of colorless liquid, b.p. 105–106° (3.0 mm.), $n^{24}D$ 1.4556, $\nu_{max}^{pure\ liquid}$ 1775 (lactone carbonyl) and 1720 cm.$^{-1}$ (ketone carbonyl).

Anal. Calcd. for $C_8H_{12}O_3$: C, 61.52; H, 7.75; equiv. wt., 156. Found: C, 61.39; H, 7.96; N, 0.00; equiv. wt., 155.

Mild Acid Hydrolysis of 1,3-Dihydro-3,5,7-trimethyl-2H-azepin-2-one.—A slurry of 3.0 g. (0.020 mole) of 1,3-dihydro-3,5,7-trimethyl-2H-azepin-2-one in 10 ml. of 3 *N* hydrochloric acid was heated on a steam bath for 10 min. with frequent swirling. The solid was converted to an oil. The solution was neutralized by the cautious addition of solid potassium carbonate and the mixture was extracted with methylene chloride. The combined organic layers were dried over magnesium sulfate, filtered, and evaporated to give a colorless oil. The infrared spectrum of this material was superimposable on that of pure 5-acetonyldihydro-3,5-dimethyl-2(3H)-furanone.

[CONTRIBUTION FROM THE DEPARTMENT OF BIOLOGICAL CHEMISTRY, UNIVERSITY OF ILLINOIS, COLLEGE OF MEDICINE, CHICAGO 12, ILL.]

Phosphonic Acid Analogs of Nucleoside Phosphates. I. The Synthesis of 5′-Adenylyl Methylenediphosphonate, a Phosphonic Acid Analog of ATP[1,2]

BY TERRELL C. MYERS, KANAME NAKAMURA, AND JAMES W. FLESHER

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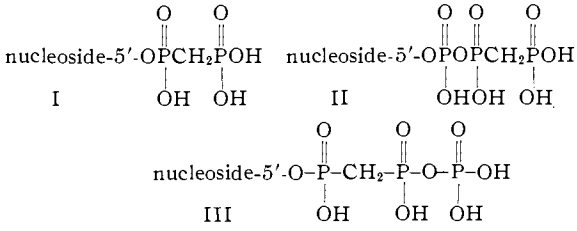
The synthesis of 5′-adenylyl methylenediphosphonate (IV) has been accomplished by the reaction of adenosine 5′-phosphoramidate with methylenediphosphonic acid and by the condensation of AMP with methylenediphosphonic acid in the presence of excess dicyclohexylcarbodiimide.

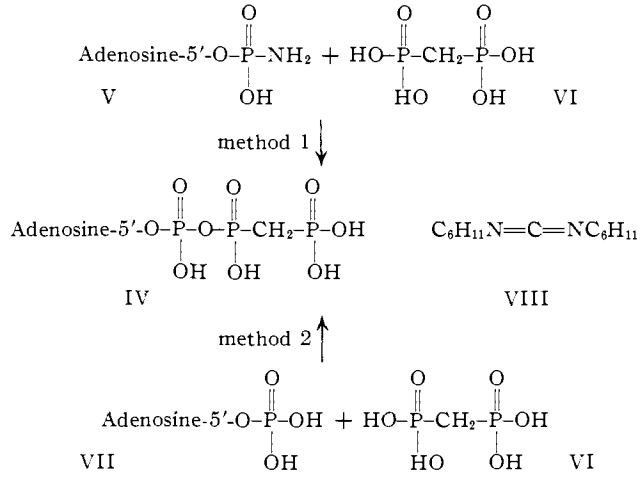
The role of the nucleoside polyphosphates in metabolism is such that studies on the metabolic effects of their analogs may provide information as to their functions. It seems especially important to investigate analogs in which the polyphosphate portion of the molecule has been altered. In particular, it would be of interest to investigate nucleoside polyphosphate analogs which have structures closely related to the polyphosphate groupings, but in which cleavage cannot readily occur, since so many of the metabolic reactions in which the nucleoside polyphosphates participate involve liberation or transfer of ortho- or pyrophosphate groups. Such analogs include compounds in which one or more of the pyrophosphate oxygens are replaced by methylene bridges. Some possible variations are depicted below for analogs of nucleoside 5′-diphosphates (I) and nucleoside 5′-triphosphates (II and III)



Since compounds of types I, II, and III are phosphonic acid derivatives, they would be expected to have physical and chemical properties similar to those of parent polyphosphates except that the C–P bonds of the analogs would be resistant to cleavage.[3] The ease of cleavage of the pyrophosphate moiety of nucleotides is well known.

