Table 1. Efficacy of phosphonomycin, tetracycline, and chloramphenicol in experimentally infected mice. The cultures are designated by numbers that are used for internal identification. Female Swiss white mice, average weight 21 to 24 g, were infected intraperitoneally with 3 to 30 LD_{50} (dose lethal for 50 percent of the mice) doses of the test organism and treated by the oral route at the time of infection and again 6 hours later. The ED_{50} (amount of drug required to protect 50 percent of the infected animals) was calculated from the survival data 7 days after infection. Fourfold dilutions of the drug were used, and six mice were tested at each concentration of the drug. The ED_{50} values for phosphonomycin are given in micrograms of disodium salt.

Organism	ED_{50} (μg/dose)		
	Phosphonomycin	Tetracycline	Chloramphenicol
Escherichia coli 2017	330	125	160
Klebsiella pneumoniae 3068	760	2020	160
Proteus vulgaris 1810	220	> 5000	320
Pseudomonas aeruginosa 3210	1110	> 10000	5000
Salmonella schottmuelleri 1814	80	1110	500
Salmonella schottmuelleri 3010	4	690	275
Staphylococcus aureus 2949	90	250	570
Streptococcus pyogenes 3009	1130	130	675

known antibiotics on the basis of antibacterial spectrum, cross-resistance (*1*), and physicochemical characteristics.

The antibiotic, a highly polar, optically active, low-molecular-weight acid, was isolated from fermentation broth by a combination of ion-exchange chromatography, gel filtration, and adsorption chromatography (*2*). Purification from broth was monitored by an agar disc diffusion assay in vitro (*3*) with *Proteus vulgaris* as indicator organism and a mouse protection test in vivo (*4*) in which *Salmonella schottmuelleri* 3010, a strain highly sensitive to phosphonomycin, was used. Both assays showed excellent agreement; a linear relation was obtained between the reciprocal of the activity in vitro and the ED_{50} (the concentration required to protect 50 percent of the infected mice).

Phosphonomycin was obtained from fermentation broth as a crystalline calcium salt. The isolation (*2*), elucidation of chemical structure, and synthesis (*5*) of the antibiotic have been described. The natural antibiotic was identical to synthetic (−)-*cis*-1,2-epoxypropylphosphonic acid having the absolute configuration 1*R*,2*S* (*5*).

Phosphonomycin is effective in vitro against a variety of bacteria, Gram-negative and Gram-positive, by both the agar diffusion and tube dilution methods. Measurement of sensitivity to the antibiotic is affected by medium constituents. As much as a 500-fold difference in end points in a tube dilution assay can be obtained depending on whether nutrient broth or brain heart infusion broth is used. The presence of glucose and phosphate in the assay medium apparently diminish the activity of phosphonomycin significantly. In studies of the accumulation of antibiotic by sensitive and resistant bacteria, it has been established that entry of phosphonomycin is mediated by an L-α-glycerophosphate transport system (*6*). This system (*7*) is repressed or inhibited by the same nutrients (glucose and phosphate, respectively) that diminish antibiotic activity in vitro.

Phosphonomycin is bactericidal in action. Its effect on the integrity of the bacterial cell wall is evident from the detection of spheroplasts with a number of bacterial strains exposed to antibiotic in media of high osmolarity. Moreover, biochemical investigations (*6*) show that phosphonomycin attaches covalently to, and thus inhibits irreversibly, pyruvate-uridine diphospho-*N*-acetylglucosamine transferase (*8*) in extracts from several Gram-positive and Gram-negative microorganisms. This enzyme catalyzes the first step in the biosynthetic pathway of the nucleotide muramyl peptides that serve as cell-wall precursors in all bacteria.

Phosphonomycin is effective orally in protecting mice against a number of infections caused by Gram-positive and Gram-negative organisms. It is an effective chemotherapeutic agent against a number of systemic infections and compares favorably with tetracycline and chloramphenicol (Table 1). Phosphonomycin shows little if any toxicity to mice; 50 percent of the mice died only after the intraperitoneal dose of the disodium salt reached 4000 mg/kg. The toxicity can be ascribed to the sodium content of the administered dose.

Phosphonomycin, as a calcium salt, is absorbed from the gastrointestinal tract in man, and adequate antibacterial concentrations appear in the serum within 2 hours. It is excreted by the kidneys in an unchanged form and appears in high concentration in the urine. It may also be administered intravenously with development of appreciably higher concentrations in the serum and with remarkably little local irritation. Toxicity has been absent by both routes of administration in clinical and pharmacological trials (*9*). On the basis that it appears to be a broad-spectrum antibiotic and because of its low toxicity and effectiveness when administered by the oral route, phosphonomycin appears to have potential as an effective chemotherapeutic agent.

