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Aggregations of Certain Nucleosides in Aqueous Solutions from Osmometric Measurements: Theory

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Abstract □ Two models, the mononuclear and multinuclear, are proposed for self-aggregating systems such as dilute aqueous solutions of 2′-deoxyadenosine, 2′-deoxyguanosine, uridine, cytidine, and thymidine, which are characterized by two linear slopes in plots of their osmolal *versus* total molal concentrations. Their first slopes indicate a monomeric behavior and a formation of critical micelle-like concentrations. By correlating the results of both models on a semiempirical basis, theoretical curves were generated that agree with previous experimental work. Equilibrium constants, osmotic coefficients, and concentrations of various associated forms can be calculated.

Keyphrases □ Nucleosides in aqueous solution, self-aggregation—osmometric measurements, theoretical curves compared to experimental data, calculation of equilibrium constants, osmotic coefficients, and species concentrations □ Osmometric measurements—self-aggregation of nucleosides in aqueous solution, theoretical curves compared to experimental data □ Aggregation of nucleosides in aqueous solution—osmometric measurements, theoretical curves compared to experimental data

The thermoelectric osmometer developed by Goyan and Johnson (1, 2) has been improved and used to study dilute aqueous solutions of caffeine and other substances of pharmaceutical interest (3). The measured osmotic property plotted against molal concentration resulted in two linear slopes for many substances. The point of break was sharp whenever it occurred and was regarded as evidence of a critical micelle concentration (CMC). The molal concentration where this break occurs is a critical concentration in that it is a property of a substance. It evidently has been overlooked by other workers, probably because of the difficulty of exploring the concentration range between 0.01 and 0.10 *M*. Later, Borazan (4) and Borazan and Goyan (5) extended past work (6, 7) into this region of concentration and included other compounds. The characteristic break between linear slopes was found for very dilute solutions of 2′-deoxyadenosine, 2′-deoxyguanosine, uridine, cytidine, and thymidine.

This paper presents some theoretical considerations in an effort to explain experimental results reported previously (3–5). An attempt is also made to recalculate equilibrium constants that might hold over a wider range of concentration than those given previously (8, 9).

THEORY

An attempt is made to explain the two intersecting linear slopes determined experimentally, when plotting a colligative property *versus* molal concentration (3–5), by using a simple mathematical model:

$$nA = A_n \qquad \text{(Eq. 1}a\text{)}$$

$$K = (A_n)/(A)^n \qquad \text{(Eq. 1}b\text{)}$$

The osmolal, o, and total molal, m, concentrations are given by the equations:

$$o = (A_n) + (A) \qquad \text{(Eq. 2)}$$

$$m = n(A_n) + (A) \qquad \text{(Eq. 3)}$$

In Eqs. 1–3, A represents the monomer and A_n represents the associated form. Parentheses indicate molal concentration. By

assigning values at intervals of 0.01 M to (A), it is easy to calculate corresponding values of (A_n) for selected values of K. When the values for o are plotted against m and compared directly with the experimental curves (3–5), the resulting curves are similar to those obtained from experimental data. In all of the graphs, the first parts were very similar to ideal solutions showing no association. This is due to the fact that the concentration of the n-mer is so low that it cannot be detected. When K is taken to be 1 or 10 and n to be 2 or 3, a few of the curves calculated in this way approximate a few of the intersecting straight lines. However, points near the intersection deviate from either line by more than can be accounted for on the basis of experimental error, except when the two slopes differ very little. To simulate the realistic slope change and an intersection in the concentration range studied with n equal to 4, it is necessary to assign large values for K. As reported previously (6, 7, 10–12), in systems of this type K is lower than 20 in most cases, and the free energy is on the order of magnitude of thermal kinetic energy. Therefore, a K value in the neighborhood of 10 with n equal to 3 seems to be a reasonable limit. With these values, a deviation of points near the intersection from straight lines is about 3 ohms. This deviation is outside the limit of experimental error by a factor of nearly 10. These facts clearly indicate that this simple mathematical model cannot be used.

A more sophisticated approach along the same lines was undertaken with the aid of a computer[1]. Based upon the assumption that two monomers react to form a dimer and that the dimer reacts with another monomer to form a trimer, *etc.*, four different association constants were postulated. The values 2, 4, 6, and 8 were assigned to K_1, K_2, K_3, and K_4 in rotation. The resulting 256 curves were calculated and plotted by the computer.

