

Infrared spectrum of N-t-BOC-Alanyl-Proline

Kamalendra Singh, R A Yadav and D K Rai†

Department of Physics, Banaras Hindu University, Varanasi-221 005, India
and

J S Yadav

Department of IST, University of Medicine and Dentistry of New Jersey,
Newark, NJ 07103, USA

Received 31 July 1990, accepted 6 November 1990

The present note deals with the interpretation of the infrared spectrum of N-t-BOC-Alanyl-Proline recorded in the regions 35-350 and 300-4000 cm^{-1} for the first time.

N-t-BOC-Ala-Pro was obtained from Bachem Feinchemikalien AG (Switzerland) and was used as such. It forms a solid compound at room temperature. The far-infrared spectrum[‡] was recorded in the spectral range 35-350 cm^{-1} on a Beckman IR-11 spectrometer using high density polyethylene pellet. The infrared spectrum[‡] was recorded in the region 300-4000 cm^{-1} on a Beckman IR-12 spectrometer using KBr pellet. The resolution of the spectrometers was of the order of 2 cm^{-1} and the bands reported are accurate to within $\pm 3 \text{ cm}^{-1}$.

N-t-BOC-Ala-Pro is a non-planar molecule with C_1 symmetry. N-t-BOC-Ala-Pro, being a 42 atomic molecule would give rise to 120 normal modes of vibration all active both in the Raman and in the infrared spectra.

Out of the 120 normal modes 20 correspond to the $\text{CH}_3/\text{CH}_2/\text{CH}$ stretching vibrations which fall in a very narrow spectral range 2850-3000 cm^{-1} . There are only two modes namely, the OH and the NH stretching vibrations expected to appear on the higher frequency side of the $\text{CH}_3/\text{CH}_2/\text{CH}$ stretching modes. The remaining 98 modes all lie in the spectral range 50-1800 cm^{-1} . As a consequence the spectrum of N-t-BOC-Ala-Pro is expected to be quite complicated both in the range 2850-3000 cm^{-1} as well as 50-1800 cm^{-1} . The infrared spectra show that these are rather complex spectra full of bands in the above mentioned frequency ranges. Therefore, it is rather difficult to analyse the infrared bands. Table 1 enlists all the observed infrared bands together with their relative intensities and proposed vibrational assignments.

†Author for correspondence.

*The spectra were recorded at the Institute of Crystallography, Free University of Berlin, while one (JSY) of the authors was there.

It is difficult to assign an infrared band in the range 2850-3000 cm^{-1} to a particular $\text{CH}_3/\text{CH}_2/\text{CH}$ stretching mode. However, some tentative assignments can be made from the comparison with the reported spectra by Colthup *et al* (1964), Pitzer and Kilpatrick (1946), Evans and Bernstein (1956), Korppi-Tommola (1978), Susi and Byler (1980), Machida *et al* (1978), Herlinger and Long (1970), Krishnan and Sankaranarayanan (1973). For N-t-BOC-Ala-Pro infrared bands are observed

Table I. Far-infrared and infrared frequencies (cm^{-1}) and vibrational assignments for N-t-BOC-Alanyl-Proline.

Obs. frequencies*	Proposed assignments†	Obs. frequencies*	Proposed assignments†
71 (mw)	τ (CC_s)	870 (42)	ν (ring)
74 (mw)		921 (42)	ρ (CH_2)
76 (mw)		963 (31)	ρ (CH_3)
80 (w)		992 (sh)	
120 (mb)	ν (O...H)	1016 (74)	ν (ring)
200 (s)	τ (COOH)	1035 (57)	ρ (CH_2)
211 (s)	τ (CH_2)	1041 (58)	ν (ring)
218 (s)		1065 (77)	ν (C- CH_2)
227 (s)		1094 (43)	ν (C-N)
233 (s)		1111 (66)	ν (ring)
248 (vs)		1154 (s-sh)	ν (C-N)
268 (ms)	δ (CCN)	1171 (93)	ν (C-OH) + β (O-H)
298 (vs)		1200 (69)	ν_{as} (CC_s)
307 (36)	δ (CCC)	1251 (89)	ν (C-O)
326 (41)	δ_{as} (CC_3)	1264 (88)	ν_{as} (CC_3)
336 (sh)		1276 (92)	ν (C-OH) + β (O-H)
374 (sh)	δ (CCN)		
392 (73)	δ (ring)	1285 (sh)	τ (CH_2)
417 (33)	δ_s (CC_2)	1308 (82)	δ (C-H)
443 (31)	ρ (CC_3)	1324 (74)	ω (CH_2)
461 (22)		1338 (70)	
519 (42)	ρ (COOH)	1344 (70)	δ (C-H)
567 (67)		1358 (77)	
584 (61b)	γ (C=O) / β (C=O)	1366 (81)	δ_s (CH_3)
602 (61)		1376 (74)	
		1388 (61)	
648 (sh)	δ (COOH)	1436 (86)	δ_{as} (CH_3)
674 (sh)	β (C=O)	1440 (sh)	
721 (24)	δ (ring)	1468 (87)	δ (CH_2)
741 (35)	ν_s (CC_3)	1385 (sh)	
757 (s-sh)	δ (ring)	1534 (95)	δ (NH)
767 (29)	ω (COOH)	1650 (100)	ν (C=O)
785 (33)	δ (ring)	1696 (100)	
824 (26)	ρ (CH_2)	1942 (60b)	584 + 1358 ;
842 (49)	γ (O-H)		

