Applications of Laser Raman Spectroscopy to Natural Products Research

Stanley K. Freeman

The value of Raman spectroscopy in natural products research lies principally in its unique aspects rather than its complementarity to infrared spectroscopy. Commercially available laser sources have enabled spectra to be generated conveniently and rapidly on microgram quantities. In general, sample handling techniques for Raman spectroscopy are simple and applicable to a wide range of materials. Raman spectral data often yield structural information not readily available from other spectral disciplines. Raman spectroscopy is an invaluable probe for functional groups such as alkenes, mono-, di-, and trisulfides, as well as ring systems. Biologically important compounds may be studied by Raman spectroscopy, yielding insights into conformational preferences, solvent effects, etc.

The potential of Raman spectroscopy in molecular structure studies, although long appreciated, began to be realized only a few years ago. Many papers concerned with the Raman effect appeared between 1930 and 1945, but this spectral discipline was eclipsed in the mid 1940's by the advent of commercially available double beam infrared instrumentation capable of generating good quality spectra. The recent Raman resurgence has been made possible by laser technology, which supplied the chemist with a nearly ideal light source, emancipating Raman spectroscopy from the many limitations of mercury arc excitation (Freeman and Landon, 1968).

SAMPLE HANDLING

Sample handling techniques are very simple and in most instances presentation of a liquid or powder to the spectrometer merely involves partially filling a melting point capillary and placing it at the focal point of the laser (Figure 1). A 3-mm continuous liquid column is approximately 2 μl. Smaller bore tubing is employed for samples in the nanoliter range. Polar compounds such as water and methanol are poor Raman scatterers and therefore are excellent solvents. A spectrum obtained on 2 nl of a 20% aqueous solution of cysteine hydrochloride in a 0.1 mm i.d. capillary is shown in Figure 2 (Freeman *et al.*, 1972). Powders and crystals yield good Raman spectra in the low microgram range. Conformational studies involving comparison of Raman spectra of a particular compound in the liquid and solid states may be performed with ease employing a commercially available variable temperature cell (Miller and Harney, 1970).

A resonance Raman effect occurs when the laser exciting line approaches or nearly coincides with an electronic absorption band of the sample. Under these conditions the intensities of some Raman bands may be increased by as much as 10^6 times those observed when the exciting frequency is far from the absorption maximum. The resonance Raman spectrum of β-carotene, a constituent of raw carrot (Figure 3), was obtained by placing the intact specimen at the focal point of an Argon ion laser (Sloane, 1972).

FUNCTIONAL GROUPS

The utility of laser Raman spectroscopy in natural products investigations has been amply demonstrated for detecting ethylenic double bonds (Freeman and Mayo, 1969), thiols (Freeman and Mayo, 1972a), monosulfides (Freeman and Mayo, 1972a), disulfides (Freeman and Mayo, 1972b), trisulfides (Freeman, 1971), aromatic compounds (Kagel, 1971; Mayo and Freeman, 1972; Nyquist, 1972), and alicyclic compounds (Freeman and Mayo, 1970; Mayo and Freeman, 1970). In addition, the Raman effect has led to an understanding of polypeptide (Rippon *et al.*, 1971) and protein conformations (Careri *et al.*, 1970; Tobin, 1968).

ETHYLENIC DOUBLE BONDS

To the best of the writer's knowledge, no compound possessing a carbon–carbon double bond has failed to display a Raman band between 1500 and 1900 cm^{-1}. The lone double bond in cholesterol (mol wt 386) is clearly evident at 1668 cm^{-1} (Figure 4). Frequencies and depolarization values of C=C stretching vibrations are good diagnostics for olefin type (Tables I and II). The Raman spectrum of carveol (Figure 5) exhibits two moderate, nearly equal strength Raman bands at 1645 and 1673 cm^{-1}. With but few exceptions, the band intensities of two different kinds of C=C moieties in a molecule are similar (Freeman, 1971). If the intensity ratio of two C=C stretching bands appearing in a Raman spectrum lies outside the range 0.7–1.3, it is quite likely that one of them arises from an impurity. Conjugated dienes and trienes have very strong bands which, when coupled with a knowledge of their uv maxima, permit ready identification in *ca.* 1-nl quantity (Freeman and Mayo, 1969).

