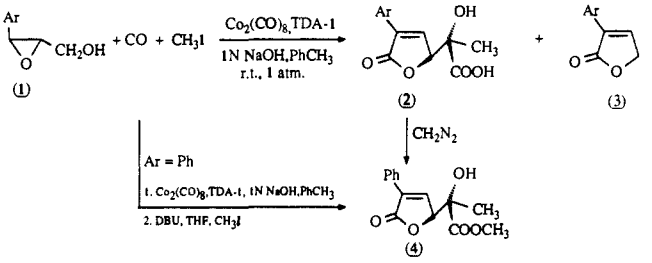
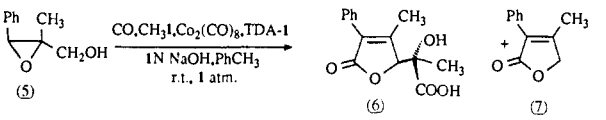
of β-epoxy alcohols as reactants, one could intercept the ring-opened organocobalt complex by the alkoxide ion (formed by deprotonation of the alcohol) and produce a different, unsaturated lactone. We now describe the novel phase-transfer-catalyzed conversion of epoxy alcohols to lactonic hydroxy acids.

Treatment of 3-(hydroxymethyl)-2-phenyloxirane (**1**, Ar = Ph) with carbon monoxide and methyl iodide, using toluene as the organic phase, 1 N NaOH as the aqueous medium, $Co_2(CO)_8$ as

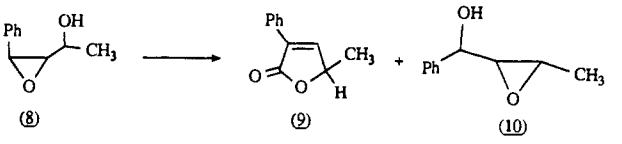


the metal catalyst [10:1 ratio of $1/Co_2(CO)_8$], and tris(polyoxaheptyl)amine (TDA-1)[6-8] as the phase-transfer agent, for 8–12 h at room temperature afforded 2-*C*-(2,5-dihydro-2-oxo-3-phenylfur-5-yl)lactic acid, (**2**, Ar = Ph), in 42% yield of pure material, with 10% 2,5-dihydro-2-oxo-3-phenylfuran (**3**, Ar = Ph)[9] formed as a byproduct. Lower yields of **2** were attained with cetyltrimethylammonium bromide (25% yield of **2**) as the phase-transfer catalyst, or at higher concentrations of base (16% with 5 N NaOH). Compound **2** (Ar = Ph) was characterized as such[10] or as its methyl ester **4**. The latter was obtained either by reaction of **2** with diazomethane (96% yield), or in 44% yield from **1** by a two-step process, the first being the carbonylation reaction, followed by exposure to 1,5-diazabicyclo[5.4.0]undec-5-ene and methyl iodide in tetrahydrofuran (THF). Excellent quality crystals of **4** were obtained and an X-ray structure determination (to be published separately)[11] provided conclusive evidence for the assigned structure. The process is diastereospecific, as X-ray and NMR analyses reveal that only the anti diastereomer is present in the solid state and in solution. Repetition of the phase-transfer reaction of **1** using ^{13}CO resulted in incorporation of the label at the lactone carbonyl, carboxylic acid carbon, as well as at the carbon bearing the hydroxyl group (^{13}C NMR: $J_{C^{13}-C^{13}}$ = 59.2 Hz for the hydroxy and acid carbons).[12]

This remarkable triple carbonylation reaction occurs with other epoxy alcohols related to **1**, including those with a 1-naphthyl (46% yield of **2**) or *p*-tolyl (50% yield) instead of a phenyl substituent ring. In addition, use of the trisubstituted oxirane **5** as the reactant



affords approximately equal amounts of the triple (**6**, 33%)[12] and single (**7**, 36%)[13] carbonylation products. Only monocarbonylation to **9**[14] and rearrangement (**10**) occurs with the secondary alcohol **8** as the reactant [**10** is also formed in the absence of $Co_2(CO)_8$].