We have therefore undertaken the synthesis of a series of such phosphonic acid analogs of nucleoside polyphosphates. The present paper describes the synthesis of 5′-adenylyl methylenediphosphonate (abbreviated AMP-PCP)[3] (IV). Assuming that the P–O–P linkage of this analog would be susceptible to enzymic cleavage or group transfer but the P–C–P bonds would not, AMP-PCP might act as: (1) an inhibitor of ATP in processes involving cleavage of the terminal P–O–P bond of ATP; (2) a metabolic substitute for ATP in processes involving cleavage of the P–O–P bonds of the second pyrophosphate oxygen of ATP; (3) a metabolic substitute for ATP in processes involving complexing or binding actions of ATP that are not accompanied by pyrophosphate bond cleavage.

AMP-PCP (IV) was synthesized by two independent methods: (1) the reaction of adenosine 5′-phosphoramidate (V) with methylenediphosphonic acid (VI)



and (2) the reaction of AMP (VII) with methylenediphosphonic acid using dicyclohexylcarbodiimide (VIII) as the condensing agent.

These methods are modifications, in which methylenediphosphonic acid is used in place of orthophosphoric or pyrophosphoric acid, of established syntheses of ATP and ADP.[4]

In method 1 the reaction between the phosphoramidate and the phosphonic acid (three molar excess) was carried out in a solution of *o*-chlorophenol and pyridine. The nucleotide derivatives in the reaction mixture were adsorbed on charcoal at pH 2, freed from excess methylenediphosphonic acid by washing the charcoal with water, and eluted with aqueous ethanolic ammonium hydroxide. AMP-PCP was purified by ion-exchange chromatography and isolated as the tetralithium salt in 22% yield.

(1) This work was supported by a grant from the National Science Foundation (G-2191) and from the National Institutes of Health (CY-2856).

(2) The following abbreviations are used: AMP-PCP, 5′-adenylyl methylenediphosphonate; ATP, adenosine 5′-triphosphate; ADP, adenosine 5′-diphosphate; AMP, adenosine 5′-phosphate; DCC, dicyclohexyl carbodiimide.

(3) G. M. Kosolapoff, "Organophosphorus Compounds," John Wiley and Sons, Inc., New York, N. Y., 1950, Chapter 7.

(4) For procedures analogous to those employed in method 1 see (a) R. W. Chambers and H. G. Khorana, *J. Am. Chem. Soc.*, **80**, 3749 (1958); (b) D. Kessler, B. Mossand, and R. W. Chambers, *Biochem. Biophys. Acta*, **38**, 549 (1960); (c) K. Tanaka, M. Honjo, Y. Sanno, and H. Moriyama, *Chem. Pharm. Bull.* (Tokyo), **10**, 220 (1962); to those employed in method 2 see (d) H. G. Khorana, *J. Am. Chem. Soc.*, **76**, 3517 (1954).

In method 2 the reaction of AMP with methylenediphosphonic acid (four molar excess) in the presence of a large excess of DCC was conducted in aqueous pyridine in a two-phase reaction mixture. The product from this reaction also was purified by adsorption on charcoal followed by ion-exchange chromatography and was isolated as the barium salt (16% yield). As might be expected, P^1,P^2-diadenosine-5′ pyrophosphate appeared as a by-product in this reaction.[4d] The use of tertiary amine salts of AMP and methylenediphosphonic acid as suggested by more recent work of Khorana[5] produced a single-phase reaction mixture but gave rise to inconsistent results with the production of higher condensation products. The details of this work will be presented in a future communication. In the present work, synthesis 2 was carried out primarily to verify the structure of the AMP-PCP obtained from method 1, which is the synthetic method of choice.

