Biochemical Pharmacology, Vol. 31, No. 2, pp. 143–148, 1982.
Printed in Great Britain.

0006-2952/82/020143-06 $03.00/0

ANTILEISHMANIAL ACTION OF 4-THIOPYRAZOLO (3,4-*d*)PYRIMIDINE AND ITS RIBONUCLEOSIDE

BIOLOGICAL EFFECTS AND METABOLISM

J. JOSEPH MARR* and RANDOLPH L. BERENS
Departments of Medicine and Microbiology, St. Louis University School of Medicine, St. Louis, MO 63104, U.S.A
and
DONALD J. NELSON, THOMAS A. KRENITSKY, THOMAS SPECTOR, STEPHEN W. LAFON and GERTRUDE B. ELION
Wellcome Research Laboratories, Research Triangle Park, NC 27709, U.S.A.

(*Received* 20 *March* 1981; *accepted* 20 *May* 1981)

Abstract—Thiopurinol [4-thiopyrazolo(3,4-*d*)pyrimidine, TPP] and its ribonucleoside (TPPR) were effective *in vitro* against the intracellular and extracellular forms of *Leishmania donovani* and the extracellular forms of *L. braziliensis* and *L. mexicana*. They also inhibited the transformation of the amastigote of *L. donovani* to the promastigote. These thio-analogues had about the same activity as allopurinol [4-hydroxypyrazolo(3,4-*d*)pyrimidine, HPP] and its ribonucleoside (HPPR). The thiopyrazolopyrimidines were converted primarily to the ribonucleoside-5'-phosphate (TPPR-MP) and to an unidentified metabolite, but not to any of the adenine ribonucleotide analogues previously shown to be formed from allopurinol and its ribonucleoside. There was an antagonism between the growth-inhibitory effects of allopurinol and thiopurinol. This is consistent with the findings that the intracellular concentrations of TPP and TPPR-MP are sufficient to inhibit the conversion of allopurinol to allopurinol ribonucleotide (HPPR-MP) by the hypoxanthine-guanine phosphoribosyltransferase by 30 per cent and the amination of HPPR-MP by adenylosuccinate synthetase by 50 per cent respectively. Consequently, the incorporation of the aminated product (aminopyrazolopyrimidine) into RNA was substantially decreased. The difference in metabolism between the thio- and hydroxypyrazolopyrimidines suggests a difference in their mechanisms of action against the pathogenic leishmania.

Allopurinol [4-hydroxypyrazolo(3,4-*d*)pyrimidine, HPP] is an antileishmanial agent *in vitro*. It inhibits the growth of *Leishmania donovani*, *L. braziliensis*, and *L. mexicana*. This inhibition can be reversed by adenine and its metabolic congeners [1, 2]. Similar results were shown in *Trypanosoma cruzi* [3], *T. rhodesiense* and *T. brucei* [4]. Biochemical investigations demonstrated metabolites of allopurinol that are unique to these organisms [3–7]. The adenine nucleotide analogues which are formed from allopurinol are incorporated into RNA. Because of the encouraging results obtained with allopurinol and with its ribonucleoside (HPPR) against the extracellular forms of the three pathogenic leishmanias [6], these compounds were tested against the intracellular forms of *L. donovani*. Both compounds eliminated amastigotes from cultured murine macrophages. The intracellular forms of *L. donovani* also transformed allopurinol into the metabolic products described above [7].

* Author to whom all correspondence should be addressed: Dr. J. Joseph Marr, Division of Infectious Diseases, Department of Internal Medicine, St. Louis University School of Medicine, 1325 South Grand Boulevard, St. Louis, MO 63104, U.S.A.

The above experimental data have given rise to several investigations by other laboratories which have borne out the effectiveness of allopurinol against several hemoflagellates. Two *in vitro* studies have shown the effectiveness of allopurinol against *T. brucei* and *T. rhodesiense* [8, 9]. More recently, Peters *et al.* [10] have shown the effectiveness of allopurinol in the treatment of visceral leishmaniasis, caused by *L. tropica major*, in mice. One small clinical study which used allopurinol in the treatment of patients who had pentostam-resistant visceral leishmaniasis found that allopurinol was useful in treatment of this disease [11]. A much larger clinical trial of allopurinol or its ribonucleoside will be necessary to derive conclusive results. One study documenting the effectiveness of allopurinol in the treatment of acute Chagas' disease in animals has appeared [12].

