The Measurement of the Raman Spectra of Organic Compounds in Solution*,[1]

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A technique is described for the routine measurement of the Raman spectra of organic compounds. The spectra are obtained from 50–400-mg. samples in the liquid state or in solution, at concentrations that are similar to those commonly used for the measurement of infrared spectra. A Cary Model 81 Raman spectrophotometer is used with capillary sample tubes and a modified filter system. The wave-number range is 4000–280/100 cm.$^{-1}$ depending on the solvent. The band intensities are referred to the peak height of the carbon tetrachloride band at 459 cm.$^{-1}$, using internal standards. The solvents employed are carbon disulfide, carbon tetrachloride, chloroform, methylene dichloride, tetrachloroethylene, acetonitrile, water, and deuterium oxide. The solvent obscuration regions are reported, and solvent bands suitable for use as secondary intensity standards have been selected. The factors for relating the measurements in other solvents to the primary intensity standard vary with the experimental conditions; the influence of the spectral slit width and the scanning rate has been evaluated, and other considerations that affect the comparisons of band intensities measured in different solvents are discussed. Techniques for the determination of the corrected depolarization ratios are described. Representative measurements on several types of compounds in different solvents are illustrated.

Raman spectroscopists have dealt mainly with small molecules in which the differences between the Raman and infrared spectra are directly interpretable from the molecular symmetry. Since the introduction of recording infrared spectrometers, organic chemists have shown little interest in Raman spectrometry as an aid to the structural analysis of more complex compounds, though the Raman technique has not been wholly neglected. Notable work on the Raman spectra of amino acids and polypeptides has appeared from Edsall's laboratory,[4] to cite one example, and the Raman spectra of the simpler types of organic compounds, particularly hydrocarbons, have continued to receive attention.[5-9] By and large, however, Raman spectrometry has failed to maintain the promise that it demonstrated in the 1930's under the stimulus of the Kohlrausch school,[10] and has yet to become one of the regular tools of organic structural analysis along with infrared, ultraviolet, and proton magnetic resonance spectroscopy. This is mainly because several grams of material have been needed to obtain a Raman spectrum, and the spectrum can only be observed in the total absence of fluorescence.

There is no virtue in using the Raman technique where the same structural information can be obtained from the infrared spectrum. However, some molecular groups are better characterized by their Raman than by their infrared spectra; these include tetrasubstituted ethylenic groups, disubstituted acetylenes, and polar substances in aqueous solution. Raman and infrared spectra provide complementary information about molecular vibrations, and, if both types of spectra were routinely available for comparison, the power of the group frequency vibration method of structural analysis would be greatly enhanced.

The technique described here employs the Cary Model 81 Raman spectrophotometer with capillary sample cells of 0.15–0.50-ml. capacity. It is suitable for pure liquids and solutions, and the sample size is in the range 50–400 mg. depending on the solubility and structure of the compound. The Raman spectra are obtained under conditions that conform closely with those employed for infrared spectra so that comparison of the two spectra will have maximal significance. The solutions prepared for Raman spectrometry can often be used directly for infrared spectrometry on cells of 0.1-mm. path length. The technique can be operated by a skilled laboratory assistant; it has a wave-number precision of ± 2 cm.$^{-1}$ and an intensity reproducibility of $\pm 10\%$ on the band heights. The resolution is comparable with that obtained in the infrared with a medium-sized sodium chloride prism spectrometer, and the wave-number range is 4000–280/100 cm.$^{-1}$ depending on the solvent. The problem of fluorescing impurities is the major hindrance to the wider application of Raman spectrometry and a further reduction in sample size is still necessary. Although some of the instrumental problems discussed below are specific to the Cary spectrophotometer, the principles are general, and the method should be adaptable to other types of semimicro spectrophotometric systems.[11]

Instrumentation

The design and construction of the Cary Model 81 Raman spectrophotometer have been described[12,13]; the high light-gathering capacity results primarily from the use of a beam splitter to convert the circular image of the sample tube into narrow rectangles for maximal illumination of the two parallel slits. Here we shall only note some structural modifications in-

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(4) D. Garfinkel and J. T. Edsall, *J. Am. Chem. Soc.*, **80**, 3818 (1958), and earlier publications.

(5) P. A. Bazhulin, A. V. Koperina, A. L. Liberman, V. A. Ovodova, and B. A. Kazanskii, *Chem. Abstr.*, **49**, 10866 (1955).

(6) M. M. Sushinskii, Institute of Petroleum, Molecular Spectroscopy, Report of a Conference organized by the Spectroscopy Panel of the Hydrocarbon Research Group, London, 1955, p. 271.

(7) P. P. Shorygin, *Usp. Khim.*, **19**, 419 (1950); National Research Council of Canada Technical Translation No. 228.

(8) J. Goubeau, E. Kohler, E. Lell, M. Nordmann, and E. Tschentscher, *Angew. chem.*, Beiheft No. 56 (1948).

(9) D. G. Rea, *Anal. Chem.*, **32**, 1638 (1960).

(10) K. W. F. Kohlrausch in "Hand- und Jahrbuch der Chemischen Physik," Vol. 9, Part 6, A. Eucken and K. L. Wolf, Ed., Leipzig, 1943; reprinted by Edwards Bros., Inc., Ann Arbor, Mich., 1945.

(11) B. Schrader, F. Nerdel, and G. Kresze, *Z. anal. Chem.*, **197**, 295 (1963).

(12) J. C. Evans in "Infra-red Spectroscopy and Molecular Structure," Mansel Davies, Ed., Elsevier Publishing Co., Amsterdam, 1963, Chapter VI, p. 212.

(13) J. Brandmüller and H. Moser, "Einführung in die Ramanspektroskopie," Steinkopff Verlag, Darmstadt, 1962, p. 212.

volving the source assembly and liquid filter systems. The spectrometer is supplied with three sets of source optics for use with cells of 65-, 5-, and 0.2-ml. capacity. Modifications of the 5-ml. cell system to permit more effective collection of the Raman radiation by a multiple reflection optical system have been described by Tunnicliff and Jones.[14] By this means they achieved a fivefold increase in sensitivity, but the large sample volume precludes the use of this type of cell in the applications that concern us here. We have used the 0.2-ml. optical system with capillary cells having volumes in the range 0.15–0.5 ml.

In Figure 1 is shown a cross section through the lamp housing and cell compartment in our modified instrument. The lamp helix is made from Corning 1720 glass with Pyrex electrode chambers; the Corning 1720 glass darkens much less rapidly than Pyrex, and the lamp coil remains clean and operative after more than a thousand hours of use. Starting electrodes are provided in both electrode chambers, and the polarity of the discharge is reversed periodically as electrodistillation leads to the accumulation of excessive mercury in the cathode pot.[15] A mercury vapor pump is preferable to an oil diffusion pump for evacuating the lamps prior to sealing as mercury-pumped lamps light more easily. The helix is wrapped on the outside with a wide mesh net of No. 36 Nichrome wire to facilitate starting with a Tesla coil. Coating the inside of the lamp housing with magnesium oxide produced only a trivial enhancement of the Raman intensity.

Filter System.—The filter system supplied with the spectrophotometer has been replaced by a double-walled Pyrex glass jacket, each annular compartment of which has an internal thickness of approximately 1 cm. A saturated aqueous solution of potassium nitrite is circulated through the outer annular space. The thermostatic control system of the original instrument is retained, but all copper tubing and connections have been replaced by stainless steel or Tygon.[16] A sintered-glass filter with a bubble trap is introduced into the nitrite flow line at the input point of the filter jacket. The inner annular space of the jacket contains a nonflowing aqueous solution of Rhodamine 5 DGN Extra (0.009%), slightly acidified with hydrochloric acid. This filter system will remain stable for several hundred hours of lamp operation. It cleanly isolates the 4358-Å. mercury line and transmits only one weak satellite mercury line at 4916 Å., which appears in the Raman spectrum at 2602 cm.$^{-1}$ and provides a convenient calibration check.[17] In some investigations we have used the 5461-Å. mercury green line with different filter systems.[18]

Sample Tubes.—The Pyrex capillary tubes supplied with the spectrometer have a nominal external diameter of 2 mm. We construct our own cells and have

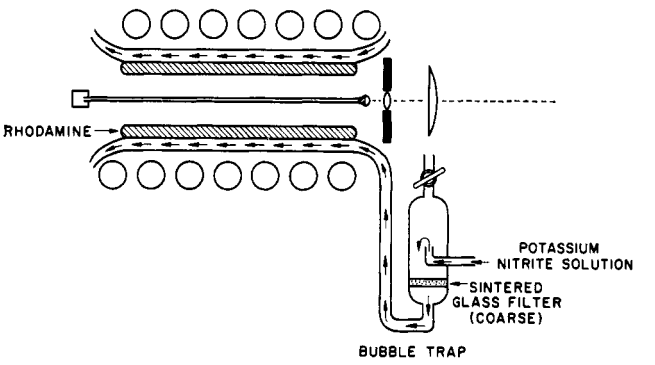

Figure 1.—Cary Model 81 Raman spectrophotometer. Cross section through the source compartment showing modifications to the filter system.

standardized on two sizes,[19] the dimensions of which are given in Table I.

