

To a solution of the trifluoroacetate in dimethylformamide (145 ml), *t*-butyloxycarbonyl-nitro-L-arginine 2,4-dinitrophenyl ester (6.0 g, prepared in the same manner as the corresponding benzyl-oxycarbonyl derivative) was added followed by triethylamine (1.0 ml). In the following 7 hr more triethylamine (a total of 1.1 ml) was added in several portions to maintain the slight alkalinity of the mixture. After about 24 hr at room temperature more active ester (2.0 g) and triethylamine (0.7 ml) were added, and after an additional day most of the solvent was removed *in vacuo*. The residue was triturated with ethyl acetate (about 1 l.) and the solid material filtered and washed with ethyl acetate and ether. After drying in air and *in vacuo* at 40°, the crude protected tetradecapeptide (13.75 g) softens at 150°, darkens from 250°, and melts at about 300° dec.

The crude material (0.75 g) was purified by countercurrent distribution in the solvent system of *n*-butyl alcohol-pyridine-acetic acid-water (4:2:1:7). The solution containing the crude product was placed in the first three tubes of a Craig apparatus (10-ml phases) and the distribution was carried out through 100 transfers. Essentially all the material was found in a band corresponding to a distribution coefficient of 11, and the experimental curve was found to be practically identical with the curve calculated for this *K* value. The recovered material (0.54 g)¹⁴ softens and darkens as the crude product; its decomposition point is about 305°. On slow evaporation of the solvents used in the distribution, crystals were obtained; $[\alpha]^{25}_D -39^\circ$ (*c* 2, acetic acid); $\lambda_{\max}^{0.5\% \text{ EtOH}} 269 \text{ m}\mu$ ($E_{1\%}^{1\text{cm}} 240$).

Anal. Calcd for C₈₈H₁₄₃N₂₉O₂₇: C, 51.8; H, 7.1; N, 19.9.

(14) In the similar purification of 12 g of the crude protected tetradecapeptide XIV, 10.75 g of purified material was recovered.

Found: C, 52.2; H, 7.8; N, 19.9; and in a second preparation: C, 51.4; H, 7.8; N, 19.9.

A solution of XIV (8.2 g) in trifluoroacetic acid (40 ml) was kept at room temperature for 15 min. The trifluoroacetic acid was removed *in vacuo* and the residue triturated with ether. The trifluoroacetate was washed with ether and dried *in vacuo* over sodium hydroxide, weight 8.1 g. It softens at 140° and melts at 250° dec. *Anal.* Calcd for NF: N, 19.8; F, 2.8. Found: N, 20.4; F, 3.3. In the system *n*-butyl alcohol-acetic acid-water (4:1:5), a single spot is detected by its ultraviolet absorption and with ninhydrin, *R_f* 0.70.

A sample of the fully protected tetradecapeptide amide XIV (200 mg) was dissolved in acetic acid (10 ml) and the mixture hydrogenated in the presence of 10% palladium on charcoal catalyst (200 mg) for 2 days. The catalyst was removed by filtration and the acetic acid removed by evaporation from the frozen state. The fluffy white residue was dissolved in trifluoroacetic acid (4 ml) and left at room temperature for 15 min. Ether was added and the precipitate (trifluoroacetate) was collected by filtration and dried *in vacuo* to give material which traveled as a single band on paper electrophoresis (pH 1.9, formic acid-acetic acid). After hydrolysis with constant boiling hydrochloric acid at 110° for 19 hr *in vacuo*, a sample gave the following ratios of amino acids: Arg, 2.7; Asp, 1.1; Ser, 0.9; Ala, 1.0; Leu, 4.0; Glu, 2.2; Gly, 1.1; and Val, 1.2.

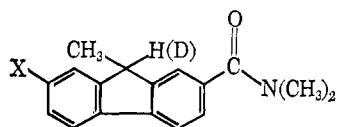
Acknowledgment. The authors express their gratitude to Mr. J. Alicino and his group for the microanalyses, to Mr. O. Kocy for the examination by paper electrophoresis of the intermediates, and to Mr. F. Russo-Alesi for the quantitative amino acid analyses.