Because of the results from our laboratories and the encouraging data published by others, we have looked for other pyrazolopyrimidines which might have equal or greater value in the treatment of leishmaniasis or trypanosomiasis. In addition to seeking agents which might be useful clinically, we wished to develop more information about the metabolism of these compounds by the parasite

in order to gain further insights into the mechanism of the chemotherapeutic effectiveness of pyrazolopyrimidines.

Thiopurinol [4-thiopyrazolo(3,4-*d*)pyrimidine, TPP] is used in place of allopurinol for the treatment of hyperuricemia in some countries. The metabolism of thiopurinol in man, although less extensively studied, appears to be analogous to that of allopurinol except that no thiopurinol ribonucleoside (TPPR) has been detected [13, 14]. This report compares the antileishmanial activity of thiopurinol and its ribonucleoside to that of allopurinol and its ribonucleoside.

MATERIALS AND METHODS

Growth experiments. The experimental organisms and the method of their growth, quantitation, and harvesting were described previously [15, 16]. Experiments in which the amastigotes were permitted to transform to the promastigote form also have been described previously [2, 17].

Amastigotes were grown in tissue culture using the P388D$_1$ macrophage line. This is a continuous murine clone, [18, 19] obtained through the courtesy of Dr. H. S. Koren, Duke University Medical Center. The ability of these cells to support the replication of the amastigotes of *L. donovani* was reported earlier [20]. Experiments to investigate the effects of TPP and TPPR on amastigotes in tissue culture were performed as described [7].

Chemicals. Chemicals were obtained from the Sigma Chemical Co., St. Louis, MO, and were used without further purification. The thiopyrazolopyrimidines were provided by the Burroughs Wellcome Co., Research Triangle Park, NC. Purine and purine analogue bases were brought into solution with 0.1 N NaOH. Ribonucleosides and ribonucleotides were dissolved in morpholinopropanesulfonic acid (30 mM, pH 7.1). Compounds tested as antagonists of TPP and TPPR were added to the medium at a final concentration ten times that of the agonist. The pH of the medium was adjusted to 7.1 and sterilized by filtration (pore diameter 0.22 μm; Millipore Corp., Bedford, MA).

Nucleotide analysis. Analysis of perchloric acid-soluble extracts of cells for purine and pyrazolopyrimidine ribonucleotides was performed by anion high performance liquid chromatography (h.p.l.c.) as described previously [16].

Enzymatic assays. Nucleoside phosphotransferase (EC 2.7.1.77) was partially purified from promastigotes of *L. donovani* and assayed as described previously [6]. The adenylosuccinate synthetase (EC 6.3.4.4) was purified from promastigotes of *L. donovani* and assayed as described elsewhere [21].

Antagonism of thiopurinol and allopurinol. Promastigotes of *L. mexicana* were grown in the presence of HPP and TPP to determine if there was a synergistic, antagonistic, or additive effect of the combination. The experiments were done according to the method of Elion *et al.* [22] and consisted of determining several growth inhibition curves with increasing concentrations of HPP. Each of these experiments contained either no TPP or one of several fixed concentrations of TPP. Ninety percent inhibition was chosen to represent a significant depression of growth. These data were recorded as fractional inhibitory concentrations of each drug in the combination necessary to produce this inhibition.

Table 1. Activities of pyrazolo(3,4-*d*)pyrimidines against *Leishmania**

Organism	Agent (μM) TPP	HPP	TPPR	HPPR
L. braziliensis	20	50	4	3
L. donovani	100	300	2	0.3
L. mexicana	110	40	30	40

* The concentrations given in the table are those required to reduce the number of promastigotes by 90 percent as compared to control cultures—that is, an inhibitory concentration (IC_{90}).

RESULTS

Effect of thiopyrazolopyrimidines on leishmania promastigotes. Table 1 compares the antileishmanial effect of TPP and TPPR to that of allopurinol and its ribonucleoside. The pyrazolopyrimidine bases are of approximately equivalent antileishmanial activities. The ribonucleosides are also of essentially equal activity and are more active than their respective bases, except in *L. mexicana* where HPP and HPPR are of equal potency.

