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Resonance Raman spectroscopic studies of 2-(4'-hydroxyphenylazo)-benzoic acid and some substituted analogs—I. pH effect on spectra

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Abstract—The resonance Raman spectrum of 2-(4'-hydroxyphenylazo)-benzoic acid (HABA) is pH dependent; the spectral changes are related to the formation of protonated and deprotonated ionic species of the dye molecule. In addition a tautomeric equilibrium process between azo and hydrazone forms of HABA in neutral aqueous solution has been demonstrated using different excitating laser lines. In alkaline, neutral and weakly acid media HABA exists mainly in the azo form.

Resonance Raman spectra between 1000 and 1700 cm^{-1} of six HABA analogs have been recorded at alkaline, neutral and acid pH. A comparison of these spectra allow us to assign most of the important bands, and to establish some intramolecular coupling effects.

1. INTRODUCTION

The resonance Raman spectrum of 2-(4'-hydroxyphenylazo)-benzoic acid (HABA) in aqueous solution depends critically on the pH: the observed pH-dependent spectral changes are presumably a reflection of protonation and deprotonation of the phenolic and carboxylic functions. In addition, tautomeric equilibrium between azo and hydrazone forms of HABA must be considered. Resonance Raman spectra of HABA and assignments for various bands have been made recently on this basis [1], but must be regarded as incomplete.

For the interpretation of our results [2] concerning changes in the resonance Raman spectrum of HABA when bound to avidin and bovine serum albumin, a more complete interpretation of the observed bands was needed. Accordingly, we have studied the resonance Raman spectra of HABA and some deuterated and substituted analogs at neutral, alkaline and acid pH. A comparison of these results allow us to identify the characteristic bands for each benzenoid ring, to detect the presence of hydrazone and azo forms, and to demonstrate intramolecular coupling effects in the HABA molecule.

The structures of the HABA analogs studied and the abbreviations used are tabulated in Fig. 1.

2. METHODS

Preparation of compounds

All the HABA analogs were prepared by coupling the appropriate diazotized anthranilic acid with the corresponding phenol at pH 9.5–10. The products were recovered by acidification, and recrystallized several times from aqueous methanol.

All compounds were homogeneous by TLC (Silica-gel G, chloroform, methanol–formic acid, 85:12:3, as solvent), and gave satisfactory analyses for C, H and N.

HABA(d_4), containing four atoms of deuterium in the phenolic ring, was prepared by coupling diazotized anthranilic acid with phenol-2, 3, 4, 5, 6-d_5 (prepared from aniline-2, 3, 4, 5, 6-d_5, obtained from Aldrich Chemicals). A low resolution mass-spectrum showed *m*-equiv 246, with fragments 125, 97 and 69. HABA showed *m*-equiv 242, with fragments 121, 93 and 65.

Dye solutions in 0.1 M phosphate buffer were adjusted to the required pH with either 0.1 M HCl or 0.1 M NaOH, using a combined pH electrode.

For the measurements in heavy water, stock solutions of analogs were prepared in a dry-box; these solutions were diluted with heavy water or 0.1 N DCl (prepared from 20% DCl solution) to the desired concentration before several measurements.

Spectral measurements

Resonance Raman spectra were recorded between 50 and 3500 cm^{-1} using a Coderg T.800 spectrometer. The main spectral features are observed from 1000 to 1700 cm^{-1}.

Laser lines at 457.9, 488.0 and 514.5 nm were obtained with an Argon Laser (Spectra Physics 164 AC); a Krypton Laser (Spectra Physics 164) was used for the 647.1 nm line. Samples were contained in 5 mm Pyrex tubes.

In all cases reproducibility of all spectral features was observed as a function of time. A continuous flow system [3] enabled convenient study of spectral changes as a function of pH without removing the sample cell from the laser beam.

Visible spectra between 200 and 600 nm were obtained with a Pye-Unicam SP 1800 instrument using 1 cm cells.

