J. Pharm. Pharmacol., 1965, **17**, 167–172 Received December 3, 1964

Nitroethylenes and related compounds as trichomonacides and candidacides

ANN CLITHEROE, D. GREEN, A. B. A. JANSEN, P. C. PHILLIPS AND A. W. RULE

Several of the compounds examined, particularly certain β-nitrostyrenes and di(2-vinyl)benzenes, were highly active *in vitro* against both *Trichomonas vaginalis* and *Candida albicans*, which are commonly found together in vaginitis. The urinary recovery of the compounds after oral administration, however, suggested that the drugs had not gained access to the circulation in amounts sufficient to ensure a worthwhile effect against the organisms and, moreover, they proved irritant to vaginal mucosa.

LOCAL drug treatment of *Trichomonas vaginalis* infection in women, where the condition is overt, has met with indifferent success and such treatment is inapplicable to men who are often symptomless carriers of the pathogen. The introduction of metronidazole [1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole (Cosar & Julou, 1959) marked a great advance in therapy for it was effective orally.

In a search for alternative drugs we were prompted by the well-known antibacterial activity of β-nitrostyrenes to examine the general class of nitroethylenes against *T. vaginalis*. Bocobo, Curtis, Block & Harrell (1954) have observed that certain nitrostyrenes were active against *Candida albicans*. This is of particular interest, since mixed infection by this pathogen and *T. vaginalis* is common.

We report both *in vitro* and *in vivo* investigations made with a number of nitroethylenes variously substituted with aromatic, heterocyclic and aliphatic groups.

Methods

CHEMISTRY

The method of preparation of the new compound is given in Table 1. The remaining compounds were made by published methods.

Method A. 2N Potassium hydroxide solution (5 ml) was added dropwise over 15 min to a stirred solution of the aldehyde (10 mmol) in nitromethane (640 mg) and ethanol (10 ml) kept at 0–2°. After a further 5 min 2N hydrochloric acid (25 ml) was added and the precipitated nitroethylene was collected by filtration, washed with water, dried *in vacuo* and purified as indicated.

Method B. A mixture of the aldehyde (10 mmol) and the equivalent amount of the appropriate nitroparaffin in absolute ethanol (5 ml) and butylamine (1 mmol) was refluxed for 6 hr. Next morning the precipitate was collected by filtration, washed with ethanol, dried *in vacuo* and purified.

Method C. (a) *Nitrocarbinol.* Methanolic potassium hydroxide solution (2·3 mmol) was added dropwise to a suspension of the aldehyde (2 mmol)

From the Research Laboratories, John Wyeth & Brother Ltd., New Lane, Havant, Hants.