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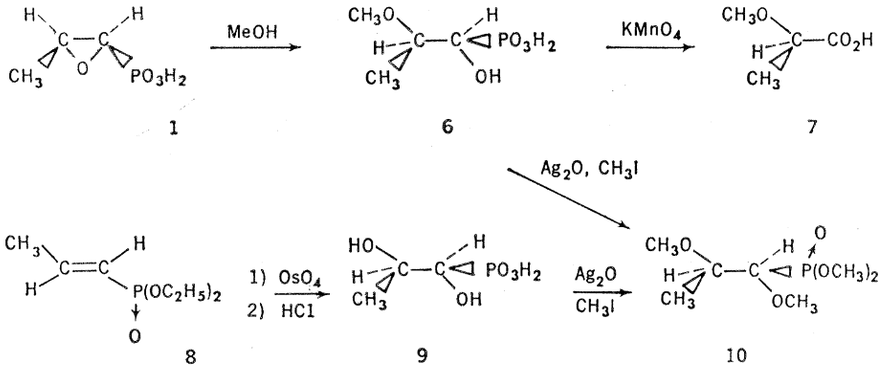
Phosphonomycin: Structure and Synthesis

Abstract. *Synthesis and resolution of the antibiotic phosphonomycin are described. The structure is (−)(1R,2S)-1,2-epoxypropylphosphonic acid.*

The biological properties and mode of action of a promising new antibiotic, phosphonomycin, have been described (*1*). This substance was first isolated from fermentation broths in which *Streptomyces fradiae* was grown. After preliminary fractionation of the broth,



the biologically active material was purified by paper chromatography with 1-butanol-acetic acid-water (4:1:1); the area of highest biological activity (R_F 0.26) was eluted with methanol.

A nuclear magnetic resonance spectrum of the antibiotic in D_2O (internal DSS) showed signals of a methyl group at 8.52 τ (d, $J = 5.4$ hz) and signals of two methine protons at 7.16 τ ($J =$ 18.5 and 5.1 hz) and 6.73 τ (multiplet). The dimethyl ester (*2*) spectrum corresponded, except for solvent and *p*H effects, to that of the biologically active salts between 6.5 and 8.5 τ. Between 6.1 and 6.3 τ, six additional methyl ester protons were recorded.

The unusually small coupling constant for the methyl protons suggested that this group was attached to a small ring. This inference, together with a consideration of the chemical shifts of the methine protons, suggested an epoxide. The large second coupling ($J =$ 18.5 hz) of the methine which appeared at 7.1 τ and the long-range coupling ($J = 0.7$ hz) which was visible in the methyl doublet required an interacting nucleus other than hydrogen. A phosphonic acid group satisfied the requirement and was consistent with the acidic nature of the antibiotic, with the observed coupling ($J = 10.5$ hz) of both methoxy signals of the ester, with a strong infrared band at 1200 cm^{-1} (*3*), and with a positive response to the molybdate reagent of Hanes and Isherwood (*4*). The coupling of 5.1 hz between the epoxide protons indicated a *cis* configuration (*5*), and the strong deshielding of the methyl protons by the P → O group agreed with this assignment. Further evidence for the *cis* relationship was provided by the observed nuclear Overhauser effect for the methyl protons upon irradiation of the phosphorus nucleus (*6*). These data led to a proposal that the antibiotic could be a *cis*-1,2-epoxypropylphosphonate. This structure was confirmed by mass spectrometric analysis of the dimethyl ester (the molecular weight calculated for $C_5H_{11}O_4P$ is 166.0395; we found 166.0398) and by synthesis as follows.

Propynylmagnesium bromide and di-*n*-butyl chlorophosphoridate reacted smoothly in benzene-tetrahydrofuran solution to afford di-*n*-butyl propynylphosphonate (**3**), which boiled at 102°C at 0.15 mm. Elemental analysis indicated (percent) C, 56.96; H, 8.99. Calculated values (percent) for $C_{11}H_{21}O_3P$ are C, 56.88; H, 9.11. Catalytic hydrogenation of **3** with Lindlar catalyst gave di-*n*-butyl *cis*-propenylphosphonate (**4**) (*7*) which boiled at 72°C at 0.12 mm. Elemental analysis indicated (percent) C, 56.52; H, 9.81. Calculated values (percent) for $C_{11}H_{23}O_3P$ are C, 56.39; H, 9.90. Compound **4** could be hydrolyzed with concentrated hydrochloric acid to the corresponding acid **5**, which was used in the next step without purification, but was characterized as the dicyclohexylammonium salt, **5a**, melting from 216° to 219°C. Elemental analyses indicated (percent) C, 59.67; H, 10.11; N, 4.83. Calculated values (percent) for $C_{15}H_{30}NO_3P$ are C, 59.39; H, 9.97; N, 4.62. Neutralization of an aqueous solution of the free acid to *p*H 5.5 with ammonia and epoxidation with an excess of 30 percent hydrogen peroxide at 60°C for 1 hour in the presence of 2 percent (by weight) of sodium tungstate (*8*) yielded ammonium (±)-*cis*-1,2-epoxypropylphosphonate (**2**).

