bonds per aggregate. The calculated thermodynamic values for the association of retinoic acid are similar to those of benzoic acid measured in nonpolar solvents.

Acknowledgments. The authors wish to thank Dr. Usama E. Younes for technical assistance at the initiation of this research. We are indebted to the National Eye Institute, National Institutes of Health (Grants EY 01777 and EY 01930), for their generous support.

References and Notes

(1) R. Hubbard and G. Wald, *J. Gen. Physiol.*, **36**, 269 (1952).
(2) D. Oesterhelt and W. Stoeckenius, *Nature (London), New Biol.*, **233**, 149 (1971).
(3) T. Hara and R. Hara, *Nature (London)*, **214**, 573 (1967).
(4) For a review see B. Honig and T. G. Ebrey, *Annu. Rev. Biophys. Bioeng.*, **3**, 151 (1974), and references cited therein.
(5) For a review see B. S. Hudson and B. E. Kohler, *Annu. Rev. Phys. Chem.*, **25**, 437 (1974), and references cited therein.
(6) For a review see B. Honig, A. Warshel, and M. Karplus, *Acc. Chem. Res.*, **8**, 92 (1975), and references cited therein.
(7) R. S. Becker, K. Inuzuka, and D. E. Balke, *J. Am. Chem. Soc.*, **93**, 38 (1971).
(8) R. L. Christensen and B. E. Kohler, *Photochem. Photobiol.*, **18**, 293 (1973).
(9) A. M. Schaffer, W. H. Waddell, and R. S. Becker, *J. Am. Chem. Soc.*, **96**, 2063 (1974).
(10) A. J. Thomson, *J. Chem. Phys.*, **51**, 4106 (1969).
(11) K. Chihara, T. Takemura, T. Yamaoka, N. Yamamoto, A. M Schaffer, and R. S. Becker, *Photochem. Photobiol.*, **29**, 1001 (1979).
(12) T. Takemura, K. Chihara, R. S. Becker, P. K. Das, and G. Hug, *J. Am. Chem. Soc.*, **102**, 2604 (1980).
(13) P.-S. Song, Q. Chae, M. Fujita, and H. Baba, *J. Am. Chem. Soc.*, **98**, 819 (1976).
(14) T. Takemura, P. K. Das, G. Hug, and R. S. Becker, *J. Am. Chem. Soc.*, **100**, 2626 (1978).
(15) T. Takemura, G. Hug, P. K. Das, and R. S. Becker, *J. Am. Chem. Soc.*, **100**, 2631 (1978).
(16) R. D. Fugate and P.-S. Song, *J. Am. Chem. Soc.*, **101**, 3408 (1979).
(17) J. P. Dalle and B. Rosenberg, *Photochem. Photobiol.*, **12**, 151 (1970).
(18) D. E. Balke and R. S. Becker, *J. Am. Chem. Soc.*, **89**, 5061 (1967).
(19) D. E. Balke and R. S. Becker, *J. Am. Chem. Soc.*, **90**, 6710 (1968).
(20) R. S. Becker, K. Inuzuka, J. King, and D. E. Balke, *J. Am. Chem. Soc.*, **93**, 43 (1971).
(21) W. H. Waddell, A. M. Schaffer, and R. S. Becker, *J. Am. Chem. Soc.*, **95**, 8223 (1973).
(22) R. L. Christensen and B. E. Kohler, *Photochem. Photobiol.*, **19**, 401 (1974).
(23) R. R. Birge, J. A. Bennett, B. M. Pierce, and T. M. Thomas, *J. Am. Chem. Soc.*, **100**, 1533 (1978).
(24) B. S. Hudson and B. E. Kohler, *Chem. Phys. Lett.*, **14**, 299 (1972).
(25) B. S. Hudson and B. E. Kohler, *J. Chem. Phys.*, **59**, 4984 (1973).
(26) R. L. Christensen and B. E. Kohler, *J. Chem. Phys.*, **63**, 1837 (1975).
(27) J. K. McVey and S. A. Rice, *J. Chem. Phys.*, **68**, 522 (1978).
(28) K. Schulten, I. Ohmine, and M. Karplus, *J. Chem. Phys.*, **64**, 4422 (1976).
(29) T. A. Moore and P.-S. Song, *Nature (London), New Biol.*, **243**, 30 (1973).
(30) W. H. Waddell, P. M. Dawson, D. L. Hopkins, K. L. Rach, M. Uemura, and J. L. West, *J. Liq. Chromatogr.*, **2**, 1205 (1979).
(31) R. S. Becker, G. Hug, P. K. Das, A. M. Schaffer, T. Takemura, N. Yamamoto, and W. H. Waddell, *J. Phys. Chem.*, **80**, 2265 (1976).
(32) R. Hubbard, *J. Am. Chem. Soc.*, **78**, 4662 (1956).
(33) For example, see N. B. Colthup, L. H. Daly, and S. E. Wiberley, "Introduction to Infrared and Raman Spectroscopy", Academic Press, New York, 1975.
(34) For example, see J. B. Lambert, H. F. Shurvell, L. Verbit, R. G. Cooks, and G. H. Stout, "Organic Structural Analysis", Macmillan, New York, 1976.
(35) For a review see R. Callender and B. Honig, *Annu. Rev. Biophys. Bioeng.*, **6**, 33 (1977).
(36) For a review see A. Warshel, *Annu. Rev. Biophys. Bioeng.*, **6**, 273 (1977).
(37) L. Rimai, D. Gill, and J. L. Parsons, *J. Am. Chem. Soc.*, **93**, 1353 (1971).
(38) A. Warshel and M. Karplus, *J. Am. Chem. Soc.*, **96**, 5677 (1974).
(39) R. E. Cookingham, A. Lewis, and A. T. Lemley, *Biochemistry*, **17**, 4699 (1978).
(40) Integrated areas were obtained by multiple planimeter tracings of the infrared absorption bands assuming Gaussian shapes. The bands are sufficiently resolved to make this approximation without introducing substantial errors.
(41) K. Chihara and R. S. Becker, unpublished results.
(42) Thermodynamic values were calculated by assuming that one hydrogen bond is formed per molecular aggregate and that the molecular aggregate is a dimer. Assuming trimer formation only, K is calculated to decrease with increasing retinol concentration.
(43) G. N. Lewis and M. Randall, revised by K. S. Pitzer and L. Brewer, "Thermodynamics", McGraw-Hill, New York, 1961.

