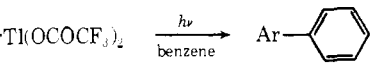
In an attempt to extend this phenylation reaction,[11] phenylthallium ditrifluoroacetate was photolyzed in pyridine. We had hoped that complex formation between the pyridine nitrogen atom and thallium[4b] might result in specific α phenylation. However, the mixture of isomeric phenylpyridines obtained (50% overall yield; isomer distribution $\alpha:\beta:\gamma$, 54:32:14) was identical in composition with that observed in other free-radical phenylations of pyridine.[12]

Table I. Photolysis of Arylthallium Ditrifluoroacetates in Benzene



Ar[a]	Yield (%) of crude biphenyl[b]
Phenyl	90
p-Tolyl	91[c]
p-Ethylphenyl	84[d]
m-Xylyl	83[e]
p-Xylyl	82[f]
p-Chlorophenyl	87[g]
Mesityl	80[h]
o-Bromo-*p*-tolyl	78[i]

[a] Unrecrystallized arylthallium ditrifluoroacetates rich in the predominant isomer were used (ref 4b). The presence of small amounts of the other positional isomers accounts for the isomeric biaryls found. [b] Purity of products was determined by glc. The identity of products was established by chromatographic comparison with authentic samples or by preparative glc followed by spectral analysis. [c] Composition: 93% *p*-methylbiphenyl, 5% *o*-methylbiphenyl, 1.5% biphenyl, 0.5% *p*-cresol. [d] Composition: 93% *p*-ethylbiphenyl, 2% of an unidentified ethylbiphenyl, 1.5% biphenyl, 3.5% *p*-ethylphenyl trifluoroacetate. [e] Composition: 98% 2,4-dimethylbiphenyl, 0.5% 2,6-dimethylbiphenyl, 1% biphenyl. [f] Composition: 99% 2,5-dimethylbiphenyl, 1% biphenyl. [g] Composition: 89% *p*-chlorobiphenyl, 8% *o*-chlorobiphenyl, 3% biphenyl. [h] Composition: 63% 2,4,6-trimethylbiphenyl, 27% mesitylene, 7% mesityl trifluoroacetate, 3% biphenyl. [i] Based on recovered starting material (20%); irradiation was carried out for only 6 hr with 3500-Å light. Composition: 93% 2-bromo-4-methylbiphenyl, 2.5% biphenyl, 2.5% 2-bromo-4-methylphenyl trifluoroacetate, 2% *m*-bromotoluene.

In the above unsymmetrical biphenyl synthesis, replacement of thallium by a phenyl group takes place cleanly without contamination by positional isomers; the same specificity of replacement was previously observed in the synthesis of aryl iodides from arylthallium ditrifluoroacetates.[13,14] Since the position of thallation can be controlled,[4b] the complementary photolytic reactions in benzene of arylthallium ditrifluoroacetates and of aryl iodides provide a simple synthesis of unsymmetrical biphenyls of predetermined orientation.

(11) Photolysis in benzene of the thallation derivatives of benzoic and phenylacetic acids (ref 4b) yielded unidentified, benzene-insoluble, brown solids, in addition to smaller amounts of crude biphenyls. For example, the mixture of biphenyls (30%) obtained from benzoic acid consisted of *o*-phenylbenzoic acid (72%) and biphenyl (18%); benzoic acid (10%) was also present. Similarly, phenylacetic acid, under the above conditions, yielded a mixture of *o*-phenylphenylacetic acid (77%), an unidentified isomer thereof (5%), *o*-methylbiphenyl (0.5%), biphenyl (8.5%), and unchanged phenylacetic acid (8%).

(12) K. Schofield, "Hetero-Aromatic Nitrogen Compounds," Butterworths, Washington, D. C., and London, 1967, p 253.

(13) A. McKillop, J. S. Fowler, M. J. Zelesko, J. D. Hunt, E. C. Taylor, and G. McGillivray, *Tetrahedron Lett.*, 2427 (1969).

(14) This is also the case in the photochemical conversion of aromatic iodides to chlorides;[15] similar observations have been made by Kharasch.[3]

(15) F. Kienzle and E. C. Taylor, *J. Org. Chem.*, **35**, 528 (1970).

(16) NRCC Postdoctoral Fellow, 1968–1970.