Both preparations of AMP-PCP, from method 1 and method 2, gave identical results on paper chromatograms in a number of solvent systems and behaved identically in several structure-determining and characterizing procedures. The assignment of structure IV to the products of both synthetic methods is supported by elemental analysis and by equivalent weight estimations as determined by ultraviolet absorbancy measurements. Electrometric titration of the free acids (as prepared from the salts of both synthetic methods by removal of lithium or of barium with Dowex-50 (H) ion-exchange resin) gave rise to essentially identical titration curves. These curves were similar to those obtained under similar conditions for ATP except in the region of the final acid dissociation, *i.e.*, the region of secondary "phosphoryl" dissociation for ATP and of secondary "phosphonyl" dissociation for AMP-PCP. Here the curve for ATP shows a pK_A' of 7.1 and the curves for the analog a pK_A' of 8.4–8.5.[6] The high pK_A' for the last ionizable hydrogen is characteristic of the phosphonic acid grouping[2] and these results are consistent with the structure proposed for the analog. Moreover the titration curves showed a ratio of strong acid groupings ($pK_A' < 5$):weak acid groupings ($pK_A' > 6$):adenine (as determined from ultraviolet absorbancy measurements on completion of the titration) of 3:1:1 (theory for AMP-PCP 3:1:1). The single acid grouping in the high pK_A' range is offered as evidence that the methylenediphosphonic acid moiety is attached to the 5′-phosphate and not to another grouping such as the 2′- or 3′-hydroxyl. This conclusion is reinforced by experiments in which positive reactions were obtained when the paper chromatograms of both products were treated with periodate–benzidine spray indicating the presence of *vic*-hydroxyl groupings.

The results from a series of hydrolytic degradations of the products from both synthetic methods support the structure assignment. The procedure for "labile phosphorus" (15-min. hydrolysis with 1 *N* HCl at 100°) did not liberate inorganic phosphate, while hydrolysis with 2.5 *N* NaOH for 30 min. at 100° caused degradation to AMP. Since one-third of the total phosphorus of AMP-PCP should be obtainable upon hydrolysis as orthophosphate, a procedure was developed for the determination of the ratio of total phosphorus to that obtainable as orthophosphate upon exhaustive hydrolysis. Aliquots after a 16-hr. hydrolysis with boiling 6 *N* H_2SO_4 (for "phosphate phosphorus") and after Kjeldahl digestion (for "total phosphorus") were analyzed for inorganic phosphate by the Fiske–SubbaRow method. The ratio of total phosphorus to phosphate phosphorus under these conditions was utilized routinely for determination of the degree of purity of individual samples and for characterization of the final product. This ratio for the analytical samples of the lithium and barium salts was 3:1 in accordance with results anticipated on the basis of structure IV.

In studies on actomyosin systems[7] AMP-PCP did not replace ATP in causing contraction of glycerinated muscle, did not produce a drop in viscosity in actomyosin solutions, and was not hydrolyzed by a homogenate of glycerinated muscle. A partial inhibition of myofibrillar adenosine triphosphatase by the analog was observed in the absence of magnesium. Inhibition by AMP-PCP in the polynucleotide phosphorylase system has been demonstrated.[8] The compound was more active than ATP in inducing vasodepressor response in the cat.[9] Other studies with AMP-PCP and related analogs in additional biological systems are currently in progress.

Experimental

Materials.—Methylenediphosphonic acid was obtained by hydrolysis in concentrated hydrochloric acid of its tetraethyl ester which was prepared by the reaction of methylene iodide with excess triethyl phosphite.[10] 1,3-Dicyclohexylguanidinium adenosine 5′-phosphoramidate was purchased from the Sigma Chemical Co., AMP dihydrate from the Pabst Laboratories, and DCC from Aldrich Chemical Co., Inc.

Methods.—Paper chromatography was performed using Whatman No. 1 filter paper. Four solvent systems were employed: (A)[11] isobutyric acid–concd. NH_4OH–water, 66:1:33 (v./v.), pH 3.7; (B)[12] isobutyric acid–1 *N* ammonia–0.1 *M* ethylenediaminetetraacetic acid, disodium salt, 100:60:1:6 (v./v.); (C)[13] 5% disodium hydrogen phosphate–isoamyl alcohol; (D)[11] 0.1 *M* sodium phosphate buffer, pH 6.8 (1 l.)–ammonium sulfate (100 g.)–1-propanol (20 ml). The descending technique was used with solvent system D, the ascending technique was used with the remaining systems. Ultraviolet-absorbing products were detected on the dried papers by means of a short-wave length ultraviolet lamp. Products bearing *vic*-dihydroxy groupings were detected on the chromatograms by use of a periodate–benzidine spray.[14]

