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Synthesis and antiprotozoal activity of some imidazoles derivatives

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The synthesis and *in vitro* antiprotozoal activity of a series of nitroimidazoles are described. A substituted phenyl group and, in many of the compounds, a *N*-substituent were also present: vinylogues of some of the compounds are described. Several of the nitroimidazoles exhibit very high *in vitro* activity against *Trichomonas vaginalis* and *T. foetus*, and good activity against *Histomonas meleagridis* and *Entamoeba histolytica*. Their antifungal action was lower than that of some of the un-nitrated imidazoles. The antiprotozoal activity of these compounds is discussed in relation to their chemical structure. 4-*p*-Acetamidophenyl-, 4-*p*-chlorophenyl- and 4-(3,4-dichlorophenyl)-1-methyl-5-nitroimidazole, and 4-*p*-chlorophenyl- and 4-(3,4-dichlorophenyl)-1-(2-hydroxyethyl)-5-nitroimidazole are the most active against *Trichomonas*.

THE failure of acinitrazole (2-acetamido-5-nitrothiazole; Tritheon) to fulfil its early promise (Cuckler, Kupferberg & Millman, 1955) as a clinically useful drug against *Trichomonas vaginalis* infections (Bushby & Copp, 1955) emphasises the need of further search for a trichomonacide for clinical and veterinary use. In such compounds as chloramphenicol, azomycin, acinitrazole and several nitrofurans the nitro-group attached to a benzene or heterocyclic ring is essential for their high chemotherapeutic efficiency. In view of the antifungal activity of some of the imidazole derivatives previously described (Ellis, Epstein, Fitzmaurice, Golberg & Lord, 1964), it was of interest to study the effect of nitration on their antiprotozoal activity. The new compounds prepared contain a nitro-group attached at either position 4 or 5 of the imidazole ring and a substituted phenyl group at the corresponding position 5 or 4. Many of the compounds also carry another substituent on the ring nitrogen atom.

The effect of the compounds described in this and the previous paper (Ellis & others, 1964) was studied on selected fungi and protozoa.

While the present work was in progress, reports on the use of metronidazole [1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole; Flagyl, 8823 R.P.] in the treatment of human trichomonad infections were published (Cosar & Julou, 1959; Sylvestre & Gallaiz, 1960; Bonzaine & Desranleau, 1960; Schnitzer, 1963).

For the preparation of the compounds studied, one of two standard methods of nitrating imidazoles was used. Either the imidazole nitrate was heated, with stirring, with concentrated sulphuric acid, or solid sodium nitrate was added to the imidazole base dissolved in sulphuric

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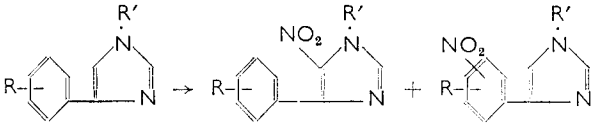
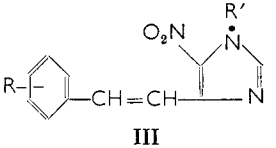
acid. Both methods gave a mixture of the nitroimidazole (I) and nitrophenyl (II) compounds, but the proportion varied with the substituent already on the benzene ring. Good yields of the almost colourless nitroimidazole were obtained only when the benzene ring carried a deactivating group. Separation of the isomers depended on the greater basicity of the yellow-coloured nitrophenyl isomer which remained in solution after dilution of the reaction mixture when the nitroimidazoles were precipitated. The structure of the nitrophenyl isomers was determined by oxidation with potassium permanganate to give a substituted benzoic acid. For example, 4-chloro-3-nitrobenzoic acid was obtained by the oxidation of the acid-soluble product of the nitration of 4(5)-*p*-chlorophenylimidazole. Nitroimidazoles unsubstituted at position 1 were



I II

alkylated by treatment with an alkyl sulphate to give the 1-alkyl-5-nitroimidazole. If an alkyl halide and potassium carbonate were used for alkylation, a mixture of the 4- and 5-nitroimidazoles was formed from which the pure 1-alkyl-4-nitroimidazole was obtained by fractional crystallization. Assignment of structures has been discussed by Ellis & others (1964). 5(4)-Nitro-4(5)-styrylimidazoles (III, R′ = H) analogous to the above phenylnitroimidazoles were readily prepared by condensing an aromatic aldehyde with 4(5)-methyl-5(4)-nitroimidazole or the 1-alkyl derivatives.