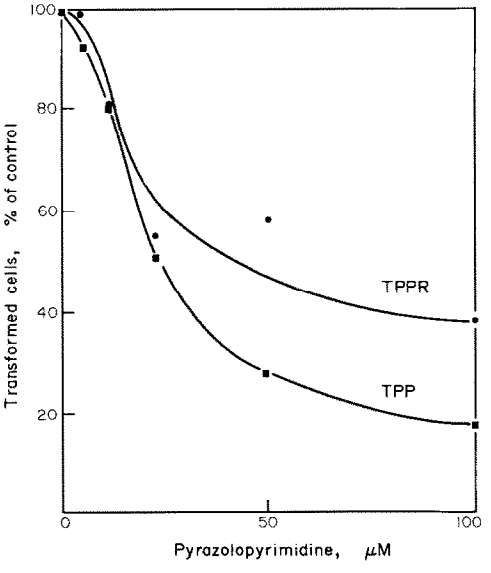

Fig. 1. Inhibition of transformation of *L. donovani* amastigotes to promastigotes. The experiment was carried out as described in Ref. 2. The percentage of transformed cells was defined as the number of cells with a visible flagellum per total cells counted, multiplied by 100. Usually 150–200 cells were counted per sample, with four replicate samples taken. The counts were made at 72 hr after exposure to the pyrazolopyrimidine. Approximately 75 percent of the amastigotes transformed to promastigotes; this was used as the reference value to determine percent transformation of those organisms exposed to TPP or TPPR.

Effect of thiopyrazolopyrimidines on the transformation of L. donovani *amastigotes to promastigotes.* Both TPP and TPPR inhibited the transformation of amastigotes of *L. donovani* to the promastigote form (Fig. 1). The concentration required to achieve 50 percent inhibition was approximately 25 μM for TPP and 50 μM for TPPR. At these concentrations a high percentage of the transformed promastigotes was significantly smaller and less motile than the controls and the remaining amastigotes had not undergone the enlargement which indicates the start of transformation [17].

Effect of thiopyrazolopyrimidines on the intracellular forms of L. donovani. Since the above experiment indicated that the amastigotes probably were susceptible to the effects of TPP and TPPR, the abilities of these compounds to inhibit the growth of amastigotes in macrophage tissue culture were tested (Fig. 2). The percentage of infected macrophages decreased to approximately 15 percent within 7 days. During the same time the infected, untreated cells had an infection rate of approximately 60 percent. At 15 days, the infected cells exposed to TPP and TPPR still had an apparent infection rate of 15 percent; untreated cells were 80 percent infected. Neither TPP nor TPPR inhibited the growth of uninfected macrophages when present at concentrations as high as 50 μg/ml (326 and 169 μM respectively). The infected, treated macrophages were placed at 26° on day 15 in an attempt to recover intracellular organisms from them. No organisms grew. Corresponding experiments with untreated, infected macrophages yielded amastigotes which transformed to promastigotes.

Metabolism of radiolabeled TPP and TPPR by L. donovani *promastigotes.* The promastigotes of *L. donovani* were incubated for 24 hr at a concentration of about 10^8 cells/ml in the presence of [4-^{35}S]TPP, [4-^{35}S]TPPR, and [U-^{14}C]ribose TPPR. All of the labeled precursors were added at a concentration of 5 μg/ml to the incubation medium; this corresponds to 33 and 17 μM for the base and ribonucleoside respectively. The perchloric acid-soluble extracts of these cells were analyzed by h.p.l.c. A radioactive metabolic product was identified which had a retention time and u.v. absorption spectrum identical to authentic 4-thiopyrazolo(3,4-*d*)pyrimidine ribonucleoside-5′-phosphate (TPPR-MP). It was present at a concentration of 39 pmoles/10^6 cells. This may be compared to an ATP concentration of 48 pmoles/10^6cells. There was no radioactivity in the di- or triphosphate region of the chromatogram, indicating that the organisms did not phosphorylate TPPR-MP beyond the 5′-monophosphate stage. There was no detectable conversion of unlabeled TPP or [^{14}C]TPPR to allopurinol ribonucleotide or aminopyrazolopyrimidine (APP) ribonucleotides. This method would detect 1 pmole/10^6 cells. The thiol group was stable for the duration of the experiment; no [^{35}S]sulfate was found when the radiolabeled sulfur compounds were used.

