orless oils **12** (0.213 g, 91%) and **17** (0.195 g, 78%).

Thujaplicin. The requisite α-chlorotropone **9** or **14** (0.207 g, 1.11 mmol) was dissolved in glatial acetic acid (10 mL) containing aqueous phosphoric acid (44%; 8 ml) and heated at reflux for 15 h.[13] The reaction mixture was then cooled and poured into water (40 mL) and the solution pH adjusted to pH 4–5 with aqueous sodium hydroxide. The aqueous phase was extracted with methylene chloride (5 × 20 mL) then the combined organics dried and the solvent removed in vacuo. Chromatographic filtration (Silica Gel; ether (50%)/pentane) afforded γ-thujaplicin (**3**) [mp 75–77 °C (lit.[16] mp 82 °C)] (0.142 g, 78%) and β-thujaplicin (**4**) [mp 44–46 °C (lit.[16] mp 50–52 °C)] (0.150 g, 83%).

Acknowledgment. The author is grateful for the financial assistance of the Vanderbilt University Natural Science Committee.

Registry No.—16, 66967-16-6; 17' 66967-17-7; 4-isopropylphenol, 99-89-8; 3-isopropylphenol, 618-45-1; triethylchlorosilane, 994-30-9.

References and Notes

(1) K. Nakanishi, T. Goto, et al., "Natural Products Chemistry", Vol. 2, Academic Press, New York, N.Y., 1975, p 144.
(2) The α-tropolone ring system occurs naturally incorporated in the fused aromatic systems of benzotropolones[2a] and tropoloisoquinolines.[2b] (a) D. Ollis, D. T. Coxon, A. Holmes, and V. C. Vora, *Tetrahedron Lett.*, 5237 (1970); (b) J. V. Silverton, C. Kabuto, K. T. Buck, and M. P. Cava, *J. Am. Chem. Soc.*, **99**, 6708 (1977).
(3) For a review covering troponoid synthesis and chemistry see: F. Pietra, *Chem. Rev.*, **73**, 293 (1973).
(4) For an alternate synthesis of the isomeric thujaplicins proceeding from the corresponding tropone see: R. Noyori, S. Makino, T. Okita, and Y. Hayakawa, *J. Org. Chem.*, **40**, 807 (1976).
(5) Such a "six to seven membered ring expanding" approach to troponoids and tropolonoids has appealed to synthetic chemists for some time. Birch and Keeton[5a,b] and Doering and Detert[6b] employed six to seven ring expansion in their syntheses of the tropone ring system and Tobinaga et al.[6a] utilized a less generalized homologation scheme in their synthesis of the α-tropolone ring in colchicine. (a) A. J. Birch and R. Keeton, *J. Chem. Soc. C*, 109 (1968); (b) A. J. Birch and R. Keeton, *Aust. J. Chem.*, **24**, 331 (1971).
(6) (a) S. Tobinaga, E. Kootani, and F. Miyazaki, *J. Chem. Soc., Chem. Commun.*, 300 (1974); (b) W. V. E. Doering and F. L. Detert, *J. Am. Chem. Soc.*, **73**, 876 (1951).
(7) The work of Donaldson and Fuchs[8] was published concurrent with our own investigation in this area. Only minor differences need be noted. To retain continuity with our earlier work on the dichlorocyclopropanation of trialkylsilyl enol ethers,[9] our studies employed triethylsilyl phenyl ethers, instead of *tert*-butyldimethylsilyl phenyl ethers. As noted by Donaldson and Fuchs,[8] trimethylsilyl phenyl ethers are too labile under the dissolving metal conditions to be useful. However, triethylsilyl phenyl ethers are at least as stable as the corresponding *tert*-butyldimethylsilyl phenyl ethers to the reaction conditions.
(8) R. E. Donaldson and P. L. Fuchs, *J. Org. Chem.*, **52**, 2032 (1977).
(9) G. Stork and T. L. Macdonald, *J. Am. Chem. Soc.*, **97**, 1264 (1975).
(10) J. M. Conia, P. Amico, and C. Blanco, *Synthesis*, 196 (1976).
(11) T. L. Macdonald, *J. Org. Chem.*, in press.
(12) Birch and Keeton[5b] have isolated two related dichlorocyclopropanols. To the best of our knowledge, these compounds represent the only previously isolated α,α-dichlorocyclopropanols.
(13) For example, see: T. Nozoe, T. Asao, E. Takahasi, and K. Takahashi, *Bull. Chem. Soc. Jpn.*, **39**, 1310 (1966).
(14) (a) M. Cavazza, R. Cabrino, and F. Pietra, *Synthesis*, 298 (1977); (b) R. Cabrino, M. Cavazza, and F. Pietra, *J. Chem. Soc., Chem. Commun.*, 721 (1976).
(15) H. Corrodi and E. Hardegger, *Helv. Chim. Acta*, **40**, 193 (1957).
(16) T. Nozoe, *Fortschr. Chem. Org. Naturst.*, **13**, 232 (1956).
(17) ^{13}C NMR data were obtained for this compound. The author would like to acknowledge the assistance of Kevin Darst. Chemical shifts (in ppm downfield from Me_4Si) 17.74, 18.42, 18.65, 28.44, 31.70, 34.92, 58.55, 58.83, 61.85, 68.30.

