[CONTRIBUTION FROM THE DEPARTMENT OF INDUSTRIAL MEDICINE, NEW YORK UNIVERSITY MEDICAL CENTER]

Solvent Effects in the Fluorescence of Indole and Substituted Indoles[1]

BENJAMIN L. VAN DUUREN[2]

Received November 30, 1960

Pronounced changes were observed in the fluorescence emission spectra of indole and substituted indoles with change of solvent. Similar shifts do not occur in the fluorescence excitation and ultraviolet absorption spectra. The possible roles of dielectric constants of the solvents and hydrogen bond formation in accounting for these shifts are discussed. The use of fluorescence spectroscopy as a diagnostic tool in chemical structural analysis in the indole series is described. Some observations on the quenching of fluorescence of indole by solvents and acids are reported.

The infrared,[3,4] ultraviolet,[5-8] and NMR[9] spectroscopic examination of indole derivatives have been the subject of many reports because of the significance of these compounds in biochemistry and natural product organic chemistry.

In the course of our studies on the fluorescence spectra of dilute solutions of aromatic compounds[10,11] indole and its derivatives were also examined. The remarkable solvent effects observed in the fluoresence spectra of the indole compounds prompted further inquiry into their fluorescence spectroscopy; the results constitute the subject of this report.

RESULTS

The ultraviolet absorption spectra of indole in various solvents are shown in Fig. 1. The fluorescence excitation spectra of indole, also, were very similar. The fluorescence emission spectra of indole in various solvents and solvent mixtures are shown in Fig. 2, 3, and 4. All these emission spectra were obtained on solutions of the same concentration, 4.7×10^{-5} moles/l., with the same instrument settings, slit width 5 mμ, sensitivity 0.1, and the same excitation wavelength, 285 mμ. A plot of concentration of indole in cyclohexane versus fluorescence intensity gave a straight line between concentrations 0.05×10^{-5} and 8.0×10^{-5} moles/l. The concentration chosen for the comparative spectra was therefore probably within the limits of linear response for all the compounds in the various solvents since the fluorescence intensities were of approximately the same order. A possible exception is 1-methyl-2-phenylindole which exhibited a higher fluorescence intensity than the other compounds in this series.

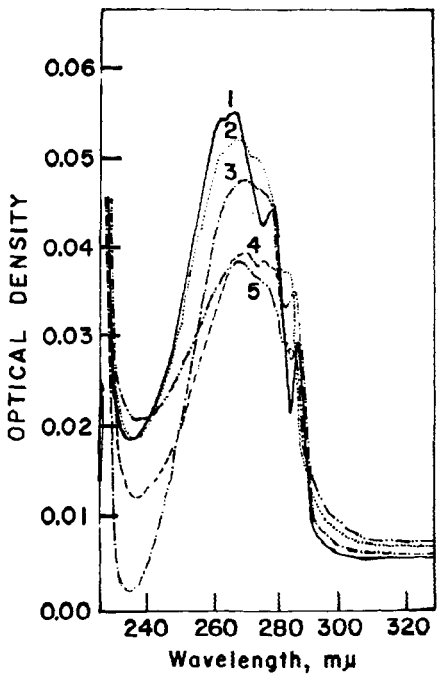


Fig. 1. Ultraviolet absorption spectra of indole, 8.7×10^{-6} mole/l.
1. ———, cyclohexane. 2. . . . . , water. 3. — · — · —, dioxane. 4. — — — —, ethanol. 5. — · · — · · —, 10% aqueous sulfuric acid

When the fluorescence of indole was measured with oxygen-free solvents the positions of the bands remained unchanged although there was a 5 to 10% increase in fluorescence intensity as compared to solvents from which oxygen had not been excluded. It was also determined that the intensity of the 297 mμ emission band of indole in cyclohexane is not changed by continuous exposure to the exciting radiation, 285 mμ, for six hours. It is conceivable

(1) Presented in part at the Eleventh Annual Pittsburgh Conference of the Spectroscopy Society of Pittsburgh and the Pittsburgh Section of the American Chemical Society, March 1960.

(2) Aided by a grant from the American Cancer Society, Inc., New York.

(3) N. Fuson, M. Josien, R. L. Powell, and E. Utterback, *J. Chem. Phys.*, **210**, 145 (1952).

