(25) A similar anomaly has been noted for methyl 3'-O-methylsulfonyl-N-methylthymidine-5'-uronate.[18] Contrastingly, such a difference is not seen in the corresponding uridine derivative **10q**.
(26) W. Saenger and D. Suck, *Nature (London)*, **242**, 610 (1973).
(27) W. Regel, E. Stengele, and H. Seliger, *Chem. Ber.*, **107**, 611 (1974.)
(28) J. Žemlička, R. Gasser, J. V. Freisler, and J. P. Horwitz, *J. Am. Chem. Soc.*, **94**, 3213 (1972).
(29) J. Defaye, M. Naumberg, and T. Reyners, *J. Heterocycl. Chem.*, **6**, 229 (1969).
(30) K. C. Murdock and R. B. Angier, *J. Am. Chem. Soc.*, **84**, 3758 (1962).
(31) J. P. Horwitz, J. Chua, M. A. DaRooge, M. Noel, and I. L. Klundt, *J. Org. Chem.*, **31**, 205 (1966).
(32) G. Giovanetti, L. Nobile, M. Amorosa, and J. Defaye, *Carbohydr. Res.*, **21**, 320 (1972).
(33) D. M. Brown, A. R. Todd, and S. Varadarajan, *J. Chem. Soc.*, 2388 (1956).
(34) D. M. Brown, A. R. Todd, and S. Varadarajan, *J. Chem. Soc.*, 868 (1957).
(35) J. P. H. Verheyden and J. G. Moffatt, *J. Org. Chem.*, **35**, 2319 (1970).
(36) J. P. H. Verheyden and J. G. Moffatt, *J. Org. Chem.*, **35**, 2868 (1970).
(37) A. F. Cook and J. G. Moffatt, *J. Am. Chem. Soc.*, **89**, 2697 (1967).
(38) A. M. Michelson and A. R. Todd, *J. Chem. Soc.*, 951 (1953).
(39) R. E. Belz and D. M. Visser, *J. Am. Chem. Soc.*, **77**, 736 (1955).
(40) P. A. Levene and R. S. Tipson, *J. Biol. Chem.*, **104**, 385 (1934).

Synthetic Spectroscopic Models. Intramolecular Stacking Interactions between Indole and Connected Nucleic Acid Bases. Hypochromism and Fluorescence[1-3]

Kiyoshi Mutai, Bruce A. Gruber, and Nelson J. Leonard*

Contribution from the Roger Adams Laboratory, School of Chemical Sciences, University of Illinois, Urbana, Illinois 61801. *Received January 27, 1975*

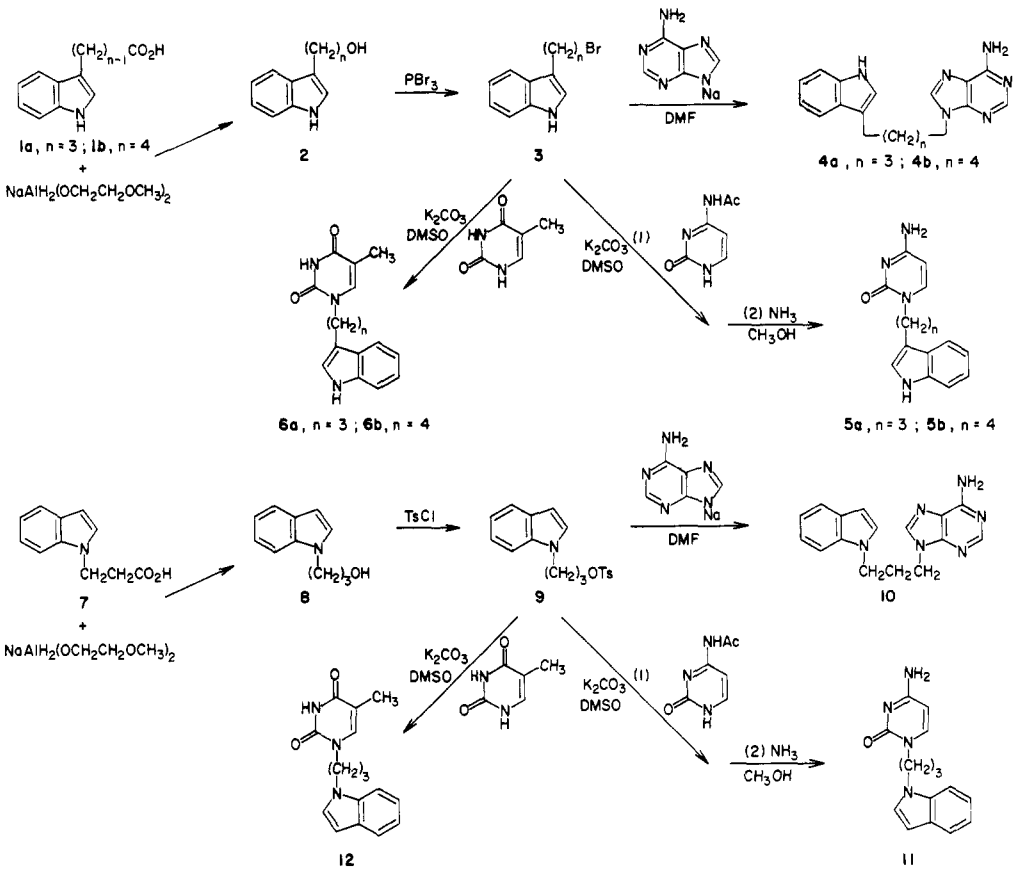
Abstract: Stacking interactions between indole, as a neutral representative of tryptophan, and the nucleic acid bases have been observed in aqueous solution at 25° by means of hypochromism and fluorescence emission. This was accomplished by synthesizing and utilizing compounds in which indole and the nucleic acid bases adenine, cytosine, guanine, and thymine are connected by a three-atom or four-atom bridge, particularly the trimethylene bridge. The degree of interaction between indole and the purine bases was found to be of the same order as that between two purine bases themselves. For the Ind-$(CH_2)_3$-Base models which allow plane parallelism of the two units, the percentage of internally stacked vs. unfolded conformations was determined from fluorescence quantum yield and lifetime measurements, which gave a decreasing order of complexation with indole of adenine ≈ guanine > thymine ≫ cytosine. The equilibrium between stacked and unfolded conformations for the indole/adenine, guanine, or thymine cases indicates ΔG near zero. On the basis of our results, total fluorescence quenching of the indole of tryptophan in a polypeptide or protein is to be expected if it comes into close proximity with a base moiety of a nucleic acid or if intercalation occurs.

The binding of proteins to nucleic acids involves electrostatic forces, hydrogen bonding, and π-overlap or stacking interactions,[4-6] all of which depend upon the accommodating sizes, shapes, and spacings of the interacting units. Among the specific stacking interactions[3,4,7-18] which may contribute to the positioning of protein with respect to nucleic acid, that of tryptophan or related indolic compounds with nucleic acid bases has been demonstrated (1) by complexation of the indole derivative with DNA, RNA, or poly A,[19-23] (2) by the quenching of tryptophan fluorescence in the binding of aminoacyl-tRNA synthetases and tRNA's,[24-28] (3) by ^{1}H NMR studies of aqueous solutions, especially acidic solutions, of tryptophan and other indole derivatives with nucleic acid bases,[29,30] and (4) by reflectance and luminescence studies of complex formation between tryptophan and nucleic acid components in aggregates formed in frozen aqueous solutions.[31-33]

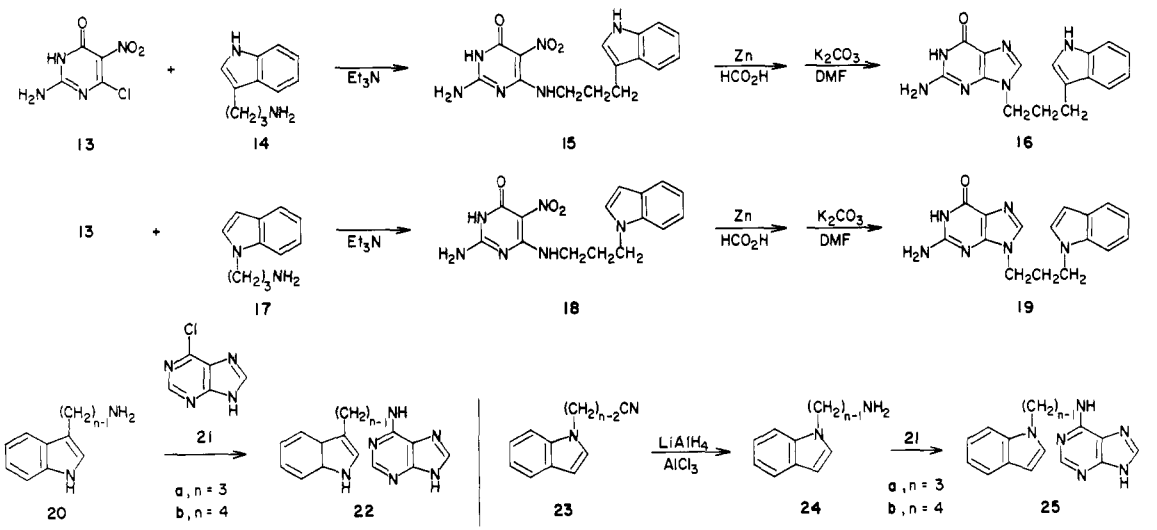
Although the accumulated information is impressive, we sought to avoid certain of the limitations inherent in each set of experiments (and no doubt substituting different limitations of our own) by selecting suitable spectroscopic models for the observation of stacking interactions between indole (as an uncharged tryptophan) and the nucleic acid bases in dilute, neutral aqueous solution. We therefore chose a system that would permit intramolecular stacking, but not hydrogen-bonding, interactions which would be detectable by both ultraviolet and fluorescence spectroscopy. In the past, we have used polymethylene bridges, and in particular the trimethylene bridge, $-(CH_2)_3-$, as synthetic spacers to study intramolecular interactions between nucleic acid bases.[34] These bridges also provide the possibility of further controlling the inter-ring interactions by attachment of the chain to different positions on the heterocyclic termini.[3,16] Accordingly, we have synthesized compounds in which indole and the nucleic acid bases adenine, cytosine, guanine, and thymine are connected by a three-atom or four-atom bridge.[35] Attachments are at the 1 or 3 position of the indole and at the 9 position of adenine and guanine, the 1 position of cytosine and thymine, and also the N^6 position of adenine. The simple bases rather than the nucleosides were chosen so that we could survey the heteroaromatic interactions in the absence of additional factors involving the carbohydrate and phosphate linkages. With these models we could determine the degree of interaction between indole and nucleic acid base with respect to that between two nucleic acid bases, the degree of quenching of indole fluorescence by a nucleic acid base, and the equilibrium between folded or stacked conformations and open conformations.