Table I. (Contd.)

Obs. frequencies*	Proposed assignments†	Obs. frequencies*	Proposed assignments†
	248 + 1696	2931 (52)	} $\nu_{as}(\text{CH}_3)$
2280 (52)	584 + 1696	2952 (68)	
2365 (53)	1696 + 674	2976 (78)	
2430 (51)	1650 + 785	2996 (sh)	} $\nu(\text{C-H})/\nu(\text{N-H})$
2862 (sh)	$\nu_s(\text{CH}_2)$	3040 (s-sh)	
2883 (56)	$\nu_s(\text{CH}_3)$	3354 (91)	} $\nu(\text{O-H})$
2926 (53)	$\nu_{ns}(\text{CH}_2)$	3405 (s-sh)	

*The number within bracket following each frequency represents the corresponding relative peak height in a scale of 100; sh - shoulder; s-sh - strong-shoulder; b - broad. Due to lack of calibration for relative peak heights far-infrared frequencies are labelled as s - strong; ms - medium-strong; vs - very-strong; m - medium; w - weak; mw - medium-weak.

† ν - stretching; δ - deformation; ρ - rocking; τ - torsion; β - in-plane deformation (relative to local plane) and γ - out-of plane deformation (relative to local plane).

at 2862, 2883, 2926, 2931, 2952, 2976 and 2996 cm^{-1} . The frequency 2883 cm^{-1} could be assigned to the $\nu_s(\text{CH}_3)$ mode(s). Likewise, the frequencies 2931, 2952, 2976 and 2996 cm^{-1} may be correlated with the $\nu_{as}(\text{CH}_3)$ modes. The frequency 2862 cm^{-1} of N-t-BOC-Ala-Pro could be associated to the $\nu_s(\text{CH}_2)$ modes and the $\nu_{ns}(\text{CH}_2)$ modes could be associated to the frequency 2926 cm^{-1} . The only frequency corresponding to the $\nu(\text{CH})$ mode for N-t-BOC-Ala-Pro is observed as a shoulder at 3040 cm^{-1} . These assignments are in agreement with the work of Pitzer and Kilpatrick (1946), Evans and Bernstein (1956), Korppi-Tommola (1978), Susi and Byler (1980) and Colthup et al (1964).

For N-t-BOC-Ala-Pro two strong infrared bands are observed at 1436 and 1468 cm^{-1} and could be assigned to the $\delta_{as}(\text{CH}_3)$ modes of the t-BOC and alanyl units respectively. In the region 1300-1400 cm^{-1} the infrared spectrum of N-t-BOC-Ala-Pro shows 8 bands at 1308, 1324, 1338, 1344, 1358, 1366, 1376 and 1388 cm^{-1} all with fairly good intensities. In comparison with the assignments for alanine (Susi and Byler 1980), the frequencies 1308 and 1358 cm^{-1} of N-t-BOC-Ala-Pro may be assigned respectively to the CH deformation and $\delta_s(\text{CH}_3)$ modes of the alanyl unit. Evans and Bernstein (1956) have assigned the $\delta_s(\text{CH}_3)$ modes all at 1373 cm^{-1} for isobutane in conformity with which the bands 1366, 1376 and 1388 cm^{-1} of N-t-BOC-Ala-Pro could be assigned to the $\delta_s(\text{CH}_3)$ modes of the t-BOC unit. Krishnan and Sankaranarayanan (1973) have assigned the proline CH deformation mode at 1349 and 1354/1340 cm^{-1} for proline and hydroxyproline which could be correlated with the 1344 cm^{-1} band of the N-t-BOC-Ala-Pro molecule.

