zero-order turnover and measuring the difference between the experimental points and the extrapolated line, the first-order rate constant of the acceleration was obtained. An independent calculation of this rate constant using the intercept, y_0, of the P_1 *vs.* t plot at $t = 0$ and the equation, $y_0 = (E_0 - E_{free})(1 - (k_3\text{CLyNPE}/k_3\text{BAEE}))$,[5] gave good agreement. This rate constant which measures the regeneration of the enzyme from an inactive but regenerable form is slightly higher than the rate constant of the trypsin-catalyzed hydrolysis of α-N-benzoyl-L-arginine ethyl ester (Table I).

The only explanation of these results is that trypsin plus α-N-benzoyl-L-arginine ethyl ester form an appreciable amount of α-N-benzoyl-L-arginyl-trypsin in a dynamic equilibrium, and that the decomposition of this acyl-enzyme, seen in the acceleration,[6] is mainly but not completely rate determining in the over-all hydrolytic reaction. Using this hypothesis the value of k_2 at pH 2.94 may be calculated in several ways. The known values of k_3 (1.25×10^{-2} sec.$^{-1}$, from the average of four acceleration determinations) and k_{cat} (from the turnover reaction) and the equation $k_2 = k_{cat}k_3/(k_3 - k_{cat})$ yields 4.5×10^{-2} sec.$^{-1}$. The magnitude of the burst, the known k_3, and the equation $k_3/k_2 = (E_0/ES')/(1 + (K_m(app)/S)) - 1$ yields 2.4×10^{-2} sec.$^{-1}$. Thus, the k_2/k_3 ratio for the trypsin-catalyzed hydrolysis of α-N-benzoyl-L-arginine ethyl ester is about 3–4 at pH 2.94, and K_s is not far different from K_m(app).

In a similar experiment, a trypsin solution was equilibrated with excess α-N-benzoyl-L-arginine, and then an aliquot of this mixture was added to the lysine ester solution. Again the burst decreased (to 55% of the blank experiment), and again an acceleration was observed before reaching a zero-order turnover identical with that in the absence of α-N-benzoyl-L-arginine (Figure 1). The first-order rate constant of this acceleration was similar to that produced from the corresponding ester, indicating that both α-N-benzoyl-L-arginine and α-N-benzoyl-L-arginine ethyl ester form the same inactive but regenerable enzyme derivative. The decrease in the initial burst of *p*-nitrophenol was dependent on the time of equilibration of trypsin with α-N-benzoyl-L-arginine (before addition to the lysine ester solution) with a rate constant of 2.1×10^{-2} sec.$^{-1}$. Both from the extent of the decrease in the burst and the rate constant of the decrease, the acylation/deacylation ratio of the acid (k_{-3}/k_3) was determined to be about 1.6. The absolute value of the k_2/k_3 of the ester and k_{-3}/k_3 of the acid and the relationship between them are closely similar to those found for the ester and acid of N-acetyl-L-tryptophan with α-chymotrypsin.[7]

The experiments reported in this paper[8] are consistent with the conclusion that the trypsin-catalyzed hydrolysis of α-N-benzoyl-L-arginine ethyl ester proceeds solely through an acyl-enzyme intermediate.

(5) The intercept of eq. 4 of ref. 4.

(6) Only a single acceleration is observed. *A priori*, the acceleration could correspond to the decomposition of either the acylenzyme, the enzyme–substrate complex, or the enzyme–product complex. The two latter possibilities, however, are ruled out by the time scale of the acceleration.

(7) F. J. Kézdy, G. E. Clement, and M. L. Bender, *J. Am. Chem. Soc.*, **86**, 3690 (1964).

(8) The k_2/k_3 ratio is independent of pH from 2.94 to 4 (Table I, footnote *h*), and the k_{cat} is dependent on a single ionizable group from pH 2 to 7. Therefore the k_2/k_3 ratio at pH 2.94 may be reasonably extrapolated to pH 7 (pK_a(acylation) = pK_a(deacylation) within experimental error).

(9) National Institutes of Health Postdoctoral Research Fellow.