Synthesis. General procedures for the linking of two different heterocyclic bases by a polymethylene bridge have been described previously,[34] including those for alkylation of adenine at the 9 position and cytosine and thymine at the 1 position. We have adapted these procedures by first preparing 3-(indol-3-yl)propyl and 4-(indol-3-yl)butyl bromides (**3a,b**) from the corresponding acids **1a,b** by reduction to the alcohols **2a,b** and displacement and then using the bromides to prepare the corresponding (indol-3-yl)alkyladenine, -cytosine, and -thymine products (**4–6**, Scheme I). While the alkylation of thymine may lead to mixtures of

Scheme I



Scheme II



1- and 3-substituted thymines, it was possible to ascertain that the products of our synthesis were 1-substituted (**6a,b**) by the observed pH dependence of their uv spectra.[36,37] 3-(Indol-1-yl)propyl *p*-toluenesulfonate (**9**), prepared by a sequence including reduction of the acid **7** to the alcohol **8**, was the preferred alkylating agent for the preparation of 3-(indol-1-yl)propyladenine, -cytosine, and -thymine (**10–12**).

The guanine derivatives **16** and **19** were prepared by ring-closure procedures similar to those reported earlier,[34] starting with the reaction of 2-amino-6-chloro-5-nitro-4(3*H*)-pyrimidinone (**13**) with indolylalkylamines (**14** and **17**) followed by reduction of the nitro compounds (**15** and **18**) and ring closure (Scheme II). The reduction with zinc dust and formic acid had to be monitored carefully because of variability in the activity of the zinc dust and because overreduction leads to contamination of the product with 2,3-dihydroindole (indoline) material. Liquid chromatography was used to ensure purity in the guanine cases **16** and **19** along with mass spectra that showed molecular ion peaks at *m/e* 308, with no intensity at *m/e* 310 above that dictated by normal isotope abundance. The N^6-(indol-3-yl)alkyladenines (**22**) and N^6-(indol-1-yl)alkyladenines (**25**) were made by the reaction of 6-chloropurine (**21**) with the appropriate indolylalkylamines [**20**, **24** (from **23**)] in excess. In all cases the final products were scrupulously purified by recrystallization and chromatography, and their purity was checked by microanalysis and by thin-layer chromatography using several solvent systems, also by mass spectrome-

try and fluorescence lifetime as necessary. The structures of the products were confirmed by their uv and NMR spectra.[38]

Experimental Section

All melting points are uncorrected. Nuclear magnetic resonance spectra were determined on a Varian Associates A-60A spectrometer using tetramethylsilane as internal standard. Electronic absorption spectra were recorded on a Cary Model 15 spectrophotometer. Microanalyses were performed by Mr. J. Nemeth and his staff, who also weighed samples for quantitative electronic absorption studies. Homogeneity of the compounds was established by TLC on silica gel with fluorescence indicator (Eastman Chromagram sheets) in the following solvent systems: A, chloroform–isopropyl alcohol (4:1); B, chloroform–methanol (5:1) with a small amount (a few drops/10 ml) of concentrated ammonia; C, chloroform–methanol with a small amount (a few drops/10 ml) of acetic acid. Mass spectra were determined by Mr. J. Wrona using a Varian-MAT Model CH-5 low-resolution spectrometer.

Electronic Absorption. For quantitative measurements, $2-4 \times 10^{-5}$ *M* 1% ethanol–99% water solutions were prepared by dissolving 0.002–0.004 mol of each compound in 10 ml of absolute ethanol and diluting 1 ml of the solution to 100 ml with distilled water. Spectra were determined in 20-mm cells in the region of 240–310 nm and in 10-mm cells in the shorter wavelength region. Spectra were measured twice at a minimum, and averaged values of ϵ_{max} are reported. In the region 190–210 nm the base line was somewhat unstable even though the spectrometer was purged with nitrogen and solutions were degassed by bubbling with nitrogen. The spectra showed reproducibility of ±5% in absorbance in the 200 ± 10-nm region. Oscillator strengths were obtained in the region of 250–310 nm for cytosine derivatives and 240–310 nm for the others by the procedure reported earlier.[3,16,34]

Fluorescence Emission. Relative quantum efficiencies were determined on a Perkin-Elmer Hitachi Model MFP-2A spectrofluorometer. The technical emission spectra obtained at 25° were not corrected for monochromator efficiency and photomultiplier response since we were dealing only with indole fluorescence and at the same level in every case. Fluorescence lifetimes were determined using the cross-correlation spectrofluorometer described previously,[39,40] using excited light modulated at 14.2 MHz. The exciting light was filtered through a monochromater and a CS-7-54 Corning filter, and emission was observed through a CS-0-54 Corning filter. The purity of the compounds for fluorescence was determined analytically by high performance liquid chromatography using an Aminex A-5 cation exchange resin, equilibrated with 0.3 *M* NH_4HCO_2, 25% DMF, pH 5. The elution solvent was the same. Fluorescence lifetimes were reproducible to ±30 psec in two or more runs for each compound.

Overlap spectra of fluorescence emission of Ind^3-C_3 and Ind^3-C_3-Ade^9 and difference spectra of emission of Ind^3-C_3 + Ade^9-C_3 and Ind^3-C_3-Ade^9 were determined on a photon counting scanning spectrofluorometer,[41,42] interfaced to a Nuclear Data ND4410 Data Acquisition System so that the spectra could be stored in memory and displayed either singly or overlapped for multiple display. Spectra could be normalized, added, subtracted, multiplied, divided, etc., by teletype commands.

Material Synthesis. 9-Propyladenine,[16,34,43,44] 1-propylcytosine,[34] 1-propylthymine,[3,34] 9-propylguanine,[34] 6-propylaminopurine,[16,45] 3-propylindole,[46] and 1-propylindole[47] had been prepared before.

3-(Indol-3-yl)propyl and 4-(Indol-3-yl)butyl Derivatives of Adenine (4), Cytosine (5), and Thymine (6). These compounds were prepared by the reaction of the corresponding (indol-3-yl)alkyl bromide (**3**)[48,49] with adenine, cytosine, and thymine in the same way as described in the literature.[34] The position of substitution on thymine was confirmed by the fact that no new peak appeared and no blue shift of the peak was observed in acidic solution (5 drops of 1 *N* hydrochloric acid/4 ml of 95% ethanol solution) which would have been indicative of 3N substitution.[36,37] The physical constants and the microanalytical data are given in Table I and the quantitative ultraviolet spectra in Table II.

3-(Indol-1-yl)propyl Alcohol (8). To a stirred solution of 250 g (70% solution in benzene, 0.87 mol) of sodium bis(methoxyethoxy)aluminum hydride, $NaAlH_2(OCH_2CH_2OCH_3)_2$, diluted with

Table I. Yields and Physical and Analytical Data

Compound	Abbreviation	Yield, %	Mp, °C	Mol formula	C, %		H, %		N, %	
					Calcd	Found	Calcd	Found	Calcd	Found
9-[3-(Indol-1-yl)propyl] adenine (**10**)	Ind^1-C_3-Ade^9	66	189–191	$C_{16}H_{16}N_6$	65.73	65.68	5.52	5.47	28.75	28.53
9-[3-(Indol-3-yl)propyl] adenine (**4a**)	Ind^3-C_3-Ade^9	19	236–238	$C_{16}H_{16}N_6$	65.73	65.53	5.52	5.50	28.75	28.63
9-[4-(Indol-3-yl)butyl] adenine (**4b**)	Ind^3-C_4-Ade^9	69	197–199	$C_{17}H_{18}N_6$	66.64	66.54	5.92	5.92	27.43	27.49
1-[3-(Indol-1-yl)propyl] cytosine (**11**)	Ind^1-C_3-Cyt^1	67	259–260	$C_{15}H_{16}N_4O$	67.14	66.87	6.01	6.00	20.88	20.99
1-[3-(Indol-3-yl)propyl] cytosine (**5a**)	Ind^3-C_3-Cyt^1	48	217–219	$C_{15}H_{16}N_4O$	67.14	66.84	6.01	6.01	20.88	20.68
1-[4-(Indol-3-yl)butyl] cytosine (**5b**)	Ind^3-C_4-Cyt^1	34	236–237	$C_{16}H_{18}N_4O$	68.06	68.08	6.43	6.45	19.85	19.56
9-[3-(Indol-1-yl)propyl] guanine (**19**)	Ind^1-C_3-Gua^9	67	265–267	$C_{16}H_{16}N_6O$	62.32	62.43	5.23	5.24	27.26	27.30
9-[3-(Indol-3-yl)propyl] guanine (**16**)	Ind^3-C_3-Gua^9	47	275–284[a]	$C_{16}H_{16}N_6O$	62.32	62.33	5.23	5.11	27.26	26.99
6-[2-(Indol-1-yl)ethylamino] purine (**25a**)	Ind^1-C_2N-Pur^6	56	204–205,[b] 210–211	$C_{15}H_{14}N_6$	64.73	64.50	5.07	5.03	30.20	30.49
6-[3-(Indol-1-yl)propylamino] purine (**25b**)	Ind^1-C_3N-Pur^6	87	203.5–205,[b] 213–213.5	$C_{16}H_{16}N_6$	65.73	65.76	5.52	5.61	28.75	28.72
6-[2-(Indol-3-yl)ethylamino] purine (**22a**)	Ind^3-C_2N-Pur^6	50	242–243[c]	$C_{15}H_{14}N_6$	64.73	64.82	5.07	5.05	30.20	30.45
6-[3-(Indol-3-yl)propylamino] purine (**22b**)	Ind^3-C_3N-Pur^6	77	216–217	$C_{16}H_{16}N_6$	65.73	65.54	5.52	5.64	28.75	28.77
1-[3-(Indol-1-yl)propyl] thymine (**12**)	Ind^1-C_3-Thy^1	11	139.5–140.5	$C_{16}H_{17}N_3O_2$	67.82	67.65	6.05	6.02	14.83	14.87
1-[3-(Indol-3-yl)propyl] thymine (**6a**)	Ind^3-C_3-Thy^1	42	213–214	$C_{16}H_{17}N_3O_2$	67.82	67.55	6.05	6.01	14.83	14.80
1-[4-(Indol-3-yl)butyl] thymine (**6b**)	Ind^3-C_4-Thy^1	33	191–193	$C_{17}H_{19}N_3O_2$	68.66	68.38	6.44	6.53	14.13	14.15

[a] Color begins to change at ca. 265°. [b] Probably polymorphs. [c] Lit.[52] 241°.

