References

Cerletti, P., Ipata, P. L., and Silipradi, N. (1957), *Anal. Chim. Acta. 16*, 548.
Greenberg, G. R. (1954), *Federation Proc. 13*, 745.
Jaenicke, L., and Brode, E. (1961), *Biochem. Z. 334*, 342.
Kay, L. D., Osborn, M. J., Hatefi, Y., and Huennekens, F. M. (1960), *J. Biol. Chem. 235*, 195.
Kornberg, A., and Pricer, W. E. (1951), *J. Biol. Chem. 193*, 481.
Krishnan, P. S. (1955), *Methods Enzymol. 2*, 591.
Layne, E. (1957), *Methods Enzymol. 3*, 447.
Lowry, O. H., and Lopez, J. A. (1946), *J. Biol. Chem. 162*, 421.
Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951), *J. Biol. Chem. 193*, 265.
May, M., Bardos, T. J., Barger, F. L., Lansford, M., Ravel, J. M., Sutherland, G. L., and Shive, W. (1951), *J. Am. Chem. Soc. 73*, 3067.
Munch-Peterson, A., and Kalckar, H. M. (1957), *Methods Enzymol. 3*, 469.
Nikiforuk, G., and Colowick, S. P. (1955), *Methods Enzymol. 2*, 469.
Peters, J. M., and Greenberg, D. M. (1957), *J. Biol. Chem. 226*, 329.
Peters, J. M., and Greenberg, D. M. (1958), *J. Am. Chem. Soc. 80*, 2719.
Peterson, E. A., and Sober, H. A. (1956), *J. Am. Chem. Soc. 78*, 751.
Rabinovitz, J. C. (1960), *Enzymes 2*, 203.
Rabinovitz, J. C., and Pricer, W. E., Jr. (1957), *J. Biol. Chem. 229*, 321.
Tabor, H., and Wyngarden, L. (1959), *J. Biol. Chem. 234*, 1830.

Countercurrent Distribution of Chemically Reacting Systems. IV. Kinetically Controlled Dimerization in a Boundary*

David F. Oberhauser, J. L. Bethune, and Gerson Kegeles

ABSTRACT: Countercurrent distribution is a discrete separation process for which any theoretical treatment is handled conveniently by digital computer methods. Useful information about other separation methods can be derived, by analogy, from the predictions obtained from such calculations. In particular, it is possible to treat the difficult problem of the separation of chemically reacting systems by this method (Bethune, J. L., and Kegeles, G., 1961, *J. Phys. Chem. 65*, 1761). This investigation includes the effects of kinetics and diffusion in a dimerizing system subjected to transport. When diffusion effects are taken into account, the distinctive features of such systems, found by solution of asymptotic equations (Belford, G. G., and Belford, R. L., 1963, *J. Chem. Phys. 37*, 1926), are altered. For example, alterations are found in the functional dependence of peak height and area upon time as well as in the range of the relevant parameters over which three boundaries are observed. In addition, it is found that either monomer or dimer can give bimodal boundaries, while the total pattern shows either one, two, or three boundaries.

The theory for moving-boundary experiments involving a single species in a countercurrent distribution apparatus has been rigorously developed by Bethune and Kegeles (1961c). In this paper, it was shown that first differences of concentration for a moving-boundary process in countercurrent distribution were identical with the corresponding concentration values, as described by binomial coefficients, for the zone process. When the number of transfers is large, the pattern described by the binomial coefficient expressions is very nearly equal to a Gaussian error function (Craig and Craig, 1950). In the case of electrophoresis, the solution of the continuity equation, starting with an infinitely sharp zone, gives the Gaussian error function for concentration, while starting with an infinite volume of solution results in the Gaussian error function for the derivative of the concentration curve. If an analogy is drawn between certain parameters in one process (for example, number of transfers), and corresponding parameters in the other process (for example, time), the results become formally identical. It is on the basis of such a correspondence that the theoretical results obtained for countercurrent distribution may be

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applied, at least qualitatively, to the experimental results obtained from the application of other transport methods.