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Identification of the Rate-Limiting Step in the Chymotrypsin-Catalyzed Hydrolysis of *N*-Acetyl-L-tryptophanamide

Sir:

The identification of the chemical changes associated with observed rate processes in enzyme-catalyzed reactions has not kept pace with the elucidation of the number and rates of such processes. In the case of the α-chymotrypsin-catalyzed hydrolysis of furylacryloyltryptophanamide,[1] the existence of no less than two intermediates prior to the acyl enzyme intermediate has been demonstrated. However, in this and in other cases[2,3] the natures of the various reaction steps have been determined only in a general way. We have previously shown[4,5] that heavy atom isotope effects in enzymatic reactions can be used to compare the rate of a step in which a bond to an isotopic atom is broken with the rates of prior steps in the enzymatic reaction sequence. In such a reaction, a heavy atom isotope effect is observed to the extent that the bond-breaking step is slow relative to steps prior to it. We have now measured the amide nitrogen isotope effect on the α-chymotrypsin-catalyzed hydrolysis of acetyl-L-tryptophanamide. The isotope effect is $k^{14}/k^{15} = 1.010$ at 25° in pH 8.0 phosphate buffer and indicates that the slowest step in the acylation of the enzyme is the step in which the carbon–nitrogen bond is broken.

For each experiment two portions of a freshly prepared solution of 0.01 *M* *N*-acetyl-L-tryptophanamide in 0.05 *M* potassium phosphate buffer at pH 8.0 were equilibrated at 25° for 30 min and an amount of desalted chymotrypsin sufficient to hydrolyze 10% of the substrate in 5–15 min was added to one of the samples and 5–10 times that amount of enzyme was added to the other. A small amount of the first sample was withdrawn for spectrophotometric monitoring at 306 mμ. After a time corresponding to approximately 10% reaction, the reaction in the first solution was stopped by the addition of Norit. The solution was filtered twice, ultra-filtered (Dia-Flo UM-2 filter), and steam distilled in all-glass apparatus. The distillate was concentrated to about 3 ml and the ammonia

(1) G. P. Hess, J. McConn, E. Ku, and G. McConkey, *Phil. Trans. Roy. Soc. London, Ser. B*, **257**, 89 (1970).

(2) M. R. Hollaway, *Annu. Rep. Progr. Chem.*, **65**, 601 (1968); M. L. Bender, M. J. Gibian, and D. J. Whelan, *Proc. Nat. Acad. Sci. U. S.*, **56**, 833 (1966); M. L. Bender, G. E. Clement, F. J. Kezdy, and H. d'A. Heck, *J. Amer. Chem. Soc.*, **86**, 3680 (1964); A. Himoe, P. C. Parks, and G. P. Hess, *J. Biol. Chem.*, **242**, 919 (1967).

(3) L. Parker and J. H. Wang, *ibid.*, **243**, 3729 (1968).

(4) M. H. O'Leary, *J. Amer. Chem. Soc.*, **91**, 6886 (1969); M. H. O'Leary and D. W. Hendrickson, *Fed. Proc., Fed. Amer. Soc. Exp. Biol.*, **29**, 407 (1970).

(5) M. H. O'Leary, D. T. Richards, and D. W. Hendrickson, *J. Amer. Chem. Soc.*, **92**, 4435 (1970).

thus prepared was oxidized to nitrogen gas with sodium hypobromite *in vacuo* as described by Bremner.[6] Isotope ratio measurements were made on a Nuclide RMS 6-60 isotope ratio mass spectrometer relative to a tank nitrogen standard. Isotope effects were calculated as described previously.[5] The results of six determinations of the isotope effect are summarized in Table I. The isotope ratios given in this table

Table I. Nitrogen Isotope Effects on the Chymotrypsin-Catalyzed Hydrolysis of *N*-Acetyl-L-tryptophanamide at pH 8.0, 25°

% reaction[a]	—Isotope ratios[b] × 10^6— Low conversion	100% conversion	k^{14}/k^{15}
11.9	9261	9333	1.0091
10.5	9274	9357	1.0095
10.0	9244	9349	1.0119
9.3	9257	9355	1.0111
9.6	9241	9335	1.0106
9.6	9265	9349	1.0095
		Mean	1.010 ±0.001

[a] Determined by spectrophotometric monitoring of the reaction at 306 mμ. 100% reaction was found to correspond to $\Delta\epsilon = 70$ M^{-1} cm^{-1}. [b] Decade settings for the ratio *m/e* 29/28, corrected to tank standard = 9200.

are not actual isotopic abundances, but are instead corrected decade settings on the isotope-ratio mass spectrometer. The decade settings are directly proportional to isotopic abundances and can be used directly in calculating isotope effects. The correctness of these results was indicated by several tests. (1) The isotope effect was constant from experiment to experiment. (2) Standard ammonia samples could be carried through our procedure and analyzed with a reproducibility of ±0.000002. (3) No ammonia was found after steam distillation and concentration of a reaction solution if either enzyme or substrate was omitted. (4) The isotope ratios for the 10% samples and for the 100% samples were constant for all experiments.