III

Some representative syntheses are given in the experimental section and the melting-point and analysis of the compounds are listed in Table 1.

Experimental

Nitration of 4(5)-*p-chlorophenylimidazole.* 4(5)-*p*-Chlorophenylimidazole (75 g) was treated with 2N nitric acid (250 ml). The solid nitrate was filtered and dried and added carefully to concentrated sulphuric acid (750 ml) with stirring. The mixture was heated for 15 hr on a steam-bath, then cooled and poured on to ice (750 g) and water (2 litres). The precipitated yellow solid was filtered off, washed with cold water and then boiled with dilute hydrochloric acid and the suspension filtered. The colourless residue was 4(5)-p-*chlorophenyl*-5(4)-*nitroimidazole* (30 g), m.p. 285°. The filtrate was basified with aqueous ammonia to yield the yellow 4(5)-(4-chloro-3-nitrophenyl)imidazole, m.p. 245° (from ethanol). The filtrate

from the nitration mixture on basifying with aqueous ammonia precipitated yellow plates of 4(5)-(4-chloro-2-nitrophenyl)imidazole, m.p. 201° (from ethanol). The analyses for these two nitrophenyl compounds were given by Ellis & others (1964). Characterisation of the three nitro-compounds was effected by oxidizing 1 g of each with potassium permanganate (4 g) in water (50 ml) containing sodium carbonate (1 g) to give *p*-chloro, 4-chloro-3-nitro-, and 4-chloro-2-nitro-benzoic acids, respectively, which did not depress the melting-points of authentic specimens.

4-p-*Chlorophenyl*-1-*methyl*-5-*nitroimidazole.* 4(5)-*p*-Chlorophenyl-5(4)-nitroimidazole (1·0 g) and dimethyl sulphate (0·6 ml) were heated together on a steam-bath for 1 hr. Water (20 ml) was added, followed by an excess of sodium bicarbonate. The mixture was extracted three times with 50 ml portions of ether, the extracts were dried (Na_2SO_4), concentrated and then diluted with light petroleum (b.p. 40–60°) (50 ml) to give the pale yellow 4-p-*chlorophenyl*-1-*methyl*-5-*nitroimidazole,* m.p. 107–108° (from ether).

5-p-*Chlorophenyl*-1-*methyl*-4-*nitroimidazole.* 4(5)-*p*-Chlorophenyl-5(4)-nitroimidazole (3·7 g), methyl iodide (2·5 g) and potassium carbonate (1·4 g) were refluxed together in acetone (200 ml) for 4 hr. The solid residue was filtered off, and the filtrate was evaporated to dryness and washed with ether (50 ml). From the residue, colourless needles of 5-p-*chlorophenyl*-1-*methyl*-4-*nitroimidazole* (1·4 g), m.p. 238–240° (from acetone) were isolated. The ether washings contained 4-*p*-chlorophenyl-1-methyl-5-nitroimidazole (0·6 g), m.p. 105°.

4(5)-(3,4-*Dichlorophenyl*)-5(4)-*nitroimidazole.* 4(5)-(3,4-Dichlorophenyl)imidazole (1 g) was suspended in water (10 ml) and treated with 10% nitric acid solution until acid to Congo Red. The suspension of the sparingly soluble salt was evaporated to dryness *in vacuo* and the residue added with cooling to concentrated sulphuric acid (5 ml). After heating the solution for 1 hr at 100°, it was poured onto ice, whereupon 4(5)-(3,4-*dichlorophenyl*)-5(4)-*nitroimidazole* (0·4 g), m.p. 302–303° (from aqueous ethanol) was precipitated. Oxidation of a sample with alkaline potassium permanganate gave 3,4-dichlorobenzoic acid, m.p. 208°.

The acid filtrate, on basification with aqueous ammonia, gave the greenish-yellow 4(5)-(4,5-dichloro-2-nitrophenyl)imidazole (0·4 g), m.p. 225–226° (Ellis & others, 1964).