The effect of chemical reactions upon the experimental records obtained from the application of transport methods such as electrophoresis, sedimentation, chromatography, countercurrent distribution, diffusion, and electrodiffusion has, in recent years, undergone extensive theoretical investigation (Gilbert, 1955, 1959; Mysels, 1956; Cann *et al.*, 1957; Bak and Kauman, 1959; Gilbert and Jenkins, 1959; Keller and Giddings, 1960; Scholten, 1961; Bethune and Kegeles, 1961a,b,c; Cann and Bailey, 1961; Van Holde, 1962; Belford and Belford, 1962; Albright, 1963). In many of these investigations it has been postulated that the chemical reaction attains equilibrium very rapidly.

Kinetically controlled reactions of only two types, isomerization and dimerization, have been investigated, however. In the case of isomerization the complete differential equations have been solved numerically for a number of cases (Scholten, 1961; Cann and Bailey, 1961). The effect of isomerization and dimerization, under kinetic control, upon sedimentation patterns has been explored (Van Holde, 1962; Belford and Belford, 1962), but with the assumption that diffusion does not occur during the transport experiment. This restriction has also been imposed in many of the cases in which an equilibrium system has been investigated (Gilbert, 1955; Gilbert and Jenkins, 1959).

A countercurrent distribution analog to moving-boundary experiments (Bethune and Kegeles, 1961c), where a boundary-spreading process analogous to diffusion does occur, has been used to investigate the effect of chemical reactions upon transport processes. This approach, which has hitherto been applied only to systems in equilibrium, has now been extended to those under kinetic control. This paper reports the results obtained for the dimerization reaction.

Theory

The Model System. The assumptions made in deriving the model for which calculations are performed are the following: (1) The solvents are immiscible; (2) no volume changes occur as a result of either extraction or reaction of the solutes; (3) all solutions are thermodynamically ideal; (4) there is instantaneous coupling across the phase boundary (i.e., all distribution equilibria are attained instantaneously); (5) the partition coefficient for each solute species is a constant over the concentration ranges employed; (6) the kinetics of the system are as represented by the equation. For a dimerization reaction in a two-phase system, the following model can be set up under these assumptions.

$$\begin{array}{lcl} 2\,M_U & \underset{k_1'}{\overset{k_2'}{\rightleftharpoons}} & D_U \quad | \quad V_U \\ K_M \big\downarrow\big\uparrow & & \big\downarrow\big\uparrow K_D \\ 2\,M_L & \underset{k_3'}{\overset{k_4'}{\rightleftharpoons}} & D_L \quad | \quad V_L \end{array}$$

Here M_U and D_U represent the monomer and dimer in the upper phase of volume V_U; M_L and D_L, the monomer and dimer in the lower phase of volume V_L; k_1' and k_2', k_3' and k_4' are the forward and reverse rate constants in the upper and lower phases; and K_M and K_D are the partition coefficients of monomer and dimer, respectively.

Mathematical Development. The relevant rate equations for chemical reaction in either phase may be written as, e.g.,

$$\left\{\frac{d[M]_U}{dt}\right\}_{chem} = -k_1'[M]_U^2 + k_2'[D]_U \qquad (1)$$

where the brackets denote concentrations measured in mass per unit volume. Since, in the calculations, the principle of conservation of matter can serve as a constant error check, these are transformed from concentration units to mass units by multiplying by the respective phase volume. The total mass change in the system, for any one species, may then be written as

$$V_U\left\{\frac{d[M]_U}{dt}\right\}_{total} + V_L\left\{\frac{d[M]_L}{dt}\right\}_{total} = \frac{dM}{dt} \qquad (2)$$

where the absence of brackets denotes mass units. Since, however,

$$V_U\left\{\frac{d[M]_U}{dt}\right\}_{partition} = -V_L\left\{\frac{d[M]_L}{dt}\right\}_{partition}$$

and

$$\left\{\frac{d[M]_U}{dt}\right\}_{total} = \left\{\frac{d[M]_U}{dt}\right\}_{chem} + \left\{\frac{d[M]_U}{dt}\right\}_{partition}$$