Table II. Quantitative Electronic Absorption Data of Indole Derivatives in 1% Ethanol[a]

Compound	λ_{max}	ϵ	λ_{min}	ϵ	λ_{max}	ϵ	λ_{min}	ϵ	λ_{max}	ϵ	λ_{max}	ϵ
Ind^1-C_3	197	21,800	204	20,000	219	33,900	240	2,100	279 (br)	5,500	~293 (sh)	4,000
Ind^1-C_3-Ade[9] (10)					212	39,800	236	7,300	262.5	16,000	~293 (sh)	3,800
Ind^1-C_3-Cyt[1] (11)	198	41,700	207.5	32,000	218	39,200	246	13,100	274	13,100		
Ind^1-C_3-Gua[9] (19)	190	40,300			205	34,300	236	8,900	254	12,700	271	11,900
					218	30,500						
Ind^1-C_3-Thy[1] (12)	200	28,800	205	28,100	218	35,900	240	4,800	272	12,600		
Ind^1-C_2N-Pur[6] (25a)					212.5	41,700	238	6,600	270	20,000		
Ind^1-C_3N-Pur[6] (25b)					213	40,700	238	6,100	269	19,100		
Ind^3-C_3	195	19,500	205	15,600	221	28,500	244	1,900			280	4,800
											288 (sh)	4,200
Ind^3-C_3-Ade[9] (4a)			204	35,000	215	37,000	237.5	6,700	266	15,300	290 (sh)	4,300
Ind^3-C_4-Ade[9] (4b)			205	35,000	215	36,900	237.5	7,200	263	15,600	290 (sh)	4,300
Ind^3-C_3-Cyt[1] (5a)	199	39,600	207.5	28,400	220	38,800	247	5,700	276	12,900	~288	9,300
Ind^3-C_4-Cyt[1] (5b)	197	40,600	208	28,200	221	39,800	246	5,600	276	13,200	~288	9,300
Ind^3-C_3-Gua[9] (16)	194	38,800	206	33,600	209	32,500	236	9,200	255	12,500	273	11,500
					~222 (sh)	29,000						
Ind^3-C_3-Thy[1] (6a)	196	26,600	205	23,500	220	34,100	241	4,300	273	12,300	~290	7,600
Ind^3-C_4-Thy[1] (6b)	196	25,500	205	22,600	221	34,800	240	3,900	273	13,100	~290	8,000
Ind^3-C_2N-Pur[6] (22a)	~197 (sh)	31,000			215	39,800	237	5,800	270	20,600		
Ind^3-C_3N-Pur[6] (22b)	198 (sh)	29,400	202	29,300	214	36,000	240	5,800	270	19,000		

[a] Abbreviations: λ, wavelength in nm; ϵ, molar extinction coefficient; sh, shoulder; br, broad.

400 ml of dry benzene was added 63 g (0.33 mol) of 3-(indol-1-yl)propionic acid (**7**)[50] in small portions at room temperature during 1 hr. The solution was heated at reflux for 2 hr, and the reaction mixture was decomposed with a mixture of 500 ml of water and 150 ml of concentrated hydrochloric acid. The organic layer was separated from the aqueous layer, and the latter was extracted with two 100-ml portions of ether. The extracts combined with the organic layer were washed with 200 ml of water, two 200-ml portions of 5% aqueous sodium carbonate, and 200 ml of water, in that order, and dried over anhydrous sodium sulfate. After removal of the solvents in vacuo, the residue was distilled under reduced pressure to yield 52.2 g (89%) of a pale yellow liquid: bp 162–163° (2.5 mm); $n^{18}D$ 1.6020.

Anal. Calcd for $C_{11}H_{13}NO$: C, 75.45; H, 7.48; N, 8.00. Found: C, 75.52; H, 7.39; N, 8.11.

3-(Indol-1-yl)propyl Derivatives of Adenine (10) and Cytosine (11). These compounds were prepared in the same way as were the 3-indolyl derivatives except that the *p*-toluenesulfonate (**9**) of the 1-indolyl derivative **8** was used instead of the bromide.

3-(Indol-1-yl)propyl *p*-Toluenesulfonate (9).[51] To a stirred solution of 5.3 g (30 mmol) of 3-(indol-1-yl)propyl alcohol (**7**) in 50 ml of dry pyridine cooled at 0° with an ice–water bath was added 8.5 g (45 mmol) of *p*-toluenesulfonyl chloride in small portions during 20 min. The resulting yellow solution was placed in a refrigerator for more than 12 hr. The entire mixture was poured with stirring into 300 ml of ice–water, and the precipitate was collected by filtration and washed with cold water. This crude ester was suspended in 60 ml of petroleum ether (bp 30–60°) and dissolved by adding a minimum amount of dichloromethane at room temperature. The solution was kept overnight at −20° and then filtered, yielding 8.35 g (84%) of analytically pure ester: mp 62.5–64°.

Anal. Calcd for $C_{18}H_{19}NO_3S$: C, 65.64; H, 5.81; N, 4.25. Found: C, 65.77; H, 5.75; N, 4.30.

1-[3-(Indol-1-yl)propyl]thymine (12). A mixture of 4.95 g (15 mmol) of 3-(indol-1-yl)propyl *p*-toluenesulfonate (**9**), 3.8 g (30 mmol) of thymine, and 2.1 g (15 mmol) of potassium carbonate in 100 ml of dimethylformamide (dried over molecular sieves) was stirred at room temperature under nitrogen for 36 hr. After filtration, solvent was removed from the filtrate in vacuo, leaving a pale brown solid. The solid was suspended in 250 ml of water, and the suspension was extracted with four 150-ml portions of chloroform. The combined extracts were dried over sodium carbonate and evaporated to dryness in vacuo, leaving a viscous brown oil. The oil was dissolved in 20 ml of hot methanol, and the solution was left overnight at −20°. The upper layer was separated by decantation from an oil precipitate, and the solution was allowed to stand at room temperature with occasional scratching of the wall of the flask to induce crystallization.

6-[2-(Indol-3-yl)ethylamino]purine (*N*⁶-12-(Indol-3-yl)ethyl]adenine) (22a).[52] A mixture of 5.8 g (36 mmol) of tryptamine and 2.8 g (18 mmol) of 6-chloropurine (**21**) in 30 ml of 2-methoxyethanol was heated at reflux for 2.5 hr. After cooling, the solvent was removed in vacuo, and the residue was poured into 100 ml of 5% aqueous sodium carbonate. The precipitate was collected by filtration and was refluxed with 100 ml of absolute ethanol. The resulting suspension was filtered while hot to collect the crude product. The amorphous solid was recrystallized from acetone–ethanol–water (approximately 1:1:1).

6-[3-(Indol-3-yl)propylamino]purine (*N*⁶-[3-(3-Indolyl)propyl]adenine) (22b). This compound was obtained by the same procedure as described for *N*⁶-[2-(indol-3-yl)ethyl]adenine (**22a**).

1-(Cyanomethyl)indole (23a).[53] To a stirred solution of 35.2 g (0.30 mol) of indole in 200 ml of dry dimethylformamide was added 12.2 g (0.30 mol) of sodium hydride (59% dispersion in mineral oil) under nitrogen. Stirring was continued for 1 hr, when the suspension became a solid mass. Chloroacetonitrile (22.6 g, 0.30 mol) in 20 ml of dimethylformamide was introduced at room temperature. Heat was evolved in the reaction, and the solid mass became a dark brown solution. The solution was stirred for 2 hr at room temperature, and the solvent was evaporated in vacuo. The residue was poured into 300 ml of water, and the suspension was extracted with three 100-ml portions of ether. The combined extracts were dried over sodium sulfate, and the solvent was removed in vacuo. The residue was distilled under reduced pressure: bp 150–152° (1.5 mm); mp 75.5–76.5° (lit.[53] 75.5–77.0°); yield, 12.3 g (26%).

6-[2-(Indol-1-yl)ethylamino]purine (N^6-[2-(Indol-1-yl)ethyl]adenine) (25a). A mixture of 1.0 g (6.5 mmol) of 6-chloropurine (**21**) and 2.5 g (15.6 mmol) of 2-(indol-1-yl)ethylamine (**24a**)[54] in 20 ml of 2-methoxyethanol was heated at reflux with stirring for 3 hr. After removal of the solvent in vacuo, the liquid residue was mixed with 20 ml of ether and the upper layer was decanted. This procedure was repeated three times. The remaining amorphous white solid was suspended in 30 ml of water containing 1.4 g (10 mmol) of potassium carbonate and was warmed with stirring for 30 min. The suspension was filtered, and the collected solid was washed with benzene and ether. Recrystallization from aqueous ethanol gave 1.0 g of microcrystalline, colorless solid.