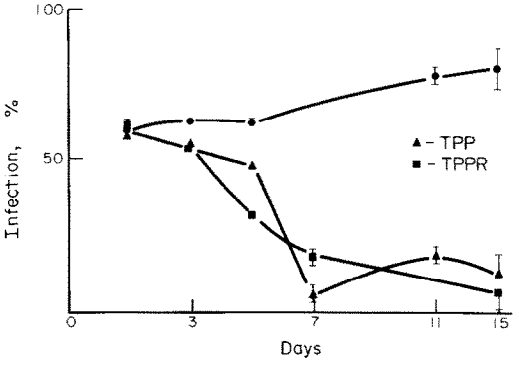

Fig. 2. Effect of thiopyrazolopyrimidines on growth of amastigotes of *L. donovani* in P338D$_1$ macrophage tissue culture. TPP was present at a concentration of 10 μg/ml and TPPR at a concentration of 20 μg/ml. These values correspond to a concentration of 63 μM. The activity of the infection was monitored using phase-contrast microscopy and, at the indicated times, cover slips were removed and stained with Giemsa stain. The percent of infected macrophages was determined using light microscopy by counting a minimum of twenty high power fields or 200 cells for each point. The variation in the method is indicated by the bars on the graph. The individual performing the counting did not know if the organisms had been exposed to drugs.

When radiolabeled TPPR was used as a precursor, labeled either in the sulfur or ribose moiety, TPPR-MP was found. The radiolabeled ribose moiety remained with the TPPR and was not present in any of the compounds in the purine or pyrimidine nucleotide pools.

In addition to TPPR-MP, a second peak appeared on the chromatogram in the region where the ribonucleoside monophosphates were eluted. This unknown metabolic product is formed from both TPP and TPPR, and contains both [^{35}S] and [^{14}C]-ribose when the appropriate radiolabeled precursors are used. This compound can be hydrolyzed by alkaline phosphatase to another product. This indicates that this unknown metabolite contains the radiolabeled sulfur moiety and the radiolabeled ribose moiety (when TPPR is used as a substrate), as well as a phosphate group. The compound has not been characterized further. It is present at approximately one-fifth the concentration of TPPR-MP. This unknown metabolic product is not 4-thio-6-aminopyrazolopyrimidine ribonucleoside monophosphate, 4-thio-6-hydroxypyrazolopyrimidine ribonucleoside monophosphate, or 4-methylthiopyrazolopyrimidine ribonucleoside monophosphate.

Enzymatic studies. Since the above metabolic investigations showed that both TPP and TPPR could be converted to the ribonucleoside monophosphate, enzymatic investigations were undertaken to clarify the metabolism of these compounds. An earlier investigation [23] demonstrated that TPP was a substrate for the hypoxanthine-guanine phosphoribosyltransferase (EC 2.4.2.8) of *L. donovani*. The K'_m for TPP was 400 μM with a V'_{max} of 27 percent of its normal substrate, guanine. The V'_{max}/K'_m ratio was 0.06 for the leishmanial enzyme, which was 10-fold greater than the ratio for the human enzyme.

Kinetic studies were performed with adenylosuccinate synthetase to establish the substrate and inhibitor activities of TPPR-MP with this enzyme. Substrate activities were determined using highly

purified [^{14}C]aspartic acid and the high voltage electrophoresis assay [21]. Inhibitor studies were performed using the spectrophotometric assay that indirectly measured the production of GDP [21, 24]. The substrate assay at 18 mM TPPR-MP revealed that the possible product, succino-APPR-MP, was not formed. This assay could have detected the production of succino-APPR-MP at a rate of 0.1 percent of the rate observed with IMP as the substrate. The inability of TPPR-MP to serve as a substrate of this enzyme corroborates the *in vivo* studies which did not detect the metabolic formation of APPR-MP. Although TPPR-MP was unable to substitute for IMP as a participant in the reaction, it was capable of inhibiting the conversion of IMP to succino-AMP. TRRP-MP produced competitive inhibition versus IMP with a $K_i \pm$ S.E of 81 ± 15 μM. The $K'_m \pm$ S.E. for IMP was 9.7 ± 1.7 μM.

