VIBRATIONAL ANALYSIS OF CROSS-LINKING AGENTS SPDP AND LC-SPDP

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Infrared and Laser Raman spectra of the compound, N-succinimydyl 3- (2- pyridyldithio) proprionate (SPDP) and N-succinimydyl [3-(2-pyridyldithio) propionate] hexanote (LC-SPDP) are recorded in the region 100 - 4000 cm^{-1}. A few observed bands are assigned to various modes of vibrations on the basis of normal coordinate calculations assuming C_s point group symmetry to the molecules. A complete set of internal coordinates used for the calculations also presented.

Key words: IR, Laser Raman Spectra, Vibrational analysis, SPDP, LC-SPDP.

Introduction

SPDP and LC-SPDP as cross-linking agents play an important role in the design of peptides and proteins. Hence, they are used to induce immune response against these compounds (Wawrzynczakand *et al* 1987; Peeters *et al* 1989). They are also used to synthesize immunotoxins and hormonotoxins. (Peeters 1989, Singh *et al* 1991, Moller 1982, Frankel 1988, Singh *et al* 1989). In order to understand the cross-linking nature of these reagents, a vibrational study particularly those involving C-S and S-S links, as they are more sensitive to local environment, is attempted in the present work. We have studied the IR and Raman spectra of SPDP and LC-SPDP with the support of theoretical calculations using normal coordinate analysis.

Experimental

The reagents, N-Succinimydyl 3- (2-pyridyldithio) proprionate (SPDP) and N-Succinimydyl (3- (2-pyridyldithio) propionate) hexanate (LC-SPDP) are purchased from Pierce Rockford, Ic, USA and used as such without any further purification. IR spectra of these compounds are recorded in the region 100-4000 cm^{-1} using the Shimadzu FTIR 8101 spectrophotometer. Raman spectra are recorded in the fused quartz capillary on a Dilor Z24 Raman spectrometer equipped with a spectra physics 165 Ar ion laser source operating with 488nm excitation source with 200mw power in the region 100-4000 cm^{-1}.

Normal coordinate analysis. The normal coordinate calculations are performed to support the assignment of the fundamental vibrational frequencies. The point group symmetry of the reagents are considered as C_s assuming the substituted pyridine ring as planar and all other molecules as point masses. The necessary bond lengths and bond angles are obtained from

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Sutton's table (Suttan 1958). The calculations are carried out partly by Willson's F-G Matrix method using the modified computer programme developed in our laboratory from Mink *et al* (1983) Program. All the atoms in the molecules are numbered in order to define the internal coordinates, which are shown in Fig. 1a & 1b. SPDP having 32 atoms, posses 90 degrees of vibrational modes. Using the standard procedure (Varsanyi 1974) the following set of internal coordinates,

$$31(r) + 30(\phi) + 12(\tau) + 17(\delta)$$

is choosen for SPDP. Under C_s symmetry only two types of vibrational modes viz. in plane mode (A') and out of plane (A") mode are possible. The coordinates $31(r) + 30(\phi)$ belong to A' species and rest are A" species. Here r, ϕ, δ and τ represent stretching, in-plane bending, out-of-plane deformation and torsion respectively. Similarly, for LC-SPDP the following set of internal coordinates,

$$50(r) + 49(\phi) + 29(\delta) + 15(\tau)$$

is choosen for representing various types of motions.

Results and Discussions

The experimentally observed frequencies in IR and Raman spectra with their relative intensities are given in Table 2, along with the calculated frequencies. The frequencies are assigned with the help of compounds like pyridine, N-hydroxy-succinimyl, etc. (Varsanyi 1974, Bellamy 1975, Oraeger 1983, Volosek *et al* 1983, Prasad *et al* 1989). CH_2-vibrations are compared with those of aliphatic chains having similar environment and amide from di-methyl acctamidc (Barnstein 1953, Suzuki 1962)).

C-H stretching vibrations. In pyridine (Varsanyi 1974, Draeger 1983, Volosek *et al* 1983, Prasad *et al* 1987), the four

bands in the region 3000-3200 cm^{-1} are assigned to C-H stretching mode. These bands are observed sharp in Raman spectrum while it is not distinct in infrared spectrum. Thus, the bands at 3010, 2997 and 2977 cm^{-1} in Raman and 3064 cm^{-1} in IR of SPDP and those at 3072, 3050 and 3029 cm^{-1} in Raman and at 3010 cm^{-1} in IR of LC-SPDP are assigned to C-H ring stretching. There are two CH$_2$ groups in SPDP and seven in LC-SPDP. Thus, there should be four stretching modes in SPDP and fourteen in LC-SPDP. However, CH$_2$ group vibrations are influenced by the adjacent group. The presence of C=O group adjacent to C-H lowers the frequency and in-

creases the intensity. The two CH$_2$ groups common to both SPDP and LC-SPDP give rise to four bands. The additional five CH$_2$ groups in LC-SPDP have similar environment except one which exists adjacent to CO group. Therefore some additional bands are also expected. We have assigned bands at 2974, 2921, 2935 and 2886 cm^{-1} in SPDP and 2939, 2927, 2935, 2908, 2870 and 2849 cm^{-1} in LC-SPDP to CH$_2$ group stretching.

