elevated temperatures. While the degradation products of phenylpropanolamine cannot be quantified by this method, a significant decrease in phenylpropanolamine was observed in stressed samples. Assay results for fresh and stressed samples by periodate oxidation correlated well with this assay, thus supporting the stability-indicating properties of this assay for phenylpropanolamine.

The recovery of the antihistamines, pyrilamine maleate[5] and pheniramine maleate, from a tablet matrix containing resinous material became increasingly difficult as the tablet was subjected to stress. Consequently, the acidic–alcoholic sample solution was exposed to heat to free the remaining antihistamines from the matrix.

In general, the chromatograms of stressed samples showed a decrease in phenylpropanolamine and methscopolamine but no loss in the pheniramine or pyrilamine content. There was no indication that excipients or degradation products interfered with the four peaks.

Careful control of buffer pH was necessary to obtain adequate separation of the pyrilamine and pheniramine peaks. The monitoring wavelength of 216.5 nm was selected as the optimum response *versus* baseline noise for the four species. At this wavelength, trace amounts of α-aminopropiophenone, a precursor and possible trace contaminant in phenylpropanolamine, do not absorb to a significant amount.

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Antiparasitic Structure–Activity Relationships of Congocidine Derivatives

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Received October 23, 1980, from the **Department of Pharmacy, School of Pharmacy, the* [‡]*Department of Parasitology, Hadassah Medical School, and the* [§]*Department of Natural Products, School of Pharmacy, Hebrew University of Jerusalem, Jerusalem, Israel.* Accepted for publication December 17, 1980.

Abstract □ Several congocidine analogs were synthesized and tested for *in vivo* activity against *Trypanosoma congolense* and *in vitro* activity against amastigotes of *Leishmania tropica*. The tripyrrole derivative, β-{[*N*-methyl-4-[*N*-methyl-4-(guanidinoacetamido)pyrrole -2- carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido}butyroamidine dihydrochloride, was less toxic and more active than congocidine. The guanidinoacetyl moiety appears to be a structural requirement for antiparasitic activity in the congocidine series.

Keyphrases □ Congocidine derivatives—tri- and monopyrrole analogs, synthesized and evaluated for antiparasitic activity in mice, *in vivo* and *in vitro* studies □ Structure–activity relationships—tripyrrole derivatives of congocidine synthesized and evaluated for antiparasitic activity in mice, *in vivo* and *in vitro* studies □ Antiparasitic activity—tripyrrole derivatives of congocidine synthesized and evaluated for activity in mice, *in vitro* and *in vivo* studies

Congocidine (I) and distamycin A (II) are basic oligopeptide antiviral antibiotics isolated from the medium of *Streptomyces* sp. (1–3). The structure of congocidine was established by a series of degradations and a total synthesis (4, 5) and was shown to be identical to that of netropsin, which was isolated from a medium of *Streptomyces netropsis* (6, 7).

BACKGROUND

Congocidine (I) and distamycin A (II) are the major components of a group of pyrrole amidine antiviral antibiotics. Unlike distamycin A and in addition to its antiviral and antibacterial activities (8, 9), congocidine also shows antiparasitic activity against *Trypanosoma congolense* (the source of its name) and other parasites (1, 2, 7). Little information is available on the structural requirements for the antiparasitic activity of congocidine. The only reported study (10) demonstrated that replacement of the *N*-methylpyrrole ring in congocidine by thiophene, pyridine, or benzene led to the loss of its trypanocidal activity.

Several studies on the distamycin series showed that when distamycin A (a tripeptide) was converted to its tetra- or pentapeptide analogs, the antiviral and antibacterial activities against some viruses and bacteria increased (11–14). At the same time, cytotoxicity was somewhat reduced, indicating that the biological and cytotoxic activities were separable.

A similar observation was made in the congocidine series. Several tripyrrole derivatives of congocidine were less cytotoxic and more active than the parent drug in three tests against Herpes simplex virus (15). The present study evaluated the antiparasitic activity of different tri- and monopyrrole derivatives of congocidine to establish some structural requirements in the congocidine molecule. Congocidine and its derivatives were tested against *Leishmania tropica*[1] *in vitro* in mouse peritoneal exudate cells and against *T. congolense*[2] *in vivo*.