Communications to the Editor

A Kinetic Model for Mechanisms of Base-Catalyzed Hydrogen-Deuterium Exchange between a Carbon Acid and Deuterated Medium¹

Sir:

Previous papers reported four stereochemical courses for the base-catalyzed hydrogen-deuterium exchange reaction at asymmetric carbon in systems I or II.²



I, X = H
II, X = NO₂

Values of k_e/k_α (one-point rate constant for isotopic exchange over that for racemization) indicated that electrophilic substitution can occur with net retention ($k_e/k_\alpha > 1$), total racemization ($k_e/k_\alpha = 1$), net inversion ($0.5 < k_e/k_\alpha < 1.0$), or net isoracemization (net racemization without exchange, $k_e/k_\alpha < 0.5$).³ In this and the following communication,⁴ we report two independent

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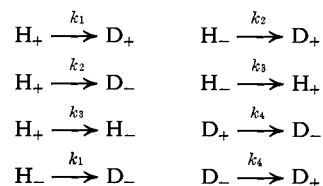
(2) (a) D. J. Cram and L. Gosser, *J. Am. Chem. Soc.*, **86**, 5445 (1964); (b) D. J. Cram and L. Gosser, *ibid.*, **86**, 2950 (1964).

(3) For a review, see D. J. Cram, "Fundamentals of Carbanion Chemistry," Academic Press Inc., New York, N. Y., 1965, Chapter 3.

(4) W. T. Ford, E. W. Graham, and D. J. Cram, *J. Am. Chem. Soc.*, **89**, 690 (1967).

methods for determining the contributions of the individual stereochemical pathways which compose the classes of k_e/k_α values.

The first dissection of the over-all stereochemical result of an exchange reaction of a protio carbon acid with a deuterated solvent (greater than 99% deuterated) utilizes a kinetic scheme which assumes only that, at low concentrations of starting protio carbon acid, the generated deuterio carbon acid never becomes re-protonated. Symbols such as H₊ and D₋ indicate the isotope attached to carbon and the sign of rotation of the particular enantiomer. All possible courses of reaction are defined by the scheme's four rate constants, k_1 (exchange with retention), k_2 (exchange with inversion), k_3 (inversion without exchange, defined as *isoinversion*), and k_4 (inversion of exchanged material). Solution of the four simultaneous first-order rate



equations of the scheme with H₊ as starting material provides eq 1-4, where [H₊] and [D₋] represent mole fractions at time *t*, $\beta = k_1 + k_2$, and $\gamma = k_1 + k_2 + 2k_3$. Substitution of eq 1-4 into an expression for

$$[H_+] = 1/2[e^{-\beta t} + e^{-\gamma t}] \quad (1)$$

$$[H_-] = 1/2[e^{-\beta t} - e^{-\gamma t}] \quad (2)$$

$$[D_+] = 1/2 \left[(1 - e^{-\beta t}) - \left(\frac{k_2 - k_1}{\gamma - 2k_4} \right) (e^{-2k_4 t} - e^{-\gamma t}) \right] \quad (3)$$

$$[D_-] = 1/2 \left[(1 - e^{-\beta t}) + \left(\frac{k_2 - k_1}{\gamma - 2k_4} \right) (e^{-2k_4 t} - e^{-\gamma t}) \right] \quad (4)$$

rotation α of a kinetic solution gives eq 5, where $\phi = k_4/(k_2 + k_3)$ (the reciprocal of the kinetic isotope effect, k_H/k_D for racemization), $\psi = (k_2 - k_1)/2(k_2 + k_3)$, $\theta = 2(k_2 + k_3)$ (the rate constant for racemization at zero time), and α_0 is the rotation at zero time. Only

$$\alpha = \alpha_0([H_+] + [D_+] - [H_-] - [D_-]) =$$

$$\left[\frac{\alpha_0}{(\phi - 1) + \psi} \right] [(\phi - 1)e^{-\theta(1-\psi)t} + \psi e^{-\theta\phi t}] \quad (5)$$

when $\phi = 1$ or $\psi = 0$ does α show simple first-order decay. Values of k_1 , k_2 , and k_3 may be calculated using eq 5, the experimentally determined dependence of α on time, and the independently measured rate constants k_4 and $k_e (= (k_1 + k_2))$ (rate constant for isotopic exchange).

We now report values of k_1 , k_2 , k_3 , and k_4 for treatment of I-H₊ with potassium methoxide in methanol-O-d. Kinetic experiments demonstrated that racemization of I is distinctly first order in potassium methoxide and first order in substrate in the concentration ranges studied. The racemization isotope effects and k_e/k_α values are shown in Table I.

