strated antitumor activity against both leukemia L1210 and Walker 256 systems. As expected, compound XI was inactive in KB cell culture test system. It is of interest that, although compound XI possesses two aziridinyl groups, it failed to show activity against the Walker (subcutaneous) 256 test system designed for the evaluation of alkylating agents.

Attempts to prepare the thione analogs of phosphazine and methylphosphazine were not successful in our hands. Phosphochlorination of 2-amino-*s*-triazine and 2-amino-*as*-triazine with thiophosphoryl chloride gave only intractable materials.

Experimental Section[14]

2-Aminopyrimidine Hydrochloride (VII).—Through a suspension of 200 g (2.01 moles) of 2-aminopyrimidine[15] (Eastman) in 1600 ml of absolute EtOH was passed, without cooling, a generous stream of dry HCl. The temperature of the reaction mixture gradually rose almost to boiling while the solid slowly dissolved. After *ca.* 30 min the hydrochloride salt started to precipitate from the hot solution. The stream of HCl was continued for another 15 min, and the resulting mixture was allowed to cool to room temperature. The solid was collected by filtration, washed well with absolute EtOH, then dried at 70–80° to give 210 g (76% yield) of VII, mp 200–202°, pure enough for the next step.

2-Pyrimidinylphosphoramidic Dichloride (VIII).—A mixture of 190 g (1.445 moles) of VII and 1 l. of $POCl_3$ was refluxed for 6 hr, then cooled to room temperature. The resulting solid was collected by filtration and washed well with C_6H_6 to give 294 g (90% yield) of VIII, mp 171–173°. This product was used as such in the next preparation after drying *in vacuo* at room temperature for 5 hr in a rotary evaporator. An analytically pure sample, mp 188–190° (lit.[10] mp 190°), can be obtained by recrystallization of the crude product from a large volume of C_6H_6.

P,P-Bis(1-aziridinyl)-N-2-pyrimidinylphosphinic amide (IXa) was prepared essentially by the procedure of Kropacheva and Sazonov:[10] λ_{max}^{EtOH} 222 mμ (ε 17,500), 276 mμ (ε 2800).

P,P-Bis(2-methyl-1-aziridinyl)-N-2-pyrimidinylphosphinic Amide (IXb).—To a stirred mixture of 190 g (0.9 mole) of VIII in 2 l. of anhydrous C_6H_6 cooled in an ice bath was added dropwise 128 g (2.24 moles) of propylenimine (Interchemical Corp., Organic Chemicals Department, Carlstadt, N. J.) and 226 g (2.24 moles) of Et_3N in 200 ml of anhydrous C_6H_6 at such a rate that the temperature of the reaction mixture did not exceed 20°. The mixture was allowed to stir for another 30 min in the ice bath and for an additional 2 hr without cooling. The solvent was removed *in vacuo* at *ca.* 50°, and the residue was swirled in 1800 ml of hot (70°) anhydrous C_6H_6. The insoluble $Et_3N \cdot HCl$ was removed by filtration and washed with 200 ml of hot C_6H_6. The combined filtrate and washings were allowed to cool, yielding the first crop of IXb. This was isolated by filtration, and the volume of the filtrate was reduced to 500 ml when another portion of IXb precipitated on cooling: total 96 g, mp 142–145°. An additional 41 g of product was isolated when the volume of the filtrate was reduced to 250 ml, mp 140–143°, total yield 60%. An analytical sample was obtained by recrystallization from C_6H_6: mp 145–147°; λ_{max}^{EtOH} 223 mμ (ε 17,000), 277 mμ (ε 2500). This compound is stable at room temperature under ordinary storage conditions.

Anal. Calcd for $C_{10}H_{18}N_5OP$: C, 47.4; H, 6.37; N, 27.7. Found: C, 47.2; H, 6.38; N, 27.4.

N,N′-Bis(2-chloroethyl)-N″-2-pyrimidinylphosphoric Triamide (X).—Phosphazine IXa (20 g) was added portionwise to 400 ml of methanolic HCl (saturated at 5°). The resulting mixture was left overnight at room temperature and evaporated under reduced pressure to a clear viscous oil. The oil was dissolved in 150 ml of H_2O, and the pH of the solution was adjusted to 4 by careful addition of 1 *N* NaOH. After 15 hr the precipitate was filtered, washed with cold H_2O, and dried at 70° for 18 hr to give 9.2 g of X, mp 103–104°. Recrystallization from H_2O afforded an analytical sample: mp 105–106°; λ_{max}^{EtOH} 223 mμ (ε 16,700), 277 mμ (ε 2700).