When the experimental slopes (5) are converted to the osmolal system by dividing by 924 from the sucrose standardization, all initial slopes for single pure substances in water show an experimental value of unity within the limits of experimental error. All computer-generated curves based on four K values, where $K_1 = 2$, show an initial slope of 0.98 based on the first plotted point and 0.96 or 0.95 based on the second point, regardless of the values assigned to K_2, K_3, and K_4. Higher values of K_1 produce lower slopes, indicating the need to try smaller values for the initial association constants.

A study of the lines generated with four association constants showed that K_4 played a minor role. It can be concluded from this study that association constants are very small and that certain combinations of K values can produce almost two linear intersecting slopes in the range of concentrations studied, with the first slopes having values approximately equal to one, thus resembling reported experimental curves (5).

In an effort to calculate equilibrium constants for the successive aggregation processes from the experimental curves reported, two models are proposed here.

Mononuclear Aggregation—A solute molecule, say A', can serve as a nucleus for other molecules of the same solute:

$$A' + A = A'A \qquad \text{(Eq. 4a)}$$

$$A'A + A = A'A_2 \qquad \text{(Eq. 4b)}$$

$$\vdots$$

$$A'A_{j-1} + A = A'A_j \qquad \text{(Eq. 4c)}$$

$$\vdots$$

$$A'A_{\sigma-1} + A = A'A_\sigma \qquad \text{(Eq. 4d)}$$

where σ is the maximum number of multiple equilibria. In the model presented here, it is assumed that no interactions among various associated forms take place and that the binding sites on the nucleus molecule are dependent; *i.e.*, the binding of a monomer to a nucleus can influence the binding of another monomer to the same nucleus. Therefore, the following equations can be written:

$$K_1 = (A'A)/(A')(A) \qquad (A'A) = K_1(A')(A) \qquad \text{(Eq. 5)}$$

$$K_2 = (A'A_2)/(A'A)(A) \qquad (A'A_2) = K_2(A'A)(A) \qquad \text{(Eq. 6)}$$

$$K_j = (A'A_j)/(A'A_{j-1})(A) \qquad (A'A_j) = K_j(A'A_{j-1})(A) \qquad \text{(Eq. 7)}$$

$$K_\sigma = (A'A_\sigma)/(A'A_{\sigma-1})(A) \qquad (A'A_\sigma) = K_\sigma(A'A_{\sigma-1})(A) \qquad \text{(Eq. 8)}$$

Combining Eqs. 5 and 6:

$$(A'A_2) = K_1K_2(A')(A)^2 \qquad \text{(Eq. 9)}$$

If one carries the same technique of substitutions for the consecutive processes, the following equation can be derived:

$$(A'A_j) = K_1K_2 \ldots K_j(A')(A)^j \qquad \text{(Eq. 10)}$$

Under the assumption that no self-association takes place below the critical concentrations, (C_0), based upon experimental results, and if (C_0) and $(A_0 - C_0)$ are considered as the total concentrations of the reactant species, A' and A, respectively, where (A_0) is the total concentration of the solute, then the following can be defined:

$$(C_0) = (A') + (A'A) + (A'A_2) + \ldots + (A'A_j) + \ldots + (A'A_\sigma) \qquad \text{(Eq. 11)}$$

$$= (A') + \sum_{j=1}^{\sigma} (A'A_j)$$

By substitution from Eq. 10 of $(A'A_j)$ in Eq. 11:

$$(C_0) = (A')\left[1 + \sum_{j=1}^{\sigma}\left(\prod_{j=1}^{j} K_j\right)(A)^j\right] \qquad \text{(Eq. 12)}$$

where:

$$\prod_{j=1}^{j} K_j = K_1K_2 \ldots K_j$$

$$(A_0 - C_0) = (A) + (A'A) + \ldots + j(A'A_j) + \ldots + \sigma(A'A_\sigma) \qquad \text{(Eq. 13)}$$

$$= (A)\left[1 + (A')\sum_{j=1}^{\sigma} j\left(\prod_{j=1}^{j} K_j\right)(A)^{j-1}\right]$$

and:

$$o = (A') + (A) + (A'A) + \ldots + (A'A_j) + \ldots + (A'A_\sigma) \qquad \text{(Eq. 14)}$$

$$= (A') + (A) + \sum_{j=1}^{\sigma} (A'A_j)$$

where o represents osmolal concentration.