Myron L. Bender, Ferenc J. Kézdy, Joseph Feder[9]
Division of Biochemistry of the Department of Chemistry
Northwestern University, Evanston, Illinois 60201
Received July 22, 1965

Secondary Deuterium Isotope Effects in Asymmetric Syntheses and Kinetic Resolutions

Sir:

We wish to report that partial asymmetric alcoholysis of α-phenylbutyric anhydride in pyridine[1] with (+)-(*S*)-2-propanol-1-d_3 (**1**)[2] affords (after hydrolysis of unreacted anhydride) α-phenylbutyric acid (**2**) with detectable optical activity and thus provides the first example of the operation of a steric isotope effect in an asymmetric synthesis or kinetic resolution.[3]

In a typical experiment, a solution of α-phenylbutyric anhydride (1.619 g., 0.0052 mole) in 12.5 ml. of pyridine was added to 0.165 g. (0.0026 mole) of **1**, and the mixture was allowed to stand at room temperature for 16 hr. The excess of unreacted anhydride was hydrolyzed by addition of 0.5 ml. of water in the cold. Titration of **2** with 1 *N* sodium hydroxide (0.0078 mole) in the presence of benzene established that the esterification reaction had been quantitative. The aqueous solution was extracted with chloroform and acidified; the acid solution was repeatedly extracted with benzene. The isolated acid **2** had $[\alpha]_D$ +0.46° before and after distillation, corresponding to a 0.48% optical yield based on the maximum rotation of **2** ($[\alpha]_D$ ±96.5° in benzene). Several repetitions of this experiment yielded essentially the same results. The observed rotations varied between +0.02 and +0.12° (depending on the conditions of measurement) and were thus well outside the limits of experimental error (±0.002°). The optical yields of **2** varied between 0.4 and 0.5%.

Although the details of the mechanism of alcoholysis have not yet been fully elucidated,[1,5,6] it has been possible to show empirically[1] that the sign of rotation of recovered **2** is related to the absolute configuration of the inducing alcohol; partial alcoholysis with alcohols of configuration **3** affords, after hydrolysis of unreacted anhydride, acid **2** containing an excess of the (+)-(*S*)-enantiomer.[7]

(1) A. Horeau, *Tetrahedron Letters*, **No. 15**, 506 (1961); **No. 21**, 965 (1962); A. Horeau and H. B. Kagan, *Tetrahedron*, **20**, 2431 (1964).

(2) K. Mislow, R. E. O'Brien, and H. Schaefer, *J. Am. Chem. Soc.*, **82**, 5512 (1960); **84**, 1940 (1962).

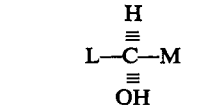
(3) It had previously been observed[2] that the partial asymmetric Meerwein–Ponndorf–Verley reduction of a racemic biphenyl ketone with **1** does not result in detectable asymmetric induction, and it was concluded that more highly crowded transition states might be required for the exhibition of steric isotope effects, *i.e.*, secondary deuterium isotope effects which could be ascribed to differences in the size of isotopic groupings. The first unambiguous kinetic evidence for the operation of steric isotope effects was subsequently provided by a study of the racemization of variously deuterated 9,10-dihydro-4,5-dimethylphenanthrenes.[4]

(4) K. Mislow, R. Graeve, A. J. Gordon, and G. H. Wahl, Jr., *J. Am. Chem. Soc.*, **85**, 1199 (1963); **86**, 1733 (1964).

(5) C. W. Bird, *Tetrahedron Letters*, **No. 3**, 117 (1962).

(6) H. Falk and K. Schlögl, *Monatsh. Chem.*, **96**, 266, 276 (1965).

(7) This generalization is *not* equivalent to the statement[6] that partial alcoholysis with alcohols having the (*R*) configuration affords acid **2** containing an excess of the (+)-(*S*) enantiomer. Stereoformula **3** corresponds to (*R*) alcohol only if the large group (L) has priority over the medium-sized group (M) in the Cahn–Ingold–Prelog nomenclatural scheme (R. S. Cahn, *J. Chem. Educ.*, **41**, 116 (1964)); however, there



L = large group
M = medium-sized group

3

Application of this generalization to the present results reveals that **3** corresponds to the (*S*) configuration of **1** if and only if CH_3 is taken as the large group and CD_3 as the medium-sized group. This finding is in harmony with the earlier conclusion that CH_3 exceeds CD_3 in effective bulk[4] and underscores the remarkable sensitivity of this kinetic resolution to differences in group size, including the capability of discriminating between isotopic substituents.