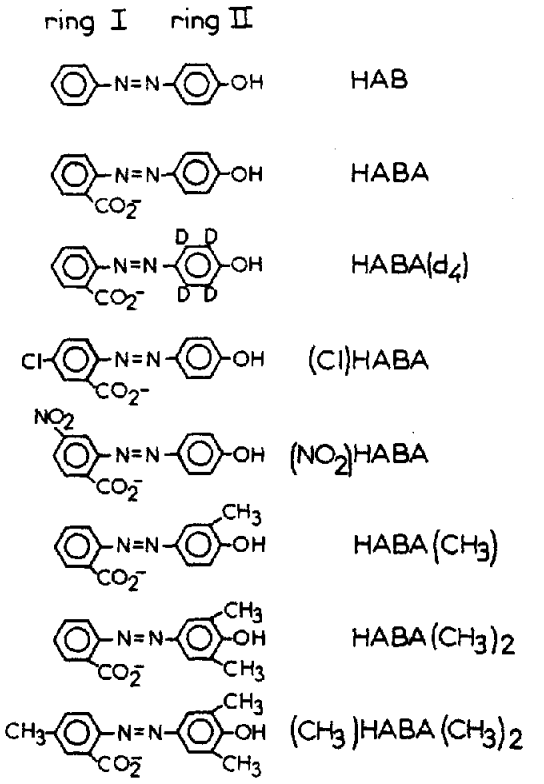

Fig. 1. Structure and abbreviations used for HABA analogs.

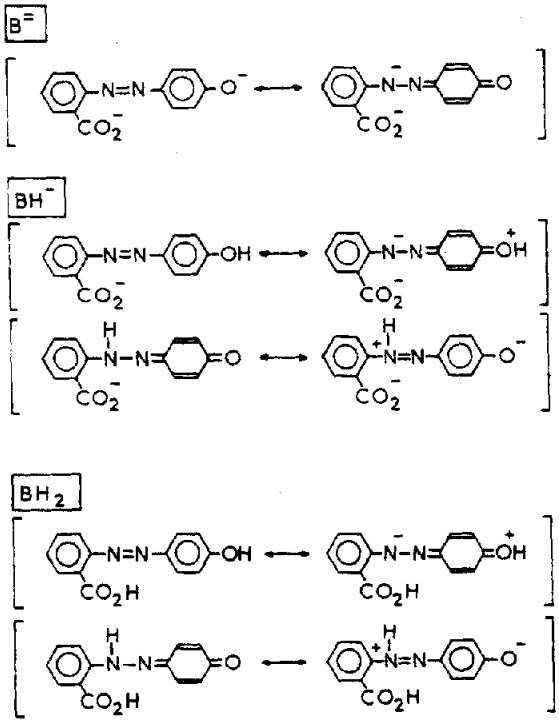

Fig. 3. Resonance hybridization for ionic species obtained by protonation and deprotonation of the HABA molecule.

3. CHANGES IN RESONANCE RAMAN SPECTRUM OF HABA AS A FUNCTION OF pH

The visible absorption spectrum of HABA at various pH values is shown in Fig. 2.

Resonance Raman spectra of HABA at several pH values obtained using the 488 nm line are shown in Fig. 4. In the pH range under study (2.5–13), two ionization equilibria are possible [4] involving the carboxylic ($pK \sim 3$), and phenolic ($pK = 8.2$) groups. Three different HABA species (BH_2, BH^- and $B^=$) are obtained from the following equilibria:

$$BH_2 \rightleftarrows BH^- + H^+$$

$$BH^- \rightleftarrows B^= + H^+.$$

The possible tautomerism and resonance hybridization for HABA and its ionic species are shown in Fig. 3.

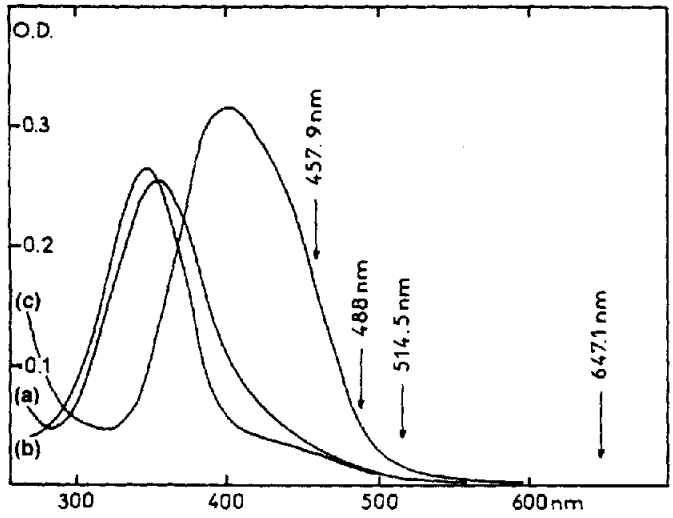

Fig. 2. Absorption spectra of HABA in aqueous solution ($2.4\ 10^{-5}$M) under acid-base conditions: (a) phosphate buffer pH 7.0; (b) 0.1 N, HCl; (c)0.1 N, NaOH.

