The Conant-Swan Fragmentation Reaction: Stereochemistry of Phosphate Ester Formation

Kim C. Calvo†

Contribution from the James Bryant Conant Laboratories, Department of Chemistry, Harvard University, Cambridge, Massachusetts 02138. *Received December 26, 1984*

Abstract: The fragmentation of β-halophosphonic acids was proposed to occur with formation of monomeric metaphosphate. In direct contrast to the hydrolysis of phosphoric acid esters, this fragmentation reaction occurs without nucleophilic assistance. Several recent studies on the stereochemistry of phosphate ester solvolysis provide unambiguous proof that metaphosphate is not a free intermediate, even though earlier kinetic data indicated a minimal amount of bond formation between the phosphorus and the leaving group in the transition state. Since the degree of stereochemical integrity in phosphorus substitution reactions provides a method of "clocking" the lifetime of an intermediate metaphosphate, the stereochemistry of ester formation during the Conant-Swan fragmentation reaction was determined. The reaction of (1,2-dibromo-2-phenylethyl)-[(*R*)-^{16}O,^{17}O,^{18}O]phosphonic acid with 1-[(1,1-dimethylethyl)dimethylsilyloxy]-(*S*)-butan-3-ol produced an ester in which the phosphorus underwent stereochemical inversion. This result implies that metaphosphate is not a free intermediate under the conditions of this reaction. The Conant-Swan fragmentation reaction necessarily proceeds through a preassociative mechanism in either a concerted or stepwise fashion.

For almost 30 years, chemists have postulated that metaphosphates are intermediates in the hydrolysis of phosphate esters[1] and represent the active phosphorylating agents of intermediary metabolism.[2] The anion[3] and methyl esters[4] of metaphosphate can exist as discrete species in the gas phase, and considerable evidence shows that metaphosphate or something similar can act as a phosphorylating agent in solution.[2]

Kinetic and stereochemical studies designed to evaluate the formation of metaphosphate in protic solutions during phosphate monoester hydrolysis are in apparent conflict. As both Jencks and Knowles have pointed out, the dichotomy centers on the question of whether an intermediate is formed with a sufficient lifetime to escape the solvent cage in which it is formed.[5,6] It appears that the mechanism of solvolysis requires the presence of the nucleophile in the transition state of the reaction, even though Brønsted plots indicate very little bond formation between the phosphorus and the nucleophile.[7] It is not surprising then that methanolysis of 2,4-dinitrophenyl phosphate occurs with inversion of configuration at phosphorus,[6] as predicted by the preassociation mechanism formalized by Jencks.[5] In the case of the second-order transfer of PO_3^- from 3-methoxypyridine or isoquinoline to a series of substituted pyridines, a linear Brønsted plot resulted, consistent with a symmetrical transition state requiring simultaneous weak bonding to the nucleophile and leaving group in a concerted preassociation mechanism.[8,9]

Westheimer and his co-workers have postulated that metaphosphate is formed in the Conant-Swan fragmentation reaction[10-12] (eq 1). The reaction produces an intermediate which

$$C_6H_5CH(Br)CH(Br)PO_3^{2-} \rightarrow C_6H_5CH{=}CHBr + PO_3^- + Br^-$$

$$PO_3^- + ROH \rightarrow ROPO_3H^- \qquad (1)$$

is an extremely reactive electrophile capable of phosphorylating ketone oxygens to generate the corresponding enol phosphates.[11,12a] In direct contrast to the reactions of phosphate esters in protic solution, β-halophosphonic acids decompose unimolecularly without nucleophilic assistance.[12b] However, the absence of kinetic assistance is not an absolute criterion for the existence of metaphosphate as a free intermediate. The reaction can still proceed through a preassociation stepwise mechanism.[5]

Knowles and co-workers have suggested that the determination of the stereochemical course of the reactions at phosphorus provides a clocking mechanism for determining the lifetime of an intermediate metaphosphate.[6,13] Were a planar, trigonal, freely solvated metaphosphate formed, then an initially chiral phosphorus group would undergo racemization. In order to determine whether metaphosphate is formed as a "free" intermediate in the Conant-Swan fragmentation, the stereochemical course at phosphorus has been determined during the capture of metaphosphate by an alcohol.

Experimental Section

General. The reagents used were of the highest purity commercially available. All solvents and liquid reagents were distilled from appropriate drying agents under nitrogen immediately before use. Trioctyl- and tributylamines were passed through a small column of neutral alumina before use. Solid reagents were recrystallized or sublimed (PCl_5 and potassium *tert*-butoxide). Deuterated solvents were obtained from Merck, Sharp & Domme. Enzymes were purchased from Sigma. Microanalyses were performed by Galbraith Laboratories.

