Charge Transfer as a Molecular Probe in Systems of Biological Interest. Intermolecular Interactions of the Indole–Pyridinium Type*

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ABSTRACT: A detailed study of intermolecular complex formation between substituted pyridinium-type acceptors and indole-type donors is presented in order to provide a basis for the use of such acceptors as conformational probes. The compounds investigated comprise the acceptors *N*-methylnicotinamide chloride and *N*-methylisonicotinamide chloride, and the donors skatole, 3-indoleacetic acid, *N*-acetyl-L-tryptophan, 3-indoleacetamide, and *N*-acetyl-L-tryptophanamide. New (charge transfer) absorption bands are observed on the long wavelength side of the spectra. These can be graphically resolved into two gaussian absorption bands located at 315 and 360 nm (*N*-methylnicotinamide chloride + donors) or 350 and 410 nm (*N*-methylisonicotinamide chloride + donors). Association constants were determined from data covering as much as possible of the titration curve and a considerable dependence on solvent composition (ethanol–water mixtures) was observed, suggesting a strong influence of hydrophobic interactions on the strength of the complex (besides, mainly, dipole–dipole interactions). The results indicate that *N*-methylnicotinamide and *N*-methylisonicotinamide chlorides and similar, derived compounds can be useful as molecular probes of the charge transfer type. The concept of approximating topography and conformation of polypeptides by observation of binary side-chain interactions with the aid of charge transfer and nuclear magnetic resonance probes is briefly reviewed.

It has recently been suggested that the solution conformation and topography of polypeptides may be approximated by demonstrating the presence or absence of interactions between specific side chains (Carrión *et al.*, 1967, 1968). As a means of visualizing such binary side-chain interactions in solution, we have suggested the use of charge transfer probes[1] (Carrión *et al.*, 1967, 1968) and nuclear magnetic resonance markers (Schwyzer and Ludescher, 1968). The latter procedure has been applied to the study of the conformers of gramicidin *s* (Schwyzer and Ludescher, 1968).

Carrión *et al.* (1968) have demonstrated that phthalimide, 4-nitrophthalimides, and tetrachlorophthalimides give colored molecular complexes with tryptophan and with *p*-dimethylaminophenylalanine. Spectroscopic evidence and Hückel molecular orbital calculations indicated the presence of charge transfer interactions between the phthalimide or substituted phthalimide rings (acceptors) and the indole or *p*-dimethylaminophenyl rings (donors). Both *intermolecular* and *intramolecular* interactions were studied. Intermolecular interactions are concentration dependent, and can thus be distinguished from concentration independent intramolecular interactions. In the former case, the association constants in organic solvents were found to be small (approximately 0.4–1.2 l./mole) and the enthalpies of complex formation varied between −0.5 and −2.5 kcal/mole, well within the range of values assumed for van der Waal's interactions between side chains.

While the use of the phthalimide-type acceptors as charge transfer probes has been shown to be feasible, the detailed evaluation of the properties of their complexes has been restricted to organic solvents for reasons of solubility. In a parallel and somewhat more detailed study, the properties of the complexes between substituted pyridinium-type acceptors and indole-type donors have been evaluated in aqueous systems in order to provide the basis for the use of such acceptors as conformational probes. This study is the subject of the present paper. In addition, a formal description of the use of binary side-chain interactions in determining the conformation and topography of polypeptides is presented in an Appendix.

Experimental Procedure

Chemicals. *N*-Methylnicotinamide chloride, *N*-methylisonicotinamide chloride, and *N*-methylpicolinamide chloride were prepared from the corresponding iodides by repeated equilibration with an excess of AgCl (Karrer *et al.*, 1936); the iodides, in turn, were obtained from the unmethylated bases by refluxing with an excess of methyl iodide in acetone. *N*-Acetyl-L-tryptophan was prepared by the method of du Vigneaud and Sealock (1932), and *N*-acetyl-L-tryptophanamide was similarly prepared starting from L-tryptophanamide (Fluka, puriss). *Anal.* Found for *N*-acetyl-L-tryptophan: C, 63.2; H, 5.9; N, 11.5. Calcd: C, 63.2; H, 5.7; N, 11.3. Found for *N*-acetyl-L-tryptophanamide:

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[1] Independently, Little and Eisen (1967) have been using the dinitrophenyl group for detection of tryptophan in antibodies to polynitrobenzenes, and Sigman and Blout (1967) have used the *p*-nitrophenacyl group as a conformational probe for chymotrypsin.

C, 63.1; H, 6.5; N, 16.9. Calcd: C, 63.2; H, 6.1; N, 17.0. Skatole, 3-indoleacetic acid, and 3-indoleacetamide were commercial products (Fluka, puriss); the latter two were recrystallized before use. Ethanol was a Spectroquality Reagent.

Absorption Spectra. Absorption measurements were made with a Beckman DK2A recording spectrophotometer fitted with a temperature regulated cell holder in which the temperature could be maintained within ±0.1°. Difference spectra were measured in tandem double cells (Herskovits and Laskowski, 1962). One compartment in the sample beam was filled with a solution of donor plus acceptor (giving complex), and the second compartment was filled with pure solvent. The two compartments in the reference beam contained, separately, donor and acceptor solutions at the appropriate concentrations.