The second linear slope, S_2 (5), can be expressed:

$$S_2 = [o - (C_0)]/(A_0 - C_0) = \frac{\text{second experimental slope}}{\text{sucrose slope}} \qquad \text{(Eq. 15)}$$

By substitution from Eqs. 14, 11, and 13 of o, C_0, and $(A_0 - C_0)$, respectively, in Eq. 15:

$$S_2 = \left[1 + (A')\sum_{j=1}^{\sigma} j\left(\prod_{j=1}^{j} K_j\right)(A)^{j-1}\right]^{-1} \qquad \text{(Eq. 16)}$$

set:

$$\left[1 + \sum_{j=1}^{\sigma}\left(\prod_{j=1}^{j} K_j\right)(A)^j\right] = \phi(K_j, A) \qquad \text{(Eq. 17)}$$

and:

$$\sum_{j=1}^{\sigma} j\left(\prod_{j=1}^{j} K_j\right)(A)^{j-1} = \frac{\partial\phi}{\partial(A)} \qquad \text{(Eq. 18)}$$

Substituting for the summation of terms, Eq. 18, by the partial derivative into Eq. 16:

$$\frac{\partial\phi}{\partial(A)} = \frac{1}{(A')}\left(\frac{1 - S_2}{S_2}\right) \qquad \text{(Eq. 19)}$$

Combining Eqs. 17 and 12 and substituting into Eq. 19:

$$\left(\frac{(C_0)S_2}{1 - S_2}\right)\frac{\partial\phi}{\partial(A)} = \phi \qquad \text{(Eq. 20)}$$

[1] The approach was suggested by Professor Leo Brewer, University of California, Berkeley. An IBM 360 computer was programmed by Mr. Phillip Johnson, University of California San Francisco Information Service, who contributed much of his own time and thought to this portion of the work.

Table I—Equilibrium Constants of Self-Association of the Nucleosides Together with Their Progression Constant

Nucleoside	Progression[a] Constant, α_s, molal	β_1, molal^{-1}	β_2, molal^{-1}	β_3, molal^{-1}
2′-Deoxyadenosine	0.0272	4.07	12.20	36.60
2′-Deoxyguanosine	0.0193	5.77	17.30	51.80
Uridine	0.330	0.33	1.00	3.00
Cytidine	0.338	0.40	1.20	3.60
Thymidine	0.238	0.55	1.65	4.95

[a] Progression constant $= \dfrac{\text{second slope} \times \text{critical concentration}}{\text{sucrose slope} - \text{second slope}}$, according to Eq. 21. The data were taken from *Reference 5*. The slope of standard solution of sucrose is 923.7 ohms molal^{-1}.

The critical concentration, (C_0), is supposed to be a constant quantity for a particular molecule; therefore:

$$\frac{(C_0)S_2}{1 - S_2} = \text{constant} = \alpha_s \qquad \text{(Eq. 21)}$$

Equation 20 has the following solution:

$$\phi = e^{(A)/\alpha_s} \qquad \text{(Eq. 22)}$$

The function ϕ can be expressed as a power series in $(A)/\alpha_s$, and according to Eq. 17:

$$\phi = 1 + K_1(A) + K_1K_2(A)^2 + \ldots + K_1 \ldots K_j(A)^j + \ldots + K_1 \ldots K_\sigma(A)^\sigma$$
$$= 1 + \frac{(A)}{\alpha_s} + \frac{1}{2!}\left(\frac{(A)}{\alpha_s}\right)^2 + \ldots + \frac{1}{j!}\left(\frac{(A)}{\alpha_s}\right)^j + \ldots + \frac{1}{\sigma!}\left(\frac{(A)}{\alpha_s}\right)^\sigma + \ldots + \frac{1}{\infty!}\left(\frac{(A)}{\alpha_s}\right)^\infty \qquad \text{(Eq. 23)}$$