(44) H. Hosaya, J. Tanaka, and S. Nagakura, *J. Mol. Spectrosc.*, **8**, 257 (1962).
(45) M. D. Joesten and L. J. Shaad, "Hydrogen Bonding", Marcel Dekker, New York, 1974.
(46) J. F. Feeney and S. M. Walker, *J. Chem. Soc. A*, 1148 (1966).
(47) L. J. Bellamy and R. J. Pace, *Spectrochim. Acta*, **22**, 525 (1966).
(48) S. N. Vinogvadov and R. H. Linnell, "Hydrogen Bonding", Van Nostrand-Reinhold, Princeton, N.J., 1971.
(49) G. Allen, J. G. Watkinson, and K. H. Webb, *Spectrochim. Acta*, **22**, 807 (1966).
(50) For a review see L. Eberson, "Chemistry of Carboxylic Acids and Esters", S. Patai, Ed., Interscience, New York, 1969.
(51) L. J. Bellamy, R. F. Lake, and R. J. Pace, *Spectrochim. Acta*, **19**, 443 (1963).

Regiospecific Coordination of Ambidentate Tetrazoles to Cobalt Oximes

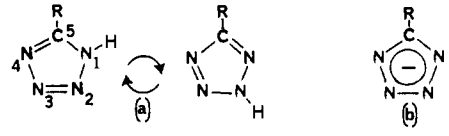
Nicholas E. Takach,[1a] Elizabeth M. Holt,[1b] Nathaniel W. Alcock,[1c] Ronald A. Henry,[1d] and John H. Nelson*[1a]

Contribution from the Department of Chemistry, University of Nevada, Reno, Nevada 89557, the Departments of Chemistry and Biochemistry, University of Georgia, Athens, Georgia 30602, the Department of Chemistry and Molecular Sciences, University of Warwick, Coventry, England CV47AL, and the Chemistry Division, China Lake Naval Weapons Center, China Lake, California 93555.
Received October 2, 1979