CH$_2$ group modes. In addition to the above symmetric and asymmetric stretching, bending, rocking, wagging and twisting motions are also expected in CH$_2$ groups. The bending and

Table 1a
Internal co-ordinates of SPDP

Stretch co-ordinates	Bending co-ordinates	Out of plane deformation	Torsion co-ordinates
r_1 C$_2$ - H$_8$	ϕ_1 C$_2$ - H$_8$	δ_1 C$_2$ - H$_8$	τ_1 H$_8$ - C$_2$ - C$_1$ - H$_7$
r_2 C$_1$ - H$_7$	ϕ_2 C$_1$ - H$_7$	δ_2 C$_1$ - H$_7$	τ_2 H$_7$ - C$_1$ - C$_6$ - H$_{10}$
r_3 C$_6$ - H$_{10}$	ϕ_3 C$_6$ - H$_{10}$	δ_3 C$_6$ - H$_{10}$	τ_3 H$_{10}$ - C$_6$ - C$_5$ - H$_9$
r_4 C$_5$ - H$_9$	ϕ_4 C$_5$ - H$_9$	δ_4 C$_5$ - H$_9$	τ_4 H$_8$ - C$_2$ - C$_3$ - S$_{11}$
r_5 C$_3$ - C$_2$	ϕ_5 C$_{14}$ - H$_{13}$	δ_5 C$_3$ - S$_{11}$	τ_5 O$_{27}$ - C$_{23}$ - N$_{22}$ - O$_{21}$
r_6 C$_2$ - C$_1$	ϕ_6 C$_{14}$ - H$_{15}$	δ_6 C$_{14}$ - H$_{13}$	τ_6 O$_{32}$ - C$_{26}$ - N$_{22}$ - O$_{21}$
r_7 C$_1$ - C$_6$	ϕ_7 C$_{17}$ - H$_{16}$	δ_7 C$_{14}$ - H$_{15}$	τ_7 O$_{27}$ - C$_{23}$ - C$_{24}$ - H$_{28}$
r_8 C$_6$ - C$_5$	ϕ_8 C$_{17}$ - H$_{18}$	δ_8 C$_{17}$ - H$_{16}$	τ_8 O$_{32}$ - C$_{26}$ - C$_{25}$ - H$_{31}$
r_9 C$_5$ - N$_4$	ϕ_9 C$_{20}$ - O$_{19}$	δ_9 C$_{17}$ - H$_{18}$	τ_9 H$_{29}$ - C$_{24}$ - C$_{25}$ - H$_{30}$
r_{10} C$_{26}$ - O$_{32}$	ϕ_{10} C$_{23}$ - O$_{27}$	δ_{10} C$_{20}$ - O$_{19}$	τ_{10} O$_{19}$ - C$_{20}$ - N$_{22}$ - C$_{27}$
r_{11} C$_3$ - S$_{11}$	ϕ_{11} C$_{24}$ - H$_{28}$	δ_{11} C$_{21}$ - N$_{22}$	τ_{11} O$_{19}$ - C$_{20}$ - N$_{22}$ - C$_{26}$
r_{12} S$_{11}$ - S$_{12}$	ϕ_{12} C$_{24}$ - H$_{29}$	δ_{12} C$_{23}$ - O$_{27}$	τ_{12} Ring Torsion
r_{13} S$_{12}$ - C$_{14}$	ϕ_{13} C$_{25}$ - H$_{30}$	δ_{13} C$_{26}$ - O$_{32}$	
r_{14} C$_{14}$ - H$_{13}$	ϕ_{14} C$_{25}$ - H$_{31}$	δ_{14} C$_{24}$ - H$_{28}$	
r_{15} C$_{14}$ - H$_{15}$	ϕ_{15} C$_{26}$ - O$_{32}$	δ_{15} C$_{24}$ - H$_{29}$	
r_{16} C$_{14}$ - C$_{17}$	ϕ_{16} C$_1$ - C$_2$ - C$_3$	δ_{16} C$_{25}$ - H$_{30}$	
r_{17} C$_{17}$ - H$_{16}$	ϕ_{17} C$_2$ - C$_1$ - C$_6$	δ_{17} C$_{25}$ - H$_{31}$	
r_{18} C$_{17}$ - H$_{18}$	ϕ_{18} C$_1$ - C$_6$ - C$_5$		
r_{19} C$_{17}$ - C$_{20}$	ϕ_{19} C$_6$ - C$_5$ - N$_4$		
r_{20} C$_{20}$ - O$_{19}$	ϕ_{20} C$_3$ - S$_{11}$ - S$_{12}$		
r_{21} C$_{20}$ - O$_{21}$	ϕ_{21} S$_{11}$ - S$_{12}$ - C$_{14}$		
r_{22} O$_{21}$ - N$_{22}$	ϕ_{22} S$_{12}$ - C$_{14}$ - C$_{17}$		
r_{23} N$_{22}$ - C$_{23}$	ϕ_{23} C$_{14}$ - C$_{17}$ - C$_{20}$		
r_{24} C$_{23}$ - O$_{27}$	ϕ_{24} C$_{17}$ - C$_{20}$ - O$_{21}$		
r_{25} C$_{23}$ - C$_{24}$	ϕ_{25} C$_{20}$ - O$_{21}$ - N$_{22}$		
r_{26} C$_{24}$ - H$_{28}$	ϕ_{26} N$_{22}$ - C$_{23}$ - C$_{24}$		
r_{27} C$_{24}$ - H$_{29}$	ϕ_{27} C$_{23}$ - C$_{24}$ - C$_{25}$		
r_{28} C$_{24}$ - C$_{25}$	ϕ_{28} C$_{24}$ - C$_{25}$ - C$_{26}$		
r_{29} C$_{25}$ - H$_{30}$	ϕ_{29} C$_{25}$ - C$_{26}$ - N$_{22}$		
r_{30} C$_{25}$ - H$_{31}$	ϕ_{30} C$_5$ - N$_4$ - C$_3$		
r_{31} C$_{25}$ - C$_{26}$			