(4) F. Millick and E. I. Becker, *J. Org. Chem.*, **23**, 1096 (1958).

(5) F. Pruckner and B. Witkop, *Ann.*, **554**, 127 (1943).

(6) S. Menczel, *Z. Phys. Chem.*, **125**, 195 (1926).

(7) F. Walls, O. Collera, and A. Sandoval, *Tetrahedron*, **2**, 173 (1958).

(8) B. G. Edwards, *Arch. Biochem. Biophys.*, **21**, 103 (1949).

(9) L. A. Cohen, J. W. Daley, H. Kny, and B. Witkop, *J. Am. Chem. Soc.*, **82**, 2184 (1960).

(10) B. L. Van Duuren, *Anal. Chem.*, **32**, 1436 (1960).

(11) B. L. Van Duuren, *J. Nat. Cancer Inst.*, **25**, 53 (1960), and preceding papers cited therein.

TABLE I

FLUORESCENCE OF INDOLE AND SUBSTITUTED INDOLES

Compound	Cyclohexane			Benzene			Dioxane			Ethyl alcohol			Water		
	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c
Indole, I	285	297	6.0	285	305	0.6	285	310	5.8	285	330	6.1	285 (280[d,e])	350 (350[d], 355[e])	4.0
2-Methylindole, II	280	306	6.2	280	305	very weak[f]	280	325	8.9	280	335	6.5	280 (280[d])	355 (355[d])	2.0
3-Methylindole, III	280	315	6.6	280	317	very weak[f]	280	329	8.0	280	350	6.0	280 (290[d])	370 (370[d])	6.3
1,2-Dimethylindole, IV	291	315	10.0	291	326	11.0	291	329	7.7	291	340	11.0	291	362	7.9
2,3-Dimethylindole, V	282	320	6.0	282	330	0.1	282	340	11.0	282	360	9.1	282	376	3.6
3-Hydroxymethylindole, VI	285	305	4.8	285	325	0.85	285	327	7.6	285	338	8.4	285	360	7.4
Indole-3-acetic acid, VII	No fluorescence			No fluorescence			290	325	8.5	290	340	6.3	290 (285[d])	360 (360[d])	5.3
2-(3-Indolyl)-2,3-dihydroindole (Indole dimer), VIII	290[g] 306	337	8.2	316	367, 390	3.2	290, 306	346	12.5	290	360	3.8	290, 306	345	0.35
1-Methyl-2-phenylindole, IX	310	360	64.0	310	370	63.0	310	370	72.0	310	370	61.0	305	380	17.0
Carbazole, X[h]	290, 318,330	332, 348	5.4, 5.3	—	—	—	—	—	—	290, 323,335	342, 355	7.0, 7.3	290, 323[i],335	342[i], 355	8.0, 8.9

[a] Fluorescence excitation maximum, mμ. [b] Fluorescence, emission maximum, mμ. [c] Relative fluorescence intensity; microammeter readings at same sensitivity setting, same slit width, 5 mμ, and, except where otherwise noted, same concentration of 4.7×10^{-5} moles/l. The intensity readings are uncorrected. [d] Reported value: H. Sprince, G. R. Rowley, and D. Jameson, *Science*, 125, 442 (1957). [e] Reported value: D. E. Duggan, R. L. Bowman, B. B. Brodie, and S. Udenfriend, *Arch. Biochem. and Biophys.*, 68, 1 (1957). [f] Observed only with 10 mμ slits. [g] Where more than one peak is observed in the spectra, the most intense peak is underlined. [h] 4.0×10^{-6} moles/l. [i] The positions of these maxima are unchanged by acidification with 10% aqueous sulfuric acid.