^1H NMR spectra were obtained by using a Varian CFT-80 spectrometer and are reference to TMS. ^{31}P NMR spectra were obtained on a Varian XL-100 (40 MHz) or a Brucker WM-300 (121.5 MHz) instrument and are reference to external phosphoric acid. ^{13}C NMR spectra were obtained on the Brucker instrument. Optical rotations were determined by using a Perkin-Elmer 241 polarimeter. An AEI MS-9 was used for mass spectra. Phosphate esters were derivatized with *N,O*-bis(trimethylsilyl)acetamide in pyridine before their mass spectra were determined. The peak ratios were corrected for ^{13}C, ^2H, ^{37}Cl, ^{29}Si, and ^{30}Si where appropriate. Melting points were taken on a Thomas Hoover melting point apparatus and are uncorrected.

Column chromatography was performed by using E. Merck silica gel 60 (63-200 μm) or 40-63 μm for flash chromatography. Ion-exchange chromatography was performed by using Bio-Rad AGl-x8 or Dowex 50-x8 for anion and cation exchange, respectively. Phosphate esters were purified by anion-exchange chromatography by using linear gradients of

† Present address: Department of Chemistry, University of Akron, Akron, OH 44325.

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triethylammonium bicarbonate (TEAB) (pH 7.4). Phosphate esters were qualitatively and quantitatively determined by the procedure of Ames[14] following enzymatic digestion with alkaline phosphatase.

Materials. Dihydro-(1,2-dibromo-2-phenyl-1-ethyl)phosphonic Acid (I). Dihydro-[(*E*)-2-phenylethenyl]phosphonic acid was synthesized by the procedure of Kenyon:[10a] mp 157–159 °C (lit.[10a] 157–159 °C); ^{31}P NMR (CD_3OD/CH_3OH, 5/1, v/v)δ 16.10 (s) (dd, J_1 = 17.7, J_2 = 21.7 Hz); ^{1}H NMR (acetone-d_6) δ 7.81–7.31 (m, 6 H), 6.66 (app. t, J = 17.4 Hz, 1 H). The unsaturated phosphonic acid (1.2 g) was dissolved in $CHCl_3$, and 1.1 equiv of bromine was added. A portion of the solvent was removed and the solution cooled to 0 °C. White crystals formed which were isolated by filtration (1.9 g): mp 141–142 °C; ^{31}P NMR (CD_3OD/CH_3OH) δ 11.82 (s), ^{1}H coupled ^{31}P NMR (dd, J_1 = 10.8, J_2 = 6.0 Hz); ^{1}H NMR (acetone-d_6) δ 7.67–7.27 (m, 5 H), 5.59 (dd, J_1 = 6.3, J_2 = 8 Hz, 1 H), 4.78 (dd, J_1 = 11.3, J_2 = 8.0 Hz, 1 H). Anal. ($C_8H_9Br_2O_3P{\cdot}H_2O$) C,H,P.

(2*R*,4*S*,5*R*)- and (2*S*,4*S*,5*R*)-3,4-Dimethyl-5-phenyl-2-[(*E*)-2-phenylethenyl]-1,3,2-oxazaphospholidin-2-one (III). Styrene (14 mmol) was dissolved in 40 mL of benzene, and 11 mmol of PCl_5 was added. The creamy yellow suspension was stirred for 2 h at room temperature. Water (11 mmol) was added in 10 portions to the stirred suspension over a period of 1.5 h. The clear solution was frozen and the solvent lyophilized. The remaining oil was distilled. The product, [(*E*)-2-phenylethenyl]phosphonic dichloride (II) crystallized on standing (1.14 g, 45%): bp 100–103 °C/0.05 mmHg (lit.[15] 107–110/0.2 mmHg); ^{31}P NMR ($CDCl_3$) 32.92 (s). A solution of 0.78 g of *l*-ephedrine and 0.91 g of triethylamine in 10 mL of benzene was slowly added to a benzene solution of the phosphonic dichloride (1 g in 8 mL of benzene). When addition was complete (1 h), the mixture was stirred for a further 30 min and filtered, and the solvent was removed. The resulting solid was redissolved in 5 mL of $CHCl_3$ and subjected to flash chromatography, in three portions (eluant, ethyl acetate). The fractions from the chromatographic separation were analyzed by TLC. Two compounds were eluted: (a) R_f = 0.4 (0.56 g, 40%) and (b) R_f = 0.23 (0.57 g, 41%). IIIa: ^{31}P NMR ($CDCl_3$) δ 29.72 (s); mp 144.5–146 °C; ^{1}H NMR ($CDCl_3$) δ 7.83–7.26 (m, 11 H), 6.32 (dd, J_1 = 17.2, J_2 = 19.3 Hz, 1 H), 5.53 (dd, J_1 = 6.1, J_2 = 4.3 Hz, 1 H), 3.68 (m, 1 H), 2.68 (d, J = 10.2 Hz, 3 H), 0.88 (d, J = 6.6 Hz, 3 H). Anal. ($C_{18}H_{20}NO_2P$) C,H,N,P. IIIb: ^{31}P NMR ($CDCl_3$) δ 27.18 (s); ^{1}H NMR ($CDCl_3$) δ 7.95–7.12 (m, 11 H), 6.17 (dd, J_1 = 17.2, J_2 = 19.6 Hz, 1 H), 5.82 (d, J = 5.9 Hz, 1 H), 3.78 (m, 1 H), 2.77 (d, J_1 = 9.4 Hz, 3 H), 0.79 (d, J = 6.6 Hz, 3 H). Anal. ($C_{18}H_{20}NO_2P$) C,H,N,P.