Anal. Calcd for $C_8H_{14}Cl_2N_5OP$: C, 32.2; H, 4.74; N, 23.5; Cl, 23.8. Found: C, 32.2; H, 4.56; N, 23.6; Cl, 23.5.

P,P-Bis(1-aziridinyl)-N-2-pyridylphosphinic amide (XI) was prepared by the known procedure[13] from 2-aminopyridine;[18] λ_{max}^{EtOH} 226 mμ (ε 12,300), 280 mμ (ε 3900).

Acknowledgments.—The authors wish to express their appreciation to Mrs. Margaret L. Rounds, Mr. John R. Gravatt, and Mr. Leland R. Lewis for the analytical and instrumental measurements. They are also grateful to Dr. Harry B. Wood, Jr., and Dr. Robert R. Engle of CCNSC for providing the screening results of cytoxan and thio-TEPA.

(14) All melting points (corrected) were taken on a Thomas–Hoover melting point apparatus. The uv absorption spectra were determined with a Perkman DK-2 spectrophotometer.

(15) S. Gabriel, *Ber.*, **34**, 3384 (1901).

(16) A. E. Tschitschibabin and O. A. Seide, *J. Russ. Phys.-Chem. Soc.*, **46**, 1216 (1914); K. Ziegler and H. Zeiser, *Ber.*, **63**, 1847 (1930).

Studies on Antiprotozoans. Synthesis and Biological Activity of Some Styrylimidazole Derivatives

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A series of 1-aminoalkyl- and 1-aminoalkyl-2-methyl-5(4)-nitro-4(5)-styrylimidazoles were synthesized and examined for biological activity. These compounds were tested on *Trichomonas vaginalis*, *Entamoeba histolytica*, and *Candida albicans*. Their *in vitro* activity against *T. vaginalis* was found particularly interesting. For the 1-aminoalkyl-5(4)-nitro-4(5)-styrylimidazoles, we have separated isomers and determined their activities. Different methods used to assign positions to the nitro group in the heterocyclic ring are described.

For several years we have been carrying out in our laboratories research on heterocyclic substances with trichomonacidal activity as reported in a previous publication.[1] Continuing our study with other heterocyclic compounds, we have investigated some imidazole derivatives, since this heterocyclic system proved to have a marked trichomonacidal activity in compounds like azomycine and metronidazole.[2]

It is well known that the introduction of a styryl group into appropriate molecules gives substances highly active against trypanosomes; styrylquinolines and styrylbenzothiazoles are also active in the presence

(1) F. Lauria, V. Vecchietti, and I. de Carneri, *Farmaco* (Pavia), *Ed. Sci.*, **22**, 479 (1967).

(2) C. Cosar and L. Julou, *Ann. Inst. Pasteur*, **96**, 238 (1959).

of serum and exhibit a particular activity in mice.[3] In the literature, a description of the 4-diethylaminoalkylamino-2-styrylquinoline derivatives is given in relation to their broad spectrum of activity against bacteria, actinomycetes, other fungi, and protozoa.[4] Also, in our laboratories during research on the amebicidal activity of dichloroacetamido derivatives, we noticed a remarkable increase in the antiprotozoal activity with N-cinnamyl-N-methyldichloroacetamide.[5] We deemed it interesting, therefore, to synthesize some imidazole derivatives containing a styryl group at position 4 or 5 and some aminoalkyl chains at position 1. Introduction of basic groups into the aromatic or heterocyclic rings can greatly enhance pharmacological properties; the polar character of the basic groups highly affect the chemical and physical properties of molecules—their solubility, their partition coefficients, and their ability to react with acid groups of proteins or enzyme systems.

Chemical Properties and Structure.—Based on the above considerations, substances of the following general formulas have been prepared. When a substituent is introduced at position 4 or 5 in the imidazole ring, the resulting compound must be designated as either a 4- or 5-substituted imidazole; its tautomeric character precludes a definite assignment of structures. A substituent replacing the imino hydrogen eliminates the possibility of tautomerism and defines the structure. Owing to this substitution, we obtain the two isomers chemically defined, with the styryl and the nitro groups at position 4 or 5. Examples of the preparative methods employed are shown in Chart I. The compounds described are shown in Table I. Compounds with R_2 = H have been separated by silica gel, column chromatography and are shown in Table II.