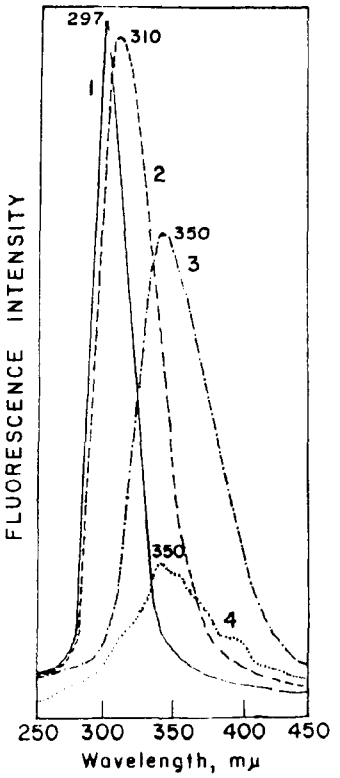


Fig. 2 Fluorescence emission spectra of indole, 4.7×10^{-5} mole/l., excitation at 285 mμ

1. ———, cyclohexane. 2. – – – –, dioxane; 3. – · – · –, water. 4. ....., 10% aqueous sulfuric acid

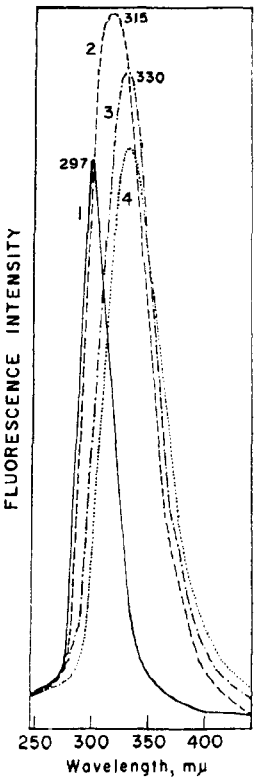


Fig. 3. Fluorescence emission spectra of indole, 4.7×10^{-5} mole/l., excitation at 285 mμ

1. ———, cyclohexane. 2. – – – –, 1% ethanol in cyclohexane. 3. – · – · –, cyclohexane-ethanol (1:1). 4. ....., ethanol

that a series of bands are present in each emission spectrum and that some of these bands are of low intensity. In order to explore this possibility the fluorescence spectra of indole in some of the solvents were examined in more concentrated solutions (up to 2×10^{-3} moles/l.) and also at higher dilutions (to 2×10^{-7} moles/l.). In the latter case the source intensity was increased by using wider slits. New bands were not observed in these experiments—i.e., the fluorescence emission wavelengths were not affected by concentration within the limits examined.

The fluorescence characteristics of a number of substituted indoles and of carbazole were measured with the same series of solvents. The results are listed in Table 1.

In order for corrected fluorescence intensity values to have real significance all possible sources of error should be considered. As this was not feasible without extensive instrumental calibrations the fluorescence intensity values given in Table I are uncorrected. Two of the most often mentioned errors in relative fluorescence intensity measurements are differences in source output with wave length and differences in phototube response with wave length. Of the compounds listed in Table I, all but compound IX were excited between 280 and 290 mμ; as the spectral output curve above 280 mμ is relatively flat, it is expected that the errors in this respect are small. Moreover, the maximum difference in phototube response between 300 and 375 mμ (which again covers the emission maxima of all compounds except IX) is 10% (data taken from typical relative response curves for standard R.C.A. 1P28 photomultiplier tubes). The solvents used were not oxygen-free and this will also have some effect on the fluorescence intensity values.

Pertinent ultraviolet absorption spectra of the indole compounds in various solvents are given in the experimental section.

The quenching properties of some substituents and solvents were examined. Thus, 3-acetylindole does not fluoresce in any of the solvents used. The fluorescence of indole is quenched in chloroform and in carbon tetrachloride solutions. The fluorescence intensity of cyclohexane solutions containing various concentrations of chloroform was measured; the results are given in Table II. It was also observed that indole does not fluoresce in acetone or in cyclohexane containing more than 1.0% (0.13 moles/l.) of acetone. As the ultraviolet absorption of acetone[12] may interfere (λ_{max} 279 mμ, ϵ_{max} 12 in hexane) the fluorescence of indole in water was examined in the presence of 1% acetone.