Abstract: A series of complexes of the type (n-Bu_3P)$Co(DH)_2$(5R-tetrazolate) (DH is the monoanion of dimethylglyoxime; R = CF_3, CH_3, C_6H_5, $C_6H_5CH_2$, $(CH_3)_2N$, 4-FC_6H_4, and 3-FC_6H_4) have been prepared and characterized by conductance studies, elemental analyses, and ^1H, $^{13}C\{^1H\}$, $^{31}P\{^1H\}$, and ^{19}F NMR spectroscopy. Quantum-mechanical calculations (MINDO/3) indicate that the N_1 nitrogens of the aromatic tetrazolate anion are slightly more nucleophilic than the N_2, yet in each complex the ambidentate tetrazolate anion is coordinated to cobalt via the N_2 nitrogen, showing that regiospecific coordination is sterically induced. This is in marked contrast to tetrazole complexes of platinum and palladium wherein both N_1 and N_2 bound tetrazoles are found in approximately equal abundances. These cobalt complexes react with alkyl halides such as CH_3I and $C_6H_5CH_2Br$ to produce exclusively 1,5-disubstituted tetrazoles. None of the isomeric 2,5-disubstituted tetrazole is detected in these reactions in marked contrast to the reactions of sodium tetrazolates or gold, palladium, or platinum tetrazolate complexes with alkyl halides which produce mixtures of the 1,5- and 2,5-disubstituted tetrazoles. The crystal structure of n-$Bu_3PCo(DH)_2$(5-CF_3-tetrazolate) was determined using three-dimensional X-ray diffraction techniques. The molecule crystallizes in the orthorhombic space group *Pbca* in a unit cell of dimensions a = 12.040 (2) Å, b = 21.531 (3) Å, c = 23.536 (4) Å, ρ_{calcd} = 1.368 g/cm^3, ρ_{obsd} = 1.374 g/cm^3. Refinement converged to 5.4% with 2525 independent reflections. The tetrazolato ring is coordinated to cobalt via N_2 and is planar. The Co–P bond (2.263 Å) is one of the shortest observed in $LCo(DH)_2X$ structures. The acute dihedral angle between the planes of the two glyoximato groups (10.4°) is among the largest dihedral angles observed in structures of this type. Surprisingly, the tetrazolato ring does not lie in the fold of the two glyoximato rings but is nearly normal to it. Thus, the regiospecificity of the alkylations of the coordinated tetrazolate with alkyl halides is sterically promoted.

Introduction

The anion that results when a 5-substituted tetrazole's tautomeric ring proton is attacked by a base has been found to possess two bonding modes (N_1 vs. N_2) that are virtually energetically equivalent.[2]



Tetrazole ring numbering scheme

Consequently, linkage isomers have been reported in solutions of 5-substituted tetrazolato ions coordinated to any of a number of transition-metal centers.[3,4] Treatment of this type of anion either as a sodium salt or a transition-metal complex with alkylating agents always produces mixtures of 1,5- and 2,5-disubstituted tetrazoles[3,5-12] in relative amounts that are affected by the electronegativity and size of the 5 substituent.[6,11] The family of tetrazoles is economically important because of the many practical applications of both a biological[13-20] and nonbiological[21] nature found for its members. For disubstituted tetrazoles, a major subclass with mainly medicinal and pharmacological applications, activity is usually isomer dependent and requires separation procedures that may be difficult and costly. This is particularly true if the 1,5 isomer is the active one[13,16,17] because steric restrictions generally cause it to be formed in lower yield[5-12] than the 2,5 isomer. Thus far no successful method has been found for directly or indirectly producing the 1,5 isomer as the exclusive product. Blocking of the N_2 position by a *tert*-butyl group has been attempted but the N_1 position was also attacked even when the 5 substituent was relatively bulky.[22] Kozima et al.[8] did obtain high selectivity in formation of the 1,5 isomer by blocking the 2 position with tri-n-butyltin prior to alkylation but the 2,5 isomer was still formed in about a 10% average yield.

Marzilli et al.[23] prepared complexes of the type $PBu_3Co(DH)_2$pur, where pur is the anion of a purine-type base such as xanthine, and showed that the heterocyclic base was regiospecifically coordinated at the least hindered of two possible nitrogen bonding sites. Alkylation of the xanthine complex cleaved the heterocycle to yield an alkylated purine and halocobaloxime according to the equation

$$PBu_3Co(DH)_2pur + RX \rightarrow PBu_3Co(DH)_2X + R\text{-}pur \quad (1)$$

Identification of the alkylated ring site led to a deduction of the bonding mode in the purine complexes. The similarities in

0002-7863/80/1502-2968$01.00/0