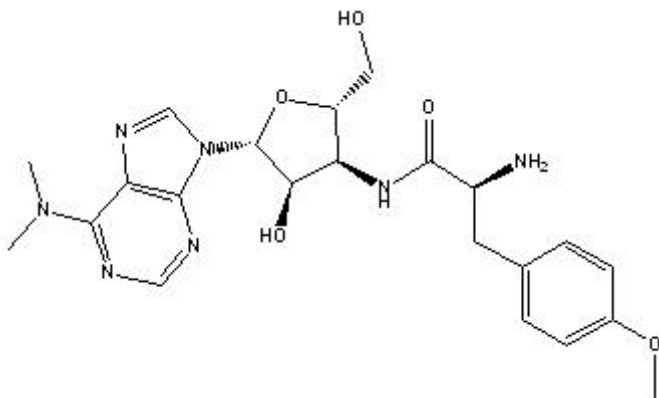


TECHNICAL INFORMATION

Catalog Number: 100552, 194539

Puromycin Dihydrochloride

Structure:



Molecular Formula: C₂₂H₂₉N₇O₅·2HCl

Molecular Weight: 544.4

CAS #: 58-58-2

Synonym: 6-dimethylamino-9-(3'-deoxy-3'-(p-methoxy-L-phenylalanylamino)- B-D-ribofuranosyl)-purine

Physical Description: White to off white powder

Solubility: Soluble in water (50 mg/ml - clear, colorless solution)

Description: Puromycin is an antibiotic of unique structure and biological activity. It is produced by fermentation using *Streptomyces albo-niger*. There are four aspects of puromycin's biological activity that are 1) antitumor effect; 2) nephrotoxic action; 3) inhibition of purine and/or protein synthesis and 4) antitrypanosome activity.

Antitumor Effect: puromycin has been tested against a variety of experimental tumors in various animals (Troy et al. 1953 and Suigiura et al. 1958). As might be expected, a range of effects from the destructive or inhibitory to mild or no activity was obtained, depending upon the type of tumor and dosage. In some cases toxicity accompanied carcinostasis, since peak activity was only apparent at maximum tolerated doses.

Puromycin showed significant cytotoxicity in vitro against a series of tissue cultures derived from normal and neoplastic human and animal cells (Cobb 1955, Eagle and Foley 1956, 1958, and Foley and Handler 1958).

In an agar plate assay procedure for antitumor agents, which employed four human cell lines and sarcoma 180 of mouse origin, it was found that the human cell lines were about 2-4 times more sensitive to puromycin than was sarcoma 180 (Schuurmans et al. 1961).

Neprosis: There is evidence of kidney damage in rats after repeated injections of puromycin for one to four weeks (Hewitt et al. 1953, Sherman et al. 1954, Borowsky et al. 1958). Mephrosis was manifested by elevated serum cholesterol, decreased total serum proteins, hypoalbuminemia, progressive proteinuria, and casts. Weights of the treated rats were somewhat lower than the controls.

A great number of references exist in regard to the experimental nephrotic syndrome, but practically all of this work is concerned with the use of the aminonucleoside derivative of puromycin, which is more active than puromycin.

Inhibition of Purine and/or Protein Synthesis: Because of its chemical structure it was natural to attribute the growth inhibitory effect of puromycin to its possible role as an inhibitor of purine, nucleic acid, or protein synthesis and metabolism in many cell systems, mainly microbial. uromycin was a competitive antagonist for guanylic acid in the growth of the protozoan *Tetrahymena pyriformis*; the complete molecule was necessary for the maximum inhibitory action (Bortle and Oleson 1954). In studies on the effects of various inhibitors on the photosynthetic reaction, it was reported that puromycin was a growth stimulant at low concentrations (10⁻⁴M) for *Chlorella pyrenoidosa*, but at higher concentrations (10⁻²) it completely inhibited growth (Tomisek et al. 1957).

Puromycin had some inhibitory action on the incorporation of radioactive glycine in disrupted *Staphylococcus aureus* strain Duncan cells, but it was the least active of the five antibiotics tested (Gale and Folkes 1957).

Puromycin showed an inhibitory activity against a purine-requiring strain of *Escherichia coli*, which was reversed by guanine but not adenine (Collier and Huskinson 1957).

Adenine by itself also was incapable of reversing the growth inhibition due to the action of puromycin on *Lactobacillus plantarum* (Hutchings 1957). However, when uracil or uridine was also added, the combination with adenine or other purines permitted growth at levels of puromycin which produced less than maximum inhibition.

Puromycin completely inhibited protein synthesis but not nucleic acid synthesis in *Pseudomonas fluorescens* (Asanuma 1953,

and Takeda et al. 1960). The latter authors further confirmed these observations by experiments in which orthophosphate -P 33 and methionine -3 35 were used as radioactive markers for ribonucleic acid and protein synthesis, respectively.