Table 1b
Internal co-ordinates of LC-SPDP

Stretch co-ordinates	Bending co-ordinates	Out of plane deformation	Torsion co-ordinates
r_1 C_3 - C_2	ϕ_1 C_2 - H_8	δ_1 C_2 - H_8	τ_1 C_3 - C_2 - C_1 - H_7
r_2 C_2 - C_1	ϕ_2 C_1 - H_7	δ_2 C_1 - H_7	τ_2 H_7 - C_1 - C_6 - H_{10}
r_3 C_1 - C_6	ϕ_3 C_6 - H_{10}	δ_3 C_6 - H_{10}	τ_3 H_{10} - C_6 - C_5 - H_9
r_4 C_6 - C_5	ϕ_4 C_5 - H_9	δ_4 C_5 - H_9	τ_4 H_8 - C_2 - C_3 - S_{11}
r_5 C_5 - N_4	ϕ_5 C_{14} - H_{13}	δ_5 C_3 - S_{11}	τ_5 O_{46} - C_{42} - N_{41} - O_{40}
r_6 C_2 - H_8	ϕ_6 C_{14} - H_{15}	δ_6 C_{14} - H_{13}	τ_6 O_{51} - C_{45} - C_{41} - H_{40}
r_7 C_1 - H_7	ϕ_7 C_{17} - H_{16}	δ_7 C_{14} - H_{15}	τ_7 O_{46} - C_{42} - N_{43} - O_{47}
r_8 C_6 - H_{10}	ϕ_8 C_{17} - H_{18}	δ_8 C_{17} - H_{16}	τ_8 O_{51} - C_{45} - C_{44} - H_{50}
r_9 C_5 - H_9	ϕ_9 C_{20} - O_{19}	δ_9 C_{17} - H_{18}	τ_9 H_{48} - C_{43} - C_{44} - H_{49}
r_{10} C_3 - S_{11}	ϕ_{10} C_{22} - H_{21}	δ_{10} C_{20} - O_{19}	τ_{10} O_{36} - C_{39} - C_{41} - H_{42}
r_{11} S_{11} - S_{12}	ϕ_{11} C_{24} - H_{23}	δ_{11} N_{22} - H_{21}	τ_{11} O_{38} - C_{39} - N_{22} - H_{21}