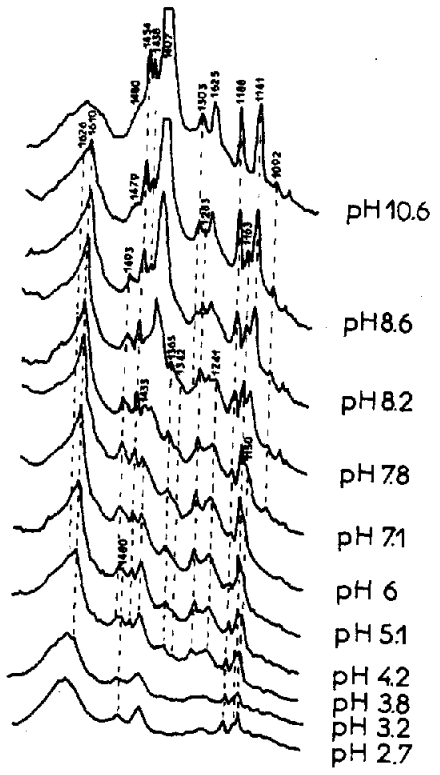

Fig. 4. Changes of resonance Raman spectra of HABA as a function of pH; 10^{-4} M in water; slitwidth 5 cm^{-1}; 488 nm.

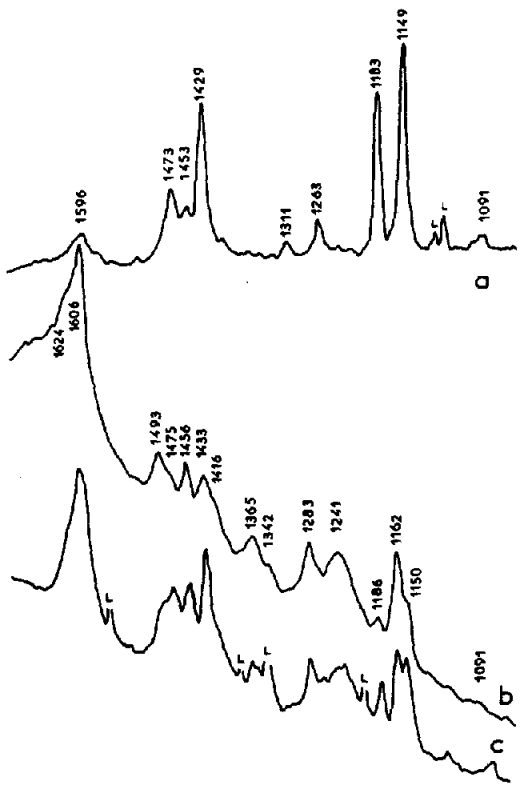

Fig. 5. Raman and resonance spectra of HABA in phosphate buffer solution (pH 6.0): (a) 5.10^{-3} M—slitwidth 8 cm^{-1}, 647.1 nm; (b) 5.10^{-5} M—slitwidth 5 cm^{-1}, 514.5 nm; (c) 5.10^{-5} M—slitwidth 5 cm^{-1}, 457.9 nm, and L = non Raman lines from the laser plasma.

Above pH 8.2, B^- is the predominant ionic species. The strong azo character of B^- is shown by the intense band at 1410 cm^{-1}, assigned to the azo group itself [1, 5–11].

At neutral pH values, BH^- will predominate, but the possibility now exists of an azo-hydrazone equilibrium. The strong band observed near 1600 cm^{-1} could be taken to indicate that BH^- is *not* predominantly in the azo form. However, the visible absorption spectrum of HABA in neutral medium shows one major band, centred at 348 nm, assignable to the azo form of HABA [12, 13].

Resonance Raman spectra obtained with several laser lines (Fig. 5) resolved this paradox. Non-enhanced Raman spectra obtained with the 647.1 nm line (Fig. 5(a)) show a strong 1410 cm^{-1} band characteristic of the azo form. On the other hand, resonance Raman spectra obtained with the 514.5 nm lines (Fig. 5(b)) showed (as for 488 nm excitation) weak bands around 1400 cm^{-1}, and a strong line in the 1600 cm^{-1} region.