Synthesis of (3*S*,4*S*)-4-Amino-3-hydroxy-6-methylheptanoic Acid Derivatives. Analysis of Diastereomeric Purity

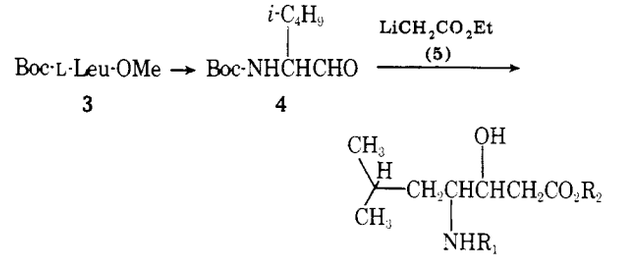
Daniel H. Rich,* Eric T. Sun, and Amrit S. Boparai

School of Pharmacy, University of Wisconsin—Madison, Madison, Wisconsin.

Received May 9, 1978.

Pepstatin, isovaleryl-L-valyl-L-valyl-(3*S*,4*S*)-statyl-L-alanyl-(3*S*,4*S*)-statine (**1**),[1] is a low molecular weight inhibitor of acid proteases, e.g., pepsin, renin, and cathepsin D.[2] Pepstatin contains the novel amino acid statine, (3*S*,4*S*)-4-amino-3-hydroxy-6-methylheptanoic acid (**2a**). Kinetic studies have shown that the (3*S*)-hydroxyl group in the statine residue in position 3 of pepstatin is necessary for tight-binding-inhibition of pepsin.[3,4] Synthetic statine is needed to further study the kinetic and biological properties of pepstatin and the importance of the (3*S*)-hydroxyl group of statine requires that its stereochemistry be rigorously established. However, while several syntheses of statine **2** have been reported,[5-8] the preparation of (3*S*,4*S*)-statine free of contamination from the (3*R*,4*S*) diastereomer is not readily achieved. We report here a convenient, high-yield synthesis of (3*S*,4*S*)-statine via a route that allows for separation of diastereomers and for determination of optical purity.

Scheme I



2a, $R_1 = R_2 = H$ (3*S*,4*S*)
b, $R_1 = R_2 = H$ (3*R*,4*S*)
5a, $R_1 = Boc$; $R_2 = C_2H_5$ (3*S*,4*S*)
b, $R_1 = Boc$; $R_2 = C_2H_5$ (3*R*,4*S*)
6a, $R_1 = Boc$; $R_2 = H$ (3*S*,4*S*)
b, $R_1 = Boc$; $R_2 = H$ (3*R*,4*S*)

Results and Discussion

The preparation of statine derivatives is outlined in Scheme I. Boc-L-leucine methyl ester (**3**) was reduced with diisobutylaluminum hydride in toluene at −78 °C for 6 min. Excess hydride was destroyed with methanol,[9] and the reaction worked up using Rochelle salt[10] to solubilize the complex. Aldehyde **4** was isolated in 85% yield. Addition of lithium ethyl acetate (**5**) at −78 °C to **4** according to a modification of the procedure of Steulmann and Klostermeyer[7] gave an 80% yield of the ester **5a,b** as a mixture of diastereomers (60% (3*S*,4*S*); 40% (3*R*,4*S*)). Diastereomers **5a** and **5b** can be separated by standard column chromatography over silica gel. A better and faster separation is achieved by using commercially prepared columns (Lobar) (3.7 × 44 cm) which can provide 1–2 g of pure **5a** from 2–4 g of the mixture in only a few hours. The overall yield of pure Boc-(3*S*,4*S*)-statine ethyl ester **5a** from ester **3** is 38–40%. Saponification of ester **5a** gives acid **6a** (86%) which, in turn, is readily converted to free statine **2a** by mild acid hydrolysis with trifluoroacetic acid.