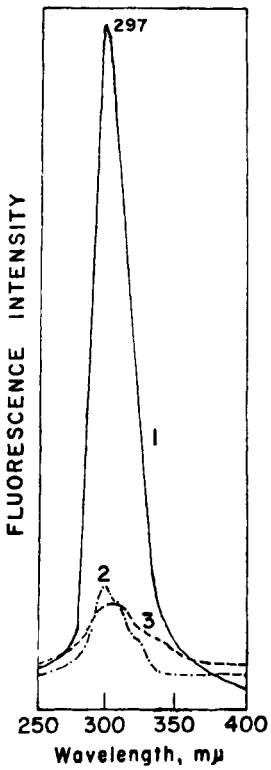


Fig. 4. Fluorescence emission spectra of indole, 4.7×10^{-6} mole/l., excitation at 285 mμ

1. ———, cyclohexane. 2. -·-·-, cyclohexane +1% trichloroacetic acid. 3. – – – –, benzene

TABLE II
QUENCHING OF INDOLE FLUORESCENCE BY CHLOROFORM[a]

Millimoles Chloroform/L. Cyclohexane	Fluorescence Intensity
438.0	0.15
365.0	0.16
292.0	0.18
219.0	0.25
146.0	0.45
73.0	0.94
58.0	0.95
43.8	1.0
29.2	1.8
14.6	3.4
7.3	4.2
3.6	4.8
0.7	5.0
0.07	5.2
0.00	5.2

[a] Indole concentration: 4.0×10^{-5} moles/l. in cyclohexane; excitation at 282 mμ; emission at 297 mμ.

The ultraviolet absorption maximum of acetone in water is at 264.5 mμ (ϵ_{max} 17.4)[12] so that its ultraviolet absorption is not expected to interfere appreciably with fluorescence measurements at excitation 290 mμ. Under these conditions it was found that, in the presence of 1% acetone, the fluorescence intensity of indole was reduced to one-tenth of its value in the absence of acetone.

(12) A. Gillam and E. S. Stern, *Electronic Absorption Spectroscopy*, E. Arnold, Ltd., London, 1950, p. 50.

DISCUSSION

Solvent polarity. Numerous studies have been made of the effect of solvents on ultraviolet absorption spectra. The shifts either to longer or to shorter wave length are usually of the order of 5 of 10 mμ and seldom more than 17 mμ.[13] These shifts have been correlated with the dielectric constants of the solvents[14-16] and have been extensively studied in relation to hydrogen bonding effects.[17-19]

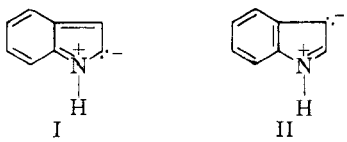
Changes in fluorescence spectra with change in *p*H are also well known and constitute the basis of the fluorescent indicators.[20] In these cases changes in the fluorescence emission maxima are accompanied by comparable changes in the ultraviolet absorption spectra. Some instances are known in which only the fluorescence emission spectra are affected by *p*H–*e.g.* in the hydroxy and aminopyrenesulfonic acids[21,22] and 1-naphthylamino-4-sulfonic acids.[23] The solvent effects described in the present work are similar to those observed by these workers in that fluorescence emission spectra were affected without comparable changes in ultraviolet absorption spectra.

Solvents have very little effect on the ultraviolet spectra of indoles as is exemplified by indole itself (Fig. 1). The resolution of bands was usually improved in hydrocarbon solvents but the λ_{max} and ϵ_{max} values were very similar in different solvents. The only exceptions are 3-acetylindole and carbazole both of which exhibited shifts in their ultraviolet absorption spectra with change of solvent (see Experimental).

The fluorescence emission maxima of the indole compounds shown in Table 1 follow the same pattern shown in Fig. 2 to 4 for indole itself—*i.e.* there is a shift to longer wave length with increased dielectric constant of the solvent in the order: cyclohexane < benzene < dioxane < ethanol < water.

(13) Reference 12, p. 265.
(14) G. Scheibe, E. Felgor, and G. Rossler, *Ber.*, **60**, 1406 (1927).
(15) H. Ungnade, *J. Am. Chem. Soc.*, **75**, 432 (1953).
(16) M. Ito, K. Inuzuka, and S. Imanishi, *J. Am. Chem. Soc.*, **82**, 1317 (1960).
(17) G. J. Brealey and M. Kasha, *J. Am. Chem. Soc.*, **77**, 4462 (1955).
(18) G. C. Pimentel, *J. Am. Chem. Soc.*, **79**, 3323 (1957).
(19) Reviewed by G. C. Pimentel and A. L. McClellan, *The Hydrogen Bond*, W. H. Freeman and Company, San Francisco and London, 1960, p. 158.
(20) W. West in "Techniques of Organic Chemistry," Vol. IX. *Chemical Applications of Spectroscopy*, A. Weissberger, ed., Interscience, New York, 1956, p. 714.
(21) T. Förster, *Naturwissenschaften*, **36**, 186 (1949).
(22) T. Förster, *Z. Elektrochem.*, **54**, 42 (1950).
(23) H. Boaz and G. K. Rollefson, *J. Am. Chem. Soc.*, **72**, 3435 (1950).