r_{12} S_{12} - C_{14}	ϕ_{12} C_{24} - H_{25}	δ_{12} C_{24} - H_{23}	τ_{12} O_{19} - C_{20} - N_{22} - H_{21}
r_{13} C_{14} - C_{17}	ϕ_{13} C_{27} - H_{26}	δ_{13} C_{24} - H_{25}	τ_{13} O_{19} - C_{20} - C_{14} - H_{13}
r_{14} C_{14} - C_{15}	ϕ_{14} C_{27} - H_{28}	δ_{14} C_{27} - H_{26}	τ_{14} O_3 - S_{11} - S_{12} - C_{14}
r_{15} C_{14} - H_{15}	ϕ_{15} C_{30} - H_{29}	δ_{15} C_{27} - H_{28}	τ_{15} Ring Torsion
r_{16} C_{17} - H_{16}	ϕ_{16} C_{30} - H_{31}	δ_{16} C_{30} - H_{29}	τ_{16} O_{38} - C_{39} - C_{20} - O_{19}
r_{17} C_{17} - H_{18}	ϕ_{17} C_{33} - H_{34}	δ_{17} C_{30} - H_{31}	τ_{17} H_{21} - N_{22} - C_{14} - H_{13}
r_{18} C_{17} - C_{20}	ϕ_{18} C_{36} - H_{35}	δ_{18} C_{33} - H_{32}	τ_{18} C_3 - S_{11} - C_{20} - O_{19}
r_{19} C_{20} - O_{19}	ϕ_{19} C_{36} - H_{37}	δ_{19} C_{33} - H_{34}	τ_{19} C_3 - S_{11} - N_{22} - H_{21}
r_{20} C_{20} - N_{22}	ϕ_{20} C_{39} - O_{38}	δ_{20} C_{36} - H_{35}	
r_{21} N_{22} - H_{21}	ϕ_{21} C_{42} - O_{46}	δ_{21} C_{36} - H_{37}	