Analysis of the above results suggested that the product of triple carbonylation may arise via **3**. Indeed, exposure of **3** to the reaction conditions described for **1** [i.e., $Co_2(CO)_8$, CH_3I, CO, 1 N NaOH, $PhCH_3$, TDA-1, room temperature, 16 h] gave **2** in 78% yield. Note that **3** does not react with 1 N NaOH, CH_3I, and TDA-1 in the absence of CO and $Co_2(CO)_8$. Similarly, **7** afforded **6** in 58% yield. A possible pathway for the double carbonylation of butenolides may involve the participation of enol cobalt intermediates (steric factors are evident in the failure of **9** to undergo double carbonylation).

In conclusion, cobalt carbonyl and TDA-1 catalyze the unique conversion of epoxy alcohols to 2-*C*-(2,5-dihydro-2-oxofur-5-yl)lactic acids in moderate yields, and under exceptionally mild conditions. The products can be considered as unusual lactic acid systems. This transformation constitutes the first example, to our knowledge, of a net triple carbonylation reaction. Furthermore, these are the first cases of double carbonylation of butenolides.

Acknowledgment. We are grateful to British Petroleum, and to the Natural Sciences and Engineering Research Council of Canada, for support of this research.

(8) Soula, G., *J. Org. Chem.* **1985**, *50*, 3717.
(9) Kayser, M. M.; Morand, P. *Can. J. Chem.* **1980**, *58*, 2484.
(10) Properties for **2** (Ar = Ph): mp 236.0–238.8 °C; IR (KBr) ν 3426 (OH), 1748 (CO) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.48 (s, 3 H, CH_3), 5.36 (d, 1 H, H5, J_{H4-H5} = 1.9 Hz), 7.43 (m, 3 H, Ph protons), 7.94 (dd, 2 H, Ph protons ortho), 8.20 (d, 1 H, CH=) ppm; ^{13}C NMR (DMSO-d_6) δ 22.6 (CH_3), 73.9 (C(OH)CH_3), 84.2 (CHO), 126.6, 128.4, 128.9, 129.6, 130.8, 146.8 (Ph and olefinic carbons), 170.9 (CO-lactone), 174.4 (COOH) ppm. **2** (Ar = *p*-$CH_3C_6H_4$): mp 240.6–242.7 °C; IR (KBr) ν 3420 (OH), 1745 (CO) cm^{-1}; ^1H NMR [$(CD_3)_2CO$] δ 1.44 (s, 3 H, CH_3), 2.31 (s, 3 H, $CH_3C_6H_4$), 5.31 (d, 1 H, H5, J_{H4-H5} = 1.7 Hz), 7.24 (d, 2 H, protons ortho to methyl-bearing carbon), 7.81 (d, 2 H, other aromatic protons), 8.09 (d, 1 H, CH=) ppm; ^{13}C NMR (DMSO-d_6) δ 20.9 (CH_3), 73.9 (C(OH)CH_3), 84.1 (CHO), 126.4, 126.7, 128.9, 130.7, 138.5, 145.6 (aromatic and olefinic carbons), 171.0 (CO-lactone), 174.4 (COOH) ppm. **2** (Ar = 1-$C_{10}H_7$): IR (KBr) 3475 (OH), 1740 (CO) cm^{-1}; ^1H NMR [$(CD_3)_2CO$] δ 1.55 (s, 3 H, CH_3), 5.57 (d, 1 H, H5, J_{H4-H5} = 1.9 Hz), 7.50–8.05 (m, 8 H aromatic and olefinic protons). **4**: mp 169.0–170.0 °C; IR (KBr) ν 3487 (OH), 1760 (CO), 1738 (CO) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.57 (s, 3 H, CH_3), 3.82 (s, 3 H, OCH_3), 5.17 (d, 1 H, H5, J_{H4-H5} = 2.1 Hz), 7.38 (m, 3 H, Ph protons), 7.55 (d, 1 H, CH=), 7.84 (dd, 2 H, Ph protons ortho) ppm; ^{13}C NMR ($CDCl_3$) δ 22.8 (CH_3), 53.2 (OCH_3), 76.0 (C(OH)CH_3), 84.9 (CHO), 128.1, 129.7, 130.2, 131.2, 133.6, 146.1 (aromatic and olefinic carbons), 172.0 (CO), 174.9 (CO) ppm. **6**: mp 223.6–226.1 °C; IR (KBr) ν 3339 (OH), 1748 (CO), 1739 (CO) cm^{-1}; ^1H NMR [$(CD_3)_2CO$] δ 1.57 (s, 3 H, CH_3COH), 2.17 (s, 3 H, CH_3C=), 5.30 (s, 1 H, CHO), 7.47 (m, 5 H, Ph) ppm; ^{13}C NMR [$(CD_3)_2CO$] δ 14.2 (CH_3), 23.2 (CH_3), 75.5 (C(OH)CH_3), 86.2 (CHO), 128.8, 128.9, 129.9, 131.1, 159.0 (Ph and olefinic carbons), 171.9 (CO-lactone), 174.9 (COOR) ppm (Note: NMR assignments for **2**, **4**, and **6** established by COSY and HETCOR techniques).
(11) Hynes, R., unpublished results.
(12) Breitmayer, E.; Voelter, W. *Carbon-13 NMR Spectroscopy*; VCH: Weinheim, West Germany, 1987; pp 147–152.
(13) Duboudin, J. G.; Ousseaume, B. J. *J. Organomet. Chem.* **1979**, *168*, 233.
(14) Hussain, S. A. M. T.; Ollis, W. D.; Smith, C.; Stoddart, J. F. *J. Chem. Soc., Perkin Trans 1* **1975**, 1480.