THIOLS, MONO-, DI-, AND TRISULFIDES

A host of organosulfur compounds are found in nature (Bacq, 1971; Gal'pern, 1971), many of which are valuable as flavors (Schwimmer and Friedman, 1972). The presence of a Raman band near 2550 cm^{-1}, originating from the S–H stretching vibration, is nearly incontrovertible evidence for a thiol. Monosulfide spectra generally contain a strong C–S stretching band between 600 and 725 cm^{-1}. An interpretively important pattern is observed for compounds of the type $CH_3S(CH_2)_nCH_3$, where n is greater than 1 (Figure 6) (Freeman and Mayo, 1972a). Sulfides of the general formula $CH_3(CH_2)_nS(CH_2)_mCH_3$, where n is greater than 1 and m is greater than 2, also display a characteristic series of bands (Figure 7) (Freeman and Mayo, 1972a).

Two bands are observed in the Raman spectra of acyclic and cyclic disulfides: C–S stretch at 600–725 cm^{-1} (moderate intensity) and S–S stretch at 450–550 cm^{-1} (intense). A typical profile for an alkyl disulfide is shown in Figure 8 (Freeman and Mayo, 1972b). The bands ascribed to the less stable conformer, marked with asterisks, do not persist in the spectrum of the solid. The Raman spectrum of a mixture of (*Z*)- and (*E*)-propenyl disulfide, isolated from onion oil, is instructive (Figure 9). The intense bands at 497 cm^{-1} (ρ = 0.06) and 694 cm^{-1} (ρ = 0.13) arise from the S–S and CH_3–S stretching modes, respectively. Disappearance of the 517 cm^{-1} band in the Raman spectrum recorded on the solid material indicates that it

International Flavors & Fragrances, Inc. (R&D), Union Beach, New Jersey 07735.

Table I. Characteristic Frequencies and Depolarization Ratios of Acyclic Alkenes

Ethylenic	Structure	=C-H stretch, cm^{-1}	$=CH_2$ stretch, cm^{-1}		C=C stretch, cm^{-1}	
Vinyl	$-CH=CH_2$	3075-3100	3010-3025	0.01	1635-1650	0.04
			3075-3095	0.5		
Vinylidene	$>C=CH_2$		3075-3100		1640-1660	0.04
Z^a	-CH=CH-	3000-3050			1635-1660	0.05
E^a	-CH=CH-	3000-3050			1665-1680	0.08
Trisubstituted	>C=CH-	2990-3050			1665-1695	0.1
Tetrasubstituted	>C=C<				1665-1685	0.1

[a] Z and E indicate cis and trans, respectively.

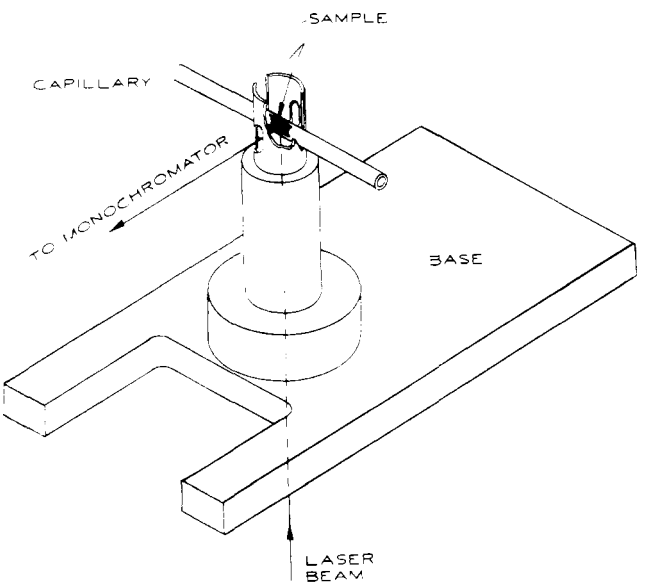


Figure 1. Capillary cell holder.