r_{22} N_{22} - C_{24}	ϕ_{22} C_{43} - H_{47}	δ_{22} C_{39} - O_{38}	
r_{23} C_{24} - H_{23}	ϕ_{23} C_{43} - H_{48}	δ_{23} O_{40} - N_{41}	
r_{24} C_{24} - H_{25}	ϕ_{24} C_{33} - H_{32}	δ_{24} O_{42} - O_{46}	
r_{25} C_{24} - C_{27}	ϕ_{25} C_{44} - H_{49}	δ_{25} C_{45} - O_{51}	
r_{26} C_{27} - H_{26}	ϕ_{26} C_{44} - H_{50}	δ_{26} C_{43} - H_{47}	
r_{27} C_{27} - H_{28}	ϕ_{27} C_{45} - O_{51}	δ_{27} C_{43} - H_{48}	
r_{28} C_{27} - C_{30}	ϕ_{28} C_3 - C_2 - C_1	δ_{28} C_{44} - H_{49}	
r_{29} C_{30} - H_{29}	ϕ_{29} C_2 - C_1 - C_6	δ_{29} C_{44} - H_{50}	
r_{30} C_{30} - H_{31}	ϕ_{30} C_1 - C_6 - C_5		
r_{31} C_{30} - H_{33}	ϕ_{31} C_6 - C_5 - N_4		
r_{32} C_{33} - H_{32}	ϕ_{32} C_5 - N_4 - C_3		
r_{33} C_{33} - H_{34}	ϕ_{33} Ring bending		
r_{34} C_{33} - C_{36}	ϕ_{34} C_3 - S_{11} - S_{12}		
r_{35} C_{36} - H_{35}	ϕ_{35} S_{11} - S_{12} - C_{14}		
r_{36} C_{36} - H_{37}	ϕ_{36} C_{14} - C_{17} - C_{20}		
r_{37} C_{36} - H_{39}	ϕ_{37} C_{17} - C_{20} - N_{22}		
r_{38} C_{39} - O_{38}	ϕ_{38} C_{20} - N_{22} - C_{24}		
r_{39} C_{39} - O_{40}	ϕ_{39} N_{22} - C_{24} - C_{27}		
