Preresonance Raman Spectra of Crystals of Retinal Isomers

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Abstract: Preresonance Raman spectra of crystals of *all-trans*-, 9-*cis*-, 11-*cis*-, and 13-*cis*-retinal are reported. The solid state spectra of these retinal isomers show a detailed vibrational fine structure observed in several instances in the resonance Raman spectra of various rhodopsins but not seen in solution spectra. A systematic variation in the C=C stretching vibration among the isomers is noted, and a splitting in the stretching vibration of the terminal carbonyl group has also been detected which is apparently sensitive to the conformation of this group. Furthermore, a doublet band is reported in the 1005–1031-cm^{-1} region which appears to arise from 5-CH_3, 9-CH_3, and 13-CH_3 vibrations with the conjugated portion of the molecule and is characteristic of each individual isomer. The vibrational frequencies of the doublet are interpreted in terms of resonance structures. In addition, various other vibrational modes between 700 and 1700 cm^{-1} are catalogued which also reflect the conformation of retinal.

I. Introduction

The retinylidene chromophore is of great importance in the study of the visual pigment rhodopsin since the energy received by the chromophore from a single photon, when coupled with available thermal energy, is sufficient to initiate the sequence of events leading to the visual response. The retinal chromophore is set in a protein matrix called opsin and our data showed that it is connected to opsin through a protonated Schiff base linkage[1-3] to a lysine residue. The retinal is originally in an 11-cis conformation in vertebrate rhodopsin. Upon the absorption of a photon, a series of conformational changes are induced which eventually cause a cis–trans isomerization of the retinal and initiate the primary ion movements leading to the visual process. It is known that there are several intermediate forms of rhodopsin in this process; however, the conformation of the retinylidene chromophore is not definitely known for any of these intermediate states. This study was undertaken to identify vibrational modes that are sensitive to the conformation of the retinylidene chromophore.

Our experiments have demonstrated[1-3] that resonance Raman spectroscopy is a powerful technique to study changes in the retinylidene chromophore by selectivity enhancing retinal's vibrational modes and those of the opsin that may be strongly coupled to the retinal. Thus, the Raman scattering from one of the principal action sites in the protein is enhanced above the background scattering due to the protein.[1-7]

Since the isomers of retinal are fundamental components in the rhodopsin molecule, a thorough understanding of their Raman spectra is essential to the interpretation of our work on rhodopsin. The Raman spectra of retinal and its isomers have been obtained in solution, and many of the observed bands can be assigned to specific vibrational modes.[8-11] This study involved looking at isomers of retinal in the solid state. Utilizing polycrystalline samples there is a smaller probability that retinal will undergo isomerization from a cis to a trans conformation. This has been verified by measurements made with high-pressure liquid chromatography. Furthermore, the availability of x-ray structures for the 11-cis and all-trans isomers allow detailed comparison between the known conformation in the crystalline state and the observed vibrational spectrum.

II. Materials and Methods

The samples of polycrystalline 9-*cis*-, 13-*cis*-, and *all-trans*-retinal were obtained from Sigma Chemical Co. and used without further purification. Paul Brown at Harvard University supplied the crystalline samples of 11-*cis*-α- and 11-*cis*-β-retinal.[12] Additional samples of 9-*cis*-, 13-*cis*-, and *all-trans*-retinal obtained from Eastman Organic Co. give identical spectra. Raman spectra were taken of polycrystalline powders of these samples. All of the crystals were stored in the dark under a nitrogen atmosphere and the samples were transferred under nitrogen to melting point capillaries for use in the experiments. At the conclusion of these experiments liquid chromatographs of the samples were taken to test for isomerization.

The crystals were illuminated with the 647.1-nm line of a krypton ion laser that had been filtered with a narrow-band interference filter and the scattering observed at 90° with a Spex Industries 1401 double monochromator. The signal was detected with an RCA Model C31034 photomultiplier. Data were recorded using photon counting techniques.

III. Results

The preresonance Raman spectra of the four isomeric forms studied are shown in Figure 1. The strong band in the 1575-cm^{-1} region of the spectrum is typically four to five times the full-scale intensity shown here. Table I is a catalogue of all of the bands observed in the spectra with a qualitative estimate of the intensity of each band.