Both Boc acids **6a** and **6b** can be crystallized from diethyl ether–petroleum ether (30–60 °C) mixtures. It was possible to isolate the less soluble **6b** by fractional crystallization of the mixture of diastereomers but further concentration of the mother liquor gave **6a** in only 80% optical purity. We were unable to crystallize either **6a** or **6b** from isopropyl alcohol.[7]

A convenient method for establishing the optical purity of the various statine diastereomers has been needed. Diastereomers **2a** and **2b** do not easily separate when subjected to standard amino acid analysis and other ion exchange conditions[8] although separation can be achieved at high temperatures.[6] We have found that the esters **5a** and **5b** are easily separated by gas-liquid chromatography (GC) on an OV-225 column. A mass spectrum of the material eluting from the GC column shows that the diastereomers are chromatographing as the intact Boc esters **5a** and **5b** and have not been degraded

0022-3263/78/1943-3624$01.00/0

Table I. Properties of Statine and Derivatives

	registry no.	^{1}H NMR, δ C-2	C-3	C-4	C-5,6	C-7,8	$[\alpha]^{24}_D$, deg	mp, °C
2a[a]	49642-07-1	2.43[c] 2.57	4.0 m	3.30 m	1.45 m	0.96 d (J = 6 Hz)	−20 (c 1, H_2O)	201–202[d]
2b[a]	49642-13-9	2.40[c] 2.43	4.2 m	3.4 m	1.45 m	0.93 d (J = 5 Hz) 1.01 d (J = 6 Hz)	−18 (c 1, H_2O)	202–203[d]
5a[b]	67010-43-9	2.49[c] 2.52	4.0 m	3.6 m	1.45 m	0.92 d (J = 6 Hz)	−37.9 (c 0.84, CH_3OH)	oil
5b[b]	67010-44-0	2.45[c] 2.49	4.0 m	3.6 m	1.45 m	0.90 d (J = 5 Hz) 0.92 d (J = 6 Hz)	−23.2 (c 0.94, CH_3OH)	oil
6a[b]	58521-49-6	2.52[c] 2.57	3.98 m	3.65 m	1.45 m	0.92 d (J = 6 Hz)	−39.6 (c 0.31, CH_3OH)	117–118
6b[b]	66967-01-9	2.48[c] 2.53	3.98 m	3.65 m	1.45 m	0.90 d (J = 5 Hz) 0.91 d (J = 6 Hz)	−27.6 (c 0.31, CH_3OH)	135–136

[a] Taken in D_2O with DSS added as internal reference. [b] Taken in $CDCl_3$ with internal Me_4Si as standard. [c] AB portion of ABX pattern. [d] Data taken from ref 5, 6, and 8.

to either cyclic carbamates or dehydro amino acids. Using the GC method to analyze the diastereomeric purity of **5a** and **5b** it has been possible to prepare the optically pure (>99%) statine derivatives listed in Table I.

The data in the table show that the optical rotation of derivatives **2a**, **2b**, **5a**, and **5b** is not a sensitive test for optical purity. However, the nuclear magnetic resonance (NMR) spectra of these diastereomers can be used to assign configuration and to estimate optical purity. In general the C-2 protons appear as an AB portion of an ABX pattern. The chemical shift of one of these protons resonates farther downfield in **2a** and **5a** than in **2b** and **5b**. This method is not as sensitive as the GC method for measuring optical purity and is probably accurate to only ±10%. In contrast to the above, the optical purity of the Boc acids **6a** and **6b** can be accurately established by optical rotation and melting point (Table I). The rotations reported in the table were obtained on analytically pure derivatives shown to be single diastereomers by GC. To be certain that no epimerization of the 3-hydroxyl group had occurred during saponification of **5a** and **5b**, the Boc acids **6a** and **6b** were converted to methyl esters by reaction with diazomethane. Each sample was analyzed by GC and shown to be homogeneous (retention times: (3*S*,4*S*) methyl ester, 10.0 min; (3*R*,4*S*) methyl ester, 11.4 min).