We suggest that the spectra obtained with the 488 nm and 514.5 nm lines are representative of the hydrazone form of HABA, although the presence of this form is not indicated in the visible spectra. Presumably its concentration is too low to observe, but its absorption maximum must be near 500 nm. The non-enhanced Raman spectrum, obtained with the 614.7 nm line, show the predominance of the azo form of HABA in neutral aqueous solution, and thus confirms the assignment of the 348 nm band to the azo form of HABA.

By using the 457.9 nm laser line (Fig. 5(c)) which is closer to the 348 nm absorption band, the Raman spectrum contains both hydrazone and azo form lines.

At more acidic pH (pH < 3.5), the species BH_2 will predominate, but can also be represented by two tautomeric forms. Both the visible and the Raman spectra indicate the predominance of the azo form.

4. RESONANCE RAMAN SPECTRA OF HABA ANALOGS

Basic forms

The visible absorption spectra of all HABA analogs show an absorption band which is centred at about 400 nm in 0.1 N NaOH. Using the 488.0 nm exciting line, a strong resonance Raman band at around 1400 cm^{-1} is observed for all the compounds (Fig. 6). This strong band can be assigned with confidence to the –N=N– stretching mode, and shows that at pH 13, all HABA analogs studied are present in the azo form.

In addition to the N=N stretching mode, we can expect to observe in the 1000–1700 cm^{-1} region characteristic modes of the benzenoid rings: C–C stretching vibrations (called 8*a*, 8*b*, 19*a*, 19*b* ac-

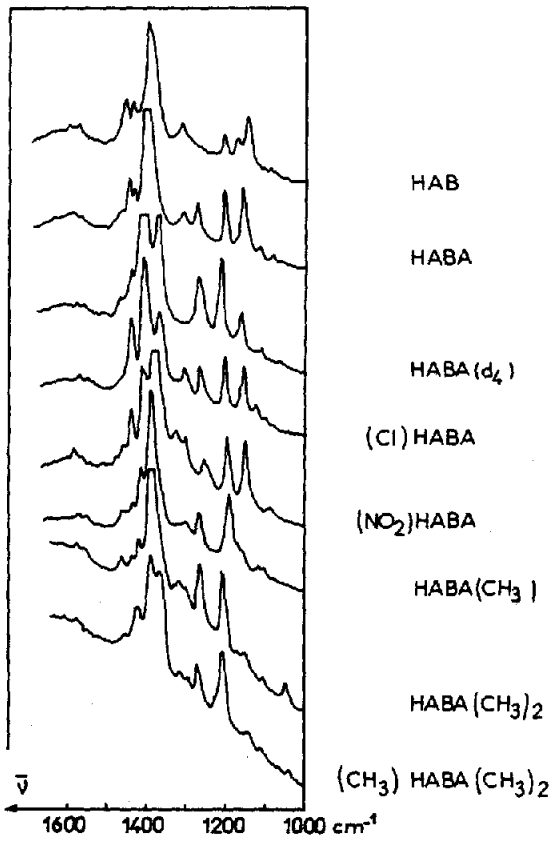

Fig. 6. Resonance Raman spectra of HABA and its analogs at alkaline pH ($\sim 10^{-4}$ M in 0.1 N NaOH solution) slitwidth 4 cm^{-1}; 488 nm.

cording to WILSON [14]), and C–H in plane bending vibrations (called 3, 9*a*, 9*b*, 18*a*, 18*b*). Stretching modes of the ring-substituents coupled with benzenoid ring modes 1 or 12 and 6*a* give rise to *e* type modes [15].

Most substituents do not take part directly in electronic delocalization and their internal modes will not be strongly resonance Raman active [5, 8, 10]. The following assignments were made by comparison with spectral data of azobenzene derivatives [5–11] and substituted benzenes [16–19]. The band frequencies and tentative assignments for each compound are tabulated in Table 1.

The bands present in the 1550–1610 cm^{-1} region are assigned to the 8(*a*, *b*) modes of the benzenoid rings. As observed for all azo compounds, these bands are very weak.

The weak bands, appearing near 1480 cm^{-1}, are attributed to ring I (substituted with a carboxylate group in the *ortho* position).