6-[3-(Indol-1-yl)propylamino]purine (N^6-[3-(Indol-1-yl)propyl]adenine) (25b). This compound was prepared in the same way as described for the lower homolog **25a.**

2-Amino-6-[3-(indol-3-yl)propylamino]-5-nitro-4(3*H*)-pyrimidinone (15). A solution of 3.8 g (20 mmol) of 2-amino-6-chloro-5-nitro-4(3*H*)-pyrimidinone (**13**), 3.5 g (20 mmol) of 3-(3-aminopropyl)indole (**14**), and 2.5 g (25 mmol) of triethylamine in 2.8 l. of methanol was stirred at room temperature for 24 hr. The solution was concentrated in vacuo to 1.0 l. and then cooled to −20°, yielding 4.8 g (73%) of pale yellow amorphous solid: mp 260–261° dec.

Anal. Calcd for $C_{15}H_{16}N_6O_3$: C, 54.87; H, 4.91; N, 25.60. Found: C, 54.61; H, 4.98; N, 25.35.

9-[3-(Indol-3-yl)propyl]guanine (16). To a suspension of 1.64 g (5 mmol) of 2-amino-6-[3-(indol-3-yl)propylamino]-5-nitro-4(3*H*)-pyrimidinone (**15**) in 250 ml of 98–100% formic acid was added 21.0 g (0.33 g-atom) of zinc dust, and the resulting suspension was stirred for 30 min at room temperature. The reaction mixture was filtered under nitrogen, and the filtrate was evaporated in vacuo, leaving a brown oil which was immediately dissolved in 400 ml of dry deoxygenated dimethylformamide. The solution was concentrated to 250 ml to ensure complete removal of formic acid and water. A 2.5-g (18 mmol) portion of anhydrous potassium carbonate was added, and the resulting suspension was stirred at reflux under nitrogen for 33 hr. The brown suspension was filtered, and the filtrate was evaporated to dryness in vacuo. The residue was dissolved in 150 ml of concentrated aqueous ammonia by warming. After filtration of insoluble material, the filtrate was neutralized with acetic acid, yielding a pale yellow amorphous solid which was recrystallized from *n*-propyl alcohol–water. Warming of the solid at the boiling point of xylene in vacuo (<1 mm) gave an analytically pure solid.

2-Amino-6-[3-(indol-1-yl)propylamino]-5-nitro-4(3*H*)-pyrimidinone (18) was prepared essentially as described for the indol-3-yl analog **15:** mp 286° dec; yield, 52%.

Anal. Calcd for $C_{15}H_{16}N_6O_3$: C, 54.87; H, 4.91; N, 25.60. Found: C, 54.98; H, 5.00; N, 25.76.

9-[3-(Indol-1-yl)propyl]guanine (19) was prepared as described for the indole-3-yl analog **16** except that the 98–100% formic acid–zinc dust suspension was stirred for 10 min at room temperature and immediately filtered under nitrogen. With longer reaction times (depending on the activity of the zinc dust), some 9-[3-(indolin-1-yl)propyl]guanine was obtained following the ring-closure step. The mixture of **19** and the corresponding indolinyl compound is separable by high performance liquid chromatography using Aminex A-5 cation exchange resin and 0.4 *M* NH_4HCO_2, pH 4.2, in 25% DMF. The low-resolution mass spectrum of the Ind^1-C_3-Gua^9 showed a molecular ion at *m/e* 308 and no greater intensity at *m/e* 310 than could be accounted for by the normal isotope abundance.

Results and Discussion

Hypochromism. One criterion for assessing interaction is the percent hypochromism, *H*,[57-59] which is dependent upon the degree and orientation of intramolecular stacking. In discussing the hypochromism results, we would first like to consider the initial conclusions that can be made and then to consider how these must be modified or refined in order to accommodate the fluorescence results. In order to examine the interaction between the indole ring and the pyrimidine or purine ring in the models (**4–6, 10–12, 16, 19, 22, 25**) by percentages of hypochromism, their electronic absorption spectra were compared quantitatively with the summation of the spectra of the constituent monomeric or half units, e.g., 1- or 3-propylindole and 1-propylcytosine, 1-propylthymine, 9-propyladenine, 9-propylguanine, or 6-propylaminopurine. The method had been used previously in this laboratory for assessing the stacking interactions between nucleic acid bases or coenzyme moieties.[3,16,34,60-68] The ultraviolet absorption spectra were determined at concentrations low enough to avoid intermolecular association and to ensure the observation of 1:1 interaction between the heterocyclic units only within the same molecule. It was not possible to guarantee dissolution of all the model compounds in water. Accordingly, solutions in 1% ethanol–99% water were employed after we first ascertained that the extinction coefficients of representative compounds were not affected perceptibly in going from aqueous solution to 99% aqueous solution. While hypochromism was thereby observable effectively in aqueous solution, it should be noted that the influence of ethanol as a denaturing agent for DNA,[69] single-stranded poly A,[70] and diribonucleoside phosphate models[3,16,34] prevailed with the present models. More specifically, the indole–nucleic acid base models Ind^1-C_3-Ade^9 (**10**), Ind^1-C_3-Cyt^1 (**11**), Ind^1-C_3-Thy^1 (**12**), and Ind^1-C_3N-Pur^6 (**25b**) showed no hypochromism in 95% ethanol but, rather, slight hyperchromism.

Table III. Computed Percent Hypochromism for the 240–310-nm Absorption Band[a]

Formula	Compound[c]	*H*,[b] %
4a	Ind^3-C_3-Ade^9	10.1
4b	Ind^3-C_4-Ade^9	9.2
10	Ind^1-C_3-Ade^9	9.4
16	Ind^3-C_3-Gua^9	13.1
19	Ind^1-C_3-Gua^9	16.5
6a	Ind^3-C_3-Thy^1	9.3
6b	Ind^3-C_4-Thy^1	6.4
12	Ind^1-C_3-Thy^1	9.0
5a	Ind^3-C_3-Cyt^1	(−0.2)
5b	Ind^3-C_4-Cyt^1	(−0.8)
11	Ind^1-C_3-Cyt^1	3.8
22a	Ind^3-C_2N-Pur^6	(−0.4)
22b	Ind^3-C_3N-Pur^6	8.5
25a	Ind^1-C_2N-Pur^6	5.7
25b	Ind^1-C_3N-Pur^6	11.3

[a] 250–310 nm for the cytosine compounds. 99% water–1% ethanol solution at 25°. [b] These values are internally reproducible within ±0.5 but probably should be regarded with a tolerance of ±1.0%. [c] See ref 35.

The percentages of hypochromism, *H*, in water at 25° have been calculated as in the past[3,16] for the long wavelength band, which is actually a composite band,[71-75] and are given in Table III. The first observation is that many of the model compounds do show appreciable hypochromism, indicative of intramolecular interaction between indole and the base and corresponding to folded or stacked conformations. Since the magnitude of *H* is dependent upon the thermodynamic strength of the interaction[76] and the relative orientation of the transition moments,[57,58] it cannot be used as a *precise* measure of interaction. Nevertheless, further general observations can be made and appropriate conclusions drawn from the data, which also indicate exceptions that will require explanation. The Ind^3 compounds are listed first in each group in Table III since, as 3-substituted indoles, they are closer analogs for tryptophan interaction. However, a comparison of the hypochromism values for the Ind^3-C_3- vs. the Ind^1-C_3-adenine, guanine, thymine, and cytosine models indicates that the pairs of *H* values are in the same range, as would be suggested by the geometry of attachment, since N-1 and C-3 of indole are nearly equivalent positions.[16,71] Extending the trimethylene bridge to a te-

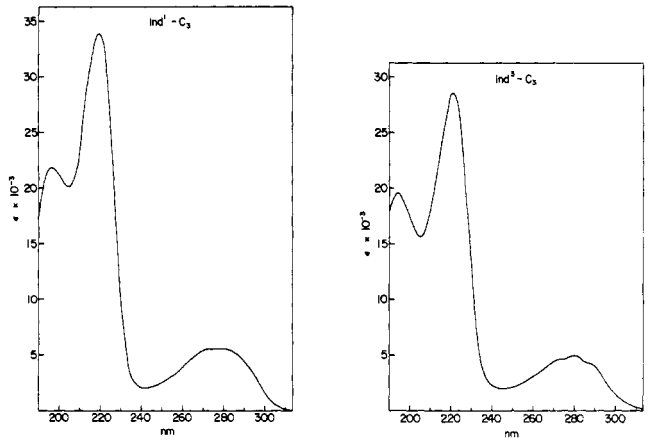


Figure 1. Ultraviolet absorption spectra in 99% water–1% ethanol solution.

tramethylene bridge between indole and base gave some reduction of hypochromism in two cases: Ind^3-C_3-Ade^9 vs. Ind^3-C_4-Ade^9 and Ind^3-C_3-Thy^1 vs. Ind^3-C_4-Thy^1, as observed previously with Thy-C_n-Thy[3] and currently in the Ade-C_n-Ade series.[77] The values of H at room temperature for Ind^3-C_3-Gua^9 and Ind^1-C_3-Gua^9 are in the same range as those observed for guanine–guanine, adenine–guanine, and adenine–adenine interaction,[34] and those for Ind^3-C_3-Ade^9 and Ind^3-C_3-Thy^1 are only slightly less. The negligible hypochromism of the indole–cytosine models is a surprise compared with the hypochromism values of the indole–guanine, adenine, and thymine models. It is also a surprise in relation to the order of increasing tendency of indole to form electron donor–acceptor complexes in aggregates in frozen aqueous solution at 77 K with guanosine < adenosine < cytidine, uridine, thymidine < adenosine cation < cytidine cation,[31,32] even though different states and different media are being compared in the two investigations. It should be mentioned, however, that zero hypochromism (for the indole–cytosine models) does not necessarily mean no interaction, as we shall see from the fluorescence quenching results.

