The Fluorescence of some Biphenyl Derivatives

By J. W. BRIDGES, P. J. CREAVEN AND R. T. WILLIAMS

Department of Biochemistry, St Mary's Hospital Medical School, London, W.2

(Received 17 February 1965)

1. The variation with pH of the excitation and fluorescence wavelengths and fluorescence intensity of several hydroxy- and amino-biphenyls has been investigated with a view to using the data for the determination of hydroxybiphenyls in biological material. 2. 2- and 3-Hydroxy-, 2,2'-dihydroxy-, and 2-, 3- and 4-amino-biphenyl exhibited excited-state ionization. 4-Hydroxy- and 4,4'-dihydroxy-biphenyl did not, and showed normal fluorescence changes with pH. 3. Biphenyl and 2- and 4-methoxybiphenyl showed no changes in fluorescence in the range pH 0–14. 4. 4,4'-Dihydroxybiphenyl showed fluorescent peaks corresponding to the un-ionized form, the monoanion and dianion at the appropriate pH values, but 2,2'-dihydroxybiphenyl showed fluorescent peaks corresponding to the un-ionized form and the monoanion only, suggesting that it did not form a dianion. 5. 3- and 4-Nitrobiphenyl were non-fluorescent. 6. These results are discussed.

Biphenyl is metabolized by liver preparations to 4-hydroxybiphenyl, which can be determined fluorimetrically (Creaven, Parke & Williams, 1965). However, in some species, 2-hydroxybiphenyl is also produced and it became necessary to examine the fluorescence of biphenyl and its hydroxy derivatives in detail. Williams (1959) reported that both phenols were fluorescent in alkaline solution, but not in acid solution. In the present paper it is shown that 2- and 4-hydroxybiphenyl are fluorescent in acid and alkaline solution and that it is possible to distinguish these isomers fluorimetrically, since the 2-isomer undergoes excited-state ionization. Other derivatives of biphenyl have also been examined.

MATERIALS AND METHODS

Materials. Biphenyl, recrystallized from ethanol, m.p. 70°, 2-hydroxybiphenyl, recrystallized from light petroleum (b.p. 100–200°), m.p. 56.5°, 4-hydroxybiphenyl, recrystallized from light petroleum (b.p. 100–120°), m.p. 166.5°, 2,2'-dihydroxybiphenyl, recrystallized from toluene, m.p. 109.5°, 4,4'-dihydroxybiphenyl, recrystallized from ethanol, m.p. 277–279° (decomp.) (all from British Drug Houses Ltd., Poole, Dorset), 2-aminobiphenyl hydrochloride, recrystallized from water, and 4-nitrobiphenyl, recrystallized from light petroleum (b.p. 100–120°) followed by ethanol, m.p. 114.5° (both from Koch–Light Laboratories Ltd., Colnbrook, Bucks.), were purchased and purified as indicated. 2-Methoxybiphenyl, recrystallized from light petroleum (b.p. 100–120°), m.p. 29°, and 4-methoxybiphenyl, recrystallized from ethanol, m.p. 89°, were prepared from the corresponding hydroxybiphenyls with dimethyl sulphate. 3-Nitrobiphenyl, recrystallized from ethanol, m.p. 58°, was prepared from *m*-nitroaniline according to the method of Bamberger (1896) (cf. Blakey & Scarborough, 1927).

3-Aminobiphenyl, recrystallized from water, m.p. 28°, was prepared by the reduction of a methanolic solution of 3-nitrobiphenyl with 10% palladium charcoal (Koch–Light Laboratories Ltd.), in a hydrogenator at room temp. and 4 atm. pressure for 2 hr. 3-Hydroxybiphenyl, recrystallized from water, m.p. 74°, was prepared by the diazotization of 3-aminobiphenyl followed by treatment with boiling dilute H₂SO₄. Melting points agreed with those quoted in the literature.