Binding Experiments and Calculations. In a typical binding experiment, a solution of the relevant charge transfer donor, at a constant total concentration $[P_0]$, was titrated with increasing amounts of a charge transfer acceptor with variable total concentration $[X_0]$ (the limited solubility of the donors used in this study prevented the experiment in which the acceptor is titrated with the donor in a similar fashion). Specifically, concentrated standard acceptor solutions (usually 1.0 and 0.45 M) containing a small amount of donor (usually 0.001 M) were diluted volumetrically with a standard solution of donor at the same concentration as in the acceptor solutions. Between 12 and 16 dilutions were made in this manner, and the complete absorption spectrum in the charge transfer region was recorded for each dilution. Each experiment was then repeated at least twice with freshly prepared standard solutions of donor and acceptor. Except as noted, all solutions contained 1% ethanol, to ensure that the donor, which in certain cases (skatole, indoleacetamide, *N*-acetyl-L-tryptophanamide) had marginal solubility in water at the concentrations employed, remained in solution and did not aggregate. Beer's law was strictly obeyed for each of the donors employed in this work-up to twice the concentration actually used in the experiment, and for each of the acceptors up to the highest concentration used.

In each experiment, a single wavelength, well separated from the normal absorption bands of donor and acceptor, was chosed for calculation of the binding constant and extinction coefficient, and at least once in every set of experiments several other wavelengths were checked to make sure that the calculated binding constant was independent of the particular wavelength chosen. Within the limits of experimental error, this independence was verified for all of the complexes reported herein. The formation constant k (in liter/mole) for the reaction $P + X \rightleftharpoons PX$, and the molar extinction coefficient of the complex ϵ (in liter/mole cm), were calculated from the slope and intercepts of a plot of $A/[P_0][X_0]$ *vs.* $A/[P_0]$ according to the relationship (Scatchard, 1949; Deranleau, 1969)

$$A/[P_0][X_0] = k[\epsilon - (A/[P_0])] \quad (1)$$

Here A is the absorbance of pure complex calculated in principle from

$$A = A_{obsd} - \epsilon_P[P_P] - \epsilon_X[X] \quad (2)$$

where [P] and [X] are the concentrations of the uncomplexed dilute (donor) and excess (acceptor) components, with extinction coefficients ϵ_P and ϵ_X, respectively, and A_{obsd} is the observed absorbance of the entire system (corrected for base line). In a region where both components absorb, the last equation cannot be used without prior knowledge of k. However, k was evaluated in a spectral region where the second term on the right is effectively zero, and since the acceptor X is in large excess $[X] = [X_0]$ with negligible error, and eq 2 reduces to

$$A = A_{obsd} - \epsilon_X[X_0] \quad (3)$$

(The error in the approximation $[X] = [X_0]$ is $([X_0] - [X])/[X_0] = s[P_0]/[X_0]$, where $s = [PX]/[P_0]$ is the saturation fraction of the dilute component, was of the order of 0.1% for the experimental conditions described herein.)

The data from the several experiments on each system were then combined, and the entire set subjected to linear regression (least-squares) analysis to determine the most probable final values of k and ϵ at the chosen wavelength. These values were found to agree reasonably well with the averaged results of the individual experiments, as expected. Using the most probable value of k, the observed spectrum of a data point lying on the regression line of the combined data was used to calculate the charge transfer difference spectrum from eq 4, which follows directly from eq 2 (with s as defined above) and the conservation relations $[X] = [X_0] - [PX]$, $[P] = [P_0] - [PX]$:

$$\Delta\epsilon = \epsilon - \epsilon_P - \epsilon_X = (A_{obsd} - \epsilon_P[P_0] - \epsilon_X[X_0])/s[P_0] \quad (4)$$

The difference extinction coefficient $\Delta\epsilon$ is supposedly that part of the extinction ϵ of the complex which is due to charge transfer alone, and is the quantity which was checked using the tandem double cells as described above. In what follows, we shall be concerned only with the charge transfer part of the spectrum, and hence shall use ϵ and $\Delta\epsilon$ interchangeably. In actual fact, the two quantities are identical over much of the spectral range studied, since ϵ_P and ϵ_X are both negligibly small with respect to ϵ except in the region where the charge transfer spectrum begins to overlap with the normal, high intensity component transitions of donor and acceptor. It will always be clear from the context, however, where the approximation to the extinction of the charge transfer band has been made in terms of $\Delta\epsilon$.

The oscillator strength f, the transition dipole strength D, and the mean frequency of the transition $\bar{\nu}$ were calculated from the area under the curves of the appropriate plots from the equations (Tinoco, 1965)

$$f = 4.318 \times 10^{-9} \int \epsilon d\nu \text{ (unitless)} \quad (5)$$

$$D = 9.180 \times 10^{-3} \int (\epsilon/\nu) d\nu \text{ (debye}^2\text{)} \quad (6)$$

$$\bar{\nu} = 2.125 \times 10^6 (f/D) \text{ (cm}^{-1}\text{)} \quad (7)$$

where $\bar{\nu}$ is the frequency in cm^{-1}. Generally speaking, on the short wavelength side of the band maximum (λ_{max}), where both donor and acceptor contribute appreciably to the total absorbance, $\Delta\epsilon$ was used in the above equations. On

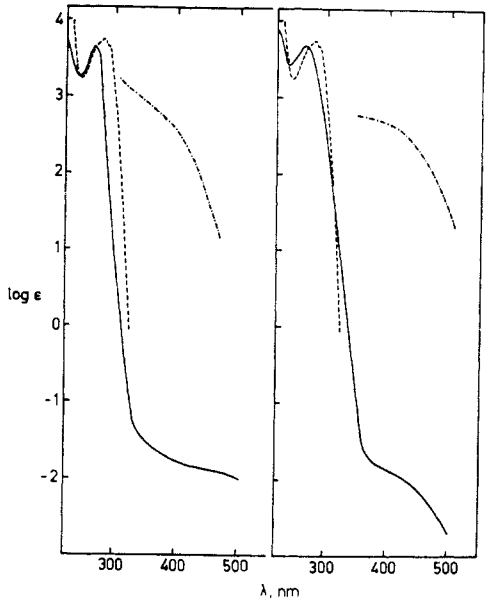

FIGURE 1: Molar extinction coefficient as a function of wavelength for the intermolecular complexes (.-·-) between, left, skatole (- - -) and *N*-methylnicotinamide chloride (—), and, right, skatole (- - -) and *N*-methylisonicotinamide chloride (——), in 1% ethanol at 25°.

