volcanic islands contain cavernicolous shrimp (*18*), indicating that habitats other than caves can support these fauna. Terrestrial cavernicolous fauna have colonized Hawaiian lava tubes as soon as 100 years after the formation of the tubes (*19*). The high tidal range and corresponding strong currents in the Jameos cave would provide a means of sweeping larvae or weakly swimming species into the cave. Since the underwater tube was at a depth of 53 m and still descending at the limit of our diving explorations, deep water species or their larvae may also be drawn directly into the cave. Furthermore, at least some deep sea species have been shown to have two distributional maxima, one in the deep sea and another in shallow marine caves (*13*). Thus, it is possible that the Jameos cave fauna could have entered the cave by local dispersion from adjacent and older caves, other suitable habitats, or even the deep sea in a relatively short period of time.

THOMAS M. ILIFFE
Bermuda Biological Station for Research, Ferry Reach 1-15, Bermuda
HORST WILKENS
JAKOB PARZEFALL
Universität Hamburg, Zoologisches Institut und Zoologisches Museum, 2000 Hamburg 13, West Germany
DENNIS WILLIAMS
Box F-931, Freeport, Bahamas

References and Notes

1. Some examples of relict species from marine caves are a new family of ostracods and a new order of peracarid Crustacea from Bermuda, and a new family of shrimps from the Caicos Islands.
2. Although most true cavernicolous marine species inhabit only a single cave system or caves from a small area, some, such as the shrimp *Barbouria cubensis* from Bermuda, Cuba, the Bahamas, and the Cayman Islands, are widely dispersed by as yet unknown mechanisms.
3. D. C. Culver, *Cave Life: Evolution and Ecology* (Harvard Univ. Press, Cambridge, Mass., 1982), pp. 96–133.
4. The period about 10^8 years before present when the continents clustered around the Tethys Sea, a paleographic sea ancestral to the present Mediterranean which came into existence in early Permian time.
5. B. Sket and T. M. Iliffe, *Int. Rev. Gesamten Hydrobiol.* **65**, 871 (1980); J. H. Carpenter, in *Proceedings of the Eighth International Congress of Speleology*, B. F. Beck, Ed. (National Speleological Society, Huntsville, Ala., 1981), vol. 1, p. 24.
6. T. M. Iliffe, C. W. Hart, Jr., R. B. Manning, *Nature (London)* **302**, 141 (1983).
7. A. Vandel, *Biospeleology: The Biology of Cavernicolous Animals* (Pergamon, Oxford, 1965), pp. 8–9.
8. K. Koelbel, *Ann. K. K. Naturhist. Hofmuseums* **7**, 105 (1892); W. T. Calman, *Ann. Mag. Nat. Hist.* **7**, 213 (1904); L. Fage and T. Monod, *Arch. Zool. Exp.* **78**, 97 (1936); W. Harms, *Zool. Anz.* **52**, 101 (1971).
9. H. Wilkens and J. Parzefall, *Ann. Speleol.* **29**, 419 (1974); J. Parzefall and H. Wilkens, *ibid.* **30**, 325 (1975).
10. J. Yager, *J. Crust. Biol.* **1**, 328 (1981).
11. _____, personal communication.
12. J. Stock, *Studies on the Fauna of Curaçao and Other Caribbean Islands* **55**, 1 (1977); L. de Castro, *Biol. Mus. Nac. Rio Janeiro* **238**, 1 (1962).
13. R. Riedl, *Biologie der Meereshöhlen* (Verlag Paul Parey, Hamburg, 1966).
14. J. Stock and J. J. Vermeulen, *Bijdr. Dierkd.* **52**, 3 (1982).
15. E. M. Driscoll, G. L. Henry, K. J. Tinkler, *Geol. J.* **4**, 321 (1964).
16. A. Abdel-Monem, N. D. Watkins, P. W. Gast, *Am. J. Sci.* **271**, 490 (1971).
17. The term anchialine refers to "pools with no surface connection with the sea, containing salt or brackish water, which fluctuates with the tides".
18. F. A. Chace and R. B. Manning, *Smithson. Contrib. Zool.* **131**, 1 (1972); L. B. Holthuis, *Zool. Verh. Rijksmus. Nat. Hist. Leiden* **128**, 1 (1973); T. Monod and P. Cals, *Miss. Zool. Belge Iles Galapagos Ecuador* **2**, 57 (1970); J. A. Maciolek, *Bull. Mar. Sci.* **33**, 606 (1983).
19. F. G. Howarth, *Science* **175**, 325 (1972).
20. We thank S. Exley, K. Fulghum, C. Pitcairn, R. Power, and M. van Soeren—all members of the Jameos del Agua International Expedition 1983—for cave diving assistance. We also thank the Cabildo Insular de Lanzarote for assistance to our scientific studies, and H. Dalens, G. Hartmann-Schroder, L. B. Holthuis, W. Sterrer, and M. Turkay for valuable information. Supported by grants from the National Science Foundation (BSR-8215672) and the National Academy of Sciences to T.M.I.