The compound Ind^3-C_2N-Pur^6, with dimethylene bridge attached from the indole C-3 to the N^6 of adenine, or a three-atom bridge between the rings, is a tryptamine derivative. This compound (**22a**) and the other three N^6-substituted models (**22b, 25a, 25b**) are unusual and perhaps should not have been selected for study. However, once they had been made and their quantitative electronic absorption and fluorescent emission spectra had been obtained, we felt compelled to include these data even though they did not lead to definitive conclusions. Among the N^6-substituted adenine compounds, the C_3N-linked pair showed greater hypochromism than the C_2N-linked pair (zero for **22a**) and the Ind^1, greater H than the Ind^3 pair. The C_3N bridge may be of the same effective length as a three-atom bridge, as we have observed for N^6,$N^{6\prime}$-trimethylenebisadenine (15.5%) and N^6,9′-trimethylenebisadenine (16.5% hypochromism).[16] Another problem in all these N^6 models, however, lies in the fact that N-9 of the adenine is unsubstituted and therefore the tautomeric form is uncontrolled. If the indole-N^6-adenine models were to exist partially as the 7-H tautomer instead of solely as the 9-H tautomer, the effect on the ultraviolet spectra would be the same as that observed in going from N^6,9-dibenzyladenine to N^6,7-dibenzyladenine.[78] The overall change would be strongly hypochromic at the absorption maximum and hyperchromic at the long wavelength edge of the 240–310-nm band, and the net effect would be hypochromic for the entire band. This is

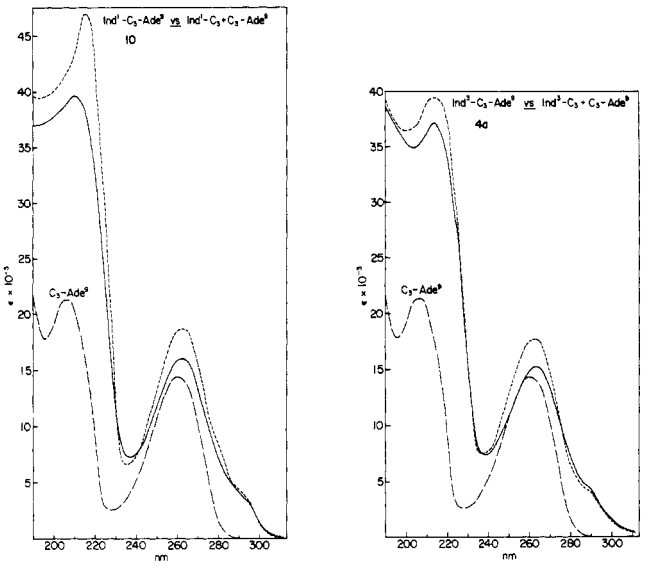


Figure 2. Ultraviolet absorption spectra in 99% water–1% ethanol solution. The full line is for the Ind-C_n-Base.

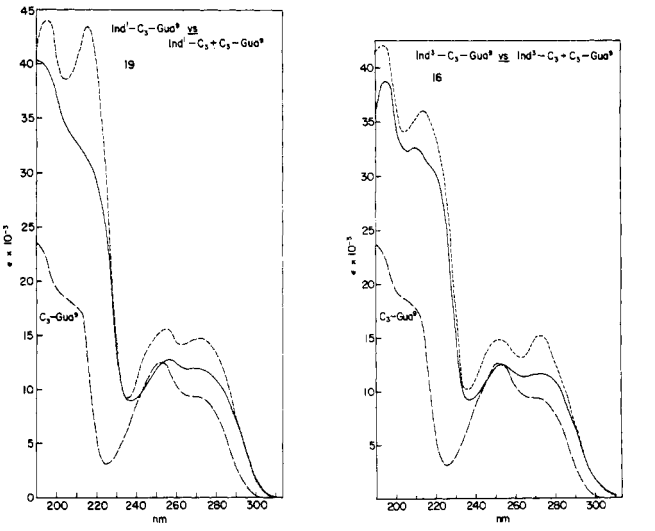


Figure 3. Ultraviolet absorption spectra in 99% water–1% ethanol solution. The full line is for the Ind-C_n-Base.

the same effect that is observed in scanning the ultraviolet curves for **22a, 25a,** and **25b** compared with the summation curves for the two halves of the molecules.

The hypochromic effects on the long wavelength band for selected indole–base models are shown graphically in Figures 1–6. In these it will be seen that in water solution there is no evidence for a charge-transfer band such as observed for indole–pyridinium[79] or indole–imidazolium[80] complexes, or for intramolecular charge-transfer complexes in compounds of types p-$NO_2C_6H_4(CH_2)_nNRAr$ and p-$NO_2C_6H_4O(CH_2)_nNRAr$ in benzene–cyclohexane.[81,82] The novelty of Figures 1–6 does not lie in the display of the hypochromism of the 240–310-nm band but rather in *the observation, for the first time, of the hypochromic effect in the short wavelength region.* The usual precautions of purging the spectrometer with nitrogen and degassing the solutions by bubbling with nitrogen were followed in order to obtain meaningful spectra in this region. We have not calculated percent hypochromism for the second intense absorption band, which is also a composite band, since the sources of error lead to greater uncertainty in the calculated oscillator strengths than for the long wavelength band.

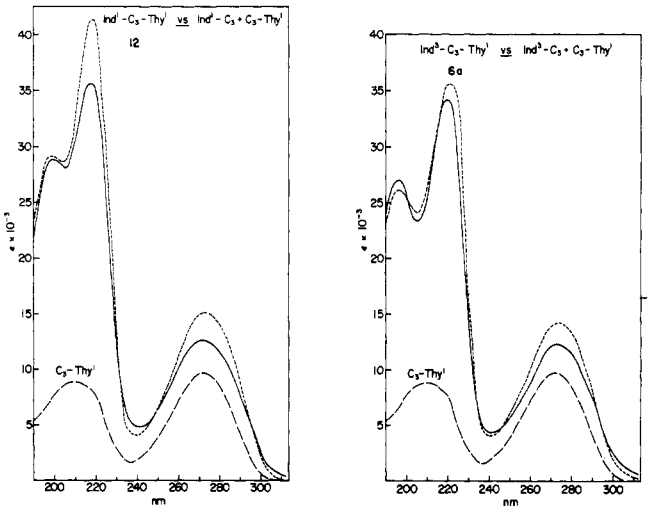


Figure 4. Ultraviolet absorption spectra in 99% water–1% ethanol solution. The full line is for the Ind-C_n-Base.

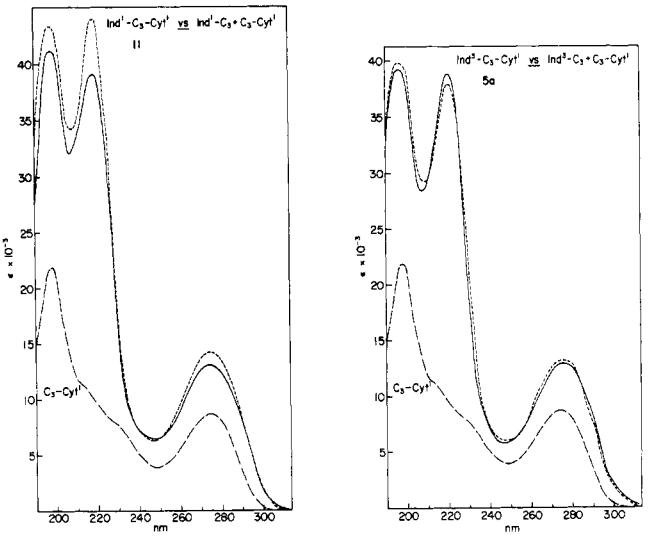


Figure 5. Ultraviolet absorption spectra in 99% water–1% ethanol solution. The full line is for the Ind-C_n-Base.

However, inspection of the spectral curves alone provides convincing indication of the general hypochromic effect in the 190–240-nm band, which appears to be greater for the Ind^1-C_3- than for the Ind^3-C_3-adenine, guanine, thymine, and cytosine models. All of the models with the exception of Ind^3-C_3-Thy^1, Ind^3-C_3-Cyt^1, and Ind^3-C_2N-Pur^6 showed appreciable hypochromism in this band, thus giving additional indication of stacking interactions. In relation to dinucleotide or DNA hypochromicity in the 190–240-nm region, theoretical predictions have not been fully resolved,[83,84] and experimentally no *hyper*chromism was observed down to 185 nm.[85,86]

Fluorescence. For the fluorescence studies, the purity of the compounds was determined analytically by high performance liquid chromatography and checked by constancy of their fluorescence lifetimes. The intramolecular quenching of the indole moiety by the nucleic acid bases in the model compounds **4a, 19, 6a, 5a, 22a,** and **22b** was studied in aqueous solution (99% water–1% ethanol) and in 95% ethanol at 25°. In general, greater quenching was observed in water than in ethanol. In water, for all the compounds with the exception of Ind^3-C_3N-Pur^6 (**22b**) the fluorescence emission of indole was quenched 95–98%. The relative quantum efficiencies, q ($=F/F_0$), given in Table IV were determined by comparing the fluorescence intensity of the Ind-C_3-Base with a solution containing equivalent concentrations of Ind-C_3 and Base-C_3. Both solutions had equal absorbance at the exciting wavelength, 280 nm, to correct for hypochromism. The reduced quantum efficiency of indole caused by the attached nucleic acid base results from two processes: (1) static quenching, a time-independent process resulting from the proximity of the quencher predating excitation, and (2) dynamic quenching, a time-dependent process resulting from quenching during the excited state lifetime.[40,87-90]

The quenching processes are illustrated in Scheme III, where Ind^1-C_n-Ade^9 is chosen arbitrarily as the example, and the particular folded or stacked conformation shown is intended to portray not necessarily a preferred overlap but rather *any* stacked conformation.

