TABLE II. The Effect of Ergotamine Tartrate and 2% Starch Solution on Epinephrine-Induced Hyperglycemia in the Rat.

Compound	No. of rats/group	Avg blood sugar in mg %: Control,† tail blood	1½ hr after epinephrine‡: Tail blood	1½ hr after epinephrine‡: Heart blood
Ergotamine tartrate	9	86.1 ± 2.6*	53.7 ± 4.8*	154.0 ± 5.0*
2% starch sol. (control)	8	85.3 ± 3.7	212.1 ± 10.6	230.8 ± 10.1

* Stand. error of mean.
† Blood sample taken after 16 hr fast, before admin. of compound.
‡ Dose 0.2 mg/kg of epinephrine hydrochloride subcut. 20 min. after admin. of ergotamine tartrate or 2% starch sol.

by cardiac puncture exhibited normal glycemic levels. This discrepancy is probably related to the prolonged vasoconstrictive action of ergotamine tartrate and the resultant interference with normal peripheral circulation. It would appear that 0.2 mg/kg of epinephrine hydrochloride had little effect on the rat peripheral circulation since the hyperglycemic response in the starch-treated controls was equally distributed between systemic and peripheral blood. The results with insulin indicated that a true hypoglycemic agent lowered both tail and heart blood sugar values to the same extent. Slight inhibition of epinephrine-induced hyperglycemia by ergotamine tartrate was evident in heart blood, but appeared complete in tail blood, a reflection no doubt of the impairment of peripheral circulation by ergotamine tartrate. These results indicate very strongly that systemic, not peripheral blood, is the better indicator of drug effects on the blood sugar level.

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In vitro Study of Antifungal Activity of Nitrostyrenes.* (20836)

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The discovery by Elson(1) of the fungistatic activity of propamidine (p,p'trimethylenedibenzamidine) against a number of pathogenic fungi led to further confirmatory investigations of other aromatic diamidines(2-6) such as pentamidine (p,p'-pentamethylenedibenzamidine) and stilbamidine (p,p'-stilbenedicarboxamidine) and to clinical trials in the treatment of systemic mycotic infections (7-12). Singular success was obtained with the use of stilbamidine in the therapy of North American blastomycosis. Curtis and Harrell(10) described 2 cases of North American blastomycosis that responded to treatment by large doses of diethylstilbestrol and noted the similarity of the chemical structures of stilbamidine and diethylstilbestrol. It was considered worthwhile to screen for antifungal activity other compounds with

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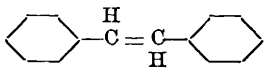
TABLE I. Antifungal Activity of Nitrostyrenes. Lowest effective concentration in mg of compound per ml medium.

	β-Nitrostyrene	p-methoxy-β-nitrostyrene	p-acetoxy-β-nitrostyrene	β-methyl-β-nitrostyrene	p-methoxy-β-methyl-β-nitrostyrene
Trichophyton mentagrophytes	.1	.1	.1	.001	.001
rubrum	.01	.01	.01	.001	.001
schoenleini	.1	.1	.1	.001	.001
violaceum	.1	.1	.1	.001	.001
ferrugineum	.1	.1	.1	.001	.01
tonsurans	.1	.1	.1	.01	.001
Microsporum gypseum	.1	.1	.1	.001	.01
audouini	.001	.01	.01	.001	.001
canis	.1	.1	.1	.001	.01
Epidermophyton floccosum	.1	.1	.1	.001	.001
Cladosporium wernecki	.1	.1	.1	.01	.01
Hormodendrum pedrosoi	.1	.1	.1	.01	.01
Monosporium apiospermum	.1	.1	.1	.01	.001
Nocardia asteroides	>.1	>.1	.1	.1	.1
Candida albicans	.1	.1	1.0	>.1	.1
Cryptococcus neoformans	.01	.01	.1	.001	.01
Sporotrichum schenckii	.01	.01	.01	.01	.001
Blastomyces dermatitidis	.001	.01	.01	.001	.001
braziliensis	.01	.01	.001	.001	.001
Histoplasma capsulatum	.1	.1	.1	.001	.001
Alternaria sp.	>.1	>.1	>.1	.1	.1
Penicillium sp.	>.1	>.1	>.1	.1	.1
Aspergillus niger	>.1	>.1	>.1	.1	.1

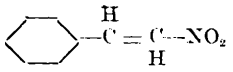
chemical formulae related to the stilbene nucleus:



Methods and materials. Antifungal activity was measured by the serial dilution agar slant method as described previously in another paper(5). This consisted essentially of setting up serial dilutions of the compound being tested in Sabouraud's dextrose agar slants in concentrations of 1 mg per ml, 0.1 mg per ml, 0.01 mg per ml, and 0.001 mg per ml medium. If complete inhibition occurred in all the tubes, the dilution was extended to 0.0001 mg per ml and 0.00001 mg per ml medium during repeat experiments. The pH of the media was determined. The solvent used was acetone so that additional acetone controls were made by adding 1% acetone to the Sabouraud's dextrose agar. The experiments were all done in duplicate and repeated when considered necessary. The test organisms used were strains of pathogenic fungi maintained in the stock collection of the Department of Dermatology of the University of Michigan Hospital. Equal amounts of suspensions of the organisms in physiological saline solution were inoculated on the agar slants. Growth was observed at room temperature and the end-point was determined at the lowest concentration of the compound where complete inhibition of the growth of the organism occurred. The 53 compounds available for the screening survey were aromatic ethylenes and included a number of cinnamic acid derivatives.

Results. In order to eliminate compounds that do not exhibit any significant antifungal powers, preliminary screening procedures were performed using as test organisms 5 fairly representative organisms: *Trichophyton rubrum, Microsporum audouini, Candida albicans, Cryptococcus neoformans,* and *Blastomyces dermatitidis.* Compounds which showed promising activity against these 5 species were tested against the remaining pathogenic fungi.

It became apparent from these preliminary results that a group of compounds belonging to the nitrostyrenes possessed a high degree of activity against pathogenic fungi. The activity of the group was higher than the other compounds tested, including stilbamidine and propamidine, whose clinical efficacy has already been demonstrated. The similarity in structure between the nitrostyrenes and the stilbene nucleus can be seen from the structural formula for β-nitrostyrene.



These 5 nitrostyrenes were 1) β-nitrostyrene, 2) p-methoxy-β-nitrostyrene, 3) p-acetoxy-β-nitrostyrene, 4) β-methyl-β-nitrostyrene and 5) p-methoxy-β-methyl-β-nitrostyrene. Their *in vitro* activity against the growth of pathogenic fungi is shown in Table I.

The literature contains some references to the antifungal properties of the nitrostyrenes. Horsfall(13) pointed out, "the excellent fungicidal and reasonably good protective powers on foliage" of β-nitrostyrene and β-methyl-β-nitrostyrene. McGowan, Brian, and Hemming(14) studying the fungistatic activity of ethylenic and acetylenic compounds, found a number of nitrostyrenes effective against *Botrytis allii, Fusarium graminearum,* and *Penicillium digitatum.* One of the components of essential oils reported to be fungistatic by Vilanova and Casanovas(15) was isoeugenol, p-hydroxy-o-methoxy-β-methylstyrene.

Summary. In screening 53 compounds with chemical formulae related to the stilbene nucleus for antifungal activity by the serial dilution agar method, a number of nitrostyrenes were found to exhibit a high degree of activity against pathogenic fungi.

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