Preparation of solutions. Compounds were dissolved in ethanol (Burrough's RR grade, which did not interfere with the fluorescence determinations). These solutions were diluted with glass-distilled water to contain 1–10 µg. of compound/ml. and 5% (v/v) of ethanol. To obtain solutions of various pH values, these aqueous solutions were titrated with solutions of the same concentration of the compound in 0.001–10 N-NaOH or 0.001–10 N-HCl according to which strength was appropriate for the pH required. The pH values of solutions between 2 and 12 were measured in a Pye Master pH-meter and pH values below 2 and above 12 were calculated from the amount of acid or alkali added.

Determination of absorption, excitation and fluorescence spectra. Absorption spectra were determined in a Unicam SP.500 spectrophotometer, and excitation and fluorescence spectra in an Aminco–Bowman spectrophotofluorimeter with a 150 w Osram xenon arc lamp. Solutions were examined for fluorescence immediately after they were prepared and all measurements of fluorescence intensity were made within 30 sec. of exposure to the exciting light to avoid possible photodecomposition and temperature effects. All measurements were made at room temperature unless otherwise stated. The data quoted are instrumental values.

RESULTS AND DISCUSSION

Stability of aqueous solutions in ultraviolet light. Solutions of 2-hydroxybiphenyl in water and 4-

hydroxybiphenyl in water or 0.1 N-sodium hydroxide were found to be relatively stable when exposed for up to 2 hr. to the light from the xenon lamp of the spectrofluorimeter. Thus 4-hydroxybiphenyl in water showed no more than a 4% variation in fluorescence intensity on being exposed to ultraviolet light (288 m μ) for 10 min. However, 2-hydroxybiphenyl in 0.1 N-sodium hydroxide was unstable in the exciting light and decomposed at a rate of about 1%/min. After 100 min. exposure, its fluorescence intensity had diminished to less than 10% of the initial value.

Effect of temperature. The fluorescence intensities of 2- and 4-hydroxybiphenyl were unaffected by slight temperature fluctuations, a temperature alteration of 1° causing less than 1% change in intensity.

Concentration and oxygen quenching. All the compounds examined showed concentration quenching of fluorescence. With 4-hydroxybiphenyl, fluorescence intensity was proportional to concentration up to 6 μ g./ml. Measurements of fluorescence were therefore carried out with concentrations where no significant self-quenching occurred, usually below 5 μ g./ml. The fluorescence of biphenyl, but of none of the other compounds studied, was slightly quenched by oxygen. Nitrogen was therefore bubbled through the biphenyl solution for 15 min. before its fluorescence intensity was measured.

Biphenyl. Benzene has a weak absorption band at 255 m μ (ϵ_{max} , 230) (see Gillam & Stern, 1954), and

therefore when it was excited by light of this wavelength it showed a weak fluorescence, which occurred at 291 m μ (Williams & Bridges, 1964). Biphenyl, however, shows an intense absorption at 246 m μ (ϵ_{max} , 16 500), characteristic of a highly resonant structure. The fluorescence of biphenyl is about 100 times as intense as that of benzene, the observed wavelength of maximum fluorescence occurring at 318 m μ and the corresponding maximum excitation wavelength being 270 m μ (see Tables 1 and 2).

4-Hydroxybiphenyl. 4-Hydroxybiphenyl shows a single intense absorption band at about 260 m μ (ϵ_{max} , 18 000) from pH -1 to 8. The absence of separate benzene and phenolic absorption bands indicates that 4-hydroxybiphenyl has a highly resonant structure compared with 2- and 3-hydroxybiphenyl (see Table 1) and that there is considerable π -electron delocalization. Above pH 10, 4-hydroxybiphenyl occurs largely as the anion (pK_a 9.55; see Kieffer & Rumpf, 1954*a,b*). At lower pH values, the fluorescence of 4-hydroxybiphenyl is very similar to that of phenol (Rosen & Williams, 1961). It is barely fluorescent at pH -1, but at pH 0 it shows a typical phenol fluorescence at λ_{max} , 340 m μ (excitation, λ_{max} , 288 m μ), and at pH 1 it fluoresces with maximum intensity, which remains constant with increasing pH up to pH 8.6. Above this pH, the fluorescence at 340 m μ progressively diminishes and a new fluorescence peak appears at 401 m μ , while the maximum excitation wavelength lengthens from 288 m μ to 311 m μ . The new fluorescence reaches its