r_{40} O_{40} - N_{41}	ϕ_{40} C_{24} - C_{27} - C_{30}		
r_{41} C_{42} - N_{41}	ϕ_{41} C_{27} - C_{30} - C_{33}		
r_{42} C_{42} - O_{46}	ϕ_{42} C_{30} - C_{33} - C_{36}		
r_{43} C_{42} - C_{43}	ϕ_{43} C_{32} - C_{36} - C_{39}		
r_{44} C_{43} - H_{47}	ϕ_{44} C_{36} - C_{35} - O_{40}		
r_{45} C_{43} - C_{44}	ϕ_{45} C_{39} - C_{40} - N_{41}		
r_{46} C_{44} - C_{45}	ϕ_{46} O_{40} - N_{41} - C_{42}		
r_{47} C_{43} - H_{48}	ϕ_{47} N_{41} - C_{42} - C_{43}		
r_{48} C_{44} - C_{49}	ϕ_{48} N_{41} - C_{45} - C_{44}		
r_{49} C_{44} - H_{50}	ϕ_{49} C_{43} - C_{44} - C_{45}		
r_{50} C_{45} - O_{51}			
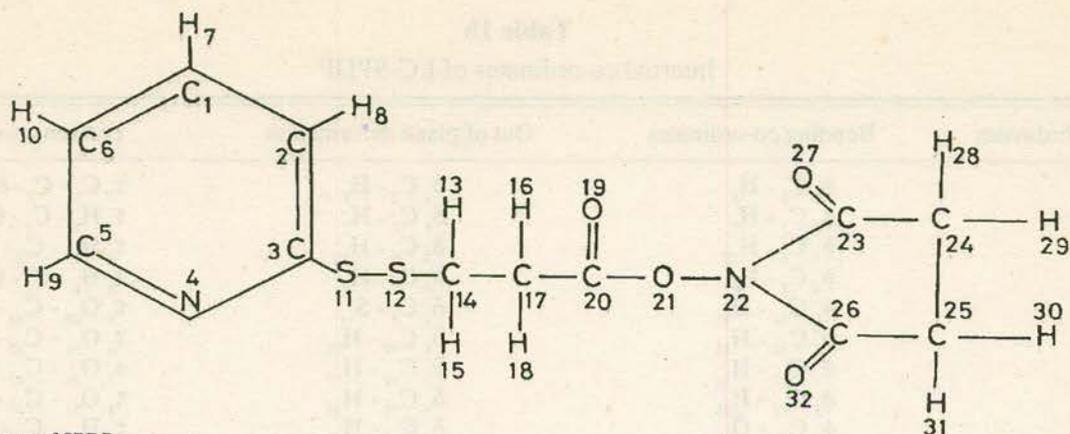
Fig 1a. Structure of SPDP.
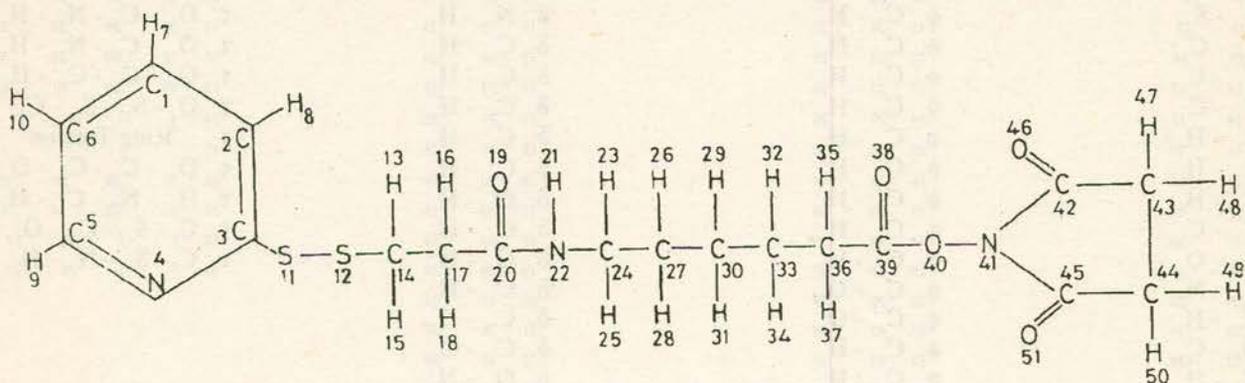
Fig 1b. Structure of LC - SPDP.
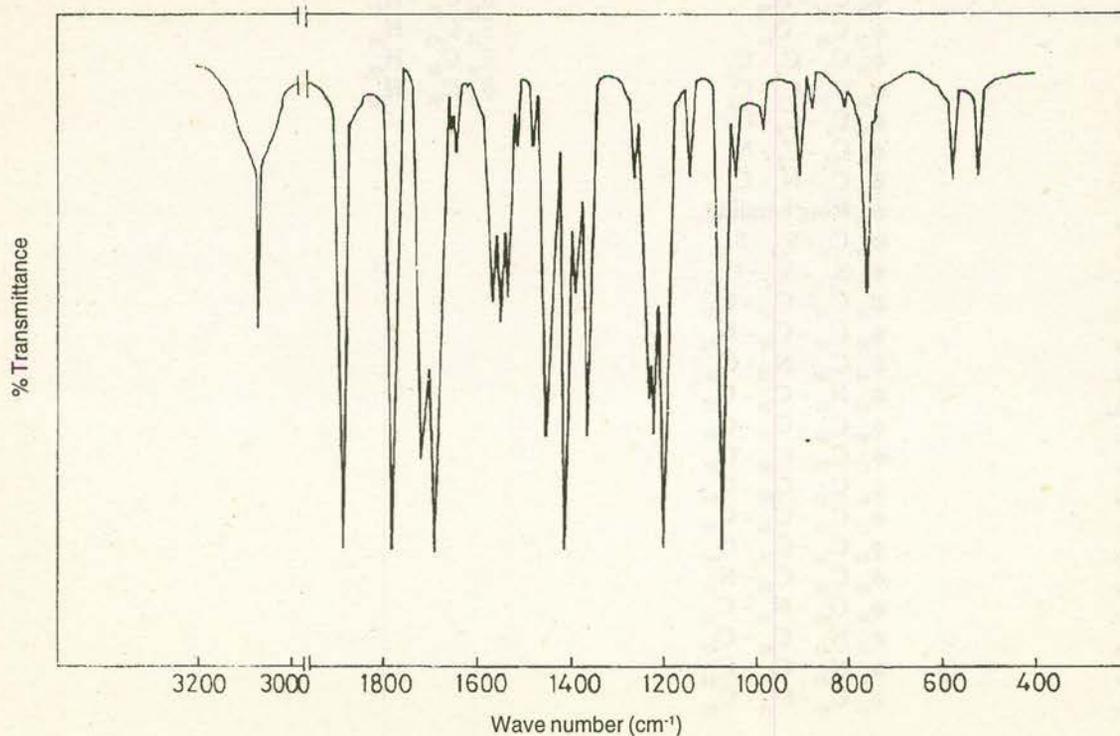
Fig 2a. FTIR spectrum of SPDP.
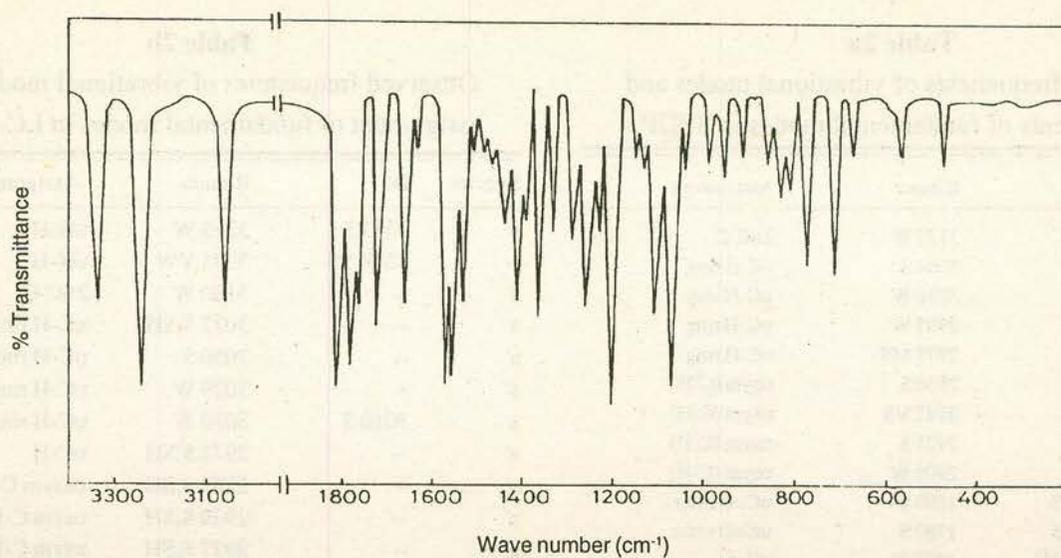
Fig 2b. FTIR spectrum of LC - SPDP.
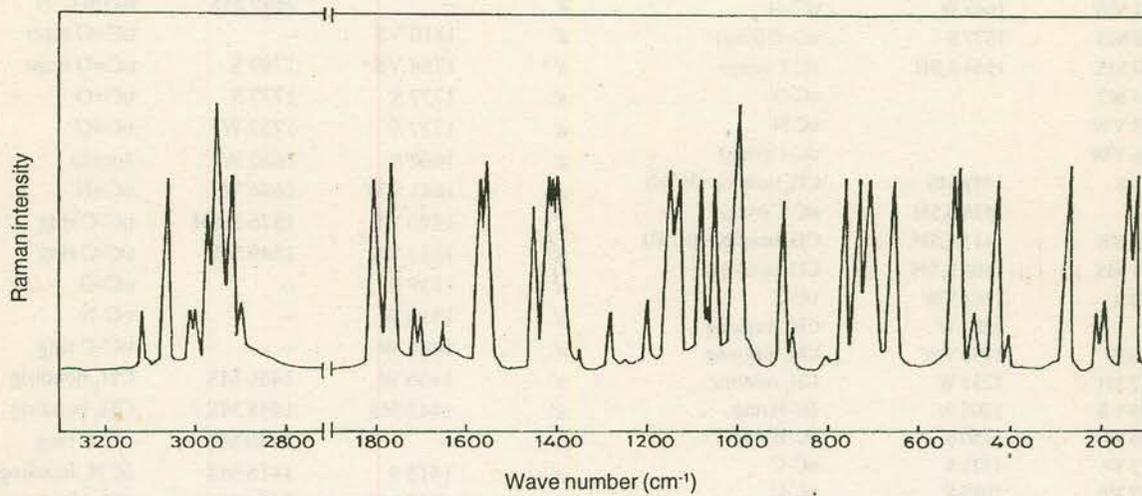
Fig 3a. Laser Raman spectrum of SPDP.
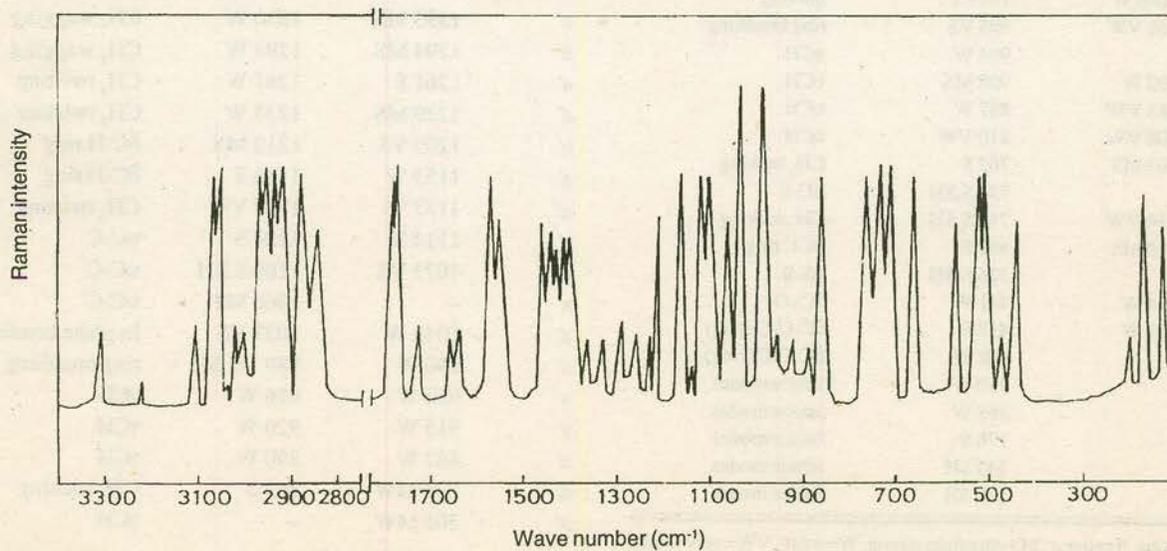
Fig 3b. Laser Raman spectrum of LC - SPDP.