If the number of multiple equilibria, σ, is large enough, one can deduce with reasonable approximation, for low concentration of A, the following by comparing coefficients of (A) from Eq. 23:

$$K_1 = 1/\alpha_s \qquad K_j = 1/j\,\alpha_s \qquad \text{(Eq. 24a)}$$

$$K_2 = 1/2\,\alpha_s \qquad K_\sigma = 1/\sigma\,\alpha_s \qquad \text{(Eq. 24b)}$$

Multinuclear Aggregation—According to this model, the binding of a monomer to $(j - 1)$-mer to produce j-mer induces an identical, independent binding probability for another monomer; any of the equivalent subunits in the j-mer can serve as a binding site for another monomer and so on. Therefore, one would expect that the overall equilibrium constants are increasing and related to one intrinsic binding constant, k.

Based upon configurational probabilities for these completely independent and identical sites, the following can be derived (13):

$$A + A = A_2 \qquad k/\sigma = \beta_1 \qquad \text{(Eq. 25a)}$$

$$A_2 + A = A_3 \qquad \left(\frac{2}{\sigma - 2 + 1}\right) k = \beta_2 \qquad \text{(Eq. 25b)}$$

$$\vdots$$

$$A_j + A = A_{j+1} \qquad \left(\frac{j}{\sigma - j + 1}\right) k = \beta_j \qquad \text{(Eq. 25c)}$$

$$\vdots$$

$$A_\sigma + A = A_{\sigma+1} \qquad \sigma k = \beta_\sigma \qquad \text{(Eq. 25d)}$$

Since no simple way is available for one to derive the precise values of k and σ, the progression constant, α_s, described by Eq. 21 was sought intuitively as a possible common constant between the overall equilibrium constants in the two models. It is, therefore, assumed empirically that the reciprocal of the progression constant which determines the first and highest equilibrium constant in the first model could also determine the highest but the last equilibrium constant in the second model. It is also assumed that the last equilibrium constant for the mononuclear aggregation model, K_σ (when all binding sites are occupied except one), may be the real intrinsic binding constant, k, in the second model. Accordingly, the following are set:

$$K_1 = \beta_\sigma = \sigma K_\sigma = \frac{1}{\alpha_s} = \frac{1 - S_2}{(C_0)S_2} = \sigma k \qquad \text{(Eq. 26)}$$

$$k = \frac{1 - S_2}{\sigma(C_0)S_2} \qquad \text{(Eq. 27)}$$

and β_j, according to Eqs. 25 and 27, is:

$$\beta_j = \frac{j(1 - S_2)}{(\sigma - j + 1)\,[\sigma(C_0)S_2]} \qquad \text{(Eq. 28)}$$

Osmolal and total molal concentrations can be defined and related to measurable quantities:

$$o = (A) + (A_2) + (A_3) + \ldots + (A_{j+1}) + \ldots + (A_{\sigma+1}) \qquad \text{(Eq. 29)}$$

Concentrations of various species can be expressed in terms of their corresponding equilibrium constants and free monomer concentration:

$$(A_2) = \beta_1(A)^2 \qquad \text{(Eq. 30a)}$$

$$(A_3) = \beta_1\beta_2(A)^3 \qquad \text{(Eq. 30b)}$$

$$(A_{j+1}) = \beta_1\beta_2 \ldots \beta_j(A)^{j+1} \qquad \text{(Eq. 30c)}$$

Thus:

$$o = (A) + \sum_{j=1}^{\sigma}\left(\prod_{j=1}^{j}\beta_j\right)(A)^{j+1} \qquad \text{(Eq. 31)}$$

Substituting for the product of β_j, according to Eq. 28:

$$o = (A) + \sum_{j=1}^{\sigma}\frac{j!(1 - S_2)^j}{\left[\prod_{j=1}^{j}(\sigma - j + 1)\right][\sigma(C_0)S_2]^j}(A)^{j+1} \qquad \text{(Eq. 32)}$$

By carrying similar substitutions, the total molal concentration will be:

$$m = (A) + \sum_{j=1}^{\sigma}(j + 1)\frac{j!(1 - S_2)^j}{\left[\prod_{j=1}^{j}(\sigma - j + 1)\right][\sigma(C_0)S_2]^j}(A)^{j+1} \qquad \text{(Eq. 33)}$$