In aqueous solution an Ind-C_3-Base can exist in a population of conformations including unstacked or unfolded and stacked or folded forms. Irradiation of those molecules that are intramolecularly folded results in immediate quenching with no fluorescence. If the complex itself were fluorescent, the emission would appear as a new band at longer wavelength.[31,32] That the emission observed is from indole alone is shown by the overlap spectra of fluorescence emission of Ind^3-C_3 and Ind^3-C_3-Ade^9 (Figure 7A). These were determined on a photon counting scanning spectrofluorometer[41,42] interfaced to a data acquisition system so that the spectra could be stored in memory and displayed according

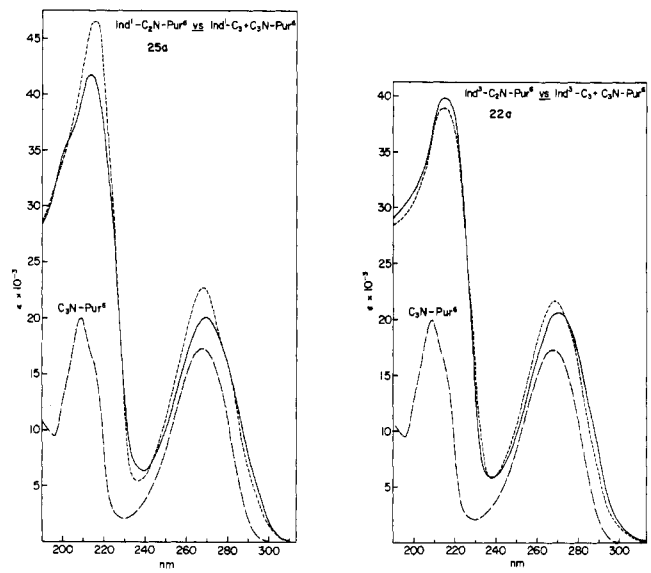


Figure 6. Ultraviolet absorption spectra in 99% water–1% ethanol solution. The full line is for the Ind-C_n-Base.

Scheme III

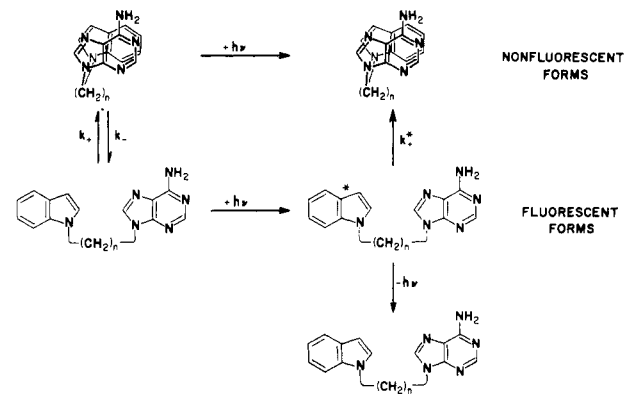


Table IV. Fluorescence Properties and Intramolecular Complexing

Compound	Fluorescence lifetime, nsec[a,b]	Rel quantum efficiency[c]	Static quenching: Efficiency, γ	Static quenching: Equilibrium constant $(1/\alpha - 1)$	Deg of internal assoc, $(1-\alpha)$, %	Dynamic quenching: Efficiency, τ/τ_0	Dynamic quenching: Rate constant, k_+*($\times 10^8$ sec^{-1}) $(1/\tau - 1/\tau_0)$
Ind^3-C$_3$-Ade^9 (4a)	0.50	0.028	0.47	1.13	*53*	0.06	1.9
Ind^1-C$_3$-Gua^9 (19)	0.66	0.045	0.48	1.08	*52*	0.09	1.4
Ind^3-C$_3$-Thy^1 (6a)	0.46	0.030	0.55	0.82	*45*	0.05	2.1
Ind^3-C$_3$-Cyt^1 (5a)	0.30	0.027	0.76	0.32	*24*	0.04	3.2
Ind^3-C$_2$N-Pur^6 (22a)	0.38	0.028	0.62	0.61	*38*	0.06	2.5
Ind^3-C$_3$N-Pur^6 (22b)	0.82	0.096	0.98	0.02	*2*	0.10	1.1

[a] Lifetimes determined in aqueous solution by phase.[39] [b] ±30 psec below 2 nsec. Excitation 290 nm. [c] Fluorescence intensity of Ind-C$_n$-Base/Ind-C$_3$ + Base-C$_3$. Excitation 280 nm.

to command. Only with this capability was it possible to obtain overlap spectra and difference spectra of emission of widely differing quantum efficiencies. Any deviation from superposability of the spectra in Figure 7A may be due to slightly imperfect subtraction of the solvent background from the sample emission since the spectrum of Ind^3-C$_3$-Ade^9 (the slightly lower dots in the figure) had to be magnified over 30-fold. The molecules in the unfolded form, when excited, can emit a photon or, if the excited state lifetime is sufficiently long, the indole may collide with its base partner at the quenching rate constant k_+*. Simultaneous measurement of quantum yield and fluorescent lifetime allows the calculation of the parameter γ, the fraction of the light absorbed by fluorescent forms, from $\gamma = q(\tau_0/\tau)$, where τ and τ_0 are the excited state lifetimes in the presence and absence of intramolecular quencher. The fluorescence lifetimes were determined by phase measurements using a subnanosecond cross-correlation spectrofluorometer[39,40] and were reproducible to ±30 psec. The fluorescent lifetime of Ind^1-C$_3$ so determined is 7.0 nsec and that of Ind^3-C$_3$ is 8.4 nsec. From the lifetime data we can calculate the quenching rate constants, k_+* = $1/\tau - 1/\tau_0$, and from the γ values, the equilibrium constant for complexation (Table IV). In the situation where the observed molar absorptivity of the solution containing Ind-C$_3$-Base, $\bar{\epsilon}$,[90] is equal to the molar absorptivity of Ind-C$_3$ plus Base-C$_3$, ϵ_0, $\gamma = \alpha$, where α is the fraction of molecules in the unfolded conformations. In our experiments we have adjusted the solutions so that the absorbance at the wavelength of excitation is equal in both cases. For Ind^3-C$_3$-Thy^1 $\bar{\epsilon}$ differs from ϵ_0 by about 15% in molar absorptivity, while for all other models the molar absorptivities are within 10%. Due to this uncertainty in the quantitative absorbance of the fluorescent species of the Ind-C$_3$-Base molecules, the short fluorescent lifetimes, and the low efficiencies, a tolerance of ±10% must be applied to the figures in column six in Table IV for $(1 - \alpha)$, the degree of internal association, which represents the extent of indole–nucleic acid base stacking. Another possible source of error, that due to resonance energy transfer according to Förster, did not have to be considered because in the cases we have studied there was essentially no spectral overlap of the absorption of Base-C$_3$ and the emission of Ind-C$_3$.[91-94]

Within the limits indicated the figures for $(1 - \alpha)$ are very useful because they indicate ranges of indole–base stacking interactions in aqueous solution at 25° and they give reasonable values for the equilibria between stacked and unstacked conformations that cannot be obtained from ^{1}H NMR and hypochromism data under the same conditions. The value of 53% for $(1 - \alpha)$ in the case of Ind^3-C$_3$-Ade^9 is similar to that obtained for ϵApϵC (58%).[90] The hypochromism data in Table III and the degree of internal association in Table IV are closely comparable, in that Ind^3-C$_3$-Ade^9 (**4a**) and Ind^1-C$_3$-Gua^9 (**19**) show the greatest stacking interactions and Ind^3-C$_3$-Thy^1 (**6a**), a little less. The cytidine compound Ind^3-C$_3$-Cyt^1 (**5a**) shows negligible hypochromism and, from fluorescence, only 24% of stacked conformations. The last feature reminds us that hypochromism is dependent upon both π-orbital overlap and the angle between the component transition moments and that a pair of heteroaromatic rings showing zero hypochromism may nonetheless show a reasonable degree of stacking, e.g., 24%. The stacking or internal association of Ind^3-C$_2$N-Pur^6 (**22a**) is greater (38%) than that of Ind^3-C$_3$N-Pur^6 (**22b**) (2%), indicating that the three-atom bridge is sufficient and conformationally satisfactory to permit stacking in the N^6-substituted series. The difficulties with regard to the *H* values for the N^6 compounds were mentioned earlier. A direct comparison with aromatic models in which electron donor–acceptor complexes are formed (in nonaqueous solution)[81,82] must await a similar treatment of fluorescent yields *and lifetimes* in those series.

The indole–base interaction models exhibited no hypochromism in 95% ethanol solution. The fluorescence quantum efficiencies in ethanol were greater than those in water solution. When a similar analysis based upon the fluorescence yields and lifetimes for compounds in Table IV was made for their ethanolic solutions, the fluorescence quenching was at least 95% dynamic. That is, there was no evidence of internal association or complexation in ethanol.