The $\delta(\text{CH}_2)$ modes of the proline ring are expected to appear in the region of the $\delta_{\text{as}}(\text{CH}_3)$ mode and therefore, the bands assigned to the $\delta_{\text{as}}(\text{CH}_3)$ modes may also be correlated with the $\delta(\text{CH}_2)$ modes of the proline unit. The wagging mode of the CH_2 groups appear in the region $1300\text{-}1340\text{ cm}^{-1}$. The frequencies 1324 and 1328 cm^{-1} of N-t-BOC-Ala-Pro could be assigned to the $\omega(\text{CH}_2)$ modes.

The $\rho_{\parallel}(\text{CH}_3)$ and $\rho_{\perp}(\text{CH}_3)$ modes interact strongly with the skeletal modes of the skeleton to which the CH_3 group is attached and appear in the region $800\text{-}1100\text{ cm}^{-1}$ whereas the modes $\tau(\text{CH}_2)$ and $\omega(\text{CH}_2)$ appear near 1300 and 900 cm^{-1} . Tentative assignments for some of these modes are given in Table 1. The frequencies 211 , 218 , 227 and 233 cm^{-1} are assigned to the $\tau(\text{CH}_2)$ for N-t-BOC-Ala-Pro in accordance with the earlier work (Susi and Byler 1980).

The $\nu(\text{OH})$ mode appears in the region near 3400 cm^{-1} characteristically separated from the regions of the other vibrational modes. The infrared peak at 3354 cm^{-1} may be assigned to the $\nu(\text{OH})$ mode. The large bandwidth and asymmetry of the band could be due to inter-/intra-molecular association.

The NH stretching modes in alanine are observed in the region $3000\text{-}3100\text{ cm}^{-1}$. In the present case the shoulder at 3040 cm^{-1} and assigned to the $\nu(\text{CH})$ mode could also be assigned to the only N-H stretching mode in the N-t-BOC-Ala-Pro molecule. In amino acids with unionized carbonyl group(s) the C=O stretching mode and the O-H in-plane (with respect to the carbonyl group) bending mode couple strongly to give rise to two frequencies in the range $1100\text{-}1400\text{ cm}^{-1}$. For N-t-BOC-Ala-Pro the carbonyl group is unionized and so one may expect to observe two frequencies corresponding to the mixed $\nu(\text{C}-\text{OH})$ and $\beta(\text{O}-\text{H})$ modes. In view of the hydrogen bonding, however, the resulting bands are expected to appear with relatively larger bandwidths. The infrared spectrum of N-t-BOC-Ala-Pro contains two bands, namely, 1171 and 1276 cm^{-1} with appropriate bandwidths and are observed to have strong intensities. These bands might be assigned to the coupled modes $\nu(\text{C}-\text{OH})$ and $\beta(\text{O}-\text{H})$. The N-H bending mode, observed as $\text{NH}_2^+/\text{NH}_3^+$ modes in proline/alanine, is a pure N-H bending mode for N-t-BOC-Ala-Pro and is assigned at 1534 cm^{-1} .

The OH non-planar (with respect to the local plane) bending mode is the OH torsional mode and absorbs very broadly and diffusely in the region $450\text{-}700\text{ cm}^{-1}$. The infrared spectrum of N-t-BOC-Ala-Pro actually contains a very broad band in the above region superimposed with three peaks and a shoulder. However, this broad band appears to have strong intensity and therefore, the assignments of these bands are deferred till the discussion of the C=O group modes.