4-(3,4-*Dichlorophenyl*)-1-*methyl*-4- and -5-*nitroimidazole.* 4(5)-(3,4-Dichlorophenyl)-5(4)-nitroimidazole (5 g), potassium carbonate (1·5 g) and methyl iodide (1·3 g) were refluxed together in acetone (100 ml) for 72 hr. The reaction mixture was filtered, the acetone distilled off, the residual solid stirred with cold ether, and the solution filtered; the filtrate, after being decolorised, concentrated and diluted with light petroleum, yielded pale yellow needles of 4-(3,4-*dichlorophenyl*)-1-*methyl*-5-*nitroimidazole* (0·5 g), m.p. 134–135° (not depressed on admixture with the product of nitration of 4-(3,4-dichlorophenyl)-1-methylimidazole). The residue from the filtration of the ether solution was extracted with hot ethyl acetate. On cooling the extract, unchanged starting material (0·25 g),

m.p. 304–305°, was deposited. After removing this, the solution was decolorized, concentrated and diluted with light petroleum to precipitate the colourless 5-(3,4-*dichlorophenyl*)-1-*methyl*-4-*nitroimidazole* (1·7 g), m.p. 195–197°.

4-*p*-*Acetamidophenyl*-1-*methyl*-5-*nitroimidazole.* 4(5)-*p*-Acetamidophenyl-5(4)-nitroimidazole (Grant & Pyman, 1921) (10 g) was heated on a steam-bath with dimethyl sulphate (10 ml) for 2 hr. The excess of dimethyl sulphate was removed *in vacuo*, and the residue was dissolved in water and basified with aqueous ammonia. Chloroform extracts of this solution were decolorised and the solvent was removed *in vacuo* without heating, leaving golden-yellow leaflets of 4-p-*acetamidophenyl*-1-*methyl*-5-*nitroimidazole* (2·1 g), m.p. 214–215°.

4-*p*-*Aminophenyl*-1-*methyl*-5-*nitroimidazole.* Repetition of the above preparation, but with prolonged heating for 16 hr, gave *the aminophenyl compound* as orange-red crystals, m.p. 141–143° (4·0 g) [from ethyl acetate-light petroleum (b.p. 60–80°)].

4(5)-p-*Chlorophenyl*-2-*methyl*-5(4)-*nitroimidazole.* 4(5)-*p*-Chlorophenyl-2-methylimidazole (1·3 g) was suspended in water (5 ml) and concentrated nitric acid (0·4 ml) was added. The mixture was warmed, cooled and filtered. The solid nitrate was dissolved in cooled concentrated sulphuric acid (5 ml), and the blue solution was heated at 100° for 1 hr, cooled, poured into water (20 ml) and filtered. After warming the solid with dilute hydrochloric acid, filtering and washing with water, it gave the *nitroimidazole* (0·4 g), m.p. 263–266° (from ethanol).

Hydroxyethylation of 4(5)-(3,4-*dichlorophenyl*)-5(4)-*nitroimidazole.* A mixture of 4(5)-(3,4-dichlorophenyl)-5(4)-nitroimidazole (5 g), 2-bromoethanol (1·5 ml), acetone (100 ml) and potassium carbonate (1·5 g) was refluxed for 72 hr and filtered. The solvent was distilled off and the solid extracted with boiling ether (4 × 100 ml); the combined extracts, on standing, deposited crystals which were added to the ether-insoluve residue, which was the pale yellow 5-(3,4-*dichlorophenyl*)-1-(2-*hydroxyethyl*)-4-*nitroimidazole* (1·8 g), m.p. 177° (from aqueous ethanol). From the ether solution, 4-(3,4-*dichlorophenyl*)-1-(2-*hydroxyethyl*)-5-*nitroimidazole* (0·5 g), m.p. 102–103° (from ethyl acetate-light petroleum, b.p. 60–80°) was obtained as salmon-coloured crystals by dilution with light petroleum.

5-(3,4-*Dichlorostyryl*)-1-*methyl*-4-*nitroimidazole.* 3,4-Dichlorobenzaldehyde (0·9 g), 1,5-dimethyl-4-nitroimidazole (0·7 g) and piperidine (0·1 ml) were heated together at 150–160° for 2 hr. The liquid was cooled, stirred with ether and filtered. Extraction of the solid with boiling water yielded 5-(3,4-*dichlorostyryl*)-1-*methyl*-4-*nitroimidazole* (0·25 g), m.p. 199–201° (from ethyl acetate).

