is tempting to attribute this shortening to the increased inductive effect of CH_3 groups compared to H atoms, which would be expected to be important if the P–N bond in these compounds had significant π character.

Although the structural data obtained on PF_2NH_2 and $(CH_3)_2NPF_2$ add considerable support to a π-bonding model, it should be mentioned that a recent infrared and Raman investigation of $N[P(CF_3)_2]_3$ suggests that this molecule does not have a planar NP_3 skeleton.[19]

The ^{15}N–H coupling constant (83.2 Hz) obtained for $PF_2{}^{15}NH_2$ is also consistent with a planar $-NH_2$ group. A relationship between $^1J_{^{15}N-H}$ and the percentage 2s character in the nitrogen orbital used in the NH bond has been developed.[20] This relationship predicts 30% 2s character in the nitrogen orbitals bonded to the hydrogen atoms in PF_2NH_2; a planar $-NH_2$ group with sp^2 hybrids would require 33% 2s character.

The fact that no broadening or splitting of the lines in the nmr spectrum of $PF_2{}^{15}NH_2$ occurs down to $-70°$ (near the freezing point) indicates that unless the chemical shifts of the two protons are accidentally identical, a rapid (on the nmr time scale) intramolecular conversion is taking place in this molecule. The conversion could be an inversion of the PF_2 group or an internal rotation about the P–N bond. Assumption of a reasonable difference in the chemical shifts of the two protons would lead to the conclusion that the potential barrier to interconversion is less than about 10 kcal/mol. This value is probably too small for a phosphorus inversion,[2b] and hence may be taken as an upper limit to the barrier to internal rotation about the P–N bond. In principle, this question can be settled by means of a vibrational analysis, and we are currently investigating the ir and Raman spectra of PF_2NH_2 with this goal in mind.

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Raman Spectra of Schiff Bases of Retinal (Models of Visual Photoreceptors)

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Abstract: The resonance-enhanced Raman (RR) spectra of *trans*-retinal and of three of its Schiff bases in various solutions are presented and correlated with the corresponding solvent-shifted absorption spectra. The frequencies of solvent-shifted vibrational modes of the conjugated segment of retinal are plotted *vs.* the corresponding λ_{max}. The degree of π-electron delocalization in a given solvent is an implicit parameter for each point on the plot. The locus of points representing acidified Schiff bases differs from that of the unacidified ones. A single point representing preliminary data on lumirhodopsin in whole bovine retina fits well into the locus of acidified Schiff bases. Stretching modes of C=O and C=N, as well as other vibrational modes, are identified. The Raman spectrum of acidified retinal looks as if contributed by a single solute species, while the corresponding absorption spectrum is characteristic of a mixture.

There exists substantial evidence that retinal binds to opsin by Schiff base condensation of the carbonyl with ϵ-amine of lysine[2-9] or, perhaps, with the primary amine of phosphatidylethanolamine.[10-12] Model systems of *trans*-retinal bonded covalently to aliphatic amines can be readily prepared, yet it is significant that these models do not reproduce the bathochromic shift of λ_{max} observed in retinal isomers bound to opsin.[6] A possible reason for this inadequacy of the models may be in some noncovalent bonding specific to opsin, which may be studied and hopefully elucidated by vibrational Raman spectroscopy *in situ*. The feasibility of observing the Raman spectrum of pigments (*e.g.*, bound retinal) in heterogeneous media[13] (*e.g.*, frozen bovine retina[14]) is a consequence of the phenomenon of resonance enhancement (RE) of the Raman effect[15] which is operative only for pigment molecules excited close to an electronic transition.[16,17]

The vibrational modes most strongly enhanced are those contributed by the stretching deformations of C=C and C—C bonds in the conjugated chain, while a somewhat attenuated RE is extended to vibrations in-

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Table I. Spectroscopic Properties (Vibrational Frequencies of Major Raman-Active Modes and λ_{max} of the Electronic Absorption) of *trans*-Retinal and Its Schiff Bases with *n*-Hexylamine, Aniline, and *p*-Bromoaniline in Solution[a]