EXPERIMENTAL

Chemistry—The syntheses of the compounds were described previously (15, 16).

Parasitology[3]—*Animals*—Male mice of the inbred strains C_3H and

[1] The *L. tropica* LRC-L137 strain was obtained from the strain collection of the World Health Organization's International Reference Center for Leishmaniasis (WHO-LRC) at the Department of Protozoology, Hadassah Medical School, Jerusalem, Israel.

[2] The *T. congolense* TREU 1183 strain was kindly supplied by Professor Curtis L. Patton, Department of Epidemiology and Public Health, Yale University, New Haven, Conn.

[3] Some of the parasitological work was presented at the annual meeting of the Israeli Society of Protozoologists (April 1980) and will appear as the following abstracts: J. El-On, C. L. Greenblat, D. T. Spira, R. Mechoulam, and M. Bialer, "The Effect of Congocidine Derivatives on *Leishmania tropica* and *Trypanosoma congolense*," in *J. Protozoology Supplement* (1981).

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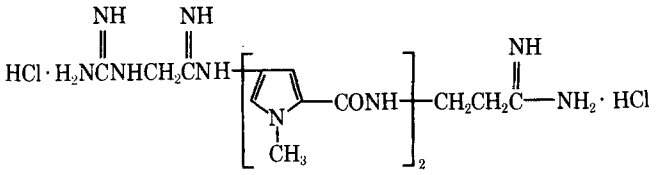

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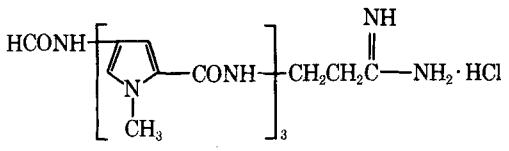

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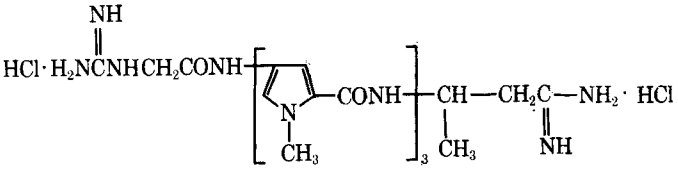

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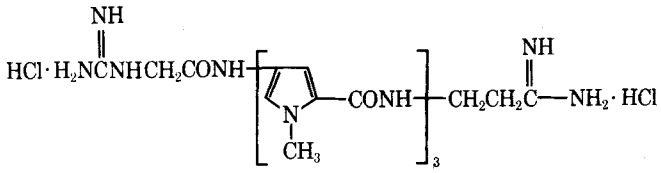

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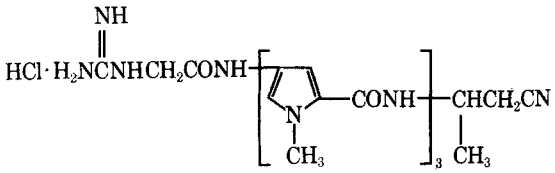

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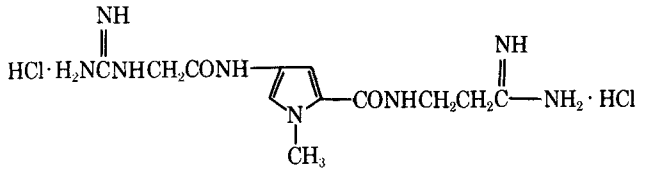

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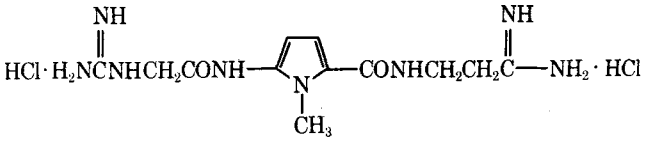

VII

Balb/c (25–35 g, 8–12 weeks old) were used. The C_3H mice were the source of peritoneal exudate cells, while the Balb/c mice were used for *in vivo* studies of *T. congolense*.

Cutaneous Leishmaniasis—Mice peritoneal exudate cells were prepared from thioglycolate-stimulated mice as described by Handman *et al.* (17). The cells were cultivated in 24-well microplates at 37° and were infected 2 hr after cultivation with promastigotes prepared from a stabilate held at liquid nitrogen. The drug was introduced 24 hr after the transformation into intracellular amastigotes. The development of the parasites was checked daily, and the parasite survival index was determined. This index is the mean number of intracellular parasites multiplied by the percent of infection, as compared with the untreated infected control.