Biological methods

In vitro ASSAY OF ANTIPROTOZOAL ACTIVITY

The following pathogenic protozoa (obtained from the sources stated) were used as test organisms: *Trichomonas vaginalis* (T 70), Liverpool Public Health Laboratory; *T. foetus* (T 69, Belfast strain), Agricultural

Research Council, Weybridge; *T. gallinae* (T 80, Wilson strain), Agricultural Research Council Veterinary Laboratories, Midlothian; *Histomonas meleagridis* (Joyner strain), Agricultural Research Council, M.A.F.F. (Weybridge; *Entamoeba histolytica* (Strain DC), Liverpool School of Tropical Medicine. The culture media used were:

Trichomonas stock culture medium. The composition w/v of a modified transport medium (Stuart, 1954) was as follows: sodium glycerophosphate 1·0, calcium chloride 0·01, sodium thioglycollate 0·1, agar 0·2, and methylene blue 0·0002%. Final pH 7·4; 12 ml of this was sterilised in ½ oz. screw-capped bottles at 15 lb/in² for 15 min. Before inoculation, the colourless medium was supplemented with 10% v/v heat-inactivated bovine serum.

Trichomonas assay medium. The three trichomonas strains were grown in the following modification (designated T.V. medium) of Kupferberg's medium (Kupferberg, Johnson & Sprince, 1948): Difco Protease peptone No. 3, 10; Difco Tryptose peptone, 10; Kerfoot D-glucose, 10; sodium chloride, 5; L-cysteine hydrochloride, 1·0; ascorbic acid, 1·5 g; 'Panamede' (Paines & Byrne) desiccated liver extract, 0·5 g, and distilled water 1 litre. Supplements to the medium included 400 I.U./ml penicillin and 0·5 mg/ml streptomycin sulphate together with 10% v/v heat-inactivated bovine serum.

The T.V. medium was prepared by dissolving separately the peptones, sodium chloride, L-cysteine hydrochloride and liver extract in 100-ml portions of hot distilled water. The warm solutions were mixed and cooled to room temperature before adding the glucose and ascorbic acid. The clear filtrate obtained by gravity filtration through a fluted Whatman No. 1 paper was diluted to 1 litre before adding the antibiotics. The medium was adjusted to pH 6·2, prefiltered through a Pyrex (porosity grade 4) sterile sintered-glass filter before final sterilisation by filtration through an Oxoid membrane. The filtrate was stored in the dark at 3–5°, and used, within 2 weeks, for maintaining the trichomonad, and preparing the inocula and assay medium. In each case it was supplemented with 10% v/v heat-inactivated bovine serum immediately before use.

Histomonas and entamoeba stock culture medium. A diphasic medium maintained continuously at 37° was used for both organisms. Sterile serum slopes (4 ml) were prepared in 6 × ⅝ inch cotton-wool-plugged test tubes for the histomonas and in ½-oz screw-capped bottles for the entamoeba. Coagulation of the serum was effected by heating at 80° for 20 min and to the cooled slope it was necessary to add 5–10 mg of heat-sterilised Difco rice starch powder. The egg-white overlay liquid medium was prepared by aseptically separating the white of a medium sized egg, mixing with 200 ml of sterile Ringer-phosphate saline (*p*H 7·4), adding 2 ml of sterile 20% w/v aqueous D-glucose solution and mixing well by means of a magnetic stirrer. The medium was then stored at 3–5° and used within 10 days.

The final medium (Rees & Reardon, 1945) consisted of 4 ml of inspissated horse serum slant covered with 8 ml of diluted egg white plus

rice starch powder. For both organisms the stock culture was maintained by subculturing every two or three days; the overlay liquid medium was preconditioned by inoculating with the mixed "natural" bacterial associates of the respective strains 4–5 hr before inoculating with approximately 50,000 viable amoeboid cells.

MAINTENANCE OF STOCK CULTURES OF TRICHOMONAD STRAINS

A 24-hr old vigorously growing, bacteria-free trichomonad culture, the flagellate of which exhibited extremely rapid motility was used. A 10% v/v inoculum was added to 12-ml volumes each of the Stewart transport medium and the T.V. medium, and then stored at 3–5° for two or three days. The cultures were unimpaired by this storage and were then incubated at 37° for 24 hr to provide once again a vigorously growing culture which could be used for either a static culture to be stored in the refrigerator or provide an inoculum for assays to determine trichomonacidal activity of new compounds.