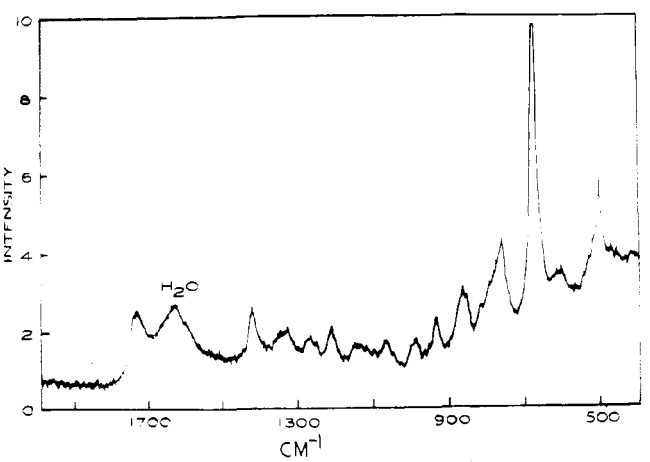


Figure 2. Raman spectrum on 2 nl of a 20% aqueous solution of cysteine HCl (200 mW Ar^+ excitation, 514.5 nm).

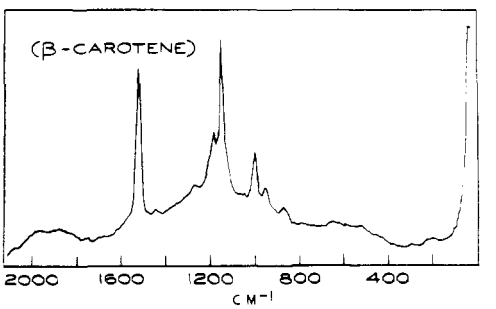


Figure 3. Raman spectrum obtained by placing a raw carrot at the focus of an Ar^+ laser (488.0 nm).

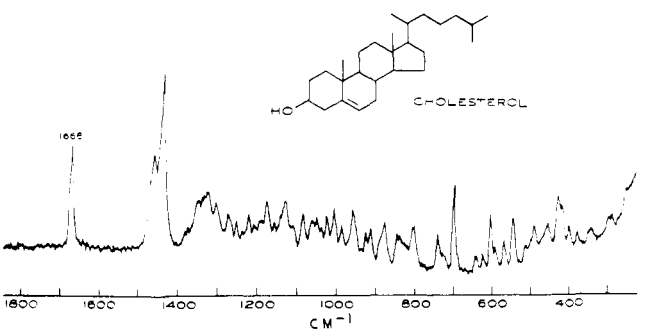


Figure 4. Raman spectrum of cholesterol (200 mW Ar^+ excitation, 514.5 nm).

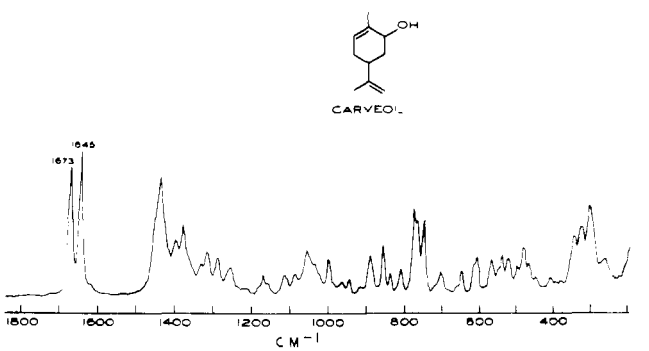


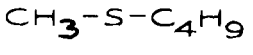
Figure 5. Raman spectrum of carveol (50 mW He-Ne excitation).

is the S-S stretch of a higher energy rotamer. From the intensity ratio of scattering at 1377/1440 cm^{-1}, it can be concluded that the molecule possesses one ethylene methyl group (Freeman and Mayo, 1972c). Proximity of the sulfur atom to the carbon-carbon double bond decreases the stretching frequency of the *Z* and *E* isomers to 1612 cm^{-1} (ρ = 0.06) and 1623 cm^{-1} ($\rho \sim 0.10$), respectively. The approximate peak height ratio indicates roughly a 2:1 mixture of *Z*/*E* forms.