Trypanosomiasis—Balb/c mice were inoculated intraperitoneally with 5×10^5 trypanosomes. Drug treatment was begun 2–3 days after infection

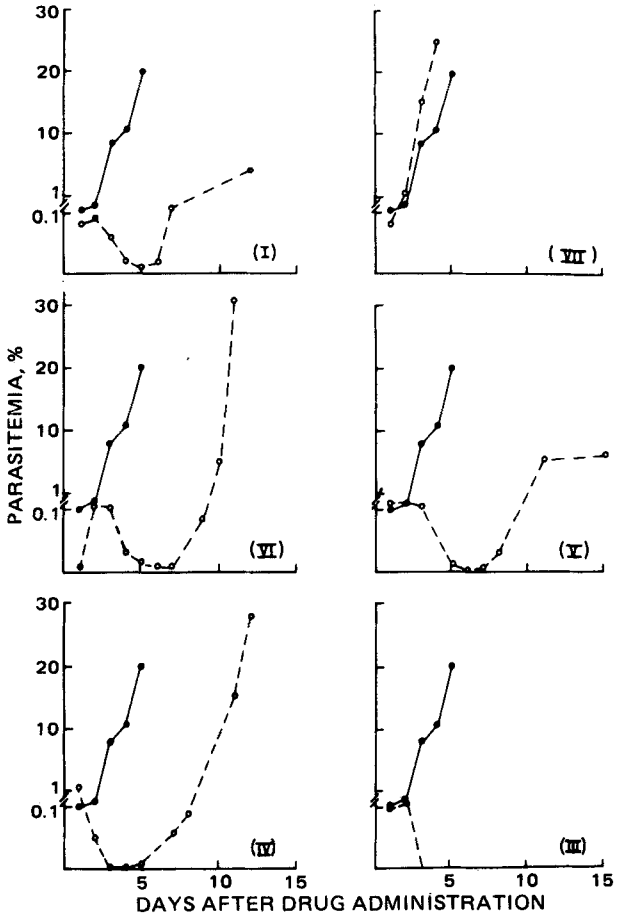

Figure 1—*Effect of congocidine derivatives on the growth of* T. congolense *in Balb/c mice. Day 1 is the 1st day of drug administration (40 mg/kg). The full-line (●) drawing indicates the results in mice receiving no drug.*

when parasites were detected in the blood. The parasites were counted daily in blood smears prepared from the mouse tail.

RESULTS

The tripyrrole analog of congocidine (III) showed the greatest growth inhibition of *T. congolense* in mice. Two injections (40 mg/kg ip) given

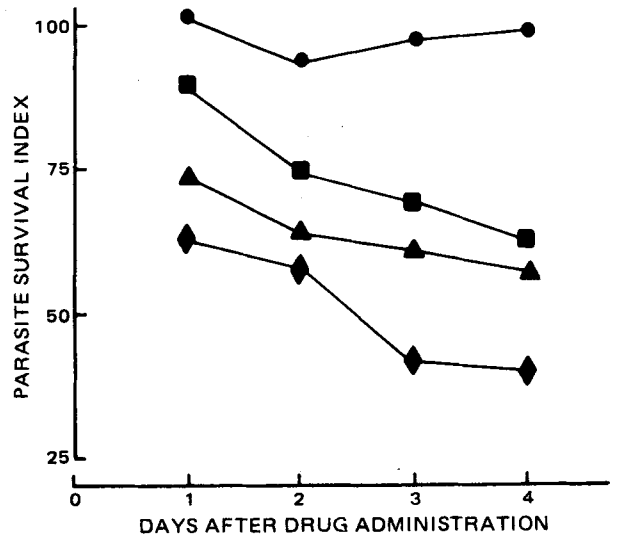

Figure 2—*Effect of distamycin A and congocidine derivatives on the growth of* L. tropica *amastigotes in peritoneal exudate cells from C_3H mice at 37°. Key: ●, 10 μg of I/ml; ▲, 100 μg of II/ml; ♦, 10 μg of III/ml; and ■, 25 μg of IV/ml.*