The osmotic coefficient can be calculated from the definitions of o and m by the following relationship:

$$\phi = o/m \qquad \text{(Eq. 34)}$$

RESULTS AND DISCUSSION

Equations 24*a* and 24*b* indicate that the highest equilibrium constant is the first and that the experimental curves can only be generated by previous knowledge of their critical concentrations. However, a given set of K values calculated from the relationships given (in Eqs. 24*a* and 24*b*), three or four K's, failed to reproduce curves obtained experimentally when plotting their calculated osmolal *versus* total molal concentration according to Eqs. 2 and 3.

Borazan (4) hypothesized that the possible maximum number of molecules in each aggregate of the nucleosides studied is four to five (all molecules in each aggreagate are not necessarily bonded at the same time, according to the theory). Thus, one would expect the number of multiple equilibria, σ, to be between three and four.

To test the second model and the validity of the assumptions made in the derivations when osmotic coefficient, ϕ, was calculated for $\sigma = 3$, several values were assigned for the monomer concentrations and Eqs. 32–34 were applied, with data taken from Borazan and Goyan (5), and compared to experimental values; reasonable agreement was found over a wide molal range for most of the nucleosides. The data are summarized in Table II. In this way, theoretical curves can be calculated from the definitions of osmolal and molal concentrations, Eqs. 32 and 33, respectively. The overall equilibrium constants calculated according to Eqs. 25 and 27 and from data taken from Borazan and Goyan (5) (Table I) can be used to calculate concentrations of various associated forms.

It was found by trial and error that slightly lower values of S_2

Table II—Comparison between Osmotic Coefficients Interpolated from Osmotic Curves Calculated According to Eqs. 32 and 33, ϕ_{calc}; Osmotic Coefficients Calculated from Experimental Data (*Reference 5*), ϕ_{exp}; and Experimental Values Reported in the Literature at 25°, ϕ_{rep}

Molal Concentration	ϕ_{calc}	ϕ_{exp}	ϕ_{rep}
	2′-Deoxyadenosine		
0.005	0.970	1.004	—
0.010	0.955	1.004	—
0.015	0.923	1.004	—
0.01725	0.913	0.961	0.84 ± 0.07[a]
0.020	0.907	0.918	—
0.025	0.871	0.864	0.900 ± 0.01[b]
0.030	0.846	0.828	—
0.0345	0.815	0.804	0.760 ± 0.04[a]
0.040	0.796	0.782	—
0.050	0.770	0.755	0.800 ± 0.005[b]
	2′-Deoxyguanosine		
0.010	—	1.001	—
0.012	—	1.001	—
0.014	0.907	0.957	—
0.015	0.896	0.931	—
0.016	0.887	0.911	—
0.017	0.877	—	—
	Uridine		
0.030	0.990	0.999	—
0.040	0.988	0.999	—
0.050	0.983	0.999	0.969[c]
0.060	—	0.991	—
0.070	—	0.972	—
0.080	0.975	0.957	—
0.094	0.968	0.942	0.952[c], 0.962[c]
0.095	0.965	0.941	0.933[c]
0.096	0.965	0.940	0.937[c]
0.100	0.960	0.936	0.943[c]
0.130	0.947	—	—
0.150	0.944	—	—
0.192	0.925	—	0.896[c], 0.903[c]
0.292	0.881	—	0.861[c]
0.395	0.837	—	0.838[c]
0.501	0.800	—	0.804[c]
0.579	0.773	—	0.768[c]
0.693	0.740	—	0.756[c]
0.710	0.735	—	0.760[c]
	Cytidine		
0.030	0.987	1.000	—
0.040	0.983	1.000	—
0.050	0.980	0.981	0.967[c]
0.070	0.961	0.955	—
0.080	0.954	0.947	—
0.0941	0.947	0.939	0.941[c]
0.0948	0.947	0.939	0.939[c]
0.0955	0.944	0.938	0.936[c]
0.100	0.943	0.936	0.935[c]
0.130	0.931	—	—
0.150	0.928	—	—
0.194	0.905	—	0.888[c]
0.291	0.855	—	0.833[c], 0.825[c]
0.397	0.804	—	0.791[c]
0.503	0.764	—	0.750[c]
0.695	0.701	—	0.697[c]
0.709	0.697	—	0.693[c]
	Thymidine		
0.040	0.977	1.001	—
0.050	0.977	1.001	—
0.070	0.975	0.963	—
0.090	0.944	0.929	—
0.100	0.930	0.917	0.905[b]
0.130	0.910	—	—
0.150	0.900	—	—
0.200	0.863	—	0.805[b]
0.250	0.830	—	—
0.300	0.800	—	—
0.344	0.775	—	—