As will be reported in detail elsewhere,[8] the partial asymmetric alcoholysis of α-phenylbutyric anhydride in pyridine with optically active α-deuterated primary alcohols (RCHDOH) also affords optically active **2** in low optical yields. If the original generalization[1] is extended so that the H in stereoformula **3** is replaced by S (= small group), the signs of rotation of **2** correctly reflect the known absolute configurations of these alcohols, provided that the bulk of hydrogen is taken to be greater than that of deuterium, *i.e.*, R (=L) > H (=M) > D (=S).

exists no strict connection between group size and the priority sequence. Thus, although stereoformula **3** *usually* corresponds to the (*R*) configuration, it does not do so *necessarily*, as exemplified by **1** in the present work.

(8) A. Horeau and A. Nouaille, unpublished results.

Alain Horeau, Andrée Nouaille
Laboratoire de Chimie Organique des Hormones
Collège de France, Paris, France

Kurt Mislow
Department of Chemistry, Princeton University
Princeton, New Jersey 08540

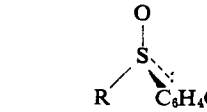
Received August 30, 1965

Light-Induced Pyramidal Inversion of Sulfoxides[1]

Sir:

The stereomutation[2] of sulfoxides has been induced catalytically (by hydrogen chloride and dinitrogen tetroxide),[3] *via* a sequence of reactions constituting a Walden cycle,[4] and by heating at elevated temperatures.[3c,5] We now wish to report the photochemical stereomutation of sulfoxides.[6] To the best of our knowledge our observations constitute the first examples of an inversion of pyramidal molecules by photochemical means.[7]

Compounds **1–6** were employed in racemization



1, R = α-$C_{10}H_7$ (naphthyl)
2, R = β-$C_{10}H_7$
3, R = mesityl
4, R = CH_3
5, R = α-$C_{10}H_7$–CH_2
6, R = α-$C_{10}H_7$–CH_2CH_2

studies.[8] Irradiation (Hanovia high-pressure quartz mercury vapor lamp (450 w.), immersed in a 300-ml. Pyrex irradiation flask with a capillary nitrogen inlet at the base) for 1 hr. of *ca.* 10^{-3} *M* ether solutions of (−)-(*S*)-**1**[9] (ϵ_{300}^{diox} 7910), (+)-(*S*)-**2**[11] (ϵ_{300}^{diox} 2050), and (−)-(*S*)-**3**[9,10] (ϵ_{300}^{diox} 1250) under oxygen-free nitrogen at room temperature, using a Pyrex filter sleeve (cut-off at wave lengths below 285 mμ), afforded completely racemized products,[12] with recoveries of 70% or greater.

Irradiation of **1** in the absence of a filter resulted in complete decomposition; naphthalene could be detected as one of the products.

Irradiation of a 0.1 *M* solution of (+)-(*R*)-**4**,[10,13] λ_{max}^{diox} 246 mμ (ε 5490), in ether (Pyrex filter) for 2 hr. resulted in 5–10% racemization and no decomposition; this result occasioned no surprise since ϵ_{300}^{diox} 5. In the absence of a filter, irradiation of 6.5×10^{-3} *M* **4** resulted in extensive decomposition. When the time of irradiation was limited to 10 min., a 40% yield of **4** could be recovered, 72% racemized.

In order to explore the possibility of photosensitization in the racemization, a 0.1 *M* solution of **4** was irradiated (Pyrex filter) for 2 hr. *in the presence of 0.02 M naphthalene.* A 99% recovery of sulfoxide was realized; the recovered material was 24% racemized. Sensitization has thus been demonstrated. Since *intra*molecular sensitization was expected to be a more efficient process than the *inter*molecular counterpart,[14]

(1) The work was supported by the National Science Foundation under Grant No. GP-3375 at Princeton University and Grant No. GP-2488 at the California Institute of Technology.