The ribonucleoside of thiopurinol was phosphorylated by a nucleoside phosphotransferase partially purified from promastigotes of *L. donovani*. The kinetic data are similar to those found previously for the ribonucleoside of allopurinol [6]. The K'_m for TPPR was 3.4 mM; the K'_m for HPPR was 4.6 mM. The V'_{max} value for TPPR was appreciably greater than that reported for HPPR: 34 vs 17 nmoles·min^{-1}·(mg protein)$^{-1}$ respectively. The ability of nucleoside phosphotransferase to phosphorylate the ribonucleoside of thiopurinol corroborated the *in vivo* results given above.

Antagonism of thiopurinol and allopurinol. Several experiments were performed to determine whether TPP and HPP had synergistic, antagonistic, or additive effects on the growth of leishmania. Inhibition curves were determined with *L. mexicana* using TPP and HPP at various concentrations. A fractional inhibition index was calculated to illustrate the relationship between the two compounds [22]. This is shown in Fig. 3. The curve indicates an antagonism between the two compounds, most marked at lower concentrations of TPP. At these concentrations of TPP, a high concentration of HPP is less effective than when HPP is used alone; as the concentration of TPP increases, the biological effect of the latter becomes important and the curve returns toward the theoretical curve for additive effects.

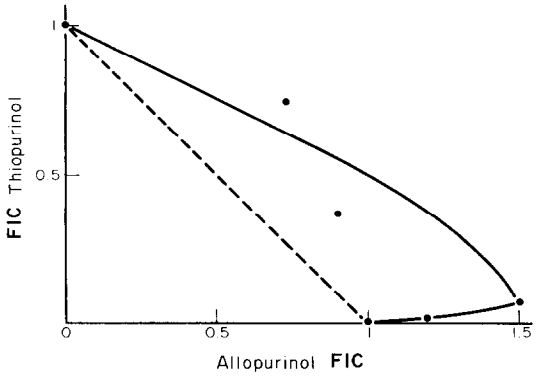

Fig. 3. Antagonistic action of thiopurinol and allopurinol in *L. mexicana*. A synergistic effect of the two drugs would produce a line which is concave and to the left of the dotted line; an additive effect would produce a line which falls on the dotted line; an antagonistic effect results in a line which is convex [22]. FIC is fractional inhibitory concentration.

Table 2. Inhibition of [^{14}C]HPP metabolism in *L. mexicana* by TPP*

Cellular nucleotides	[^{14}C]HPP +TPP	+[^{14}C]HPP only
	(pmoles/10^{6}cells)	
HPPR-MP	14.0	19.5
APPR-MP	0.4	0.8
APPR-DP	0.2	0.3
APPR-TP	0.04	0.09
Total HPP nucleotides	14.0	19.5
Total APP nucleotides	0.704	1.38
RNA (μg/10^6 cells)	12	15
APP nucleotides in RNA	0.064	0.19
Cell density (× 10^6/ml)	64	67
Concn. TPP (μM)	66	0

* Allopurinol was present at a final concentration of 7 μM and a specific activity of 52.4 mCi/mmole. Organisms were incubated for 2 hr with or without TPP and then for 4 hr with HPP.

Effect of thiopurinol on allopurinol metabolism. In view of the antagonism between TPP and HPP, *L. mexicana* and *L. donovani* promastigotes were grown to mid-log phase and then separated to three aliquots of 100 ml containing 5 × 10^6 organisms/ml. At zero time TPP was added to two of the aliquots, and all three flasks were incubated at 26° for 2 hr. At this time [6-^{14}C]HPP was added to the flask which did not contain TPP and to one of the flasks which did. All three flasks were incubated 4 hr longer, the organisms were collected by centrifugation and then processed for analysis by h.p.l.c. In agreement with the enzyme studies, there was inhibition of both the HGPRTase and the adenylosuccinate synthetase when both compounds were present (Table 2). The inhibition was not sufficient to prevent the formation of aminopyrazolopyrimidine ribonucleotides or HPPR-MP, but there was a 66 percent decrease in the amount of HPP incorporated into the RNA as 4-aminopyrazolo(3,4-*d*)pyrimidine when TPP was present.

