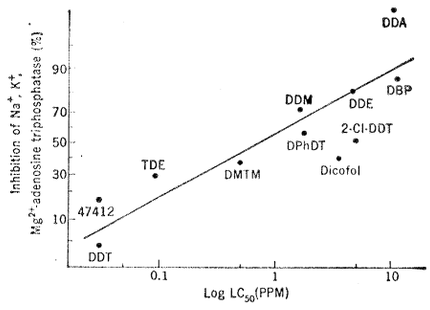

Fig. 2. Relation between the toxicities in vivo of DDT analogs (*9*) toward mosquito larvae (*Aedes aegypti*) and the amount of the Na^+,K^+,Mg^{2+}-adenosine triphosphatase activity remaining after inhibition by $10^{-5}M$ of these analogs [expressed in percent activity of the enzyme remaining in probability units (*16*)]. The toxicities are expressed in terms of insecticide concentration which gives 50 percent of mortality (LC_{50}) in the mosquito populations as tested by the World Health Organization standard method. The LC_{50} value for DDA could not be determined because of its low toxicity to mosquito larvae.

phosphatase in this preparation and that one of them was particularly sensitive to DDT. The inhibitor concentrations that resulted in 50 percent inhibition (I_{50}) of Na^+,K^+,Mg^{2+}-adenosine triphosphatase was for DDT approximately $3 \times 10^{-7}M$ as opposed to $3 \times 10^{-4}M$ for DDE.

The same enzyme source was also tested against DDT and DDE in the K^+,Mg^{2+} buffer at *p*H 7 (*3*). The inhibition curves obtained for K^+,Mg^{2+}-adenosine triphosphatases were almost identical to those in Fig. 1.

To study the relation between the actual toxicities in vivo and the extent of adenosine triphosphatase inhibition, several DDT analogs (final concentration, $10^{-5}M$) were incubated with the same enzyme preparation. The results (Fig. 2) indicated that the degrees of inhibition of the Na^+,K^+,Mg^{2+}-adenosine triphosphatases were closely related to the actual toxicities in vivo against mosquito larvae of those DDT analogs.

The insecticide DDT is much more toxic at lower temperature (negative temperature correlation). Experiments, at 37°C, indicated that the activity of Na^+,K^+,Mg^{2+}-adenosine triphosphatase in relation to the total adenosine triphosphatase activity (mainly Mg^{2+}-adenosine triphosphatases) was much less at 37°C (less than 10 percent). Accurate assessment of toxicity of DDT to this small portion of Na^+,K^+,Mg^{2+}-adenosine triphosphatase activity was, therefore, difficult. The I_{50} value appeared to be, however, higher than $10^{-5}M$. When the incubation temperature was lowered to 13°C, the activity of the Na^+,K^+,Mg^{2+}-adeonosine triphosphatase increased to 50 percent of the total adenosine triphosphatase activity and the I_{50} value toward DDT decreased to approximately $5 \times 10^{-8}M$. Moreover at this temperature, 100 percent of the Na^+,K^+,Mg^{2+}-adenosine triphosphatases were sensitive to DDT.

Electrophysiological studies (*11*) indicate that the minimum concentration for DDT to elicit the neurophysiological symptoms of DDT poisoning in the isolated abdominal nerve cord (central nervous system) of the German cockroach at 24°C is 1 to $3 \times 10^{-6}M$. Eaton and Sternburg (*12*) showed that when 1 μg (threshold or sublethal amount) of DDT was injected into a male American cockroach only a portion (25 percent) of given DDT (approximately $10^{-6}M$) reaches to the nerve cord itself, and the remainder stays with the lipid sheath. Our I_{50} value, therefore, is at least in the same order as the actual threshold concentration of DDT in the central nervous systems of the German and American cockroach.

Another piece of evidence supporting the view that this enzyme system is involved in the actual poisoning processes of DDT is the fact that the same enzyme preparation contains a component which specifically binds with C^{14}-DDT (*13*). The concentration of specific DDT-binding substances is also in the same magnitude at $3 \times 10^{-7}M$ (*13, 14*).

The Na^+,K^+,Mg^{2+}-adenosine triphosphatases are known to be present in many biological membranes and the evidence of their contribution to the process of active transport of Na^+ and K^+ is accumulating (*4*). Although the synapses are not known to be the most sensitive site for DDT attack (sensory endings are), they take part in the process of DDT poisoning (*15*) and contain significant amounts of Na^+,K^+, Mg^{+2}-adenosine triphosphatases. Therefore, the involvement of an adenosine triphosphatase or an adenosine triphosphate-utilizing system, or both, in DDT poisoning is a likely possibility.