Table 1. *Absorption spectra of substituted biphenyls*

Wavelengths are given to the nearest m μ ; for broad peaks the wavelength quoted is that for the centre of the peak; ϵ is quoted to the nearest 100; (infl.) indicates an inflexion. Many of these compounds show intense peaks in the 200–210 m μ region, but these are not quoted.

Compound	Absorption maxima							
	In ethanol		In 0.1 N-HCl		In water		In 0.1 N-NaOH	
	λ_{max} , (m μ)	$10^{-3} \times \epsilon_{\text{max}}$	λ_{max} , (m μ)	$10^{-3} \times \epsilon_{\text{max}}$	λ_{max} , (m μ)	$10^{-3} \times \epsilon_{\text{max}}$	λ_{max} , (m μ)	$10^{-3} \times \epsilon_{\text{max}}$
Biphenyl	246	16.5	—	—	—	—	—	—
2-Methoxybiphenyl	248	10.8	244	10.4	244	10.2	245	9.9
	285	4.6	282	4.1	282	4.1	283	4.0
4-Methoxybiphenyl	260	20.2	—	—	260	23.5	—	—
2-Hydroxybiphenyl	246	11.0	243	9.8	243	9.8	252	6.0
	288	5.1	283	4.0	282	4.1	309	5.6
3-Hydroxybiphenyl	251	15.0	251	14.9	250	15.1	235(infl.)	25.2
	284	4.2	280(infl.)	4.3	280	2.1	308	3.8
4-Hydroxybiphenyl	262	17.5	260	18.0	259	18.2	290	20.0
2,2'-Dihydroxybiphenyl	242	10.7	236	7.4	234	7.4	250	9.4
	286	7.2	279	6.0	279	5.8	308	8.6
4,4'-Dihydroxybiphenyl	264	21.0	262	20.4	263	20.4	290	25.6
2-Aminobiphenyl	—	—	233	12.7	220	18.6	217	29.4
	—	—	283–290	1.0	290	2.6	292	3.0
3-Aminobiphenyl	234	22.8	249	14.1	231	22.2	—	—
	300	2.6	—	—	298	3.1	—	—
4-Aminobiphenyl	280	10.5	247	18.5	272	17.7	273	19.1

Fig. 1. Variations of (a) fluorescence intensity and of (b) wavelengths of maximum excitation (A) and fluorescence (B) of 4-hydroxybiphenyl (—) and of 2-hydroxybiphenyl (---) with pH. Wavelengths and intensities are instrumental values. Intensities are given in arbitrary units based on the photomultiplier readings of the spectrofluorimeter. When excitation and fluorescence wavelengths are changing, two fluorescence peaks may be observed over a limited pH range. To avoid confusion these are not shown on the wavelength diagrams, but are indicated on the intensity curves (see also Figs. 2 and 4).