Table 2a
Observed frequencies of vibrational modes and assignments of fundamental modes in SPDP

Species	IR	Raman	Assignment
		3127 W	2υC-C
a'	3064 W	3064 S	υC-H ring
a'		3010 W	υC-H ring
a'		2997 W	υC-H ring
a'		2977 MS	υC-H ring
a'		2956 S	υsym (C-H)
a'		2947 VS	υsym (C-H)
a'		2921 S	υsym (C-H)
a'		2905 W	υsym (C-H)
a'	1890 VS	1803 S	υC=O ester
a'	1786 VS	1787 S	υC=O ester
a'	1728 S,SH	1727 W	υC=O
a'	1697 VS	1706 W	υC=O
a'	1641 VW	1649 W	υC=N
a'	1566 MS	1577 S	υC=C (ring)
a'	1554 MS	1564 S,SH	υC-C (ring)
a'	1534 MS		υC-O
a'	1513 VW		υC-N
a'	1486 VW		υC-C (ring)
a'	1448 S	1449 MS	CH₂ bending (δC-H)
		1426 S,SH	υC-C (ring)
a'	1416 VS	1411 S,SH	CH₂ bending (υC-H)
a'	1391 MS	1404 S,SH	CH₂ bending
a'	1358 S	1361 VW	υC-C
a"		1287 W	CH₂ wagging
a"	1246 W	1254 VW	CH₂ wagging
a"	1222 SH	1231 W	CH₂ twisting
a'	1199 VS	1205 W	δC-H ring
a'	1145 W	1150 S	δC-H ring
a'	1117 W	1131 S	υC-C
a'	1077 VS	1086 S	υC-C
a'		1072 W	υC-C
a'	1046 W	1053 S	ipb ring
a'	988 VW	995 VS	ring breathing
a"		964 W	υCH
a"	902 W	908 MS	υCH
a"	883 VW	887 W	υCH
a"	808 VW	810 VW	υCH
a'	763 MS	762 S	CH₂ rocking
a'		732 S,SH	υC-S
a'	714 VW	717 S,SH	CH₂ rocking
a'	669 MS	669 S	υCC ring
a'		526 S,SH	υS-S
a'	474 W	481 W	δC=O
a'	421 W	432 S	δC-C-C (ring)
a'		402 W	δC-C-C (ring)
		210 W	lattice modes
		198 W	lattice modes
		176 S	lattice modes
		145 SH	lattice modes
		125 SH	lattice modes

VS=very strong, S=strong, MS=medium strong, W=weak, VW=very weak, SH=shoulder.

Table 2b
Observed frequencies of vibrational modes and assignment of fundamental modes in LC-SPDP.