IV. Discussion

The dominant feature in each spectrum is the very strong band in the 1575-cm^{-1} region. This band is generally interpreted as one of the high-frequency intramolecular collective modes of a C=C stretch of the polyene portion of the molecule.[8,9] Table II lists the measured frequencies of this band for the different isomers including the standard deviation error estimates. Notice the trend for the wavenumber of the band to increase as the position of the cis bend moves away from the aldehyde group at the end of the conjugated chain. This trend is not present in data available on solution spectra of the isomers, possibly due to solvent effects and/or isomerization. A comparison is shown between reported values obtained for this band in various solvents by Rimai and his co-workers.[8,9] The solution and solid state values tend to be different by several wavenumbers. This may indicate that the C=C stretch is sensitive to the environment in which the molecule is located and thus may be useful in investigating rhodopsin. In addition, we observe several sidebands and shoulders along with the main C=C band in the spectrum of each isomer, which could possibly be due to the five different carbon double bonds in this polyene.

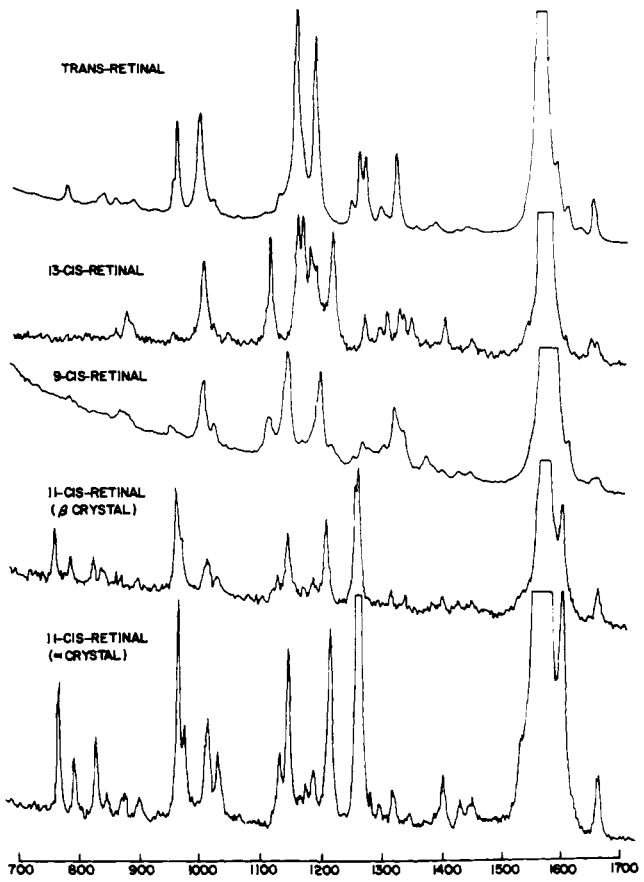

Figure 1. Raman spectra of four retinal isomers. Laser line 647.1 nm, 2-cm^{-1} resolution. True zero is shown for lowest, 11-cis-α spectrum; all other spectra have approximately identical background intensities.

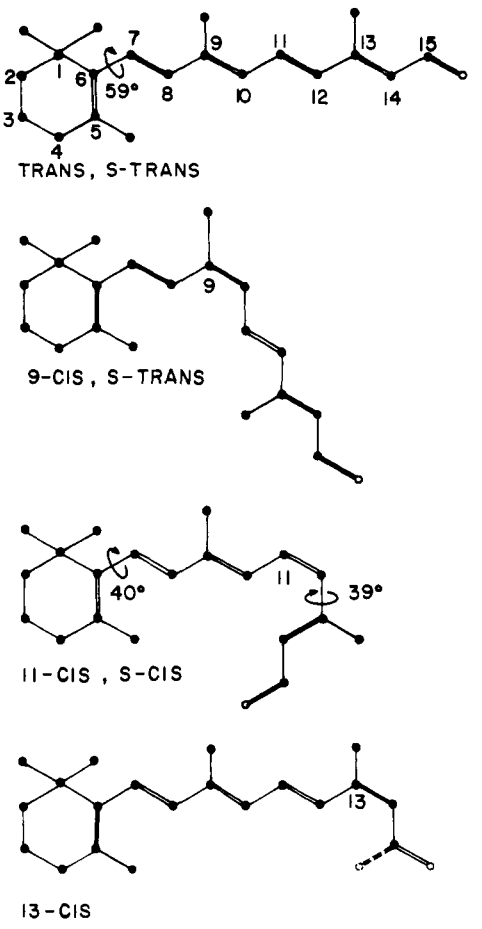

Figure 2. Schematic plane representations of the isomers of retinal studied. The dotted lines represent the 13-cis, 14-s-cis isomeric configuration.