Intramolecular Conversions of Acyllithium. Cyclization in the Reaction of Carbon Monoxide with [1-(Silyl)vinyl]lithium

Ilhyong Ryu, Yoshihiko Hayama, Akira Hirai, Noboru Sonoda, Akihiro Orita, Kouichi Ohe, and Shinji Murai*

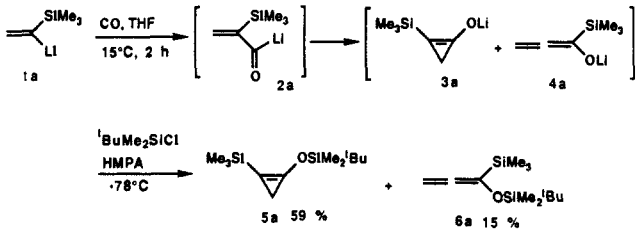
Department of Applied Chemistry, Faculty of Engineering Osaka University, Suita, Osaka 565, Japan
Received November 21, 1989

The use of unstable, exceedingly reactive acyl anions in synthesis represents a challenge of intense interest.[1] In order to realize selective transformations from acyl anions, Seyferth et al. have studied *intermolecular* reactions involving direct trapping with electrophiles under carefully controlled reaction conditions.[2] On the other hand we have devised an *intramolecular* reaction involving the conversion of [2-(silyl)acyl]lithiums to acylsilane