TABLE I
DIMENSIONS OF CAPILLARY SAMPLE TUBES

	Effective length, mm.	Diameter, mm. Inside	Diameter, mm. Outside	Volume, ml.	Solute required in different solvents, mg.[a] CS_2	$CHCl_3$	H_2O
Small	225	0.9 ± 0.1	2.0 ± 0.2	0.15	100	200	300
Large	225	1.7 ± 0.1	2.4 ± 0.2	0.50	200	300	300

[a] This includes allowance for the parasitic loss in the transfer syringe. Under favorable circumstances acceptable spectra have been obtained from half these amounts.

One end of the capillary is sealed, care being taken to obtain a symmetric meniscus seal free of entrapped air. The cell holder and source optical unit supplied by the manufacturer is used. This maintains the closed end of the tube in contact with a wide-angle hemispherical collecting lens using a glycerol immersion contact. A Teflon diaphram with a small central hole accurately locates the end of the sample tube on the axis of the collecting lens. We have found it necessary to use separate cell holders and source optical systems for the small and large tubes, because the small tube will not center accurately enough in the enlarged diaphram hole of the large tube holder. It is important to prevent seepage of glycerol from the lens contact point to the intersurface between the tube and the Teflon diaphram, as this can lead to serious energy loss.

A vertically mounted, permanently clamped, syringe is used to fill the tube. The syringe consists of a 300-mm. length of no. 20 wire gauge flexible steel tubing, which is sealed at the top to a reservoir made from an 80-mm. length of 8-mm. Pyrex tubing. This in turn is attached by rubber tubing to a 2-ml. hypodermic syringe. The movement of the syringe plunger is controlled by a micrometer screw. The sample solution or neat liquid is withdrawn from its vial into the syringe, and the steel tubing is threaded down the cell to make contact with the bottom. The sample is next slowly transferred, the cell being progressively withdrawn so that the tip of the steel tube remains below the surface of the liquid. In manipulating concentrated solutions it is advisable to warm the syringe before beginning the transfer. For reasons discussed

(14) D. D. Tunnicliff and A. C. Jones, *Spectrochim. Acta*, **18**, 569 (1962).

(15) Lamps constructed of Corning 1720 glass are now available from the Applied Physics Corp., Monrovia, Calif.

(16) Because of the corrosive action of the potassium nitrite solution, the mechanical seal assembly on the stainless steel pump supplied with the instrument was replaced by a "Viton A" mechanical seal; a stellited impeller was also used. These modifications were provided by the pump manufacturer (Eastern Industries, Inc.).

(17) Although the positions of Raman bands are correctly reported as wave-number *displacements* ($\Delta\nu$), it is often convenient to overlook this nicety, particularly in making comparisons with infrared spectra.

(18) K. Noack and R. N. Jones, *Z. Elektrochem.*, **64**, 707 (1960).

(19) Suitable glass tubing, drawn to close diameter tolerances, is obtainable from Jencon Ltd., Hemel Hempstead, England.

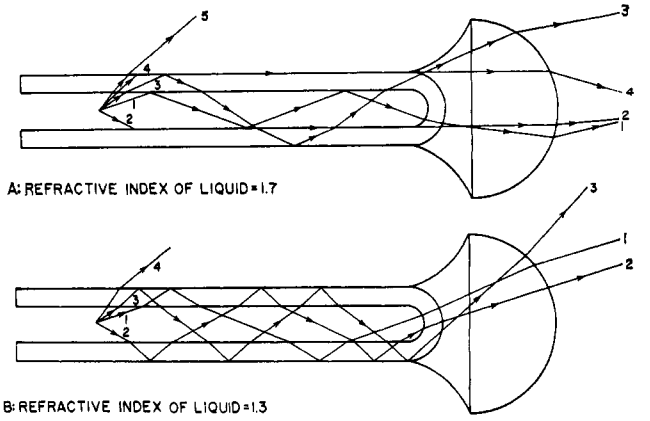

Figure 2.—Paths of typical rays in the capillary sample tube.

in the section on the external reference standard, the optical efficiency of the large tube is greater than that of the small one, and the need to employ a larger volume of solution can be partly offset by working at a higher dilution (See Table I). The open end of the sample tube is closed with a small Teflon stopper. To ensure a good seal, a strip of Parafilm M[20] is wrapped around the stopper and upper segment of the tube. Samples can be stored indefinitely without solvent loss in tubes sealed in this manner.

Wave-Number Calibration.—The two wave-number scales of the spectrophotometer read ν and $\Delta\nu$ in cm.$^{-1}$. The ν scale is calibrated against the emission lines of argon, mercury, and neon. Since the line positions are tabulated as wave length in air,[21,22] they must be vacuum corrected before conversion to wave number.[23] With the wave-number scale adjusted to 22,938 cm.$^{-1}$ and $\Delta\nu$ set at zero on the peak of the 4358.35-Å. mercury line, the cam error (δ_1) was found to increase linearly to -3 cm.$^{-1}$ at $\Delta\nu = 4000$ cm.$^{-1}$. The spectrophotometer was calibrated under conditions of fast-recorder response (damping period 10 sec.), but in normal operation signal to noise considerations necessitate a slower response (30–40 sec.). This introduces a lag of about 5 cm.$^{-1}$ in the recorded peak position when scanning the exciting line. It is convenient to reset the $\Delta\nu$ scale, and the fiducial chart marker, so that both read 0 $\pm$ 2 cm.$^{-1}$ when the exciting line is scanned under normal operating conditions. The exciting line is rescanned at the beginning of each run; this further small correction (δ_2) within the range of ± 2 cm.$^{-1}$ is applied to allow for day by day variation in location of the exciting line. Using this calibration program it was observed that the well-documented positions recorded for benzene and the solvents shown in Figures 2–5[24] can be reproduced within a range of $+4$ to 0 cm.$^{-1}$. This tendency for the peak positions to read slightly high probably reflects a residual recorder lag due to the bands of the liquid phase samples being wider than the exciting line. To allow for this we apply an addition correction of -2 cm.$^{-1}$. The final peak value, $\Delta\nu_{true}$, is therefore given by

$$\Delta\nu_{true} = \Delta\nu_{obsd} + \delta_1 + \delta_2 - 2 \quad \ldots\ldots\ldots \quad (1)$$

Following this procedure we are able to reproduce the literature values for the standard solvent peak positions, and for benzene, within ± 2 cm.$^{-1}$.

Spectral Response Calibration.—We employ one 1P21 and one 1P28 photomultiplier tubes to record the Raman radiation. These tubes were selected for their good signal to noise performance, and the use of two tubes of different spectral response is inconsequential. In making Raman intensity measurements it is necessary to correct for the change in the wave-length sensitivity of the detector. The detector system is therefore calibrated for spectral response against a magnesium oxide coated diffuse reflectant surface illuminated with a standard tungsten lamp.

To make these measurements the filter jacket was removed from the lamp housing and a magnesium oxide coated screen was placed obliquely on the optical axis beyond the end of the Toronto arc coil. The screen was illuminated with a General Electric Tungsten ribbon filament lamp (rated at 6 v. and 15 amp.), operated from a Sorensen 6-30-Å. stabilized d.c. power supply at 5.00 v. and approximately 15 amp. Care was taken that no direct radiation from the lamp entered the spectrometer, and the measurements were carried out in a darkened room. This arrangement provides a uniform diffuse illumination of the spectrometer optics, and the spectral distribution of the light scattered from the magnesium oxide is insensitive to the exact geometrical relationships of lamp, screen, and spectrometer. The spectral distribution from the screen was determined in a separate experiment against a calibrated primary source.[25]

The emission from the screen was scanned and the apparent intensity (i_ν) normalized with respect to the intensity at 4358 Å. (i_0), as measured on the recorder chart. The spectral sensitivity σ_ν is given by the relationship

$$\sigma_\nu = \frac{i_\nu E_0}{i_0 E_\nu} \quad (2)$$

where E is the spectral energy distribution of the radiation from the screen in arbitrary units. The spectral response curve of our spectrophotometer is shown in Figure 6 in which the Raman frequency ranges excited both with the 4358- and the 5461-Å. mercury lines are indicated.[26] It will be seen that with 4358-Å. excitation the maximum spectral sensitivity corresponds to a Raman wave number $\Delta\nu \approx 2000$ cm.$^{-1}$ and nowhere falls below 0.85 in the range under consideration. The 1P28 and 1P21 tubes are much less efficient detectors when 5461-Å. excitation is used, particularly where $\Delta\nu > 2000$ cm.$^{-1}$. This is somewhat mitigated by the fact that the C–H stretching vibrations are strong Raman scatterers. Thus we were able to observe the C–H stretching bands of biacetyl at $\Delta\nu =$ 3018 cm.$^{-1}$ without difficulty using 5461-Å. excitation,

(20) Manufactured by the Marathon Division of the American Can Co., Menasha, Wis.