Steulmann and Klostermeyer described the first synthesis of (3*S*,4*S*)-Boc-Sta **6a** and reported that fractional crystallization from isopropyl alcohol gave pure **6a** ($[\alpha]^{20}_D$ −27.8°; mp 95 °C).[7] However, we observe both a more negative rotation and a higher melting point for **6a** (Table I). These differences could result from different experimental procedures or could indicate that the Boc-Sta **6a** reported by Steulmann and Klostermeyer contains only 77% of the (3*S*,4*S*) diastereomer. We found that a synthetic mixture of **6a** and **6b**, which contained 77% of the (3*S*,4*S*) diastereomer by GC, gave a rotation of −28° and melted over the temperature range 97–102 °C. These results suggest that their fractional crystallization procedure may not provide an optically pure sample of **6a**.

Experimental Section

Melting points were determined on a Fisher-Johns melting point apparatus and are corrected. The ^{1}H NMR spectra were recorded on a Bruker HX-90E-pulse Fourier transform NMR spectrometer interfaced with a Nicolet 1080 computer and disk unit. Optical rotations were measured at the sodium D line using a Perkin-Elmer 241 polarimeter. Mass spectra were determined on a Finnigan Model 1015 mass spectrometer. Microanalyses were performed by Galbraith Laboratory, Knoxville, Tenn.

Gas chromatography was carried out on a Nuclear Chicago Selectra System 5000 gas chromatograph using glass columns (4 ft × 5 mm) packed with 1% OV-225 on gas Chromosorb Q (110–120 mesh) at 165 °C. The injection temperature was 255 °C and the flow rate was 35 mL/min.

Thin-layer chromatography (TLC) was performed on silica gel G plates using 20% ethyl acetate in benzene as eluant [R_f (1)].

Preparative HPLC was carried out using a Lobar Lichroprep Si 60 column, obtained from E. Merck, Darmstadt, Germany, eluting with 20% ethyl acetate in hexane or benzene.

Boc-L-leucinal (4). To a stirred solution of Boc-L-leucine methyl ester **3** (4.0 g, 16.3 mmol) in dry toluene (70 mL) was added a hexane solution of diisobutylaluminum hydride (40.8 mmol) at −78 °C under a nitrogen atmosphere. After 6 min, the reaction was quenched with methanol (4 mL)[9] and Rochelle salt solution[10] was added immediately. The mixture was allowed to warm to 25 °C and ether (100 mL) was added. The etheral layer was separated and combined with ether extracts of the aqueous layer. The combined layers were dried ($MgSO_4$) and concentrated under reduced pressure.

The crude product (oil) was passed through a short pad of silica gel, eluting with 4% ethyl acetate in benzene to remove the alcohol side product. The weight of Boc-leucinal obtained was about 2.98 g (85%): R_f(1) = 0.47; ^{1}H NMR ($CDCl_3$) δ 9.57 (s, 1 H), 5.28 (1 H), 4.15 (1 H), 1.15–2.0 (m, 12 H, with singlet at δ 1.47), 0.96 (d, 6 H, J = 6 Hz). This product was used without further purification.[11]

(3*RS*,4*S*)-*N*-Boc-4-amino-3-hydroxy-6-methylheptanoic Acid Ethyl Ester (5). To 5 mL of dry tetrahydrofuran cooled in dry ice–CCl_4 was added diisopropylamine (15 mmol) under a nitrogen atmosphere, followed by a solution of n-butyllithium in hexane (15 mmol). After 1 h the bath temperature was lowered to −78 °C and dry ethyl acetate (15 mmol) was added via syringe and stirred for 15 min. Boc-leucinal **4** (2.15 g, 10 mmol) in 10 mL of tetrahydrofuran was added and the reaction mixture was stirred for 5 min before 1 N HCl was added. The flask was warmed to room temperature and the reaction mixture acidified with cold 1 N HCl to pH 2–3, then extracted with ethyl acetate three times. The organic layer was washed with saturated NaCl and dried ($MgSO_4$). Evaporation under reduced pressure gave an oil which after silica gel column chromatography gave 2.42 g of Boc-Sta-OEt (80%) as a mixture of diasteromers (**5a,b**).

Chromatography of mixture **5a,b** on silica gel eluting with a gradient of 10% ethyl acetate in benzene to 50% ethyl acetate in benzene separated **5a** [R_f(1), 0.21] from **5b** [R_f(1), 0.17].

(3*S*,4*S*)-*N*-Boc-4-amino-3-hydroxy-6-methylheptanoic acid ethyl ester (5a) was isolated in 38–40% yield as an oil: GC retention time, 11.3 min; mass spectrum m/e, 303 (0.5), 230 (8.3), 202 (6.1), 187 (14), 186 (32), 158 (10), 140 (5.7), 131 (14.9), 130 (84), 129 (6.2), 117 (13), 86 (100), and 57 (95). See Table I for other physical constants.