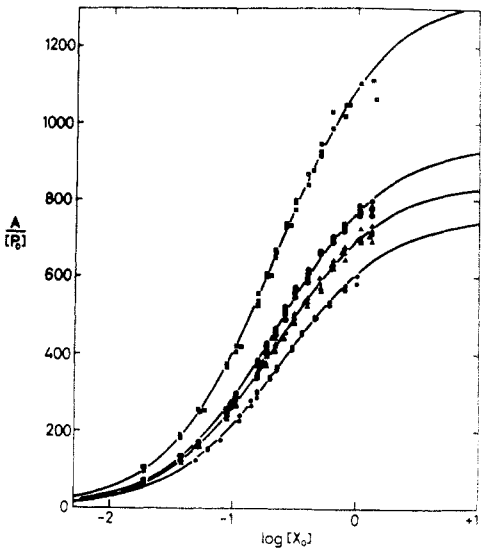

FIGURE 2: Representative formation function curves for *N*-methylnicotinamide chloride complexes with skatole (■), 3-indoleacetic acid (●), *N*-acetyl-L-tryptophan (▲), and *N*-acetyl-L-tryptophanamide (●), in 1% ethanol at 25° λ, 350 nm.

the long wavelength side of λ_{max}, ϵ and $\Delta\epsilon$ are identical for all practical purposes for the absorption bands described herein, as discussed above.

The binding experiments all satisfied criteria recently detailed by Deranleau (1969) for the measurement of weak molecular complexes. Roughly 80% of the available saturation range ($0 \leqslant s \leqslant 1$) was measured in each experiment, except for the experiments with *N*-methylpicolinamide chloride, where the smaller association constant precluded measurements at high saturation fractions. Even in this case, however, more than 50% of the saturation curve was explored.

Results and Discussion

The left-hand portion of Figure 1 shows the experimental absorption spectra of skatole, *N*-methylnicotinamide chloride, and the complex between them. While the observed charge transfer spectrum is broad and almost featureless, as is common for such complexes, it may be considered as the sum of two roughly symmetrical, overlapping bands: a narrow band with a maximum at approximately 315 nm, and a broad band with a maximum at approximately 360 nm[2] (see below). The right-hand portion of Figure 1 shows the observed complex spectrum resulting when *N*-methylisonicotinamide chloride is used as an acceptor. The extremely weak long wavelength absorption band of the uncomplexed acceptor present in each case varies somewhat from preparation to preparation, and may be the result of interaction of the ring with chloride ion. In this connection, a minute contamination by iodide ion has a large effect, and the iodide complexes of both acceptors are intensely yellow colored substances with long wavelength absorption bands several orders of magnitude larger than those shown in the figure. Also apparent from Figure 1, and important for comparison purposes to be discussed later, is the fact that the difference spectrum between the complex and its components can be carried closer to the absorption edge of the donor in the case of *N*-methylnicotinamide chloride complexes. The absorption spectrum of the normal 265-nm band of *N*-methylisonicotinamide chloride is broadened with respect to that of *N*-methylnicotinamide chloride, and is such that inaccuracies in the difference spectrum become relatively more important near the donor absorption edge, when it is remembered that the acceptor is present in large excess and makes a major contribution to the total absorbance in this region.

N-Methylnicotinamide Chloride. The experimental curves for the binding of the acceptor *N*-methylnicotinamide chloride to several related donors are shown in Figures 2 and 3 in two different forms. Figure 2 is the direct formation function, or "titration curve," and Figure 3 is the derived plot (Scatchard, 1948; Deranleau, 1969) which puts the data in a convenient form for calculating the association constant and molar extinction coefficient of the complex (see Experimental Procedure). Both plots are particularly useful in gaining insight into the stoichiometry of the binding and the reliability of the calculated constants (Edsall and Wyman, 1958; Weber, 1965; Deranleau, 1969).

The molar extinction coefficient decreases with the introduction (on the indole moiety) of side chains containing a polar or highly polarizable group. The largest effect is observed when the first such group is introduced, as can be seen from the high concentration asymptotes of Figure 2 (lim $A/[P_0]$ as $[X_0] \rightarrow \infty$ equals ϵ), or from the $A/[P_0]$ axis intercepts

[2] Energy level calculations (Pullman and Pullman, 1959; Moser, personal communication, 1968) indicate the acceptor has two relatively close-lying empty levels differing by only 0.2 β unit. This would predict two possible charge transfer transitions differing by about 50–60 nm in the near-ultraviolet portion of the spectrum.

TABLE I: Properties of *N*-Methylnicotinamide Chloride Complexes.[a]

Donor	Association Constant k (l./mole)	Oscillator Strength f	Dipole Strength D (debye²)	Calcd Mean Wavelength[b] $\bar{\lambda}$ (nm)	Obsd Band Maximum λ_{max} (nm)	Extinction[c] at λ_{max} $\epsilon(\lambda_{max})$
Skatole	4.4 ± 0.2	0.053	3.9	345	320	1720
3-Indoleacetic acid	4.4 ± 0.2	0.037	2.7	345	314	1440
N-Acetyl-L-tryptophan	4.5 ± 0.2	0.035	2.5	340	314	1330
3-Indoleacetamide	3.4 ± 0.2	0.032	2.4	345	318	1320
N-Acetyl-L-tryptophanamide	4.0 ± 0.2	0.029	2.2	355	314	1180

[a] 25°, 1% ethanol. [b] ±5 nm. [c] Liter/mole cm.

of Figure 3. The effect of a second polar group is relatively smaller. The same intensity decreases are preserved over the entire charge transfer spectra of these complexes, as shown in Figure 4. In addition, a shift toward shorter wavelength is observed in the band maximum when a polar or polarizable group is introduced.