	λ_{max}, nm	Isomer	Fingerprint		Vibrational frequencies							$\bar{\nu}$(C=C)	$\bar{\nu}$(C=N)	$\bar{\nu}$(C=O)
					Unacidified Solutions									
trans-Retinal in C_6H_{14}	370	1162		1198	1224	1271	1282		1453			1583		1673
trans-Retinal in C_6H_6	381	1165	1179	1199		1271	1283	1336			1549	1580		1662
trans-Retinal in CCl_4	382	1163	1176	1198		1270	1281	1331	1450			1578		1665
trans-Retinal in C_2H_5OH	385	1665		1202	1225	1272	1282		1453			1574		1654
trans-Retinal in $C_8H_{17}OH$	387	1167	1177	1200	1226	1272	1283	1336	1450			1574		1654
trans-Retinal + *n*-$C_6H_{13}NH_2$ in C_6H_{14}	360	1167		1197	1221	1269	1281		1453			1583	1627	
trans-Retinal + *n*-$C_6H_{13}NH_2$ in C_6H_6	365	1168	1177	1195	1221	1269	1282	1325				1580	1623	
trans-Retinal + *n*-$C_6H_{13}NH_2$ in C_2H_5OH	365	1170		1201	1230	1272	1282		1454			1582	1625	
trans-Retinal + *n*-$C_5H_{13}NH_2$ in $C_8H_{17}OH$	368	1165	1175	1201	1229	1271	1280	1327	1450			1583	1621	
trans-Retinal + aniline in *n*-C_6H_{14}	384	1167		1198	1228	1271	1284	1328	1451	1485	1557	1571	1618	
trans-Retinal + aniline in C_6H_6	393	1166.5	1178	1198	1227	1271	1284	1329		1485	1555	1569	1614	
trans-Retinal + aniline in C_2H_5OH	395	1169	1179	1199	1231.5	1273	1287	1333	1457	1488	1553 1527	1569	1613	
trans-Retinal + *p*-bromoaniline in C_2H_5OH	404	1168	1174	1198	1230	1273	1279	1332	1455	1484	1550	1564	1612	
					Acidified Solutions									
trans-Retinal + hexylamine in *n*-C_6H_{14}	435	1161		1201	1238	1271	1281		1450			1560	1654	
trans-Retinal + hexylamine in $C_8H_{12}OH$	442											1559		
trans-Retinal + hexylamine in C_2H_5OH	448		1175	1200	1234	1272	1282		1454			1557	1646	
trans-Retinal + *p*-bromoaniline in C_2H_5OH	512	1161	1175	1190		1273	1281					1547	1650	
trans-Retinal + aniline in C_2H_5OH	514											1547 1584		
trans-Retinal in C_2H_5OH	347 368 370	1162		1188	1229		1281		1453			1584 1531		

[a] The modes denoted by $\bar{\nu}$(C=C), ν(C=C), and ν(C=N) have a dominant component of C=C, C=O, and C=N stretching, respectively. The samples denoted as retinal + amine refer to the Schiff bases of the respective constituents.

volving heteroatoms bonded to the conjugated chain. The RE of the C=O stretching mode in retinal (1655–1675 cm^{-1}) and of the C=N mode in the Schiff bases (1625–1645 cm^{-1}) is evidenced by comparing the intensities of these modes with those of C–H stretching. When retinal or its derivatives are excited in the visible, the C=O or C=N modes are at least an order of magnitude more intense than the C–H modes, but when this excitation is applied to small conjugated molecules which absorb at shorter wavelengths the corresponding ratio of intensities is reversed.

The Raman spectra have, in addition to resonance enhancement, the advantage of identifying the stereoisomers of retinal by well-resolved fingerprint modes.[18]

We present the Raman spectra of a number of normal and of acidified *trans*-retinylidene Schiff bases in various solvents, excited by laser sources in the visible (Table I). (Excitation profiles, *i.e.*, the dependence of Raman intensities on the wavelength of excitation, will be reported separately.)

The pigments were: (1) *trans*-retinal;[19] (2) *trans*-retinylidene-*n*-hexylamine, in which the aliphatic amine is a substitute for the ϵ-amine of lysine; (3) Schiff bases of *trans*-retinal with the aromatic amines, aniline, and *p*-bromoaniline. The aromatic amines,[20] which have no counterpart in the retina, were bonded to retinal in order to probe the influence of the interaction of two covalently bonded conjugated π systems on the RE Raman spectra.