Ultraviolet absorption measurements were made with a Zeiss spectrophotometer, model PMQ 11. Fractionation during column chromatography was followed by absorbancy measurements at 260 mμ. The molar concentrations of adenine-containing compounds in a given fraction or solution as well as the "spectral equivalent weights" of the final products were also determined from absorbancy measurements at pH 7 at 260 mμ as described in the Pabst Laboratories Circular OR-17. A molar absorbancy index for AMP-PCP of 15,400 at pH 7 (*i.e.*, that reported for AMP, ADP, and ATP) was assumed in these determinations.[11]

Electrometric titrations were conducted using a Leeds and Northrup pH meter, model 7664. The samples of nucleotide, which had been converted from their lithium or barium salts to the free acids by treatment with Dowex 50(H) cation-exchange resin, were present at about 1 m*M* concentration. The titrations were carried out with 0.1 *M* NaOH[15] in 0.2 *M* tetraethylammonium bromide as background electrolyte. At the end of each titration the exact amount of nucleotide in the mixture was determined by absorbancy measurements at 260 mμ.

(5) M. Smith and H. G. Khorana, *J. Am. Chem. Soc.*, **80**, 1141 (1958).

(6) Part of the rationale for the design of the analog was centered around this anticipated difference between its pK_A' and that of ATP. This property is expected to be of interest in connection with kinetic studies conducted over a range of pH's in the event that AMP-PCP is a substitute for or an inhibitor of ATP in a given enzyme system.

(7) C. Moos, N. R. Alpert, and T. C. Myers, *Arch. Biochem. Biophys.*, **88**, 183 (1960).

(8) L. N. Simon and T. C. Myers, *Biochim. Biophys. Acta*, **51**, 178 (1961).

(9) J. W. Flesher, Y. T. Oester, and T. C. Myers, *Nature*, **185**, 772 (1960).

(10) G. M. Kosolapoff, *J. Am. Chem. Soc.*, **75**, 1500 (1953).

(11) Circular OR-17, Pabst Laboratories, Division of Pabst Brewing Co., Milwaukee, Wisc., 1961.

(12) H. A. Krebs and R. Hems, *Biochem. Biophys. Acta*, **12**, 172 (1953).

(13) W. E. Cohen and C. E. Carter, *J. Am. Chem. Soc.*, **72**, 4273 (1950).

(14) M. Viscontini, D. Hoch, and P. Kaiser, *Helv. Chim. Acta*, **38**, 642 (1955).

(15) The use of sodium hydroxide as a titrant introduces a slight error toward the acid side in pK_a' determinations because of the tendency of the analog to form complexes with the sodium ions. The association constants of AMP-PCP and other related analogs with a number of metal ions of biological interest will be the subject of a future publication.

The results are reported in the preparative experiments in terms of pK_A's of the adenine ammonium grouping and the "secondary phosphonyl" grouping and the ratios of adenine (determined spectrophotometrically): strong acid ($pK_A' < 5$): weak acid ($pK_A' > 6$) (theory for AMP-PCP, adenine:strong acid:weak acid = 1:3:1).

Phosphorus Analysis.—Three types of phosphorus determinations were utilized in the characterization of AMP–PCP: *"labile phosphorus,"* phosphorus released as orthophosphate in 15 min. at 100° by 0.1 *N* HCl; *"phosphate phosphorus,"* phosphorus released as orthophosphate in 16 hr. by refluxing with 6 *N* H_2SO_4; *"total phosphorus"* phosphorus obtainable as orthophosphate after Kjeldahl digestion with concentrated sulfuric acid and hydrogen peroxide for 3 hr. Phosphate was estimated colorimetrically after the respective degradative procedures by the method of Fiske and SubbaRow.[16] The determinations were performed directly on the lithium salts of AMP–PCP. In the case of the barium salts, barium was removed by treatment with Dowex 50(H) cation-exchange resin and analyses were run on aliquots of the resulting solutions. The ratios of phosphate phosphorus:total phosphorus as determined on aliquots were used as an index of the degree of purity of preparations of AMP–PCP (theory for AMP–PCP, phosphate-P:total P = 1:3).