8 December 1983; accepted 17 February 1984

Raman Spectroscopy of a Coal Liquid Shows That Fluorescence Interference Is Minimized with Ultraviolet Excitation

Abstract. *The first ultraviolet resonance Raman measurements of a coal liquid are reported. The spectra detail the presence of numerous polycyclic aromatic hydrocarbons with ring systems similar to those of naphthalene, fluorene, phenanthrene, pyrene, and triphenylene. The ultraviolet resonance Raman measurements of this highly complex sample show no significant interference from fluorescence. The lack of fluorescence interference and the high selectivity indicate that ultraviolet resonance Raman spectroscopy is a powerful new technique for characterizing highly complex samples and mixtures.*

Raman spectroscopy represents only one of a myriad of spectroscopic techniques used in the study of molecular structure (*1*). However, resonance Raman spectroscopy is unique among these techniques because of its selectivity, since the resonance phenomenon permits the specific enhancement of the vibrational spectrum of one particular molecular species within a complex solution or mixture (*2*). Other species in the mixture not resonantly enhanced show Raman spectra of much lower intensity (often by a factor of 10^{-5}). Raman spectroscopy has shown particular promise for biological investigations because the weak Raman scattering of water does not significantly interfere with measure-

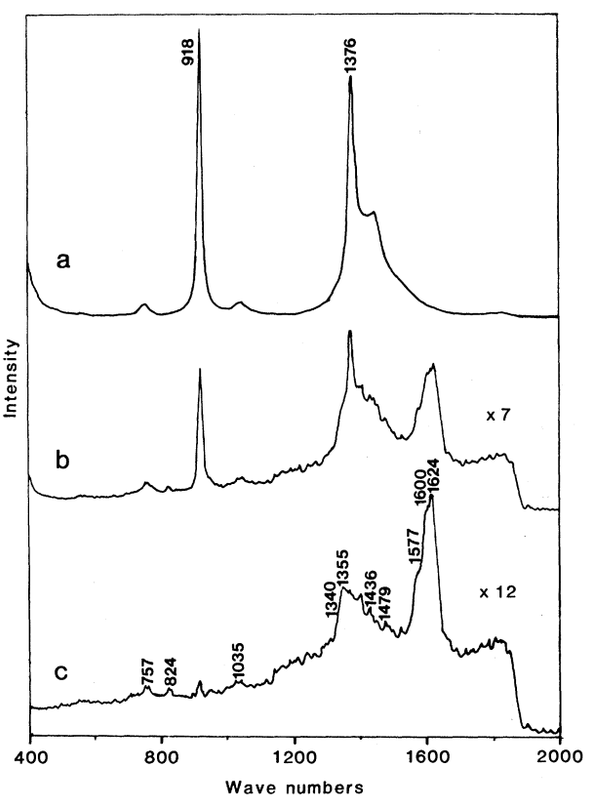

Fig. 1. Ultraviolet resonance Raman spectrum of (a) acetonitrile; (b) a coal liquid sample in acetonitrile diluted ~500 times (volume/volume); the intensity scale of (b) is expanded by a factor of 7 as compared to (a); (c) difference spectrum (b − a) showing the coal liquid resonance Raman spectrum; the intensity scale is expanded by a factor of ~12 as compared to (a). The measurement parameters were as follows: excitation wavelength, 256 nm; number of laser pulses averaged, 18,000 (15-minute scan at 20 Hz); average power, 2.0 mW; spectrometer bandpass, ~6 cm^{-1}.

ments of proteins, nucleic acids, and other biomolecules. The information content of the spectra is high, comparable to or greater than that available from infrared spectrometry, especially when the excitation wavelength is varied. However, resonance Raman spectroscopy has not been extensively used for investigations of complex systems because instrumentation was not available to resonantly excite the majority of compounds that have their electronic absorption bands in the ultraviolet and often sample or matrix fluorescence obscured the Raman spectra. This matrix fluorescence often prevents Raman studies of complex systems such as catalysts, coal, and biological tissues, where the large variety of compounds present almost ensures that fluorescence will swamp the Raman scattering.