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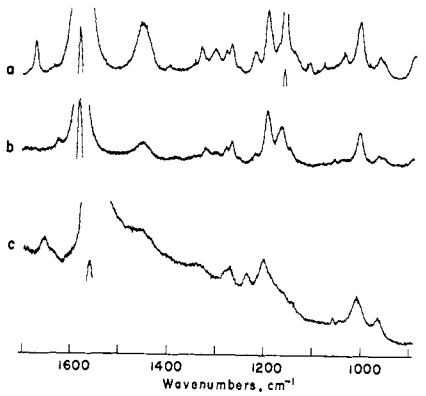

Figure 1. Raman spectra of retinal and retinylidenehexylamine in hexane. The excitation is at 4880 Å, the spectral slit width is ~5 cm^{-1}, and the scan speed is 20 cm^{-1}/min: (a) *trans*-retinal in hexane (the C=O stretching mode at 1673 cm^{-1} is sharp), (b) retinylidenehexylamine in hexane (the C=N stretching mode at 1627 cm^{-1} has completely replaced that of the carbonyl), (c) retinylidenehexylamine in acidified hexane.

Experimental Section

trans-Retinylidene amines were prepared in various organic solvents, listed in Table I, which were 15 m*M* in *trans*-retinal (Sigma Chemical Co.) and 375 m*M* in either *n*-hexylamine (Eastman Organic Chemicals), redistilled aniline (Mallinckrodt), or *p*-bromoaniline (Eastman). The solutes were allowed to react in the dark for approximately 2 hr under a stream of N_2. The Schiff bases in ethanol were acidified by the addition of a few drops of concentrated aqueous HCl, and the hexane solutions were acidified by addition of a drop of a mixture of EtOH and concentrated aqueous HCl. There was no indication that the Schiff bases hydrolyzed to any appreciable extent in the presence of this small quantity of water; the absorption spectra were identical with those reported in the literature for protonated retinylidene amines[21] and the corresponding Raman spectra showed a mode characteristic of the protonated C=N⁺—H linkage (see discussion below). Moreover, the presence of a significant quantity of acidified retinal (an obvious hydrolysis product), which should have been detectable in the Raman spectra, was clearly not observed (see below).

Results and Discussion

Representative Raman spectra are shown in Figures 1–3, and the major vibrational modes are listed in Table I. In addition to the changes in the uv and visible absorption spectra[21,22] (see column 2 in Table I), the conversion of retinal to the Schiff base can be monitored by changes in vibrational modes, as was shown earlier from infrared absorption studies.[22] In the case of Raman spectra, this is most clearly demonstrated for the formation of retinylidenehexylamine in hexane (Figure 1). Upon condensation, the intense carbonyl band of retinal at 1673 cm^{-1} (Figure 1a and Table I) is replaced by a mode at 1627, which is characteristic of the C=N bond stretching.[22] Upon protonation of the Schiff base, this mode is replaced by a broader band which peaks at 1654 cm^{-1}, as expected for a protonated C=N⁺—H bond. In ethanol solutions (Figure 2), the situation is similar but less clear, because of a lower signal-to-noise ratio. In Figure 2a the C=O line is visible at 1654 cm^{-1}, whereas the C=N vibration of the Schiff base (Figure 2c) appears as a shoulder at 1625 cm^{-1} on the intense ethylenic mode. The protonated Schiff base (Figure 2d) shows

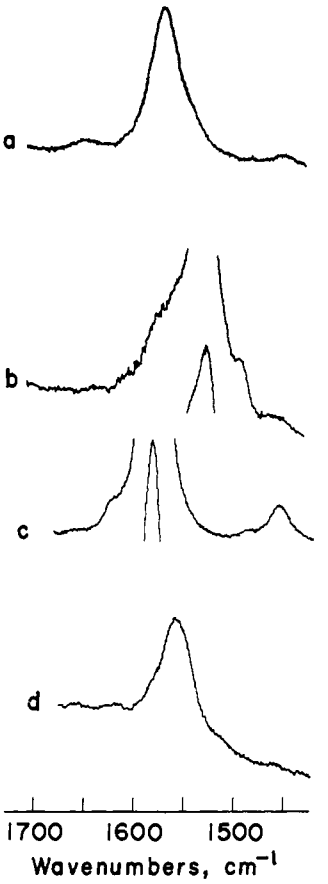

Figure 2. Details of Raman spectra in the region of the ethylenic mode, taken in ethanol solutions: (a) *trans*-retinal in ethanol (the stretching mode of C=O at 1654 cm^{-1} is broadened by hydrogen bonding to the solvent), (b) *trans*-retinal in acidified ethanol (acidification brought about the lowering of $\bar{\nu}$(C=C) and the disappearance of the carbonyl mode), (c) *trans*-retinylidenehexylamine in ethanol (a broadened C=N stretching mode appears as a shoulder at 1625 cm^{-1}), (d) *trans*-retinylidenehexylamine in acidified ethanol.