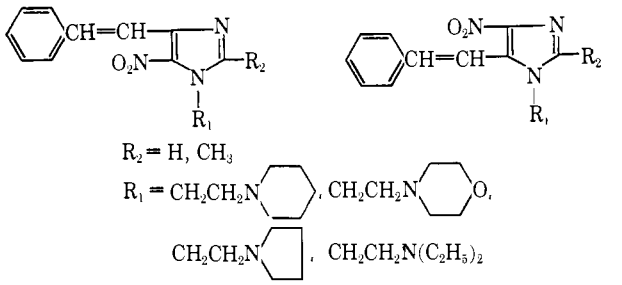


CHART I

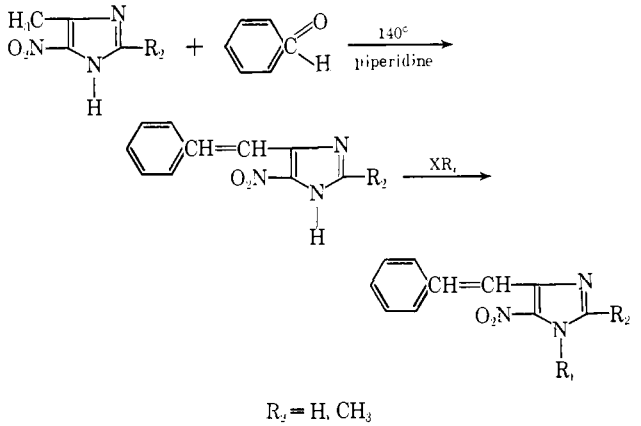
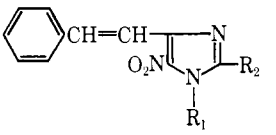
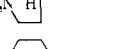
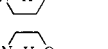
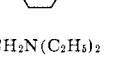
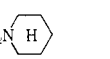
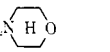
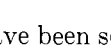


TABLE I

BIOLOGICAL ACTIVITY OF 1-AMINOALKYL-5(4)-NITRO-4(5)-STYRYLIMIDAZOLES



No.	R_2	R_1	*In vitro* act. (μg/ml) *vs.* *T. vaginalis*	*E. histolytica*	*C. albicans*
I	H	$CH_2CH_2N(C_2H_5)_2$	0.9–1.9	8.2	62.5
II	H		0.4–0.9	8.2	250
III	H		3.9	6	125
IV	H		1.9	37–24.6	500
V	CH_3	$CH_2CH_2N(C_2H_5)_2$	31.2	6	500–250
VI	CH_3		7.8	4	250
VII	CH_3		31.2	8.2	500
VIII	CH_3		62.5	8.2–13	500

The positions of the nitro group have been assigned by various methods. Of each pair of isomers, we determined the equivalent weight by titration in a nonaqueous solvent with perchloric acid, using crystal violet as indicator. It must be pointed out that the basic nature of imidazole is related to the ability of a pyridine-like nitrogen to accept a proton. Electron-attracting substituents, like nitro or halogens, decrease the basic strength. When the nitro group is located in close proximity to the electron-releasing system, the resulting product will be the weaker base. In isomers with the nitro group at position 4, only nitrogen in the aminoalkyl chain at position 1 will be practicably titratable. One of the two isomers gave, constantly, an equivalent weight equal to the molecular weight. Therefore, the nitro group of this isomer has been assigned to position 4. Consequently, the other isomer has the nitro group at position 5. For the latter, however, the strength of the yellow color negatively affects the toning of the indicator used and makes titration practically impossible.

The position of the nitro group can be further confirmed by ultraviolet spectra. The isomers with the nitro group at position 4 have one of two maxima, that, in relation to the imidazole ring, is shifted to lower wavelengths (Figure 1). This is in accordance with results obtained by other authors[6] with nitrostyrylimidazole derivatives, and it is a characteristic capable of differentiating the position of styryl and nitro groups on the imidazole ring. Also, ultraviolet spectra in acid solution confirm the different basic strengths as well as the different structure of the compounds under study. In fact, in 4-nitro the wavelength of the maximum absorption is not shifted by passing from neutral to acid medium, while a small but consistent shift is revealed in 5-nitro under the same conditions (Table III).

(3) Th. Wagner-Jauregg, "Therapeutische Chemie, Medizin," Verlag Huber, Bern, 1958, pp 151–152.