The calculated parameters characterizing the complex between *N*-methylnicotinamide chloride and various related donors are summarized in Table I. A carboxamide group seems to be more effective than a carboxyl group in reducing the oscillator strength relative to the skatole complex, the difference between the two groups being of the order of 10% of the oscillator strength of the skatole complex, and the *N*-acetyl group has a smaller effect (*ca.* 5%). While the figure of 5% may be within experimental error, and even the figure of 10% marginal in this respect, the data concerning the introduction of at least one such polar or polarizable group clearly point up the importance of local environment on the charge transfer properties of a given donor–acceptor pair. Thus a 30% decrease in oscillator strength is observed relative to skatole for 3-indoleacetic acid, a 34% decrease for *N*-acetyl-L-tryptophan, a 40% decrease for 3-indoleacetamide, and a 45% decrease for *N*-acetyl-L-tryptophanamide.

With respect to the equilibrium between donor and acceptor[4] the carboxyl group has little or no apparent effect on the binding (Table I), whereas the carboxamide group, with its large permanent dipole moment (3.7 Debyes, Kurland and Wilson, 1957), lowers the association constant. Further, the magnitude of the effect is apparently related to the distance of the permanent dipole from the ring, as one would expect from consideration of the nature of dipole–dipole interactions (see, for example, Edsall and Wyman, 1958; Wiberg, 1963). The central atom (carbon) of the amide dipole is only one atom removed from the ring in 3-indoleacetamide, and two atoms removed from the ring in the C-terminal carboxamide group of *N*-acetyl-L-tryptophanamide. The carboxyl carbon of the *N*-acetyl group is three atoms removed in both *N*-acetyl-L-tryptophan and *N*-acetyl-L-tryptophan-

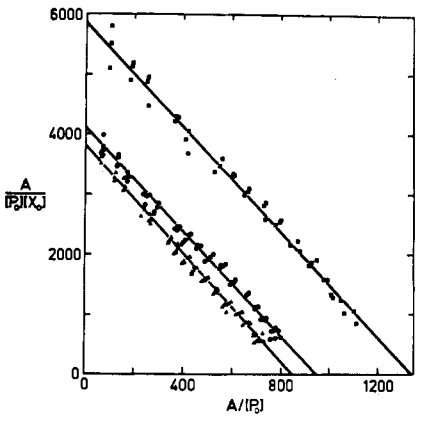

FIGURE 3: Representative Scatchard plots for *N*-methylnicotinamide chloride complexes with skatole (■), 3-indoleacetic acid (●), and *N*-acetyl-L-tryptophan (▲), in 1% ethanol at 25°, λ 350 nm.

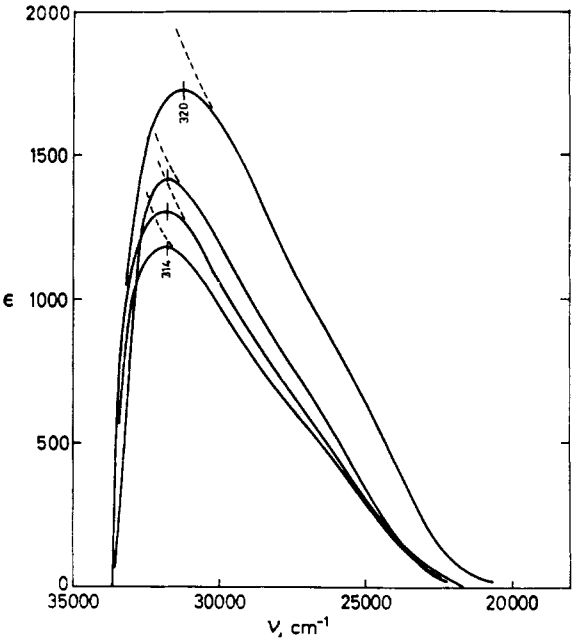

FIGURE 4: Charge transfer difference spectra (solid lines, $\Delta\epsilon$) for the complexes between *N*-methylnicotinamide chloride and, in decreasing order of intensity, skatole, 3-indoleacetic acid, *N*-acetyl-L-tryptophan, and *N*-acetyl-L-tryptophanamide. Dashed lines are ϵ of the entire complex; see text for details.

TABLE II: Properties of *N*-Methylisonicotinamide Chloride Complexes.[a]

Donor	Association Constant k (l./mole)	Oscillator Strength f	Dipole Strength D (debye^2)	Calcd Mean Wavelength[b] $\bar{\lambda}$ (nm)	Obsd Band Maximum λ_{max} (nm)	Extinction[c] at λ_{max} $\epsilon(\lambda_{max})$
Skatole	3.8 ± 0.2	0.029	2.4	390	365	830
3-Indoleacetic acid	4.0 ± 0.2	0.020	1.6	375	355	670
N-Acetyl-L-tryptophan	3.9 ± 0.2	0.018	1.5	385	355	580

[a] 25°, 1% ethanol. [b] ±5 nm. [c] Liters/mole cm.

amide; this is apparently already far enough away from the indole moiety so as to produce little or no effect. It is of interest to point out that the carboxyl carbon of the *N*-acetyl group is twice as far away *in space* from the ring as that of the C-terminal carboxamide group when the "peptide backbone" is arranged as in an α helix.