246 $m\mu$ ($\epsilon_{\max}$ 11 000) and 288 $m\mu$ ($\epsilon_{\max}$ 5 100), the former being due to the biphenyl nucleus and the latter being the phenolic absorption (see Table 1). This indicates that 2-hydroxybiphenyl is not capable of the same high degree of resonance as the 4-isomer and suggests that the electron contribution of the hydroxyl group is not delocalized over the entire biphenyl molecule to the same extent as in 4-hydroxybiphenyl. This could be due to loss of co-planarity in the molecule arising from steric hindrance and leading to a reduction in resonance energy (Braude & Forbes, 1955). In 0.1 N-sodium hydroxide, 2-hydroxybiphenyl occurs as an anion, as indicated by the longer wavelengths of the absorption peaks (Table 1). At pH -1, 2-hydroxybiphenyl is barely fluorescent, but at pH 0 it shows a typical phenol fluorescence with $\lambda_{\max}$ 348 $m\mu$, which is excited maximally at two wavelengths, namely 262 and 295 $m\mu$, the latter giving the more intense fluorescence. However, on reaching pH 1, the fluorescence wavelength lengthens and at pH 2 it has a value of 415 $m\mu$, which is characteristic of the anion, but the excitation wavelengths remain unchanged. Thus at pH 2 the absorbing species is the molecular form, whereas the fluorescent species is the anion. 2-Hydroxybiphenyl therefore exhibits the phenomenon of excited-state ionization. The wavelength of fluorescence of 2-hydroxybiphenyl remains at 415 $m\mu$ from pH 2 to 14, but the excitation wavelengths begin to change at pH 9.6, until at 10.4 they have lengthened to 272 and 320 $m\mu$. This change in excitation wavelengths corresponds to the normal ionization of 2-hydroxybiphenyl, which has pK_a 10.01 (Kieffer & Rumpf, 1954a,b). The most intense fluorescence exhibited by 2-hydroxybiphenyl occurs at pH 12–14 (see Fig. 1).

3-Hydroxybiphenyl. This compound has two absorption peaks, at 251 $m\mu$ ($\epsilon_{\max}$ 15 000) and 282–286 $m\mu$ ($\epsilon_{\max}$ 4250), indicating a less resonant structure than 4-hydroxybiphenyl. The fluorescence of 3-hydroxybiphenyl differs in some details from that of 2- and 4-hydroxybiphenyl, although, like the 2-isomer, this compound shows excited-state ionization. At low pH values, it shows two fluorescence peaks, one at 345 $m\mu$ corresponding to the unionized molecule and one at 420 $m\mu$ due to excited-state ionization. At pH -1, it shows feeble fluorescence at 345 $m\mu$ and 420 $m\mu$, both excited maximally by wavelengths of 270 and 290 $m\mu$ (see Fig. 2). At pH 0, fluorescence is relatively intense, but

maximum intensity at pH 10.4 and remains more or less constant up to pH 14. The fall in intensity of the fluorescence at 340 $m\mu$ and the appearance of the fluorescence at 401 $m\mu$ correspond to the ionization of 4-hydroxybiphenyl. The changes in excitation wavelengths also correspond to the occurrence of ionization (see Fig. 1). 4-Hydroxybiphenyl differs from phenol in that its anion is fluorescent whereas the anion of phenol is non-fluorescent (Williams, 1959). For practical purposes (see Fig. 1) the fluorescence of the anion of 4-hydroxybiphenyl is slightly more intense than that of the unionized form, but when allowances are made for the variation in energy output of the xenon lamp at various wavelengths it can be calculated that the intensity of the fluorescence of the anion is actually 70% of that of the molecular form. Above pH 14, the excitation and fluorescence wavelengths of 4-hydroxybiphenyl lengthen (λ_{exc} 320 $m\mu$; λ_a 410 $m\mu$). This may be due to the formation of a quinonoid structure such as (I).