[a] From *Reference 14*. [b] From *Reference 7*. [c] From *Reference 6*.

for cytidine and thymidine should be used to generate curves that almost duplicate experimental curves within the limit of accuracy. Accordingly, values of 0.87 and 0.782 were used instead of 0.891 and 0.807 for cytidine and thymidine, respectively.

It was also observed that the shapes of the calculated curves were similar to those obtained experimentally and that all points fell on almost two linear slopes. The deviation of the data from the first ideal slopes is considered just about the limit of experimental error.

Ts'o and Chan (9) also based their calculations of K on multiple-equilibria processes (of the type of Eqs. 25*a*–25*d*) and on the assumption that all K's are equal. Assuming that the multiple equilibria go to infinity, they derived the following equation, which is based upon the work of Schellman (8):

$$K = \frac{1 - \phi}{M\phi^2} \qquad \text{(Eq. 35)}$$

where M = total molal concentration, and ϕ = osmotic coefficient.

Attempts to use Eq. 35 with experimental data (5) at 0.1 molal gave values for K of 0.705, 0.705, and 0.973 for uridine, cytidine, and thymidine, respectively. Ts'o *et al.* (6) calculated 0.61 and 0.87 for uridine and cytidine, respectively, while Solie (10) calculated 0.91 for thymidine at the same concentration and showed that the values held fairly well at higher concentrations. However, the low concentration values of Ts'o *et al.* (6) were somewhat influenced by curve smoothing and fitted to preselected polynomial. Attempts to calculate K values at some of the low accurately measured values (5) show that Eq. 35 fails to yield nearly constant values, even well above the critical concentration. Broom *et al.* (7) agreed that in some cases Eq. 35 fails even at higher concentrations using data taken from smoothed curves. It should be emphasized that Eq. 35 worked well at concentrations higher than 0.1 molal, while it failed to produce constant K values at concentrations lower than 0.1 molal.

Therefore, the results presented in this paper seem to be more meaningful and realistic, in that several consecutive equilibrium constants with increasing order and limited associated forms are to be considered, and theoretical values that agree with experimental data (Table II) can be generated over a far greater range than with the model previously reported (8, 9).

It should also be emphasized that generating theoretical data that agree with experimental data from a model does not mean that the model is absolutely correct. The actual situation could be different from that hypothesized, knowing that the results presented were subject to various approximations and empirical assumptions, for example, ignoring some interactions, mathematical approximations, *etc.*

CONCLUSION

Theoretical curves have been generated to reproduce experimental results within the limit of accuracy. The model in question assumes the generation of equivalent and independent binding sites. The overall equilibrium constants are increasing, and limited associated forms have to be considered, in the neighborhood of four for the nucleosides studied.

The theoretical osmotic coefficient values (Table II) agree fairly well with those reported in the literature.

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This work is dedicated to Professor Frank M. Goyan, Department of Pharmaceutical Chemistry, University of California, San Francisco, Calif., on his retirement to honor him for his contribution to pharmacy.

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Synthesis of Substituted Cinnamides: Relationship between Anticonvulsant and Monoamine Oxidase Inhibitory Properties

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Abstract □ Several esters and hydrazides of substituted cinnamides were synthesized and characterized. Evaluation of their anticonvulsant activity indicated appreciable activity of hydrazides where no correlation could be observed between anticonvulsant activity possessed by these hydrazides and their ability to inhibit monoamine oxidase.