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References and Notes

1. T. Yamasaki and T. Narahashi, *Botyu-Kaguku* **22**, 296 (1957); W. E. Dale, T. B. Gaines, W. J. Hayes, G. W. Pearce, *Science* **142**, 1474 (1963); T. Narahashi and H. G. Haas, *ibid.* **157**, 1438 (1967).
2. R. B. Koch, *J. Neurochem.* **16**, 269 (1969).
3. F. Matsumura, T. A. Bratkowski, K. C. Patil, *Bull. Environ. Contam. Toxicol.*, in press.
4. J. C. Skou, *Physiol. Rev.* **45**, 597 (1965).
5. S. Puszkin, S. Berl, E. Puszkin, D. D. Clarke, *Science* **161**, 179 (1968).
6. O. Gonda, A. Kaluszymer, Y. Avi-Dor, *Biochem. J.* **73**, 583 (1959).
7. J. C. Skou, *Biochim. Biophys. Acta* **58**, 314 (1962).
8. ———, *Biochem. Biophys. Res. Comm.* **10**, 79 (1963).
9. Abbreviations are: DDT, 1,1,1-trichloro-2,2-*bis*(*p*-chlorophenyl)ethane; and DDE, 1,1-dichloro - 2,2 - *bis*(*p* - chlorophenyl)ethylene. Chemical names of uncommon DDT analogs are: DDM, *bis*(*p*-chlorophenyl)methane; DPhDT, 1,1 - diphenyl - 2,2,2 - trichloroethane; DBP, *p*-chlorophenyl-*p*-chlorobenzophenone; CP-47412, 1,1-*bis*(*p*-chlorophenyl)-2,3-dichlorocyclopropane; DMTM, 1,1-*bis*(*p*-methoxyphenyl)-2,2,2-trimethylethane; 1-Cl-DDT, 1,1-*bis*(*p*-chlorophenyl)-1-chloro-2,2,2-trichloroethane; DDA, 1,1-*bis*(*p*-chlorophenyl) acetic acid; TDE, 1,1-dichloro-2,2-*bis*(*p*-chlorophenyl)ethane; and dicofol, 1,1,1-trichloro-2,2-*bis*(*p*-chlorophenyl)ethanol.
10. B. B. Marsh, *Biochim. Biophys. Acta* **32**, 357 (1959).
11. C. M. Wang and F. Matsumura, unpublished data.
12. J. L. Eaton and J. G. Sternburg, *J. Econ. Entomol.* **60**, 1699 (1967).
13. H. Brunnert and F. Matsumura, *Biochem. J.*, in press.
14. F. Matsumura and R. D. O'Brien, *J. Agr. Food Chem.* **14**, 36 (1966).
15. J. L. Eaton and J. G. Sternburg. *J. Econ. Entomol.* **60**, 1358 (1967)
16. C. I. Bliss, *Science* **79**, 38 (1935).
17. Supported in part by a PHS research grant CC-00252 from the National Communicable Disease Center, Atlanta, Ga. We thank Dr. D. J. Hennessy for some of the analogs of DDT.

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Phosphonomycin, a New Antibiotic Produced by Strains of Streptomyces

Abstract. *Phosphonomycin is a newly discovered antibiotic produced by streptomycetes. It is effective, when administered by the oral route, to mice infected with Gram-positive or Gram-negative microorganisms. The antibiotic is bactericidal and inhibits cell-wall synthesis.*

A new antibiotic, phosphonomycin, was discovered during a search for organisms producing broad-spectrum antibiotics. We report here some of the biological properties of the agent.

Phosphonomycin is produced in aerated, submerged cultures of strains of *Streptomyces fradiae* (ATCC 21096), *Streptomyces viridochromogenes* (ATCC 21240), and *Streptomyces wedmorensis* (ATCC 21239) grown on a variety of media. One medium which produced activity and was employed for the isolation studies had the following composition: oatmeal, 20 g; tomato paste, 20 g; and water to 1 liter. Phosphonomycin was differentiated from

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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References and Notes

1. E. O. Stapley, *Appl. Microbiol.* **6**, 392 (1958).
2. L. Chaiet, T. W. Miller, A. Kempf, R. Goegelman, F. J. Wolf, in preparation.
3. J. G. Vincent and H. W. Vincent, *Proc. Soc. Exp. Biol. Med.* **55**, 162 (1944).
4. A. K. Miller, *Chemotherapia* **8**, 154 (1964).
5. B. G. Christensen, W. J. Leanza, T. R. Beattie, A. A. Patchett, B. H. Arison, R. E. Ormond, F. A. Kuehl, Jr., G. Albers-Schonberg, O. Jardetzky, *Science*, this issue.
6. F. M. Kahan and J. E. Kahan, in preparation.
7. J. P. Koch, S. Hayashi, E. C. C. Lin, *J. Biol. Chem.* **239**, 3106 (1964).
8. J. L. Strominger, *Biochim. Biophys. Acta* **30**, 645 (1958); K. G. Gunetileke and R. A. Anwar, *J. Biol. Chem.* **243**, 5770 (1968).
9. E. L. Foltz and H. Wallick, in preparation.

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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,