Bands between 1430 and 1460 cm^{-1}, insensitive to deuteration of ring II of both HAB [6] and HABA, are assigned to ring I modes. The participation of ring II modes in this region can be expected; some spectral shifts and intensities changes occur when ring II is substituted.

When ring I is substituted one strong band appears in the 1370 cm^{-1} region. On the one hand we can assign this band to a 9*a* mode of ring I: heavy substituents can decrease the frequency of this mode [18]; on the other hand the CH_3 group increases the frequency of skeletal ring vibrations [16]; in the 1379 cm^{-1} band, observed in

Table 1. Line positions (cm^{-1}) and tentative assignments of Raman bands of HABA and its analogs in alkaline solution

HAB	HABA	HABA(d_4)	(Cl)HABA	(NO_2)HABA	HABA(CH_3)	HABA(CH_3)$_2$	(CH_3)HABA(CH_3)$_2$	TENTATIVE ASSIGNMENT
	1605(w)	1599(w)	1591(w)	1607(w)	1599(w)	1598(w)	1600(w)	} 8 a,b modes of benzene rings
1587(v.w)	1581(w)	1581(w)	1570(w)		1576(w)	1580(w)		
				1495(w)				
	1480(v.w)	1475(v.w)		1474(v.w)	1474(v.w)	1480(v.w)	1477(w)	ring I } 19 a,b modes
1459(m)	1454(m)	1449(w)	1447(S)	1447(m)	1450(v.w)	1452(w)		ring I,II } 19 a,b modes
1438(w)	1438(w)	1430(sh)			1427(m)	1433(m)	1436(m)	ring I } 19 a,b modes
1400(v.S)	1407(v.S)	1410(v.S)	1410(v.S)	1416(S)	1397(v.S)	1394(v.S)	1401(v.S)	N=N stretching
			1373(S)				1379(S)	ring I } 19 a,b modes
				1383(v.S)				
		1373(S)						ring II } 19 a,b modes
				1327(m)	1326(v.w)	1327(w)	1323(w)	
1310(w)	1303(m)		1303(m)	1302(m)	1303(w)	1308(w)	1304(w)	ring II(3)+e(Ph-O$^-$)
	1265(m)	1260(S)	1258(m)	1252(m)	1265(m)	1262(m)	1265(m)	e (Ph-CO_2^-)?
				1242(sh)			1223(sh)	
1189(m)	1189(S)	1201(S)	1190(S)	1188(S)	1184(S)	1202(S)	1204(S)	e (Ph-N=)9a(ringI)
1160(w)		1160(v.w)	1151(v.w)		1162(v.w)	1165(v.w)		9 b
1138(S)	1141(S)	1145(m)	1141(S)	1140(S)	1147(w)	1145(w)	1134(w)	e(Ph-N=)9a(ringII)
	1092(w)	1091(w)	1111(w)		1106(w)			9 b
			1083(w)	1072(w)	1092(w)	1095(w)	1095(v.w)	18 a,b
		1037(v.w)	1059(v.w)			1034(w)	1034(w)	

sh = shoulder, v.w. = very weak, w = weak, m = medium, s = strong, v.s. = very strong.
* Both benzene rings could contribute to 19(*a*, *b*) feature.

$(CH_3)HABA(CH_3)_2$ we could therefore expect some participation of a δ, CH_3 mode.

The shift in frequency toward the low frequency range of the –N=N– stretching mode in comparison with azobenzene, is indicative of a reduction in bond order for the azo group.

The 1303 cm^{-1} band in the HABA spectrum, disappears upon deuteration of ring II; it is presumably a 3 mode of ring II, shifting to 1010 cm^{-1} in the HABA (d_4) spectrum. The 1265 cm^{-1} band of the HABA spectrum observed in all analogs except for HAB, is insensitive to deuteration of ring II and substitution of both ring I and ring II; this band is assigned to the carboxylate *e* mode. The increase of the intensity of the 1260 cm^{-1} line and its broadening upon deuteration of ring II may indicate that another band is present: possibly the characteristic line of the phenolate *e* mode is overlapped by the 1303 cm^{-1} band, and shifts to 1260 cm^{-1} in the HABA (d_4) spectrum.

The 1190 cm^{-1} band in the HABA spectrum, seen in all analogs, has been observed in azobenzene [7] and HAB. This band shifts markedly on deuteration of the azobenzene rings and on deuterating the phenolic ring of HAB [6]. This deuteration studies are consistent with an assignment of the 1190 cm^{-1} band to the 9(*a*, *b*) modes of ring I.