(3*R*,4*S*)-*N*-Boc-4-amino-3-hydroxy-6-methyl heptanoic acid ethyl ester (5b): GC retention time, 13.5 min; mass spectrum m/e, 303 (0.3), 230 (8.3), 202 (4.1), 187 (14), 186 (32), 158 (10), 140 (5.7), 131 (15), 130 (83), 129 (4.2), 86 (100), 57 (95). See Table I for other physical constants.

(3*S*,4*S*)-*N*-Boc-4-amino-3-hydroxy-6-methylheptanoic Acid (6a). A solution of ester **5a** (548 mg, 1.8 mmol) in aqueous dioxane was maintained at pH 10 for 30 min. The solution was acidified (pH 2.5) with cold 1 N hydrochloric acid and the aqueous layer washed with ethyl acetate. The organic layer was dried ($MgSO_4$) and evaporated to give 428 mg (86%) of acid **6a**. See Table I for physical constants. Anal. Calcd for $C_{13}H_{25}NO_5$: C, 56.70; H, 9.08; N, 5.09. Found: C, 56.66; H, 9.32; N, 5.05.

(3*R*,4*S*)-*N*-Boc-4-amino-3-hydroxy-6-methyl Heptanoic Acid (6b). This compound was prepared from ester **5b** using the procedure for **6a** and was isolated in 90% yield. See Table I for physical constants. Anal. Calcd for $C_{13}H_{25}NO_5$: C, 56.70; H, 9.08; N, 5.09. Found: C, 56.68, H, 9.28; N, 5.11.

Acknowledgments. This work was supported by a grant from the Graduate School of the University of Wisconsin—Madison.

Registry No.—3, 63096-02-6; 4, 58521-45-2.

References and Notes

(1) H. Umezawa, T. Aoyagi, H. Morishima, M. Matzusaki, H. Hamada, and T. Takeuchi, *J. Antibiot.*, **23**, 259 (1970).
(2) T. Aoyagi and H. Umezawa, *Cold Spring Harbor Conf. Cell Proliferation*, **2**, 429 (1975).
(3) D. H. Rich, E. Sun, and J. Singh, *Biochem. Biophys. Res. Commun.*, **74**, 762 (1977).
(4) D. H. Rich and E. Sun, "Peptides: Proceedings of the Fifth American Peptide Symposium, San Diego", J. Meienhofer and M. Goodman, Eds., Halsted Press, New York, N.Y., 1977, pp 209–212.
(5) H. Morishima, T. Takita, and H. Umezawa, *J. Antibiot.*, **26**, 115 (1973).
(6) M. Kinoshita, A. Hagiwara, and S. Aburaki, *Bull. Chem. Soc. Jpn.*, **48**, 570 (1975).
(7) R. Steulmann and H. Klostermeyer, *Justus Liebigs Ann. Chem.*, 2245 (1975).
(8) W. S. Liu and G. I. Glover, *J. Org. Chem.*, **43**, 754 (1978).
(9) When water was used to quench the reaction the yield of aldehyde was only 55%.
(10) L. F. Fieser and M. Fieser, "Reagents for Organic Synthesis", Vol. 1, Wiley, New York, N.Y., 1967, p 983.
(11) Passage through silica gel must be rapid since the Boc-aldehyde **4** racemizes (about 5% per h) on contact with silica gel. See: A. Ito, R. Takahashi, and Y. Baba, *Chem. Pharm. Bull. Jpn.*, **23**, 3081 (1975), for the synthesis of (*Z*)-L-leucinal and its racemization on silica gel.

Communications

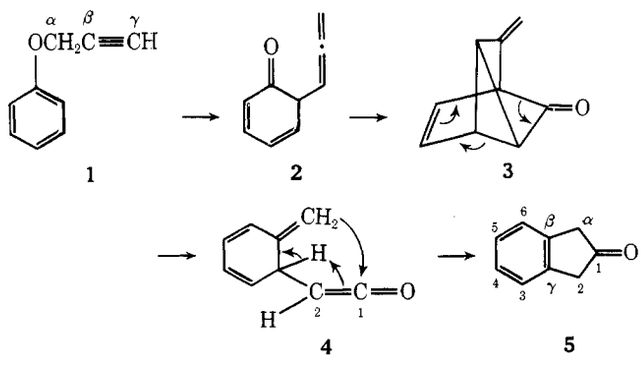
On the Mechanism of Flash Vacuum Pyrolysis of Phenyl Propargyl Ether. Intramolecular Deuterium Kinetic Isotope Effect on Claisen Rearrangement[1]

Summary: We wish to report the first intramolecular deuterium kinetic isotope effect observed in the Claisen type rearrangement of 2-deuteriophenyl propargyl ether (**8**), which is interpreted in terms of a nonsynchronous mechanism.