An intense S-S-S stretching band at 475-490 cm^{-1} is observed in the spectra of cyclic and acyclic trisulfides (Figure 10). Not only can mono-, di-, and trisulfides be detected by Raman spectroscopy, but analysis of their mixtures is also possible. For example, as little as *ca.* 0.3% cystine hydrochloride can be determined in cysteine hydrochloride by measuring the intensity of the 498 cm^{-1} S-S stretching band (Freeman, 1971). Lenthionine, an odorous compound occurring in the Shiitake mushroom,

Table II. Frequencies and Depolarization Values of Some Cyclic Alkenes

	$\nu_{C=C}$, cm^{-1}	Depolarization value
	1654	0.05
	1650	0.08
	1612	0.01
	1655	0.03
	1650	0.02
	1674	0.09
	1570	0.03
	a, 1616 b, 1574	0.03



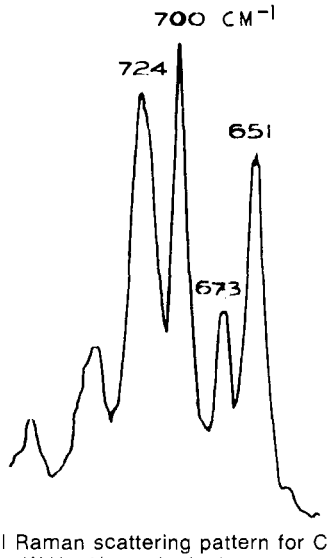
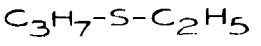


Figure 6. Typical Raman scattering pattern for $CH_3S(CH_2)_nCH_3$, where $n > 1$ (50 mW He-Ne excitation).

contains both a di- and trisulfide linkage (Morita and Kobayashi, 1966). Its Raman spectrum clearly shows the presence of these moieties (Figure 11).

AROMATIC COMPOUNDS

Raman spectra of aromatics usually contain fewer bands than their infrared counterparts, thereby easing the task of interpreting Raman data. When meta-directing groups are substituted on a benzene ring, the out-of-plane hydrogen vibrations, which give rise to characteristic infrared bands, are perturbed (Kagel, 1971). Often the intensities of bands associated with these vibrations decrease, and they may be shifted to higher frequencies where other vibrations occur. As a result, it is difficult to determine ring substitution of this type by infrared spectroscopy. No such anomalies are encountered in the Raman effect. Pyridines exhibit diagnostically valuable Raman bands (Freeman, 1971; Green *et al.*, 1969; Spinner, 1963) and Raman spectra-structure correlations of



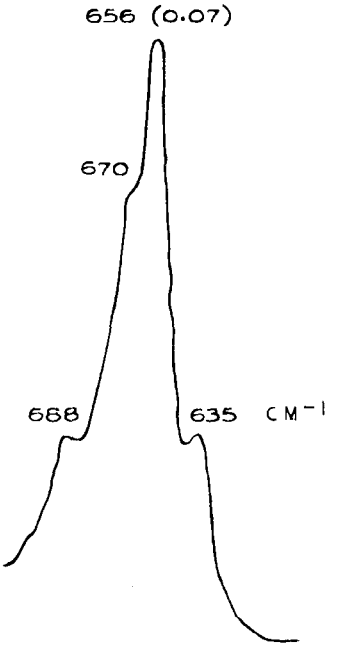
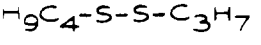


Figure 7. Typical Raman scattering pattern for $CH_3(CH_2)_nS(CH_2)_mCH_3$, where $n > 1$, $m > 2$ (50 mW He-Ne excitation).