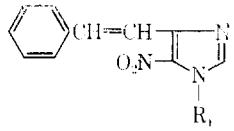
(4) M. V. Rubstov and G. N. Pershin, *J. Med. Pharm. Chem.*, **2**, 113 (1960).

(5) L. Almirante, I. de Carneri, G. Coppi, and W. Logemann, *Antibiot. Chemotherapy*, **10**, 667 (1959).

(6) J. Baddiley, J. G. Buchanan, F. E. Hardy, and J. Stewart, *J. Chem. Soc.*, 2893 (1959).

TABLE II

BIOLOGICAL ACTIVITY OF 1-AMINOALKYL-4-NITRO-5-STYRYLIMIDAZOLES AND 1-AMINOALKYL-5-NITRO-4-STYRYLIMIDAZOLES



No.	R_1	Position of NO_2 group	Mol wt	Equiv wt	*In vitro* act. (μg/ml) vs. *T. vaginalis*	*E. histolytica*	*C. albicans*
Ia	$CH_2CH_2N(C_2H_5)_2$	4	314.38	316	0.24–0.12	4	500
Ib		5			1.9–3.9	12	31.2
IIa		4	312.37	309	0.06–0.03	4	250–500
IIb		5			0.62	12	125
IIIa		4	324.39	318	0.06	5	250
IIIb		5			1.9	10	31.2
IVa		4	328.36	321	0.24–0.48	18	500
IVb		5			3.9	30	500

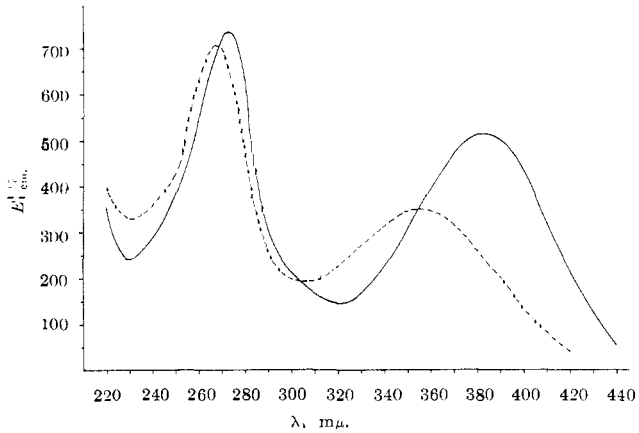


Figure 1.—Uv spectra of 1-(2-morpholinoethyl)-5-styryl-4-nitroimidazole (IVa) (– – – –) and 1-(2-morpholinoethyl)-4-styryl-5-nitroimidazole (IVb) (————) in 95% EtOH.

Infrared spectra of the two isomers, with regard to the –C═C– stretching of the styryl group, reveal rather constant differences of frequency according to whether the group is at position 4 or 5 (Table III). The band of the 5-styryl derivatives regularly appears at a frequency higher by 7–10 cm^{-1} than that of the 4-styryl isomers. This can be explained by the +M effect caused by the nitrogen at position 3 on the ethylenic bond of the styryl group.

By observing the nmr spectra of the IVa and IVb in deuteriochloroform, we note for the methylene at position 1 a shift of 0.22 ppm (4.16 → 4.38) (Figure 2). In order to ascertain whether such a shift might be imputed to a different position of the nitro group on the imidazole ring, we determined the nmr spectra of 1-methyl-4-nitroimidazole and of 1-methyl-5-nitroimidazole.[7] These spectra also revealed a shift of 0.12 ppm (3.80 → 3.92) for the methyl group at position 1. Spectra determined in deuteriobenzene showed a signal in the aromatic hydrogen range having a coupling constant J = 16 cps. The large value of this constant suggests a *trans* configuration of the ethylenic hydrogen in the styryl group (Figure 3).

In order to ascertain the position of the styryl group on the imidazole ring, we determined the nmr spectrum, in DMSO, of the reaction product between 2,4(2,5)-dimethyl-5(4)-nitroimidazole and benzaldehyde (Chart I). This compound shows a sharp singlet due to the methyl group at 2.29 ppm.

The nmr spectra of 2-methyl-4(5)-nitroimidazole and of 4(5)-methyl-5(4)-nitroimidazole show sharp singlets at 2.27 and at 2.48 ppm, respectively, due to the methyl group. We confirmed in this way the presence of the methyl group at position 2 in the compound under examination.

