A New Synthesis of the Antibiotic Phosphonomycin

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A new, total synthesis of the antibiotic phosphonomycin is described. Thermal rearrangement of di-*t*-butyl 2-propynyl phosphite yields the di-*t*-butyl propadienylphosphonate ester. A selective hydrogenation, followed by acid-catalyzed cleavage of the *t*-butyl groups, affords *cis*-propenylphosphonic acid in high yield. This olefin acid is epoxidized and the product is resolved in essentially one step to furnish phosphonomycin.

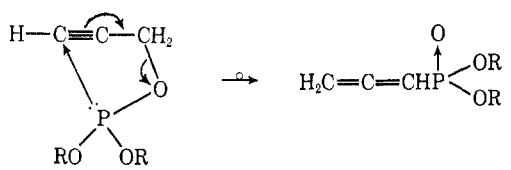
Phosphonomycin is a promising new antibiotic of unusual structure originally isolated from fermentation broths of *Streptomyces fradiae*. It has been found to be orally effective against both Gram-positive and Gram-negative infections in mice. Its bactericidal mode of action is *via* irreversible binding to the enzyme pyruvate–uridine diphospho-N-acetylglucosamine, thereby inhibiting cell wall synthesis.[1]

Phosphonomycin has been shown to be (−)-(1*R*,2*S*)-1,2-epoxypropylphosphonic acid. Proof of structure was obtained by synthesis together with a chemical determination of the absolute configuration.[2]

We wish to describe a different total synthesis of phosphonomycin which is simple and elegant, and which affords the antibiotic in much higher yield. Our approach is based on a five-step *in situ* preparation of *cis*-propenylphosphonic acid followed by a one-step epoxidation and resolution. The overall sequence leading to (−)-*cis*-1,2-epoxypropylphosphonic acid as its mono-(+)-α-phenethylammonium salt is shown in Scheme I.

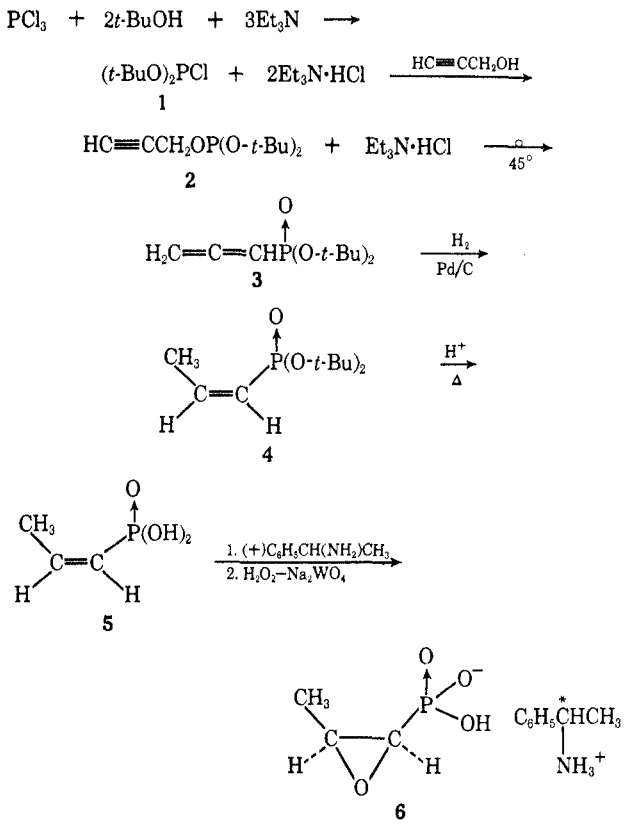
Di-*t*-butyl phosphorochloridite (**1**) was prepared by adding 2 equiv of *t*-butyl alcohol to a benzene solution of phosphorus trichloride containing triethylamine as hydrogen chloride acceptor. Propargyl alcohol was then added at 5–10° to form di-*t*-butyl 2-propynyl phosphite (**2**). Immediately after the addition was completed, the reaction mixture was analyzed by infrared spectroscopy. Absorption bands at 3.00 and 4.65 μ characterized the mixed phosphite **2**. In addition, a doublet at 5.08, 5.14 μ indicated its partial rearrangement to the allene **3**.

The thermal rearrangement of 2-alkynyl phosphites to 1,2-alkadienyl phosphonates has been the subject of intensive study in recent years.[3] The reaction has been shown to be of first order and follows an intramolecular pathway of the SNi′ type.