As the ultraviolet absorption and fluorescence excitation spectra remain unchanged with change of solvent, it follows that there is interaction between solvent and the activated indole molecule but not between solvent and the ground state of the indole molecule. Thus, it seems probable that polar contributing mesomeric states of the indole molecule, such as I and II, make a larger contribution in the activated indole molecule than in the ground state. Such polar structures would be more sensitive to the dielectric constant of the surrounding solvent and this would account for pronounced shifts in the fluorescence emission spectra that are not observed in the ultraviolet absorption spectra.



I II

Hydrogen bonding. Solvent effects observed in the ultraviolet absorption[18,19] and fluorescence emission spectra[24] of certain compounds have been ascribed to hydrogen bonding. However, the relative significance of hydrogen bonding and dielectric effects have not been unequivocally established.[19] This situation applies also in the solvent effects observed in the indole series. Indole can behave as both proton donor or as proton acceptor with hydrogen bonding solvents such as dioxane, ethanol, and water. A test for the possibility of hydrogen bonding in this series lies in an examination of the fluorescence emission maxima of 1,2-dimethylindole and 1-methyl-2-phenylindole in various solvents. These substances cannot undergo hydrogen bonding with dioxane, nevertheless their fluorescence emission maxima follow the same shift to longer wave length in going from cyclohexane to dioxane as do the other indoles that are not substituted on the nitrogen atom. This implies that the dielectric properties of the solvents are of overriding importance in bringing about shifts to longer wave length.

Fluorescence quenching. For compounds I to VIII the fluorescence intensities are of the same order of magnitude in cyclohexane, dioxane, and ethanol. On the other hand, there is, except in the case of 1,2-dimethylindole, a generally lower fluorescence intensity in water and a much lower fluorescence intensity in benzene. This quenching effect of benzene is not observed when benzene is used as a solvent in the fluorescence spectroscopy of aromatic hydrocarbons. Moodie[25] in discussing energy transfer processes in hydrocarbon systems points out that wider recognition should be given to so called "dispersion force" complexes and that this phenomenon may explain certain types of fluorescence quenching. It is possible that such complex formation takes place in the indole compounds in benzene solution. It is noteworthy that the fluorescence intensities of 1,2-dimethylindole and 1-methyl-2-phenylindole are not decreased in benzene solution compared to other solvents which would imply that the free >NH is essential for such complex formation.

In polarizable solvents molecular interactions of the Van der Waals type are expected to play an important role and this may account for quenching by chloroform and carbon tetrachloride. By plotting fluorescence intensity versus quencher concentration from the data given in Table II, a value of 0.021 mole/l. is obtained for the quenching efficiency of chloroform. This value ("halbwertskonzentration"[26]) represents that concentration of chloroform which reduces the fluorescence intensity of the pure compound in solution by one-half. The quenching efficiency of chloroform for indole in cyclohexane is of the same order as that found for inorganic ions on the fluorescence of quinine[23] and other organic compounds.[26]

In the indole cation the aromaticity of the molecule is destroyed and this will account for the very weak fluorescence of indole in dilute aqueous sulfuric acid and in cyclohexane in the presence of trichloracetic acid (Fig. 2 and 4). This behavior is, of course, different from the fluorescence quenching brought about by chloroform and carbon tetrachloride. Tryptophane and tryptamine, both of which fluoresce in neutral solutions do not fluoresce in acid solution.[27]

2-(3-Indolyl)-2,3-dihydroindole. Fluorescence spectra of this compound were examined because of the possibility that it may be formed in the photochemical dimerization of indole. Examination of the fluorescence spectra of this compound in various solvents indicated that such dimerization does not take place. Nevertheless, indole dimer did exhibit unique solvent effects. The ultraviolet absorption spectra and fluorescence excitation spectra are the same in all solvents except benzene. Pronounced shifts were observed in the fluorescence emission maxima with change in solvent but these shifts do not conform to the pattern exhibited by the other indoles in going from solvents of low to solvents of high dielectric constant. As in the case of indole the fluorescence of indole dimer is reduced in acid aqueous solution and the position of the fluorescence emission maximum remains unchanged from that in neutral aqueous solution.