(7) K. E. Hazeldine, F. L. Pyman, and Winchester, *J. Chem. Soc.*, **125**, 1431 (1924).

TABLE III

ULTRAVIOLET AND INFRARED SPECTRA (>C═C< STRETCHING OF THE STYRYL GROUP) OF 1-AMINOALKYL-4-NITRO-5-STYRYL- AND OF 1-AMINOALKYL-4-STYRYL-5-NITROIMIDAZOLES

Styrylimidazole deriv	$\lambda^{1\,N\,HSO_4}_{max}$, mμ	ε	$\lambda^{95\%\ EtOH}_{max}$, mμ	ε	ν^{KBr}_{max}, cm^{-1}
Ia	268	21,590	268	21,950	1632
	357	9,303	356	10,850	
Ib	270	19,070	273	23,390	1622
	380	16,450	384	16,040	
IIa	268	24,280	268	22,170	1627
	358	10,500	358	10,880	
IIb	270	18,600	274	23,020	1620
	380	16,010	384	16,170	
IIIa	268	20,750	268	19,150	1629
	355	8,920	355	9,188	
IIIb	272	17,640	273	23,150	1619
	380	15,500	383	16,020	
IVa	267	21,290	267	21,590	1633
	360	9,030	359	10,620	
IVb	271	18,650	274	22,660	1624
	380	16,210	383	15,800	
1-Methyl-4-styryl-5-nitro	272	20,800	275	20,795	1625
	379	13,810	382	13,800	
1-Methyl-4-nitro-5-styryl	270	27,830	270	22,350	1636
	367	10,175	365	11,140	

Biological Methods and Results.—The substances under study have been tested on *Trichomonas vaginalis*, *Entamoeba histolytica*, and *Candida albicans*. Trichomonacidal activity was tested *in vitro* on a suspension of 30,000 *T. vaginalis* for each millimeter of CPLM medium,[8] results being read after 3 days at 37°. From the positive test tubes, subcultures were systematically made and read 5 days later.

In vitro amebicidal activity was determined at 37° by performing microscopic examination and test-tube subcultures with 4 ml of the dilutions in Pavlova medium, 48 hr after seeding with 40,000 *E. histolytica*

(8) I. de Carneri, *Farmaco* (Pavia), *Ed. Sci.*, **11**, 926 (1956)