We have found that when R = *t*-butyl, the nucleophilicity of the trivalent phosphorus atom is so enhanced that even at 0° rearrangement slowly takes place. The acetylene → allene rearrangement was completed *in situ* by heating the reaction mixture at 45° for 2 hr to afford di-*t*-butyl propadienylphosphonate (**3**). Longer heating periods, even at reflux temperature (80–82°), did not cause the prototropic further rearrangement of **3** to the α,β-propynyl phosphonate ester.[3]

SCHEME I



The heavy precipitate of triethylamine hydrochloride salt was removed at this stage by extraction into water. The benzene phase containing allene **3** was dried and then hydrogenated at 15 psi with Pd–C catalyst. The reduction in benzene solution was extremely selective and stereospecific, with hydrogen attacking only the terminal double bond of the allene moiety to give the *cis* olefin.[4] This selectivity was confirmed by a reduction with D_2 which showed that more than 96% of the deuterium had entered the β,γ positions. In addition, the hydrogenation conveniently stopped when 1 equiv of hydrogen was taken up to give di-*t*-butyl *cis*-pro-

(1) D. Hendlin, E. O. Stapley, M. Jackson, H. Wallick, A. K. Miller, F. J. Wolf, T. W. Miller, L. Chaiet, F. M. Kahan, E. L. Foltz, H. B. Woodruff, J. M. Mata, S. Hernandez, and S. Mochales, *Science*, **166**, 122 (1969).

(2) B. G. Christensen, W. J. Leanza, T. R. Beattie, A. A. Patchett, B. H. Arison, R. E. Ormond, F. H. Kuehl, Jr., G. Albers-Schonberg, and O. Jardetzky, *ibid.*, **166**, 123 (1969).

(3) See V. Mark in "Mechanisms of Molecular Migrations," Vol. II, B. S. Thyagarajan, Ed., Interscience, New York, N. Y., 1969, p 319, for leading references.

(4) After completion of our work, the selective hydrogenation of 1,2-diene phosphonic esters in alcohol solution with 5% Pd–$CaCO_3$ was reported by A. A. Petrov, B. I. Ionin, and V. M. Ignatyev, *Tetrahedron Lett.*, 15 (1968).

penylphosphonate (4). Continuation of the reduction for several hours longer resulted in no further absorption of hydrogen. The rapid removal of the two *t*-butyl groups was accomplished by refluxing the benzene solution containing **4** with a strong acid catalyst. A particularly elegant procedure is to use *cis*-propenylphosphonic acid as catalyst to avoid contaminating the product with a foreign acid. Removal of solvent leaves *cis*-propenylphosphonic acid (**5**) in 81% overall yield. This was the first intermediate isolated after five *in situ* chemical reactions.

We have found that *t*-butyl is an especially advantageous blocking group and deserves to be more widely used in organophosphorus chemistry. We estimate the combined yield of di-*t*-butyl phosphorochloridite (**1**) and mixed phosphite (**2**) to be extremely high, in the range of 90–95%. Furthermore, acid catalyzed cleavage of both *t*-butyl groups in phosphonate ester **4** is a very clean and rapid reaction, requiring moderate temperatures and with the blocking groups leaving the reaction medium as gaseous isobutylene. The deblocking process can also be carried out thermally, after a short induction period, by heating the neat di-*t*-butyl phosphonate ester to 100°, and maintaining that temperature during the strongly endothermic process. In either case, the rate of gas evolution accelerates as the reaction proceeds because the product being formed is itself a strong acid which can serve as catalyst.

When ethyl and isopropyl were used as blocking groups in this synthesis, the yield and purity of *cis*-propenylphosphonic acid was decidedly lower. This was due in part to a poorer yield at the mixed phosphite (of type **2**) stage, as well as partial attack at the olefinic and C–P bonds of ester **4** by the hot aqueous hydrochloric acid required to cleave these alkyl groups.

Conversion of *cis*-propenylphosphonic acid (**5**) to phosphonomycin was accomplished in essentially one step, as follows. Slightly more than 1 equiv of a mixture of resolving base, (+)-α-phenethylamine, and triethylamine was added to a propanol solution of *cis*-propenylphosphonic acid to attain the desired pH range for epoxidation. The warm solution was then treated with hydrogen peroxide, with sodium tungstate as catalyst.[2,5] After 1 hr at 50–55°, the epoxidation was complete. Cooling the reaction mixture produced a crystalline precipitate of (+)-α-phenethylammonium (−)-*cis*-1,2-epoxypropylphosphonate (**6**) which was found to be 92% optically pure. The undesired triethylammonium salt of the (+) isomer remained in solution. A single recrystallization from aqueous propanol afforded phosphonomycin (salt) of 100% optical purity. The yield in this combined epoxidation and resolution step was 32.5%, or 65% based only on the (−) form.