(3*S*)-1-[(1,1-Dimethylethyl)dimethylsilyloxy]-3-butanol (IV). To 4 g of (*S*)-1,3-butanediol (Aldrich, lot No. JK 3027 EK, $[\alpha]^{22}_D$ 25.0° (*c* 1, ETOH) was added 6.64 g of imidazole in 10 mL of dry DMF. The solution was cooled to 0 °C and 6.69 g of *tert*-butyldimethylsilyl chloride was added in four portions at 10-min intervals. The reaction mixture was stirred for an additional 1 h at 0 °C and then 12 h at room temperature. Ether was added (20 mL) and the organic layer extracted with H_2O. The ether layer was dried over Na_2SO_4, and the solvent was removed. The product was fractionally distilled twice from CaH_2: bp 44 °C/0.06 mmHg; yield 7.56 g (85%); ^{1}H NMR ($CDCl_3$) δ 4.15–3.70 (m, 3 H), 3.19 (s, 1 H), 1.65 (q, J = 6 Hz, 2 H), 1.19 (d, J = 6.2 Hz, 3 H), 0.90 (s, 9 H), 0.07 (s, 6 H); ^{13}C NMR ($CDCl_3$) δ 68.00, 62.57, 40.09, 25.08, 23.33, 18.08, −5.58. (Compare to ref 16).

[(*E*)-2-Phenylethenyl]-[^{18}O]phosphonic Dichloride (II-^{18}O). PCl_5 was sublimed into a tared, dry 50-mL flask (16.35 mmol), and 15 mL of freshly distilled benzene was added. Styrene (8.175 mmol) was added via syringe, and the creamy yellow suspension was stirred for 2.5 h. $H_2{}^{18}O$ (Biorad, 95% ^{18}O) was added in portions (12 × 25 μL). After the final addition, the clear solution was stirred for 20 min, the mixture was frozen, and the solvent and $P^{18}OCl_3$ were removed in vacuo. The material remaining was vacuum-distilled bulb to bulb, yield 1.75 g (48% based on $H_2{}^{18}O$): ^{1}H NMR ($CDCl_3$) δ 7.69 (dd, J_1 = 17.1, J_2 = 30.3 Hz, 1 H), 7.59–7.26 (m, 5 H), 6.67 (dd, J_1 = 34.4, J_2 = 17.1 Hz, 1 H); mass spectrum, m/z (M^+) (corrected for ^{37}Cl) 222 (92.8%), 220 (7.2%).

(2*S*,4*S*,5*R*)- and (2*R*,4*S*,5*R*)-3,4-Dimethyl-5-phenyl-2-[(*E*)-2-phenylethenyl]-1,3,2-oxazaphospholidin-2-[^{18}O]one (IIIa-^{18}O,IIIb-^{18}O). II-^{18}O (0.875 g) was dissolved in 10 mL of benzene. To 29.4 mL of benzene was added 3.90 mmol *l*-ephedrine and 7.82 mmol of triethylamine. The ephedrine solution was slowly added to the phosphonic dichloride solution over 1.5 h at room temperature. After 2 h of stirring, the solution was filtered and the amine hydrochloride was washed with 5 mL of benzene. The combined filtrates were frozen, and the benzene was removed by lyophilization, leaving a fluffy white powder. This was dissolved in $CHCl_3$ and subjected to flash chromatography (ethyl acetate/hexane, 9/1, v/v) in three portions. The two diastereomers were completely separated. (IIIa-^{18}O): ^{1}H NMR ($CDCl_3$) δ 7.60 (dd, J_1 = 19.5, J_2 = 17.2 Hz, 1 H), 7.57–7.23 (m, 10 H), 6.32 (dd, J_1 = 19.3, J_2 = 17.2 Hz, 1 H), 5.53 (dd, J_1 = 6.1, J_2 = 4.3 Hz, 1 H), 3.68 (m, 1 H), 2.70 (d, J = 10.1 Hz, 3 H), 0.88 (d, J = 6.6 Hz, 3 H); yield 0.410 g. (IIIb-^{18}O): ^{1}H NMR ($CDCl_3$) δ 7.68 (dd, J_1 = 22.8, J_2 = 17.2 Hz, 1 H), 7.63–7.23 (m, 10 H), 6.16 (dd, J_1 = 19.6, J_2 = 17.1 Hz, 1 H), 5.82 (d, J = 6.0 Hz, 1 H), 3.78 (m, 1 H), 2.77 (d, J = 9.4 Hz, 3 H), 0.78 (d, J = 6.6 Hz, 3 H); ^{31}P NMR ($CDCl_3$) δ 33.006, 32.960 (^{18}O shift, ratio 1/10); yield 0.421 g; mass spectrum, m/z (M^+) 313 (10.0%), 315 (90.0%).