(21) M.I.T. Wave-length Tables, G. R. Harrison, Ed., John Wiley and Sons, Inc., New York, N. Y., 1939.

(22) "Handbook of Chemistry and Physics," 41st Ed., Chemical Rubber Publishing Co., Cleveland, Ohio, 1959, pp. 2759, 2828, 2832, 2833.

(23) See ref. 13, pp. 157, 230, 231.

(24) G. Herzberg, "Infrared and Raman Spectra of Polyatomic Molecules," D. Van Nostrand Co., Inc., New York, N. Y., 1945, pp. 277, 311, 316, 317, 329, 333, 364.

(25) We are grateful to Dr. C. L. Sanders of the Applied Physics Division of the National Research Council of Canada for recommending this procedure, and for determining the spectral distribution of the lamp–screen combination.

(26) Since σ_ν always appears in our calculations as a ratio at two wave numbers, it is not necessary to alter the normalization factor when the exciting wave length is changed from 4358 to 5461 Å.

in spite of the fact that $\sigma = 0.01$ under these conditions.[18]

Operational Variables.—In measuring Raman spectra on small samples it is necessary to operate the spectrophotometer under severely energy-limited conditions and, to maintain the maximal reproducibility of the data, the operating conditions must be carefully chosen and standardized. The instrumental variables and the optimal settings we employ are listed in Table II. In our experience these provide the best signal to noise ratio and resolution compatible with reproducibility of band position and intensity at a reasonable rate of scan.

TABLE II

OPTIMUM SETTINGS OF THE OPERATIONAL VARIABLES OF THE SPECTROPHOTOMETER

	Range	Optimal
Slit width, cm.$^{-1}$, at 459 cm.$^{-1}$	0.1–30	5
Slit height, cm.	2.5, 5, 10	10
Recorder period, sec.	0.5–60	30 or 40[a]
Scanning speed, cm.$^{-1}$/sec.	0.005–50	0.25
Chart speed, cm./min.	...	≈1
Sensitivity	1–1000	30–600

[a] A recorder period of 30 sec. is normally used for measurements in organic solvents and 40 sec. for solutions in water and deuterium oxide.

Standardization of the Intensity Scale

Several workers have concerned themselves with the absolute measurement of Raman band intensities. Most of this work has been motivated by a desire to eliminate all instrumental factors and arrive at an expression relating the scattered radiation intensity to the electrical characteristics of the normal vibration. The publications of Bernstein and Allen,[27] Michel and Gueibe,[28] Naberukhin,[29] Rea,[30,31] and Ryason[32] and also the monograph of Brandmüller and Moser[13] should be consulted in this connection.

The separation of the solute–solvent interaction effects on the intensity from the purely instrumental effects of the refractive index has posed a problem that has not yet been completely solved. For reasons discussed below, the use of capillary sample tubes complicates this problem, and our studies of Raman band intensity measurements have had a more limited objective. We have sought only to reduce the raw experimental data to a common ordinate scale, recognizing that the resultant band heights remain subject to internal solvent field effects that it is not practicable to factor out. Similar field effects also influence the absolute intensity measurements of infrared spectra, but this has not prevented the organic chemist from making considerable use of infrared intensity measurements in investigating small structural changes in the spectra of molecules of related structure. The problem is somewhat more complex in Raman spectrophotometry, because an absolute light intensity must be measured, whereas the evaluation of the infrared molecular extinction coefficient only requires us to measure the ratio of two radiation intensities.

(27) H. J. Bernstein and G. Allen, *J. Opt. Soc. Am.*, **45**, 237 (1955).
(28) G. Michel and R. Gueibe, *Bull. soc. chim. Belges*, **70**, 323 (1961).
(29) Y. Y. I. Naberukhin, *Opt. Spectry.* (USSR), **13**, 278 (1962).
(30) D. G. Rea, *J. Opt. Soc. Am.*, **49**, 90 (1959).
(31) D. G. Rea, *J. Mol. Spectry.*, **4**, 507 (1962).
(32) P. R. Ryason, *ibid.*, **8**, 164 (1962).

The more theoretically oriented studies of Raman band intensities are concerned almost exclusively with the measurement of the areas under isolated bands. We have limited our measurements to peak height determinations, since these are the only experimentally significant data we can obtain from the overlapping band envelopes of the Raman spectra of complex organic compounds.

The External Reference Standard.—It is customary to express Raman band intensities as ratios with respect to a selected standard band. The widely accepted standard is the 459-cm.$^{-1}$ band of carbon tetrachloride, which is associated with the symmetric C–Cl stretching vibration.[33] The expression derived by Bernstein and Allen[27] for the "standard intensity" of a Raman band is as follows, where $I = \int_{\text{band}} i_\nu d\nu$,

$$S = \frac{I_{\Delta\nu}}{I_{459}^{CCl_4}} \frac{1 + \rho_{459}^{CCl_4}}{1 + \rho_{obsd}} \frac{n^2}{n_{CCl_4}^2} \frac{\sigma_{459}}{\sigma_{\Delta\nu}} \frac{M}{d} \left(\frac{d}{M}\right)_{CCl_4} \frac{\Delta\nu}{459} \times \left(\frac{\nu - 459}{\nu - \Delta\nu}\right)^4 \frac{(1 - e^{-(1.44\Delta\nu/T)})}{(1 - e^{-(1.44 \times 459/T)})} \quad (3)$$

in which i_ν is the measured intensity at the wave number ν, and $I_{459}^{CCl_4}$ is the integrated intensity of the standard band, ρ_{obsd} and $\rho_{459}^{CCl_4}$ are the respective observed depolarization ratios, n and n_{CCl_4} the refractive indices, σ the spectral sensitivity as defined by eq. 2,[34] M the molecular weight, d the density, $\Delta\nu$ the Raman wavenumber displacement, and T the absolute temperature. The original expression of Bernstein and Allen also contained a reflectivity term (R) which has since been deleted.[35]

The evaluation of S requires that I and $I_{459}^{CCl_4}$ be measured separately under identical operating conditions, using the same sample cell so that the carbon tetrachloride is used as an external standard. Precision measurements of Raman band intensities have been so far confined to simple substances that are available in quantity, and cells of large volume (50–65 ml.) have been employed. The spectrum is observed under conditions where only Raman radiation generated within the bulk of the liquid sample is recorded, and Raman radiation reflected from the walls of the cell does not enter the spectrometer. If the cell optics permit such reflected Raman radiation to be recorded, the refractive index dependence of the measured radiation intensity becomes extremely complicated, and the use of an external standard becomes of doubtful efficacy. This is already apparent in the Cary spectrophotometer with the 5-ml. cell optical system.[14] In the capillary cell the internal reflections both at the liquid–glass and the glass–air surfaces control the transfer of radiation to the spectrometer, since the tube performs as a light pipe.

A proper appreciation of the optical behavior of the capillary sample tube is necessary for its efficient employment. The paths of representative rays emitted at an arbitrarily chosen point on the optical axis are shown in Figure 2. The upper diagram of Figure 2

(33) The position of this band is also reported as 458 cm.$^{-1}$; a precise measurement and formal standardization is desirable. We have used this band with some misgiving because it is strongly polarized and has asymmetry and fine structure due to the presence of the chlorine isotopes. The depolarized carbon tetrachloride band at 313 cm.$^{-1}$ has also been used as a standard[7] and has advantages.
(34) Our definition of σ is the reciprocal of that used by Bernstein and Allen.[27]
(35) Personal communication from Dr. Bernstein; see also ref. 30.

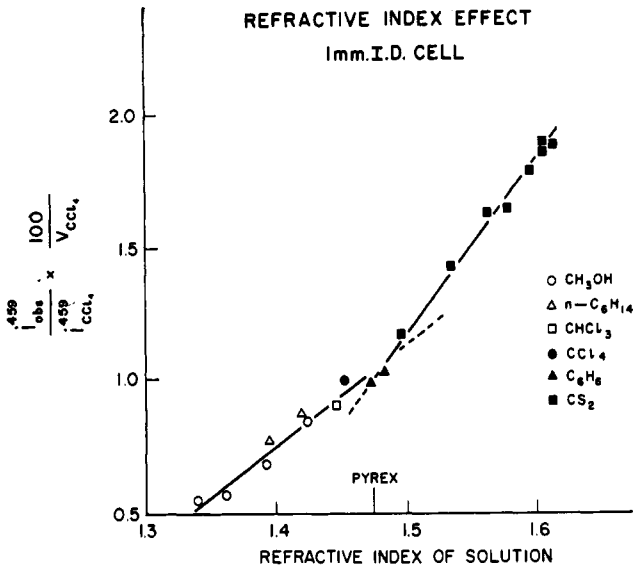

Figure 3.—Effect of the refractive index of the solution of the relative intensity of the 459-cm.$^{-1}$ band of carbon tetrachloride in a variety of solvents, using the capillary cell of 1-mm. internal diameter.