The bands that appear in the 1655-cm^{-1} region of the retinal spectrum are generally attributed to the stretching mode of the 15-C to a terminal group.[8,9] In rhodopsin, the terminal group of the chromophore is a protonated Schiff base[2,3] while in the case of retinal this is a carbon double bonded to an oxygen. Notice how the band in the 1655-cm^{-1} region is split only in the case of the 13-*cis*-retinal isomer in the spectra shown in Figure 1.

Table I lists the measured positions of the relevant bands. We feel that this splitting can be interpreted as confirmation of the assignment of this band to the carbon-oxygen double-bond stretching frequency. 13-*cis*-Retinal can possibly exist in two isomeric forms. Thus, the carbon-oxygen bond could be oriented in either of the two conformations shown in Figure 2. The terminal oxygen could interact with the hydrogen on 12-C in the 13-cis, 14-s-cis configuration depicted with the dotted lines in Figure 2. In the configuration illustrated with the solid lines (13-cis, 14-s-trans) such an interaction would be unlikely, and the frequency of the C=O vibration should be different. Our samples may contain a mixture of 13-*cis*-retinal in both configurations and thus the spectrum could be a superposition of contributions from both forms. The 13-*cis*-retinal isomer is the only one in which this splitting is observed. It is important to point out that the opsin pocket of bacteriorhodopsin can accept either the trans or the 13-cis isomer of retinal.[13] Bacteriorhodopsin is a light-driven proton pump, and its biological role is to form a proton gradient across the bacterial cell membrane.[13] We have shown that the protonation-deprotonation of the Schiff base linkage is an intrinsic part of the molecular events which generate this gradient.[3] The splitting we have observed in the case of the 13-*cis*-retinylidene chromophore may provide us with an additional handle to help monitor and understand the conformational and electronic changes which play a part in the protonation-deprotonation of this terminal Schiff base nitrogen in bacteriorhodopsin.

To confirm this assignment, a low-temperature study of the 13-*cis*-retinal isomer is also underway to study the temperature dependence of the double peak observed in the 1655-cm^{-1} region of the spectrum. Variations in intensity of the twin peaks as the temperature is varied to liquid helium temperature should identify one conformation as a lower energy state if our interpretation is indeed correct.

A very interesting pair of bands was found in the 1005- to 1031-cm^{-1} region of the spectrum. The band between 1005 and 1015 cm^{-1} labeled peak 1 in Table III and shown in Figure 3 appears in all of the isomers. A second band between 1028 cm^{-1} and 1031 cm^{-1} labeled as peak 2 in Table III, although weaker, appears with varying intensity in all of the isomers except 13-cis. Notice how the ratio of the intensity of peak 1 as compared to peak 2 varies from isomer to isomer. Such data are important in understanding the isomerization process in rhodopsin. Similar vibrations have been observed in the spectra that have been obtained on the retinylidene chromophore of visual pigments.[2,7] Another important feature of these spectra is the variation in frequency of the band listed as peak 1 from 1005 to 1015 cm^{-1} and the band called peak 2 from 1028 to 1031 $cm.^{-1}$

Previous workers, using solutions of retinal, reported only a single band in this region except in the case of the 11-cis isomer. A doublet was reported in 11-*cis*-retinal but with the bands at 999 and 1018 cm^{-1}. The single band was assigned to the degenerate n-C-CH_3 (n = 9, 13) methyl side group stretching vibrations. In the case of 11-*cis*-retinal, the doublet was attributed to the absence of any degeneracy in the stretching frequencies of the methyl side groups because of the

Table I. A Catalogue of All the Raman Bands Measured in the Spectra of the Retinal Isomers[a]