2-Hydroxybiphenyl. The fluorescence behaviour of 2-hydroxybiphenyl is completely different from that of its 4-isomer. The absorption spectrum of 2-hydroxybiphenyl in ethanol shows two peaks, at

Fig. 2. Variations of (a) fluorescence intensity and (b) wavelengths of maximum excitation (A) and fluorescence (B) of 3-hydroxybiphenyl with pH. The excitation produces two fluorescence peaks from pH—1 to about 9.9, but only one above pH about 10.5. The wavelengths and intensities of these two fluorescences are shown by the unbroken and broken lines. 3-Hydroxybiphenyl shows two excitation maxima, each one producing two fluorescence peaks. This Figure shows only the excitation producing the more intense fluorescences. Wavelengths and intensities are instrumental values, the intensities being in arbitrary units based on the photomultiplier readings of the spectrofluorimeter.

the fluorescence at 345 $m\mu$ is ten times as intense as that at 420 $m\mu$. However, as the pH rises, the 420 $m\mu$ fluorescence becomes more intense whereas the 345 $m\mu$ fluorescence diminishes, so that at pH 4 the fluorescence due to excited-state ionization is twice as intense as that due to the un-ionized form. This means that only a proportion of the excited 3-hydroxybiphenyl molecules undergo excited-state ionization and that this proportion increases with pH. The fluorescence of the un-ionized molecule does not completely disappear until pH 10 is reached. At pH 9.6, 3-hydroxybiphenyl ionizes normally (Kieffer & Rumpf, 1954a,b) and the excitation and fluorescence of the normally formed anion are observed, these occurring at 320 and 420 $m\mu$ respectively. At pH 12 the fluorescence at 345 $m\mu$ has completely disappeared, and the 420 $m\mu$ fluorescence reaches a maximum that persists with constant intensity up to pH 15. From a practical standpoint, the most intense fluorescence of 3-hydroxybiphenyl occurs between pH 0 and 1 at

Fig. 3. Variations of (a) fluorescence intensity and of (b) wavelengths of excitation (A) and fluorescence (B) of 2,2'-dihydroxybiphenyl (----) and 4,4'-dihydroxybiphenyl (—) with pH. Wavelengths and intensities are instrumental values. Intensities are given in arbitrary units based on the photomultiplier readings of the spectrofluorimeter.

345 $m\mu$, whereas with 2- and 4-hydroxybiphenyl it occurs at pH 12–14 at 415 and 401 $m\mu$ respectively.

4,4'-Dihydroxybiphenyl. This compound shows absorption and fluorescence characteristics similar to that of 4-hydroxybiphenyl except that above pH 12 it shows new fluorescence as a result of ionization of the second hydroxyl group (see Fig. 3). Below pH 8, the compound is similar to 4-hydroxybiphenyl, showing fluorescence at 354 $m\mu$ ($\lambda_{\text{exc.}}$ 282 $m\mu$). Above pH 8, a new excitation and fluorescence appears at 300 and 438 $m\mu$ respectively, due to the monoanion of 4,4'-dihydroxybiphenyl. Above pH 11, the 438 $m\mu$ fluorescence diminishes and is replaced by a new weak fluorescence at 400 $m\mu$ ($\lambda_{\text{exc.}}$ 310 $m\mu$) that is probably due to the dianion. The most intense fluorescence of 4,4'-dihydroxybiphenyl occurs at 354 $m\mu$ at pH 1–8, where the molecule is un-ionized.

2,2'-Dihydroxybiphenyl. This compound behaves like 2-hydroxybiphenyl, showing excited-state ionization that is completely developed at pH 1 and persists up to about pH 6.4. 2,2'-Dihydroxybiphenyl ionizes between pH 6 and 7, and just over

Table 2. *Relative fluorescence intensities of some hydroxybiphenyls*

Compounds were examined at a concentration of about $10\mu\text{M}$ in water containing 5% (v/v) of ethanol. For comparing fluorescence intensity that of biphenyl was taken as 100. Wavelengths are instrumental values. The fluorescence intensity values quoted in this Table have been corrected for the variation in energy output of the xenon lamp with wavelength. The lamp was calibrated by the chemical actinometer method of Hatchard & Parker (1956).