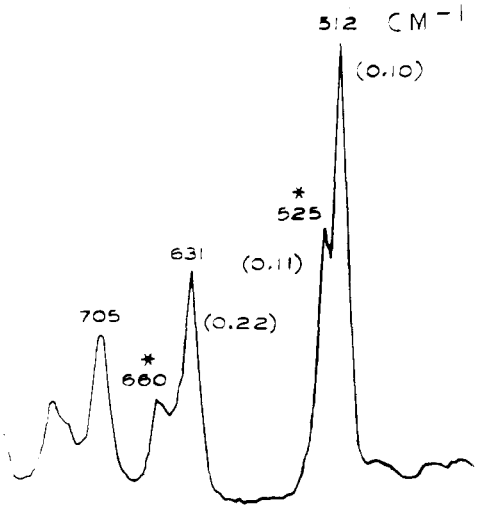


Figure 8. Typical Raman scattering pattern for $CH_3(CH_2)SS(CH_2)_mCH_3$, where $n > 1$, $m > 2$ (50 mW He-Ne excitation).

pyrazines, important flavor constituents of many natural heat-processed foods, allow simple and unequivocal determination of many substitution patterns (Mayo and Freeman, 1970; Nakel and Haynes, 1972; Oertel and Myrhe, 1972).

The Raman effect is an excellent probe for furans (Bardet *et al.*, 1971; Freeman, 1971; Katrizky and Lagowski, 1959) and thiophenes (Peron *et al.*, 1967; Treschova *et al.*, 1964). Their 2-substituted derivatives display characteristic bands which differ significantly from these observed

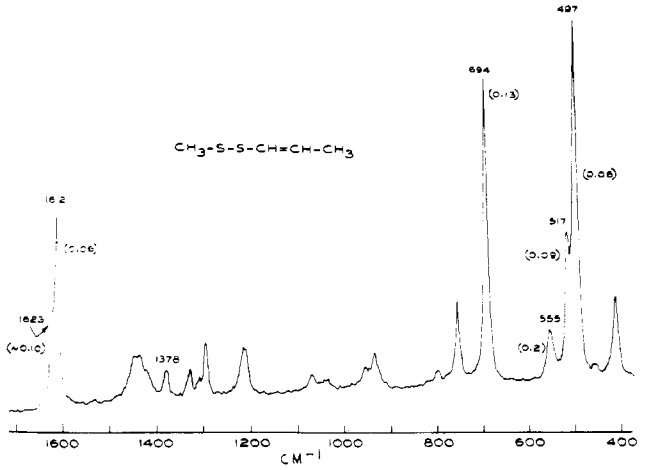


Figure 9. Raman spectrum of a mixture of (*Z*)- and (*E*)-propenyl disulfides (50 mW He–Ne excitation).

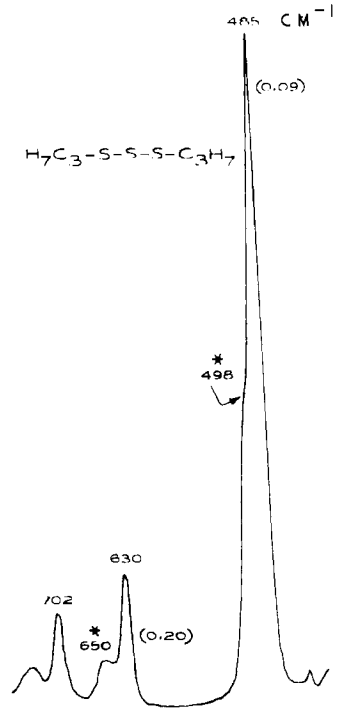


Figure 10. Typical Raman scattering pattern for $CH_3(CH_2)_nSSSCH_3(CH_2)_nCH_3$, where $n > 2$ (50 mW He–Ne excitation).

for the 3- and 5-monosubstituted and 2,5-disubstituted derivatives (Freeman, 1971). A spectrum of furfuryl thiol is presented in Figure 12; bands marked with an asterisk are those characteristic for a 2-substituted furan.