Finally, we looked for possible excimer fluorescence emission from Ind^3-C$_3$-Ade^9 (**4a**) and Ind^3-C$_3$-Cyt^1 (**5a**) at room temperature in aqueous solution, although it may have been too much to expect. In the case of dinucleotides in ethylene glycol–water glass at 77 K, fluorescence emission was shown to be red-shifted due to excimer formation, indicative of the heterocyclic bases being in close proximity in the ground state.[95] For tryptophan or tryptamine and nucleic acid components in aggregates in frozen aqueous solutions at 77 K, a new, red-shifted fluorescence band was observed by reflectance.[31,32] For the case of Ind^3-C$_3$-Ade^9 (**4a**) 10^{-5} *M* in aqueous solution at 25°, the emission of the compound was solely from indole (Figure 7A). The same compound **4a** at 3° gave the spectrum reproduced in Figure 7B from the photon counting scanning spectrofluorometer. The expanded scale spectrum (Figure 7C) clearly shows the exponential decay characteristic of only one fluorophore, the indole, and the ln scale spectrum (Figure 7D) confirms this. The same result was obtained with Ind^3-C$_3$-Cyt^1 (**5a**) in neutral and acidic aqueous solution, at pH 4 and 2. Thus, it appears unlikely that excimer fluorescence from an indole–nucleic acid base combination in more complicated natural systems will be detectable at room temperature.[96]

Conclusion

We have been able to observe indole–nucleic acid base stacking interaction in neutral aqueous solution at 25° by

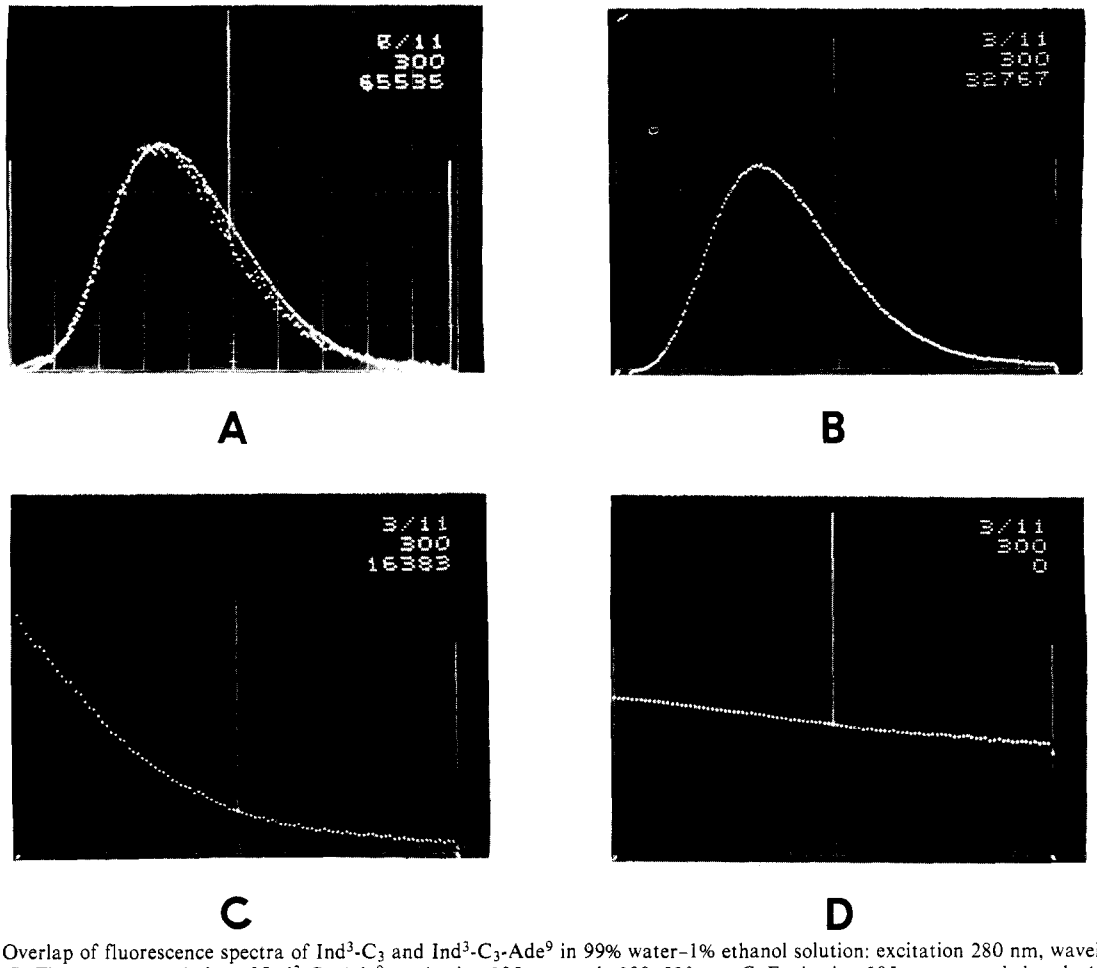


Figure 7. A. Overlap of fluorescence spectra of Ind^3-C_3 and Ind^3-C_3-Ade^9 in 99% water–1% ethanol solution: excitation 280 nm, wavelength scale 300–500 nm. B. Fluorescence emission of Ind^3-C_3-Ade^9: excitation 305 nm, scale 300–500 nm. C. Excitation 305 nm, expanded scale 400–500 nm. D. Excitation 305 nm, expanded scale 400–500 nm, ln intensity scale.

means of hypochromism and fluorescence emission. The degree of interaction between indole and the purine bases is of the same order as that between two purine bases themselves. For the Ind-C_3-Base models, the percentage of internally stacked vs. unfolded conformations was determined from fluorescence quantum yield and lifetime measurements, which give a decreasing order of complexation with indole of adenine $\approx$ guanine $>$ thymine $\gg$ cytosine in the models that allow, but do not dictate, plane parallelism of the two units. The equilibrium between stacked and unfolded conformations for the indole–adenine, guanine, or thymine cases indicates ΔG near zero, comprising negative enthalpy and entropy terms[97] as for nucleic acid base stacking in aqueous solution. The results indicate that total fluorescence quenching of the indole of tryptophan in a polypeptide or protein is to be expected (see also ref 24–28) if it comes into close proximity with a base moiety of a nucleic acid or if intercalation occurs. No evidence has been found for a new fluorescent species emitting at longer wavelength than indole in the Ind-C_3-Ade or Ind-C_3-Cyt models at 25 or 3°. Ethanol has been shown to be a denaturing solvent for the stacked conformations of indole with nucleic acid bases as in the case of the stacked bases themselves. We hope that our findings will be of use in the examination of interactions between proteins and nucleic acids.

Acknowledgment. We are particularly grateful to Dr. A. A. Lamola and Dr. J. Eisinger of Bell Laboratories, Murray Hill, N.J., for their early encouragement of this project and for their sustained interest and assistance. We also thank Dr. Claude Hélène of Centre de Biophysique Moleculaire, La Source, 45-Orléans, France, for valuable discussion and suggestions. Finally, we wish to thank our colleagues Professor Gregorio Weber and Mr. David Jameson for their help and guidance with the fluorescence studies.

Supplementary Material Available. Listings of NMR chemical shift values for the intermediates and final products will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 24× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N.W., Washington, D.C. 20036. Remit check or money order for $4.00 for photocopy or $2.50 for microfiche, referring to code number JACS-75-4095.

References and Notes

(1) This work was supported by Research Grant GM 05829 from the National Institutes of Health, U.S. Public Health Service.
(2) The present paper is No. XIV in the series on Synthetic Spectroscopic Models.
(3) For the preceding paper in this series, see N. J. Leonard and R. L. Cundall, *J. Am. Chem. Soc.*, **96,** 5904 (1974).
(4) For review, see P. C. Huang, *Prog. Biophys. Mol. Biol.*, **23,** 103 (1971).
(5) For review, see M. Yarus, *Annu. Rev. Biochem.*, **38,** 841 (1969).
(6) S.-H. Kim, at the 4th Harry Steenbock Symposium, University of Wisconsin, Madison, Wis., June 16–19, 1974, discussed the fit of a protein β-sheet with the narrow groove of a DNA double helix.
(7) H. De Voe and I. Tinoco, Jr., *J. Mol. Biol.*, **4,** 500 (1962).
(8) D. O. Jordan in "Molecular Association in Biology", B. Pullman, Ed., Academic Press, New York, N.Y., 1968, p 221.
(9) N. S. Kondo, H. M. Holmes, L. M. Stempel, and P. O. P. Ts'o, *Biochemistry*, **9,** 3479 (1970).
(10) A. D. Broom, M. P. Schweizer, and P. O. P. Ts'o, *J. Am. Chem. Soc.*, **89,** 3612 (1967).
(11) C. E. Bugg, J. M. Thomas, M. Sundaralingam, and S. T. Rao, *Biopolymers*, **10,** 175 (1971).
(12) C. E. Bugg and U. Thewalt, *Biochem. Biophys. Res. Commun.*, **37,** 623