Dihydro-(1,2-dibromo-2-phenyl-1-ethyl)[(*R*)-^{16}O,^{17}O,^{18}O]phosphonic Acid (I-^{13}O,^{18}O). IIIb-^{18}O (281 mg) was dried at 0.01 mm for 12 h. Dioxane (10 mL) containing 1.5 mL of $H_2{}^{17}O$ (Monsanto Research Corp., ^{16}O = 28.4%, ^{17}O = 41.8%, ^{18}O = 29.8%) was added followed immediately by 63 μL of trifluoroacetic anhydride. After 15 min, the solution was frozen, and the solvent was removed by lyophilization. Dioxane (1 mL) was added and the mixture freeze-dried again. The remaining white powder was dissolved in 6 mL of $CHCl_3$, and 0.71 g of bromine was added. After 4 days at room temperature, the solvent and excess bromine were removed by rotary evaporation. Benzene (5 mL) was added and lyophilized. The remaining organish oil was transferred as a chloroform solution to a dry, amber bottle. Bromotrimethylsilane (4 mL) was added and the bottle sealed with a Teflon-lined cap. After 4 days at 35 °C, the solvent was blown off under a stream of nitrogen and ether was added. The ether solution was filtered, and the solvent was removed from the filtrate. Methanol was added to the remaining brown oil and then evaporated. Benzene (5 mL) was added and lyophilized. The pale brown oil was dissolved in 2 mL of $CHCl_3$, and 0.5 mL of hexane was added. The white precipitate which formed was collected by centrifugation: ^{1}H NMR (acetone-d_6) δ 7.66–7.28 (m, 5 H), 5.62 (dd, J_1 = 7.9, J_2 = 6.4 Hz, 1 H), 4.79 (dd, J_1 = 11.3, J_2 = 7.8 Hz, 1 H); ^{31}P NMR (acetone-d_6) δ 15.88 (s); yield, 0.103 g (34% based on IIIb-^{18}O).

Methods. Transfer of Phosphorus from I-^{17}O,^{18}O to (3*S*)-1-[(1,1-Dimethylethyl)dimethylsilyloxy]-3-butanol. The I-^{17}O,^{18}O from above was dissolved in 5 mL of $CHCl_3$. Freshly distilled IV was added (2 mL) followed immediately by 2,2,6,6-tetramethylpiperidine (1 mL). After 5 minutes, the solvent and excess IV were removed by evaporation at 20 mmHg and then at 0.01 mmHg. The remaining material was partitioned between water (5 mL) and ether (20 mL). The aqueous layer (^{31}P NMR (D_2O) δ −1.18 (d, J = 8.2 Hz)) was adjusted to 10 mL with water, and 2 mL of a suspension of Dowex-50 (H^+) was added. After 30 min, the solution was filtered and the resin was washed with 20 mL of H_2O. The pH of the filtrate was then adjusted to 8 by using triethylamine. The monoester was purified on an AG1 column (25 mL) by using a linear gradient of TEAB (0.025–0.25 M): ^{31}P NMR (D_2O) δ 0.07 (d, J = 8.3 Hz). The yield was 10 μmol. A portion of the bis(triethylammonium)(1-hydroxybutyl)[^{16}O,^{17}O,^{18}O]phosphate was removed and derivatized for MS analysis: mass spectrum, m/z (M^+ − 15) 371 (3.2%), 372 (5.2%), 373 (28.6%), 374 (36.8%), 375 (26.2%).

^{31}P NMR Analysis of the Configuration at Phosphorus. The monoester was cyclized and purified by the procedure described by Begley et al.[17] for a related system: ^{31}P NMR (D_2O) δ −2.0498, −2.0802, 2.1093 (^{18}O shift, ratio 1.8:3.9:1); ^{1}H coupled ^{31}P NMR (D_2O) δ − 2.07 (d, J = 19 Hz). The cyclic diester was methylated by the procedure of Begley et al.,[17] and the configuration at phosphorus was determined by a modification of the procedure used by Buchwald et al.[6,18]

Results

Synthesis of I-^{17}O,^{18}O. The synthesis of the chiral phosphonic acid (Scheme I) parallels that developed for chiral phosphate esters.[19b] The synthesis is convenient in the sense that the oxygen isotopes can be incorporated directly from isotopically labeled water. Unlike the reaction of $POCl_3$ with *l*-ephedrine, the phosphonic dichloride reacted to form an approximate equal molar mixture (55:45) of the diastereomeric oxazaphospholadinones. ^{31}P NMR investigation of the reaction indicated that this was the thermodynamic mixture, as no change in the ratio of the diastereomers occurred over 48 h at room temperature. Complete chromatographic separation of the diastereomers was achieved based on TCL and NMR analysis. A slow exchange of the