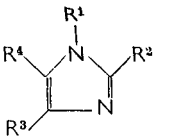
In vitro ASSAYS TO COMPARE THE ACTIVITIES OF NEW COMPOUNDS WITH STANDARD DRUGS

Trichomonacides. The minimum concentration of a compound which suppressed completely the growth of trichomonads at 37° over a period of 48 hr was determined. The end-points were ascertained by microscopic examination of the culture, death being indicated by morphological change from pear-shaped to rounded cells and complete cessation of motility. The standard drugs employed were (1) acinitrazole and (2) metronidazole. Nithiazide and dimetridazole were also included for comparison. Stock solutions (1% w/v) of standard and new compounds were prepared in either glycerol formal or liquid macrogol (Carbowax 300). Predilutions of the drugs were prepared in T.V. medium in wide-mouthed tubes, covering serial two-fold dilutions over the ranges 100–3 μg/ml, and 10–0·3 μg/ml; 5 ml portions of these dilutions were then transferred to $\frac{1}{4}$ oz screw-capped bottles.

The assay inoculum was prepared from a 24-hr old vigorous culture of the trichomonad strain in T.V. medium which was diluted in this medium to give approximately 500,000 viable protozoa per ml, 0·2 ml of which was added aseptically to each bottle so that each bottle received 100,000 trichomonads.

Amoebicides. Single-phase medium (Jones, 1946) containing 0·1% w/v autolysed yeast extract in buffered phosphate saline, pH 7·3, with rice powder supplement, along with 10% v/v heat-inactivated horse serum was used for screening new compounds. The Jones medium was preconditioned with the appropriate bacterial associates incubated for 4–5 hr at 37°, and two-fold serial dilutions of the drugs were prepared so that finally the medium plus rice powder in 5 ml portions were contained in $\frac{1}{4}$ oz screw-capped bottles. Each bottle then received 50,000 viable amoeboid cells as inoculum. After incubation for 48 hr at 37°, the sedimented layers in each bottle were examined microscopically and the minimum cidal concentration (MCC) was ascertained.