One might expect to observe three frequencies corresponding to the $\nu(\text{C}=\text{O})$ modes of three carbonyl groups. However, the infrared spectrum of N-t-BOC-Ala-Pro shows only two bands in the $\nu(\text{C}=\text{O})$ region, namely, 1650 and 1696 cm^{-1} . This could be explained as follows. The carbon atom of the carbonyl group of the alanyl unit is attached with a carbon and a nitrogen atoms and that of the

proline unit with a carbon and an oxygen atoms. Since the oxygen and nitrogen atoms are both electron withdrawing groups of slightly different electronegativity, the magnitudes of the carbonyl stretching modes of the alanyl and proline units are expected to have nearly equal magnitudes. However, in case of the the t-BOC unit the carbon atom of the carbonyl group is attached with an oxygen and a nitrogen atoms the both of which are electron withdrawing groups. As a consequence the carbon atom of the carbonyl group of the t-BOC unit would be more positive making the C=O bond shorter and thereby stronger as compared to those of the alanyl and/or proline units. Therefore, the $\nu(\text{C}=\text{O})$ mode for the t-BOC unit would be expected to have a little bit higher magnitude. Thus, the band 1650 cm^{-1} could be assigned to the $\nu(\text{C}=\text{O})$ modes of the alanyl and proline units and the band 1696 cm^{-1} to the $\nu(\text{C}=\text{O})$ mode of the t-BOC unit.

For N-t-BOC-Ala-Pro a broad band centred around 584 cm^{-1} is observed to be superimposed with three peaks at 519 , 567 and 602 cm^{-1} and is followed by shoulders at 648 and 674 cm^{-1} . The band centred around 584 cm^{-1} along with the two frequencies 567 and 602 cm^{-1} may be assigned to the C=O deformation modes. However, it is difficult to distinguish between the planar and non-planar C=O deformation frequencies in the present case.

If the CH_3 groups are replaced by atoms X, the $\text{C}-(\text{CH}_3)_3$ moiety of the t-BOC unit is equivalent to a CX_3 group whose vibrations are in fact the nine CC_3 group normal modes. The assignments of the ν_s , ν_{as} , δ_s and δ_{as} modes of the CC_3 group may be made by comparison with the assignments of these modes of isobutane $(\text{CH}_3)_3\text{CH}$ (Pitzer and Kilpatrick 1946, Evans and Bernstein 1956). However, as the isobutane molecule does not have the modes ρ_1 , ρ_2 and τ , the assignments for these modes can be made by comparing the spectra reported for t-butyl alcohol reported by Korppi-Tommola (1978). The frequency 741 cm^{-1} is assigned to the $\nu_s(\text{CC}_3)$ mode and the frequencies 1200 and 1264 cm^{-1} are assigned to the two components of the $\nu_{as}(\text{CC}_3)$ mode. Contrary to the CC_3 stretching modes the $\delta_s(\text{CC}_3)$ mode is observed by Pitzer and Kilpatrick (1946) and Korppi-Tommola (1978) to have higher magnitude ($\sim 425\text{ cm}^{-1}$) than the $\delta_{as}(\text{CC}_3)$ mode ($\sim 370\text{ cm}^{-1}$). For N-t-BOC-Ala-Pro the frequencies 326 and 336 cm^{-1} are assigned to the $\delta_{as}(\text{CC}_3)$ modes and the frequency 417 cm^{-1} is assigned to the $\delta_s(\text{CC}_3)$ mode. The other three CC_3 modes are tentatively assigned and assignments are given in Table 1.

The frequencies 767 , 648 , 519 and 200 cm^{-1} may be correlated with the ω , δ , ρ and τ modes of the COOH group of N-t-BOC-Ala-Pro, respectively. The O...H bonding results in a weak and broad absorption in the infrared in the neighbourhood of 100 cm^{-1} due to the $\nu(\text{O}\cdots\text{H})$ mode. The frequency at

120 cm^{-1} in the far-infrared spectrum of N-t-BOC-Ala-Pro with such characteristics is assigned to the $\nu(\text{O}\cdots\text{H})$ mode.

References

- Colthup N B, Daly L H and Wiberley S E 1964 *Introduction to Infrared and Raman spectroscopy* (New York: Academic Press)
- Evans J C and Bernstein H J 1956 *Can. J. Phys.* **34** 1037
- Herlinger A W and Long T V 1970 *J. Am. Chem. Soc.* **92** 6481
- Korppi-Tommola J 1978 *Spectrochim. Acta* **34A** 1077
- Krishnan R S and Sankaranarayanan V N 1973 *J. Ind. Inst. Sc.* **52** 66
- Machida K, Kagayama A, Saito Y and Uno T 1978 *Spectrochim. Acta* **34A** 909
- Pitzer K S and Kilpatrick J E 1946 *Chem. Rev.* **39** 435
- Susi H and Byler D M 1980 *J. Mol. Struct.* **63** 1