Resolution was accomplished by way of the quinine salt; neutralization of the resulting acid with one equivalent of benzylamine gave benzylammonium (−)-*cis*-1,2-epoxypropylphosphonate (**1a**), melting from 170° to 4°C (capillary tube, rapid heating). The specific rotation $[\alpha]_{405}$ was −9.1° (5 percent solution in H_2O). Elemental analysis indicated (percent) C, 49.40; H, 6.45; N, 5.54. Calculated values (percent) for $C_{10}H_{16}NO_4P$ are C, 48.98; H, 6.58; N, 5.71. The identity of this product with the benzylammonium salt of the natural product was established by direct comparison.

The absolute configuration of phosphonomycin was determined chemically. A solution of the free acid **1** in methanol was heated at 40°C for 18 hours to give *threo*-1-hydroxy-2-methoxypropylphosphonic acid (**6**), melting from 168° to 171°C; $[\alpha]_{405}$ was −54° (5 percent solution in H_2O). Elemental analyses (percent) showed C, 28.27; H, 6.43. Calculated values (percent) for $C_4H_{11}PO_5$ are C, 28.24; H, 6.52. Neutral permanganate oxidation of the sodium salt **6** gave D-2-methoxypropionic acid (**7**); $[\alpha]_D$ was +72° (*9*). Thus, the β-carbon atom of phosphonomycin (**1**) is configurationally related to L-lactic acid if inversion at the β-carbon atom occurred during opening of the ring. Evidence for the expected inversion during methanolysis was provided by completely methylating compound **6** with silver oxide and methyl iodide to give dimethyl *threo*-1,2-dimethoxypropylphosphonate (**10**) boiling at 66°C at 0.1 mm. Elemental analysis indicated (percent) C, 39.54; H, 7.83. Calculated values (percent) for $C_7H_{17}PO_5$ are C, 39.62; H, 8.08. The infrared and nuclear magnetic resonance spectra and gas-liquid chromatography retention time of **10** were identical with those of the ester prepared by osmium tetroxide hydroxylation, hydrolysis, and subsequent methylation of diethyl *trans*-1-propenylphosphonate (**10**). This series of chemical transformations unambiguously defines the absolute stereochemistry of phosphonomycin as (−)-(1*R*,2*S*)-1,2-epoxypropylphosphonic acid (**1**) (*11*).

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2. The dimethyl ester was prepared from the crude antibiotic by brief reaction in aqueous methanol with ethereal diazomethane at 0°C, acidified to a *p*H of less than 2 with dilute hydrochloric acid.
3. We thank R. W. Walker for the infrared spectra, and R. N. Boos and his associates den Heuvel for gas-liquid chromatography data which were of assistance in the isolation of phosphonomycin, Dr. J. Beck for the mass

spectra, and R. N. Boos and his associates for the microanalyses.

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7. Under different conditions, varying amounts of the *cis* and *trans* isomers were obtained. The *trans* isomer was synthesized by an independent method and converted to sodium *trans*-1,2-epoxypropylphosphonate.
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11. The name phosphonomycin has been applied to the free phosphonic acid. The antibiotic, however, exists as the mono- or divalent phosphonate anion depending upon *p*H.

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Attention Shifts in a Maintained Discrimination

Abstract. *Pigeons received lights of varying wavelengths paired with sounds of varying frequencies; pecking was reinforced only at one stimulus combination. Then either the light or the sound was held constant at its reinforced value, while the other stimulus continued to vary. Subsequent tests showed that the constant stimulus had lost much of its control over the birds' responses.*

"We know a lot more about the conditions which make a stimulus 'relevant' than about those which make a stimulus 'irrelevant'." This remark by Thomas (*1*) is justified by the animal literature on attention, partly because most of this research has used a learning paradigm. Typically, naive subjects learn a task involving several stimuli; then they are tested to see which stimuli control their behavior. This paradigm emphasizes variables that favor the development of stimulus control, but it may obscure variables that affect loss of control or shifts in attention. Such matters have been clarified in human subjects by the use of difficult but well-learned discriminations that involve several classes of stimuli (*2*). My study applies some aspects of this approach to animal experimentation.