DISCUSSION

Both TPP and TPPR were effective against the three pathogenic leishmania tested (Table 1). The sensitivities of these organisms to the base were approximately the same as to allopurinol. Their sensitivities to TPPR were approximately the same as to allopurinol ribonucleoside.

Evidence for the antileishmanial effect of TPP and TPPR on the amastigote forms of *L. donovani* is shown in Fig. 1. Both compounds prevented transformation of the amastigote to the promastigote. These data are similar to those obtained earlier with allopurinol and allopurinol ribonucleoside, both of which could prevent transformation [2, 6]. Since these organisms were not in their usual intracellular

environment, their metabolism of pyrazolopyrimidines might not reflect that of the intracellular form and might actually be closer to the metabolism of the promastigote. The data in Fig. 2, however, argue against this possibility and show the effectiveness of these compounds in reducing the percentage of infected host cells in a P388D$_1$ macrophage tissue culture system. The antiparasitic effects of TPP and TPPR were evident by 5 days, and at 7 days the infection reached an apparent minimum of 10–15 percent of cells. Counting by light microscopy was not accurate below this; however, the data support the conclusion that all of the organisms were eliminated from the infected cells. At the completion of the experiment the cells were placed at 26°. This killed the macrophages and would have allowed any viable amastigote to transform in the overlying medium. There was no growth. Like allopurinol and allopurinol ribonucleoside [7], the thio-compounds have an antileishmanial effect against the intracellular form of *L. donovani*.

The metabolic studies with intact cells and the studies with purified enzymes demonstrated that TPP can be activated directly by the HGPRTase to TPPR-MP and that TPPR can be activated by a nucleoside phosphotransferase to the same product. There was no conversion beyond this to the ribonucleoside di- or triphosphates and no incorporation into nucleic acid. It is of interest that there was no conversion of TPPR-MP to the aminopyrazolopyrimidine ribonucleotides. The latter could not be detected in either the metabolic studies or by assay of the adenylosuccinate synthetase *in vitro*. This indicates that the mechanism of action of the thio-analogue of allopurinol may be different from that of allopurinol. Allopurinol is converted by these organisms to the aminopyrazolopyrimidine ribonucleotides, and incorporated into the RNA [5, 6]. A similar metabolic sequence occurs in *T. cruzi* [3] and the African trypanosomes [4].

To investigate the relationship between allopurinol and thiopurinol, the two agents were added together to growing cultures of *L. mexicana*. Marked antagonism was noted at low concentrations of thiopurinol. At higher concentrations of TPP the biological effect of this compound became more prominent and the antagonism decreased. These data suggested that TPP and HPP might compete at the same locus with the cell, and subsequent experiments were done using radiolabeled HPP to determine if TPP could interrupt the metabolism of this compound. The data presented in Table 2 indicate that there was a 30 percent inhibition of the HGPRTase and a 50 percent inhibition of the adenylosuccinate synthetase by TPP and TPPR-MP as determined by their abilities to form HPPR-MP and APPR-MP, respectively. The concentration of allopurinol ribonucleoside monophosphate was decreased about 30 percent in the cells to which TPP was added. It is problematic whether this decrease was adequate to explain the antagonism of the two agents. Since the intracellular concentrations of TPPR-MP exceeded its K_i by at least 10-fold, it was not surprising to see significant inhibition of adenylosuccinate synthetase. Although this locus of inhibition may have contributed at least in part to the antiprotozoal activity of TPP, it does not account for the selectivity. TPPR-MP was also an inhibitor of mammalian adenylosuccinate synthetase with a K_i to $K_{m(IMP)}$ ratio of 1.5 compared to a ratio of 8.4 with the enzyme from *L. donovani* (T. Spector, unpublished data).