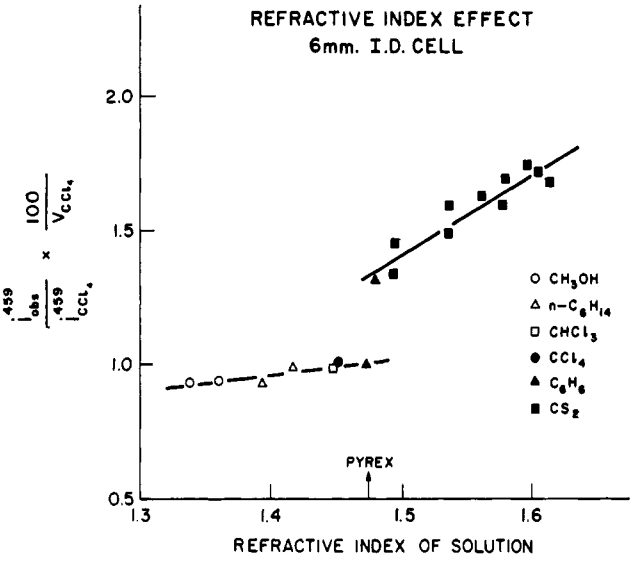

Figure 4.—Behavior of the same solutions in a cell of 6-mm. internal diameter.

illustrates a favorable situation where the refractive index of the liquid (n_l = 1.7) is greater than that of the glass (n_g = 1.5). Rays incident on the inner cell wall at an angle less than 27° suffer total internal reflection and travel within the liquid column. Rays incident at angles between 27 and 53° penetrate the glass cell wall, but are internally reflected at the glass–air surface. Only rays that strike the liquid–glass wall at an angle exceeding 53° pass out and are lost from the system. Paths of the same rays, lower diagram, are traced when the refractive index of the liquid is less than that of the glass (n_l = 1.3). Under these conditions all rays penetrate the glass and those that strike the liquid–glass wall at an angle greater than 38° pass through. The efficiency of the cell as a light pipe therefore diminishes with reduction in the refractive index on two counts. There is increasing loss through the sides of the tube, and a greater portion of the trapped radiation travels in the glass instead of the liquid column. This emphasizes the importance of using capillary tubes of the highest optical quality, and avoiding contamination of the outer wall of the tube with scratch marks, fingerprints, and other blemishes.[36]

The practical effect of refraction is shown in Figure 3 for measurements on the 459-cm.$^{-1}$ band of carbon tetrachloride in a range of solutions of different refractive index, using a Pyrex cell of 1-mm. internal diameter. The peak height of the band is plotted against the refractive index of the solution after correction for dilution. The intensities are normalized with respect to pure carbon tetrachloride. The observed intensities range from 0.55 in dilute solution in methanol to 1.93 in dilute solution in carbon disulfide, a variation by a factor of 3.5. Measurements on the same solutions in a cell of 6-mm. internal diameter are shown in Figure 4. The results are qualitatively similar, but the lines slope less steeply. Both sets of data show a sharp discontinuity near the refractive index of Pyrex glass (n_g = 1.47).

It is difficult to assess the extent to which the cell geometry alone is responsible for these effects. Experiments we have carried out with capillary tubes made of Corning 1720 glass (n_g = 1.53) show the expected displacement of the discontinuity to higher refractive index. However, Michel and Gueibe,[28] Rea,[31] and Tare and Thompson[37] observe qualitatively similar discontinuities for a variety of solute–solvent systems using large tubes in which the geometrical optical effects are presumed to have been already factored out. These residual discontinuities show no obvious relationship to the polar character of the solvent: they seem to depend only on the refractive index, and it is still questionable whether they are to be attributed to an instrumental factor that has been overlooked, or to an intrinsic solute–solvent interaction within the body of the solutions, as proposed by Ryason.[32]

The practical consequence of this refractive index effect is that a Raman spectrum can be obtained from a smaller sample of a compound in a solvent of high refractive index than of low refractive index. At the low end of the refractive index scale small changes in the internal diameter of the sample tube have a large effect on the minimum sample size. The normal sample weights we employ in carbon disulfide, chloroform and aqueous solutions are recorded in Table I for both the small and large capillary tubes. In carbon disulfide, where the refractive index is high, the minimum solute weight increases considerably with the tube volume, whereas for aqueous solutions at the opposite end of the refractive index scale, the greater optical efficiency of the larger tube effectively compensates for the greater volume, and the spectrum can be obtained from the same amount of solute at a lower concentration. The use of capillary tubes made from a glass of lower refractive index could reduce the minimum sample size. Such glasses with refractive indices as low as 1.27 have recently been reported, but are not

(36) In a personal communication R. C. Hawes of the Applied Physics Corp. has pointed out that at the end of the tube a liquid of lower refractive index than glass will produce a negative lens effect at its contact with the internal surface of the glass meniscus; this will also reduce the efficiency of the radiation transfer from the cell to the collecting lens system. This is observable in the lower diagram in Figure 2.

(37) S. A. Tare and H. W. Thompson, *Spectrochim. Acta*, **18**, 1095 (1962)

as yet available for test.[38] If satisfactory in other respects they could cut the minimum sample size by a factor of about three.

The Internal Reference Standard.—Since the refractive index corrections cannot be evaluated with capillary tubes, an internal standard method is used, in which carbon tetrachloride, or some alternative standard is added to the sample so that the standard band is measured at the same refractive index as the sample. In adopting this practice, it must be assumed that the intrinsic intensity of the Raman band is not altered by its environment. This is certainly not true, but the errors introduced by this assumption will be less than those incurred with the external standard technique, and in our experience the intensities measured in this way are useful for comparing Raman spectra of related substances in solvents of similar general character, provided one does not attempt to read undue significance into small intensity differences.

The expression we employ for calculating the *apparent Raman scattering coefficient* ($k_A^{\Delta\nu}$) is

$$k_A^{\Delta\nu} = \frac{i_A^{\Delta\nu}}{i_S^{\Delta\nu^1}} \frac{\sigma_{\nu^1}}{\sigma_\nu} \frac{g_S}{g_A} \frac{N_S}{N_A} \beta 100 \qquad (4)$$

where $i_A^{\Delta\nu}$ is the observed intensity for the substance A at a Raman shift $\Delta\nu$, $i_S^{\Delta\nu^1}$ is the corresponding value for the standard band at $\Delta\nu^1$, σ is the spectral sensitivity as defined by eq. 2, g is the sensitivity setting of the recorder amplifier, N_A and N_S are the mole fractions of the substance and standard, and β is a factor that converts the intensity scale of the secondary standard to the basic carbon tetrachloride scale. The significance of β will be considered further in a later paragraph.

Solvents

The solvents used are carbon disulfide, carbon tetrachloride, chloroform, methylene chloride, tetrachloroethylene, acetonitrile, water, and deuterium oxide. Their Raman spectra, determined by the above technique are shown in Figures 5, 7–9. In Figure 10 their solute obscuration ranges are compared with their obscuration as solvents for infrared measurements. In general the loss of significant regions of the solute spectrum is less serious for the Raman than the infrared spectra. With the exception of tetrachloroethylene they all permit measurement through the difficult region between 1650 and 1500 cm.$^{-1}$ where the olefinic C=C stretching bands occur. For the chlorinated solvents and carbon disulfide, obscuration becomes serious below 800 cm.$^{-1}$, but there is a valuable partial window in carbon disulfide between 600 and 250 cm.$^{-1}$. The complete Raman spectrum can be obtained by a judicious combination of measurements in carbon disulfide, chloroform, and tetrachloroethylene. Methylene chloride also has a useful window between 690 and 260 cm.$^{-1}$, but its low refractive index is a disadvantage. Customarily we measure spectra in carbon disulfide where solubility conditions permit, with chloroform as the second choice. Typical steroid spectra obtained in these two solvents are shown in Figures 11 and 12.

Aqueous solutions show no sharp characteristic solvent bands, but there is broad "background" scattering

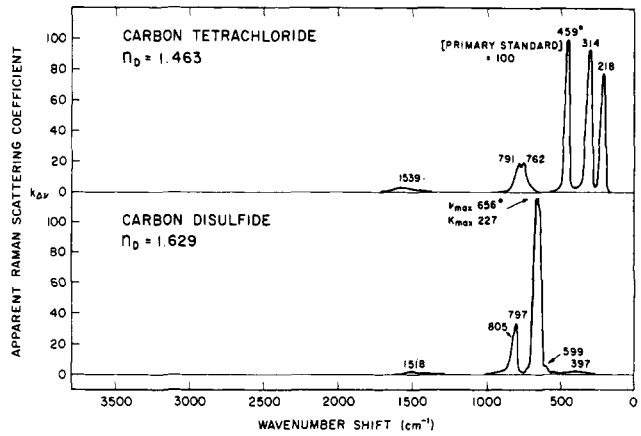

Figure 5.—Raman spectra of carbon disulfide and carbon tetrachloride. The primary and secondary standard bands are indicated by asterisks.