equation (2) may be written, by use of equation (1), as

$$\frac{dM}{dt} = V_U\{-k_1'[M]_U^2 + k_2'[D]_U\} + V_L\{-k_3'[M]_L^2 + k_4'[D]_L\} \qquad (3)$$

and with partition coefficients defined as, e.g.,

$$K_M = \frac{V_U}{V_L} \times \frac{[M]_U}{[M]_L} = \frac{V_U}{V_L} K_M'$$

equation (3) becomes

$$\frac{dM}{dt} = (-k_1'/V_U - k_3'/V_L K_M^2)M_U^2 + (k_2' + k_4'/K_D)D_U \qquad (4)$$

Since partition equilibrium is assumed, the fraction of M or D in the upper phase can be represented by, e.g.,

$$\frac{M_U}{M} = \frac{K_M}{1 + K_M}$$

Equation (4) can be written in terms of total species masses as

$$\frac{dM}{dt} = \{(-k_1'K_M^2/V_U - k_3'/V_L)/(1 + K_M)^2\}M^2 + \{(k_2'K_D + k_4')/(1 + K_D)\}D \quad (5)$$

Considering the process in any given tube as a single reaction, we may define two new rate constants by

$$k_f = -(k_1'K_M^2/V_U + k_3'/V_L)/(1 + K_M)^2$$

and

$$k_r = (k_2'K_D + k_4')/(1 + K_D)$$

Equation (5) may then be written as

$$\frac{dM}{dt} = -k_fM^2 + k_rD \quad (6)$$

and, since

$$-\frac{dM}{dt} = \frac{dD}{dt}$$

it follows that

$$\frac{dD}{dt} = k_fM^2 - k_rD \quad (7)$$

If, at the start of the reaction, the masses of monomer and dimer are M^0 and D^0, respectively, then reaction changes these by a *weight z*. Equations (6) and (7) then become, since $M^0 - z = D^0 + z$,

$$\frac{dz}{dt} = k_f(M^0 - z)^2 - k_r(D^0 + z)$$

which may be immediately integrated, with $z = 0$ at $t = 0$, to give

$$z = \frac{(b + w)\Psi - (b - w)}{2k_f(1 - \Psi)} \quad (8)$$

where

$$w = \sqrt{b^2 - 4ac}$$

$$\Psi = \frac{b - w}{b + w}e^{wt}$$

$$a = k_f$$

$$b = (-2k_fM^0 - k_r)$$

$$c = (k_f(M^0)^2 - k_r)D^0$$

The equilibrium amounts are found from the expressions

$$M = \frac{1}{2K}\{\sqrt{1 + 4TK} - 1\} \quad (9)$$

$$D = T - M$$

where

T = total amount of material
$K = k_f/k_r$

Computation

The FORTRAN program was structured as before (Bethune and Kegeles, 1961a), with equation (8) in place of the equilibrium equation used there.

Construction of the Analog. A hypothetical countercurrent-distribution train is coded into the computer, each tube being loaded with the equilibrium concentration of monomer and dimer, as calculated from equation (9). The upper phase is symbolically removed from the first two hypothetical tubes and replaced by pure upper-phase solvent, forming the boundary. The calculation is then begun and the boundary is moved through the tube train as a function of the number of transfers, *n*. This corresponds to the situation found in sedimentation or the descending boundary in electrophoresis.

Method of Checking Computer Results. Several different methods were used to check the results. A number of these were built into the computer program. For example, if the total amount of solute material present in a tube before calculation differed from the total after calculation by an amount greater than a preset factor, a diagnostic was printed. For the results reported here, the preset value was 1.0×10^{-5}.

Again, since the machine was loaded with the equilibrium system at the beginning, a plateau region existed to which the computer finally came as it calculated along the train. There was a statement in the program comparing the total mass in the tube for which calculation was being carried out with that in the plateau. If the absolute value of the difference between them was no greater than 1.0×10^{-8}, the computer passed to the next tube, without going through the calculation loop. Thus, as the plateau region was approached, the values obtained approximated the plateau values for the equilibrium mixture. These values agreed to one part in 1.0×10^8.

