

## Laser Raman Spectra of Some Non-conjugated Steroid Olefins

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The Raman spectra of 27 steroids containing non-conjugated olefinic bonds in a range of positions have been measured for solid samples, for chloroform solutions and, in some cases, for carbon tetrachloride solutions, particular attention being paid to the 3 000—3 100  $\text{cm}^{-1}$  region (olefinic C—H stretching vibrations) and 1 600—1 700  $\text{cm}^{-1}$  region (olefinic double bond stretching vibrations). All the nuclear disubstituted olefins gave a band between 3 013 and 3 028  $\text{cm}^{-1}$ . Some trisubstituted olefins gave a band between 3 035 and 3 040  $\text{cm}^{-1}$ , but the others, like tetrasubstituted olefins, did not give any observable band in this region of the spectrum. All the olefins, including the tetrasubstituted olefins, gave strong bands between 1 632 and 1 676  $\text{cm}^{-1}$ . The ranges of frequencies for the various double bond positions often overlap, but the ranges for  $\Delta^8$ -,  $\Delta^9(11)$ -, and  $\Delta^{11}$ -olefins are different from those of other tetra-, tri-, and di-substituted double bonds respectively.

THE measurement of Raman spectra has been greatly simplified by the recent introduction of laser light sources and the spectra of many compounds can now be measured routinely using only 3—15 mg of sample. We report here the Raman spectra of 27 steroids which between them contain non-conjugated double bonds in all possible positions in rings A—C except  $\Delta^9$ .<sup>1</sup> These are of interest because the stretching mode of this common functional group gives rise to only weak or even zero absorption in the i.r. whereas in the Raman it gives rise to strong sharp

bands. Raman spectroscopy therefore provides a simple way of detecting this group. The differences in the intensities of the bands due to this stretching mode for some representative examples of di-, tri-, and the tetra-substituted olefins studied in the present work are shown in Figure 1. There have been other studies of the Raman spectra of steroids<sup>2-4</sup> but these were not primarily concerned with non-conjugated olefins and few of the compounds studied contained double bonds in other than the common 5-position.

<sup>1</sup> A fuller account of this work is given in the M.Sc. Thesis of M. N. Khan, University of Lancaster, 1974.

<sup>2</sup> B. Schrader and E. Steigner in 'Modern Methods of Steroid Analysis,' ed. E. Heftmann, Academic Press, New York, 1973, ch. 9.

<sup>3</sup> R. N. Jones, J. B. DiGriorgio, J. J. Elliot, and G. A. A. Nonnenmacher, *J. Org. Chem.*, 1965, **30**, 1822.

<sup>4</sup> I. R. Beattie, J. Hudec, and K. M. S. Livingston, *J. Chem. Soc. (C)*, 1971, 2521.

Our results are summarised in the Table. Spectra were generally measured both for solid samples and for solutions in chloroform, this solvent being chosen because it dissolves a wide range of steroids. In some cases spectra were also measured for carbon tetrachloride solutions since this solvent permits study of the 3 000—3 100  $\text{cm}^{-1}$  region. The samples were purified by recrystallisation; no special efforts were made to remove traces of fluorescent impurities.<sup>3,5</sup> It is apparent that changes of phase and solvent can result in frequency differences of up to 7  $\text{cm}^{-1}$ , although in most cases they are much less.

**Olefinic C-H Stretching Bands (3 000—3 100  $\text{cm}^{-1}$  Region).**—Di- and tri-substituted double bonds but not tetrasubstituted double bonds would be expected to show bands in this region. All the disubstituted olefins studied gave a relatively strong band between 3 013 and 3 028

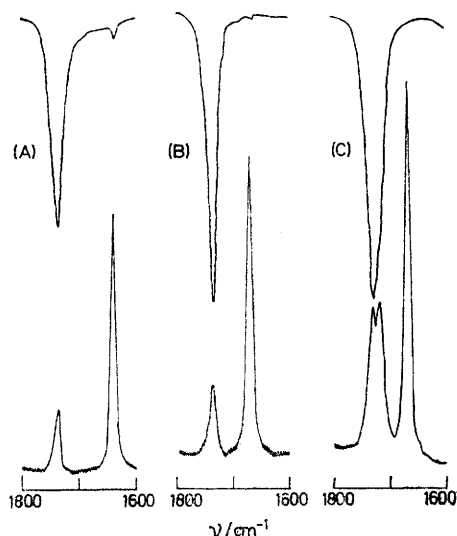


FIGURE 1 I.R. (upper trace) and Raman (lower trace) spectra of (A) di- [compound (8), i.r. Nujol mull, Raman, solid]; (B) tri- [compound (15),  $\text{CCl}_4$  solution]; and (C) tetra- [compound (23),  $\text{CHCl}_3$  solution] substituted olefins

$\text{cm}^{-1}$ . Values for specific olefins differ somewhat from those observed in i.r. spectra<sup>6,7</sup> probably because it is the symmetric vibrational mode that is Raman active and the antisymmetric mode that is i.r. active. Several of the Raman spectra contain a much weaker band between 3 052 and 3 068  $\text{cm}^{-1}$ , the origin of which is not clear.