(1) For a review, see: Narayana, C.; Periasamy, M. *Synthesis* **1985**, 253.
(2) (a) Seyferth, D.; Weinstein, R. M. *J. Am. Chem. Soc.* **1982**, *104*, 5534. (b) Seyferth, D.; Weinstein, R. M.; Wang, W.-L. *J. Org. Chem.* **1983**, *48*, 1144. (c) Weinstein, R. M.; Wang, W.-L.; Seyferth, D. *Ibid.* **1983**, *48*, 3367. (d) Seyferth, D.; Weinstein, R. M.; Wang, W.-L.; Hui, R. C. *Tetrahedron Lett.* **1983**, *24*, 4903. (e) Seyferth, D.; Hui, R. C. *Tetrahedron Lett.* **1984**, *25*, 5251. (f) Seyferth, D.; Weinstein, R. M.; Wang, W.-L.; Hui, R. C. *Tetrahedron Lett.* **1984**, *25*, 2623. (g) Seyferth, D.; Wang, W.-L.; Hui, R. C. *Tetrahedron Lett.* **1984**, *25*, 1651. (h) Seyferth, D.; Hui, R. C. *Organometallics* **1984**, *3*, 327. (i) Seyferth, D.; Hui, R. C. *J. Org. Chem.* **1985**, *50*, 1985. (j) Seyferth, D.; Hui, R. C. *J. Am. Chem. Soc.* **1985**, *107*, 4551.

0002-7863/90/1512-7061$02.50/0

Scheme I



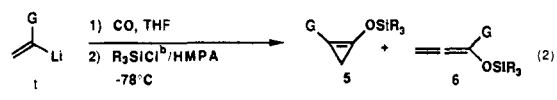
enolates.[3] This intramolecular transformation of acyllithium is achieved through anionic 1,2-silicon rearrangement. We report herein the results of the reaction of carbon monoxide with [1-(silyl)vinyl]lithium and [2-phenyl-1-(silyl)vinyl]lithium, which resulted in a new class of *intramolecular* transformations of acyllithiums, i.e., cyclization. Most notably, the former reaction corresponds to a formal [2 + 1] cycloaddition of CO with a vinyl anion to give a cyclopropanone enolate (eq 1).[4]

(1)

We have discovered that the lithium enolate of 2-(trimethylsilyl)cyclopropanone is formed by the reaction of [1-(trimethylsilyl)vinyl]lithium (**1a**)[5] with CO. Treatment of **1a** with an atmospheric pressure of CO in THF at 15 °C for 2 h followed by quenching with chlorotrimethylsilane at −78 °C afforded a somewhat labile product, which decomposed during the ordinary hydrolytic workup. Quenching with *tert*-butyldimethylchlorosilane/HMPA instead allowed the isolation of the product.[6,7] The product was a silylated cyclopropanone enolate **5a**. The overall sequence from **1a** and CO to **3a** follows a formal [2 + 1] cycloaddition (eq 1), while the conversion of **2a** to **3a** may occur via the formation of a ketene β-carbanion and the subsequent nucleophilic 3-exo cyclization (Scheme I).[8] Silylated allenolate **6a** was also formed as a byproduct. A pathway involving an anionic 1,2-shift of silicon[3] from the vinylic carbon of **2a** to the carbonyl carbon would account for **4a**. As shown in Table I, the reactions should be conducted at ambient temperatures; otherwise only poor yields were attained owing to the competitive intermolecular reactions from **2a**.

The reaction of [2-phenyl-1-(silyl)vinyl]lithium **9** and CO at 15 °C also underwent clean *intramolecular* transformation of the acyllithium, whereas the cyclization to a five-membered ring was

Table I. Reaction of [1-(Silyl)vinyl]lithiums **1** with Carbon Monoxide[a]

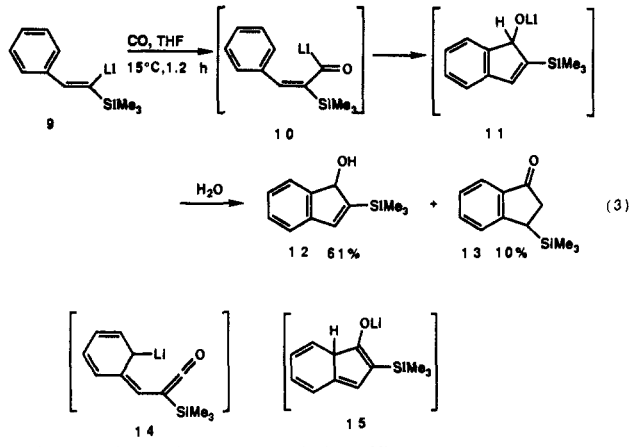


(2)