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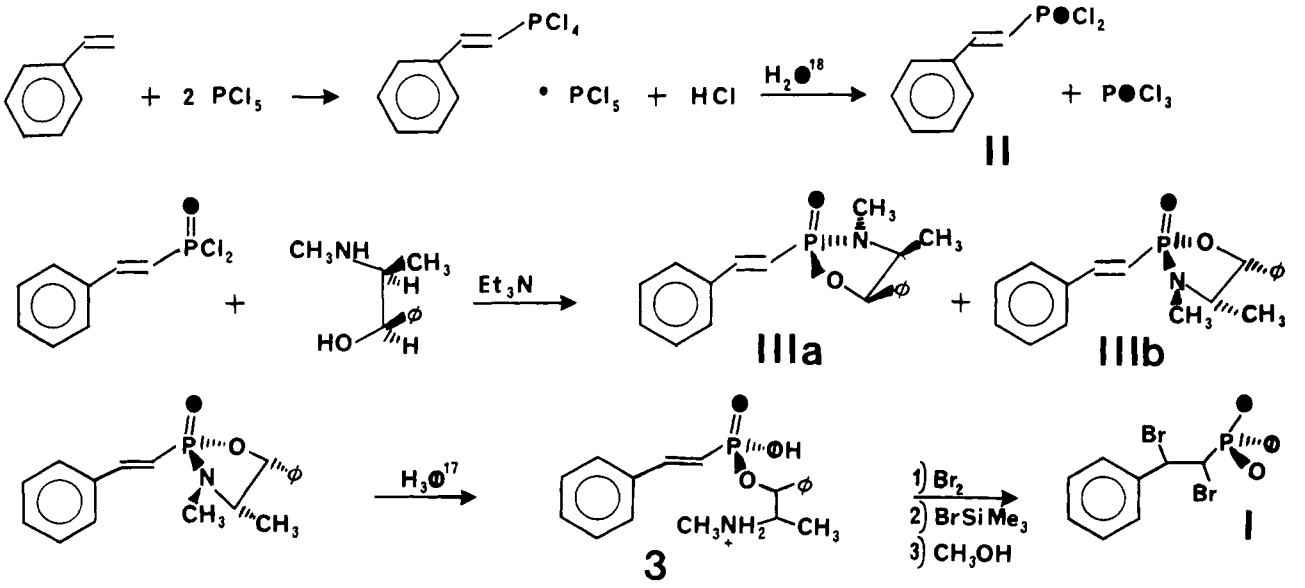
Scheme I. Synthesis of 1,2-Dibromo-2-phenyl-1-ethyl-[(*R*)-^{16}O,^{17}O,^{18}O]phosphonic Acid



phosphoryl oxygen was noted during chromatography. The resulting loss of the ^{18}O label was minimized by the use of flash chromatography.

The acid-catalyzed ring opening to form **3** (Scheme I) has been shown to proceed with inversion of configuration at phosphorus in a number of systems,[19] including phosphonic acid derivatives. Acid-catalyzed methanolysis of IIIa or IIIb proceeds with the formation of distinguishable diastereomers,[20] which, when considered in light of the hydrolysis data, is most easily interpreted as an inversion. The assignment of configuration to IIIa and IIIb is made on the basis of ^{1}H NMR shifts. There is considerable literature data on the conformations of 1,3,2-oxazaphospholidinones.[19,21] Based on stereochemical studies, Inch and his coworkers[19a] conclude that, when the phosphoryl oxygen is in a 1,3-cis relation to a proton in this heterocyclic system, the proton is considerably deshielded. When both diastereomers can be prepared and separated from each other, the ^{1}H NMR resonances of H-5 and H-4 can be used to assign the configuration at phosphorus. Using this criterion, the H-4 resonances in IIIa and IIIb are at δ 3.67 and 3.78 respectively, and the H-5 resonances are at δ 5.53 and 5.82, respectively. This indicates that the P=O is cis to the H-5 and H-4 protons in IIIb and trans in IIIa. On the basis of this analysis, the configuration of IIIb is that of the 2*R* compound. Since the acid-catalyzed ring opening reaction proceeds with inversion, the configuration at phosphorus in **3** must be *R*. Buchwald and Knowles[13] used bromotrimethylsilane to cleave the C–O bond of a phosphate diester in the synthesis of 2,4-dinitrophenyl [(*R*)-^{16}O,^{17}O,^{18}O]phosphate. The stereochemical analysis they performed confirmed the C–O cleavage mechanism. Since C–O and Si–O cleavage and bromination does not involve reaction at phosphorus, the configuration at phosphorus in I-^{17}O,^{18}O must be R_P.