Preparation of 5'-Adenylyl Methylenediphosphonate (AMP-PCP) (Method 1).—1,3-Dicyclohexylguanidinium adenosine 5'-phosphoramidate (2.04 g., 3.6 mmoles) and methylenediphosphonic acid (1.88 g., 10.8 mmoles) were treated with 54 ml. of freshly distilled *o*-chlorophenol. The mixture was cooled in ice, 36 ml. of dry pyridine was added, and the resulting clear solution was allowed to stand at room temperature with occasional shaking. After 48 hr. the reaction mixture (now cloudy) was treated with 300 ml. of water with cooling in an ice-bath and extracted six times with ether (total volume, 850 ml.).

The aqueous solution was adjusted to pH 2 with 1 *N* HCl and then was treated with 30 g. of acid-washed Norit A. After stirring for 30 min. the charcoal was collected on a Celite filter-bed and washed exhaustively with water (total volume, 5 l.) in order to remove excess methylenediphosphonic acid. The nucleotide derivatives were then eluted with 50% aqueous ethanol containing 5% concentrated ammonium hydroxide (total volume of eluent, 3 l.) and the eluate was concentrated to a volume of 400 ml. on a flash evaporator at 35°. The recovery of adenine-containing compounds at this stage, estimated spectrophotometrically, was 79%.

The concentrated eluate was applied to a Dowex-2 (chloride) column (8% cross linking) 31 cm. long × 2.7 cm. diameter. After a water wash (total volume 1 l.), gradient elution was begun using a linear technique similar to that described by Moffatt and Khorana.[17] The mixing vessel contained 2 l. of 0.003 *N* HCl and the reservoir an equal volume of 0.003 *N* HCl + 0.45 *N* LiCl. Elution was conducted at an average flow rate of 2 ml./min.; 10-ml. fractions were collected. The mixture was separated into three major ultraviolet-absorbing components: adenosine 5'-phosphoramidate (12%), tubes 150–195; AMP (14%), tubes 200–220; and AMP-PCP (53%) tubes 225–330. The yields (from adenosine 5'-phosphoramidate) were estimated from absorbancy measurements on the pooled fractions. AMP and the amidate were identified by paper chromatography in solvent A in parallel with authentic samples (R_f's: adenosine 5'-phosphoramidate, 0.48; AMP, 0.45).

The AMP-PCP fraction was neutralized (pH 7.3) with 1 *N* lithium hydroxide and concentrated to a thick sirup in a flash evaporator at 30°. The sirup was treated with 250 ml. of acetone containing 10% methanol. The white solid which separated was collected by centrifugation and washed with the acetone–methanol mixture until no chloride could be detected in the supernatant liquid (silver nitrate test). The yield of white powder after drying *in vacuo* was 1.0 g. Paper chromatography of this material in solvent system A produced a single ultraviolet-absorbing spot (R_f 0.26 with slight tailing).

In order to obtain an analytically pure product, the lithium salt (in solution in 100 ml. of water adjusted to pH 8 with lithium hydroxide) was rechromatographed in the manner just described on a Dowex-2 (chloride) column (24 × 2.7 cm.) using 1.5 l. of 0.003 *N* HCl in the mixing chamber and 1.5 l. of 0.003 *N* HCl + 0.45 *N* LiCl in the reservoir. This procedure gave rise to a small fraction of AMP (<1%), tubes 110–113, and the major fraction of AMP-PCP (49% from starting adenosine 5'-phosphoramidate), tubes 145–245. The tetralithium salt of AMP-PCP was isolated as a hygroscopic white powder by concentration *in vacuo* of this second fraction and treatment of the resulting sirup with 1.5 l. of acetone containing 10% methanol as described above. The yield, after drying at 0.1 mm. over P_2O_5 for 12 hr. at room temperature, was 0.97 g.[18] This material was dissolved in 6 ml. of water and reprecipitated by the addition of 40 ml. of methanol. The precipitate was collected by filtration, redissolved in 15 ml. of water, and the solution was freed from a small amount of insoluble material by centrifugation and lyophilized to give a white solid which was dried at room temperature at 0.1 mm. over P_2O_5 for 12 hr. The yield of tetralithium 5'-adenylyl methylenediphosphonate dihydrate was 0.45 g. (22% from starting adenosine 5'-phosphoramidate).