3-Acetylindole. The red shift observed in the ultraviolet absorption spectra of 3-acetylindole in going from solvents of low to solvents of higher dielectric contants (see Experimental) is not un-

(24) N. Mataga and S. Tsuno, *Bull. Chem. Soc., Japan*, 30, 368 (1957).

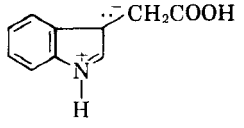
(25) M. M. Moodie and C. Reid, *J. Chem. Phys.*, 20, 1510 (1952).

(26) T. Förster, *Fluoreszenz Organischer Verbindungen*, Vandenhoeck and Ruprecht, Göttingen, 1951, p. 182.

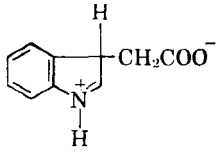
(27) S. Udenfriend, D. F. Bogdanski, and H. Weissbach, *Science*, 122, 972 (1955).

usual. This shift, associated with π-π^* transitions, has been observed for other ketones[16] and explained in terms of electrostatic interaction and hydrogen bonding effects between solute and solvent. The lack of fluorescence of 3-acetylindole is unexpected; however, it is reasonable that the electron-withdrawing carbonyl group will tend to localize the unshared electrons of the imino nitrogen on the carbonyl oxygen; such electron withdrawal will essentially destroy the aromaticity of the indole nucleus and hence no fluorescence is observed. Hydrogen bonding between 3-acetylindole and solvents, although possible in solvents such as ethanol and water, will not account for the absence of fluorescence in other solvents.

Indole-3-acetic acid. This compound shows no measurable fluorescence in cyclohexane and benzene but does fluoresce in dioxane, ethanol, and water with shifts to longer wave lengths in that order. The fluorescence intensities in these solvents are comparable to that of the other indole derivatives and the ultraviolet spectra remain essentially unchanged with change of solvent. The lack of fluorescence in non-polar solvents is reminiscent of the fluorescence of acridine. This compound, also, does not fluoresce in nonpolar solvents but fluoresces strongly in polar solvents.[10,24] In both these compounds the nature and probably also the lifetime of the excited states are profoundly influenced by the solvent. Thus, one may argue, a polarized form such as III would exist in the excited state in polar solvents where the carboxyl group is involved in stabilizing hydrogen bonding and/or electrostatic influences. In nonpolar solvents structure IV will be more likely for the excited state; this structure implies localization of the unshared electrons of the imino nitrogen, loss of aromaticity and



III



IV

hence absence of fluorescence. It should be noted that construction of a molecular model of indole-3-acetic acid shows that intramolecular hydrogen bond formation in this molecule is not possible.

1-Methyl-2-phenylindole. Compared to the other indole derivatives this compound shows much smaller shifts in fluorescence emission maxima with change of solvent. The occurrence of fluorescence emission of this compound at longer wave lengths than the other compounds in this series and the increased fluorescence intensities in all solvents compared to the other indoles is similar to that observed in the fluorescence spectra of phenyl substituted aromatic hydrocarbons[10] compared to their parent hydrocarbons.

Carbazole. **The unshared electrons of the nitrogen** atom of carbazole contribute to the π-electronic state of the whole molecule, as in the indole molecule; hence polarized forms corresponding to I and II are expected to exist in the excited state of the carbazole molecule. One would therefore expect similar fluorescence behavior in these two compounds. Carbazole does exhibit shifts in fluorescence emission maxima to longer wave length with increasing dielectric constant of the solvent; however, these shifts are smaller than in the indoles and in addition comparable shifs are observed in the ultraviolet absorption spectra with change of solvent.

The methylindoles. From Table I it is seen that in all the solvents used there is a shift to shorter wave length in the order: V > IV > III > II > I. This order is maintained except in the emission maxima of 1,2-dimethylindole in ethanol and in water. This behavior can be attributed to hyperconjugative effects of the methyl substituents. A similar effect is operative in the alkylbenzenes which show in their ultraviolet spectra shifts to longer wave lengths compared to benzene.