Raman bands have also been observed in various azonaphtalenes and in azobisisobutyronitrile (at 1190 cm^{-1} and 1179 cm^{-1} respectively) [9], and some authors [9, 11] assign them to a C–N bending mode. This assignment cannot be considered as correct, because C–N bending vibrations are expected in the low frequency range (600–1000 cm^{-1}) [20]. One of the C–N modes may however be present in this spectral region, and the appearance of the 1190 cm^{-1} feature in all the HABA analogs and several other disubstituted azo compounds suggests the following assignment: A 9*a* mode of ring I, which appears in this spectral region for an *ortho*-disubstituted benzene ring, coupled with *e* mode characteristic of the ring I–N bond.

The 1140 cm^{-1} band, seen in numerous azo compounds, is attributed to a *e*(C–N) mode, according to previous work [1, 7, 11]. Deuteration of ring II of HABA does not affect the frequency, but substitution in ring II affects it considerably. The 1140 cm^{-1} band is probably a (ring II–N) *e* mode coupled to the 9*a* mode of the ring II, which appears near 1150 cm^{-1} for *para* disubstituted benzene rings [17, 18].

Neutral forms

The visible spectra indicate that the azo form predominates for HABA (348 nm), (Cl)HABA (352 nm), and $(NO_2)HABA$ (353 nm), while an approximately 50:50 mixture of azo and hydrazone forms is present in solution for $(CH_3)HABA(CH_3)_2$ (350–478 nm) and $HABA(CH_3)_2$ (357–490 nm). The resonance Raman spectra obtained and deuteration effect are shown in Fig. 7. Results are shown in Table 2.

Hydrazone forms

In addition to ring I modes, characteristic bands of ring II in its quinoid form, and N–N, C–N stretching modes assignable to the hydrazone function are apparent.

In the 1580–1630 cm^{-1} region, strong bands appear assignable to quinoid ring modes. In HABA, there is a mixing of the C=O, C=C and C=N stretches which gives rise to the 1606 cm^{-1} line. When ring II is substituted by CH_3 group, a decrease of C=C stretching frequency gives rise to a line near 1590 cm^{-1}; the C=O and C=N stretching vibration appears in the 1620–1630 cm^{-1} range.

The 1493 cm^{-1} band in HABA is not sensitive to deuteration or substitution in ring II, but shifts to 1475 cm^{-1} in (Cl)HABA or $(NO_2)HABA$. The 1456 cm^{-1} band behaves in the same way. Both 1493 cm^{-1} and 1456 cm^{-1} bands are therefore attributed to a ring I mode. The 1433 cm^{-1} band in the HABA spectrum disappears in D_2O (Fig. 7) or

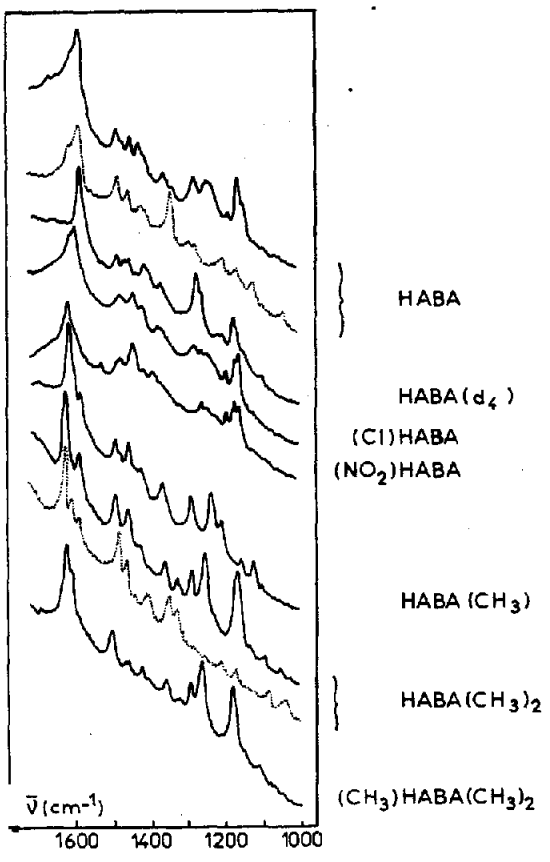

Fig. 7. Resonance Raman spectra of HABA and its analogs at neutral pH ($\sim 10^{-4}$ M in phosphate buffer pH 6.0); slitwidth 6 cm^{-1}; 488 nm) ····· in heavy water pH 6.0.