Anal.[19] Calcd. for $C_{11}H_{14}N_5O_{12}P_3Li_4 \cdot 2H_2O$: C, 23.36; H, 3.19; N, 12.39; total P, 16.46; phosphate P, 5.48; labile P, 0.0; mol. wt., 565; adenine:strong acid:weak acid, 1.0:3.0:1.0. Found: C, 23.16; H, 3.37; N, 12.16; total P, 16.27; phosphate-P, 5.42; labile P, 0.0; equiv. wt., 573 (by ultraviolet measurements, λ_{max} 259 mμ, λ_{min} 227 mμ at pH 7); titration data, adenine:strong acid:weak acid, 1.03:3.0:1.0 (pK_A's 4.2, 8.4).[20]

Paper chromatography using solutions of the lithium salt in several solvent systems gave single clean spots (solvent system A, R_f 0.26, R_{ATP} 1.14[21]; solvent system B, R_f 0.40, R_{ATP} 1.06; solvent system C, R_f 0.84, R_{ATP} 0.98; solvent system D, R_f 0.36, R_{ATP} 0.87). The product gave a positive reaction when the chromatograms were sprayed with periodate–benzidine spray.[14]

Hydrolysis of the product with 2.5 *N* NaOH (*ca.* 10 mg. of the lithium salt per ml.) for 30 min. at 100° caused degradation to AMP which was detected by paper chromatography (solvent system A, R_f 0.45; solvent system D, R_f 0.29).

Preparation of 5'-Adenylyl Methylenediphosphonate. (Method 2).—Methylenediphosphonic acid (2.0 g., 11.4 mmoles) and AMP (1.0 g. of the dihydrate, 2.6 mmoles) were dissolved in pyridine (30 ml.) and water (4 ml.) to produce a mixture which consisted of two liquid phases. DCC was added to the mixture at room temperature with vigorous stirring in the following manner: at the start of the reaction 6 g. (29 mmoles); after 4 hr., 10 g. (48 mmoles); after 12 hr., 4 g. (19 mmoles). No appreciable temperature rise was noted during the reaction. Dicyclohexylurea began to precipitate about 15 min. after the first DCC addition. The total reaction time was 24 hr. The urea was filtered off and washed with water. The combined filtrate and washings were diluted further with water (total volume, 150 ml.), extracted five times with ether (total volume, 300 ml.), frozen, and stored at −20°.

The solution was chromatographed in three portions, on a Dowex-1 (formate) column (2% cross linking) 2.5 cm. in diameter × 17.5 cm. long. The solution was applied to the column at pH 8 and after washing with water (total volume, 1500 ml.) to remove pyridine, fractionation was carried out by the gradient elution technique. The following solutions were successively added to the mixing chamber which initially contained 500 ml. of water: 4 *N* formic acid (500 ml.), 4 *N* formic acid + 0.1 *M* ammonium formate (500 ml.), 4 *N* formic acid + 0.2 *M* ammonium formate (1500 ml.). Elution was conducted at an average flow rate of 2 ml./min.; 15-ml. fractions were collected. Three major ultraviolet-absorbing fractions were obtained: AMP (50%), tubes 15–20; P^1,P^2-diadenosine-5' pyrophosphate (9%), tubes 23–30; and AMP-PCP (25%), tubes 115–134. The yields (from starting AMP) were based on absorbancy measurements on the pooled fractions.

The fractions containing the product were combined, frozen, and evaporated *in vacuo* to a volume of 200 ml. The concentrated solution was treated with 7 g. of acid-washed Norit A. After stirring for 15 min., the charcoal was collected on a Celite filterbed and washed with water (total volume, 800 ml.). The product was then eluted with 50% aqueous ethanol containing 5% concentrated ammonium hydroxide (total volume of eluent, 600 ml.). The eluate was concentrated on a flash evaporator at a bath temperature of 20° to a volume of 200 ml., filtered to remove traces of charcoal, and lyophilized to a hygroscopic white solid. The solid (0.58 mmole of product as estimated spectrophotometrically) was dissolved in 4 ml. of water and the solution was treated with excess 1 *M* barium acetate. The precipitate was collected by centrifugation, washed with a small amount of water, and redissolved in a minimum of 0.1 *N* HBr at 0°. After removal of a small amount of insoluble material by centrifugation, the pH of the supernatant solution was adjusted to 6.5 with 1 *N* sodium hydroxide and the resulting precipitate was collected by centrifugation, washed with water (2 × 2 ml.), with 95% ethanol (2 × 2 ml.), and then with ether (2 × 2 ml.). The sample was dried at room temperature over P_2O_5 for 12 hr. at 0.3 mm. to yield 370 mg. (0.42 mmole) of dibarium 5'-adenylyl methylenediphosphonate hexahydrate (16% from AMP).