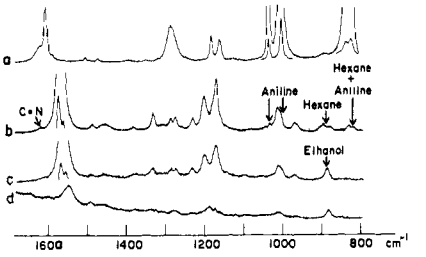

Figure 3. Raman spectra of retinal–aromatic amine Schiff bases: (a) a reference spectrum, aniline in ethanol; (b) Schiff base of retinal and aniline, dissolved in hexane; (c) the same dissolved in ethanol; (d) the acidified Schiff base of retinal and bromoaniline dissolved in ethanol. The Raman spectrum of acidified solution (c) was very poor, and only the ethylenic mode could be discerned.

only traces of two broad lines, roughly centered at 1646 and 1618. The line at ~1650 could correspond to the protonated C=N⁺—H; the identity of the protonated Schiff base yielding the spectrum in Figure 2d is substantiated both by the wavelength of its visible absorption peak (Table I) and the lowering of the vibrational frequency of the ethylenic mode. In the Schiff bases of the aromatic amines (Figure 3), the C=N frequency is clearly evidenced at 1618 cm^{-1} in

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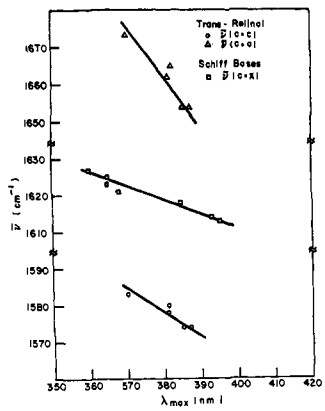

Figure 4. Correlation plot of solvent-induced shifts. The ordinate of each point is the vibrational frequency of a Raman-active mode, and the abscissa is the λ_{max} of the absorption spectra in the corresponding solvent. The coordinates of the points and the listing of their corresponding solvents are given in Table I. The straight lines are the loci of the points pertaining to a particular vibrational mode. The modes represented in this figure have dominant contributions of C=C, C=O, or C=N stretching deformations, respectively. The rigidity of the bonds is partly contributed by the π-electron density localized at the (double) bond sites. The negative slopes of the loci in this figure correlate the *weakening* of the double bonds caused by π-electron delocalization with the concomitant bathochromic shift of λ_{max}.

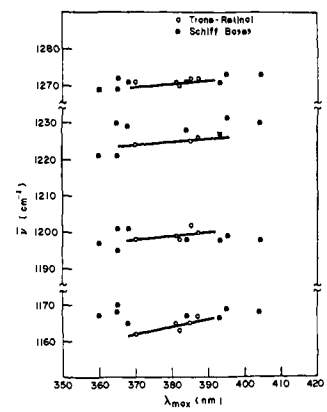

Figure 5. Correlation plot (see Figure 4) of solvent shifts for Raman-active modes in the 1100–1400-cm^{-1} range. The slightly *positive* slopes of the loci reflect the *strengthening* of the single C–C bonds, which stems from increased π-electron delocalization. Thus the slight predominance of the contribution of the C–C stretching deformations to these modes is deduced; the C–C stretching, however, is strongly admixed with other deformations, such as C–H bending.

the hexane solution and as a shoulder at 1614 cm^{-1} in the ethanol solution (Figures 3b and 3c); also the $C{=}N^{+}{-}H$ band of protonated retinylidene-*p*-bromoaniline is apparent as a peak at 1650 cm^{-1} (Figure 3d).