Compound	State	Excitation λ_{max} , (m μ)	Fluorescence λ_{max} , (m μ)	Maximum value of fluorescence intensity	pH range of maximum fluorescence
Biphenyl	Molecular	270	318	100	0-14
2-Hydroxybiphenyl	Un-ionized	{ 262 295 }	348	{ 39 83 }	0-1
	Excited-state ion	{ 262 295 }	415	{ 67 154 }	2.5-9.8
	Anion	{ 272 320 }	415	{ 99 481 }	12-14
3-Hydroxybiphenyl	Un-ionized	{ 270 290 }	345	{ 70 78 }	0-1
	Excited-state ion	{ 270 290 }	420	{ 23 25 }	1.6-9.2
	Anion	{ 270 320 }	420	{ 23 60 }	10.1-14.2
4-Hydroxybiphenyl	Un-ionized	288	340	184	1-9
	Anion	311	401	136	10-14
2-Methoxybiphenyl	Molecular	{ 262 295 }	348	{ 114 229 }	0-14
4-Methoxybiphenyl	Molecular	280	338	440	0-14
2,2'-Dihydroxybiphenyl	Un-ionized	{ 256 290 }	356	{ 2 6 }	-1 to -0.8
	Excited-state ion	{ 256 290 }	400	{ 41 385 }	1.0-6.6
	Monoanion	{ 262 320 }	400	{ 266 1593 }	8.6-13
4,4'-Dihydroxybiphenyl	Un-ionized	282	354	271	1-7
	Monoanion	300	438	19	10.9-11.3
	Dianion	310	400	7	12.1-14

pH 7 the excitation changes but not the fluorescence (see Table 2 and Fig. 3). The compound shows a fluorescence (λ_{max} 400 m μ) in the range pH 0-15 and the only change in fluorescence wavelength occurs below pH 0. At pH -1.0 to -0.8, the fluorescence is at 356 m μ , which is probably that of the un-ionized form. The only change in excitation wavelength occurs at pH 7.3-7.7, when it changes from 290 to 320 m μ . The most intense fluorescence occurs at pH 9-13. Fluorescence observations do not indicate that 2,2'-dihydroxybiphenyl undergoes a second ionization, for there are no changes in excitation or fluorescence wavelength at high pH values, i.e. pH 13-15. This suggests that the monoanion may be stabilized by hydrogen-bonding between the ionized and un-ionized phenolic groups (II).

Methoxybiphenyls. 4-Methoxybiphenyl shows a fluorescence of constant intensity at λ_{max} 338 m μ (λ_{exc} 280 m μ) in the range pH 1-14. At pH -1 it is barely fluorescent and above pH 14 the wavelength shortens, possibly owing to conversion into a quinonoid structure. 2-Methoxybiphenyl also fluoresces with constant intensity in the range pH 0-14 at λ_{max} 348 m μ (λ_{exc} 262 and 295 m μ). Unlike 2-hydroxybiphenyl this compound cannot ionize

Table 3. *Relative fluorescence intensities of some aminobiphenyls*

Compounds were examined at a concentration of about $10\ \mu\text{M}$ in water containing 5% (v/v) of ethanol. The wavelengths quoted are instrumental values. Fluorescence intensities are corrected values (see Table 2).

Compound	State	Excitation $\lambda_{\text{max.}}$ (m μ)	Fluorescence $\lambda_{\text{max.}}$ (m μ)	Maximum value of fluorescence intensity	pH range of maximum fluorescence
Biphenyl	Molecular	270	318	100	0-14
2-Aminobiphenyl	Cation	255	318	16	-1 to -0.5
	Excited-state un-ionized base	255	405	40	0.4-3
	Un-ionized base	{ 265 } { 305 }	405	{ 87 } { 275 }	5.1-11.0
3-Aminobiphenyl	Cation	270	320	54	-0.6 to -0.5
	Excited-state un-ionized base	270	410	100	1.0-4.2
	Un-ionized base	{ 270 } { 310 }	410	{ 99 } { 119 }	5.1-11.0
4-Aminobiphenyl	Cation	270	318	14	-0.8 to -0.6
	Excited-state un-ionized base	270	382	316	2.5-3.0
	Un-ionized base	295	382	1106	5.1-10.3

Fig. 4. Variations of (a) fluorescence intensity and of (b) wavelengths of excitation (A) and fluorescence (B) of 2-aminobiphenyl (---), 3-aminobiphenyl (-.-.-) and 4-aminobiphenyl (—) with pH. Wavelengths and intensities are instrumental values. Intensities are given in arbitrary units based on the photomultiplier readings of the spectrofluorimeter.