TABLE 1. ARYLNITROIMIDAZOLE DERIVATIVES



Compound No.	R^1	R^2	R^3	R^4	m.p. °C	Formula	Analysis							
							Found				Required			
							C	H	Cl or Br	N	C	H	Cl or Br	N
1[a]	H	H	*p*-$NH_2{\cdot}C_6H_4$, HCl	NO_2	245–246	Grant and Pyman (1921)								
2	Me	H	*p*-$NH_2{\cdot}C_6H_4$	NO_2	141–143	$C_{10}H_{10}N_4O_2$	54·9	4·5	—	25·2	55·0	4·6	—	25·7
3	Me	H	*p*-Ac·NH·C_6H_4	NO_2	214–215	$C_{12}H_{12}N_4O_3$	55·2	4·9	—	21·9	55·4	4·6	—	21·5
4	Me	H	*p*-($Cl_3CH{\cdot}CO{\cdot}NH$), C_6H_4	NO_2	241–243	$C_{12}H_{10}Cl_3N_4O_3$	43·5	3·2	21·1	—	43·7	3·0	21·5	—
5[a]	H	H	*p*-Br·C_6H_4	NO_2	281–283	$C_9H_8BrN_3O_2$	40·5	2·4	—	15·5	40·3	2·2	—	15·7
6	Me	H	*p*-Br·C_6H_4	NO_2	99	$C_{10}H_9BrN_3O_2$	42·8	2·9	—	14·9	42·6	2·9	—	14·9
7	Me	H	NO_2	*p*-Br·C_6H_4	170	$C_{10}H_9BrN_3O_2$	42·8	2·8	—	14·9	42·6	2·9	—	14·9
8[a]	H	H	*p*-Cl·C_6H_4	NO_2	285	$C_9H_8ClN_3O_2$	48·6	3·1	15·4	18·2	48·4	2·7	15·7	18·8
9[a]	H	Me	*p*-Cl·C_6H_4	NO_2	263–266	$C_{10}H_9ClN_3O_2$	50·0	3·4	15·1	—	50·5	3·4	14·9	—
10	Me	H	*p*-Cl·C_6H_4	NO_2	107–108	$C_{10}H_9ClN_3O_2$	50·4	2·9	14·9	17·9	50·5	3·4	14·9	17·7
11	Me	H	NO_2	*p*-Cl·C_6H_4	238–240	$C_{10}H_9ClN_3O_2$	50·6	3·0	15·3	17·9	50·5	3·4	14·9	17·7
12	Me	Me	*p*-Cl·C_6H_4	NO_2	145–147	$C_{11}H_{10}ClN_3O_2$	52·1	4·1	14·4	16·8	52·5	4·0	14·1	16·7
13	Me	Me	NO_2	*p*-Cl·C_6H_4	200	$C_{11}H_{10}ClN_3O_2$	52·7	4·2	13·9	16·4	52·5	4·0	14·1	16·7
14	$CH_2{\cdot}CH_2{\cdot}CN$	H	*p*-Cl·C_6H_4	NO_2	105–106	$C_{12}H_9ClN_4O_2$	—	—	13·1	20·6	—	—	12·9	20·2
15	$CH_2{\cdot}CH_2{\cdot}CO{\cdot}OEt$	H	NO_2	*p*-Cl·C_6H_4	132–134	$C_{13}H_{12}ClN_3O_4$	50·4	4·0	11·7	—	50·1	3·9	11·5	—
16	$CH_2{\cdot}CH_2OH$	H	*p*-Cl·C_6H_4	NO_2	108–109	$C_{11}H_{10}ClN_3O_3$	49·4	3·7	13·5	16·0	49·3	3·8	13·3	15·7
17	$CH_2{\cdot}CH_2OH$	H	NO_2	*p*-Cl·C_6H_4	140–141	$C_{11}H_{10}ClN_3O_3$	49·1	3·8	13·9	15·8	49·4	3·8	13·3	15·7
18[a]	H	H	2-$CO_2H{\cdot}C_6H_3{\cdot}Cl$-H	NO_2	252–254	$C_{10}H_8ClN_3O_4$	44·9	2·4	—	15·4	44·9	2·3	—	15·7
19[a]	H	H	*p*-$SO_3H{\cdot}C_6H_4$	NO_2	>300	$C_9H_7N_3O_5S$	40·0	2·6	—	15·2	40·1	2·6	—	15·6
20[a]	H	H	3,4-Cl_2:C_6H_3	NO_2	302–303	$C_9H_6Cl_2N_3O_2$	42·4	2·1	—	—	41·9	1·9	—	—
21	Me	H	3,4-Cl_2:C_6H_3	NO_2	134–135	$C_{10}H_7Cl_2N_3O_2$	44·3	2·7	26·0	15·2	44·1	2·6	26·1	15·4
22	Me	H	NO_2	3,4-Cl_2:C_6H_3	195–197	$C_{10}H_7Cl_2N_3O_2$	43·8	2·5	25·7	15·5	44·1	2·6	26·1	15·4
23	Et	H	3,4-Cl_2:C_6H_3	NO_2	63–65	$C_{11}H_9Cl_2N_3O_2$	45·8	3·2	24·8	14·2	46·1	3·2	24·8	14·7
