RESONANCE RAMAN SPECTROSCOPY AS AN ANALYTICAL PROBE FOR BIOLOGICAL CHROMOPHORES: SPECTRA OF FOUR Cu-ETIOPORPHYRINS*

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Summary

Resonance Raman spectra have been obtained for the position isomers Cu-etioporphyrins I, II, III, and IV, as solids dispersed in KBr matrices. The spectra of the four molecules differ markedly in the 650-850 cm^{-1} region, enabling the substances to be distinguished. The potential of resonance Raman spectroscopy as a sensitive and non-destructive analytical tool is illustrated.

Introduction

Resonance Raman scattering may occur when the frequency of the exciting radiation falls within an electronic absorption band of the scattering molecule (1-2). The intensity of Raman lines arising from vibrations coupled to the electronic transition may be enhanced by several orders of magnitude compared with their intensity in normal Raman spectra (for example, see references 2-8). This enables Raman spectroscopic investigations to be carried out at very low concentrations of the scattering moiety. The technique has recently been applied to a variety of molecules of biological importance. Several studies, from this laboratory and others, have probed the structure of the chromophore in a number of heme proteins and metalloporphyrins. In the current work, we wish to illustrate the analytical potential of this technique, and report the resonance Raman spectra of four position isomers of Cu-etioporphyrin (9) (Cu-etio I, II, III, and IV). The molecules differ from one another only in the relative positions of the

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methyl and ethyl substituents on the different pyrrole rings (the structural formulae are indicated in fig. 1). The molecules are not easily distinguished by conventional physico-chemical methods.

In an earlier publication, we have reported (10) spectra for solutions of Cu-etio I and IV. However, solution studies are often complicated by Raman bands of the solvent, which is present at a very much higher concentration than the solute. In addition luminescence and/or photolysis problems are encountered for certain molecules when one attempts to record their solution spectra. These problems are minimized in the KBr matrix spectra (7).

Experimental

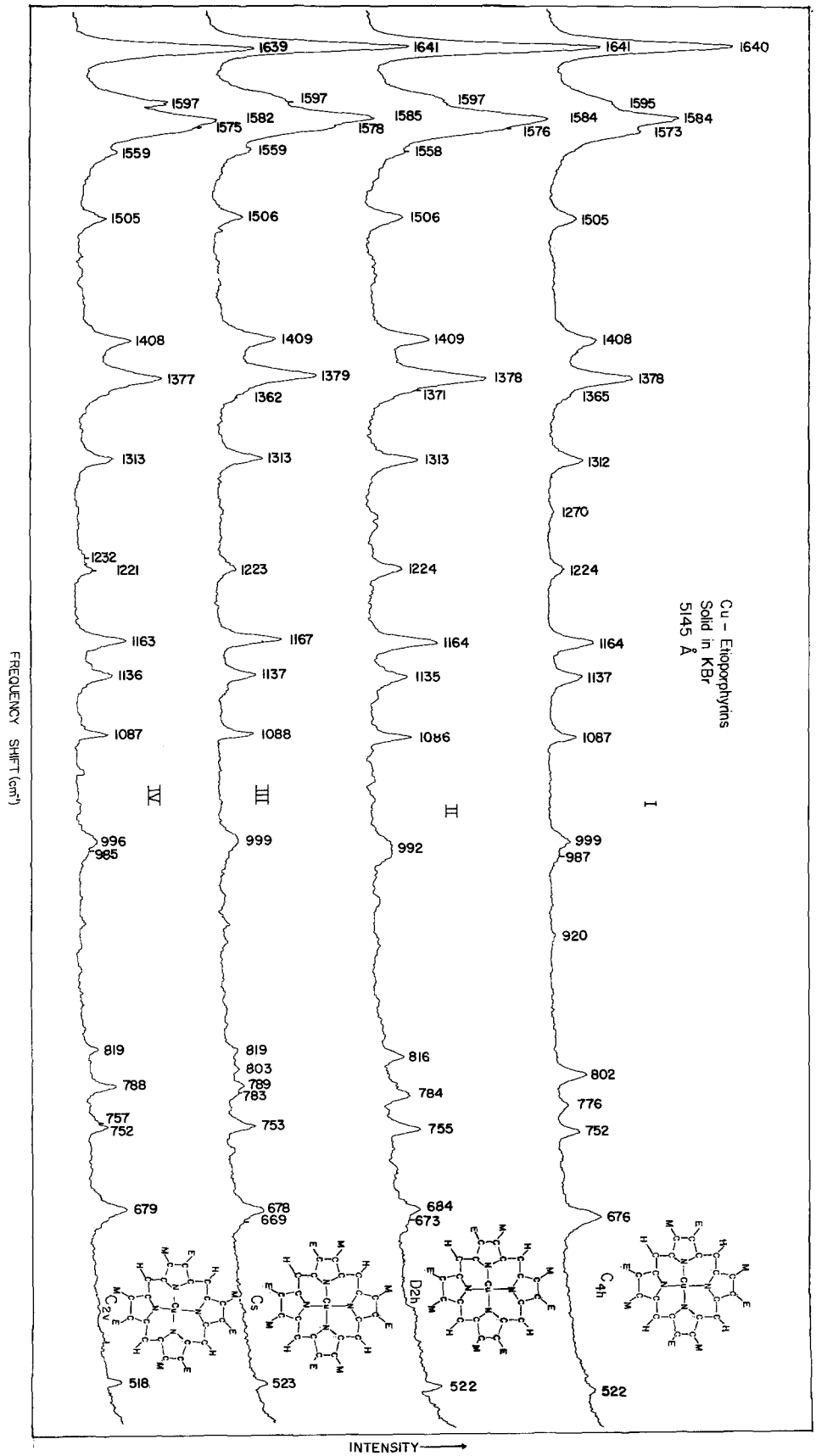
Pure crystalline Cu salts of the etioporphyrin isomers were kindly synthesized and supplied by Dr. S. F. MacDonald of N.R.C.C. Their UV and visible absorption spectra showed no impurity bands and their elemental analyses agreed with the calculated values.