(1969).
(13) B. Pullman and A. Pullman, *Prog. Nucl. Acid. Res. Mol. Biol.*, **9**, 327 (1969).
(14) P. O. P. Ts'o in "Basic Principles in Nucleic Acid Chemistry", Vol. I, P. O. P. Ts'o, Ed., Academic Press, New York, N.Y., 1974, p 558.
(15) F. Jordan and H. D. Sostman, *J. Am. Chem. Soc.*, **94,** 7898 (1972).
(16) N. J. Leonard and K. Ito, *J. Am. Chem. Soc.*, **95,** 4010 (1973).
(17) L. S. Rosen and A. Hybl, *Acta Crystallogr., Sect. B*, **27,** 952 (1971).
(18) J. K. Frank and I. C. Paul, *J. Am. Chem. Soc.*, **95,** 2324 (1973).
(19) T. E. Wagner, *Nature (London)*, **222,** 1170 (1969).
(20) J. R. Smythies and F. Antun, *Nature (London)*, **223,** 1061 (1969).
(21) M. Raszka and M. Mandel, *Proc. Natl. Acad. Sci. U.S.A.*, **68,** 1190 (1971).
(22) C. Hélène, J.-L. Dimicoli, and F. Brun, *Biochemistry*, **10,** 3802 (1971).
(23) C. Hélène, *Nature (London), New Biol.*, **234,** 120 (1971).
(24) J. G. Farrelly, J. W. Longworth, and M. P. Stulberg, *J. Biol. Chem.*, **246,** 1266 (1971).
(25) C. Hélène, F. Brun, and M. Yaniv, *Biochem. Biophys. Res. Commun.*, **37,** 393 (1969).
(26) C. Hélène, *Stud. Biophys.*, **24/25,** 369 (1970).
(27) C. Hélène, F. Brun, and M. Yaniv, *J. Mol. Biol.*, **58,** 349 (1971).
(28) C. Hélène, *FEBS Lett.*, **17,** 73 (1971).
(29) J.-L. Dimicoli and C. Hélène, *Biochimie*, **53,** 331 (1971).
(30) (a) J.-L. Dimicoli and C. Hélène, *J. Am. Chem. Soc.*, **95,** 1036 (1973); (b) C. Hélène, *Abstracts of VIth International Conference on* Magnetic Resonance in Biological Systems, Kandersteg, Switzerland, Sept 16–21, 1974, F.3.
(31) T. Montenay-Garestier and C. Hélène, *Nature (London)*, **217,** 844 (1968).
(32) T. Montenay-Garestier and C. Hélène, *Biochemistry*, **10,** 300 (1971).
(33) G. Hui Bon Hoa and P. Douzou, *J. Chim. Phys., Suppl.*, 197 (1970).
(34) D. T. Browne, J. Eisinger, and N. J. Leonard, *J. Am. Chem. Soc.*, **90,** 7302 (1968).
(35) Abbreviations used are the approved IUPAC–IUB symbols [*Biochemistry*, **9,** 4022 (1970)] and the linkage symbols proposed by W. E. Cohn, N. J. Leonard, and S. Y. Wang, *Photochem. Photobiol.*, **19,** 89 (1974); e.g., Ind^1-C_3-Ade^9 or Ind[1(CH_2)$_3$9]Ade for 9-[3-(indol-1-yl)propyl]adenine; Ind^3-C_4-Ade^9 for 9-[4-(indol-3-yl)butyl]adenine; Ind^1-C_2N-Pur^6 for 6-[2-(indol-1-yl)ethylamino]purine; Ind^3-C_3 for 3-propylindole; Cyt^1-C_3 for 1-propylcytosine, etc. (see Table I).
(36) D. Shugar and J. J. Fox, *Biochim. Biophys. Acta*, **9,** 199 (1952).
(37) E. Wittenburg, *Chem. Ber.*, **99,** 2391 (1966).
(38) The NMR chemical shift data for the intermediates and final products are included in the microfilm edition. See paragraph at end of paper regarding supplementary material.
(39) R. D. Spencer and G. Weber, *Ann. N.Y. Acad. Sci.*, **158,** 361 (1969).
(40) R. D. Spencer, W. M. Vaughn, and G. Weber in "Molecular Luminescence", E. C. Kim, Ed., W. A. Benjamin, New York, N.Y., 1969, p 607.
(41) D. M. Jameson, R. D. Spencer, and G. Weber, *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, **33,** Abstr. 557 (1974).
(42) R. D. Spencer, G. Weber, G. L. Tolman, J. R. Barrio, and N. J. Leonard, *Eur. J. Biochem.*, **45,** 425 (1974), illustrate one type of application of this spectrofluorometer.
(43) C. Temple, Jr., C. L. Kussner, and J. A. Montgomery, *J. Med. Pharm. Chem.*, **5,** 866 (1962).
(44) N. J. Leonard and R. F. Lambert, *J. Org. Chem.*, **34,** 3240 (1969).
(45) C. G. Skinner, W. Shive, R. G. Ham, D. C. Fitzgerald, Jr., and R. E. Eakin, *J. Am. Chem. Soc.*, **78,** 5097 (1956).
(46) J. B. Brown, H. B. Henbest, and E. R. H. Jones, *J. Chem. Soc.*, 3172 (1952).
(47) B. Cardillo, G. Casnati, A. Pochini, and A. Ricea, *Tetrahedron*, **23,** 3771 (1967).
(48) (a) F. Lingens and K. H. Weiler, *Justus Liebigs Ann. Chem.*, **662,** 139 (1963); (b) T. Hoshino and K. Shimodaira, *ibid.*, **552,** 19 (1935).
(49) 4-(Indol-3-yl)butyl bromide (**3b**) was prepared by the same procedure as applied to the preparation of lower homologs. Since the bromide was unstable to air and heat, no effort was made to isolate it in the pure state.
(50) H. E. Fritz, *J. Org. Chem.*, **28,** 1384 (1963).
(51) The general procedure described in the literature was slightly modified from L. F. Fieser and M. Fieser, "Reagents for Organic Synthesis", Vol. 1, Wiley, New York, N.Y., 1967, p 1180.
(52) H. Lettré and H. Ballweg, *Justus Liebigs. Ann. Chem.*, **633,** 171 (1960).
(53) M. R. Bell, Belgium Patent 659,467 (1965); *Chem. Abstr.*, **64,** 2098 (1966).
(54) This compound was prepared by the reduction of 1-(cyanomethyl)indole (**23a**) with the lithium aluminum hydride–aluminium chloride system[55,56] in 86% yield.
(55) E. Pfeil and U. Harder, *Angew. Chem., Int. Ed. Engl.*, **6,** 178 (1967).
(56) R. F. Nystrom, *J. Am. Chem. Soc.*, **77,** 2544 (1955).
(57) I. Tinoco, Jr., *J. Am. Chem. Soc.*, **82,** 4785 (1960); **83,** 5047 (1961).
(58) I. Tinoco, Jr., *J. Chem. Phys.*, **33,** 1332 (1960); **34,** 1067 (1961).
(59) W. Rhodes, *J. Am. Chem. Soc.*, **83,** 3609 (1961).
(60) N. J. Leonard, R. S. McCredie, M. W. Logue, and R. L. Cundall, *J. Am. Chem. Soc.*, **95,** 2320 (1973).
(61) J. H. Craig, P. C. Huang, T. G. Scott, and N. J. Leonard, *J. Am. Chem. Soc.*, **94,** 5872 (1972).
(62) M. W. Logue and N. J. Leonard, *J. Am. Chem. Soc.*, **94,** 2842 (1972).
(63) J. A. Secrist III and N. J. Leonard, *J. Am. Chem. Soc.*, **94,** 1702 (1972).
(64) H. Iwamura, N. J. Leonard, and J. Eisinger, *Proc. Natl. Acad. Sci. U.S.A.*, **65,** 1025 (1970).
(65) T. G. Scott, R. D. Spencer, N. J. Leonard, and G. Weber, *J. Am. Chem. Soc.*, **92,** 687 (1970).
(66) N. J. Leonard, H. Iwamura, and J. Eisinger, *Proc. Natl. Acad. Sci. U.S.A.*, **64,** 352 (1969).
(67) N. J. Leonard, K. Golankiewicz, R. S. McCredie, S. M. Johnson, and I. C. Paul, *J. Am. Chem. Soc.*, **91,** 5855 (1969).
(68) N. J. Leonard, T. G. Scott, and P. C. Huang, *J. Am. Chem. Soc.*, **89,** 7137 (1967).
(69) (a) T. T. Herskovits, S. J. Singer, and E. P. Geiduschek, *Arch. Biochem. Biophys.*, **94,** 99 (1961); (b) T. T. Herskovits, *ibid.*, **97,** 474 (1962).
(70) (a) J. Brahms, A. M. Michelson, and K. E. Van Holde, *J. Mol. Biol.*, **15,** 467 (1966); (b) J. Massoulie and A. M. Michelson, *C. R. Acad. Sci.*, **259,** 2923 (1964).
(71) H. Zimmermann and N. Joop, *Z. Elektrochem.*, **65,** 61 (1961).
(72) S. F. Mason, *J. Chem. Soc.*, 2071 (1954).
(73) R. F. Stewart and N. Davidson, *J. Chem. Phys.*, **39,** 255 (1963); **40,** 2071 (1964).
(74) P. R. Callis and W. T. Simpson, *J. Am. Chem. Soc.*, **92,** 3593 (1970).
(75) W. Hug and I. Tinoco, Jr., *J. Am. Chem. Soc.*, **95,** 2803 (1973).
(76) D. Poland, J. N. Vournakis, and H. A. Sheraga, *Biopolymers*, **4,** 223 (1966).
(77) C. F. Otto, unpublished results in this laboratory.
(78) N. J. Leonard, K. L. Carraway, and J. P. Helgeson, *J. Heterocycl. Chem.*, **2,** 291 (1965).
(79) S. Shifrin, *Biochim. Biophys. Acta*, **81,** 205 (1964).
(80) M. Shinitzky and E. Katchalski in "Molecular Associations in Biology", B. Pullman, Ed., Academic Press, New York, N.Y., 1968, p 361.
(81) K. Mutai, *Bull. Chem. Soc. Jpn.*, **45,** 2635 (1972).
(82) K. Mutai, *Tetrahedron Lett.*, 1125 (1971).
(83) J. Ladik and K. Sundaram, *J. Mol. Spectrosc.*, **29,** 146 (1969).
(84) K. Sundaram and J. Ladik, *Physiol. Chem. Phys.*, **4,** 483 (1972).
(85) D. Voet, W. B. Gratzer, R. A. Cox, and P. Doty, *Biopolymers*, **1,** 193 (1963).
(86) M. Falk, *J. Am. Chem. Soc.*, **86,** 1226 (1964).
(87) R. D. Spencer, Ph.D. Thesis, University of Illinois, Urbana, Ill., 1970.
(88) R. D. Spencer and G. Weber in "Structure and Function of Oxidation Reduction Enzymes", Å. Åkeson and Å. Ehrenberg, Ed., Pergamon Press, Oxford and New York, 1972, pp 393–399.
(89) J. R. Barrio, G. L. Tolman, N. J. Leonard, R. D. Spencer, and G. Weber, *Proc. Natl. Acad. Sci. U.S.A.*, **70,** 941 (1973).
(90) G. L. Tolman, J. R. Barrio, and N. J. Leonard, *Biochemistry*, **13,** 4869 (1974).
(91) This is in contrast with the overlap observed for guanosine, cytidine, and thymidine with tryptophan.[92,93]
(92) C. Hélène, at the International Conference on "Excited States of Biological Molecules", Lisbon, April 18–24, 1974.
(93) T. Montenay-Garestier, *Photochem. Photobiol.*, in press.
(94) We are indebted to Dr. Hélène and Dr. Montenay-Garestier for making their manuscripts containing the calculations on resonance energy transfer between the ribonucleosides and tryptophan available to us prior to publication.
(95) M. Gueron, R. G. Shulman, and J. Eisinger, *Proc. Natl. Acad. Sci. U.S.A.*, **56,** 814 (1966).
(96) See ref 33, especially p 202, under Discussion.
(97) Unpublished results from this laboratory.