Another method used to check the accuracy of the results produced by the program for any particular case consisted of setting the reverse rate constant equal to zero, then running the program with the values of all other parameters unchanged. The system should then behave like a single solute with the characteristics of the dimer, and the appearance of the pattern as well as the position of the maximum should be predicted from the equations which govern single ideal solute distributions

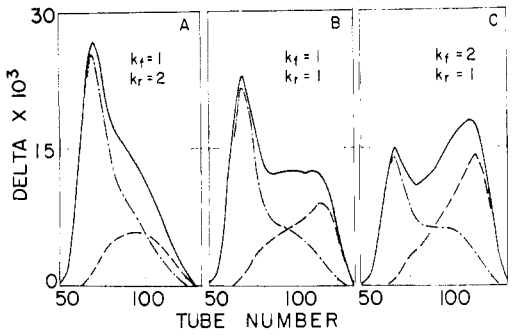

FIGURE 1: Effect of varying rate constants, k_f and k_r. Here $K_M = 0.5$; $K_D = 1.5$; initial mass per tube, T, = 1 unit; time, $t = 8 \times 10^{-3}$ unit; $n = 200$. Total, ———; monomer, –·–·; dimer, – – –.

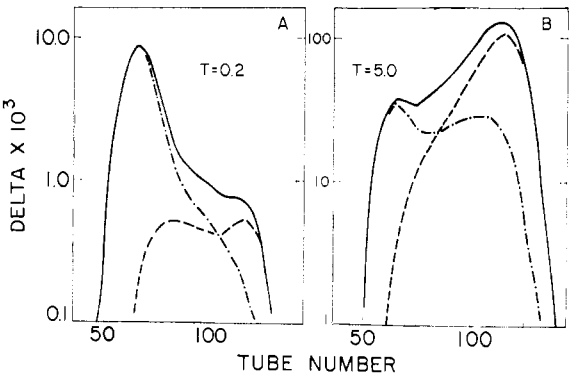

FIGURE 2: Effect of varying the initial mass per tube, T. Here $K_M = 0.5$; $K_D = 1.5$; $k_f = k_r = 1$; time, $t = 8 \times 10^{-3}$ unit; $n = 200$. Total, ———; monomer, –·–·; dimer, – – – –.

(Craig and Craig, 1950). This criterion was satisfied by all programs used here.

Method of Plotting Results. The ordinate in plots of distribution patterns is Δ, the difference between the total mass of solute in tube M and that in tube $M-1$. When this first difference is plotted against the tube number, r, as abscissa, analogs to Schlieren patterns are obtained. In addition a number of the plots show the Schlieren analog patterns obtained for the individual species. While the gradient of the total mass distribution will not change if the machine is stopped and the pattern determined after different intervals of time, the gradients for the species will change because of the approach to chemical equilibrium. These individual patterns, therefore, represent instantaneous concentration distributions.

Results

There are six obvious parameters to which values may be assigned: the forward and reverse rate constants, the partition coefficients of the monomer and dimer, the time of reaction in any tube, and the initial total mass of material per tube. There is, however, another parameter: the number of transfers that are accomplished. This is the analog of time of separation in a continuous method (Bethune and Kegeles, 1961c). A wide range of values of these parameters has been investigated. As might be expected, when the rate constants and the time of reaction are large, one obtains patterns which correspond to those for a dimerizing system at equilibrium, a single boundary moving with a predictable velocity (Bethune and Kegeles, 1961a). When the time of reaction and the rate constants are small, two boundaries are found, as expected for a system in which no interaction is occurring. As the rate constants and the time of reaction achieve intermediate values, however, a different situation begins to appear. The results of this investigation are summarized in Figures 1–5, where the effect of the variation of any one of the parameters is investigated separately.