The i.r. spectra of steroids containing a trisubstituted double bond generally show one and often two bands in this region.<sup>7,8</sup> In contrast, the Raman spectra of the  $\Delta^4$ -,  $\Delta^5$ -, and  $\Delta^7$ -olefins we studied gave just a single very weak band or no observable band in this region, whilst the  $\Delta^9(11)$ -olefins gave one moderately strong band. In all cases where a definite band was observed the fre-

<sup>5</sup> Ref. 2, p. 234.

<sup>6</sup> H. B. Henbest, G. D. Meakins, and G. W. Wood, *J. Chem. Soc.*, 1954, 800.

<sup>7</sup> G. M. L. Cragg, C. W. Davey, D. N. Hall, G. D. Meakins, E. E. Richards, and T. L. Whateley, *J. Chem. Soc. (C)*, 1966, 1266.

<sup>8</sup> P. Bladon, J. M. Fabian, H. B. Henbest, H. P. Koch, and G. W. Wood, *J. Chem. Soc.*, 1951, 2402.

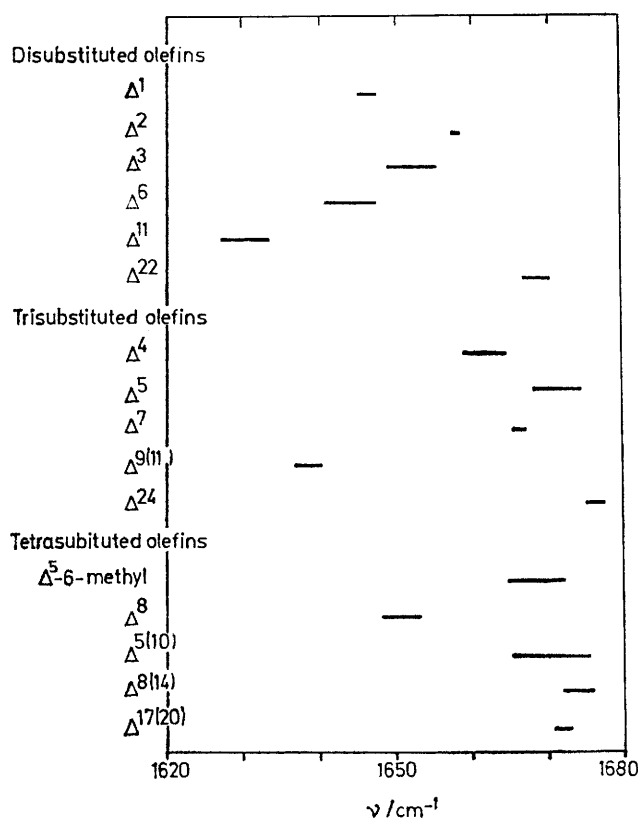


FIGURE 2 Frequency ranges of  $\nu(\text{C}=\text{C})$  for various types of olefins

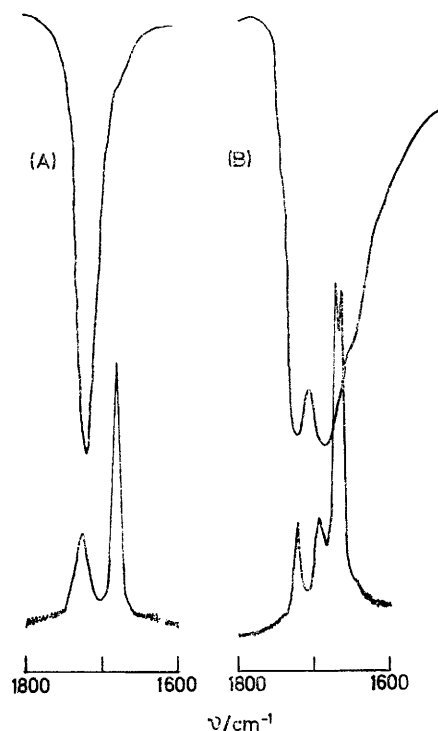


FIGURE 3 I.R. (upper trace) and Raman (lower trace) spectra of compound (24) recorded in  $\text{CHCl}_3$  solution (A) and in the solid phase (B)

quency fell between 3 035 and 3 040  $\text{cm}^{-1}$ , a range somewhat higher than that found for disubstituted olefins. For specific olefins the frequency was close to that of the i.r. band or, where there were two i.r. bands, to the higher of the two bands and these bands in both spectra probably result from the same vibrational mode.