Keyphrases □ Cinnamides of substituted amino acid esters and their hydrazides—synthesis, relationship between anticonvulsant and monoamine oxidase inhibitory properties □ Monoamine oxidase inhibitors, cinnamides of amino acid esters and their hydrazides—synthesis, anticonvulsant activity □ Anticonvulsant activity—cinnamides of substituted amino acid esters and their hydrazides, relationship to monoamine oxidase inhibitory properties □ Structure-activity relationships—cinnamides, relationship between anticonvulsant and monoamine oxidase inhibitory properties

Presence of a styryl group, responsible for high π-electron density, has been postulated to account for monoamine oxidase [EC 1.4.3.4 monoamine-O_2 oxido reductase (deaminating)] inhibitory property of styrylquinoliniums (1). The ability of monoamine oxidase inhibitors to possess anticonvulsant activity (2, 3) led to the synthesis of α-benzoylamino-*N*-[*p*-(4-aryl semicarbazide/thiosemicarbazide) - benzoyl] - *p* - substituted cinnamides possessing both an ethylenic moiety and the hydrazide group in an attempt to correlate their anticonvulsant activity with *in vitro* monoamine oxidase inhibitory effectiveness (4). In the present study, substituted cinnamides of amino acid esters and their corresponding hydrazides were synthesized as possible anticonvulsants to investigate correlation between anticonvulsant activity possessed by these hydrazides with their ability to inhibit monoamine oxidase.

CHEMISTRY

The various cinnamides of substituted amino acid esters and their corresponding hydrazides (Tables I and II) were synthesized by following the methods outlined in Scheme I.

Substituted oxazolones (*Ia*) were obtained by treatment by hippuric acid with appropriate aromatic aldehydes and acetic anhydride in the presence of anhydrous sodium acetate. Compounds *Ia* were treated with ethyl esters of amino acids (*Ib*) in the presence of 2–3 drops of triethylamine to give esters (I–XII) which were converted into the corresponding hydrazides (XIII–XXIV) by refluxing with 99–100% hydrazine hydrates (1:2 molar ratio) in absolute ethanol for 6–8 hr.

EXPERIMENTAL

Ethyl Esters of Amino Acids (*Ib*)—These were prepared by following the method of Kupryszewski and Sokolowska (5). Thionyl chloride, 0.1 mole, was added at 5° to 50 ml. of an absolute ethanolic solution of the appropriate amino acid (0.1 mole) and the mixture was refluxed for 3 hr. The excess thionyl chloride was removed under reduced pressure. The free ester, which precipitated by passing a slow stream of dry ammonia into a suspension of the ester hydrochloride in chloroform, was filtered, and excess chloroform was removed by distillation under reduced pressure.

2-Phenyl-4-(substituted benzylidene)-oxazole-5-ones (*Ia*)—The various oxazolones were prepared by heating a mixture of an appropriate aromatic aldehyde (0.96 mole), powdered dry hippuric acid (1.07 moles), freshly powdered sodium acetate (0.98 mole), and acetic anhydride (2.9 moles) in a flask on an electric hot plate (6, 7). As soon as the material had liquefied completely, the flask was transferred to a steam bath and heated for 2 hr. The reaction mixture was allowed to stand overnight, and the solid mass which precipitated was filtered with suction and washed first with two 100-ml. portions of ice-cold ethanol and finally with two 100-ml. portions of boiling water. After drying, the various oxazolones were used without further purification.

α-Benzoylamino-*N*-(substituted esters)-substituted Cinnamides (I–XII)—Equimolar quantities (1 mole) of the appropriate 2-phenyl-4-(substituted benzylidene)-oxazole-5-ones and ethyl esters of amino acids were refluxed in absolute ethanol in the presence of 2–3 drops of triethylamine on a steam bath for 6–8 hr. Excess ethanol was distilled, and the cinnamides which separated on cooling were filtered and recrystallized with either ethanol or the mixture of ethanol and water. The cinnamides were characterized by their sharp melting points and elemental analyses (Table I).

α-Benzoylamino-*N*-(substituted hydrazides)-substituted Cinnamides (XIII–XXIV)—A mixture of the appropriate α-benzoylamino-*N*-(substituted esters)-substituted cinnamides (0.1 mole) and 99–100% hydrazine hydrate (0.12 mole) in absolute ethanol was refluxed on a water bath for 6–8 hr. The solid mass which separated on cooling was filtered and recrystallized from suitable solvents (Table II).