The fluorescence spectra of alkyl derivatives of aromatic compounds are usually very similar to that of their parent compounds. In the methylindoles, however, there are shifts of 5 to 25 mμ from one compound to the next in the same solvent—e.g., in water indole shows a maximum at 350 mμ whereas 2,3-dimethylindole shows a maximum at 376 mμ. In dioxane solution indole can be readily distinguished from the various methylindoles and in cyclohexane 2- and 3-methylindole are readily distinguished.

Apart from the obvious utility of these fluorescence studies for identification purposes, there is a broader implication in the results discussed above; thus the indole compounds offer new possibilities for examining the interactions between solute and solvent and the effects of substituents on the excited states of these molecules.

EXPERIMENTAL[28]

With J. A. Bilbao.

Purification of indole compounds. Compounds I, II, III, VI, VII, IX, X, and 3-acetylindole were obtained commercially and purified by column chromatography, recrystallization, and vacuum sublimation. The ultraviolet absorption spectra of indole in various solvents are given in Fig. 1. The other seven compounds gave the following ultraviolet data: λ_{max} in mμ (ϵ_{max}).

2-Methylindole (II). Ultraviolet in cyclohexane: 263 (7028); 276 (5522); 289 (3765).

3-Methylindole (III). Ultraviolet in cyclohexane: 272 (5300); 279 (5535); 282 (5107); 290 (4447). Ultraviolet in ethanol:[8] 275 (4550); 282.5 (4910); 290 (4120).

3-Hydroxymethylindole (VI). Ultraviolet in cyclohexane: 265 (5810); 288 (3740). Ultraviolet in dioxane: 270 (5610); 288 (4540). Ultraviolet in ethanol: 270 (5080); 288 (4010). Ultraviolet in water: 270 (5350); 285 (4010).

Indole-3-acetic acid (VII). Ultraviolet in cyclohexane: 266 (4293); 277 (3975); 288 (3180). Ultraviolet in ethanol:

(28) All melting points are corrected.

280 (8427); 287 (7155). Ultraviolet in water: 280 (8745); 287 (7473).

1-Methyl-2-phenylindole (IX). Ultraviolet in cyclohexane: 297 (22,910). Ultraviolet in dioxane: 297 (22,550). Ultraviolet in ethanol: 297 (21,840).

Carbazole (X). Ultraviolet in cyclohexane: 231 (35,160); 243 (19,780); 253 (12,750); 258 (4400); 280 (8800); 285 (10,560); 289 (15,380); 306 (2510); 317 (3160); 330 (2940). Ultraviolet in hexane[6]: 242 (24,000); 255 (12,000); 273 (4300); 279.5 (12,650); 285.3 (14,000); 291 (19,000); 316.8 (3000). The positions of the 289, 317, and 330 mμ bands in the cyclohexane ultraviolet spectrum agree well with the three fluorescence excitation peaks of carbazole in cyclohexane.

3-Acetylindole. Ultraviolet in cyclohexane: 233 (7250); 249 (weak shoulder); 280 (5800). Ultraviolet in dioxane: 237 (9400); 252 (6380); 288 (11,020). Ultraviolet in ethanol: 240 (12,850); 257 (9300); 293 (12,620). Ultraviolet in water: 241 (7970); 257 (8250); 296 (11,740).

1,2-Dimethylindole (IV). The procedure used by Kissman *et al.*[29] for the synthesis of several alkylindoles (but not including 1,2-dimethylindole) was used to prepare this compound from acetone and 1-methyl-1-phenylhydrazine in the presence of polyphosphoric acid. The product was purified by chromatography on activated alumina with petroleum ether (b.p. 30–60°) as eluent followed by vacuum sublimation at 25°/0.05 mm. to give colorless crystals, m.p. 55° (reported[30] m.p. 56°).

Anal. Calcd. for $C_{10}H_{11}N$: C, 82.83; H, 7.65. Found: C, 83.07; H, 7.90.

Ultraviolet in cyclohexane: 272 (10,810); 280 (11,340); 290 (9210). Ultraviolet in dioxane: 272 (shoulder); 280 (11,100); 290 (9100). Ultraviolet in ethanol: 272 (shoulder); 280 (11,100); 290 (8680). Ultraviolet in water: 272 (shoulder); 280 (10,540); 290 (shoulder).