G, **1**	temp, °C	time, h	yield, % **5**[c]	yield, % **6**[c]
Me_3Si, **1a**	−78	1.5	6	2
	−40	2	20	4
	0	2	31	9
	15	2	59 (35)[d]	15
	25	2	43	11
Me_2PhSi, **1b**	25	1.5	44 (31)[e]	28

[a] Reactions were carried out on a 2-mmol scale using a CO balloon: **1** (2 mmol), THF (25 mL), R_3SiCl (2.09 mmol). For details, see footnote 6. [b] R_3 = $^tBuMe_2$. [c] GLC yield. [d] Isolated yield by bulb-to-bulb distillation (GLC purity, 76%). [e] Isolated yield by bulb-to-bulb distillation (GLC purity, 74%).

observed in this case. Treatment of a THF solution of **9**, generated from (*Z*)-2-phenyl-1-(trimethylsilyl)vinyl bromide (**8**) and *t*-BuLi, with carbon monoxide at 15 °C for 1.2 h followed by proton quenching yielded 2-(silyl)-1-indenol **12** in 61% yield together with 3-(silyl)indanone **13** (10%)[9] after purification by preparative TLC.[10] The cyclization process leading to **15**, which can be regarded as a formal [4 + 1] cycloaddition of CO, may involve the internal nucleophilic attack of ketene carbanion **14**, a tautomer of **10**,[8] followed by aromatization to **11** by 1,5-hydrogen shift.



(3)

Thus we have demonstrated the efficient intramolecular cyclizations via the α-silyl-substituted α,β-unsaturated acyllithium. To our knowledge 1-(siloxy)cyclopropenes produced in this study are the first example of enol silyl ethers having a three-membered ring except for the bis(silyl ether) of dihydroxycyclopropenone.[11] Mechanistically, lithioxy carbene character is an attractive possibility of the acyllithium.[12] The detailed mechanism of these cyclization processes which occurred with α-silyl substitution will require further study.[13]

(3) Murai, S.; Ryu, I.; Iriguchi, J.; Sonoda, N. *J. Am. Chem. Soc.* **1984**, *106*, 2440.

(4) Cyclopropanone enolates are elusive molecules. For a reference suggesting the intermediacy, see: Ciabattoni, J.; Kocienski, P. J.; Melloni, G. *Tetrahedron Lett.* **1969**, 1883.

(5) Grobel, B.-T.; Seebach, D. *Chem. Ber.* **1977**, *110*, 867.

(6) Typically, under an atmosphere of argon, [1-(trimethylsilyl)vinyl]-lithium (**1a**) was generated from 1-(trimethylsilyl)vinyl bromide (1.96 mmol) and *t*-BuLi (2.2 equiv) in anhydrous THF (25 mL) at −120 °C. After evacuation of argon gas under vacuum, the reaction mixture was exposed to carbon monoxide at 1 atm at 15 °C for 1 h. Then 0.5 mL of HMPA and ca. 5 mL of a THF solution of *t*-$BuMe_2SiCl$ (0.314 g, 2.09 mmol) were added to the reddish reaction mixture at −78 °C via a syringe. After warming to room temperature, the reaction mixture was diluted with pentane, washed with cold saturated aqueous sodium bicarbonate solution, and dried over anhydrous magnesium sulfate. The solvents were removed under reduced pressure to provide the crude mixture, which contained 1-(siloxy)cyclopropene **5a** (59%, GLC yield) and allenol silyl ether **6a** (15%, GLC yield) as the major components. Bulb-to-bulb distillation under vacuum gave 0.220 g of the first fraction including 76% GLC purity of **5a** [i.e., 35% yield of **5a**, bath temperature 120–150 °C (110 Torr)] with a high viscous residue (0.154 g). The product could not be isolated by chromatography on silica gel. We obtained pure **5a** (100%) by preparative GLC. **5a**: IR (neat) 1810 cm^{-1}; ^1H NMR (CCl_4) δ 0.12 (s, 9 H), 0.22 (s, 6 H), 0.94 (s, 9 H), 1.28 (s, 2 H); ^{13}C NMR ($CDCl_3$) δ −4.6 (q), −1.0 (q), 14.5 (t), 18.1 (s), 25.5 (q), 71.7 (s), 145.8 (s); MS *m/e* 242 (M^+, 1), 185 (35), 171 (93), 147 (70), 157 (32), 133 (38), 117 (31), 73 (100), 45 (40). Anal. Calcd for $C_{12}H_{26}OSi_2$: C, 59.43; H, 10.80. Found: C, 59.40; H, 11.00. For **6a**, see supplementary material.