on the 5th and 6th days of infection (when the parasitemia was >0.01%) were trypanocidal to such an extent that no parasites were detected over 90 days. The mice were tested every 15 days (Fig. 1). The other drugs, including the parent drugs (I and II), were less effective; they lowered parasitemias significantly but did not eliminate the parasites totally. Mice administered congocidine (I) or IV (40 mg/kg) three or more times died either from high parasitemia or from the toxic effect of the drug. Compound II was less toxic but also less effective against the parasite as compared with I. Six doses of II (40 mg/kg) did not affect the growth of the parasites, while five doses of 80 mg/kg inhibited the growth only partially.

In vitro studies indicated that III was also the most effective compound against *L. tropica* amastigotes in C_3H mouse mononuclear phagocytes (Fig. 2). In cultures treated with 10 μg of III/ml, the parasite survival index indicated 50% survival on the 2nd day and 20% by the 4th day. Compounds IV (25 μg/ml) and II (100 μg/ml) also reduced growth but were not as effective as III. Cytotoxicity to host cells was observed at 25, 50, 50, and 200 μg/ml of I, III, IV, and II, respectively.

The doses used were the minimal effective nontoxic ones. Thus, no inhibitory effects on the growth of *L. tropica* were seen when II and IV were given in a concentration of <100 and 25 μg/ml, respectively. The monopyrrole analog of congocidine (VI) had the same effect against *T. congolense* as the parent drug. Its position isomer (VII) was completely inactive.

DISCUSSION

In two antiparasitic tests, the tripyrrole derivative of congocidine (III) was more potent and less toxic than congocidine and more potent than its analog (IV). Although less toxic than congocidine, IV demonstrated the same inhibitory activity as the parent drug in the two systems tested. These results showed that there are considerable structural requirements in the congocidine series for their antiparasitic activity.

It was reported previously (15) that the tripeptide (IV) is more active in some antiviral tests than the dipeptide congocidine (I). However, this modification alone did not improve the antiparasitic activity, although it improved the therapeutic index. Surprisingly, the addition of a methyl group to the β-aminopropionamidine moiety in IV, leading to III, increased the antiparasitic activity and further improved the therapeutic index. The fact that distamycin A was totally inactive in the two antiparasitic tests demonstrated that the guanidinoacetyl moiety in the congocidine molecule cannot be replaced by a formamido moiety, contrary to observations in the antiviral field.

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High-Performance Liquid Chromatographic Analysis of Carbenicillin and Its Degradation Products

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Received November 19, 1980, from the *Penicillin Recovery Department, Pfizer Inc., Groton, CT 06340.* Accepted for publication December 22, 1980.

Abstract □ An ion-pair reversed-phase high-performance liquid chromatographic system is used to separate carbenicillin from carbenicillin penicilloic acid, carbenicillin penilloic acid, penicillin G, penicillin G penicilloic acid, and penicillin G penilloic acid. Conditions are provided for resolution of the carbenicillin diastereomer pair and for elution as a single peak, facilitating quantitation.

Keyphrases □ Carbenicillin—degradation products, high-performance liquid chromatographic analysis, mobile phase modifications □ High-performance liquid chromatography—analysis, carbenicillin, resolution of diastereomer pair or single-peak elution by mobile phase modifications □ Antibiotics—carbenicillin, high-performance liquid chromatographic analysis, mobile phase modifications □ Degradation—carbenicillin, high-performance liquid chromatographic analysis

Carbenicillin, a broad-spectrum semisynthetic penicillin-type antibiotic, is important because of its antipseudomonal and antiproteus effect. Most classical analytical methods available for penicillin purity or potency determinations are applicable to carbenicillin; the iodometric and hydroxylamine methods and the microbiological agar diffusion assay are the most widely used. The latter method is the one specified by the Antibiotic Regulations of the Food and Drug Administration (1). Carbenicillin monosodium monohydrate is used as a reference standard for this assay (2).

BACKGROUND

Recent literature (3–6) indicated that high-performance liquid chromatography (HPLC), especially reversed-phase or ion-pair reversed-phase HPLC on microparticulate (≤10 μm) bonded packing materials, is amenable to the analysis of penicillins. In many penicillin analyses,

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