Raman spectra were obtained using 5145 Å radiation from a Coherent Radiation 52 GA argon ion laser, Spex 1400 double monochromator and EMI 6256 photomultiplier and photon counting electronics. The laser output was filtered to remove unwanted plasma lines and the power at the sample was about 200 mw. Spectra were obtained using the rotating cell technique for solid coloured samples (11). A layer of powdered KBr was pressed in the groove of the spinning cell and the mixture of the porphyrin in KBr (about 1/50 w/w) was pressed on top. In this fashion it was possible to obtain spectra using only a few milligrams of material.

UV and visible absorption spectra were recorded using a Cary 14 Spectrophotometer.

Results and Discussion

Resonance Raman spectra of the four isomers of Cu-etioporphyrin are shown in fig. 1, along with their structural formulae and their molecular point group symmetries. Marked differences are evident in the 650-850 cm^{-1}


Cu – Etioporphyrins
Solid in KBr
5145 Å
FREQUENCY SHIFT (cm⁻¹)
INTENSITY
IV
III
II
I
1639
1597
1575
1559
1505
1408
1377
1313
1232
1221
1163
1136
1087
996
985
819
788
757
752
679
518
1641
1597
1582
1578
1559
1506
1409
1379
1362
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1223
1167
1137
1088
999
819
803
789
783
753
678
669
523
1641
1597
1585
1576
1558
1506
1409
1378
1371
1313
1224
1164
1135
1086
992
816
784
755
684
673
522
1640
1595
1584
1573
1584
1505
1408
1378
1365
1312
1270
1224
1164
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1087
999
987
920
802
776
752
676
522
C2v
Cs
D2h
C4h

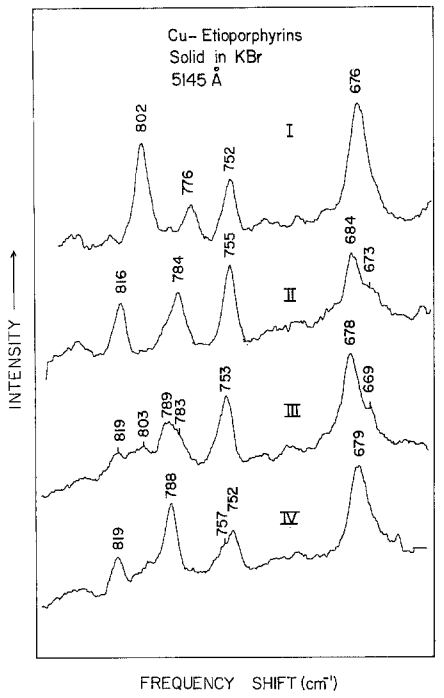

Figure 2. Resonance Raman spectra of the four Cu-etioporphyrins for the region between 600-850 cm^{-1}, spectral slitwidth $\sim$ 4 cm^{-1}.

spectral region and this region is presented on an expanded scale in fig. 2. Frequencies of the observed vibrations are marked in figs. 1 and 2 and are accurate to ± 2 cm^{-1}. The spectra of Cu-etio I and III differ from each other as well as from Cu-etio II and IV both in the positions as well as the relative intensities of the features shown in fig. 2. Although the frequencies of Cu-etio II and IV are similar, the relative intensities are sufficiently different so that the molecules can be distinguished. Thus one can easily distinguish the four isomers from their Resonance Raman spectra. It is clear from fig. 2 that Cu-etio III shows a more complex spectrum than the other isomers. The fact that more vibrations appear to be Raman active for this isomer is consistent with the fact that Cu-etio III is the least symmetrical of the four etioporphyrins. The symmetries of the etioporphyrins

Figure 1. Resonance Raman spectra of Cu-etioporphyrin I, II, III and IV as solids dispersed in KBr, spectral slitwidth $\sim$ 4 cm^{-1}. The symbols M and E stand for the methyl and ethyl groups respectively.

are such that certain vibrations which are not active in the I(C_{4h}), II(D_{2h}) derivatives become allowed in the C_s symmetry of Cu-etio III molecule. For IV(C_{2v}) and III(C_s) the number of Raman active modes should be the same but the relative intensities could differ.

It is of interest to note that the spectral region most sensitive to the structural differences present in the Cu-etioporphyrins is the one normally associated with vibrations involving mainly skeletal bending modes. The sensitivity of this spectral region has been noted previously in studies (7) of the tetramethyl esters of coproporphyrins III and IV. Although detailed understanding of the observed spectral features is not possible at this point, it is clear from the current work that the resonance Raman effect is a powerful, nondestructive, and probably quite a generally useful technique to identify and distinguish between closely related chromophoric molecules which are difficult to distinguish by other means.

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