(7) Quenching with EtOH (1 equiv) gave ethyl 3-(silyl)propionate as a major product. The formation process of this compound may involve the protonation of the enolate **3a** by EtOH to give 2-(silyl)cyclopropanone and the subsequent ring opening caused by EtOLi.

(8) The intermediacy of metal oxy carbene would be an alternative path. See the case of acylsamarium: Evans, W. J.; Hughes, L. A.; Drummond, D. K.; Zhang, H.; Atwood, J. L. *J. Am. Chem. Soc.* **1986**, *108*, 1722.

(9) With the prolonged reaction time (24 h), 3-(silyl)indanone was obtained as a sole product in 60% (70%) isolated yield (GLC yield) after proton quenching, indicated that 3-(silyl)indanone enolate was formed via further isomerization from **13** (1,5-hydrogen and 1,5-silicon shifts from **11**). This isomerization is remarkably facile in comparison with that of 2-(trimethylsilyl)indene to 3-(trimethylsilyl)indene, which was reported to require heating at 155 °C for 118 h.[9b] For fluxional behavior of the silylindenyl system, see: (a) Ashe, A. J., III; *Tetrahedron Lett.* **1970**, 2105. (b) Larrabee, R. B.; Dowden, B. F. *Tetrahedron Lett.* **1970**, 915. (c) Kisin, A. V.; Korenevsky, V. A.; Sergeyev, N. M.; Ustynyuk, Y. A. *J. Organomet. Chem.* **1972**, *34*, 893. (d) Andrews, M. N.; Rakita, P. E.; Taylor, G. A. *Tetrahedron Lett.* **1973**, 1851. Also see a review: (e) Spengler, C. W. *Chem. Rev.* **1976**, *76*, 187.

(10) Starting with the corresponding (*E*)-vinyl bromide **8'**, a nearly identical result was obtained. This is due to the predominant formation of vinyllithium **9** having *E* geometry (*E*/*Z* = 94/6 after silylation at 25 °C) regardless of the stereochemistry of the starting bromides. Cf.: Negishi, E.; Takahashi, T. *J. Am. Chem. Soc.* **1986**, *108*, 3402.

(11) Eggerding, D.; West, R. *J. Am. Chem. Soc.* **1976**, *98*, 3641.

(12) β-Effect of silicon on carbenic centers, see: Creary, X.; Wang, Y.-X. *Tetrahedron Lett.* **1989**, *30*, 2493.

Acknowledgment. This work was supported by Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan. We thank Shin-Etsu Chemical Co. Ltd. for a gift of chlorosilanes.

Supplementary Material Available: Typical experimental procedures for the reactions of **1a** with CO and **9** with CO and spectral data for **5a,b**, **6a,b**, **12**, and **13** (3 pages). Ordering information is given on any current masthead page.