TABLE 1. PREPARATION, PHYSICAL CONSTANTS AND ANALYSES OF NEW COMPOUNDS

Compound	Method	Yield %	M.p. or (b.p.)	Analyses: Carbon % Calculated	Carbon % Found	Hydrogen % Calculated	Hydrogen % Found	Nitrogen % Calculated	Nitrogen % Found
m-Fluoro-β-nitrostyrene	A[1]	56	44·5–46·5°	57·5	57·6	3·6	3·6	8·4	8·6
p-Fluoro-β-nitrostyrene	A[1]	55	99·5–101°	57·5	58·3	3·6	3·8	8·4	8·5
p-(2-Nitrovinyl)benzoic acid	A[2]	23	285° (dec.)	56·0	56·4	3·65	3·9	7·25	7·2
p-(2-Nitrovinyl)cinnamic acid	A[4]	60	286–287° (dec.)	60·3	60·2	4·1	4·3	6·4	6·2
β-(*p*-2-Nitrovinylphenyl)propionic acid	A[3]	36	188–190°	59·7	59·9	5·0	5·2	6·3	6·2
o-(2-Nitrovinyl)phenoxyacetic acid	A[3]	—	173–175°	53·8	54·0	4·1	4·2	6·3	6·4
2,5-Di(2-nitrovinyl)furan	A[4]	30	225° (dec.)	45·7	46·5	2·9	3·0	—	—
4-(2-Nitrovinyl)-1-phenylpyrazole	A[3]	67	146–148°	61·4	61·75	4·2	3·7	19·5	19·5
1-Methyl-4-(2-nitrovinyl)pyrazole	A[3]	45	129–129·5°	47·05	47·3	4·6	5·05	27·4	27·05
2-Methylthio-4-(2-nitrovinyl)-1-*p*-tolylimidazole	A[3]	22	189–190°	56·7	57·1	4·8	4·8	15·3	14·3
m-Di(2-nitroprop-1-enyl)benzene	B[3]	16	104°	58·1	58·2	4·9	4·5	11·3	11·2
1,3,5-Tri(2-nitroprop-1-enyl)benzene	B[3]	12	201°	54·05	54·8	4·5	4·4	12·6	12·5
o-Methoxy-β-nitrostyrene	B[1]	8	34·5–35·5°	60·3	60·5	5·1	5·2	7·8	8·1
1-Methyl-2-(2-nitrovinyl)pyrrole	B[3,5]	24	[4] 101–102°	55·25	55·6	5·3	5·55	18·4	18·1
4-(2-Nitrovinyl)quinoline	B[3,5,6]	27	166·5–167·5° (dec.)	—	—	—	—	14·0	13·6
1-Methyl-5-(2-nitrovinyl)imidazole	B[3,5,7]	28	157–158°	47·05	46·8	4·6	4·6	27·4	27·55
4-(2-Nitrovinyl)thiazole	B[3,5]	9	108–111°	38·5	38·5	2·6	2·85	17·95	17·8
5-(2-Nitrovinyl)thiazole	B[3,5]	23	176·5–179°	38·5	38·35	2·6	2·5	17·95	17·2
4-(2-Nitrovinyl)-2-phenyl-2*H*-1,2,3-triazole	B[3,5,7]	34	128–130·5°	55·55	55·9	3·7	4·2	25·9	25·5
4-(2-Nitrovinyl)-1-propyl-1*H*-1,2,3-triazole	B[3,5,7]	9	115–116°	46·15	47·0	5·5	5·7	30·8	29·9
4-(1-Hydroxy-2-nitroethyl)-1-*p*-tolylimidazole	C(a)[1]	73	151–152·5°	58·3	58·85	5·3	5·6	—	—
4-(2-Nitrovinyl)-1-*p*-tolylimidazole	C(b)[3]	98	154–155° (dec.)	62·9	62·8	4·8	4·8	18·3	18·35
4-(1-Hydroxy-2-nitroethyl)-2-methanesulphonyl-1-*p*-tolylimidazole	C(a)[1]	70	165–168°	48·0	48·1	4·65	4·6	12·9	12·8
2-Methanesulphonyl-4-(2-nitrovinyl)-1-*p*-tolylimidazole	C(b)[3]	80	150–151°	50·8	50·8	4·3	4·1	13·7	13·4
1-*p*-Cyanophenyl-2-nitroethanol	C(a)[1]	24	96–98°	56·25	56·3	4·2	4·1	14·6	14·6
1-Methyl-2-nitroethyl chloroacetate	D	72	112–115·5°/ 3·0 mm	33·1	32·8	4·4	4·5	7·7	7·6
1-(Nitromethyl)propyl chloroacetate	D	60	102–118°/ 3·8 mm	36·8	37·5	5·1	5·3	7·2	6·3
1-(Nitromethyl)butyl chloroacetate	D	69	(128–137°/ 0·3 mm)	40·1	40·3	5·8	5·9	6·7	6·8
1-Methyl-2-nitroethyl hemisuccinate	E	4	70–71°	41·0	41·1	5·4	5·6	6·8	6·9
1-(Nitromethyl)propyl hemisuccinate	E	10	76–77°	43·8	44·0	6·0	6·0	6·4	6·4
1-(Nitromethyl)butyl hemisuccinate	E	5	71–72°	46·35	46·55	6·5	6·6	6·0	6·0

[1] Purified from sublimation. Recrystallised from: [2] light petroleum (60–80°): [3] ethanol: [4] ethanol - dimethylformamide. [5] Reaction conducted at room temperature.
[6] Methylamine used as a catalyst in place of butylamine. [7] Propylamine used as a catalyst in place of butylamine.

in ethanol (9 ml) and nitromethane (183 mg) at 0°. The mixture was brought to pH 8 with dilute hydrochloric acid and the precipitate was collected by filtration, washed with water, dried *in vacuo* and purified.