The subjects, three white Carneaux pigeons, were maintained at approximately 80 percent of their free-feeding weight. All had a long history of visual discrimination training, and prior to this experiment they had, for 6 months, daily sessions on variations of the visual and auditory tasks required in this study. The pigeon pecked at a plastic disk in an insulated chamber. Pecking produced reinforcement (2-second access to mixed grain) intermittently in the presence of one specific combination of visual and auditory stimuli. The visual stimuli were provided by a spot of monochromatic light about 0.9 cm in diameter on the plastic disk. This spot assumed one of seven wavelengths (576 to 582 nm in 1-nm steps), with a half-width dispersion of 6.6 nm and a luminance of about 7.0 millilamberts. The auditory stimuli were provided by tones from a 7.6-cm loudspeaker set in one side of the chamber. The tone assumed seven frequencies (3370 to 3990 hz in approximately equal steps) of somewhat distorted sinusoidal form. Though the chamber walls were lined with absorbent material, standing waves were prominent in the chamber; the stimulus intensity varied over a range of about 6 db near the pigeon's head, with a mean of 92 db. In addition to these variable stimuli, a small bulb dimly illuminated the chamber with indirect light, and a white noise at about 90 db was supplied through the stimulus loudspeaker at all times except during the presentation of tone stimuli.

Each day the pigeons received many brief trials during which a light and a sound came on simultaneously. On an average of 78 of these trials, a peck at the plastic disk produced reinforcement. On these trials, the sound was always set at 3990 hz and the light at 582 nm. The first trial in a session was a reinforced trial; thereafter, such trials occurred randomly, with a probability of 1/32. The remainder of the session comprised 2499 unreinforced trials. These consisted, in most sessions, of all 49 possible combinations of the seven tones and seven lights. The combination to occur on a given trial was chosen randomly, except that no combination reappeared until the entire series of 49 had appeared. This series was repeated 51 times.

During some sessions, as noted below, either the visual or the auditory stimulus remained at its reinforced value on all trials. In all other respects the procedure was the same as before. In particular, the birds were reinforced equally often and at the same stimulus

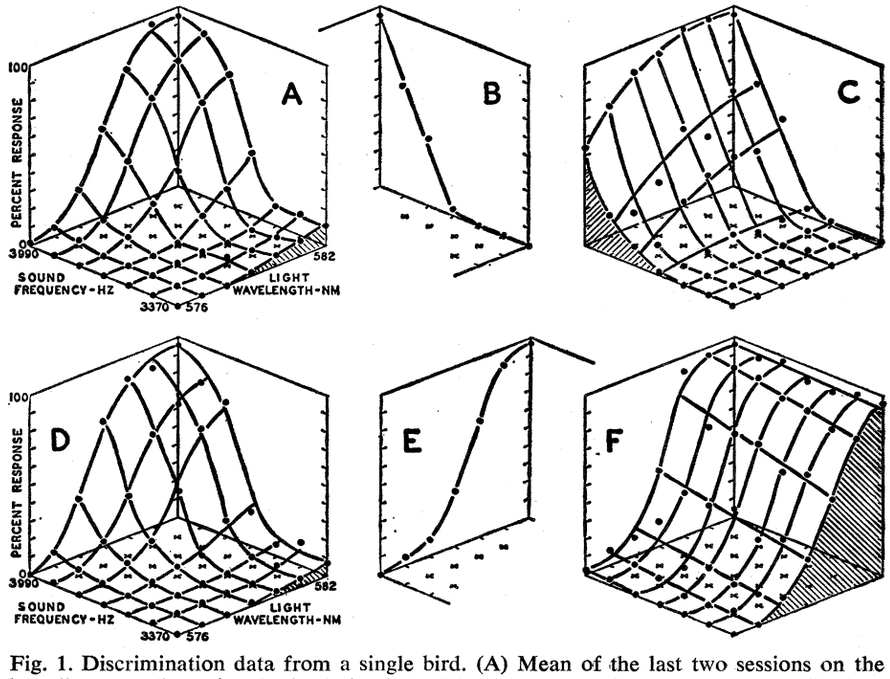

Fig. 1. Discrimination data from a single bird. (A) Mean of the last two sessions on the base-line two-dimensional discrimination; (B) last two sessions on auditory discrimination with visual stimulus constant at its reinforced value (582 nm); (C) first session on base-line procedure after visual constant training; (D) last two sessions on second base-line series; (E) last two sessions on visual discrimination with auditory stimulus constant at its reinforced value (3990 hz); (F) first session on base-line procedure after auditory constant training.