Species	IR	Raman	Assignment
a'	3343 S	3315 W	υN-H
--	3248 VS	3238 VW	υN-H
a'	--	3130 W	2υC-C
a'	--	3072 S,SH	υC-H ring
a'	--	3050 S	υC-H ring
a'	--	3029 W	υC-H ring
a'	3010 S	3010 W	υC-H ring
a'	--	2972 S,SH	υC-H
a'	--	2962 S,SH	υasym C-H
a'	--	2939 S,SH	υsym C-H
a'	--	2927 S,SH	υsym C-H
a'	--	2870 S	υsym C-H
a'	--	2849 MS	υsym C-H
a'	1810 VS	--	υC=O ester
a'	1784 VS	1789 S	υC=O ester
a'	1777 S	1777 S	υC=O
a'	1727 S	1732 W	υC=O
a'	1662 S	1662 W	Amide I
a'	1641 VW	1646 W	υC=N
a'	1570 VS	1576 S,SH	υC-C ring
a'	1553 VS	1549 MS	υC-C ring
a'	1539 S	--	υC-O
a'	1514 W	--	υC-N
a'	1486 W	--	υC-C ring
a'	1466 W	1466 MS	CH₂ bending
a'	1442 MS	1448 MS	CH₂ bending
a'	--	1440 MS	υC-C ring
a'	1415 S	1418 MS	δCH₂ bending
a'	1397 MS	1405 MS	CH₂ bending
a'	1367 S	1368 W	υC-C
a'	1333 MS	1330 W	CH₂ wagging
a'	1294 MS	1294 W	CH₂ wagging
a'	1261 S	1261 W	CH₂ twisting
a'	1239 MS	1235 W	CH₂ twisting
a'	1209 VS	1212 MS	δC-H ring
a'	1153 W	1162 S	δC-H ring
a"	1137 W	1137 VW	CH₂ twisting
a'	1114 S	1124 S	υC-C
a'	1073 VS	1100 S,SH	υC-C
a'	--	1066 MS	υC-C
a'	1048 W	1037 VS	In plane bending ring
a'	990 W	989 VS,SH	ring breathing
a'	953 W	956 W	γCH
a'	915 W	920 W	γCH
a'	867 W	890 W	γCH
a'	831 MW	863 S	CH₂ rocking
a'	806 MW	--	γCH

(Contd...)

(Table 2 cont'd...)

a'	770 MS	764 S	CH$_2$ rocking
a'	--	736 S	υC-S
a'	713 MS	717 S,SH	CH$_2$ rocking
a'	672 W	669 S	γCC ring
a'	570 W	573 MS	γCC ring
a'	--	523 S,SH	υS-S
a'	478 W	476 W	δC=O
a'	--	422 MS	δC-C-C ring
	--	198 W	lattice mode
	--	172 S	lattice mode
	--	130 SH	lattice mode

rocking modes constitute the in-plane motion while the wagging and twisting modes constitute the out-of-plane motions. The bands due to wagging and twisting are very weak in Raman while that of bending and rocking are strong. This indicates that all these modes of vibrations may not have observable intensity. Thus in the present work, the bands at 1449, 1411, 1404 cm^{-1}; 1287, 1254 cm^{-1}; 763, 714 cm^{-1} and 1231 cm^{-1} in SPDP and 1466, 1448, 1418, 1405 cm^{-1}; 1330, 1294cm^{-1}; 863, 764, 717 cm^{-1} and 1261, 1235, 1137 cm^{-1} in LC-SPDP are assigned to bending, wagging, rocking and twisting modes respectively.

Ring vibrations. The substituted aromatic ring exhibit C-H in plane and C-C skeletal modes of vibrations. The bands corresponding to these motions, assigned in comparison with related molecules are shown in Table 2a and 2b. In Wilson's notations, the C-C skeletal modes are correlated to 8a, 8b, 19a, 14 and 1 modes of benzene. These modes in different molecules are assigned at 1600, 1585, 1485, 1430, 1375 and 1000 cm^{-1}. In the present study, we assign these modes at 1577, 1549, 1564, 1486, 1426 and 1440 cm^{-1}.

Carbonyl group vibrations. The C=O stretching of esters are assigned at 1893 and 1787 cm^{-1} in SPDP and at 1810 and 1789 cm^{-1} in LC-SPDP, while that of carbonyl groups on the ring have been assigned at 1727 and 1706 cm^{-1} in SPDP and at 1777 and 1732 cm^{-1} LC-SPDP. In LC-SPDP, the presence of CONH (amide) group also gives rise to additional mode viz. a predominant C=O stretching. This is assigned at 1662 cm^{-1}. The C=O stretching is assigned at 1534 and 1539 cm^{-1} in SPDP and LC-SPDP respectively, which is observed only in infrared spectrum.

The bands which are considered important in this study are due to S-S and C-S bonds. These modes are quite unambiguously assigned. A very strong band at 526 cm^{-1} in SPDP and at 523 cm^{-1} in LC-SPDP are assigned to S-S stretching and the bands at 732 cm^{-1} in SPDP and at 736 cm^{-1} in LC-SPDP are assigned to C-S stretching. It may also be mentioned that the position of these bands are conformation sensitive. Particu-

larly, the position of v_{s-s} depends upon the diherdal angle between the adjacent C-S planes. Once the cross linker is linked with protein, the change in the frequency of this mode indicates the change in the conformation. The assignment of remaining modes, which are done with the help of literatures of similar systems are given in Table 2.

However, the frequencies of all the coordinates as suggested by the group theoretical consideration are not obtained due to the instrument limitations. Hence the potential energy distribution calculations are not carried out in the present work.

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