Table 2. Line positions (cm^{-1}) and tentative assignments of Raman bands of HABA and its analogs in neutral solution

HABA	HABA(d_4)	(Cl)HABA	(NO_2)HABA	HABA(CH_3)	HABA(CH_3)$_2$	(CH_3)HABA(CH_3)$_2$	TENTATIVE ASSIGNMENT	
							Hydrazone	Azo
1624(sh)		1622(sh)	1620(S)	1620(S)	1630(S)	1629(S)	ν(C=N)+ν(C=O)	
1606(S)	1593(S)	1604(S)				1617(sh)	ν(C=C)	
		1593(sh)		1586(w)	1585(w)	1599(sh)		
			1531(w)					$\nu_s(NO_2)$?
1493(m)	1488(m)			1488(m)	1489(m)	1502(m)	ringI 19(a,b)	
		1476(m)	1475(w)					
1475(w)*	1472(w)*							
1456(m)*								ringI 19(a,b)
1456(m)	1455(m)	1442(m)	1442(m)	1451(m)	1458(m)	1455(w)	rings I+II	
1433(m)	1411(m)			1417(w)	1425(w)	1424(w)		
1416(v.w)*		1412(w)*	1414(v.w)*					
			1384(w)					$\nu(NO_2)$
1365(w)	1372(w)	1371(w)		1354(m)	1356(m)	1353(m)	δ (N-H) ?	
1342(v.w)		1351(w)						
					1322(w)	1319(w)	ring II	
	1298(v.w)							
1283(m)	1269(S)	1272(w)		1280(m)	1281(m)	1283(m)	(Ph-NH)stretching	
1262(v.w)*		1251(v.w)*	1252(w)*					ring I
	1269(S)*							ring II
	1263(v.w)							
1241(m)		1234(w)	1235(v.w)	1230(m)	1247(m)	1253(m)	ring II, (Ph-NH-N)	
	1212(w)							
1186(v.w)*	1198(w)*	1183(w)*	1181(w)*					ringI (9a), e (Ph-N)
1162(m)	1170(m)	1159(m)	1160(m)		1156(m)	1160(m)	(N-N)stretching	
1150(v.w)*	1145(v.w)*	1140(m)	1145(m)	1142(w)				ringII (9a), e (Ph-N)
		1108(w)	1108(w)	1110(w)				
1091(v.w)*	1092(v.w)*							ring I, 18(a,b)
				1086(v.w)	1086(v.w)	1090(v.w)	ring II 18(a,b)	

* The Raman lines due to the azo form.

by substitution of ring I. We assign this band to a ring I mode, possibly sensitive to an intramolecular interaction between the ionized carboxylate group of HABA and the N–H proton of the hydrazone function (see below). The intensity and frequency of this band are also sensitive to the substitution of ring II.

The 1365 cm^{-1} band in HABA is insensitive to deuteration or substitution in ring II, and is assigned to a ring I mode. Its disappearance in spectra obtained with D_2O solution suggests an influence of the intramolecular H bond on the ring I mode as for the 1433 cm^{-1} band.

The 1283 cm^{-1} band in HABA is also seen in all the analogs; it is sensitive to deuteration of ring II and disappears in spectra obtained with D_2O solution. We assign this band to (ring I–N)*e* mode. As for secondary amines, this mode is extremely sensitive to deuteration on nitrogen. The band shifts to 1337 cm^{-1} in HABA, and to 1340 cm^{-1} in HABA(CH_3)$_2$. This behaviour implies a strong coupling between the C–N and N–H vibrations [18, 20].

The 1241 cm^{-1} band in HABA is sensitive to deuteration of ring II; it is observed in this region for all analogs, and disappears when either HABA or HABA(CH_3)$_2$ are examined in D_2O solution. In comparison with *p*-benzoquinone analogs [21] we assign this band to an in plane CH deformation mode of ring II. As for *p*-benzoquinone 1,4-imines, strong participation of the hydrazone (–NH–N=) function is expected.

The 1162 cm^{-1} band is relatively insensitive to substitution or deuteration of ring II. It is assigned to N–N stretching vibration of the hydrazone group. Deuteration of the hydrazone group has only a slight effect on the intensity of this mode.