(b) *Dehydration.* A mixture of the nitrocarbinol (1 mmol), anhydrous sodium acetate (330 mg) and acetic anhydride (1·5 g) was heated to 100° for 20 min and then poured into water (50 ml). Dilute sodium hydroxide solution was added until pH 8 was reached and the product was isolated as above.

Method D. A mixture of the nitrocarbinol and a molar equivalent of chloroacetyl chloride was kept at room temperature for 6 days and then fractionally distilled.

Method E. A mixture of the nitrocarbinol (5 mmol), succinic anhydride (600 mg) and concentrated sulphuric acid (2 drops) was stirred for 2 hr at 40°, then treated with benzene, and the whole was poured onto ice. Succinic acid was filtered off and the separated benzene layer was extracted with ice-cold sodium bicarbonate solution. Acidification of the aqueous extract liberated the hemisuccinate, which was extracted in benzene. After evaporation of the solvent, the product was crystallised from isopropyl ether/light petroleum (60–80°).

MICROBIOLOGY

Trichomonacidal assay. Essentially the conditions described by Trussell (1947) for the comparative evaluation of trichomonacides were followed. A sterile stock solution of each of the compounds was prepared in a liver medium (Feinberg & Whittington, 1957) from which two-fold dilutions were taken to give a range from 500 to 1 μg/ml. Compounds insoluble in water were first dissolved in a minimum volume of dimethylformamide. Control studies showed that this solvent had no effect on the organisms at the dilutions employed. The final pH of all solutions was adjusted to 5·8. Two ml volumes of each concentration were transferred to sterile tubes and inoculated with 0·1 ml of a culture of *T. vaginalis* (Strain W3). The organisms were maintained in Feinberg's medium and subcultured daily so that the maximum number of actively multiplying organisms was achieved in a 24-hr culture. It is known that the minimal lethal concentration of some trichomonacidal drugs varies with the size of the inoculum, hence throughout the screening the inoculum was kept to a standard of approximately 250,000 organisms per tube. All the experiments were duplicated. The tubes were incubated at 37° for 24 hr. If the organisms were non-motile microscopically and failed to grow within nine days of being subcultured into the same medium, they were presumed to be dead.

Candidacidal assay. A range of dilutions of each compound was prepared as above. Tubes containing 2 ml of Sabouraud fluid medium (Oxoid), which was used for all cultures, were inoculated with 0·02 ml of a suspension of *C. albicans* equivalent to approximately 20,000 cells per inoculum. After incubation at 37° for 24 hr the tubes were subcultured by plating an aliquot onto Sabouraud agar; failure of the subcultures to grow within five days indicated death.

ANIMAL STUDIES

Urinary excretion. The test compounds were administered orally to rats at a dose of 1/10th of their LD50 or 200 mg/kg if the LD50 was in excess of 2 g/kg.

The rats were placed in stainless steel metabolism cages and the urine was collected in glass vessels containing approximately 10 mg of streptomycin and 10 mg of benzylpenicillin potassium to prevent the heavy bacterial overgrowth which would otherwise interfere with the microbiological assay. Urine was collected from 0–6 hr and from 6–24 hr after dosing and its trichomonacidal activity determined as described above.

Irritancy. Doses (12·5–100 mg) of the test compounds incorporated into 0·1 ml of a polyethyleneglycol base were administered intravaginally to rats. Two days later 0·5 ml of an aqueous suspension (4 mg/ml) of azovan blue was injected intravenously and after 2 hr the rats were killed. The vaginae were opened and examined for signs of dye-leakage; stained sections were prepared in the normal way and examined microscopically for signs of inflammation or damage.