ALICYCLIC COMPOUNDS

Vibrations associated with ring systems generally give rise to intense Raman emissions and weak infrared absorptions. Polar functional groups, which may be located on the ring, scatter poorly in the Raman effect, and fingerprint assignments often allow confident assignment of the molecular backbone. Several ring systems have been characterized in this way; *e.g.*, pinane (Freeman and Mayo, 1970), cedrane (Mayo and Freeman, 1970), patchoulane (Freeman, 1971), and inosine (Medeiros and Thomas, 1971). The Raman spectra of pinane and cedrane are shown in Figure 13. Asterisks mark the characteristic bands.

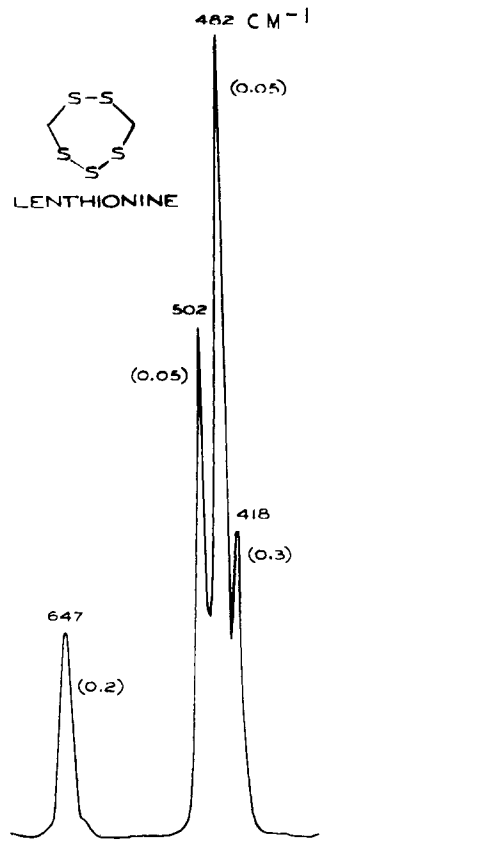


Figure 11. Raman spectrum of lenthionine (50 mW He–Ne excitation).

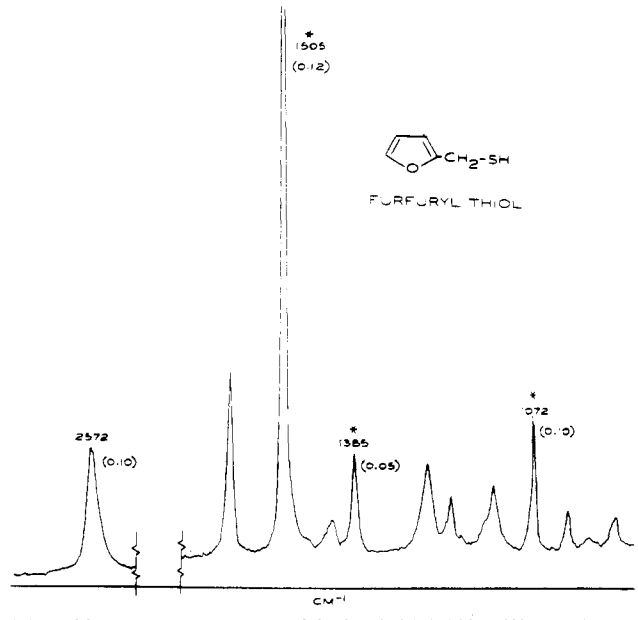


Figure 12. Raman spectrum of furfuryl thiol (50 mW He–Ne excitation). Asterisks mark the bands characteristic of 2-substituted furans.