24	Et	H	NO_2	3,4-Cl_2:C_6H_3	175–176	$C_{11}H_9Cl_2N_3O_2$	45·9	3·2	24·9	14·4	46·1	3·2	24·8	14·7
25	Pr^n	H	3,4-Cl_2:C_6H_3	NO_2	49–51	$C_{12}H_{11}Cl_2N_3O_2$	48·8	4·0	23·8	13·7	48·0	3·7	23·7	14·0
26	Pr^n	H	NO_2	3,4-Cl_2:C_6H_3	165–167	$C_{12}H_{11}Cl_2N_3O_2$	48·6	3·5	23·1	13·8	48·0	3·7	23·7	14·0
27	$n\text{-}C_8H_{17}$	H	3,4-Cl_2:C_6H_3	NO_2	34–35	$C_{17}H_{31}Cl_2N_3O_2$	55·3	5·7	19·2	11·2	55·1	5·7	19·2	11·3
28	$n\text{-}C_8H_{17}$	H	NO_2	3,4-Cl_2:C_6H_3	89–90	$C_{17}H_{31}Cl_2N_3O_2$	54·2	5·7	19·2	11·4	55·1	5·7	19·2	11·3
29	$CH_2{\cdot}CO_2H$	H	NO_2	3,4-Cl_2:C_6H_3	225–226	$C_{11}H_7Cl_2N_3O_4{\cdot}H_2O$	39·7	3·0	21·0	—	39·5	2·7	21·3	—
30	$CH_2{\cdot}CO{\cdot}OEt$	H	3,4-Cl_2:C_6H_3	NO_2	113	$C_{13}H_{11}Cl_2N_3O_4$	45·6	3·3	20·8	11·8	45·3	3·2	20·6	12·2
31	$CH_2{\cdot}CO{\cdot}OEt$	H	NO_2	3,4-Cl_2:C_6H_3	139–140	$C_{13}H_{11}Cl_2N_3O_4$	45·1	3·4	20·7	12·2	45·3	3·2	20·6	12·2
32	$CH_2{\cdot}CO{\cdot}NH{\cdot}NH_2$	H	NO_2	3,4-Cl_2:C_6H_3	195–197	$C_{11}H_9Cl_2N_5O_3$	—	—	22·7	—	—	—	22·9	—
33	$CH_2{\cdot}CH_2OH$	H	3,4-Cl_2:C_6H_3	NO_2	102–103	$C_{11}H_9Cl_2N_3O_3$	43·3	2·8	24·2	14·3	43·7	3·0	23·5	13·9
34	$CH_2{\cdot}CH_2OH$	H	NO_2	3,4-Cl_2:C_6H_3	177	$C_{11}H_9Cl_2N_3O_3$	43·4	2·8	24·1	14·4	43·7	3·0	23·5	13·9
35	$CH_2{\cdot}CH_2OEt$	H	NO_2	3,4-Cl_2:C_6H_3	127–128	$C_{13}H_{13}Cl_2N_3O_3$	47·0	4·1	21·3	12·5	47·2	3·9	21·5	12·5
36	$CH_2{\cdot}CH_2{\cdot}NEt_2$	H	3,4-Cl_2:C_6H_3	NO_2	48–49	$C_{15}H_{18}Cl_2N_4O_2$	49·5	5·1	19·8	—	50·4	5·0	19·9	—
37	$CH_2{\cdot}CH_2{\cdot}NEt_2$, 2HCl	H	NO_2	3,4-Cl_2:C_6H_3	180–184	$C_{15}H_{20}Cl_4N_4O_2$	41·5	4·7	31·6	12·9	41·6	4·7	33·0	13·0
38	2-(pyrid-2-yl)ethyl, HCl	H	3,4-Cl_2:C_6H_3	NO_2	222–223	$C_{12}H_{11}Cl_3N_4O_4$	—	—	9·0[b]	—	—	—	8·9[b]	—
39	$CH_2{\cdot}CH(OH){\cdot}CH_2OH$	H	NO_2	3,4-Cl_2:C_6H_3	197–198	$C_{12}H_{11}Cl_2N_3O_4$	—	—	21·4	12·8	—	—	21·4	12·7
40[a]	H	H	2,4,5-Cl_3:C_6H_2	NO_2	207–208	$C_9H_4Cl_3N_3O_2$	37·6	1·8	—	14·8	37·0	1·4	—	14·4
41[a]	H	H	*p*-Cl·$C_6H_4{\cdot}CH$:CH	NO_2	315	$C_{11}H_8ClN_3O_2$	—	—	13·9	—	—	—	14·2	—
42[a]	H	H	3,4-Cl_2:$C_6H_3{\cdot}CH$:CH	NO_2	303–304	$C_{11}H_7Cl_2N_3O_2$	46·3	2·5	24·5	14·6	46·5	2·5	25·0	14·8
43	Me	H	3,4-Cl_2:$C_6H_3{\cdot}CH$:CH	NO_2	231–232	$C_{12}H_9Cl_2N_3O_2$	48·0	3·1	24·3	14·2	48·3	3·0	23·8	14·1
44	Me	H	NO_2	3,4-Cl_2:$C_6H_3{\cdot}CH$:CH	199–201	$C_{12}H_9Cl_2N_3O_2$	47·5	3·5	23·6	13·6	48·3	3·0	23·8	14·1