N-Methylisonicotinamide Chloride. The data for the titration of skatole, 3-indoleacetic acid, and *N*-acetyl-L-tryptophan with *N*-methylisonicotinamide chloride are given in Figures 5 and 6, and the corresponding charge transfer absorption spectra in Figure 7. In addition to the obvious spectral differences—decreased intensity and red-shifted absorption bands—relative to the corresponding *N*-methylnicotinamide chloride complexes, the binding constants are somewhat smaller. It is important that complexes with *N*-methylisonicotinamide chloride parallel those with *N*-methylnicotinamide chloride in their spectral and binding behavior, regardless of the actual magnitude of the various measured parameters. As in the case of the *N*-methylnicotinamide chloride complexes with skatole, 3-indoleacetic acid, and *N*-acetyl-L-tryptophan, the introduction of the carboxyl moiety decreases the oscillator strength relative to skatole, without a significant change in binding constant, when *N*-methylisonicotinamide chloride is the acceptor. Again, the *N*-acetylcarboxamide group, three atoms removed from the ring, has no observable effect on the binding or on the spectral properties. The results are summarized in Table II in terms of the binding constants, oscillator strengths, and band positions and intensities. Note that the decreases in oscillator strength from the skatole complex to 3-indoleacetic acid and to *N*-acetyl-L-tryptophan complexes are 30 and 38%, respectively, in excellent agreement with the decreases noted when *N*-methylnicotinamide is the acceptor.

N-Methylpicolinamide Chloride. This acceptor was tested with skatole as the donor, and both the binding constant (k = 1.5 ± 1 l./mole) and the oscillator strength (f = 0.026) were smaller than with the other acceptors. The calculated mean wavelength of the band was intermediate between the other two acceptors ($\bar{\lambda} \simeq$ 370 nm), but the position of the band maximum was the same as with *N*-methylnicotinamide

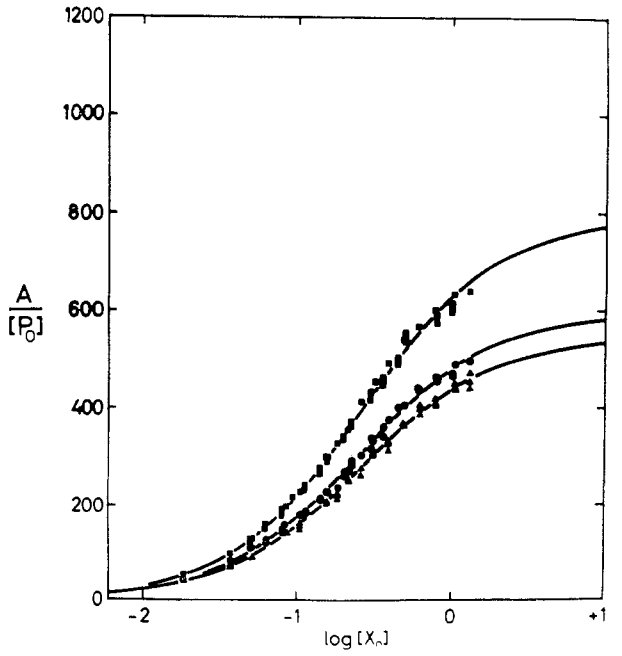

FIGURE 5: Formation function curves for *N*-methylisonicotinamide chloride complexes with skatole (■), 3-indoleacetic acid (●), and *N*-acetyl-L-tryptophan (▲), in 1% ethanol at 25°, λ 375 nm.

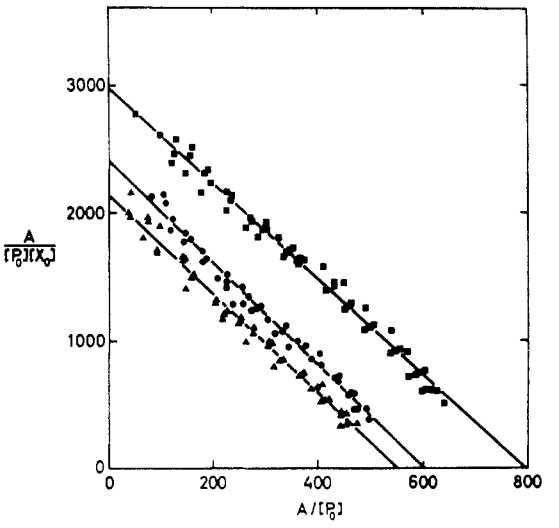

FIGURE 6: Scatchard plots for N-methylisonicotinamide chloride complexes with skatole (■), 3-indoleacetic acid (●), and *N*-acetyl-L-tryptophan (▲), in 1% ethanol at 25°, λ 375 nm.

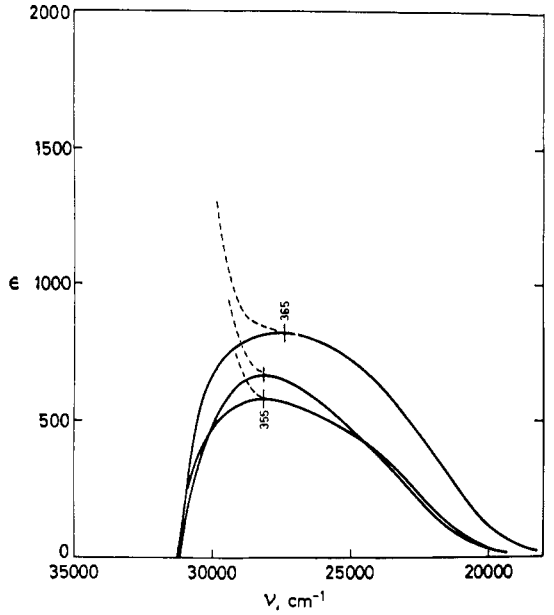

FIGURE 7: Charge transfer difference spectra (solid lines, $\Delta\epsilon$) for the complexes between *N*-methylisonicotinamide chloride and, in decreasing order of intensity, skatole, 3-indoleacetic acid, and *N*-acetyl-L-tryptophan. Dashed lines are ϵ of the complex; see text for details.