Results and discussion

The trichomonacidal and candidacidal activities of a collection of nitroethylenes variously substituted with aromatic, heterocyclic and aliphatic groups are shown in Table 2. For comparison, metronidazole killed *T. vaginalis* at 2 μg/ml and nystatin killed *C. albicans* at 32 μg/ml. Neither drug was active against both organisms.

In the aromatic series generally, although differences are neither marked nor wholly consistent, substituents on the ring, whether electro-positive or -negative, tend to enhance activity against both pathogens. Salt-forming groups which enhance water solubility have a variable effect. A dimethylamino-group weakens, and a quaternary ammonium group totally abolishes, the activity. A side chain bearing a carboxyl group reduces candidacidal activity but has a less predictable effect on trichomonacidal activity. Polysubstitution of the aromatic ring, particularly with additional nitrovinyl groups, increases the activity against *Trichomonas* but, with two exceptions, weakens it against *Candida.*

Replacement of the benzene ring with various heterocyclic rings did not lead to any compounds with outstanding activity. They were usually inferior to the nitrostyrenes. In all instances examined, quaternisation of a ring nitrogen atom rendered the compound inactive.

Aliphatic nitro-olefins had weak activity and the carbinols from which they were prepared were even less active—similar effects, denoted with an asterisk in Table 2, were observed in the aromatic and heterocyclic series—but their esters were as active, or more so, than the related olefins.

The acute oral toxicity of the more active compounds in mice was low, ranging from 500 to 2000 mg/kg, but the recovery from urine, at best only 1–2% of that of metronidazole, suggested that absorption was poor and an adequate serum concentration of the drugs was unlikely to have been achieved. (A reliable assay of these drugs in blood or serum is

lacking). To assess the potentiality of the compounds as topical candidacides—there is no satisfactory systemic treatment for this condition—they were administered intravaginally to rats in an ointment base. Subsequent examination of the vaginal tissues revealed greater damage than was observed with nystatin.

TABLE 2. ACTIVITY OF SOME NITRO-ETHYLENES AGAINST *Trichomonas vaginalis* AND *Candida albicans*