Figure 1 illustrates the effect of varying rate constants. When the reverse rate constant is greater than the forward rate constant (Figure 1a) only one boundary with a shoulder is seen in the total mass pattern. The peak maximum is in tube 67, as expected after 200 transfers for a single substance distributing with a partition coefficient of 0.5. The distributions of the individual species reveal, however, that while the maximum is due almost exclusively to a gradient of monomer the shoulder is contributed both by a gradient of dimer and by a long trailing gradient of monomer. While the position of the monomer boundary has not been affected by the reaction, the position of the dimer boundary is drastically affected, its maximum concentration gradient being in tube 95 rather than in tube 120, as expected for a substance with a partition coefficient of 1.5.

When the rate constants are equal (Figure 1b), three maxima appear in the curve. One is located in the position expected for monomer, another is located near the position expected for the dimer (maximum in tube 113 rather than 120), but the third is at an intermediate position (tube 97). An examination of the patterns for the individual species shows that this intermediate boundary is not contributed by a second boundary in either of the two species, but rather by the tailing effect imposed by the reaction upon each of the individual species.

When the forward reaction rate is more rapid than that of the backward reaction (Figure 1c) only two boundaries appear. One, again, is at the position of the monomer; the other is again near the position expected for the dimer. There is no intermediate maximum seen in this case. An examination of the patterns of the species alone reveals why this is so.

In the region in which the third maximum formerly occurred there is now a virtually constant gradient of monomer, while the gradient of dimer is rising steadily. The superposition of these two curves gives only an elevated pattern corresponding to the dimer curve. From the appearance of this pattern it might be inferred that

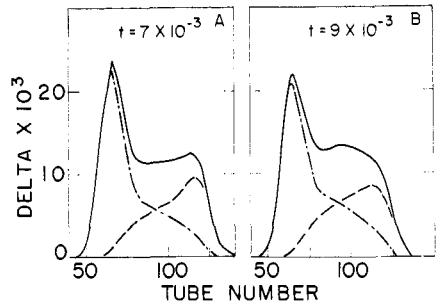

FIGURE 3: Effect of varying the reaction time, t. Here $K_M = 0.5$; $K_D = 1.5$; initial mass per tube, $T_1 = 1$ unit; $k_f = k_r = 1$; $n = 200$. Total, ———; monomer, -·-·; dimer, – – – –.

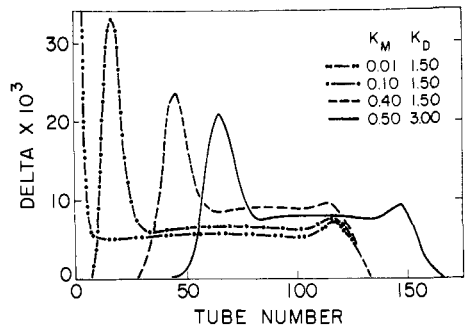

FIGURE 4: Effect of varying partition coefficients, K_M and K_D. Here $k_f = k_r = 1$; time, $t = 8 \times 10^{-3}$ units; initial mass per tube, $T_1 = 1$ unit; $n = 200$.

it would be possible for either of the individual components to develop two boundaries. That this can occur is shown in Figure 2, where the total amount loaded to each individual tube is varied. When the total is 0.2 mass unit per tube (Figure 2a) only a single boundary with a shoulder appears. Examination of the distribution of the different species in this case, however, reveals a profound difference from the case of Figure 1a, where one boundary also occurred. The main boundary is again due to the monomer alone; but the shoulder, while due to a combination of monomer and dimer, is now composed of a double boundary of the dimer, one maximum in tube 80, the other in tube 118, which is very near the position expected for the dimer alone. When the mass is increased to 1 unit per tube (Figure 1b) neither of the two individual species has developed a double boundary. When the mass is increased to 5 units per tube, as in Figure 2b, there are again two boundaries, one occurring at the position expected for monomer, the other near the position expected for the dimer; but now an examination of the individual species present reveals that the monomer boundary is bimodal, the dimer giving one maximum with a trailing edge.