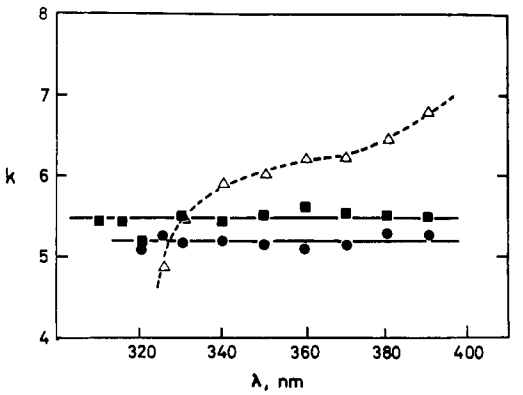

FIGURE 8: Wavelength dependence of the association constant k for the *N*-acetyl-L-tryptophan complex with *N*-methylnicotinamide chloride in water (■) and in sodium phosphate buffer, 0.1 M, pH 7.0 (●), and for the *N*-acetyl-L-tryptophan complex with *N*-benzylnicotinamide chloride in sodium phosphate buffer, 0.1 M, pH 7.0 (△).

chloride (320 nm). Both the binding constant and the oscillator strength in this complex indicate that relative ring orientation effects (rotation of rings in parallel planes) may also be of importance in these complexes. The permanent dipole in *N*-methylpicolinamide chloride deviates significantly from the direction it takes in either of the other two acceptors, and this may affect the orientation and overlap of the donor and acceptor ring systems, depending on the relative strength of the dipole orientation and mutual polarization forces.

Variation of k with Wavelength. Stoichiometry. While all of the complexes reported in this paper were checked at several wavelengths to show the constancy of k over the band, which is evidence for only a single type of complex, the system *N*-methylnicotinamide chloride was more or less exhaustively examined in this respect. Figure 8 shows the results (solid figures) in water and in 0.1 M sodium phosphate buffer (pH 7) of the wavelength dependence of the binding constant. For comparison, the curve for *N*-benzylnicotinamide chloride is shown. The latter compound was used by Cilento and Giusti (1959) and Cilento and Tedeschi (1961) in an investigation of the charge transfer between several related donors. As can be seen from the figure, more than one complex is obviously involved in the association reaction between donor and *N*-benzylnicotinamide chloride, which explains partly why Cilento and Tedeschi (1961) were unable to obtain reproducible results with this substance. The problem is of course easily resolved when consideration is made of the fact that the benzene moiety is also a donor, albeit a weak one. The results shown in Figure 8 were measured in water without 1% ethanol, such that the observed binding constant (k = 5.2 ± 0.3 for more than 80 points on the saturation curve) is higher than that shown in Table I; all other parameters are, however, identical within very narrow limits. The apparent difference between the water and buffer curves is within the error of determination of the constants.

As a check on the stoichiometry, *N*-(2-indol-3-yl-ethyl)-nicotinamide chloride, an *intra*molecular charge transfer complex (Shifrin, 1964) involving the nicotinamide positive ion and indole, was titrated with *N*-methylnicotinamide chloride. This attempt to pick up the binding of a second mole of the acceptor could not be unequivocally interpreted. The binding, if any, was very weak, and the plotted data closely resembled a portion of the theoretical error curve (Deranleau, 1969). It can probably be safely concluded that there is negligible binding by a second mole at the concentrations available (up to 1.5 M in acceptor) for measurement. The evidence for 1:1 complexes as the major species in our experiments is then: (a) straight lines are obtained on the Scatchard plot, (b) Beer's law is strictly obeyed by the components (see Experimental Procedure), (c) k is invariant with wavelength, and (d) the *intra*molecular donor–acceptor complex does not bind a second mole of acceptor.

Effect of Temperature and Solvent. The complex between *N*-acetyl-L-tryptophan and *N*-methylnicotinamide chloride was examined in water as a function of temperature. The temperature dependence of the equilibrium constant is shown in Figure 9 (van't Hoff plot), and the thermodynamic parameters derived from the plot and referred to 25° are $G°$ = −0.98 kcal/mole, $H°$ = −3.5 kcal/mole, and $S°$ = −8.3 eu. These are in the range of values previously reported for charge transfer complexes (Briegleb, 1961). The temperature dependence of the extinction coefficient calculated at 350 nm (the calculation wavelength for k in Figure 9) is shown in Figure 10. As expected for well-behaved complexes, this dependence is small, amounting to a decrease of approximately 1 ϵ unit/degree. Figure 11 shows the temperature variation of the entire spectrum at three different temperatures; the solid curve is the spectrum in 1% ethanol at 25°. The maximum observed temperature difference, at the short wavelength end of the plot, amounts to 2 ϵ units/degree.

The solvent dependence of the binding is shown in Figure 12 on the Scatchard plot, where it can be seen that increasing the ethanol concentration markedly decreases the association

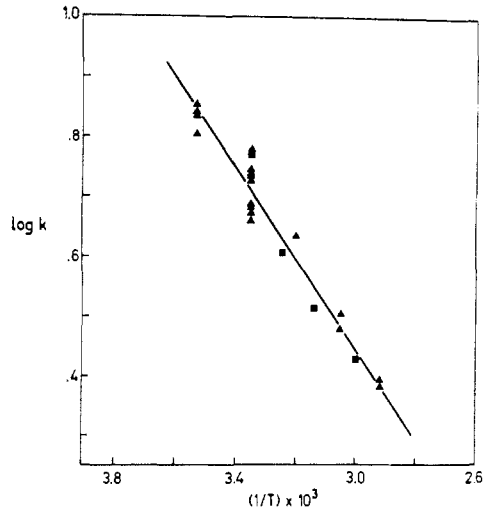

FIGURE 9: van't Hoff plot for the *N*-acetyl-L-tryptophan–*N*-methylnicotinamide chloride complex in water (temperature variation +10° to +70°).