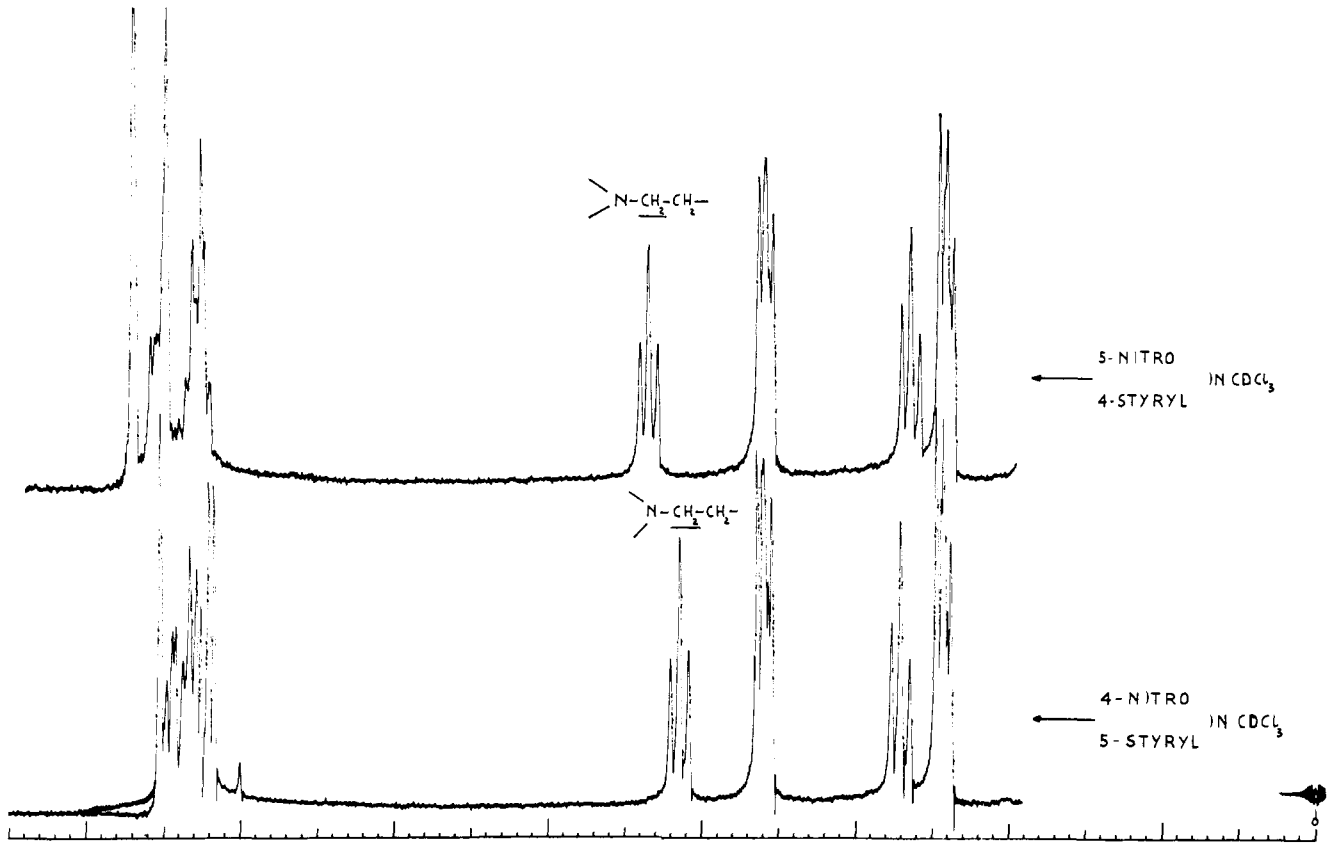


Figure 2.—Nmr spectra of 1-(2-morpholinoethyl)-5-styryl-4-nitroimidazole and 1-(2-morpholinoethyl)-4-styryl-5-nitroimidazole.

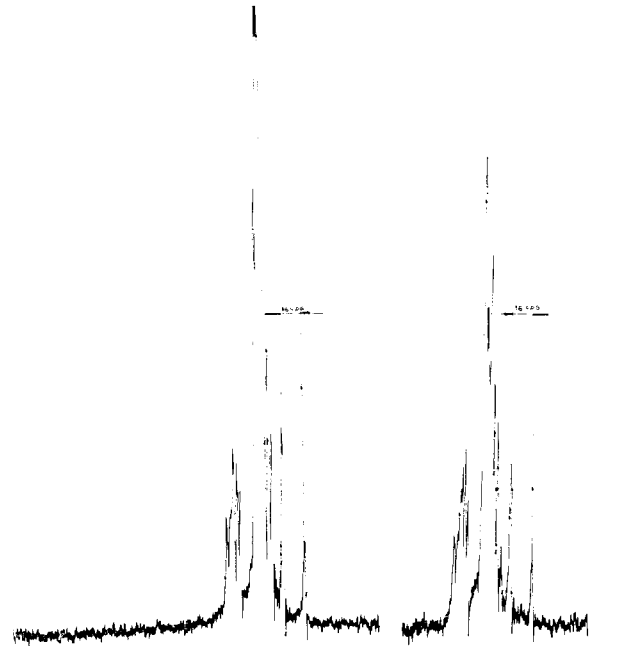


Figure 3.—Nmr spectra (aromatic range) of 1-(2-morpholinoethyl)-5-styryl-4-nitroimidazole and 1-(2-morpholinoethyl)-4-styryl-5-nitroimidazole.

trophozoites strain EdM.[9] Antimycotic activity *in vitro* was tested on *C. albicans* ATCC 2091 by performing serial dilutions in Sabouraud's broth, seeding with 10,000 microorganisms/ml and reading the results after 3 days at 27°. While the antimycotic activity is rather low, antiprotozoal activity is more evident and particularly high against *T. vaginalis in vitro*. The activity data for a mixture of isomers are shown in Table I. Activity is especially remarkable when R = H. Separation of the isomers has been done in order to ascertain the relationship between structure and trichomonacidal activity. Data for the single pairs of isomers are reported in Table II.

According to the literature,[10,11] 5 nitroimidazoles, where position 4 is free, display a higher trichomonacidal activity than corresponding 4 isomers unsubstituted at 5. Our isomers show, instead, higher activity when the nitro group is at position 4. As regards the influence on activity when the nitro group is at position 1, we note for both pairs of isomers the following order: pyrrolidine > piperidine > diethylamine > morpholine.