2,3-Dimethylindole (V). The procedure of Kissman *et al.*[29] was used to prepare this compound. The product was purified by crystallization from cyclohexane followed by vacuum sublimation, m.p. 100–102° (reported[29] m.p. 100–102°). Ultraviolet in cyclohexane: 271 (6412); 282 (6254); 290 (4335). Ultraviolet in ethanol[8]: 283 (6860); 290 (5980).

2-(3-Indolyl)-2,3-dihydroindole (VIII). The previously described method[31,32] was used to prepare this compound. The base was purified by crystallization from cyclohexane, m.p. 107–108° (reported[31] m.p. 108°).

Anal. Calcd. for $C_{16}H_{14}N_2$: C, 82.12; H, 6.03; N, 11.97. Found: C, 82.37, H, 6.29; N, 11.76.

Ultraviolet in cyclohexane: 245 (13,170); 282 (9350); 286 (9350); 305 (shoulder). Ultraviolet in dioxane: 245 (13,170); 282 (9350); 286 (9350). Ultraviolet in ethanol: 245 (11,470); 282 (9350); 286 (9350). Ultraviolet in water: 282 (10,620); 286 (9770).

Solvents. Spectroscopically pure cyclohexane, chloroform, dioxane (Matheson, Coleman, and Bell), benzene and ethanol (prepared in this laboratory) were used in this work. All solvents were regularly checked for purity by fluorescence and ultraviolet absorption.

Instrumentation. The Farrand automatic recording spectrofluorometer, used in this work, is equipped with a Hanovia, 150-watt, xenon arc source and a R.C.A. 1P28 photomultiplier tube. The sample can be irradiated at any desired wave length between 220 and 650 mμ through a grating monochromator. The measuring monochromator can be used at any chosen wave length in the same range. Except where stated otherwise, 5-mμ slits were used. A quartz fluorescence macrocell (10 × 20 × 50 mm.), requiring approximately 7 ml. of solution, was used for all measurements. All the spectra were obtained at room temperature and oxygen was not excluded. The instrument was calibrated daily for wave length and fluorescence intensity with quinine sulfate in 0.1*N* sulfuric acid. Ultraviolet absorption spectra were obtained with a Beckman DU spectrophotometer equipped with a Process and Instruments Co. automatic recording unit.

Acknowledgment. This work was aided by a grant from the American Cancer Society, Inc., New York. The spectrofluorometer used in the work was purchased through a field investigation grant, No. CS-9577 from the Public Health Service, National Institutes of Health of the U. S. Department of Health, Education and Welfare, National Cancer Institute.

NEW YORK 16, N. Y.

(29) H. M. Kissman, D. W. Farnsworth, and B. Witkop, *J. Am. Chem. Soc.*, 74, 3948 (1952).

(30) L. Marion and C. W. Oldfield, *Can. J. Res.*, 25B, 1 (1947).

(31) O. Schmitz-Dumont and B. Nicolojannis, *Ber.*, 63, 323 (1930).

(32) For structure proof of this compound see: A. F. Hodson and G. F. Smith, *J. Chem. Soc.*, 3544 (1957).

[CONTRIBUTION FROM THE PHYSICS LABORATORY, MATERIALS CENTRAL OF WRIGHT AIR DEVELOPMENT DIVISION]

Effect of *cis-trans* Isomerism on the Urea Inclusion Compound Forming Ability of a Molecule; Study of the Maleate-Fumarate System

JACK RADELL, JOSEPH W. CONNOLLY, AND WILLIAM R. COSGROVE, JR.

Received October 24, 1960

Several maleates and fumarates were investigated to determine their ability to form urea inclusion compounds. Olefinic esters of both acids will form inclusion compounds. However, the shortest chain required to stabilize a particular ester was related to the over-all cross-sectional diameter of the ester. The fumarates, having the more slender conformation, form complexes more readily than the maleates.

Urea inclusion compounds are combinations of two or more compounds, one (guest) of which is contained within the crystalline framework of the other (host). The guest and host are capable of existing separately, and do not unite chemically. They are held together by secondary valence forces and by hydrogen bonding. Unlike ordinary hydrogen-bonded complexes, the size and shape of the