Figure 3 illustrates the effect of varying the time of reaction. In Figure 3a the time of reaction is 7×10^{-3} and in Figure 3b, 9×10^{-3} time unit. Figure 1b illustrates the pattern for 8×10^{-3} unit. At the shorter time, as would be expected, only two boundaries appear, one corresponding to the position of the monomer and the other corresponding to the position of the dimer. At the intermediate time three boundaries are seen (Figure 1b), but when the time of reaction is made greater, as in Figure 3b, the situation is changed. There are still two boundaries, but now the one corresponding to the position of the dimer has disappeared and a diffuse boundary appears at an intermediate position, corresponding roughly to the position of the intermediate boundary found when the time parameter is 8×10^{-3} unit.

Figure 4 shows the effect of varying the partition coefficient. This is analogous to changing the velocity with which the different species travel (Bethune and Kegeles, 1961c). All the other parameters in this case correspond to those used in Figure 1b. It is seen that, as the partition coefficient of the monomer is decreased from 0.4 to 0.01, with that of the dimer held constant, the essential features of the pattern are retained. As the velocity of the monomer becomes lower with respect to that of the dimer, the intermediate zone in which a maximum is found becomes much broader and the intermediate maximum occurs at lower tube numbers.

It should be noted that this change corresponds, in the analogous continuous-transport experiments, to a constant diffusion coefficient for the dimer but an increasing diffusion coefficient for the monomer (Bethune and Kegeles, 1961c). This corresponds, as the partition coefficient of the monomer is raised from 0.01 to 0.4, to a 20-fold increase in the diffusion coefficient.

The last pattern of Figure 4 shows the effect of varying the partition coefficient of the dimer. It is again seen that the main effect of this change is a broadening of the intermediate zone.

Figure 5 shows the result of varying the number of transfers. At low numbers of transfers only two boundaries appear, but as the number of transfers increases three boundaries are resolved. Increase in the number of transfers causes simultaneously a diminution of the leading boundary, finally leaving in prominent view only the boundary at the position of the monomer and the long diffuse intermediate boundary.

Discussion

While one report of the influence of a dimerization reaction that is kinetically controlled has appeared (Belford and Belford, 1962), the present investigation differs in two respects: (a) The effects of an analog of diffusion are incorporated in the calculations and (b) the gradient of the distribution of the individual species has been recorded.

In agreement with previous publications on this subject (Cann *et al.*, 1957; Cann and Bailey, 1961; Scholten, 1961; Van Holde, 1962; Belford and Belford, 1962), the parameters most significantly affecting the resultant patterns are, first, the rate constants and

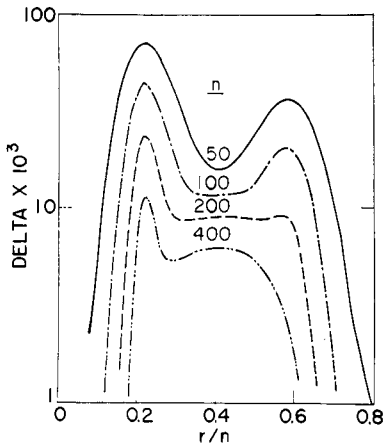

FIGURE 5: Effect of varying the number of transfers, n. Here $K_M = 0.3$; $K_D = 1.5$; $k_f = k_r = 1$; time, $t = 8 \times 10^{-3}$ unit; initial mass per tube, $T_1 = 1$ unit.

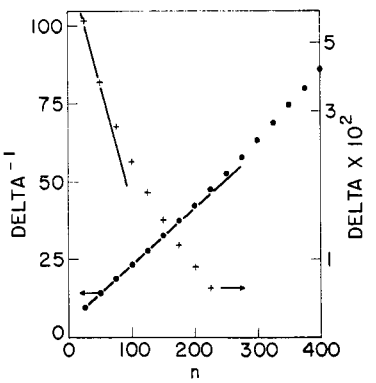

FIGURE 6: Height of the slow maximum (·) and of the rapid maximum (+) as a function of n, the number of transfers, for the data graphed in Figure 5.