Comparing our results concerning activity against *T. vaginalis* with those described in the literature, we obse ve how the introduction of a styryl group in the appropriate molecule leads to extremely active compounds.

Experimental Section

All melting points (capillary) are uncorrected. The R_f values were obtained by thin layer chromatography on silica gel; running phase, benzene–cyclohexane–methanol–diethylamine (100:100:20:5). For the chromatographic separation of the mixture of isomers a column of 1-m length and 5-cm i.d. filled with 500 g of silica gel was used. Ultraviolet spectra were obtained on Optica CF_4. Infrared spectra were recorded on a Perkin-Elmer 21 spectrophotometer. Nmr spectra were taken with a Varian spectrophotometer, Model A-110.

(9) I. de Carneri, G. Coppi, L. Almirante, and W. Logemann, *Antibiot. Chemotherapy*, **10**, 626 (1960).

(10) G. N. Pershin, P. M. Kochergin, A.M. Tsyganova, N. A. Novotskaja, L. B. Blinova, and V. Slikhunova, *Med. Prom. SSSR*, **18**, 11 (1964).

(11) C Cosar, *et al.*, *Arzneimittel-Forsch.*, **16**, 23 (1966).

1-(2-Diethylaminoethyl)-4(5)-styryl-5(4)-nitroimidazole (I).—To 0.53 g of Na in 20 ml of ethanol was added 5 g of 4(5)-styryl-5(4)-nitroimidazole[12] in 100 ml of anhydrous EtOH (stirring). Solvent was removed under reduced pressure, and the resultant orange sodium salt was recrystallized from 2-propanol-petroleum ether (bp 40–60°). A mixture of 10 g of this sodium salt and 8.9 g of 2-diethylaminoethyl chloride in 50 ml of dry N,N-dimethylformamide (DMF) was refluxed for 90 min, cooled, and poured into water. This solid was collected and recrystallized from EtOH; mp 95–96°, yield 9.93 g (75%).

Anal. Calcd for $C_{17}H_{22}N_4O_2$: C, 64.95; H, 7.05; O, 10.18; N, 17.82. Found: C, 64.48; H, 7.17; O, 10.54; N, 17.45.

Chromatographic separation of the mixture on a column gave Ia, mp 67–68°, R_f 0.52, and Ib, mp 100–101°, R_f 0.74. See Table II.

1-(2-Pyrrolidinoethyl)-4(5)-styryl-5(4)-nitroimidazole (II).—This compound was prepared in the same way as I and recrystallized from 2-propanol; mp 112–113°, yield 11.78 (89.5%).

Anal. Calcd for $C_{17}H_{20}N_4O_2$: C, 65.37; H, 6.41; O, 10.24; N, 17.94. Found: C, 65.20; H, 6.51; O, 10.45; N, 17.75.

Chromatographic separation of the mixture on a column gave IIa, mp 115–116°, R_f 0.40, and IIb, mp 95–96°, R_f 0.65.

1-(2-Piperidinoethyl)-4(5)-styryl-5(4)-nitroimidazole (III) was prepared in the same way as I and recrystallized from EtOH; mp 110–111°, yield 8.25 g (60%).

Anal. Calcd for $C_{18}H_{22}N_4O_2$: C, 66.23; H, 6.79; O, 9.80; N, 17.16. Found: C, 66.07; H, 6.97; O, 9.73; N, 16.99.

Chromatographic separation of the mixture on a column gave IIIa, mp 88–89°, R_f 0.43, and IIIb, mp 116–117°, R_f 0.69.

1-(2-Morpholinoethyl)-4(5)-styryl-5(4)-nitroimidazole (IV) was prepared as described for I and recrystallized from EtOH; mp 127–128°, yield 8.99 g (65%).

Anal. Calcd for $C_{17}H_{20}N_4O_3$: C, 62.18; H, 6.14; O, 14.62; N, 17.06. Found: C, 61.86; H, 6.23; O, 14.79; N, 16.80.

Chromatographic separation of the mixture on a column gave IVa, mp 138–139°, R_f 0.32, and IVb, mp 140–141°, R_f 0.59.