The decrease in aminopyrazolopyrimidine ribonucleoside was about 50 percent. This was reflected in the 66 percent decrease of APP incorporation into RNA in the presence of TPP. Recent investigations [25] have shown that HPP is an inhibitor of RNA and protein synthesis in these organisms. The observation that HPP–TPP antagonism was related to a decreased incorporation of APP into RNA supports the hypothesis that the mechanism of action of HPP in these organisms is to inhibit RNA synthesis or function. The mechanism of action of TPP remains unknown, but may be related to the inhibition of these two enzymes and a secondary decrease in RNA synthesis.

Acknowledgements—This investigation was supported by the Wellcome Research Laboratories and grants from the UNDP/World Bank/WHO and NIH (1 RO1 AI15663-01). We thank Mrs. Kathleen Lee and Miss Claire Burrus for assistance in preparation of the manuscript. Mr. Wayne Miller synthesized the TPPR-MP.

REFERENCES

1. M. A. Pfaller and J. J. Marr, *Antimicrob. Agents Chemother.* **5**, 469 (1974).
2. J. J. Marr and R. L. Berens, *J. infect. Dis.* **136**, 724 (1977).
3. J. J. Marr, R. L. Berens and D. J. Nelson, *Science* **201**, 1018 (1978).
4. R. L. Berens, J. J. Marr and R. Brun, *Molec. biochem. Parasit.* **1**, 69 (1980).
5. D. J. Nelson, C. J. L. Bugge, G. B. Elion, R. L. Berens and J. J. Marr, *J. biol. Chem.* **254**, 3959 (1978).
6. D. J. Nelson, S. W. LaFon, J. V. Tuttle, W. H. Miller, R. L. Miller, T. A. Krenitsky, G. B. Elion, R. L. Berens and J. J. Marr, *J. biol. Chem.* **254**, 11,544 (1979).
7. R. L. Berens, J. J. Marr and D. J. Nelson, *Biochem. Pharmac.* **29**, 2397 (1980).
8. J. Williamson and T. J. Scott-Finnegan, *Antimicrob. Agents Chemother.* **13**, 735 (1978).
9. R. E. Desjardins, R. A. Casero, G. P. Willet, G. E. Childs and C. J. Canfield, *Expl. Parasit.* **50**, 260 (1980).
10. W. Peters, E. R. Trotter and B. L. Robinson, *Ann. trop. Med. Parasit.* **74**, 321 (1980).
11. P. A. Kager, P. H. Rees, B. T. Wellde, W. T. Hockmeyer and W. H. Lyerly, Jr., *Trans. R. Soc. trop. Med. Hyg.*, in press.
12. J. L. Avila and A. Avila, *Expl Parasit.* **51**, 204 (1981).
13. R. Grahame, H. A. Simmonds, A. Cadenhead and B. M. Bean, *Adv. exp. Med. Biol.* **41**, 597 (1974).
14. R. Grahame, H. A. Simmonds, J. S. Cameron and A. Cadenhead, *J. clin. Chem. clin. Biochem.* **14**, 291 (1976).
15. R. L. Berens and J. J. Marr, *J. Parasit.* **64**, 160 (1978).
16. J. J. Marr, R. L. Berens and D. J. Nelson, *Biochim. biophys. Acta* **544**, 360 (1979).
17. R. Brun, R. L. Berens and S. M. Krassner, *Nature, Lond.* **262**, 689 (1976).
18. C. J. Dawe and M. Potter, *Am. J. Path.* **33**, 603 (1957).
19. H. S. Koren, B. S. Handwerger and J. R. Wunderlich, *J. Immun.* **114**, 894 (1975).
20. R. L. Berens and J. J. Marr, *J. Protozool.* **26**, 453 (1979).

21. T. Spector, T. E. Jones and G. B. Elion, *J. biol. Chem.* **254**, 8422 (1969).
22. G. B. Elion, S. Singer and G. H. Hitchings, *J. biol. Chem.* **208**, 477 (1954).
23. J. V. Tuttle and T. A. Krenitsky, *J. biol. Chem.* **255**, 909 (1980).
24. T. Spector and R. L. Miller, *Biochim. biophys. Acta* **419**, 509 (1976).
25. D. L. Looker, R. L. Berens and J. J. Marr, Abstr. *International Conference on Antimicrobial Agents and Chemotherapy* (September 1980).