Table I. Rate Ratios for Racemization and Exchange of I^a in Methanol with Potassium Methoxide^b at $24.9 \pm 0.1^\circ$

| | |
|---|---------------------|
| $(k_H^{CH_3OH}/k_D^{CH_3OH})^c$ | $= 6.66 \pm 0.27^c$ |
| $(k_H^{CH_3OD}/k_D^{CH_3OD})^{c,d}$ | $= 6.50 \pm 0.22^e$ |
| $\left(\frac{k_e}{k_\alpha} \right)^{I-H}$ CH ₃ OD | $= 0.92 \pm 0.05^e$ |
| $\left(\frac{k_e}{k_\alpha} \right)^{I-D}$ CH ₃ OH | $= 0.82 \pm 0.05^e$ |

^a 0.060 M. ^b 0.07–0.08 M in KOCH₃. ^c Substrate isotope effect for racemization. ^d CH₃OD used contained greater than 0.99 atom of D per molecule by nmr. ^e All ratios are of second-order constants obtained by dividing base concentrations into least-squares first-order constants. We thank Dr. Paul Haake for use of a least-squares program. Errors reported are standard deviations.

Previous interpretation of the k_e/k_α value (0.82) for treatment of I-D₊ with potassium methoxide in methanol implied that, for racemization of I-H₊ in methanol-O-d, $k_2 > k_1$.^{2a} By eq 5, if $k_2 > k_1$ ($\psi > 0$) and $\phi < 1$ ($k_H/k_D > 1$), then experimentally the rotation of a kinetic solution of H₊ as starting material in methanol-O-d changes from (+) to (–) at some time during the run as the more reactive H₊ produces less reactive D_– by net exchange with inversion. In spite of the large isotope effect ($\phi = 0.15$), we observed no (–) rotation at any time. Calculations indicate that if the value of $k_2 - k_1$ was just 1% of the mean of k_1 and k_2 , a distinctly negative rotation ($\alpha_{obsd} - 0.003^\circ$ at the 436-m μ Hg line on a Perkin-Elmer Model 421 polarimeter) would have been observed during the

kinetic run, and that k_1 and k_2 most likely differ by less than 0.2% of their mean value. Therefore, $k_1 = k_2$, and the over-all net inversion with exchange indicated by the k_e/k_α value (0.92) for I-H₊ in methanol-O-d with potassium methoxide must be due to an *isoinversion* component (governed by k_3) in the mechanism for isotopic exchange and racemization. Second-order rate constants k_1 , k_2 , k_3 , and k_4 calculated from our data are given in Table II. The following

Table II. Second-Order Kinetic Model Rate Constants for Racemization of I-H₊ in CH₃OD,^a 0.0739 M in CH₃OK at $24.9 \pm 0.1^\circ$

| Constant | $10^3 k$, l. mole ⁻¹ sec ⁻¹ |
|----------|--|
| k_1 | 3.02 ± 0.17^b |
| k_2 | 3.02 ± 0.17^b |
| k_3 | 0.26 ± 0.17^b |
| k_4 | 0.51 ± 0.01^b |

^a CH₃OD used contained greater than 0.99 atom of D per molecule by nmr. ^b Errors reported represent standard deviations.

communication contains a more detailed discussion of the mechanism for hydrogen–deuterium exchange of I in hydroxylic solvents.⁴

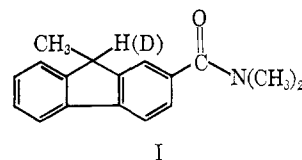
(5) National Science Foundation Trainee at UCLA, 1965–1966.

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Mechanistic Components in Base-Catalyzed Hydrogen–Deuterium Exchange between a Carbon Acid and Hydroxylic Solvents¹

Sir:

The preceding communication described a kinetic model for dissecting the mechanistic components in the base-catalyzed hydrogen–deuterium exchange reaction at asymmetric carbon between system I-H and methanol-O-d.² This communication describes an



experimentally independent method based on the kinetic equations which define the contributions to the over-all results of exchange with retention (rate constant k_1), of exchange with inversion (rate constant k_2), and of *isoinversion* (inversion without exchange, rate constant k_3). The method has been applied to reactions of I in two solvent–base systems.

The model assumes only that the substrate, once exchanged with isotopically labeled solvent, never regains its original isotope. Equations 1–5 provide solutions for rate constants k_1 , k_2 , and k_3 in terms of the isotopic and enantiomeric composition of the substrate at reaction time t and an independently measured²

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(2) W. T. Ford, E. W. Graham, and D. J. Cram, *J. Am. Chem. Soc.*, **89**, 689 (1967).