In a continuing study we have developed new instrumentation for ultraviolet resonance Raman spectroscopy (*3*) and have recently demonstrated that this system has a high sensitivity and selectivity for ultraviolet Raman measurements of polycyclic aromatic hydrocarbons such as naphthalenes, substituted anthracenes, and pyrene (*4*). Low concentrations of these species could be monitored down to 200 parts per billion, and the spectra differentiated between species as similar as 2-methyl- and 9-methylanthracene. In this report we demonstrate that the ultraviolet resonance Raman spectra of compounds excited below 260 nm are much less plagued by fluorescence than conventional visible-wavelength Raman spectra; the number of compounds that fluoresce below 260 nm is limited, and their quantum yields for fluorescence are, in general, low.

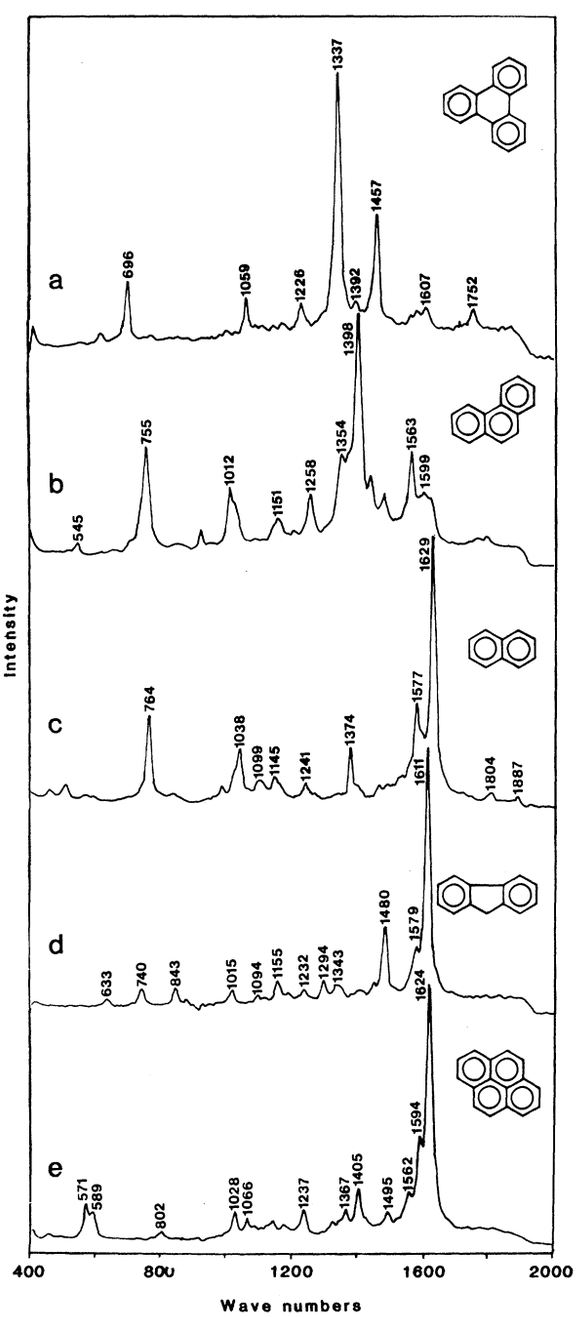

Fig. 2. Ultraviolet resonance Raman spectra of polycyclic aromatic hydrocarbons dissolved in acetonitrile and excited at 255 nm: (a) $5 \times 10^{-3}M$ triphenylene; (b) $5 \times 10^{-3}M$ phenanthrene; (c) $5 \times 10^{-3}M$ naphthalene; (d) $5 \times 10^{-3}M$ fluorene; (e) $5 \times 10^{-3}M$ pyrene. The peaks from the solvent have been numerically subtracted; average power, 2.5 mW; number of laser pulses averaged, 36,000 (30-minute scan); spectrometer bandpass, ~ 12 cm^{-1}.