(cf. fluorescence of anisole and phenol; Rosen & Williams, 1961) and therefore excited-state ionization does not occur.

Aminobiphenyls. The three aminobiphenyl isomers show excited-state ionization. At pH -1, where they exist as cations, they show a relatively weak fluorescence at $\lambda_{\text{max.}}$ 318-320 m μ , which is characteristic of biphenyl itself (see Table 3), but at pH 1 the fluorescence wavelength in each case has lengthened to 405 m μ for the 2-isomer, to 410 m μ for the 3-isomer and to 382 m μ for the 4-isomer. However, the excitation wavelengths do not change until pH 3.0-3.5 is reached (see Fig. 4), where one would expect the aminobiphenyl cation to lose a proton to yield molecular aminobiphenyl. The aminobiphenyl cation thus undergoes excited-state ionization at about pH 0. The fluorescence of uncharged aminobiphenyl can be observed in the range pH 0-14, but the excitation wavelength corresponding to this species is only observed in the range pH 3.5-14, for below pH 3 the excitation corresponds to that of the cation. The most intense fluorescence for all three isomers occurs in the range pH 5-11. The 4-isomer is nine times as fluorescent as the 3-isomer and four times as fluorescent as the 2-isomer. The fluorescence ($\lambda_{\text{max.}}$ 320 m μ) of the cationic form of 3-aminobiphenyl is more intense than that of the 2- and 4-isomers and seems to have maximum intensity at about pH -0.5 (i.e. in 3 N-acid) (see Fig. 4).

3- and 4-Nitrobiphenyl were non-fluorescent, as would be expected on theoretical grounds (see Williams & Bridges, 1964).

Comment. Biphenyl and 12 of its derivatives were examined fluorimetrically in the Aminco-Bowman spectrofluorimeter. Six of these derivatives, namely 2- and 3-hydroxy-, 2,2'-dihydroxy-, and 2-, 3- and 4-amino-biphenyl, exhibited excited-state ionization or deprotonation. This phenomenon was first discovered by Förster (1950) and simplified accounts of it and its possible analytical application have been given by Bridges (1964) and Williams & Bridges (1964). In the excited state, 2- and 3-hydroxybiphenyl ionize to form anions at about pH 1–2, whereas in the ground state they ionize at pH 9–11. These phenols are therefore 10^8 times stronger acids in the excited-state than in the ground state. With 2,2'-dihydroxybiphenyl, excited-state ionization occurs at pH 0–1, compared with pH 6–7 for the ground-state ionization, and here the increase in acid strength is about 10^6 . The bases, on the other hand, tend to become weaker in the excited state, for the 2-, 3- and 4-amino-biphenyl cations show excited-state deprotonation at about pH 0, whereas ground-state deprotonation of these cations would be expected to occur at about pH 4.