second, the time of reaction (Figures 1, 3, and 5). In this analog, however, there are two different time parameters; the first is the reaction time in any one tube and the second is the number of transfers applied. It might be expected that the product of these two parameters should be the controlling factor. Thus, in Figure 3, where the patterns are shown after 200 transfers, it might be expected that when the time constant is 7×10^{-3} the application of 230 transfers would give the same pattern as seen after 200 transfers when the time constant is 8×10^{-3} unit. This, however, is not so, since a change in the reaction time in any tube changes the masses of each of the solutes that are transported from that tube to the succeeding tube when a transfer is made.

What has not been emphasized previously is the diagnostic value of variation in the initial concentration of the material undergoing the transport process. Figure 2 shows that changes in the total concentration of material can induce remarkable changes in the patterns observed. As in the case of a rapidly reversible polymerization (Gilbert, 1955; Bethune and Kegeles, 1961a) or in a reaction of the type A + B $\rightleftharpoons$ C (Gilbert and Jenkins, 1959; Bethune and Kegeles, 1961b), this is probably the most valuable diagnostic test for a reacting system. If the distribution of the *individual* species can be detected, this test is even more valuable, as demonstrated in Figure 2, where the individual gradients of the different species are represented.

The presence of bimodal boundaries in the individual species distribution is startling. It suggests that if the right conditions are chosen it is possible to have bimodal boundaries for each species (Bethune and Kegeles, 1961b) while the total pattern shows only two or three maxima.

The influence of the introduction of an analog of a diffusion coefficient is apparent in Figure 4. As the partition coefficient of the monomer is increased, corresponding to an increase in the diffusion coefficient, not only do the maxima themselves broaden but the intermediate boundary becomes much more prominent. It is also noticeable that it is only over a narrow range of the values of the rate constants, time of reaction, and concentrations that the third maximum can be detected at all. This suggests that the introduction of an analog of diffusion wipes out the distinguishing features of such a system much more rapidly than the calculations based upon an asymptotic solution of the relevant equations would suggest (Belford and Belford, 1962). In the asymptotic solution the reciprocal of the height of the slower-moving maximum and the logarithm of that of the leading boundary are linear functions of time. The relevant data, from Figure 5, are plotted against the number of transfers in Figure 6. Neither plot is linear, nor are similar plots of the areas under the boundaries. This behavior suggests that the inclusion of diffusion effects has perturbed the patterns from those found when these effects are neglected.

References

Albright, J. G. (1963), *J. Phys. Chem. 67*, 2628.

Bak, T. A., and Kauman, W. G. (1959), *Trans. Faraday Soc. 55*, 1109.

Belford, G. G., and Belford, R. L. (1962), *J. Chem. Phys. 37*, 1926.

Bethune, J. L., and Kegeles, G. (1961a), *J. Phys. Chem. 65*, 433.

Bethune, J. L., and Kegeles, G. (1961b), *J. Phys. Chem. 65*, 1755.

Bethune, J. L., and Kegeles, G. (1961c), *J. Phys. Chem. 65*, 1761.

Cann, J. R., and Bailey, H. R. (1961), *Arch. Biochem. Biophys. 93*, 576.

Cann, J. R., Kirkwood, J. G., and Brown, R. A. (1957), *Arch. Biochem. Biophys. 72*, 37.

Craig, L. C., and Craig, D. (1950), *Tech. Org. Chem. 3*, 171.

Gilbert, G. A. (1955), *Discussions Faraday Soc. 20*, 68.
Gilbert, G. A. (1959), *Proc. Roy. Soc. (London) Ser. A: 250*, 377.
Gilbert, G. A., and Jenkins, R. C. Ll. (1959), *Proc. Roy. Soc. (London) Ser. A: 253*, 420.
Keller, R. A., and Giddings, J. C. (1960), *J. Chromatog. 3*, 205.
Mysels, K. L. (1956), *J. Chem. Phys. 24*, 371.
Scholten, P. C. (1961), *Arch. Biochem. Biophys. 93*, 568.
Van Holde, K. E. (1962), *J. Chem. Phys. 37*, 1922.