Although guanine and adenine stimulated riboflavin synthesis by *Eremothecium ashbyii*, puromycin did not inhibit such synthesis when used in concentrations subinhibitory to growth (Brown et al. 1958).

After treatment with puromycin the uredospores of the corn rust fungus, *Puccinia sorghi*, incorporated radioactivity from L-leucine-C 14 and from acetate-2-C 14 into the protein fractions more than twice as fast as spores treated with water (Staples et al. 1961).

In experiments with *Escherichia coli* it was concluded that puromycin prevented the final condensation of activated amino acids to peptides (Mathans and Lipman 192, and vonShronstein and Lipman 1961).

Similarly, in earlier work, it was found that puromycin inhibited the incorporation of L-Leucine-C14 into protein in a cell-free preparation from rat liver. This inhibition was believed to be due to the fact that no leucine-C14 was transferred from soluble (or transfer) ribonucleic acid-leucine C14 to microsomal protein (Yarmolinsky and de la Haba 1959).

Administration of puromycin to ovariectomized rats blocked the incorporation of glycine-3-C14 into uterine protein *in vivo* without inhibiting its incorporation into the adenine of the mixed nucleic acids or the incorporation of orthophosphate-P32 into ribonucleic acids and ethanolamine phosphatides. Under the same conditions, puromycin treatment blocked protein synthesis, prevented imbibition of water, and prohibited the early acceleration of phospholipid and ribonucleic acid synthesis in the uteri of estradiol-treated rats (Mueller et al. 1961).

As a further indication of interference with protein synthesis, puromycin at growth inhibitory concentrations (10 micrograms per ml.) completely inhibited B-galactosidase formation in washed cells of *Staphylococcus aureus* strain Duncan (Creaser 1955), but no inhibitory action of puromycin on pancreatic ribonuclease was detected (Heymann et al. 1958).

It has been found that with *Trypanosoma cruzi* in Warburg flasks puromycin has little or no effect on the rate of incorporation of glycine-1-C14 into protein and acid soluble adenine nucleotides, or the incorporation of adenine-S-C14 into acid soluble adenine compounds and mixed nucleic acids purines (Fernandes and Castellani 1958). These results are of interest: 1.) because puromycin has a trypanostatic effect on another species of trypanosoma, namely, *Trypanosoma equiperdum* that can be counteracted by adenine or various substituted purines (Hewitt et al. 1954, Agosin and von Brand 1954); 2.) while the intact puromycin molecule has no such action against *Trypanosoma cruzi*, its aminonucleoside moiety is active as an adenine antagonist for this organism, and no difference in permeability of *Trypanosoma cruzi* to puromycin or its aminonucleoside could be found (Fernandes and Castellani 1959).

Antitrypanosome Activity: Puromycin cured *Trypanosoma equiperdum* infections in mice and rabbits, and was partially effective against *T. cruzi*. Multiple doses were more effective and less toxic than single doses (Hewitt et al. 1953). These results were confirmed in *T. gambiense* and *T. rhodesiense* infections in mice and *in vitro* (Trincao et al. 1955 and 1956), and *T. equiperdum* infections in mice (Agolini 1957).

Puromycin was tested for its trypanocidal properties against six species in mice (Tobie 1954). When treatment was begun approximately four hours after inoculation with the trypanosomes, or even at the height of infection, puromycin had a strong suppressive effect against all species except *T. congolense*. Administration of the antibiotic four days prior to inoculation did not prevent infections from progressing.

In two experiments with white rats infected with *T. rhodesiense* it was found that best results with puromycin were obtained with total doses of 430-500 mg. per kg., given in ten equal daily intraperitoneal doses commencing one or more days after the trypanosomes were visible in the peripheral blood. Some of the treated rats died without parasitaemia, presumably due to the toxicity of the antibiotic (Baker 1957).

Availability:

Catalog Number	Description	Size
100552	Puromycin dihydrochloride	10 mg 25 mg 100 mg 250 mg 500 mg 1 g
194539	Puromycin dihydrochloride, cell culture reagent	10 mg 25 mg 100 mg

References:

- Agolini, G. "Confronto di attivita per alcuni chemioterapici verso la infezione sperimentale da *Trypanosoma equiperdum*." *Boll. Soc. Ital. Biol. Sper.*, v. 33, 877-880, (1957).
- Agosin, M., and von Brand, T. "The influence of puromycin on the carbohydrate metabolism of *Trypanosoma equiperdum*." *Antibiotics & Chemother.*, v. 4, 624-632, (1954).
- Asamuna, K. "Effect of puromycin on ribonucleic acid and protein biosynthesis in *Pseudomonas fluorescens*." *Osaka Kaigaku Igaku Zasshi*, v. 10, 2089-2094, (1958) *Chem. Abstr.* v. 53, 7320d, (1959).
- Baker, B.R., Scaub, R.E., Joseph, J.P., and Williams, J.H. "Total synthesis of the antibiotic puromycin." *J. Am. Chem. Soc.*, v. 76, 4044-4045, (1954).
- Baker, B.R., Schaub, R.E., Joseph, J.P., and Williams, J.H. "Puromycin Synthetic studies IX. Total synthesis." *J. Am. Chem. Soc.*, v. 77, 12-15, (1955).
- Baker, J.R. "Stylomycin as a therapeutic agent in *Trypanosoma rhodesiense* infections in white rats." *Trans. Roy. Soc. Trop. Med. & Hyg.*, v 51, 183-185, (1957).
- Borowsky, B.A., Kessner, D.M., and Recant, L. "Structural analogues of puromycin in production of experimental nephrosis in rats." *Proc. Soc. Exptl. Biol. & Med.*, v. 97, 857-860, (1958).
- Bortle, L. and Oleson, J. J. "Effect of puromycin on *Tetrahymena Pyriformis* nutrition." *Antibiotics annual 1954-1955*, 770-776.
- Brown, E.G., Goodwin, T.W., and Jones, O.T.G. "Biosynthesis of riboflavin. IV. Purine metabolism and riboflavin synthesis in *Eremothecium ashbyii*." *Biochem. J.*, v. 68, 40-49, (1958).
- Cobb, J.P. "Tissue culture observations of the effects of chemotherapeutic agents on human tumors." *Trans. N.Y. Acad. Sci.*