Sir: It has been reported by Trahanovsky and Mullen[2] that flash vacuum pyrolysis (FVP) of phenyl propargyl ether (**1**) gives rise to benzocyclobutene and 2-indanone (**5**). Based on their mechanistic studies, they proposed[2] the mechanism shown in Scheme I for the formation of **5**. Kinetic studies on the thermal rearrangement of **1** indicated that the step **1** to **2** is rate determining.[3] Furthermore, rearrangement of **1** to **2** has been classified as a [3,3] sigmatropic process.[3] Very recently, Dewar[4] has presented results of MINDO calculations on some pericyclic reactions and has concluded that two-bond ractions are never synchronous, with the exception of a number of ene reactions, but are two-stage or two-step processes which involve unsymmetrical transition states. Furthermore, it has been indicated that the Cope rearrangement of 1,5-hexadienes, a [3,3] sigmatropic process according to Woodward–Hoffmann rules,[5] is not a pericyclic reaction but follows a different mechanism which involves reaction intermediates.[4]

The study of secondary deuterium kinetic isotope effects provides a useful method to estimate the degree of force constant changes at the isotopic position between the ground and transition states,[6] and consequently is a powerful tool in determining the degree of bond cleavage–bond formation that occurs at the transition states of two-bond reactions.

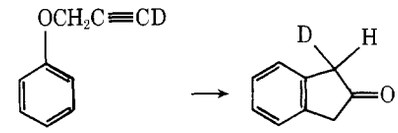
Scheme I



1 2 3 4 5

It is the purpose of this work to further test the mechanism outlined in Scheme I, and to determine the relative timing of bond cleavage–bond formation at the transition state for the rearrangement of **1** to **2**.

The mechanism suggested for the FVP of **1** implies, among other things, transfer of the acetylenic hydrogen (γ hydrogen) of the reactant, during the rearrangement, to a position which ends up as nonaromatic in the product 2-indanone (**5**, Scheme I). Therefore, by substituting the acetylenic hydrogen in **1** by deuterium, subsequent FVP of the resulting deuterated ether should produce an indanone with all deuterium bonded to the nonaromatic position. Thus, phenyl γ-deuteriopropargyl ether (**6**) was synthesized by five successive exchanges of the γ hydrogen in **1**[7] using D_2O/NaOD in dried diethyl ether. The NMR analysis of **6** revealed that 87% of deuterium is incorporated in the desired position. The FVP[7] of **6** was carried out



6 7

at 460 °C and 0.02 Torr. The NMR spectrum of the 2-indanone (**5**) in CCl_4 displays peaks at δ 7.15 (singlet, aromatic H's) and 3.25 (singlet, nonaromatic H's) and with an integration ratio of 1.00:1.00. The NMR spectrum of the deuterated 2-indanone derived from **6** gave an aromatic protons/nonaromatic protons ratio of 1.30:1.00, consistent with the prediction and the structure given by **7**. Thus, our observation further substantiates the mechanism proposed[2] for the FVP of **1** (Scheme I).

The problem of gaining an insight into the structure of the transition state for the rearrangement of **1** to **2** could be carried out by examining the magnitude of the intramolecular deuterium kinetic isotope effect involved in the FVP of 2-deuteriophenyl propargyl ether (**8**). Considering Scheme II, if bond formation is taking place at the rate-determining step, then due to the rehybridization change (sp^2 to sp^3) of the ortho C–H and C–D bonds an inverse isotope effect would be expected in the FVP of **8**. If on the other hand, bond formation is occurring in a subsequent fast step, then k_H would be equal to k_D. The FVP of **8** should give rise to dienones **9** and **10**, which subsequently lead to products **11** and **7**, respectively. The proportion of **9** and **10** would depend on the magnitude of the k_H/k_D involved, and would be reflected in the ratio of **11** to **7** as determined by NMR analysis. Synthesis of **8** was accomplished from the reaction of 2-deuteriophenol[9] with

0022-3263/78/1943-3626$01.00/0