(16) P. B. Hawk, B. L. Oser, and W. H. Summerson, "Practical Physiological Chemistry," Thirteenth Edition, McGraw–Hill Book Co., Inc., New York, N. Y., 1954, p. 630.

(17) J. G. Moffatt and H. G. Khorana, *J. Am. Chem. Soc.*, **83**, 649 (1961).

(18) Elemental analysis of the product from previous runs at this stage of purification gave inconsistent results and indicated contamination with methanol.

(19) Analyses for C, H, and N were conducted by the Microtech Laboratories of Skokie, Ill.

(20) Titration under similar conditions of a solution of ATP prepared from crystalline disodium ATP (Pabst Research Biochemicals) gave adenine: strong acid:weak acid, 1.0:3.0:1.0 (pK_A's 4.2, 7.1).

(21) R_{ATP} is defined as mobility with respect to that of ATP.

Anal.[22] Calcd. for $C_{11}H_{14}N_6O_{12}P_3Ba_2 \cdot 6H_2O$: C, 14.94; H, 2.97; N, 7.92; total P, 10.51; phosphate P, 3.50; labile P, 0.0; mol. wt., 884; adenine:strong acid:weak acid, 1.0:3.0:1.0. Found: C, 14.90; H, 3.13; N, 8.23; total P, 10.56; phosphate P, 3.53; labile P, 0.0; equiv. wt., 892 (by ultraviolet measurements; λ_{max} 259 mμ, λ_{min} 227 mμ at pH 7); titration data, adenine:strong acid:weak acid, 0.97:3.0:1.0 (pK_A's 4.2, 8.5).[20]

Paper chromatography using solutions of the free acid (prepared by treatment of aqueous suspensions of the barium salt with Dowex-50(H) resin) gave single clean spots (solvent system A, R_f 0.26, R_{ATP} 1.20; solvent system C, R_f 0.81, R_{ATP} 0.97; solvent system D, R_f 0.39, R_{ATP} 0.82). The product gave a positive reaction when the chromatograms were sprayed with periodate–benzidine spray.

Hydrolysis of the product (as the free acid prepared from the barium salt with use of Dowex-50(H) resin, *ca.* 10 mg. per ml.) with 2.5 *N* NaOH for 30 min. at 100° caused degradation to AMP which was detected by paper chromatography (solvent system A, R_f 0.46; solvent system D, R_f 0.30).

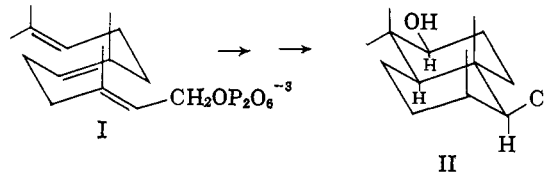
(22) Analyses for C, H, and N were conducted by the Midwest Microlab, Inc., of Indianapolis, Ind.

COMMUNICATIONS TO THE EDITOR

The Biogenetically Patterned *in vitro* Oxidation–Cyclization of Farnesyl Acetate

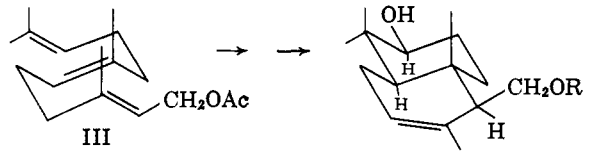
Sir:

By virtue of incisive tracer and isolation studies, it has been demonstrated that farnesyl pyrophosphate (I), derived from mevalonic acid *via* isopentenyl pyrophosphate, serves as a natural precursor of squalene, lanosterol, cholesterol, and, by implication, all of the many varieties of steroids, as well as sesqui-, di-, and



I II

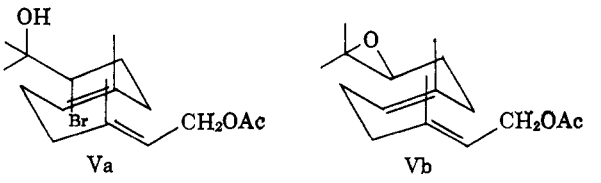
triterpenoids, which usually possess the characteristic part ring system and stereochemistry depicted in II.[1] We wish to report the nonenzymatic selective terminal oxidation of (*trans-trans*) farnesyl acetate (III) and