For example, Fig. 1a shows the ultraviolet Raman spectrum of acetonitrile (CH_3CN) at 256 nm. We measured this spectrum in a flowing liquid jet, using instrumentation described elsewhere (*3*). Figure 1b shows a Raman spectrum of a dilute solution of a coal liquid in acetonitrile. This coal liquid sample was prepared by the direct hydrogenation of coal and is a heavy distillate recovered between 340° and 510°C. Such coal liquids are known from other studies (*5*) to contain large concentrations of polycyclic aromatic hydrocarbons with two, three, or four fused rings. The additional features in Fig. 1b, especially the intense Raman peaks from the coal liquid sample at ~ 1400 and ~ 1600 cm^{-1}, can be most easily seen in the difference spectrum shown in Fig. 1c. These additional features derive mainly from the resonance Raman spectra of polycyclic aromatic hydrocarbons, which contain between two and four fused rings as can be seen if one compares the coal liquid spectrum to the spectra of triphenylene, phenanthrene, naphthalene, fluorene, and pyrene (Fig. 2). For example, the peak from triphenylene at 1337 cm^{-1} may contribute to the shoulder at 1340 cm^{-1} in the coal liquid spectrum, whereas the peak at 757 cm^{-1} and the intensity at ~ 1400 cm^{-1} in the coal liquid spectrum may derive from the presence of phenanthrene-like structures. The intense broad peak between 1560 and 1630 cm^{-1} in the coal liquid spectrum may derive from substituted ring systems based on naphthalene (1577 and 1629 cm^{-1}), fluorene (1611 cm^{-1}), and pyrene (1624 cm^{-1}).

By changing the excitation wavelength, it is possible to selectively enhance different polycyclic aromatic hydrocarbons. For example (*6*), excitation of the coal liquid sample at 235 nm results in intense peaks at 1380 cm and 1628 cm^{-1}; the frequencies of these peaks and their relative intensities are essentially the same as that of naphthalene excited at 235 nm. Thus, changing the excitation wavelength enhances the spectra of different species within the coal liquid sample.

The most interesting feature of the coal liquid spectrum (Fig. 1b) is the lack of a luminescent background from fluorescence from the myriad of species present in the coal liquid (*5*). The acetonitrile Raman intensities from the coal

liquid solution are decreased by a factor of ~16 as compared to that of pure acetonitrile as a result of the absorption of the laser excitation and Raman light by the coal liquid. The background intensity observed in Fig. 1b is similar to that found for the high-purity ultraviolet-grade acetonitrile (Fig. 1a). Indeed, no fluorescence is observed even with excitation at shorter wavelengths (down to 220 nm). The lack of fluorescence interference in the Raman spectrum of this complex coal liquid sample is consistent with all our ultraviolet Raman studies of samples excited below 260 nm; significant fluorescence interference has not been observed for any sample yet examined.

The reason for this initially surprising result is that the smallest conjugated polyene or aromatic molecule to show significant fluorescence from its first excited singlet state is benzene, which shows fluorescence at a wavelength >260 nm. Larger aromatics show absorption bands below 260 nm but excitation into these bands results in fast internal conversion of this energy into the lowest energy singlet or triplet excited state, and fluorescence or phosphorescence occurs at much longer wavelengths. Other molecules or functional groups that have their lowest singlet states below 250 nm, such as butadiene and hexatriene, show extremely low fluorescence quantum yields (*7*). Thus, it appears that few if any organic compounds will show fluorescence in the spectral region below 260 nm. The obvious conclusion is that ultraviolet resonance Raman measurements will show little fluorescence interference as compared to conventional visible-wavelength Raman measurements. Thus, it is possible, perhaps likely, that complex samples such as coal liquids, biological samples, and complex organic reactions can be best studied by excitation in the ultraviolet; the only obvious complicating factor in ultraviolet Raman measurements is photochemical decomposition. However, photochemical decomposition did not pose a major problem for our aromatic amino acid studies (*8*) or for studies of benzene derivatives (*9*) or for our polycyclic aromatic hydrocarbon studies (*4*) or for excitation profile studies in the $n \rightarrow \pi^*$ transition of the carbonyl group in acetone (*10*). Thus, ultraviolet resonance Raman spectroscopy appears to be a uniquely sensitive and selective new technique for characterizing highly complex systems. These systems are obviously most tractable when a technique is used with high selectivity for one or a limited number of the species present. In the case of resonance Raman spectroscopy, the molecular selectivity can be tuned by changing the excitation wavelength.