Experimental Section[6]

Di-*t*-butyl Propadienylphosphonate (3).—A stirred solution of phosphorus trichloride (68.7 g, 0.50 mol) in anhydrous benzene (750 ml) was cooled to 5° under N_2, and then triethylamine (154.4 g, 1.525 mol) was added at 5–10° over a 20-min period. After stirring for 20 min more, a solution of *t*-butyl alcohol (74.1 g, 1.00 mol) in anhydrous benzene (74 ml) was added dropwise, with good agitation, while maintaining the reaction temperature between 5 and 10° with an ice–methanol bath. The thick reaction mixture containing di-*t*-butyl phosphorochloridite[7] (**1**) was stirred for 1.5 hr at 5–10°, and then a solution of propargyl alcohol (28.0 g, 0.50 mol) in benzene (40 ml) was added at 5–10° over a 30-min period. When the addition was complete, a filtered aliquot was analyzed by infrared spectroscopy: bands at 3.00 (HC≡) and 4.65 μ (HC≡C) characterized the product, di-*t*-butyl 2-propynyl phosphite (**2**), while a doublet at 5.08, 5.14 μ indicated its partial rearrangement to **3**.

After stirring for 1 hr at 5–10°, the mixture was warmed to 40–45° and kept at that temperature for 2 hr to complete the rearrangement. The reaction mixture was then cooled to room temperature and water (185 ml) was added in portions. The triethylamine hydrochloride precipitate dissolved, and the aqueous layer was separated and reextracted with benzene (50 ml). The combined benzene layers were dried over sodium sulfate (60 g) to afford a solution of di-*t*-butyl propadienylphosphonate (**3**) which was used directly in the next step. An analytical sample was obtained by distillation: bp 54–56° (0.1 mm); ir (neat) 5.08, 5.14 (C=C=C), 7.90 (P→O), 9.62 (P–O–C), and 12.10 μ (=CH_2).

Anal. Calcd for $C_{11}H_{21}O_3P$: C, 57.31; H, 9.11; P, 13.33. Found: C, 57.06; H, 9.32; P, 12.97.

Di-*t*-butyl *cis*-Propenylphosphonate (4).—The dried benzene solution containing **3** was treated with 5.0 g of 5% Pd–C and reduced at 16–18° in a jacketed steel vessel equipped with "Magnadrive" stirrer (1500 rpm) at a constant 15 psi of H_2. The theoretical amount of H_2 was taken up in 1–1.5 hr. The catalyst was removed by filtration and the filtrate (1.15 l.) containing **4** was used directly for the deblocking step. The infrared showed a strong band at 6.14 μ characteristic of the *cis* olefin, while absence of the allene doublet at 5.0–5.2 μ indicated the completeness of the reduction. An analytical sample was obtained by distillation: bp 45–46° (0.1 mm); ir (neat) 6.14 (*cis* C=C), 7.98 (P→O), and 9.62 μ (P–O–C).

Anal. Calcd for $C_{11}H_{23}O_3P$: C, 56.38; H, 9.89; P, 13.22. Found: C, 56.54; H, 10.09; P, 13.18.

***cis*-Propenylphosphonic Acid (5). A.**—A 500-ml flask, equipped with motor stirrer, thermometer, addition funnel, and a short distilling head with horizontal condenser connected to a receiver with a gas outlet tube, was charged with *cis*-propenylphosphonic acid (5 g) as catalyst and a 50-ml aliquot of the benzene solution containing **4**. The two-phase mixture was heated to reflux whereupon cleavage of the *t*-butyl groups began, liberating more of acid **5** and isobutylene gas which exited from the system. The remaining portion (1.1 l.) of the benzene solution of **4** was then added over 100 min, with simultaneous distillation of benzene to maintain the reaction mixture between 50 and 150 ml by balancing the addition and distillation rates. When the addition was complete, the remaining benzene was removed *in vacuo* to afford crude **5** of sufficient purity to be used in the next step. This brown oil (which sometimes crystallized) weighed 56.9 g net, having C=C content of 87% by Br_2 titration. The overall yield, therefore, from propargyl alcohol to **5** was 81%. A pure sample (hygroscopic) was prepared by recrystallization of the monobenzylammonium salt, mp 155–157°, followed by ion exchange on Amberlite IR-120 resin: mp 55–57°.