(13) The reaction of (1-*tert*-butylvinyl)lithium with CO in THF was relatively sluggish, presumably due to the proximate steric effect, and did not afford products similar to **5** and **6** but gave an enediol disilyl ether arising from intermolecular reaction with incorporation of two molecules of CO. It has been reported that the reaction of unsubstituted vinyllithium with CO gave polymeric product, see: Sawa, Y.; Miki, T.; Ryang, M.; Tsutsumi, S. *Technol. Rep. Osaka Univ.* **1963**, *13*, No. 561.

Confirmation of the Secondary Deuterium Isotope Effect for the Peptidyl Prolyl Cis-Trans Isomerase Activity of Cyclophilin by a Competitive, Double-Label Technique

Richard K. Harrison,*,† Charles G. Caldwell,‡ Avery Rosegay,§ David Melillo,§ and Ross L. Stein†

Departments of Enzymology, Medicinal Chemistry, and Metabolism, Merck Research Laboratories P.O. Box 2000, Rahway, New Jersey 07065

Received April 16, 1990

Cyclophilin, the binding protein for the immunosuppressive drug cyclosporin, has recently been shown to catalyze the cis-trans isomerization of proline imide bonds in peptides and proteins.[1,2] Since these initial reports, there has been intense interest in determining the link between the immunoregulatory and isomerase activity that this protein posses. The ability to create potent immunosuppressants based on cyclophilin's isomerase activity is an attractive target for the rational design of immunosuppressive drugs. Critical to rational design is an understanding of the mechanism of action of the isomerase activity. In attempts to definitively characterize the mechanism of this enzyme, two groups have determined the secondary β-deuterium (β-D) kinetic isotope effect (defined as the ratio of rate constants, k_H/k_D, and abbreviated as ^Dk) for the cis-to-trans isomerization of Suc-Ala-Gly-(L,L)-*cis*-Pro-Phe-pNA (L = H, D). While Fischer and his co-workers[3] report a β-D effect of 0.91 and claim formation of a tetrahedral intermediate in the transition state, we report an effect of 1.13[4] and claim catalysis by distortion. A clear distinction between the two is imperative if successful rational inhibitor design is to be realized.

To distinguish the two mechanisms, we have used a competitive double-label technique.[5] To apply this technique, isotopically substituted substrates are labeled with a second, distinct reporting group. In the present case, the ^{14}C- and ^3H-methyl esters of Suc-Ala-Gly-Pro-Phe-pNA and Suc-Ala-Gly-(D,D)-Pro-Phe-pNA, respectively, were used. The isotope effect is then calculated from the dependence of the $^{14}C/^3H$ ratio of a mixture of the substrates on the fraction of reaction.

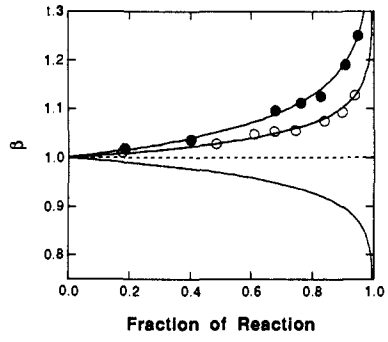


Figure 1. The dependence of β on the fraction of reaction for the nonenzymatic (open circles) and cyclophilin-catalyzed (filled circles) isomerization of MeOSuc-Ala-Gly-(L,L)-Pro-Phe-pNA. The lines through the data are the nonlinear least-squares fit to eq 3. The line in the bottom half of the figure was drawn according to eq 3 with $\alpha = 1.047$. Methyl esters of the peptide Suc-Ala-Gly-(L,L)-Pro-Phe-pNA (L = H, D; Bachem) were prepared by reaction with diazomethane and radiolabeled methyl iodide. Specific activities were as follows: $H_3{}^{14}CO$-Suc-Ala-Gly-Pro-Phe-pNA, 80 μCi/mg; ^3H_3CO-Suc-Ala-Gly-(D,D)-Pro-Phe-pNA, 108 μCi/mg. Cyclophilin from calf thymus was purified by the method of Harding et al.[7] and kindly supplied to us by Dr. John Siekierka (Immunology Department, Merck Research Laboratories). Determination of active-site concentration was reported elsewhere[6] and was 56 nM in these experiments. For isotope-effect determinations, 1.2 mg/mL solutions of each substrate in DMSO were mixed to a final volume of 300 μL so that $^{14}C/^3H$ was near 1. The 300-μL mixtures were then added to 10 mL of assay buffer (50 mM HEPES, pH 7.8) for a final substrate concentration of 70 μM. Values of β were determined as described in the text.