The stereoisomer fingerprints are located in the spectral range of ~1100–1400 cm^{-1}. Careful inspection of this range reveals that the stereoisomers do not interconvert when exposed to laser irradiation in the red.[18] The Raman spectroscopy of cis isomers using blue or green excitation invariably yields the spectrum of the trans isomer. When, however, the spectrum of this sample is subsequently taken with red excitation, it is still characteristic of the trans isomer,[19] indicating that *trans*-retinal is the most abundant photoproduct of blue-green illumination. Consequently, we studied the trans isomer in detail and took advantage of the intensity enhancement achieved by excitation at shorter wavelengths in the visible.

The spectra of acidified samples showed increased line widths in the fingerprint region. This may be related to the fact that several modes in the 1100–1400-cm^{-1} range strongly admix bending deformations of the allylic protons with C–C stretching, and the former modes would be very sensitive to the association of extra protons with the polyene chain.

The main feature of the spectra is the intense line in the range of 1530–1580 cm^{-1}, contributed mainly by C=C stretching in the conjugated segment of retinal [the mode has been described as "ethylenic" by some authors; we denote its frequency by $\bar{\nu}$(C=C)].[23-27] The stationary pattern of alternating C=C and C—C bonds in conjugated polyenes is well known to exert the influence of a periodic potential, and thus maintain a finite energy gap h/λ_{max} between the highest occupied and the lowest vacant π orbitals in polyenes of unlimited length.[28-33] Solvent interactions may tend to equalize the carbon–carbon bond orders, in which case the C=C force constants decrease, the C—C force constants increase, and the h/λ_{max} gap at the zone boundary narrows; *i.e.*, λ_{max} shifts toward the infrared.[6,34,35] In order to correlate the intensity of the periodic potential with the π–π^* energy gap, the Raman and the absorption spectra were taken for each solution. On the plots shown in Figures 4 and 6, the vibrational frequency of the ethylenic mode is the ordinate and λ_{max} is the abscissa. The position of the point reflects the degree of π-electron delocalization in the conjugated chain.

As seen in Figure 4, the modes assigned to C=C, C=O, and C=N stretching undergo (in a decreasing order) the largest solvent-induced shifts in the vibrational frequency, while the corresponding shifts of all the other modes are much smaller. The data in Figures 4 and 6 demonstrate that all of these shifting modes are valuable indicators of the degree of π delocalization. This may be practically important in the study of π conjugation in strongly scattering or opaque samples, for which the acquisition of a resonance-enhanced Raman spectrum is much easier than the alternative of reflection spectroscopy.

Figure 5 represents some solvent-shifted modes in the wavelength range of 1100–1400 cm^{-1}. The positive slopes of the loci of these modes, as opposed to the slope of the ethylenic mode, indicate a dominant contribution of C–C stretching to the modes. This is because the same solvent influence that tends to weaken the C=C

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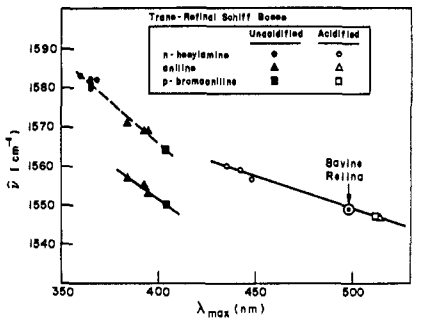


Figure 6. Correlation plot (see Figure 4) for the "ethylenic mode" in Schiff bases of retinal. Again the frequency ν(C=C) of the ethylenic mode is plotted *vs.* the λ_{max} of the absorption spectrum in the corresponding solvent. The samples are the Schiff bases of *trans*-retinal with *n*-hexylamine, aniline, and *p*-bromoaniline, in unacidified as well as in acidified solutions. Bonding to aromatic amines gives rise to a subsidiary ethylenic mode, represented by points located at the lower left end of the plot. Acidification results in the disappearance of the subsidiary mode and in a change of slope. $\bar{\nu}$(C=C) of the "retina" sample was obtained by Raman scattering from frozen bovine retina, while the corresponding λ_{max} is that of lumirhodopsin, presumably the most abundant component in the sample. The retina point fits well on the locus of acidified Schiff bases.

bonds increases the rigidity of the C—C bonds. The smallness of the positive slopes (Figure 5) indicates that the component of C–C stretching in each individual mode is markedly diluted by the admixture of other deformations such as proton bending. This conclusion is supported by the Raman spectra of fully deuterated carotenoids.