- Soc., v. 2, 17, 237-249, (1955).
- Collier, H.O. J., and Huskinson, P. L. "The effects of potential antipurines on a purine-requiring strain of Escherichia coli." *Ciba Foundation Symposium. Chem. and Biol. of Purines* **1957**, 146-156.
 - Creaser, E. H. "The induced (adaptive) biosynthesis of B-galactosidase in *Staphylococcus aureus*," *J. Gen. Microbiol.*, v. **12**, 283-295, (1955).
 - Eagle, W., and Foley, G. E. "The cytotoxic action of carcinolytic agents in Tissue culture." *Am. J. Med.*, v. **21**, 739-749, (1956).
 - Eagle, H., and Foley, G.E. "Cytotoxicity in Human Cell cultures as a primary screen for the detection of anti-tumor agents." *Cancer Res.*, v. **18**, 1017-1023, (1958).
 - von Ehrenstein, G., and Lipmann, F. "Experiments on hemoglobin biosynthesis." *Proc. Natl. Acad. Sci. U.S.*, v. **47**, 941-950, (1961).
 - Fernandes, J.J., and Castellani, O. "Nucleotide and polynucleotide synthesis in *Trypanosoma cruzi* I. Precursors of purine compounds." *Exptl. Parasitol.*, v. **7**, 224-235, (1958).
 - Fernandes, J. F. and Castellani, O. "Nucleotide and polynucleotide synthesis in *Trypanosoma cruzi*. II. In Vitro effect of tioguanine and of the aminonucleoside of styromycin." *Exptl. Parasitol.*, v. **8**, 480-485, (1959).
 - Foley, G. E., and Handler, A. H. "Tumorigenic activity of tissue cell cultures." *Ann. N.Y. Acad. Sci.*, v. **76**, 506-512, (1958).
 - Fryth, P.W., Walker, C.W., Hutchings, B.L. and Williams, H.J. "The structure of the antibiotic puromycin." *J. Am. Chem. Soc.*, v. **80**, 2736-2740, (1958).
 - Gale, E.F., and Folkes, J.P. "The assimilation of amino acids by bacteria. 24. Inhibitors of incorporation of glycine in disrupted stahylococcal cells." *Biochem. J.*, v. **67**, 507-517, (1957).
 - Hesseltine, C.W., Porter, J. N., Deduck, N., Hauck, M., Bohonos, N., and Williams, J.H. "A new species of streptomycetes." *Mycologia*, v. **46**, 16-23, (1954).
 - Hewitt, R. I., Wallace, W.S., Gumble, A.R., Gill, E.R., and Williams, J.H. "Experimental chemotherapy of trypanosomiasis. III. Effect of achromycin against *Trypanosoma equiperdum* and *Trypanosoma cruzi*." *Am. J. Trop. Med.*, v. **2**,
 - Hewitt, R.I., Gumble, A.R., Wallace, W.S., and Williams, J.H. "Experimental chemotherapy of trypanosomiasis. IV. Reversal by purines of the in vivo activity of puromycin, and an amino nucleoside analog. against *Trypanosoma equiperdum*". *Antibiotics and Chemother.*, v. **4**, 1222-1227, (1958).
 - Heymann, H., Gulick, Z.R., DeBoer, C.J., deStevens, G., and Mayer, R.L. "The inhibition of ribonuclease by acidic polymers and their use as possible antiviral agents." *Arch. Biochem.*, v. **73**, 366-383, (1958).
 - Hutchings, B.L. "Puromycin." *Ciba Foundation Symposium, Chem. and Biol. of Purines* **1957**, 177-178.
 - Mueller, G.C., Gorski, J., and Aizawa, Y. "The role of protein synthesis in early estrogen action." *Proc. Natl. Acad. Sci. U.S.*, v. **47**, 164-169, (1961).
 - Nathans, D. and Lipmann, F. "Amino Acid transfer from aminoacyl-ribonucleic acids to protein on ribosomes of *E. coli*." *Proc. Natl. Acad. Sci. U.S.*, v. **47**, 497-504, (1961).
 - Porter, J.M., Hewitt, R.I., Hesseltine, C.W., Krupa, G., Lowery, J.A., Wallace, W.S., Bohonos, N., and Williams, J.H. "Achromycin: a new antibiotic having trypanocidal properties." *Antibiotics and Chemother.*, v. **2**, 409-410, (1952).
 - Porter, J.N., Krupa, G.C., and Bohonos, N. (to American Cynamid Co.) "Puromycin." **U.S. Patent 2,763,642**. Sept. 18, 1956; *Chem. Abstr.* **51**, 3936b, (1957).
 - Schuurmans, D.M., Duncan, D.T., and Olson, B.H. "Variations in drug sensitivity among five mammalian cell lines used in an agar plate assay." *Cancer Res.*, v. **21**, 773-777, (1961).
 - Sherman, J.F., Taylor, D.J., and Bond, H.W. "Puromycin. III. Toxicology and Pharmacology." *Antibiotics Annual* **1954-1955**, 757-765.
 - Staples, R.C., Syamananda, R., and Block, R.J. "Puromycin-induced changes in uredospores of *Puccinia sorgh*." *Schw. Science*, v. **134**, 739, (1961).
 - Sugiura, K., Stock, C.C., Reilly, H.C., and Schmid, M.M. "Studies in a tumor spectrum. VII. The effect of antibiotics on the growth of a variety of mouse, rat, and hamster tumors." *Cancer Res.*, v. **18**, 66-77, (1958).
 - Szumski, S.A., and Goodman, J.J. (to American Cynamid Co.) "Puromycin." **U.S. Patent 2,797,187**, June 25, 1957; *Chem Abstr.* **51**, 1421e, (1957).
 - Takeda, Y., Havashi, S., Nakagawa, H., and Suzuki, F. "The effect of puromycin on ribonucleic acid and protein synthesis." *J. Biochem (Japan)*, v. **48**, 169-177, (1960).
 - Tobio, E.J. "The effect of puromycin on six species of *Trypanosoma* in mice." *Am. J. Trop. Med.*, v. **3**, 852-859, (1954).
 - Tomisek, A., Reid, N.R., Short, W.A., and Skipper, H.E. "Studies on the photosynthetic reaction. II. The effects of various inhibitors upon growth and carbonatfixation in *Chlorella pyrenoidosa*." *Plant Physiol.*, v. **32**, 7-10, (1957).
 - Trincao, C., Nogueira, A.A.R., and Franco, T.A. "Quelques essais sur l'activate therapeutique de la stilemycine (ex achromycine et puromycine) dans la trypanosomiase experimentale chez la souris (*T. gambiense* et *rhodosiense*)." *Compt. Rend. Soc. Biol.*, v. **149**, 1815-1817, (1955); *Antibiotics & Chemother.*, v. **5**, 505-507, (1955).
 - Trincao, C., Nogueira, A.R. and Del Almeda Franco, L.T. "Accao de puromicina (estilomicina) sobre as culturas "in Vitro" do "Trypanozoma gambiense." Action of puromycin (Stylomycin) on cultures of *Trypanosoma gambiense* in vitro." *Anais Inst. Med. Trop. Lisbon*, v. **13**, 429-431, (1956); *Trop Dis. Bull.*, v. **54**, (Abs.) 271, (1957).
 - Troy, W., Smith, s., Personeus, G., Moser, L., James, E., Sparks, S.J., Stevens, M., Halliday, s., McKenzie, d., and Oleson, J.J. "The effect of puromycin on experimental tumors." *Antibiotics Annual* **1953-1954**, 186-190.
 - Waller, C.W., Fryth, P.W., Hutchings, B.L., and Williams, J.H. Achromycin. "The structure of the antibiotic puromycin." *I.J. Am. Chem. Soc.*, v. **75**, 2025, (1953).
 - Yarmolinsky, M.B., and Haba, G.L. de la. "Inhiibition by puromycin of amino acid incorporation into protein." *Proc. Natl. Acad. Sci. U.S.*, v. **45**, 1721-1729, (1959).