ing the position of a double bond in cases where it is not known, but within their substitution type, the ranges for  $\Delta^{11}$ -,  $\Delta^{9(11)}$ - and  $\Delta^8$ -olefins appear to be characteristic. Apart from the  $\Delta^{11}$ -olefin the nuclear disubstituted olefins have values between 1 641 and 1 658  $\text{cm}^{-1}$ , but  $\Delta^{22}$ -olefins and the tri- and tetra-substituted olefins other

Raman bands of non-conjugated steroid olefins

No.	Compound	$\nu(\text{C-H})/\text{cm}^{-1}$		$\nu(\text{C=C})^a/\text{cm}^{-1}$		
		Solid sample	$\text{CCl}_4$ soln.	Solid sample	$\text{CHCl}_3$ soln.	$\text{CCl}_4$ Soln.
(i) Disubstituted olefins						
(1)	5 $\alpha$ -Androst-1-en-3 $\beta$ -ol	3 024m		1 645	1 647	
(2)	5 $\alpha$ -Cholest-1-en-3 $\beta$ -ol	3 026w		1 646	1 646	
(3)	5 $\alpha$ -Cholest-2-ene	{ 3 020m 3 058vw	3 025m	1 657	1 657	1 658
(4)	5 $\alpha$ -Cholest-2-en-6-one	{ 3 024m 3 068vw		1 658		
(5)	5 $\alpha$ -Pregn-2-en-20-one	3 017m		1 657		
(6)	5 $\alpha$ -Cholest-3-ene	3 017m	3 019m	1 652	1 649	1 651
(7)	Methyl 5 $\beta$ -chol-3-enoate	{ 3 013m 3 064vw		1 655	1 653	
(8)	5 $\alpha$ -Cholest-6-en-3 $\beta$ -yl acetate	{ 3 017m 3 052vw	3 017m	1 641	1 647	1 644
(9)	3 $\alpha$ -Hydroxy-5 $\beta$ -chol-11-enoic acid	3 028m		1 627	1 627	
(10)	(25R)-5 $\alpha$ -Spirost-11-en-3 $\beta$ -yl <sup>a</sup> acetate	{ 3 016m 3 053vw		1 633	1 632	
(ii) Trisubstituted olefins						
(11)	Cholest-4-enc	*	*	1 661	1 660	1 661
(12)	Cholest-4-en-3 $\beta$ -yl acetate	*	*	1 659	1 662	1 664
(13)	Cholest-5-ene	3 035vw	*	1 672	1 668	1 669
(14)	Cholest-5-en-3-one	3 035vw	*	1 674	1 672	1 670
(15)	Cholest-5-en-3 $\beta$ -yl acetate	*	*	1 671	1 672	1 671
(16)	3 $\beta$ -Chlorocholest-5-ene	3 035vw	*	1 671	1 670	1 670
(17)	20-Methylpregna-5,17(20)-dien-3 $\beta$ -yl acetate	*		1 671 <sup>b</sup>	1 673 <sup>b</sup>	
(18)	Stigmasta-5,22-dien-3 $\beta$ -yl acetate	*		1 670 <sup>c</sup>	1 670 <sup>c</sup>	
(19)	5 $\alpha$ -Cholest-7-en-3 $\beta$ -ol	3 040vw		1 666	1 666	
(20)	5 $\alpha$ -Androst-9(11)-ene-3 $\beta$ ,17 $\beta$ -diol	3 038w		1 640	Insoluble	
(21)	3 $\beta$ -Acetoxy-5 $\alpha$ -androst-9(11)-en-17-one	3 038w		1 638	1 637	
(iii) Tetrasubstituted olefins						
(22)	(25R)-6-Methylspirost-5-en-3 $\beta$ -yl acetate			1 672	<sup>d</sup>	1 665
(23)	3 $\beta$ ,17 $\alpha$ -Diacetoxy-6-methylpregn-5-en-20-one			1 671	1 669	
(24)	17 $\alpha$ -Ethylnyl-17 $\beta$ -hydroxyestr-5(10)-en-3-one			{ 1 666 1 673 <sup>e</sup>	1 675 <sup>e</sup>	
(25)	5 $\alpha$ -Lanost-8-en-3 $\beta$ -ol			1 652	1 653	
(26)	5 $\alpha$ -Lanosta-8,24-dien-3 $\beta$ -ol	*		1 647 <sup>f</sup>	1 650 <sup>g</sup>	
(27)	5 $\alpha$ -Cholest-8(14)-en-3 $\beta$ -ol			1 672	1 676	

<sup>a</sup> Nuclear double bonds only. <sup>b</sup> Band due to  $\Delta^{17(20)}$ -double bond occurs at same frequency. <sup>c</sup> Band due to  $\Delta^{22}$ -double bond occurs at same frequency. <sup>d</sup> Background fluorescence too high to permit measurement of spectrum. <sup>e</sup>  $\nu(\text{C=C})$  at 2 105  $\text{cm}^{-1}$  for solid sample and 2 112  $\text{cm}^{-1}$  for solution. <sup>f</sup> Band due to  $\Delta^{24}$ -double bond appears at 1 675  $\text{cm}^{-1}$ . <sup>g</sup> Band due to  $\Delta^{24}$ -double bond appears at 1 677  $\text{cm}^{-1}$ .