Anal. Calcd for $C_3H_7O_3P$: C, 29.52; H, 5.78; P, 25.38. Found: C, 29.48; H, 5.89; P, 25.54.

B.—Neat di-*t*-butyl ester **4** was treated with a catalytic amount of concentrated hydrochloric acid (1 ml acid/50 g ester), and heated on the steam bath in the hood until evolution of isobutylene ceased (30 min). Gas evolution was slow in the beginning, but became extremely vigorous toward the end. The weight yield of crude acid **5** was similar to that obtained by method A, but the purity was somewhat lower.

(+)-α-Phenethylammonium (−)-*cis*-1,2-Epoxypropylphosphonate (6).—*cis*-Propenylphosphonic acid having C=C purity of 83% (148 g, 1.00 mol "pure") was dissolved in propanol (800 ml). To the stirred solution was added (+)-α-phenethylamine[8] (80.5 g, 0.665 mol), followed by enough triethylamine (56 g,

(5) G. B. Payne and P. H. Williams, *J. Org. Chem.*, **24**, 54 (1959).

(6) Melting points were determined with a Thomas–Hoover Uni-Melt apparatus in unsealed capillary tubes and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer Model 621 grating infrared spectrophotometer. We are grateful to Mr. R. N. Boos and associates for microanalyses, and Mr. J. Gilbert and colleagues for all other assays. Solvents were dried to less than 0.1 mg/ml of H_2O over molecular sieves. All reagents used had less than 1.5 mg/ml of H_2O as determined by Karl Fischer assay.

(7) V. Mark and J. R. Van Wazer, *J. Org. Chem.*, **29**, 1006 (1964).

(8) Practical grade of 95% optical purity from Norse Laboratories, Santa Barbara, Calif.

0.553 mol) to reach a pH of 5.8–5.9. The resulting warm solution was treated in one portion with sodium tungstate dihydrate (5.0 g, 0.015 mol) and disodium ethylenediaminetetraacetic acid (1.0 g) dissolved together in 15 ml of warm (65–70°) water. Hydrogen peroxide (1.53 mol, 157 ml of a 30% solution) was then added dropwise with stirring over a 15-min period while maintaining the temperature between 40 and 55°. After the addition was complete, the reaction was kept at 50–55° for 1 hr to complete the epoxidation. The solution was then cooled to −5° over a 30-min period to initiate crystallization. After stirring for 2 hr at −5°, the product was filtered and the cake washed with cold propanol (four 50-ml portions). This salt when dried weighed 106 g, and was about 92% optically pure. To complete the resolution, the salt was dissolved in 770 ml of hot (75–80°) propanol. The slightly turbid solution was charcoal treated (2.5 g) and filtered while hot through a preheated funnel. To the hot filtrate was added 80 ml of warm (60–70°) water. Crystallization of the monohydrate began within a few minutes. After stirring the mixture at 0° for 2 hr the product was filtered, washed with cold propanol (three 25-ml portions), and dried *in vacuo* at 45°. The yield of phosphonomycin salt **6** was 90.1 g (32.5%): mp 132–134° dec; $[\alpha]^{25}{}^{\circ}{}_{405}$ −2.6° (*c* 5, H_2O) or +18.7° (*c* 3, DMF); Karl Fischer 6.6% (theory 6.5%); equiv wt 278.7 (theory 277.3).

Anal. Calcd for $C_{11}H_{13}NO_4P \cdot H_2O$: C, 47.64; H, 7.27; N, 5.05; P, 11.17. Found: C, 47.66; H, 7.00; N, 5.29; P, 11.09.

Registry No.—**3**, 25383-48-6; **4**, 25383-05-5; **5**, 25383-06-6; **6**, 25383-07-7; phosphonomycin, 23155-02-4.