BIOLOGICAL MATERIALS

In addition to the complementary aspects of Raman and infrared spectroscopy, the Raman spectrum of a compound can be more useful to biological studies for several reasons (Rippon *et al.*, 1971). Materials can be studied in their natural state by Raman spectroscopy and isotopic exchange data can be gained by comparing spectra in H_2O and D_2O. Comparison of spectra obtained from solids and

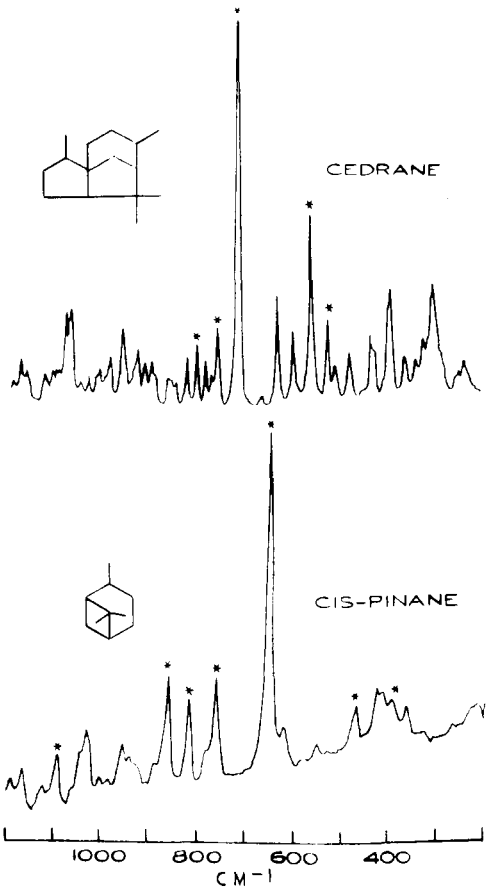


Figure 13. Raman spectra of cedrane and *cis*-pinane (50 mW He–Ne excitation). Asterisks mark the characteristic ring bands.

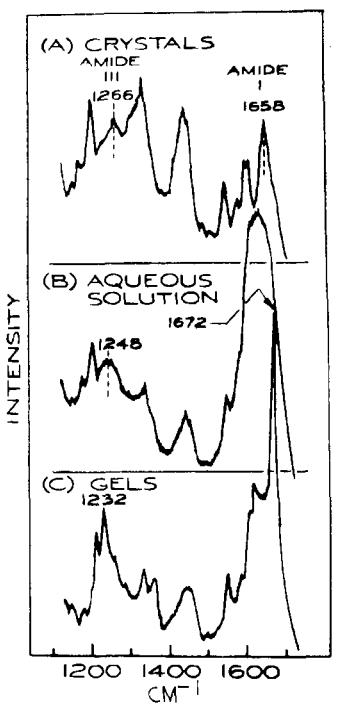


Figure 14. Raman spectra of glucagon. (A), α-helix; (B), random coil; (C), β-pleated sheet conformations.

aqueous solutions often yields insights into solvent effects on conformational states. Some interesting investigations pertaining to conformational changes (*i.e.*, alterations in secondary, tertiary, or quarternary structures of polypeptides and proteins) have been reported (*e.g.*, Careri *et al.*, 1970; Tobin, 1968). Two spectral regions are useful for characterizing the polypeptide backbone: 1630–1700 cm^{-1} (amide I) and 1220–1300 cm^{-1} (amide III). Studies of Raman spectra of synthetic polypeptides indicate that the amide I and amide III frequencies are quite different for α-helical, β-pleated sheet, and random coil forms (Fanconi *et al.*, 1969; Lord and Yu, 1970; Small *et al.*, 1970). Recently, it has been shown that these frequencies can be used to assess conformation in proteins (Yu and Liu, 1972). The partial Raman spectra of crystalline, aqueous, and gel glucagon, a protein of pancreatic origin, appear in Figure 14. The conformational changes from α-helix (A) to random coil (B) to β-pleated sheet (C) may be observed from the amide I–amide III band frequency shifts. The strong water band near 1640 cm^{-1} obscures the amide I frequency of glucagon in aqueous solution.

CONCLUSION

There is little doubt that the Raman effect is a valuable tool for structure elucidation of naturally occurring compounds. Rather than stressing the complementarity of Raman and infrared spectroscopy, the chemist should recognize that the former technique can be employed as a powerful independent probe for many functional groups and for the investigation of biological materials.