4-Hydroxy- and 4,4'-dihydroxy-biphenyl did not show excited-state deprotonation, and it may be pertinent to ask why the excited-state phenomenon occurs with 2- and 3-hydroxybiphenyl, but not with 4-hydroxybiphenyl. No adequate answer to this type of question can yet be given. It has been shown (see Reid, 1957) that ionization can take place in 10^{-12} sec. and the life-time of the excited state is 10^{-8} sec., so there is ample time for ionization to occur during the life-time of the excited molecule. The excitation of a molecule leads to the promotion of a π -electron to a higher energy level and this results in an asymmetric distribution of electrons in the electron cloud surrounding the molecule. The remaining unexcited electrons then redistribute themselves to compensate for the loss of the excited electron. If electrons are withdrawn from the substituent group to assist in this redistribution it is then conceivable that the bond between the oxygen and hydrogen atoms in the hydroxyl group in the hydroxy compounds would be weakened and there would be an increased tendency for the hydroxyl group to lose a proton. Presumably the extent of excited-state deprotonation would depend on the extent to which electrons are withdrawn from the substituent group to re-establish the electron cloud. This apparently would not occur to any large extent in 4-hydroxybiphenyl, which has a highly resonant structure, judged from its absorption spectrum, and does not exhibit excited-state deprotonation. However, with 2-hydroxybiphenyl it would occur readily, whereas

with 3-hydroxybiphenyl it would occur to a smaller extent for in this case fluorescences due both to excited-state ionization and to the un-ionized molecule occur together (see Fig. 3).

In the excited aminobiphenyl cations, electrons would apparently be withdrawn from the NH_3^+ group in such a way as to facilitate the loss of a proton. Other cations that have been shown to exhibit excited-state deprotonation are those of pyridoxine, pyridoxal and its 5'-phosphate, and pyridoxamine (Bridges, Creaven, Davies & Williams, 1963; J. W. Bridges, D. S. Davies & R. T. Williams, unpublished work; Williams & Bridges, 1964). However, there does not appear to be as yet a simple explanation of why one compound exhibits excited-state deprotonation and another does not.

The fluorescent 2- and 4-methoxybiphenyl do not contain ionizable groups and therefore they do not exhibit excited-state ionization. 3- and 4-Nitrobiphenyl did not show fluorescence under our conditions. This result is expected since the nitro group tends to suppress fluorescence by tending to withdraw electrons from the ring system and increasing their localization (Bridges & Williams, 1962; Williams & Bridges, 1964).

P. J. C. is grateful to the Medical Research Council for a scholarship.

REFERENCES

- Bamberger, C. (1896). *Ber. dtsch. chem. Ges.* **29**, 446.
- Blakey, W. & Scarborough, H. A. (1927). *J. chem. Soc.* p. 3000.
- Braude, E. A. & Forbes, W. F. (1955). *J. chem. Soc.* p. 3776.
- Bridges, J. W. (1964). In *Methods in Polyphenol Chemistry*, p. 59. Ed. by Pridham, J. B. London: Pergamon Press Ltd.
- Bridges, J. W., Creaven, P. J., Davies, D. S. & Williams, R. T. (1963). *Biochem. J.* **88**, 65P.
- Bridges, J. W. & Williams, R. T. (1962). *Nature, Lond.*, **196**, 59.
- Creaven, P. J., Parke, D. V. & Williams, R. T. (1965). *Biochem. J.* **96**, 879.
- Förster, T. (1950). *Z. Elektrochem.* **54**, 531.
- Gillam, A. E. & Stern, E. S. (1954). *Electronic Absorption Spectroscopy*, p. 116. London: Edward Arnold Ltd.
- Hatchard, C. G. & Parker, C. A. (1956). *Proc. Roy. Soc. A*, **235**, 518.
- Kieffer, F. & Rumpf, P. (1954a). *C.R. Acad. Sci., Paris*, **238**, 360.
- Kieffer, F. & Rumpf, P. (1954b). *C.R. Acad. Sci., Paris*, **238**, 700.
- Reid, C. (1957). *Excited States in Chemistry and Biology*, p. 67. London: Butterworths Scientific Publications.
- Rosen, A. & Williams, R. T. (1961). *Bull. photoelect. Spectrom. Grp*, no. 13, p. 339.
- Williams, R. T. (1959). *J. roy. Inst. Chem.* **83**, 611.
- Williams, R. T. & Bridges, J. W. (1964). *J. clin. Path.* **17**, 371.