2-Methyl-4(5)-styryl-5(4)-nitroimidazole.—A mixture of 15 g of 2,4(2,5)-dimethyl-5(4)-nitroimidazole,[13] 30 ml of benzaldehyde, and 2 ml of piperidine was heated (oil bath) to 140–150°. After cooling, the solid was collected and washed first with H_2O to remove the unreacted 2,4(2,5)-dimethyl-5(4)-nitroimidazole, then with EtOH to remove the colored material. Recrystallization from HOAc gave 10 g (41.4%) of 2-methyl-4(5)-styryl-5(4)-nitroimidazole, mp 245–246°.

Anal. Calcd for $C_{12}H_{11}N_3O_2$: C, 62.87; H, 4.84; O, 13.96; N, 18.33. Found: C, 62.98; H, 4.95; O, 14.07; N, 18.3.

1-(2-Diethylaminoethyl)-2-methyl-4(5)-styryl-5(4)-nitroimidazole (V).—To 3 g of the sodium salt of 2-methyl-4(5)-styryl-5(4)-nitroimidazole in 20 ml of dry DMF was added 2.29 g of 2-diethylaminoethyl chloride. The mixture was refluxed for 90 min, cooled, and poured into water; the solid was collected. Recrystallization from EtOH gave V, mp 93–94°, yield 2.74 g (70%).

Anal. Calcd for $C_{18}H_{24}N_4O_2$: C, 65.82; H, 7.36; O, 9.75; N, 17.06. Found: C, 65.75; H, 7.53; O, 9.98; N, 16.97.

The **sodium salt** was prepared as described for I.

1-(2-Pyrrolidinoethyl)-2-methyl-4(5)-styryl-5(4)-nitroimidazole (VI) was prepared as described for I; mp 100–101°, yield 2.84 g (73%).

Anal. Calcd for $C_{18}H_{22}N_4O_2$: C, 66.24; H, 6.79; O, 9.80; N, 17.16. Found: C, 65.71; H, 6.71; O, 9.84; N, 17.72.

1-(2-Piperidinoethyl)-2-methyl-4(5)-styryl-5(4)-nitroimidazole (VII) was prepared as described for I; mp 122–123°, yield 3.08 g (76%).

Anal. Calcd for $C_{19}H_{24}N_4O_2$: C, 67.04; H, 7.11; O, 9.40; N, 16.46. Found: C, 67.04; H, 7.22; O, 9.81; N, 16.28.

1-(2-Morpholinoethyl)-2-methyl-4(5)-styryl-5(4)-nitroimidazole (VIII) was prepared as described for I; mp 125–126°, yield 1.76 g (43%).

Anal. Calcd for $C_{18}H_{22}N_4O_3$: C, 63.14; H, 6.48; O, 14.02; N, 16.36. Found: C, 62.97; H, 6.52; O, 14.29; N, 16.20.

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Penicillins and Cephalosporins from Isothiazolylacetic Acids

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A number of isothiazolylacetic acids have been synthesized including the three isomeric unsubstituted isothiazolylacetic acids, 4-chloro-3-isothiazolylacetic acid, 4-bromo-3-isothiazolylacetic acid, 3-methyl-4-isothiazolylacetic acid, and 3-methyl-5-isothiazolylacetic acid. The penicillins and cephalosporins prepared from these acetic acids by the activated ester method exhibit good antibacterial activity, particularly the unsubstituted derivatives. The antibacterial activity of the unsubstituted potassium 6-(isothiazolylacetamido)penicillanates against gram-negative microorganisms is considerably higher than that of benzylpenicillin.

Little recent information is reported in the literature on analogs of benzylpenicillin[1] (penicillin G). As part of a study on the effect of the aryl group on the antibacterial activity of heteroarylmethylpenicillins we synthesized several isothiazolylacetic acids[2] and from these the corresponding penicillins (I) and cephalosporins (II).

3-Isothiazolylacetic acids (III) were prepared from the 3-methylisothiazoles by a sequence involving bromination with N-bromosuccinimide, cyanation, methanolysis, and hydrolysis. The required 3-methylisothiazoles were obtained by reductive deamination of the corresponding 3-methyl-5-aminoisothiazoles.[3,4] 4- and 5-isothiazolylacetic acids (IV and V) were all successfully prepared from the corresponding carboxylic acids *via* the Arndt–Eistert synthesis[5]. Isothiazole-4-carboxylic acid could be prepared either by a permanganate oxidation of 4-methylisothiazole or by bromination of isothiazole, followed by cyanation and hydrolysis. Table I lists the various isothiazolylacetic acids prepared and their nmr parameters.

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