LITERATURE CITED

Bacq, Z. M., *Int. J. Sulfur Chem. Part B* **6(2)**, 93 (1971).
Bardet, L., Maillols, J., Fabre, G., *J. Chim. Phys. Physicochim. Biol.* **68**, 984 (1971).
Careri, G., Mazzacurati, V., Signorelli, G., *Phys. Lett. A* **31**, 425 (1970).
Fanconi, B., Tomlinson, B., Nafie, L. A., Small, W., Peticolas, W. L., *J. Chem. Phys.* **51**, 3993 (1969).
Freeman, S. K., unpublished work, 1971.
Freeman, S. K., Landon, D. O., "The Spex Speaker," Vol. VIII, No. 4, Spex Industries, Metuchen, N. J., 1968.
Freeman, S. K., Mayo, D. W., *Appl. Spectrosc.* **23**, 610 (1969).
Freeman, S. K., Mayo, D. W., *Appl. Spectrosc.* **24**, 595 (1970).
Freeman, S. K., Mayo, D. W., Abstracts Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, Cleveland, Ohio, March 1972a.
Freeman, S. K., Mayo, D. W., paper presented before the Eastern Analytical Symposium, Atlantic City, N. J., Nov 1972b.
Freeman, S. K., Mayo, D. W., *Appl. Spectrosc.* **26**, 543 (1972c).
Freeman, S. K., Reed, P. R., Landon, D. O., *Mikrochim. Acta* 288 (1972).
Gal'pern, G. D., *Int. J. Sulfur Chem. Part B* **6(2)**, 115 (1971).
Green, J. H. S., Harrison, D. H., Kynaston, W., Paisley, H. M., *Spectrochim. Acta Part A* **26**, 2139 (1969).
Kagel, R. O., Dow Chemical Company, Midland, Mich., unpublished work, 1971.
Katrizky, A. R., Lagowski, J. M., *J. Chem. Soc.* 657 (1959).
Lord, R. C., Yu, N.-T., *J. Mol. Biol.* **50**, 509 (1970).
Mayo, D. W., Freeman, S. K., *Appl. Spectrosc.* **24**, 591 (1970).
Mayo, D. W., Freeman, S. K., Abstracts Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, Cleveland, Ohio, March 1972.
Medeiros, G. C., Thomas, G. J., Jr., *Biochim. Biophys. Acta* **247**, 449 (1971).
Miller, F. A., Harney, B. M., *Appl. Spectrosc.* **24**, 291 (1970).
Morita, K., Kobayashi, S., *Tetrahedron Lett.* **no. 6**, 573 (1966).
Nakel, G. M., Haynes, L. V., *J. Agr. Food Chem.* **20**, 683 (1972).
Nyquist, R. A., *Appl. Spectrosc.* **26**, 81 (1972).
Oertel, R. P., Myhre, D. V., *Anal. Chem.* **44**, 1589 (1972).
Peron, J. J., Saumagne, P., Lebas, J. M., *C. R. Acad. Sci. Ser. B* **264**(10), 797 (1967).
Rippon, W. B., Koenig, J. L., Watson, A. G., *J. Agr. Food Chem.* **19**, 692 (1971).
Schwimmer, S. S., Friedman, M., *Flavour Ind.* **3**, 137 (1972).
Sloane, H., Cary Instruments Co., Monrovia, Calif., unpublished work, 1972.
Small, E. W., Fanconi, B., Peticolas, W. L., *J. Chem. Phys.* **52**, 4369 (1970).
Spinner, E., *J. Chem. Soc.* 3860 (1963).
Tobin, M. C., *Science* **161**, 68 (1968).
Treschova, E. G., Ekkhardt, D., Yur'ev, Yu. K., *Zh. Fiz. Khim.* **38**(2), 295 (1964).
Yu, N.-T., Liu, C. S., *J. Amer. Chem. Soc.* **94**, 5127 (1972).

Received for review November 9, 1972. Accepted February 9, 1973. Presented at symposium on Non-Vapor Phase Techniques for the Isolation and Identification of Flavor Compounds, 164th National Meeting of the American Chemical Society, New York, N. Y., September 1972.