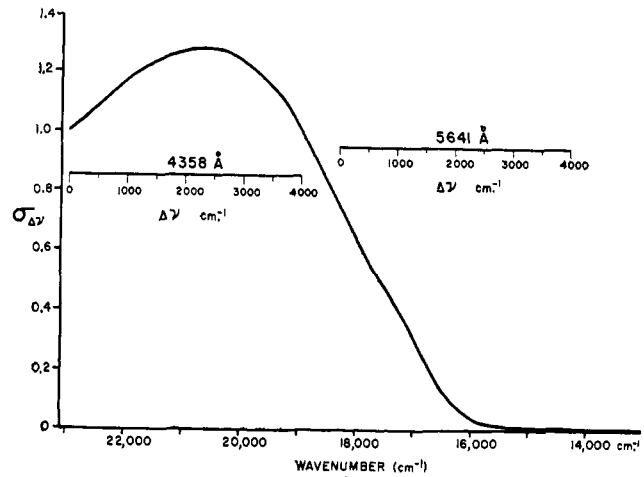

Figure 6.—Spectral sensitivity curve using one 1P21 and one 1P28 photomultiplier tubes to measure the Raman radiation.

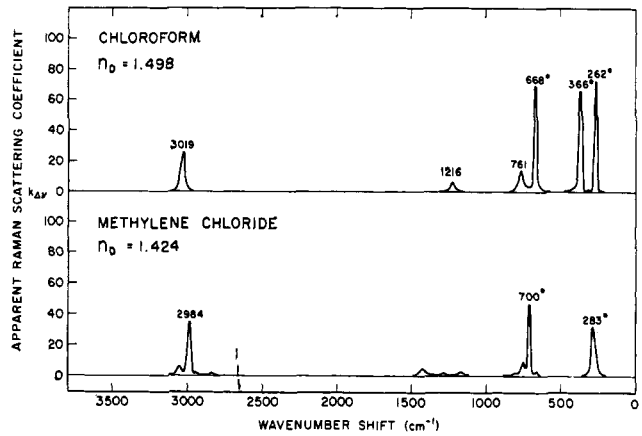

Figure 7.—Raman spectra of chloroform and methylene chloride. The secondary standard bands are indicated by asterisks.

below 700 cm.$^{-1}$ and a very broad hydrogen bonded O–H band above 3100 cm.$^{-1}$ (Figure 9). With aqueous solutions it is sometimes convenient to add 10% by weight of acetonitrile and use the C≡N stretching band at 2255 cm.$^{-1}$ as an internal reference standard. The Raman spectrum of an aqueous solution of α-methyl-D-xylopyranoside is shown in Figure 13.[39]

Neat liquids can be investigated down to 180 cm.$^{-1}$ under normal operating conditions. The cut off

(38) J. Schröder, *Angew. Chem., Intern. Ed. Engl.*, **3**, 376 (1964).

(39) We wish to thank Dr. W. Mitura for providing this spectrum.

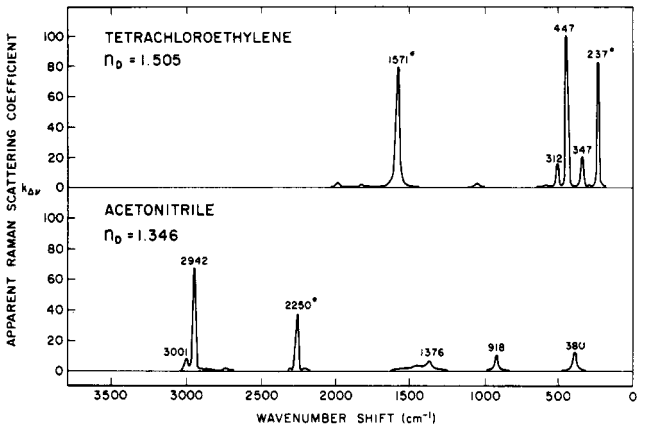

Figure 8.—Raman spectra of tetrachloroethylene and acetonitrile. The secondary standard bands are indicated by asterisks.

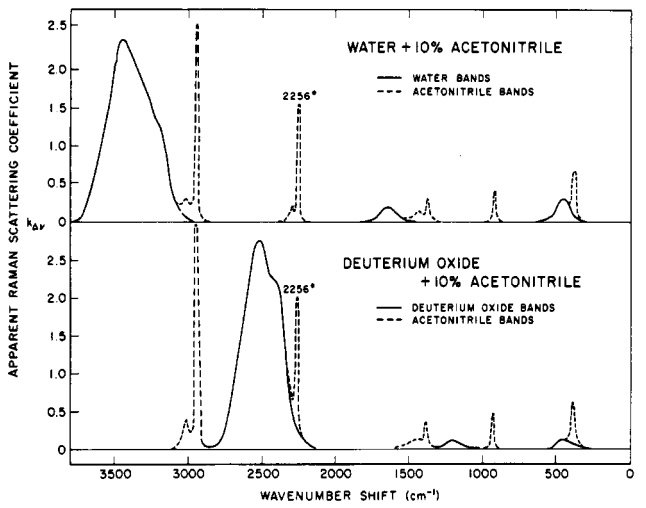

Figure 9.—The Raman spectra of water and deuterium oxide containing 10% by weight of acetonitrile. The acetonitrile bands are indicated by dashed lines.

observed near 175 cm.$^{-1}$ is the edge of a diffraction band resulting from the use of parallel slits in the spectrometer. This is eliminated by closing one of the slits and the spectrum can then be measured to 100 cm.$^{-1}$ at a sacrifice of 50% of the sensitivity.

Evaluation of the β Constants.—For measurements on neat liquids it is usually possible to add 10% by weight of carbon tetrachloride and standardize the intensities directly against the 459-cm.$^{-1}$ band. For the Raman spectra of solids measured in solution in other solvents we employ one of the solvent bands as a secondary standard and apply the β conversion factor as indicated in eq. 4. This factor can only be regarded as a scale adjustment parameter; it cannot take account of the solvent effects influencing the band intensity. Instrumentally β is a function of the spectral slit width, the amplifier time constant, and the scanning rate. These relationships are shown in Figures 14 and 15 for the 657-cm.$^{-1}$ band of carbon disulfide and the 668-cm.$^{-1}$ band of chloroform. The solvent peaks that we employ as reference standards are indicated by asterisks in Figures 5, 7–9 and the β constants are listed in Table III.

The β constants can be evaluated in two ways. The direct method is to determine the relative intensities

TABLE III

β CONSTANTS FOR SECONDARY STANDARD BANDS

Solvent	Band position, cm.$^{-1}$	β Constant: From binary solution with CCl_4	β Constant: By indirect measurement on common solute	β Constant: Accepted value
CCl_4	459	...	...	1.00
CS_2	656	2.27	1.92,[a] 1.85,[b] 1.85[c]	1.90
$CHCl_3$	668	0.70	0.70[b]	0.70
	366	0.66	...	0.66
	262	0.72	...	0.72
CH_2Cl_2	700	0.46	...	0.46
	283	0.33	0.32[b]	0.33
C_2Cl_4	1571		1.07[b]	
			...	
C_2H_3N	2250	0.36	...	0.36

[a] Solute methyl benzoate. [b] Solute dodecane. [c] Solute etiocholane.

of the primary and secondary standard peaks in binary mixtures of the secondary solvent with carbon tetrachloride. This should be averaged over a range of concentrations. Internal field effects will influence the ratio of such peak height measurements; quantitative information on this subject tends to be limited and inconsistent, but suggests that the relative peak heights in the binary mixtures of the common solvents will vary by $\pm 10\%$ depending on the relative concentration of the two components.[28,40] Recent work of Bernstein and Koningstein[41] indicates that the variation is greater for the system CCl_4–CS_2 than for CCl_4–$CHCl_3$. Within the limitations of our technique we have not observed any systematic intensity changes that can be attributed to changes in the β values with concentration.