Conant–Swan Fragmentation Reaction of I and I-^{17}O,^{18}O. To determine whether the dianion of I undergoes Conant–Swan fragmentation, I was decomposed in an acetophenone solution to yield the corresponding enol phosphate.[11b,12a] The formation of this product is consistent with a free metaphosphate as an intermediate in the reaction.[2] To determine the stereochemistry of the reaction of this metaphosphate, I-^{17}O,^{18}O was decomposed in a chloroform solution containing 1-*t*-BDMS-3-(*S*)-butanol as phosphoryl group acceptor (Scheme II). After acid-catalyzed removal of the protecting group and purification of the monoester, the configuration at phosphorus was determined by the ^{31}P NMR method developed in Knowles' group[18] (Figure 1). Based upon the known isotopic composition of I-^{17}O,^{18}O and the known enantiomeric excess of the (*S*)-1,3-butanediol,[22] the relative peak areas in the final ^{31}P NMR spectrum can be calculated (Table I) for the various possible stereochemical outcomes. The NMR peak areas were determined by cutting the peaks out and weighing

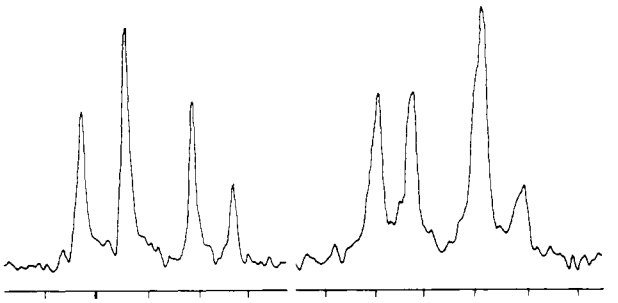


Figure 1. ^{31}P NMR spectrum of the products from the "in-line" ring closure and methylation[6] of 3-[^{16}O,^{17}O,^{18}O]phospho-(*S*)-butane-1,3-diol obtained from the reaction of I-^{17}O,^{18}O and 1-(*t*-BDMS)-(*S*)-butane-1,3-diol in chloroform containing 2,2,6,6-tetramethylpiperidine. The spectrum was obtained on a Brucker WM-300 instrument at 121.5 MHz with a deuterium lock and broad-band decoupling: spectral width 500 Hz, acquisition time 16.4 s, pulse width 23.5 μs, number of transients 2716, Gaussian multiplication (Gaussian broadening 0.045 Hz; line broadening −0.28 Hz), and Fourier transfer in 16K. The chemical shifts are −4.8745, −4.8905, −4.917, and −4.9337 for the equatorial triester and −5.8816, −5.8962, −5.9232, and −5.9382 for the axial triester. The scale used is 0.02 ppm per division.

Table I. Peak Integrations for the ^{31}P NMR Spectrum of Figure 1

	peak no.[a]							
predicted for	1	2	3	4	5	6	7	8
inversion[b]	22.0	38.7	27.4	11.8	22.0	27.4	38.7	11.8
retention[b]	22.0	27.4	38.7	11.8	22.0	38.7	27.4	11.8
racemization[b]	22.0	33.0	33.0	11.8	22.0	33.0	33.0	11.8
obsd[c]	26.1	37.8	24.0	12.1	22.3	25.1	41.8	10.8

[a] Reading from low field up. [b] Based upon the known isotopic composition of the (*S*)-3-[^{16}O,^{17}O,^{18}O]phosphobutane-1,3-diol and the known enantiomeric excess of the (*S*)-1,3-butanediol. [c] Integration by cutting out peaks and weighing.

(20) Methanol/HCl reacts with IIIa to give a compound with a ^{1}H NMR resonance at δ 3.84 corresponding to the P–O–CH_3. The same reaction with IIIB gives a P–OCH_3 ^{1}H NMR resonance at δ 3.63.

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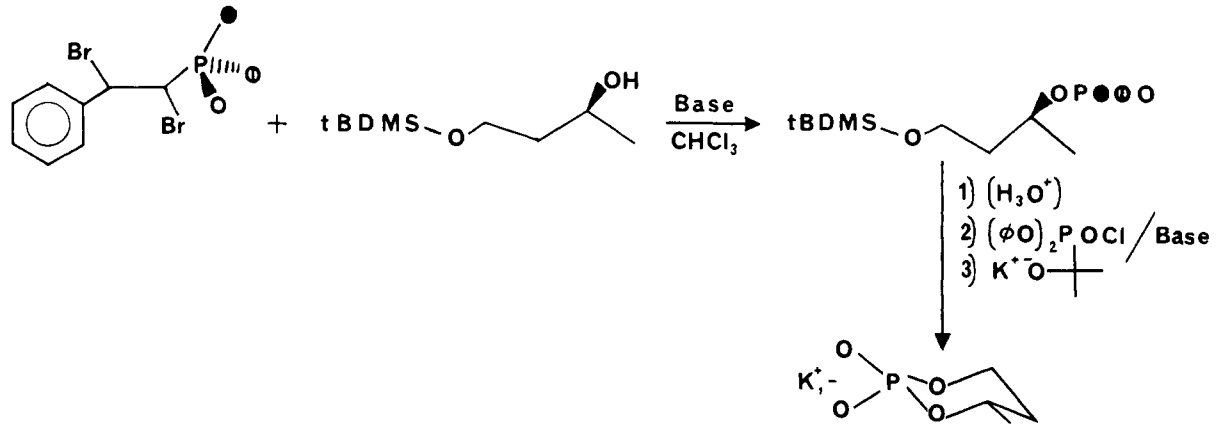
(22) The Aldrich material has $[\alpha]^{22}_D$ 25.0 (*c* 1, EtOH). Murakami[23] reports $[\alpha]^{22}_D$ 29.6 (*c* 1, EtOH). This leads to an enantiomeric excess of the (*S*)-1,3-butanediol used here of 84%.