Results are mean values of at least 2 estimations

Compound	Minimal lethal concentration (μg/ml)	
	T. vaginalis	*C. albicans*
Aromatic		
β-Nitrostyrene	8	10
2-Nitroprop-1-enylbenzene	8	8
o-Fluoro-β-nitrostyrene	4	8
m-Fluoro-β-nitrostyrene	4	2
p-Fluoro-β-nitrostyrene	4	8
o-Chloro-β-nitrostyrene	8	4
m-Chloro-β-nitrostyrene	16	8
p-Chloro-β-nitrostyrene	8	2
p-Bromo-β-nitrostyrene	4	1
p-Iodo-β-nitrostyrene	8	2
o,β-Dinitrostyrene	4	4
m,β-Dinitrostyrene	4	2
p,β-Dinitrostyrene	4	16
p-Cyano-β-nitrostyrene	8	8
*1-*p*-Cyanophenyl-2-nitroethanol	64	64
o-Hydroxy-β-nitrostyrene	4	16
m-Hydroxy-β-nitrostyrene	2	4
p-Hydroxy-β-nitrostyrene	8	16
o-Methoxy-β-nitrostyrene	8	8
m-Methoxy-β-nitrostyrene	8	16
p-Methoxy-β-nitrostyrene	4	4
p-(2-Nitrovinyl)benzoic acid	8	125
p-(2-Nitrovinyl)cinnamic acid	4	125
β-(*p*-2-Nitrovinylphenyl)propionic acid	4	64
o-2-Nitrovinylphenoxyacetic acid	32	250
p-Dimethylamino-β-nitrostyrene	16	125
5-Fluoro-2,β-dinitrostyrene	4	500
4-Chloro-3,β-dinitrostyrene	4	32
m-Di(2-nitrovinyl)benzene	2	8
m-Di(2-nitroprop-1-enyl)benzene	2	64
p-Di(2-nitrovinyl)benzene	1	1
p-Di(2-nitroprop-1-enyl)benzene	1	16
1,3,5-Tri(2-nitrovinyl)benzene	4	500
1,3,5-Tri(2-nitroprop-1-enyl)benzene	1	500
Heterocyclic		
2-(2-Nitrovinyl)furan	16	32
5-Nitro-2-(2-nitrovinyl)furan	32	16
2,5-Di(2-nitrovinyl)furan	4	8
2-(2-Nitrovinyl)thiophen	16	16
1-Methyl-2-(2-nitrovinyl)pyrrole	16	64
3-(2-Nitrovinyl)indole	16	125
3-(2-Nitrovinyl)pyridine	8	16
3-(2-Nitroprop-1-enyl)pyridine	4	8
4-(2-Nitrovinyl)quinoline	8	32
4-(2-Nitrovinyl)-2-phenyloxazole	4	>500
5-(2-Nitrovinyl)-3-phenylisoxazole	4	>500
4-(2-Nitrovinyl)-1-phenylpyrazole	16	16
1-Methyl-4-(2-nitrovinyl)pyrazole	125	32
1-Methyl-5-(2-nitrovinyl)imidazole	16	125
4-(2-Nitrovinyl)-1-*p*-tolylimidazole	8	32
*4-(1-Hydroxy-2-nitroethyl)-1-*p*-tolylimidazole	8	64
2-Methylthio-4-(2-nitrovinyl)-1-*p*-tolylimidazole	4	>500
2-Methanesulphonyl-4-(2-nitrovinyl)-1-*p*-tolylimidazole	16	250
*4-(1-Hydroxy-2-nitroethyl)-2-methanesulphonyl-1-*p*-tolylimidazole	125	500
4-(2-Nitrovinyl)thiazole	16	4
4-(2-Nitroprop-1-enyl)thiazole	8	8
5-(2-Nitrovinyl)thiazole	32	8
5-(2-Nitroprop-1-enyl)thiazole	8	16
4-(2-Nitrovinyl)-2-phenyl-2*H*-1,2,3-triazole	2	8
4-(2-Nitrovinyl)-1-propyl-1*H*-1,2,3-triazole	16	250

TABLE 2—*continued*

Compound	Minimal lethal concentration (μg/ml)	
	T. vaginalis	*C. albicans*
Aliphatic		
1-Nitropropene	16	—
1-Nitrobut-1-ene	64	64
1-Nitropent-1-ene	250	8
1-Nitropropan-2-ol	250	125
1-Nitrobutan-2-ol	125	125
1-(Nitromethyl)butyl acetate	4	16
1-(Nitromethyl)propyl acetate	64	32
1-Methyl-2-nitroethyl acetate	32	32
1-Methyl-2-nitroethyl chloroacetate	32	64
1-(Nitromethyl)propyl chloroacetate	64	32
1-(Nitromethyl)butyl chloroacetate	32	16
1-Methyl-2-nitroethyl hemisuccinate	32	125
1-(Nitromethyl)propyl hemisuccinate	64	64
1-(Nitromethyl)butyl hemisuccinate	64	32

Acknowledgements. The *Trichomonas vaginalis* strain W3 was kindly supplied by Dr. J. G. Feinberg.

References

Bocobo, F. C., Curtis, A. C., Block, W. D. & Harrell, E. R. (1954). *Proc. Soc. exp. Biol. N.Y.*, **85**, 220–222.

Catterall, R. D. & Nicol, C. S. (1957). *Brit. med. J.*, **2**, 29–31.

Cazemier, C., Goslings, W. R. O., Houwert, K. A. F., van Leeuwen, D. P., Lubbers, G. J. & Kok, P. C. (1959). *Antibiotic Med. Clin. Ther.*, **6**, 601–605.

Cosar, C. & Julou, L. (1959). *Ann. Inst. Pasteur.*, **96**, 238–341.

Feinberg, J. G. & Whittington, M. S. (1957). *J. chem. Path.*, **10**, 327–329.

Trussell, R. E. (1947). *Trichomonas vaginalis and Trichomoniasis*, 1st ed., p. 54. Illinois: Charles C. Thomas.