Of the three or more steps involved in the acylation of chymotrypsin by *N*-acetyl-L-tryptophanamide, only the rate of the step in which the carbon–nitrogen bond is broken is expected to be affected appreciably by isotopic substitution.[7] A nitrogen isotope effect will be observed in this reaction only if the steps leading from the carbon–nitrogen cleavage step back to the starting materials are not slow compared to the cleavage step.[5] The nitrogen isotope effect observed in this case is similar to those observed in other cases where carbon–nitrogen single bond breaking occurs in the rate-determining step[8] and indicates that the above condition in fact obtains—that is, the carbon–nitrogen bond-breaking step is rate determining.

(6) J. M. Bremner, in "Methods of Soil Analysis," American Society of Agronomy, Madison, Wis., 1965, p 1256.

(7) There might also be a small isotope effect on some other step due to the loss of the extra zero-point energy associated with the partial double bond character of the amide carbon–nitrogen bond. Such might be the case, for example, if a tetrahedral intermediate is formed during the reaction. However, until further information is available about the structures of the intermediates involved in this reaction, we are unable to estimate the importance of such an effect.

(8) P. J. Smith and A. N. Bourns, *Can. J. Chem.*, **48**, 125 (1970); G. Ayrey, A. N. Bourns, and V. A. Vyas, *ibid.*, **41**, 1759 (1963); S. Seltzer and S. G. Mylonakis, *J. Amer. Chem. Soc.*, **89**, 6584 (1967).

If we assume that the rate constants for the chymotrypsin-catalyzed hydrolysis of *N*-acetyl-L-tryptophanamide are approximately the same as those of the very similar substrate furylacryloyltryptophanamide, our results show that the slow step in the kinetic scheme of Hess, *et al.*,[2] is, as they suggested, the step in which the carbon–nitrogen bond is broken.[9] This conclusion is also consistent with a number of previous observations concerning the chymotrypsin-catalyzed hydrolysis of specific substrate amides, for example, the large substituent effect on both the rate and the solvent isotope effect in the hydrolysis of acetyltyrosine anilides.[3] Such an effect is most easily explicable if carbon–nitrogen bond cleavage occurs in the slow step of the reaction.[10]

Acknowledgment. This research was supported by grants from the National Institutes of Health (NS-07657), the University of Wisconsin Graduate School, and the Research Corporation.

(9) Our results do not exclude the possibility that there might be more intermediates in this reaction than have been observed. Such a possibility would not change our conclusion that the carbon–nitrogen bond breaking is occurring in a slow step.

(10) The observation that the solvent isotope effect in this series of compounds varies from 1.5 to 2.8 with various substituted anilides lays to rest forever the objection that has repeatedly been raised that solvent isotope effects in enzymatic reactions may merely be indicators of solvent-sensitive enzyme conformational changes, rather than of transition state-structure and solvation. The former factor should be nearly constant for various substrates, and therefore can have a maximum value of 1.5 for this series of compounds. The remaining isotope effect (up to a factor of 2) must be due to the second factor.

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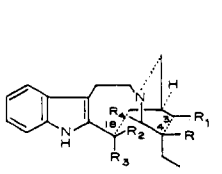
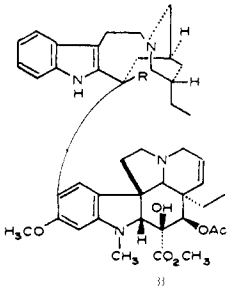
Studies on the Synthesis of Monomeric and Dimeric Vinca Alkaloids. The Total Synthesis of Isovelbanamine, Velbanamine, Cleavamine, 18β-Carbomethoxycleavamine, and Catharanthine

Sir:

Previous publications from our laboratory have demonstrated the general utility of the chloroindolenines of the cleavamine derivatives (I, R = R_1 = R_2 = R_3 = R_4 = H and R = R_1 = R_2 = R_4 = H; R_3 = $COOCH_3$) in the synthesis of monomeric[1,2] and dimeric[3] indole and dihydroindole alkaloids.





(1) J. P. Kutney, W. J. Cretney, P. Le Quesne, B. McKague, and E. Piers, *J. Amer. Chem. Soc.*, **88**, 4756 (1966).

(2) J. P. Kutney, W. J. Cretney, P. Le Quesne, B. McKague, and E. Piers, *ibid.*, **92**, 1712 (1970).

(3) J. P. Kutney, J. Beck, F. Bylsma, and W. J. Cretney, *ibid.*, **90**, 4504 (1968).