An alternative method of obtaining β is to measure the spectrum of a compound both in carbon tetrachloride and in the secondary solvent. The intensities of the more prominent bands in carbon tetrachloride solution are directly evaluated from eq. 4, using $\beta = 1$. The intensities in the secondary solvent are initially calculated with respect to an interim scattering coefficient, k', based on the secondary solvent standard band taken as 100 units. The ratio k/k' gives a value for β. A separate estimate of β is obtained from each peak measurement and these can be averaged. This method has the advantage that the ratio is determined for several bands, each of which is likely to have a different internal field effect, and the field effects for different types of solute molecules can also be compared. A series of such measurements for *n*-dodecane in a range of solvents is given in Table IV. The β constants

TABLE IV

COMPARISON OF APPARENT RAMAN SCATTERING COEFFICIENTS FOR *n*-DODECANE IN VARIOUS SOLVENTS

Band position,[a] cm.$^{-1}$	Intensity (k): CCl_4	CS_2	$CHCl_3$	CH_2Cl_2
2892	134	141	134	137
2852	123	128	126	127
1442	33	34	34	31
1302	19	19	19	18
1133	6.2	6.5	6.5	6.2
1080	11.4	11.8	12.4	11.3
891	7.7	7.4	7.9	6.8

[a] For curve see ref. 42.

(40) D. D. Tunnicliff and A. C. Jones, *Spectrochim. Acta*, **18**, 579 (1962).
(41) H. J. Bernstein and J. A. Koningstein, unpublished observations.

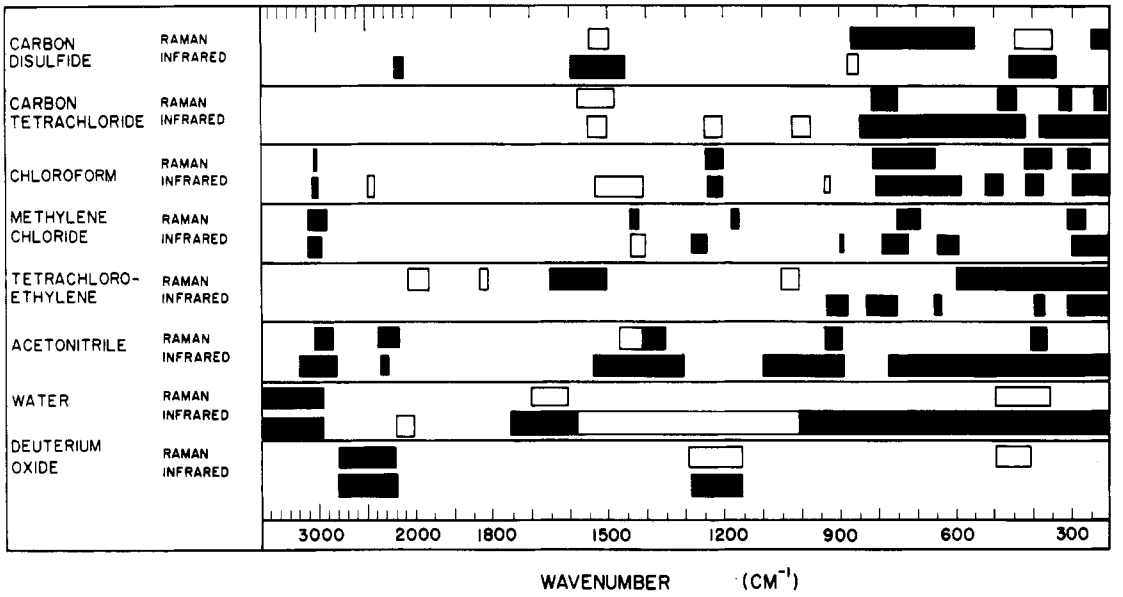

Figure 10.—Comparison of solvent obscuration ranges for Raman and infrared spectra. The infrared data are for the following path lengths: CS_2, CCl_4, $CHCl_3$, CH_2Cl_2, C_2Cl_4, C_2H_3N, above 600 cm.$^{-1}$, 0.1 mm., below 600 cm.,$^{-1}$ 1 mm.; H_2O, D_2O, 0.01 mm. Partial obscuration is indicated by open rectangles.

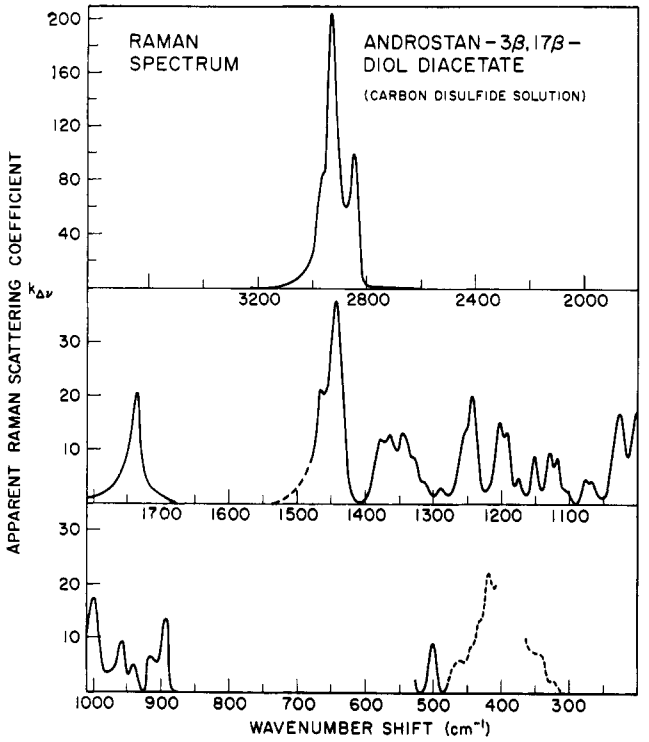

Figure 11.—Raman spectrum of androstane-3β,17β-diol diacetate: 190 mg. in 770 mg. of CS_2 in the "large" capillary cell.

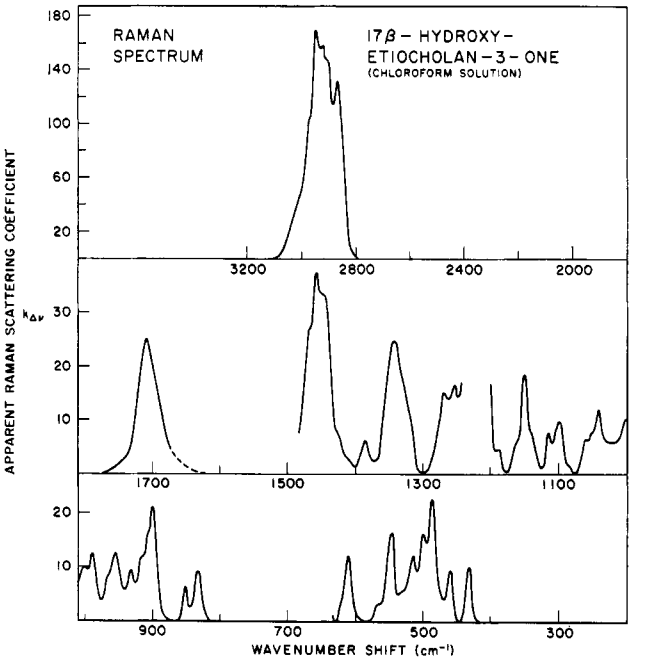

Figure 12.—Raman spectrum of 17β-hydroxyetiocholan-3-one: 395 mg. in 1190 mg. of $CHCl_3$ in the "large" capillary cell.

obtained by this method and by direct evaluation from the binary solvent mixtures with carbon tetrachloride are included in Table III. For the chlorinated solvents the differences are small. They are larger for carbon disulfide, bearing out the conclusions of Bernstein and Koningstein.[41]

In complex spectra where there is considerable band overlap, the major uncertainty in the intensity measurements is the location of the tangential base line, and this limits the reproducibility of the peak height measurements to ±10%. Only when greater accuracy of measurement is achieved will a more elaborate treatment of the band intensities be justified.

Depolarization Ratios

Infrared and Raman spectra often complement each other with respect to band intensities and the structural information they provide about characteristic group vibrations. This occurs even in complex molecules where the symmetry considerations of group theory do not apply, as in long-chain aliphatic esters in the liquid state.[42] Additional information about the nature of the group vibration should be obtainable

(42) R. N. Jones and R. A. Ripley, *Can. J. Chem.*, **42**, 305 (1964).

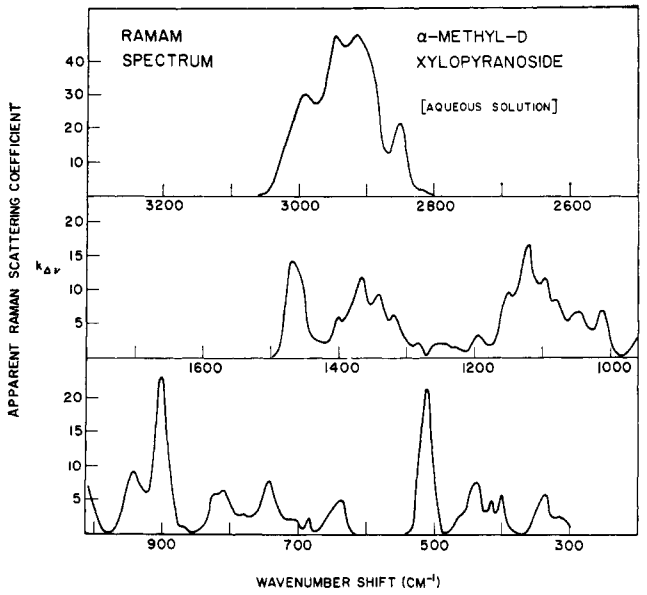

Figure 13.—Raman spectrum of α-methyl-D-xylopyranoside: 200 mg. in 195 mg. of water.