11-Cis-α	11-Cis-β	9-Cis	13-Cis	Trans
769 s	767 s			
794 m	794 m			788 m
831 m	830 m	875 m	866 w	849 m
847 w	844 w	884 w	885 m	868 w
866 w	866 w	890 w	893 w	884 vw
877 w	876 w			893 w
				898 w
902 w	903 w			930 vw
967 s	965 s	957 w	958 w ± 2.1	963 m
976 s	975 s	963 w	966 w ± 2.2	969 s
1014 s	1015 m	1010 s	1011 s	1005 s
1031 m	1030 m	1028 m		1028 w
			1049 w	
1132 m	1130 w	1117 m	1119 s	
1146 s	1146 s	1147 s	1165 s	1136 w
		1201 s	1173 s	1163 s
				1173 m
1173 vw	1172 w ± 2.2	1219 w	1185 s	1194 s
1187 w	1190 w	1255 w	1194 m	1254 m
1211 s ± 2.9	1210 s	1272 m	1222 s	1267 s
1261 s	1262 s	1290 w	1274 m	1278 s
1279 w	1317 w	1306 m	1298 m	1304 m
1295 w	1337 w ± 2.8	1324 s	1310 m	1329 s
1317 w		1339 m	1331 m	
1339 w ± 3.0			1337 m	
			1350 m	
		1377 m		1361 w
				1385 w
1402 m	1401 w		1405 m	1394 w
1428 w	1427 w	1429 w		1429 w
1449 w	1450 w	1449 w	1450 w	1447 w
1530 w	1533 w			
1558 s ± 3.6	1559 s ± 3.4	1555 w ± 4.9	1545 w ± 3.3	1547 w
1574 vs	1574 vs	1586 vs	1570 vs	1568 vs
1600s	1600 s	1612 m	1582 s	1594 s
				1611 m
		1636 w		1634 w
1659 m	1660 m	1651 w ± 2.4	1649 m	1654 w
			1659 m	

[a] Each band was calibrated against krypton ion laser emission lines. The standard deviation error estimate for each band is 2 cm^{-1} or less unless a specific value is given. The small letters indicate a qualitative estimate of the strength of each band: w = weak, m = medium, s = strong, v = very.

Table II. Measured Frequencies of the C=C Stretching Mode[a]

	Cookingham et al. (solid)	Gill et al. (1971)[9] (in CCl_4)	Rimai et al. (1971)[8] (in 1-octanol)
trans-Retinal	1568.4 ± 1.5	1578	1570
13-*cis*-Retinal	1570.0 ± 0.6	1584	1579
11-*cis*-Retinal (α crystal)	1574.0 ± 1.4		
(β crystal)	1574.3 ± 1.5	1577	
9-*cis*-Retinal	1586.0 ± 1.8		1579

[a] A comparison is shown to previous measurements on retinal isomers in various solvents.

presence of the strong perturbing forces from the 11-cis, 12-s-cis or -trans bend in the isoprenoid chain between C_{11} and C_{13}.[8,9]

Our observations of the doublet structure in the isomers of retinal suggest that the conformation of the isoprenoid chain may cause one or both of the methyl side groups attached to 9-C and 13-C to couple and modulate the π electron density of the conjugated portion of retinal. This produces an enhanced methyl stretching vibration. We also feel that the methyl group attached to 5-C may be coupled in the preresonance enhanced spectra we have obtained. It is interesting to note that NMR data are also available which suggest that the environment of the methyl groups varies from isomer to isomer.[14]

We do not at present fully understand the interactions that

Table III. Measured Frequencies of the n-CCH_3 (n = 5, 9, 13) Stretching Frequencies and the Ratio of Their Relative Intensities

	11-*cis*-Retinal (α crystal)	11-*cis*-Retinal (β crystal)	9-*cis*-Retinal	*trans*-Retinal	13-*cis*-Retinal
Peak 1	1014 ± 0.8	1015 ± 1.0	1010 ± 0.5	1005 ± 0.5	1011 ± 0.6
Peak 2	1031 ± 1.7	1030 ± 0.5	1028 ± 0.7	1028 ± 1.4	
Ratio of peak intensities (peak 2/peak 1)	0.66 ± 0.03	0.54 ± 0.11	0.35 ± 0.03	0.14 ± .02	0.00 ± 0.05

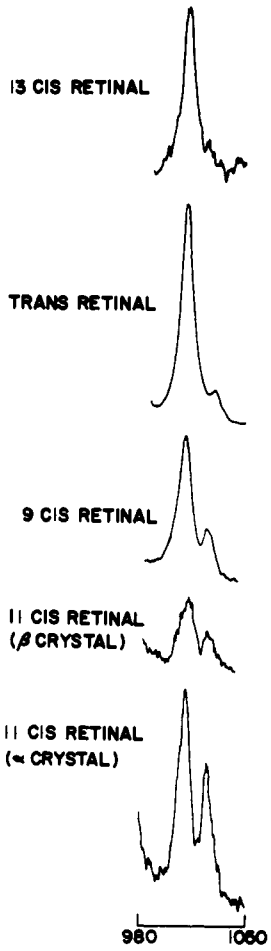

Figure 3. An expanded view of the Raman spectra of the four isomers showing the 980-cm^{-1} to 1050-cm^{-1} region. Spectra were taken with 647.1-nm excitation and 2-cm^{-1} resolution. Notice the relative intensity of the two peaks.