SANFORD A. ASHER
CRAIG R. JOHNSON

Department of Chemistry,
University of Pittsburgh,
Pittsburgh, Pennsylvania 15260

References and Notes

1. D. A. Long, *Raman Spectroscopy* (McGraw-Hill, New York, 1977).
2. R. J. H. Clark and B. Stewart, *Struct. Bonding (Berlin)* **36**, 1 (1979).
3. S. A. Asher, C. R. Johnson, J. Murtaugh, *Rev. Sci. Instrum.* **54**, 1657 (1983).
4. S. A. Asher, *Anal. Chem.* **56**, 720 (1984).
5. C. M. White, in *Handbook of Polycyclic Aromatic Hydrocarbons*, A. Bjorseth, Ed. (Dekker, New York, 1983), p. 525.
6. C. R. Johnson and S. A. Asher, *J. Am. Chem. Soc.*, in press.
7. B. S. Hudson, B. E. Kohler, K. Shulten, in *Excited States*, E. C. Lim, Ed. (Academic Press, New York, 1982), vol. 6, p. 2.
8. C. R. Johnson, M. Ludwig, S. E. O'Donnell, S. A. Asher, *J. Am. Chem. Soc.*, in press.
9. S. A. Asher and C. R. Johnson, in preparation.
10. J. M. Dudik, C. R. Johnson, S. A. Asher, in preparation.
11. We thank G. Gibbon, C. White, and B. Blaustein, Pittsburgh Energy Technology Center, Department of Energy, for the gift of the coal liquid sample and for helpful discussions. This study was supported under NSF instrumentation grant PCM-8115738, NIH grant 1R01 GM 30741-01, and a Cottrell Research Corporation grant.

2 March 1984; accepted 2 May 1984

A Novel Photosynthetic Purple Bacterium Isolated from a Yellowstone Hot Spring

Abstract. *A thermophilic photosynthetic purple bacterium was isolated from the waters of a hot spring in Yellowstone National Park, Wyoming. The organism differs from all known purple bacteria in that it grows optimally at a temperature of about 50°C. The isolate contains bacteriochlorophyll a and grows autotrophically, oxidizing sulfide to elemental sulfur which is then stored as globules inside the cell. These properties indicate that the phototroph is a member of the Chromatiaceae (purple sulfur bacteria).*

A variety of photosynthetic prokaryotes inhabit thermal springs whose water temperatures may be as high as 73°C (*1*, *2*). The only photosynthetic bacterium that has been isolated from thermal environments is *Chloroflexus aurantiacus*, a filamentous bacterium that shares physiological and biochemical properties with both the purple and green anoxygenic phototrophs (*3–6*). The composition and location of pigments in *Chloroflexus*, however, more closely resemble those of the green bacteria, and the organism is generally considered to be a member of this group (*3–7*). Purple sulfur bacteria growing in thermal waters have been reported in a number of field observations (*2*, *8*, *9*), but the characteristics of pure cultures of these phototrophs have not been reported. I now describe the general properties of a truly thermophilic purple bacterium.

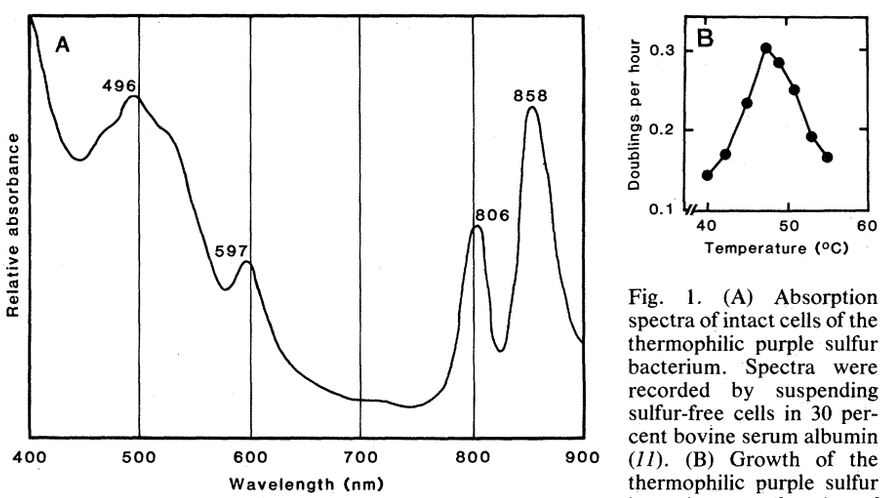

Fig. 1. (A) Absorption spectra of intact cells of the thermophilic purple sulfur bacterium. Spectra were recorded by suspending sulfur-free cells in 30 percent bovine serum albumin (*11*). (B) Growth of the thermophilic purple sulfur bacterium as a function of temperature. Cells were grown in the medium described (*16*). Cultures (250-ml bottles incubated in a water bath) were illuminated with 4300 lux of incandescent light. Cell suspensions were removed anaerobically at intervals with a sterile sampling device (*17*), and the number of cells was determined by direct microscopic counts with a Petroff-Hauser counting chamber. Growth rates (doublings per hour) were calculated from semilogarithmic plots of cell numbers against time.