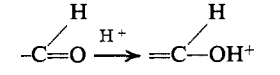
The Raman spectra of the aromatic Schiff bases differ in several respects from the spectra of the aliphatic Schiff base. Most conspicuous is the appearance of a subsidiary ethylenic mode in the Schiff bases of aniline and *p*-bromoaniline. Also the bathochromic shifts of λ_{max} are very large in these compounds. It is significant, though, that none of the modes specific to aniline or *p*-bromoaniline is resonance enhanced.

The very intense subsidiary ethylenic mode may be assigned either to a ring vibration of aniline, thus implying the transference of resonance Raman enhancement from retinal to aniline across the C=N bond, or to a normally forbidden ethylenic mode pertaining to the retinal segment alone. The observed Raman spectra do not support the first assignment because aniline has no intense Raman active mode at $\sim$1500 cm^{-1}. Furthermore, in retinal the intense ethylenic mode is not isolated, but rather belongs to a band, in which most of the components yield weak Raman lines at room temperature. Some of the weaker ethylenic modes are revealed in the Raman spectrum of free retinal cooled to $-100°$.[14] It seems that the formation of aromatic Schiff bases of retinal activates one of these forbidden modes of retinal. When the aromatic Schiff bases are acidified, the subsidiary ethylenic mode disappears and a very large bathochromic shift of λ_{max} takes place.

The single point in Figure 6 representing "whole bovine retina" deserves special comment. $\bar{\nu}$(C=C) was obtained from the Raman spectrum of whole bovine retina frozen at $-70°$.[14] We chose the corresponding λ_{max} 497 nm to be that of lumirhodopsin, which is the most abundant photoproduct in the whole cattle retina having the temperature and illumination conditions of the experiment.[3] The point fits markedly well into the line of acidified Schiff bases of retinal, thus supporting the accepted model of retinal binding to opsin. The proximity of this point to that representing the aromatic Schiff bases may perhaps indicate that some interaction, noncovalent of course, is taking place between retinal and an aromatic moiety of the opsin.[20,36]

Another result of interest was obtained when an ethanol solution of retinal was acidified with a drop of concentrated aqueous HCl. The spectroscopic characteristics of the system were as follows. (a) The main absorption peak[21,22] of *trans*-retinal at 385 nm appeared to break down into a number of subsidiary peaks, with λ_{max} 347, 368, 390, as if a mixture of different reaction products were present. This differed markedly from the corresponding spectra of the similarly acidified ethanol solutions of Schiff bases, for which a single well-defined λ_{max} in the region of 430–520 nm was observed (Table I, column 2). (b) The down-shifted (1530 cm^{-1}) ethylenic mode and the absence of a C=O line were the only major differences between the Raman spectrum of this sample and that of *trans*-retinal in ethanol. The fingerprint lines were somewhat broadened, and the whole spectrum (including the ethanol lines) was somewhat weaker because of the enhanced absorption in the visible. (c) The lowered frequency of the ν(C=C) mode is the major characteristic of this species and can be used for monitoring its presence under various conditions. For example, the acidification of retinal in *hexane* by a H_2O–HCl–C_2H_5OH solution resulted in the appearance of a small peak at 1530 cm^{-1} superimposed on the large peak characteristic of unacidified retinal in hexane. Probably HCl partitioned between the immiscible hexane and ethanol–water phases, both of which contained retinal, so that the HCl concentration in hexane was insufficient for complete acidification. In strongly acidified octanol the two peaks assumed equal intensities, indicating approximately a limiting conversion of 50%.

A possible model for these phenomena in retinal is that of proton binding to the carbonyl and also along the conjugated chain. The proton attaches to the carbonyl, causing enolization



as a result of which the C=O mode vanishes. The extra double bond thus added to the conjugated chain delocalizes the π electrons and lowers $\bar{\nu}$(C=C).

Furthermore, it is conceivable that protons bind directly to the hydrocarbon chain, giving rise to a number of cis-isomerized products. These products may contribute the multiplicity of peaks observed in the absorption spectrum, while the respective contribution to the Raman spectrum may be below threshold for the conditions under which this spectrum is taken. The alternative possibility that the Raman lines of these isomers coincide with those of the trans isomer, which is protonated solely at the C=O linkage, cannot be completely excluded either.

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