(2) By *stereomutation* we mean the interconversion of stereoisomers, *i.e.*, of enantiomers (inversion, racemization) or of diastereomers (epimerization, *cis–trans* interconversion). The term "diastereomer" is here used in its most general sense: K. Mislow, "Introduction to Stereochemistry," W. A. Benjamin, Inc., New York, N. Y., 1965, p. 51.

(3) (a) B. Iselin, *Helv. Chim. Acta*, **44**, 62 (1960); (b) K. Mislow, T. Simmons, J. T. Melillo, and A. L. Ternay, Jr., *J. Am. Chem. Soc.*, **86**, 1452 (1964); (c) C. R. Johnson and D. McCants, Jr., *ibid.*, **86**, 2935 (1964); **87**, 1109 (1965).

(4) C. R. Johnson, *ibid.*, **85**, 1020 (1963); C. R. Johnson and J. B. Sapp, 145th National Meeting of the American Chemical Society, New York, N. Y., Sept. 1963, Abstracts, p. 23Q.

(5) (a) K. Fries and W. Vogt, *Ber.*, **44**, 756 (1911); (b) H. Baw, G. M Bennett, and P. Dearns, *J. Chem. Soc.*, 680 (1934); (c) G. Farina, F. Montanari, and A. Negrini, *Gazz. chim. ital.*, **89**, 1548 (1959); (d) H. B. Henbest and S. A. Khan, *Proc. Chem. Soc.*, 56 (1964); (e) K. Mislow, P. Schneider, and A. L. Ternay, Jr., *J. Am. Chem. Soc.*, **86**, 2957 (1964).

(6) Prior work on the photochemistry of sulfoxides seems to have been restricted to the photosensitized oxidation of sulfoxides to sulfones (G. O. Schenck and C. H. Krauch, *Chem. Ber.*, **96**, 517 (1963)) and to the formation of sulfoxides by photooxidation of thioethers (O. Hinsberg, *Ber.*, **45**, 2337 (1912); G. Hirohashi, *Nagasaki Igakkai Zasshi*, **31**, 706, 761 (1956); W. F. Forbes and W. E. Savige, *Photochem. Photobiol.*, **1**, 77 (1962); W. F. Forbes, D. E. Rivett, and W. E. Savige, *ibid.*, **1**, 97, 217 (1962); J. E. Eager, C. M. Roxburgh, and W. E. Savige, *ibid.*, **3**, 129 (1964)).

(7) As in the photoracemization of biphenyls (K. Mislow and A. J. Gordon, *J. Am. Chem. Soc.*, **85**, 3521 (1963)), where the interconversion of enantiomers involves a torsional vibration, the mechanism of these transformations may not require bond breaking and bond re-formation but may merely involve a vibrational change, in this case an inversion of the sulfoxide pyramid. This question is presently under investigation.

(8) These compounds were prepared by the Grignard reaction of alkyl- or arylmagnesium halides with (−)-menthyl (−)-*p*-toluenesulfinate.[9,10] All new compounds gave satisfactory elemental analyses.

(9) K. K. Andersen, W. Gaffield, N. E. Papanikolaou, J. W. Foley, and R. I. Perkins, *J. Am. Chem. Soc.*, **86**, 5637 (1964).

(10) K. Mislow, M. M. Green, P. Laur, J. T. Melillo, T. Simmons, and A. L. Ternay, Jr., *ibid.*, **87**, 1958 (1965).

(11) M.p. 134–135°, $[\alpha]^{23}D$ +47° (chloroform). The racemic form melts at 111–113°.

(12) Racemic products were isolated by chromatography and were identified by comparison of their solution infrared spectra and their thin layer chromatograms with those of the active precursors. Elemental analyses were satisfactory in all cases.

(13) The maximum rotation observed for this compound, $[\alpha]^{25}D$ +156° (ethanol), was somewhat higher than that previously reported,[10] $[\alpha]^{26}D$ +141° (ethanol).

(14) It is well established that photoinduced stereomutation[2] may occur by intramolecular processes in which the absorbing chromophore is formally insulated from (although it may be spectroscopically coupled with) the center undergoing stereomutation; *cf.*, *e.g.*, W. von E. Doering and M. Jones, Jr., *Tetrahedron Letters*, **No. 12**, 791 (1963); Mislow and Gordon[7]; H. Morrison, *J. Am. Chem. Soc.*, **87**, 932 (1965).