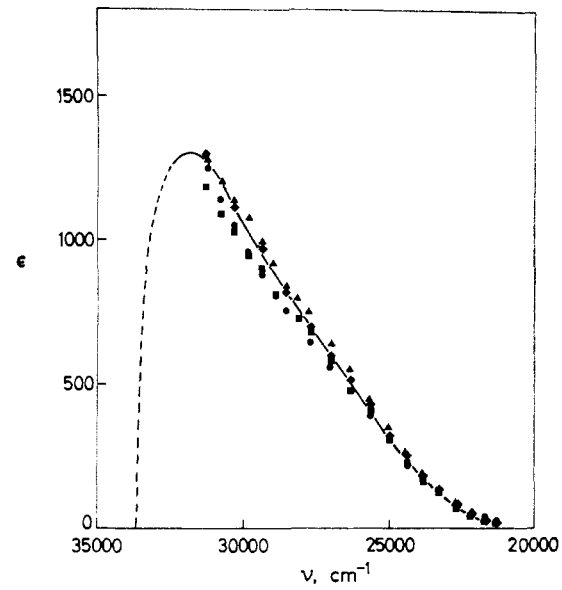

FIGURE 11: The charge transfer spectrum of the *N*-acetyl-L-tryptophan–*N*-methylnicotinamide chloride complex in water at various temperatures: 10° (▲), 25° (◆), 60° (●), 70° (■). Drawn curve: spectrum in 1% ethanol at 25°.

constant of the complex, while not appreciably affecting the extinction coefficient. The various constants are listed in Table III. As a matter of record, there is an apparent decrease in the intensity of the short wavelength part of the spectrum, and the long wavelength part of the spectrum is slightly red-shifted as the ethanol concentration is increased. The calculation wavelength (350 nm) is in a region where the effect is minimal, although it is small in any case.

Gaussian Approximation to the Charge Transfer Spectra. There is reason to believe that the charge transfer spectra of substituted indoles with *N*-methylnicotinamide and its isomers consists of at least two bands. The evidence for this is sketchy, but quantum-mechanical energy level calculations and the existence of a distinct shoulder on the spectra of the *N*-methylnicotinamide complexes (Figures 1 and 4) both indicate that more than one electronic transition is involved. Indeed, Moser (1968) has been able to demonstrate the existence of two bands in similar complexes utilizing the phthalyl group as a charge transfer acceptor. The spectra of the complexes reported herein can be fitted as the sum of two overlapping Gaussian bands, and the results are shown in Table IV. While approximations made in this manner can only be considered as crude in the absence of experimental evidence

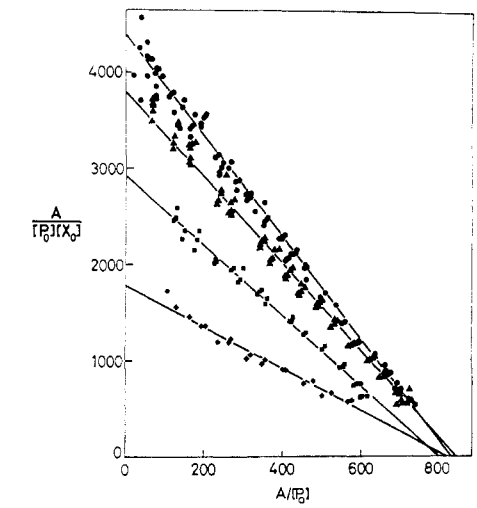

FIGURE 12: Scatchard plots for the *N*-acetyl-L-tryptophan–*N*-methylnicotinamide chloride complex formation at various ethanol concentrations: in water (●), 1% ethanol (▲), 20% ethanol (■), 40% ethanol (◆). See also Table III.

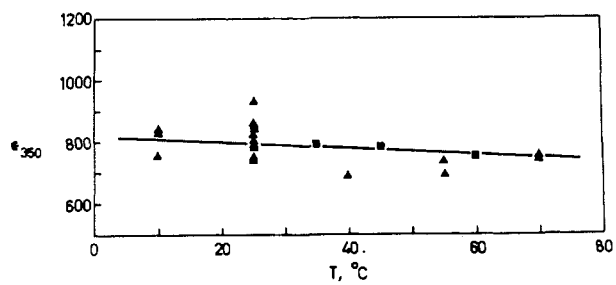

FIGURE 10: Temperature dependence of the extinction at 350 nm of the *N*-acetyl-L-tryptophan–*N*-methylnicotinamide chloride complex in water.

supporting the width, position, and relative intensity of the bands, the agreement with the quantum model of the transitions is good. We should note, however, that only the peak and long wavelength portion of the spectra were fitted in this manner. The short wavelength portion of the spectra, which is of questionable accuracy in any case, was ignored in the fitting procedure.

Acknowledgment

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TABLE III: Properties of the *N*-Methylnicotinamide Chloride–*N*-Acetyl-L-tryptophan Complex in Ethanol–Water Mixtures.