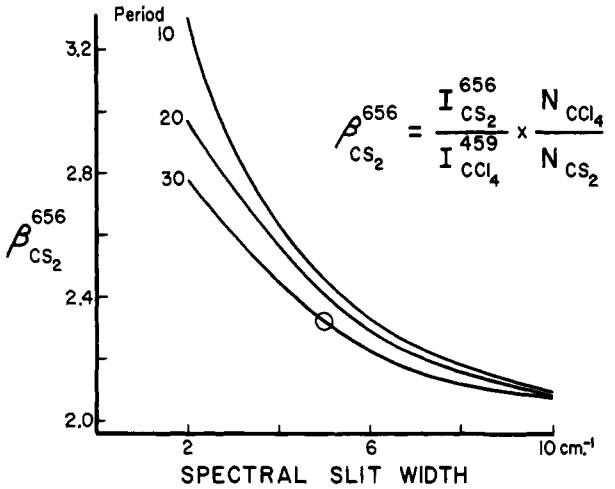

Figure 14.—Effect of amplifier time constant and spectral slit width on the β value for the 656-cm.$^{-1}$ band of carbon disulfide. The open circle indicates the standard operating conditions.

from the polarization of the Raman radiation, particularly if this can be measured with sufficient accuracy.

The apparent depolarization ratio can be obtained with the capillary optical system by inserting cylindrical Polaroid filters between the filter jacket and the sample tube. If i_{par} and i_{per} are the measured chart displacements using Polaroid filters that transmit the electric vector oriented parallel with and perpendicular to the cell axis, respectively, then the apparent depolarization ratio ρ_{obsd} is given by

$$\rho_{obsd} = i_{par}/i_{per} \qquad (5)$$

Provided the illumination is strictly perpendicular to the sample tube, this quantity should have a value lying between 0.857 and 0. In practice, a part of the radiation strikes the sample tube obliquely, and a correction must be made for this "convergence error." The obliquity of the incident radiation can be reduced by inserting transverse baffles around the sample tube,

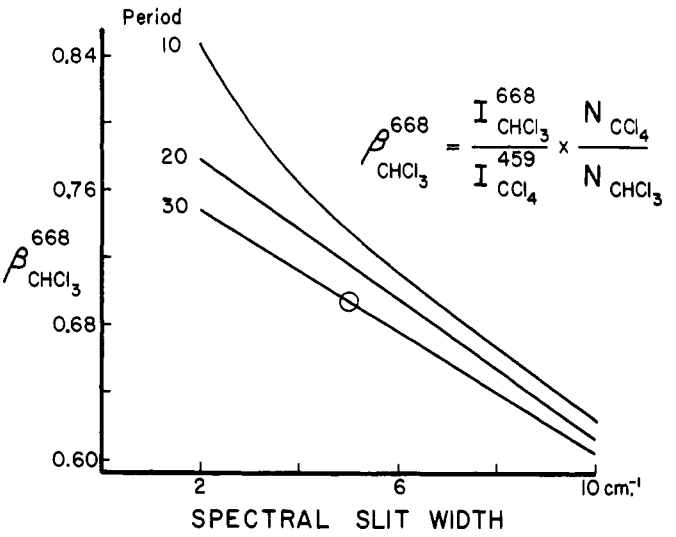

Figure 15.—Effect of amplifier time constant and spectral slit width on the β value for the 668-cm.$^{-1}$ band of chloroform. The open circle indicates the standard operating conditions.

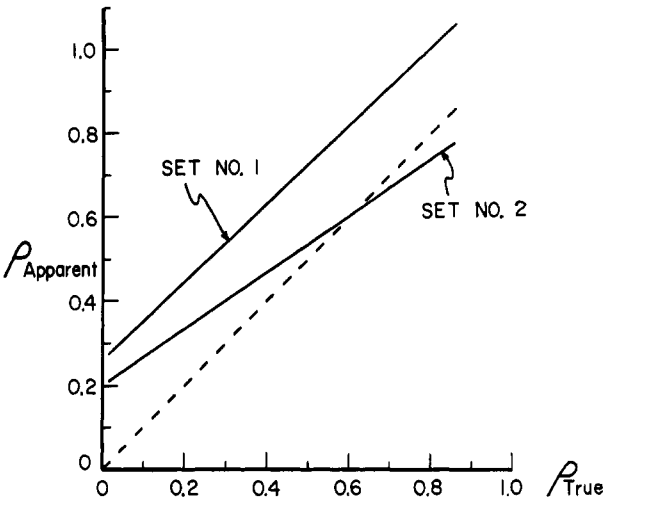

Figure 16.—Graphical correction for depolarization convergence errors by the method of Edsall and Wilson for two sets of polarizers. The theoretical line is indicated by dashes.

but this seriously diminishes the intensity of irradiation. Koningstein and Bernstein[43] have derived the relationship

$$\rho_{obsd} = T\rho_{true}\left(1 - \frac{\sin^2\bar{\theta}}{n^2}\right) + T\frac{\sin^2\bar{\theta}}{n^2} \qquad (6)$$

where $\bar{\theta}$ is an averaged angle of incidence, T is a transmission factor that takes account of the optical transmission of the polarizers and transmission effects within the optical system of the spectrophotometer, and n is the refractive index of the sample.[44]

The values of $\bar{\theta}$ and T can be obtained from measurements on bands of known polarization. The completely polarized and completely depolarized bands of tetra-

(43) J. A. Koningstein and H. J. Bernstein, *Spectrochim. Acta*, **18**, 1249 (1962).

(44) Anisotropic polarization in the optical system can also influence band-intensity measurements of polarized bands in ordinary measurements made without the use of polarizers. A method to correct for this by means of a quarter-wave plate has been described by Tunnicliff and Jones.[45] We have not used this because it leads to some loss of energy. On the 459-cm.$^{-1}$ band of carbon tetrachloride the correction amounts to about 3% in the Cary instrument.

(45) D. D. Tunnicliff and A. C. Jones, *J. Opt. Soc. Am.*, **51**, 1430 (1961).

TABLE V

MEASUREMENT OF DEPOLARIZATION RATIOS BY THE METHOD OF KONINGSTEIN AND BERNSTEIN WITH A CAPILLARY CELL[a]

Substance	n^{27}_{4340}	$\Delta\nu$, cm.$^{-1}$	ρ_{true}	ρ_{obsd}	T	$T\sin^2\bar{\theta}$	ρ^*	$\rho^* - \rho_{obsd}$
CCl_4	1.463	317	0.857	1.001	1.127	0.548	1.031	+0.030
		459	0.013	0.27			0.260	−0.006
$SiCl_4$	1.413	221	0.857	1.04	1.169	0.517	1.034	−0.005
		424	0.013	0.27			0.277	+0.007
$Si(CH_3)_4$	1.356	2962	0.857	1.06	1.189	0.526	1.037	−0.023
		598	0.000	0.26			0.288	+0.002

[a] The averaged values of T (1.162) and $T\sin^2\bar{\theta}$ (0.530) were used to calculate ρ^* from the equation $\rho^* = 1.162\rho_{true}\left(1 - \frac{0.530}{1.162n^2}\right) + \frac{0.530}{n^2}$. The small positive values for ρ_{true} in the CCl_4 band at 459 cm.$^{-1}$ and the 424-cm.$^{-1}$ band of $SiCl_4$ result from the isotopic asymmetry: see A. E. Douglas and D. H. Rank, *J. Opt. Soc. Am.*, **38**, 281 (1948).

hedrally symmetric molecules such as carbon tetrachloride, silicon tetrachloride, germanium tetrachloride, and tetramethylsilane are suitable for this purpose,[46] and the results of a set of calibration measurements are shown in Table V. The last column gives the apparent ratios as calculated back using the averaged derived values for $T\sin^2\bar{\theta}$ and T. These measurements show that with the capillary cell system depolarization ratios can be measured with a precision approaching that obtained from larger sample volumes. The measurements require the removal of the sample holder from the spectrophotometer to change the polarizers. This causes some variation in the measured peak heights. To overcome this several measurements should be made with each polarizer, and the results should be averaged. For well-isolated bands depolarization measurements based on band area or peak height ratios agree well. For many purposes a more approximate graphical correction will suffice, following the method of Edsall and Wilson.[47] The calibration curves for two sets of polarizers are shown in Figure 16. The difference in the slopes of the two curves is caused by differences in the relative transmissions of the perpendicular and parallel polarizers, and the y-intercept is a measure of the convergence effect.