4-β-Alanine-oxytocin: An Oxytocin Analog Containing a Twenty-one-membered Disulfide Ring*

Maurice Manning and Vincent du Vigneaud

ABSTRACT: 4-β-Alanine-oxytocin, an analog of oxytocin containing a β-alanine residue in place of the glutamine residue in the 4 position in the hormone, has been synthesized and tested for various pharmacological activities in comparison with 4-glycine-oxytocin. Both of these analogs lack the carboxamide-containing side chain attached to the ring in oxytocin at position 4, but the β-alanine-oxytocin contains a ring larger by one methylene unit than the 20-membered ring present in 4-glycine-oxytocin and oxytocin. This increase in ring size resulted in a marked decrease in oxytocic, avian depressor, and milk-ejecting activities.

The synthesis of oxytocin (du Vigneaud *et al.*, 1953, 1954), the principal oxytocic and milk-ejecting hormone of the posterior pituitary gland, has provided a means of studying the relationship of chemical structure to biological activity in this octapeptide hormone, the structure of which is shown in Figure 1. Efforts to elucidate this relationship in this and other laboratories during the past decade have centered on the total synthesis of numerous analogs of the hormone, incorporating various modifications of its structure, and the comparison of the pharmacological properties of these synthetic analogs with those of oxytocin. Along with some other aspects of the general problem, the studies in our laboratory have been focused particularly on the importance of the presence of the individual chemical functional groups, the specificity of the 20-membered disulfide ring, and the relation of the stereostructure of the seven optically active amino acid residues to the pharmacological manifestations of oxytocin.

Recently Drabarek (1964) in this laboratory synthesized three analogs of oxytocin in which the tyrosine, isoleucine, and glutamine residues in positions 2, 3, and 4 were replaced, respectively, by a glycine residue. The only one of these compounds that showed pharmacological activity was 4-glycine-oxytocin, which possessed 5.5 units/mg of oxytocic activity, 17 units/mg of milk-ejecting activity, and 2.8 units/mg of avian depressor activity. Oxytocin possesses approximately 500 units/mg of oxytocic and avian depressor activities, and approximately 400 units/mg of milk-ejecting activity (Chan and du Vigneaud, 1962). In 4-glycine-oxytocin the $-CH_2CH_2CONH_2$ portion of the glutamine residue at the 4 position in oxytocin is replaced by hydrogen, thus eliminating the carboxamide-containing side chain attached to the ring at this position. Even though the activities of this analog were low, we decided to determine whether the activity of the 4-glycine-oxytocin would be further diminished by increasing the ring size from 20 to 21 members through the insertion of a methylene unit at the 4 position. The present paper describes the synthesis and study of such an analog of oxytocin, namely 4-β-alanine-oxytocin. By replacing the glutamine residue in the 4 position of oxytocin by a β-alanine residue the ring size is increased by one methylene unit from 20 to 21 members. This analog also lacks the side chain at position 4 containing the carboxamide group and, as in 4-glycine-oxytocin, no asymmetric carbon is present at this position.

The key intermediate for the synthesis of 4-β-alanine-oxytocin was the protected nonapeptide *S*-benzyl-*N*-carbobenzoxy-L-cysteinyl-L-tyrosyl-L-isoleucyl-β-alanyl-L-asparaginyl-*S*-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide. This protected nonapeptide intermediate was prepared by the stepwise *p*-nitrophenyl ester procedure used previously in this laboratory for the synthesis of oxytocin (Bodanszky and du Vigneaud, 1959). Carbobenzoxy-L-asparaginyl-*S*-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide served as starting material. After removal of the carbobenzoxy group, the

* From the Department of Biochemistry, Cornell University Medical College, New York, New York 10021. *Received May 3, 1965.* This work was supported in part by Grant HE-01675 from the National Heart Institute, U.S. Public Health Service. Dr. Manning wishes to acknowledge a Fulbright Travel Grant.