EtOH (% v/v)	k (l./mole)	ϵ(350 nm) (l./mole cm)
0	5.2 ± 0.3	830
1	4.5 ± 0.2	848
20	3.6 ± 0.2	800
40	2.2 ± 0.2	818

TABLE IV: Two Band Gaussian Approximation to the Charge Transfer Spectra of Intermolecular Complexes.[a]

Complex	Short Wavelength Band λ_{max} (nm)	Short Wavelength Band Area (%)	Long Wavelength Band λ_{max} (nm)	Long Wavelength Band Area (%)
N-Methylnicotinamide plus:				
Skatole	314	63	361	37
Indoleacetic acid	314	65	361	35
Indoleacetamide	318	64	362	36
N-Acetyl-L-tryptophan	313	62	361	38
N-Acetyl-L-tryptophanamide	314	63	361	37
N-Methylisonicotinamide plus:				
Skatole	350	73	415	27
Indoleacetic acid	351	75	408	25
N-Acetyl-L-tryptophan	348	73	408	27

[a] Determined on a DuPont Model 310 curve resolver, DuPont and Co., Wilmington, Del.

Appendix

Any set $\mathbf{C}_r^{p,t,s}$ of *conformers* (*C*) *realized* (*r*) under given conditions of pressure (*p*), temperature (*t*), and surroundings (*s*) (for example, crystal structure, solvent, presence of transport proteins, receptor molecules, antibodies, enzymes, membranes, etc.) contains, and is characterized by, a unique set of *binary side-chain interactions*, $\{I_r\}^{p,t,s}$; these are defined as "bilateral (yes or no) contacts," (i–j), between the side chains of any two amino acids i and j.[3] This definition is valid as well for any individual conformer $C_{r1}, C_{r2}, \ldots C_{rn}$:

$$C_{rn}^{p,t,s} = \{I_{rn}\}^{p,t,s}$$

if

$$C_{rn}^{p,t,s} \in \mathbf{C}_r^{p,t,s}$$

then

$$\{I_{rn}\}^{p,t,s} \subset \{I_r\}^{p,t,s}$$

Obviously, one cannot only describe conformers of single molecules in this manner, but also specific molecular associations, where we are dealing both with *intramolecular* side-chain interactions (defining the conformations of the individual molecules) and with *intermolecular* interactions, characterizing the contact surface between them (Carrión *et al.*, 1968).

In an arbitrary specific case, where different *possible* (*p*) *conformers*, $C_{p1}, C_{p2}, \ldots C_{pn}$, have been suggested by existing methods (hydrodynamic, spectroscopic, model studies, etc.), the detection of specific side-chain interactions could help in deciding which one(s) of the different possibilities is realized under given circumstances. Let us assume that C_{p1}, C_{p2}, and C_{p3} are possible conformers. Inspection of molecular models yields, for example, the following relationships

$$C_{p1} = \{I_{p1}\} = \{1\text{–}5, 1\text{–}12, 2\text{–}3, \ldots, n\text{–}m\}$$

$$C_{p2} = \{I_{p2}\} = \{1\text{–}7, 1\text{–}12, 2\text{–}13, \ldots, n\text{–}m\}$$

$$C_{p3} = \{I_{p3}\} = \{1\text{–}5, 1\text{–}9, 2\text{–}3, \ldots, n\text{–}m\}$$

Analytical detection of side-chain interactions shows contacts between the side chains of amino acid residues 1 and 5 (thus excluding C_{p2}) and 1 and 9 (thus excluding C_{p1}). Conformer C_{p3} appears, under the circumstances of the experiment, to be the most likely description of the conformer actually present, $\mathbf{C}_r^{p,t,s}$. By increasing the number of observations on existing and nonexisting binary side-chain interactions, the validity of our statement regarding the identity of C_{p3} and $\mathbf{C}_r^{p,t,s}$ can be further enhanced until, after all binary contacts have been established, the maximum of information available by this method is reached. The total information could be further increased if not only "yes or no" answers regarding the contact could be extracted, but if the specific method would allow us to determine the geometry of the binary interactions. It might be pointed out that if contact 1–12, as well as others pertaining to C_{p1}, were established, we would have to assume that both C_{p1} and C_{p2} were present in the proportions indicated by the quantitative relationship between contacts 1–12 and 1–9.

It should be stressed that the $\{I_r\}^{p,t,s}$ we are observing are always those of the compounds containing the probe, and that one should be very careful in drawing conclusions about the $\mathbf{C}_r$ of the unsubstituted polypeptide. Judicious and relevant application of probes would be facilitated if: (1) the energy of interaction between the probe and a side chain were of the same order of magnitude as ordinary *van der Waals* attraction between amino acid side chains and if it were enhanced in a quantitatively similar manner by "hydrophobic" interactions (Carrión *et al.*, 1968; review by Némethy, 1967); (2) the probe had similar dimensions as the (aromatic) residues of the amino acids phenylalanine, tyrosine, or tryptophan; (3) the probe could serve as an analog of an aromatic amino

[3] Such a simple, scalar description of side-chain interactions will not distinguish between two conformations with mirror-image relationship. The "contact distance" depends on the method of detection and may vary from 3 to about 6 Å using charge transfer or nuclear magnetic resonance techniques (Carrión *et al.*, 1968; Schwyzer and Ludescher, 1968) to over 30 Å using energy transfer (Foerster, 1946, 1960; Latt *et al.*, 1965; Stryer and Haugland, 1967).

acid and be incorporated into polypeptides without affecting their biological activity; (4) conclusions regarding the geometry of the interaction could be drawn from the spectral data.

Points 1, 2, and 3 would help ensure us that, upon intramolecular or intermolecular application of the probe, no gross distortions of the original $C_r^{p,t,s}$ (without subsequent changes of $\{I_r\}^{p,t,s}$) would be introduced. Point 4 would increase the amount of information to be gained by these methods; with nuclear magnetic resonance markers, conclusions regarding the geometry can already be drawn (Schwyzer and Ludescher, 1968); in the case of charge transfer probes, very little is known (White, 1959; Shifrin, 1964), but progress is being made (D. A. Deranleau, H. Bosshard, and R. Schwyzer, submitted for publication).

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