For a first-order reaction, where

$$[S]_t = [S]_0 \exp(-kt) \tag{1}$$

we can define k_H and k_D as the rate constants for the protio and deuterio substrates, and if we let $\alpha = k_D/k_H$, the following equation holds:

$$\frac{[S_H]_t/[S_H]_0}{[S_D]_t/[S_D]_0} = \frac{\exp(-k_H t)}{\exp(-\alpha k_H t)} \tag{2}$$

If we now define f, the fraction of reaction, as $1 - \exp(-kt)$, and a term β as $([S_H]/[S_D])_0/([S]_H/[S_D])_t$, eq 2 can be rewritten as

$$\beta = \frac{(1-f)^\alpha}{(1-f)} = (1-f)^{\alpha-1} \tag{3}$$

A plot of β vs f will increase exponentially from 1.0 for a normal kinetic isotope effect ($\alpha < 1$) and decrease exponentially from 1.0 for an inverse kinetic isotope effect ($\alpha > 1$). In the double-label experiments of this study, β is equal to $(^{14}C/^3H)_0/(^{14}C/^3H)_t$ for the substrate.

Isotope-effect determinations were performed at 4 °C in a chymotrypsin-coupled assay described elsewhere.[4] In a typical determination, the reaction was initiated by addition of chymotrypsin ($[CT]_0 = 70$ μM) to a thermally equilibrated solution of substrate ($[S]_0 = 70$ μM) and enzyme ($[PPI]_0 = 56$ nM). At 30-s intervals, 150-μL aliquots of the reaction mixture were withdrawn and added to 100-μL aliquots of a 0.7 mM solution of α_1-proteinase inhibitor (Sigma A-9024), and the resultant solution was stored at room temperature until chromatographed. Substrate and products were completely separated by HPLC. $^{14}C/^3H$ ratios for unreacted substrate were determined by liquid scintillation counting of 2.0-mL samples of the appropriate column fraction. Finally, β values were calculated from $^{14}C/^3H$ ratios for the unreacted substrate at several times throughout the course of the reaction.

Figure 1 is a plot of β as a function of f. The filled circles correspond to reaction in the presence of cyclophilin. An observed isotope effect, $^D(k_{obsd})$, of 1.080 ± 0.002 is obtained from a nonlinear least-squares fit of the data to eq 3. In a second de-

† Department of Enzymology.
‡ Department of Medicinal Chemistry.
§ Department of Metabolism.

(1) Fischer, G.; Wittman-Leibold, B., Lang, K.; Kufhaber, T.; Schmid, F. X. *Nature* **1989**, *337*, 476-478.
(2) Takahashi, N.; Hayano, J.; Suzuki, M. *Nature* **1989**, *337*, 473-475.
(3) Fischer, G.; Berger, E.; Bang, H. *FEBS Lett.* **1989**, *250*, 267-270.
(4) Harrison, R. K.; Stein, R. L. *Biochemistry* **1990**, *29*, 1684-1689.
(5) Dahlquist, F. W.; Rand-Meir, T.; Raferty, M. A. *Proc. Natl. Acad. Sci. U.S.A.* **1969**, *61*, 1194-1198.
(6) Harrison, K.; Stein, R. L. *Biochemistry* **1990**, *29*, 3813-3816.

0002-7863/90/1512-7063$02.50/0