The Raman spectra of HABA and HABA(CH_3)$_2$ obtained in D_2O solution shows that the 1100–1300 cm^{-1} spectral range, where Ph–NH–N= vibrational modes appear, is very sensitive to the deuteration on nitrogen. A strong coupling effect between N–H bending and Ph–NH–N= group vibrations can explain these spectral changes. The most significant effect is observed for the C–N vibrational mode.

Another explanation depends on the possible presence of an intramolecular hydrogen bond between the ionised carboxylate group and the NH group of the neighbouring hydrazone function. Such an interaction would be admittedly weak in aqueous solution [13], but might afford an important stabilizing influence for the hydrazone forms. Perturbation of the strength of this hydrogen bond as a consequence of deuteration could then account for the above result.

Azo forms

The Raman spectrum of HABA obtained by using the 647.1 nm line is similar to that obtained

for the basic form of HABA using the 488 nm line.

The spectrum of $HABA(CH_3)_2$, obtained with the red exciting line, does not result in the appearance of strong characteristic bands of the azo form as for HABA. However four new lines characteristic of azo form are observed at 1472, 1456, 1191 and 1130 cm^{-1}, respectively assigned to ring mode, N=N stretching vibration, and 9*a* modes coupled with *e*(C–N) modes.

Assignments for characteristic lines of neutral azo forms are tabulated in Table 2.

Acid forms

A band around 345 nm is observed in the absorption spectra of HABA analogs, in acidic solution (pH < 3).

The Raman lines were similar to those seen in the spectra of HABA in its neutral and alkaline forms (Fig. 4). The 1480 cm^{-1} line is assigned to ring vibration modes; the 1433 cm^{-1} line is characteristic of –N=N– stretching vibration; the 1190 and 1150 cm^{-1} lines are assigned, like the other azo forms, to 9*a* modes coupled with *e*(C–N) modes. The *e* mode of the carboxylate function, observed at 1265 cm^{-1} for alkaline and neutral azo forms, is shifted to 1279 cm^{-1} upon protonation. The disappearance of the 1279 cm^{-1} Raman line when 0.1 N DCl solution is used confirms the assignment to the *e*(CO_2H) mode.

5. CONCLUSION

These studies show that HABA and its substituted analogs exist in both azo and hydrazone forms in solution.

In agreement with absorption spectroscopy, resonance Raman spectroscopy indicates that azo forms of HABA predominate in basic, neutral and slight acid medium. In neutral aqueous solution, a tautomeric equilibrium can be demonstrated by using different exciting laser lines. Due to a more significant resonance enhancement effect for the hydrazone form, it gives rise to an intense resonance Raman spectrum. The position of the tautomeric equilibrium strongly depends on the ring substitution pattern; it is shifted to the azo form when the carboxylic ring is substituted by NO_2 or Cl; substitution of the phenolic ring by methyl groups favours the hydrazone form.

Resonance Raman spectra obtained for BH^- species in the hydrazone form mainly shows benzenoid ring modes (ring I); the quinoid ring (ring II) is characterized by C=C stretching modes which are probably coupled with C=O and C=N stretching vibrations.

Studies in heavy water solution demonstrate the strong influence of the N–H bond on the resonance Raman spectral features. The modifications observed in the Raman spectra of both HABA and $HABA(CH_3)_2$ when nitrogen is deuterated can be explained either by a vibrational coupling effect between N–H bending and (C–NH–N) group vibrations, or by a change in the strength of the intramolecular hydrogen bond formed between the protonated nitrogen of the hydrazone function and the carboxylate group. This latter effect would perturb mainly the carboxylic ring modes. The characteristic vibrations of ring II are not very sensitive to the deuteration of nitrogen.

The different degrees of protonation of the HABA molecule do not affect markedly the Raman lines of azo forms. The most significant effect is observed for the N=N stretching frequency: 1407 cm^{-1} for the deprotonated species ($B^=$) 1429 cm^{-1} for the neutral form (BH^-), and 1433 cm^{-1} for the HABA molecule in acid solution (BH_2). The shift of N=N stretching frequency toward lower frequencies when the dye molecule is deprotonated could tentatively indicate a decrease in bond order in the N=N linkage, but the corresponding shift toward high frequencies expected for the C–N stretching mode is not observed [10]. Other effects, like variations in vibrational coupling, could be considered.

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