III IVa, R=Ac IVb, R=H

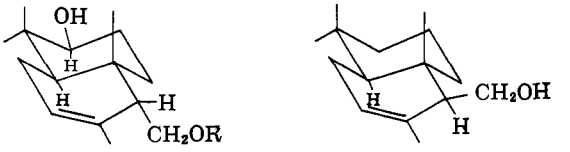
subsequent acid-catalyzed, stereo-directed cyclization to the bicyclic diol monoacetate (IVa), which duplicates, in respect to carbon framework, oxidation site, and stereochemistry at all of *four* asymmetric centers, the familiar 2-hydroxylated A–B ring system present in most polycyclic, di-, and triterpenoid systems.

Through the action of N-bromosuccinimide in aqueous glyme, *trans-trans*[2] farnesyl acetate was selectively—and exclusively, for all practical purposes—oxidized at the terminal nonallylic of the three trisubstituted double bonds,[3] giving rise to bromohydrin Va.[4] The latter, after chromatographic (silica gel) purification, was converted by means of base to epoxide Vb.[4] On treatment with boron trifluoride etherate in benzene,[5] the epoxide was transformed into a variety of



Va Vb

products, from which there could be isolated after extensive chromatographic purification a modest yield of bicyclic diol monoacetate, shown by vapor phase chromatography to consist of 85% stereoisomer IVa and 15% of the epimer VIa.[6] The constitution and stereochemistry of product VIa was proved by (1) chromic anhydride oxidation to the acetoxy ketone VIIa and



VIa, R=Ac VIb, R=H VIII

lithium aluminum hydride reduction to the same diol (VIb) (m.p. 150–151°) produced by hydrolysis of VIa, thus indicating the equatorial nature of the hydroxyl group, and (2) conversion, by means of Raney nickel desulfurization of VIIa ethylene dithio ketal, to *dl*-epidrimenol (m.p. 65.5–66.5°), an authentic sample of which was prepared by lithium aluminum hydride reduction of the methyl ester of the known corresponding acid (m.p. 138°).[7,8] In parallel experiments, the diol acetate IVa was (1) oxidized to ketone VIIb and reduced to authentic IVb (m.p. 113–114°) and (2) converted to monohydric alcohol identical with *dl*-drimenol (m.p. 61–62°).[7–9] When a mixture (65% *trans;* 35% *cis*) of farnesyl acetate was subjected to the oxidation–cyclization sequence described before, a mixture of 55% IVa and 45% VIa (by v.p.c.) was generated,[6] thus indicating that, under the specified conditions and to the extent that IVa and VIa are formed, geometry determines stereochemistry of product, at

(1) For reviews, see, *e.g.*, (a) F. Lynen and U. Henning, *Angew. Chem.*, **72**, 820 (1960); (b) L. Ruzicka, *Proc. Chem. Soc.*, 341 (1959).

(2) See R. Bates, *J. Org. Chem.*, **28**, 1086 (1963).

(3) *Cf.* the selective *in vitro* oxidation of the terminal double bonds in squalene: E. F. van Tamelen and T. J. Curphey, *Tetrahedron Letters*, **No. 3**, 121 (1962).

(4) This intermediate was not crystalline; but after suitable operations (chromatography on bromohydrin; short path distillaton of epoxide), analytically pure material was secured.

(5) For the acid-catalyzed cyclization of 2,6-dimethyl-5,6-epoxyheptene-1, see D. J. Goldsmith, *J. Am. Chem. Soc.*, **84**, 3913 (1962).

(6) Vapor phase chromatography data secured on acetoxy ketone VIIa,b (*vide infra*).

(7) P. A. Stadler, A. Eschenmoser, H. Schinz, and G. Stork, *Helv. Chim. Acta*, **40**, 2191 (1957).

(8) Comparison of bicyclic diol thus obtained with authentic material rests on identical v.p.c., infrared spectral, m.p. (and m.m.p.) behavior (as diol *per se*, or as suitable derivatives).

(9) We are indebted to Professor Dr. A. Eschenmoser (ETH) for an authentic sample.