\* No clear maximum.

C=C *Stretching Bands* (1 600—1 700  $\text{cm}^{-1}$  Region).—All the compounds studied gave a strong band between 1 632 and 1 676  $\text{cm}^{-1}$ . The frequency ranges for the various double bond locations are summarised in Figure 2, the values used being those obtained from the spectra of both solids and solutions. Bearing in mind the small number of examples in some instances and the possible effects on the band positions of changes of state, solvent, and of substituents,<sup>6,9</sup> the ranges for the di- and tri-substituted olefin agree fairly well with those obtained from i.r. spectra<sup>10,11</sup> which are much more difficult to measure. The frequencies of the tetrasubstituted double bond vibrations are not available from i.r. spectroscopy. The closeness of the ranges limits their value for identify-

<sup>9</sup> H. B. Henbest, G. D. Meakins, B. Nichols, and R. A. L. Wilson, *J. Chem. Soc.*, 1957, 997.

<sup>10</sup> R. N. Jones and F. Herling, *J. Org. Chem.*, 1954, **19**, 1252.

than the  $\Delta^{9(11)}$ - and  $\Delta^8$ -compounds have values between 1 659 and 1 676  $\text{cm}^{-1}$ . Olefins in ring D would be expected to have lower frequencies than these because the ring is only five-membered.<sup>8</sup>

The spectra of compound (24) provide an interesting example of factor group splitting. In chloroform solution only one C=O and C=C vibration frequency is observed in both the i.r. and Raman spectra, but in both spectra of the solid the bands are split into doublets. This is shown in Figure 3.

*Other Bands.*—The out-of-plane C-H bending region, 650—800  $\text{cm}^{-1}$ , of the i.r. spectra of steroid olefins yields useful information about the position of the double bond.<sup>7</sup> Unlike the i.r. spectra, the Raman spectra generally have

<sup>11</sup> G. Roberts, B. S. Gallagher, and R. N. Jones, 'Infrared Absorption Spectra of Steroids,' Interscience, New York, 1958, vol. 2, p. 26.

numerous bands of moderate intensity in this region, many of which are probably ring breathing vibrations,<sup>12</sup> and it is difficult to make assignments or even to determine whether any of the bands seen in the i.r. spectra are Raman active.

Of the bands due to other functional groups, those due to O-H and C=O vibrations give weaker bands in the Raman spectrum than in the i.r. spectrum and are most conveniently studied by the latter technique. The triple-bond vibration of the ethynyl group in compound (24) gives rise to only a very weak band in the i.r. but to a strong band at  $2105\text{ cm}^{-1}$  in the Raman.

#### EXPERIMENTAL

The steroid samples were either prepared at Lancaster using standard methods,<sup>1,7</sup> or were kindly supplied by Dr. J. Elks of Glaxo Research or Dr. G. D. Meakins of Oxford University. The samples were purified by recrystallisation.

<sup>12</sup> S. K. Freeman, 'Applications of Laser Raman Spectroscopy,' Wiley-Interscience, New York, 1974, p. 139.

*Raman Spectra.*—The spectra were excited using the 520.8 nm line of a CRL model 52K argon-krypton laser and were recorded on a Cary 81 spectrometer. Solids were examined in the standard solid holder which requires *ca.* 3 mg of sample. The solutions in chloroform or carbon tetrachloride were *ca.* 20% w/v, *ca.* 15 mg of sample being used, and they were examined by the standard capillary cell technique which requires *ca.* 60  $\mu\text{l}$  of solution. The instrument was calibrated with krypton emission lines. Most of the steroid samples gave a high background fluorescence initially but after irradiation with the laser for *ca.* 2 h satisfactory spectra could be obtained.<sup>13</sup> In one instance the background remained high and a satisfactory spectrum could not be recorded.

*I.r. Spectra.*—These were measured for Nujol mulls or *ca.* 5% solutions (w/v) in chloroform or carbon tetrachloride on a Perkin-Elmer model 225 spectrometer. The instrument was calibrated with a polystyrene film.

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<sup>13</sup> M. J. Gall, P. J. Hendra, D. S. Watson, and C. J. Peacock, *Appl. Spectroscopy*, 1971, **25**, 423.