Scheme II. Reaction for the Capture of Metaphosphate Generated by the Conant-Swan Fragmentation Reaction by a Chiral Secondary Alcohol[a]



[a] The product monoester was cyclized as in ref 17, methylated, and analyzed by ^{31}P NMR. The oxygen isotopes of the cyclic diester and the double bonds and hydrogens of the phosphonic acid have been omitted for clarity.

them in triplicate. The results obtained in this manner are in accord with the integration given by the NMR spectrometer. Clearly, within experimental error (which admittedly is about 10%), the capture of the chiral metaphosphate by alcohol occurs with inversion at phosphorus. This result adds further support[25] to our original assignment of configuration to IIIb which had been made solely on the basis of NMR coupling constants.

Discussion

The question of whether metaphosphate is an intermediate in the hydrolysis of phosphate esters has been addressed by kinetic and stereochemical analyses. In aqueous solutions, phosphate monoester dianions react with nucleophiles with second-order kinetics.[7] However, the reactions demonstrate a very small dependence on the basicity of the nucleophile and a large dependence on the pK_a of the leaving group. For the reaction of 2,4-dinitrophenyl phosphate dianion with amines, the β_{nuc} value is 0.02. For substituted phenyl phosphates, the β_{lg} value is between −1.0 and −1.2 for aminolysis and hydrolysis. The second-order kinetics require the presence of the nucleophile in the rate-limiting step, even though the rate is only marginally affected by the nucleophilicity of the catalyst. Clearly, there is substantial bond cleavage to the leaving group and minimal bond formation to the nucleophile in the transition state. This kinetic information, when combined with the fact that the methanolysis of 2,4-dinitrophenyl phosphate dianion occurs with stereochemical inversion[6] at phosphorus, necessarily requires that the solvolysis of phosphate monoester dianions occurs by a preassociative mechanism,[5,6] without the need to postulate a metaphosphate as an intermediate.

For several reasons, the Conant–Swan fragmentation reaction is believed to generate metaphosphate as an intermediate. The experimental observations are the following. (1) The reaction occurs through the dianion of the phosphonic acid, an ionization state which on electrostatic considerations should not favor nucleophilic attack at phosphorus.[11a,24] (2) The intermediate phosphorylating agent is extremely reactive. Enol phosphates can be produced when the fragmentation reaction is carried out in ketone solutions. The reaction presumably occurs through a zwitterionic phosphorylated ketone oxygen.[11b] This is in direct contrast to the reaction of the dianion of 2,4-dinitrophenyl phosphate in ketone solutions where the only product formed is trimetaphosphate.[12a] (3) Kinetically, the fragmentation reaction is first order with no nucleophilic enhancement of the rate of alkene formation.[24] Specifically, the rate constant for fragmentation of (2-bromo-1,3-diphenyl-3-oxo-1-propyl)phosphonic acid (V) in acetone containing 0.1 M ethanol was the same as the rate constant for fragmentation of V in methanol (provided that the rate constant in acetone is corrected for the fraction of the dianion of V present). In methanol as solvent, the addition of triethylenediamine to a concentration of 1.0 M did not affect the rate of fragmentation of V. (4) The solvent deuterium isotope effect on the fragmentation of V is negligible, indicating the absence of proton transfer in the rate-limiting step.[24] (5) Substituent effects on the decomposition of V are consistent with a fragmentation reaction in which the alkene is formed in the rate-limiting transition state.[24] This indicates that PO_3^-, alkene, and Br^- are formed in a concerted reaction.

The data summarized provide sufficient evidence to postulate the existence of metaphosphate as an intermediate. However, as Westheimer and I point out,[24] if an intermediate must survive a collision with a nucleophile as a necessary condition for being an intermediate, then the data presented above tell us nothing about the "intermediateness" of metaphosphate generated in the Conant–Swan fragmentation. Knowles' criterion, that a metaphosphate must undergo stereochemical racemization if it is a true intermediate[6] leads to the conclusion that metaphosphate cannot exist as a free intermediate in the presence of a primary or secondary alcohol. The Conant–Swan fragmentation reaction in the presence of an alcohol must then occur through a preassociative mechanism. By Jencks' definition of preassociation,[5] the intermediate PO_3^- must either not exist and reaction occurs through an "exploded" S_N2 transition state or PO_3^- must be so reactive that once formed, if it is not immediately captured by the alcohol, reacts with Br^- and alkene to regenerate starting material. The data do not allow a determination of whether the reaction is concerted or stepwise preassociative, that is, whether the reaction proceeds through a transition state in which PO_3^- is simultaneously bonded to both the alcohol and leaving group, or whether the PO_3^- has a short but definite lifetime. Clearly, the data are consistent with almost total P–C bond cleavage in the rate-limiting process.