Alkaloids of *Sceletium* Species.[1] III.[2] The Structures of Four New Alkaloids from *S. strictum*

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The isolation and structures of four new alkaloids, mesembrenol (**5**, R = Me; R′ = H), *O*-acetylmesembrenol (**5**, R = Me; R′ = Ac), 4′-*O*-demethylmesembrenol (**5**, R = R′ = H), and 4′-*O*-demethylmesembranol (**4**, R = H) are reported. The position of the phenolic hydroxyl in 4′-*O*-demethylmesembranol is determined by the application of a radioisotope dilution method. A discussion of the circular dichroism and nuclear magnetic resonance spectra of (+)-mesembrenone (**2**) and the nmr spectra of related alcohols mesembrenol and 6-epimesembrenol (**7**) is presented in providing information on the conformational preference of ring C in which it is shown that the equivalent forms of the half-chair, as represented in structures **2a**, **5a**, and **7a**, is preferred.

Certain *Sceletium* species (*Fam. Aizoaceae*) are used for the preparation of the drug known as *Channa* or *Kougoed*. Previous studies on *S. namaquense*, *S. tortuosum*, and *S. anatomicum* have led to the isolation and characterization of the alkaloids mesembrine (**1**), mesembrenone (**2**), and mesembranol (**4**, R = Me).[4]

Structural Studies.—In the course of a study of the biosynthesis of these alkaloids we have examined the major alkaloids of *Sceletium strictum* L. Bol.[5] Preliminary examination of the total alkaloid fraction by gas liquid chromatography (glpc) on several columns (see Experimental Section) showed it to contain one major component and several minor constituents. The major component proved to be a new alkaloid, mesembrenol (**5**, R = Me; R′ = H), $C_{17}H_{23}NO_3$, mp 145°, $[\alpha]D$ +90°, which could be isolated on occasions by crystallization of the total alkaloid fraction from acetone but was usually obtained only after chromatography over alumina. The infrared spectrum of mesembrenol shows absorption bands at 3630 and 3450 cm^{-1} characteristic of an alcoholic hydroxyl group and the presence of this group was substantiated by the formation of an O-acetyl derivative (**5**, R = Me; R′ = Ac). An N-methyl, two aromatic methoxyls, three aromatic hydrogens and two olefinic hydrogens signals are present in the nmr spectrum and a comparison with the spectra of mesembranol and mesembrine suggested it could be assigned as a member of the octahydroindole class of mesembrine-type alkaloids. This conclusion is supported by the mass spectrum which shows a molecular ion at *m/e* 289, and an intense peak at *m/e* 219. A detailed study of the mass spectra of the mesembrine alkaloids[6] has established that alkaloids of this ring system which possess a 3a-dimethoxyphenyl substituent all show a prominent peak at *m/e* 219 which is attributed to an ion of structure **6** (R = Me). The occurrence of the *m/e* 219 ion in the mass spectrum of mesembrenol implies that the double bond and hydroxyl group have to be situated in ring B and their placement as shown in structure **5** (R = Me, R′ = H) is provided by its oxidation to (±)-mesembrenone (**2**) with Jones reagent. The racemic nature of the product in this reaction is not exceptional and occurs as a consequence of the acidic conditions of the reaction which lead to the intervention of an equilibrium involving the protonated form of **2** and the symmetrical dienone **3**.

Elucidation of the remaining structural features of (+)-mesembrenol, namely, the stereochemistry of the C-6 hydroxyl and the absolute configuration was established by the hydrogenation of the new base to (−)-mesembranol (**4**, R = H; R′ = Me). The relative and absolute stereochemistry of the latter has been firmly established by a recent X-ray analysis of 6-epimesembranol methiodide.[2]

(1) Supported by the National Science Foundation Grant GB4361 and the National Institutes of Health through Grant AM13977-01 and a Research Career Program Award 1K04GM42342-01 to P. W. J.

(2) See P. Coggon, D. S. Farrier, P. W. Jeffs, and A. T. McPhail, *J. Chem. Soc. A*, in press, for paper II in this series.

(3) (a) National Science Foundation Undergraduate Research Participant, 1967; (b) National DefenseE ducation Act Fellow, 1966–1969, Du Pont Fellow, 1969–1970; (c) National Aeronautics and Space Administration Act Fellow, 1965–1968.

(4) For a review, see A. Popelak and G. Lettenbauer, "The Alkaloids," Vol. IX, R. H. F. Manske, Ed., Academic Press, New York, N. Y., 1968, p 467.

(5) Identified by Dr. L. Bolus, Bolus Herbarium, University of Cape Town, South Africa, through the courtesy of Mr. Herre, Stellenbosch, South Africa.

(6) P. W. Jeffs and N. Martin, unpublished observations.