[a] Where R^1 = H, R^3 and R^4 are interchangeable. [b] Ionic chlorine.

In vitro ASSAY OF ANTIFUNGAL ACTIVITY

The procedure used was the same as that already described by Ellis & others, 1964.

Results

Of the nitro-compounds described, five (compounds 6, 10, 21, 23 and 25, Table 1) showed antifungal activity at a concentration of 25 μg/ml or less. These compounds, and others which inhibited the growth of trichomonads at 1 μg/ml or less, are listed in Table 2. There was no

TABLE 2. ANTIPROTOZOAL ACTIVITY OF IMIDAZOLE DERIVATIVES AFTER 48 HR AT 28°

Compound No.	Minimum cidal concentration μg/ml			M.I.C. μg/ml
	Trichomonas	Histomonas	Entamoeba	Trichophyton
2	0·5		10	
3	0·075	1·5	2·5	
4	1	7·5	20	
6	0·1			25
7	1			
10	0·1	1·25	2·5	25
12	1		10	
16	0·05	1·5	1·5	
20	1			
21	0·1	2		20
23	0·5			10
25	1			10
30	1			
33	0·15			
34	1			
36	1			
42	0·5			
43	1			
44	1			
Metronidazole	0·3	2·5	2·5	
Acinitrazole	1	1	—	
*Nithiazide (Hepzide) ..	1	3	—	
†Dimetridazole (8595 R.P.) ..	0·2	0·5	2	

* 1-Ethyl-3-(5-nitro-2-thiazolyl)urea.
† 1,2-Dimethyl-5-nitroimidazole.

significant difference between the three species of trichomonads in their sensitivity to a drug.

Discussion

Microbiological tests on the nitro-compounds show them to have a pronounced difference in their spectrum of *in vitro* antimicrobial activity from that of their un-nitrated precursors (Ellis & others, 1964). Some of the latter possessed good antifungal action but no trichomonacidal properties. After nitration in position 4 or 5 of the imidazole ring, some of these compounds exhibited high inhibitory activity against several protozoa but their antifungal potency decreased; for example, on nitrating 4-(3,4-dichlorophenyl)-1-propylimidazole (compound 25), its antifungal activity fell from 4 to 10 μg/ml, while its trichomonacidal efficacy increased from a negligible value to 1 μg/ml. 4(5)-(2,4,5-Trichlorophenyl)imidazole inhibited the *in vitro* growth of *Trichophyton* species at 25 μg/ml and of trichomonads at 50 μg/ml, but nitration (to compound 40) changed these values to 100 and 2 μg/ml respectively. In general, the most potent trichomonacidal compounds showed little or no antifungal activity.

Alkylation of the ring nitrogen atom led to a considerable improvement

in trichomonacidal activity only if (*a*) the 5-nitro-isomer was formed and (*b*) the alkyl group was small. Thus, methylation of 4(5)-(3,4-dichlorophenyl)-5(4)-nitroimidazole (compound 20) produced the 1-methyl-5-nitro-derivative (compound 21), which was twenty times more potent than its 1-methyl-4-nitro-isomer (compound 22). On n-propylation of compound 20, however, the more active isomer (compound 25) was no better than the parent compound. Octylation (to compounds 27 and 28) destroyed the activity of compound 20. A similar effect was observed by Bushby & Copp (1955) when they acylated 2-amino-5-nitrothiazole with fatty acids of varying chain-length; the 2-octanoylamido-compound possessed only one-tenth of the activity of acinitriazoles.

Compounds which possessed water-solubilising groups attached to either the imidazole ring (compounds 16, 17, 29, 33, 34, 36, 37, 39) or the phenyl ring (compounds 1, 18, 19) exhibited poor *in vitro* anti-protozoal action. There was little or no difference in the activities of compounds with a chlorine or a bromine atom in the *p*-position of the benzene ring, or in the activity of a compound with a chlorine atom in the *p*-position and that of one with two chlorine atoms in positions 3 and 4. The high activity of the *p*-aminophenyl- and *p*-acetamidophenyl-imidazoles (compounds 1, 2, 3) parallels that of the corresponding nitrothiazole compounds (Bushby & Copp, 1955). Replacing the *p*-acetamido-group (compound 3) by a dichloroacetamido-substituent (compound 4) produced a decrease in activity which is not unexpected in view of the inactivity of chloramphenicol towards trichomonads (Schnitzer, 1963).

The vinylogous compounds (41–44) exhibited very good trichomonacidal activity but they differed from the phenylnitroimidazoles in two respects: first, alkylation of the ring nitrogen atom decreased potency slightly (compare compounds 42 and 43), in contrast to a ten-fold improvement between compounds 20 and 21. Secondly, the two positional isomers (43 and 44) were equally active, while the corresponding compounds 21 and 22 differed by a factor of twenty.

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