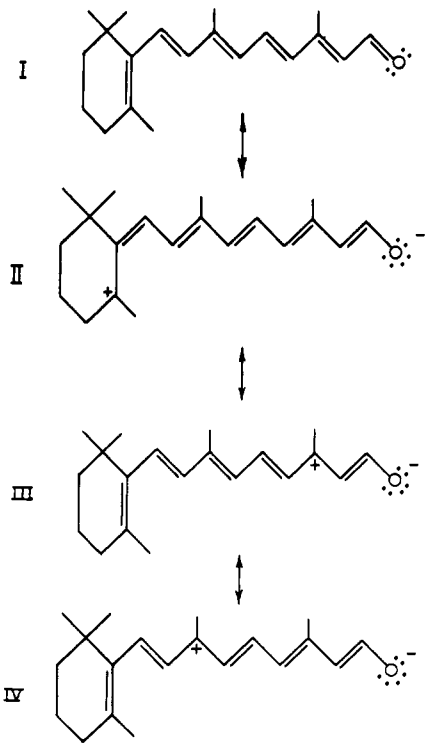

Figure 4. Four possible resonance structures for *all-trans*-retinal where a positive charge is stabilized by a tertiary center.

are revealed in the Raman spectra of retinal isomers, but various resonance forms which can be drawn for the isomers of retinal do appear to affect the frequency and possibly the intensity of the bands observed in our Raman spectra. Four resonance structures are shown in Figure 4 for *all-trans*-retinal. These are only those structures where the positive charge is stabilized at a tertiary center. These four resonance forms should be the most important ones to consider although other structures with the positive charge at any of the eight remaining carbon atoms in the polyene chain may also contribute.

The intensity of a vibrational mode in a resonance Raman spectrum can be due to the degree of modulation of π-electron density (mathematically expressed in Albrecht's theory[15] as the *B* term) and/or due to Franck-Condon overlap factors (the Albrecht *A* and *B* terms). For example, the intensity of the band labeled peak 1 in Table III which Rimai has assigned to the C_9-CH_3 and C_{13}-CH_3 vibration[8,9] can be understood based on the resonance structures drawn. If, as Figure 4 suggests, a partial positive charge can be localized at carbons 5, 9, and 13, then the C-CH_3 bond is effectively brought into the π system and would compare in its intensity to the other modes observed. Franck-Condon overlap factors should also be very important in the intensity of this mode, and more detailed calculations in the vein of Warshel and Karplus[16] will have to be undertaken before the contribution of Franck-Condon overlap factors can be understood fully. Preliminary work has shown that the intensity of bands in a resonance Raman spectrum is strongly dependent on Franck-Condon overlap factors.[15-17] If this is borne out by future experiments, then the resonance Raman spectroscopy of photochemically important molecules may selectively enhance those vibrational modes of the chromophore which exhibit large changes in their nuclear coordinates in going from the ground to the excited state and therefore lead the molecule into its photochemical pathway.

The frequency of the vibrations we have observed is dependent on the force constants which reflect the electron density in the bonds. The amount of π electron density in the bonds is governed by which resonance structures are important for a particular isomer. For example the 12-13 single bond rotation in the crystalline 11-cis,12-s-cis[18,20,21] isomer will only allow resonance forms I and III to contribute whereas the lack of such a single bond rotation in crystals of the trans isomer[18-20] will allow all four resonance structures to affect the spectrum. Similar arguments can be made for crystals of 9-*cis*- and 13-*cis*-retinal. In comparing the spectra of different isomeric configurations, changes in the coupling of various vibrations should also be important in affecting the vibrational frequencies observed. Further work is essential before any detailed assignments can be made, but the concept of resonance structures has suggested experiments which should lead to a meaningful assignment of the vibrational spectrum of retinal. One of these experiments involves a study of unprotonated and protonated Schiff bases of retinal isomers in the crystalline state on which x-ray structures are available. In these investigations, various counterions are being used to form the crystals of the protonated Schiff bases in each of the isomeric forms of retinal. These counterions should modulate the relative importance of the resonance forms depicted in Figure 4. Furthermore we have also begun a number of studies on synthetic retinals with key CH_3 groups replaced by hydrogens and butyl groups.