Because of the loss of more than 50% of the incident radiation when using polarizers, it is only practicable to obtain the depolarization ratios for strong Raman bands. For complex molecules with extensive overlap one can only observe the general effect of polarization on the shape of the band envelope and characterize the individual peaks as weakly or strongly polarized. Where a weakly polarized band is overlapped by a strongly polarized band, the polarization spectra are helpful in revealing the underlying band structure. All C=O stretching bands appear to be strongly polarized, as also are the symmetric CH_2, CH_3, CD_2, and CD_3 stretching vibrations. Polarization measurements have proved to be very helpful in sorting out the C–H and C–D stretching modes in complex spectra.[42]

Preparation of Compounds for Analysis

The main difficulty in making Raman spectra measurements is the initial purification of the compounds. It is important that they be completely nonfluorescent. This excludes measurements on such compounds as polynuclear aromatic hydrocarbons. Some colored substances that exhibit electronic absorption at the exciting frequency, but subsequently lose their excess energy by processes not involving fluorescence, give strong "resonance Raman" spectra. The theory of these spectra is still incompletely understood, but they can be easily measured, and their empirical study and correlation with molecular structure and ultraviolet absorption is of interest to organic chemists. Aromatic and aliphatic nitro compounds belong to this class.[48]

It is essential that all apparatus coming in contact with the sample should be free from fluorescing impurities. All glassware should be cleaned with dichromate–sulfuric acid cleaning solution and organic detergents should be avoided. Where possible all-glass apparatus should be used in the final stages of sample purification; joints should be made with Teflon sleeves or greaseless stopcocks and connectors. In handling Raman sample tubes care must be taken not to leave fingermarks on them. All solvents used in the final preparation of the sample should be freshly distilled and checked for fluorescence in the Raman spectrophotometer. Before beginning to scan a spectrum the sample should be allowed to remain irradiated in the instrument until any transient fluorescence has died away. This may take from 1 or 2 min. to more than an hour.

To obtain the complete spectrum of a neat liquid, four separate scans must be made. The spectrum of the liquid is first determined, followed by two scans with polarizers, and a final run is made with the addition of the calibration standard. In dealing with solutions the procedure is similar except that the fourth run can be omitted. Appropriate modification of the procedure will be necessary if the sample has a band overlapping the solvent calibration band, and runs in more than one solvent may be necessary to cover all regions of the spectrum.

Concluding Remarks

It will be apparent that the determination of a Raman spectrum still remains a complicated procedure, and, although the technique described here permits the reduction of the actual instrumental measurements to a routine laboratory process, the application of Raman spectrometry is still limited to certain classes of organic compounds, and the purification and preparation of each individual compound poses a miniature research problem of its own. It is to be anticipated

(46) Germanium tetrachloride is not satisfactory for use with Pyrex tubes because it has a similar refractive index to that of the glass. We have used it with a lead glass tube for which $n_g = 1.693$.

(47) J. T. Edsall and E. B. Wilson, Jr., *J. Phys. Chem.*, **6**, 124 (1939).

(48) H. W. Schrötter, *Z. Elektrochem.*, **64**, 853 (1960).

that the introduction of laser sources, which can be expected soon, will help to alleviate some of the difficulties by shifting the excitation frequency down the spectrum into the red or near-infrared region. It should also help to reduce the minimum sample size.

Most Raman studies of organic compounds have hitherto dealt with simple molecules and we are now trying to apply the technique to natural products and their derivatives. Measurements on mono- and disubstituted steroids indicate that the spectra are entirely different from the infrared spectra; they are more characteristic of the skeletal structure and less affected by functional groups that impart the strong identifying features on the infrared spectra. Measurements on aqueous solutions of methyl furanosides and methyl pyranosides show that the spectra are simpler than the infrared spectra obtained from solid samples, though we have not yet progressed far in interpreting them.

Molecular spectroscopists would have made little progress in the vibrational analysis of small symmetric molecules if they had been dependent on the infrared spectra alone, and one cannot expect to achieve a full interpretation of the vibrational behavior of more complex organic compounds until our understanding of the Raman spectra comes into better balance with our knowledge of the infrared spectra.

Acknowledgment.—We wish to thank Mr. George Ensell for his invaluable aid with the modification of the Raman source unit and the construction and maintenance of the lamps. Our thanks are also due to Mrs. M. A. MacKenzie for making many of the measurements and checking our results. Dr. Walter Mitura has contributed to the studies with aqueous solutions. We also wish to acknowledge the valuable contributions of Dr. P. J. Krueger and Dr. K. Noack who participated in the preliminary phases of this work, and to Dr. H. J. Bernstein with whom we have had many enlightening discussions. It is not inappropriate to add in conclusion that the trials and tribulations we have encountered in the course of these studies have recalled nostalgic memories of Room MB-4 at Mallinckrodt Laboratory at Harvard University. There, during the period 1938–1942, the enthusiasm of Professor Fieser encouraged the senior author to persevere in the then new field of ultraviolet spectrometry, where problems of a similar character were encountered.

2,2,4-Trimethyl-1,2-dihydroquinolines. Preparation and Nuclear Magnetic Resonance Studies*,[1]

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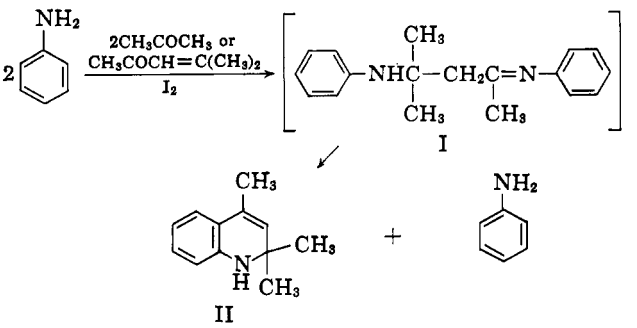
The structures of the "acetone anils" of *m*-toluidine, *m*-chloroaniline, and *m*-anisidine have been established to be 2,2,4,7-tetramethyl-1,2-dihydroquinoline (IVa), 7-chloro-2,2,4-trimethyl-1,2-dihydroquinoline (IVb), and 7-methoxy-2,2,4-trimethyl-1,2-dihydroquinoline (IVc), respectively, on the basis of n.m.r. spectrometric evidence. An analysis of the chemical shifts and splitting patterns of the aromatic protons in these and other related 1,2-dihydroquinoline derivatives has been carried out, with special emphasis on several substituent effects and, in one instance, on the influence of solvent.

2,2,4-Trimethyl-1,2-dihydroquinoline (II) and its analogs, readily prepared by condensation of the appropriate substituted aniline with acetone[2,3] or mesityl oxide[4] in the presence of iodine as a catalyst, were first formulated incorrectly by Knoevenagel[2] in 1921 as "acetone anils," or simple Schiff bases of acetone. The currently accepted structure of these products was first proposed independently on purely chemical grounds by Reddelien and Thurm,[5] by Cliffe,[6] and by Murray and co-workers,[7] and has since been substantiated by a variety of ultraviolet,[8,9] infrared,[10] and n.m.r.[10-13] spectrometric evidence. Following an earlier suggestion of Rosser and Ritter,[14] Tung[11] has recently shown that the formation of II probably proceeds by way of intermediate adduct I, which cyclizes with elimination of a molecule of arylamine. The novel conversion of II·HCl into 2-guanidino-4-methylquinazoline hydrochloride (III·HCl) on treatment with dicyandiamide,



* To Professor Louis F. Fieser.

(1) This investigation was supported in part by Research Grant C6516 and Research Career Development Award K3-CA-22,151 from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service.

(2) E. Knoevenagel, *Ber.*, **54**, 1722 (1921).

(3) W. R. Vaughan, "Organic Syntheses," Coll. Vol. III, John Wiley and Sons, Inc., New York, N. Y., 1955, p. 329.

(4) E. Knoevenagel, *Ber.*, **56**, 2414 (1923).

(5) J. Reddelien and A. Thurm, *ibid.*, **65**, 1511 (1932).

(6) W. H. Cliffe, *J. Chem. Soc.*, 1327 (1933).

(7) J. T. Murray, W. F. Short, and R. Stansfield, *J. Am. Chem. Soc.*, **55**, 2805 (1933).

(8) W. S. Johnson and B. G. Buell, *ibid.*, **74**, 4517 (1952).

(9) D. Craig and E. C. Gregg, Jr., *ibid.*, **75**, 2252 (1953).

(10) I. W. Elliott, Jr., and P. Yates, *J. Org. Chem.*, **26**, 1287 (1961).

(11) C. C. Tung, *Tetrahedron*, **19**, 1685 (1963).

(12) E. J. Zobian, W. S. Kelley, and H. C. Dunathan, *J. Org. Chem.*, **29**, 584 (1964).

(13) J. P. Brown and L. M. Jackman, *J. Chem. Soc.*, 3132 (1964).

(14) C. M. Rosser and J. J. Ritter, *J. Am. Chem. Soc.*, **59**, 2179 (1937).