The choice of nucleophile in the present experiment was made in order to simplify the stereochemical analysis. It is possible that in chloroform solution, specific solvation of the phosphonic acid dianion by the alcohol hydroxyl may have predisposed the reaction toward a preassociation mechanism. However, the nucleophilicity of the solvent can be decreased so that the lifetime of the putative metaphosphate is increased. Using stereochemical analysis as a "clock" should determine whether metaphosphate can exist as an intermediate at all.

Acknowledgment. I thank Prof. Westheimer for helpful dis-

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(25) If our original assignment had been incorrect, then the phosphate ester produced from reaction of I-^{17}O,^{18}O and IV would have to have been produced by retention. Although phosphorus is capable of reacting through a trigonal-bipyramidal intermediate, which after pseudorotation results in retention at phosphorus, carbon is notably unsuitable as an axial ligand in such a transformation.[26]

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cussions of this research. Prof. Westheimer is in entire agreement with the use of the stereochemical outcome at phosphorus as a criterion for free metaphosphate and with the conclusion from the experiments reported here, i.e., that the Conant-Swan reaction does not produce free metaphosphate but that the reaction must proceed with preassociation between the incipient metaphosphate and the alcoholic nucleophile. Support for this work was provided to Prof. F. H. Westheimer through NSF Grant 3CHE-7922045.

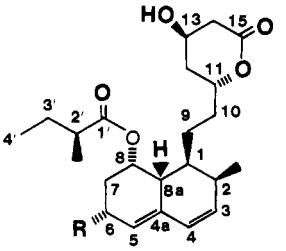
Biosynthesis of the Hypocholesterolemic Agent Mevinolin by *Aspergillus terreus*. Determination of the Origin of Carbon, Hydrogen, and Oxygen Atoms by ^{13}C NMR and Mass Spectrometry

Richard N. Moore, Glen Bigam, Jean K. Chan, Alan M. Hogg, Thomas T. Nakashima, and John C. Vederas*

Contribution from the Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2G2. *Received July 27, 1984*

Abstract: The ^1H and ^{13}C NMR spectra of mevinolin (**1**), an inhibitor of hydroxymethylglutaryl-coenzyme A reductase, were fully assigned by using selective homonuclear and heteronuclear decoupling and two-dimensional DEPT heteronuclear shift correlation. Sodium [1-^{13}C]-, [2-^{13}C]-, [1,2-$^{13}C_2$]-, [1-^{13}C,$^{18}O_2$]-, [1-^{13}C,$^2H_3$]-, and [2-^{13}C,$^2H_3$]acetate as well as $^{18}O_2$ and [*methyl*-^{13}C]methionine were incorporated into mevinolin (**1**) by cultures of *Aspergillus terreus* ATCC 20542. Double quantum coherence (2D INADEQUATE) NMR spectra of **1** derived from sodium [1,2-^{13}C]acetate independently confirmed the ^{13}C NMR assignment and provided the location of intact carbon-carbon bonds from acetate. Mevinolin (**1**) is formed from two polyketide chains (4-carbon and 18-carbon) of acetate units coupled in head to tail fashion, with each chain bearing a methionine-derived methyl group. Material obtained from ^2H and ^{18}O incorporations was analyzed by mass spectrometry and by ^{13}C NMR detection of α and β isotope shifts. The results show that oxygen atoms on the main chain are introduced by aerobic oxidation of a deoxygenated precursor. Several mechanisms, including a biological Diels-Alder reaction, are proposed to account for formation of bicyclic ring systems in mevinolin (**1**), compactin (**2**), and related metabolites.

Mevinolin (**1**)[1,2a] and compactin (**2**)[2b,c] are potent inhibitors of cholesterol biosynthesis in humans and possess potential in treatment of atherosclerosis and coronary heart disease.[3] These



1 R=CH_3

2 R=H

fungal metabolites and their corresponding 4a,5-dihydro derivatives (**3** and **4**)[4] block isoprenoid formation because of competitive inhibition of the key enzyme in the pathway, 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase: EC 1.1.1.34), by the corresponding lactone-opened forms (e.g., **5** and **6**).[5] The resulting decrease in availability of mevalonate caused by these compounds not only lowers mammalian sterol levels[3,5a,6] but also interferes with production of ubiquinone,[7] sea urchin dolichol,[8] insect juvenile hormones,[9] plant sterols and pigments,[10] and fungal gibberellins.[11] The importance of mevinolin (**1**) and compactin (**2**) as biochemical tools[12] and their unusual structures

(1) Mevinolin (**1**) from *Aspergillus terreus* and monacolin K from *Monascus ruber* (Endo, A. *J. Antibiot.* **1979**, *32*, 852-854. Endo, A. *Ibid.* **1980**, *33*, 334-336) appear to be the same compound; compactin (**2**) is also known as ML-236B.

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