The interpretation of the spectra reported in this paper is an essential element in understanding the resonance Raman spectra of the retinylidene chromophore of rhodopsin. Only when a vibrational analysis has been completed will we be able to fully interpret the detailed in situ structural information

which is being obtained for the first time through the application of resonance Raman spectroscopy to visual pigments.[1-5,7]

Note Added in Proof. As mentioned in the text of the paper, ground-state resonance structures are important in predicting the ground-state vibrational frequencies observed but not the observed intensities. Our recent data on unprotonated Schiff bases support this hypothesis. These results show, for example, that the C—CH_3 vibrational frequencies move from 1005 cm^{-1} in crystalline *all-trans*-retinal (which has an electron-withdrawing terminal carbonyl) to 1012 cm^{-1} in crystalline *all-trans-N*-retinylidene-*n*-butylamine in which the terminal oxygen has been replaced by an *n*-butylamine group. The *n*-butylamine group has a reduced electron affinity relative to oxygen. This reduces the δ^+ character on C_5, C_9, and C_{13} by making resonance structures II-IV less likely (see Figure 4), thus increasing the C-CH_3 vibrational frequencies.

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Resonance Raman Spectra of Tetra(*n*-butylammonium) Salts of the Octachlorodirhenate(III) and Octabromodirhenate(III) Ions

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Abstract: The metal-metal bonded species $[(C_4H_9)_4N]_2Re_2Cl_8$ and $[(C_4H_9)_4N]_2Re_2Br_8$ both display a resonance Raman spectrum when irradiated in the solid state with exciting lines which approach the wavenumber (ca. 14 000 cm^{-1}) of the $\delta^* \leftarrow \delta$ electronic band maximum of each anion. Two overtone progressions in the metal-metal stretching frequency, ν_1(Re-Re)a_{1g}, have been observed for each ion; these reach $4\nu_1$ for the first and $\nu_2 + 2\nu_1$ for the second, where ν_2 is the ν_2(Re-X)a_{1g} fundamental (X = Cl or Br). Further members of these progressions were obscured by strong underlying fluorescence. The results indicate that the resonant electronic transition is electric dipole allowed. The members of each overtone progression are seen to decrease in intensity and increase in half-bandwidth with increase in the vibrational quantum number of the ν_1 fundamental. The observation of overtone progressions permits the determination of the spectroscopic constants ω_1 and x_{11} for each anion. By use of a tunable dye laser, partial excitation profiles of various fundamentals of the octahalodirhenate ions have also been determined.

It is now well established that under conditions in which a molecule is excited with a laser line whose frequency corresponds or closely corresponds with the band maximum of an allowed electronic transition, a resonance Raman (RR) spectrum may be obtained. Such spectra are characterized by a large increase in the intensity of a totally symmetric fundamental of the scattering molecule, together with the appearance of high-intensity overtone progressions in this same fundamental.[1-6]

Metal-metal bonded complexes are known to display the RR effect, partly because of the highly polarizable nature of many metal-metal bonds, especially where multiple, and partly because of their generally low-lying, and therefore accessible, allowed electronic transitions. A previous RR study of the $Mo_2Cl_8^{4-}$ ion has shown[7,8] that long overtone progressions in the ν_1(Mo-Mo)a_{1g} fundamental may be observed by exciting a Raman spectrum of the ion with a laser line which corresponds in wavenumber (actually ca. 19 000 cm^{-1}) with that of the intense "metal-metal" transition $2b_{1u} \leftarrow 2b_{2g}$ ($\delta^* \leftarrow \delta$)[8-10] of the ion. The isoelectronic and isostructural $Re_2Cl_8^{2-}$ and $Re_2Br_8^{2-}$ ions also possess an intense electronic transition in the visible region (at ca. 14 000 cm^{-1}).[11,12] This band was originally considered to arise from the $^1a_{2u} \leftarrow {}^1b_{2g}$ ($\sigma_n(1) \leftarrow \delta$) transition,[13] and thus be electric dipole forbidden, but recent single crystal electronic studies[12] and X_α scattered wave calculations[14] indicate that is is correctly assigned as the $2b_{1u} \leftarrow 2b_{2g}$ ($\delta^* \leftarrow \delta$) transition; the latter would be an electric dipole allowed transition of the Mulliken charge transfer type.[15] In so far as RR spectra have only been observed,[6] and are only ex-