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Synthesis and antiprotozoal activity of some nitro(nitroaryl)imidazoles

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A series of 5(4)-nitro-4(5)-nitroarylimidazoles has been synthesized and their *in vitro* antifungal and antiprotozoal activities have been studied. The compounds may be prepared by dinitration of an arylimidazole or by mononitration of either an arylnitroimidazole or a (nitroaryl)imidazole. In some of the compounds the ring nitrogen atom has been substituted. Several compounds exhibit high *in vitro* antitrichomonal activity against *Trichomonas vaginalis* and *T. foetus*, and a moderate activity against *Histomonas meleagridis* and *Entamoeba histolytica*. The chemical structure and antimicrobial activity of these and other imidazoles previously studied are briefly discussed.

IN earlier papers we have shown that some arylimidazoles possess high *in vitro* antimicrobial action (Ellis, Epstein, Fitzmaurice, Golberg & Lord, 1964a,b); several compounds were fungicidal towards pathogenic organisms but introduction of a nitro-group into the imidazole ring largely destroyed this activity and simultaneously bestowed on some of the compounds very high antitrichomonal properties. The work is now extended to examine arylimidazoles containing two nitro-groups, one in each ring. Few representatives of this type of compound are known; the first such compound to be described was 4(5)-nitro-5(4)-*p*-nitrophenylimidazole (Grant & Pyman, 1921). Its two isomeric 1-methyl derivatives were prepared later (Hazeldine, Pyman & Winchester, 1924).

Most of the dinitro-compounds now described were prepared (see Fig. 1) from either the un-nitrated or mononitrated imidazoles but introduction of a substituent on the ring nitrogen atom of a nitro(nitroaryl)-imidazole also provided a useful route in some instances, for example, by reaction with an alkyl halide or sulphate or by an addition reaction with an activated alkene. Some *N*-2-cyanoethyl and *N*-pyrid-2-ylethyl compounds prepared by this method were described earlier (Ellis & others, 1964a). The reactions of but-1-en-3-one (methyl vinyl ketone) and 2-vinylpyridine with nitro(nitroaryl)imidazoles are described but no attempt was made to find optimum conditions for these reactions. Mononitration of an arylimidazole usually gives a mixture of nitroaryl- and nitro-imidazole derivatives (Ellis & others, 1964b); further nitration of either of these produces the same dinitro-compound, which usually has a lower water-solubility than its precursors.

The orientation of the nitro-group in the benzene ring of the 4-chloro-2- and -3-nitrophenyl compounds was determined by oxidation of the dinitro-compound with alkaline potassium permanganate to the known substituted benzoic acid. This was unnecessary for 4-(4,5-dichloro-2-nitrophenyl)-1-methyl-5-nitroimidazole, since the constitution of 4-(4,5-

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dichloro-2-nitrophenyl)-1-methylimidazole was established (Ellis & others, 1964a) by synthesis from 4,5-dichloro-2-nitroacetophenone (Keneford & Simpson, 1947).

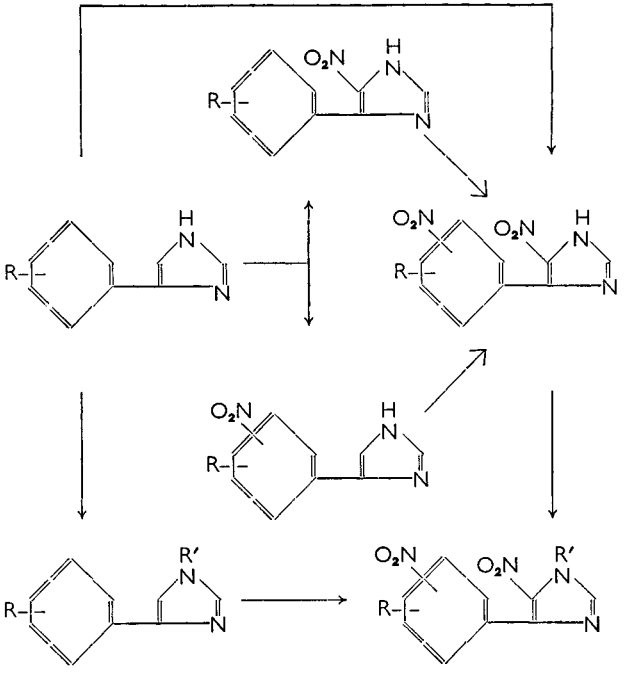


FIG. 1. The synthesis of nitro(nitroaryl)imidazoles.

Experimental

CHEMICAL

General methods. Two methods of nitration (Ellis & others, 1964b) were used: in *method A* the nitrate salt of the imidazole was treated with sulphuric acid; in *method B* the imidazole was heated for up to 2 hr in concentrated sulphuric acid with finely powdered sodium nitrate. The reaction mixture was poured into ice water, and the precipitate was filtered off and recrystallized from a suitable solvent.

4(5)-(4-*Chloro*-3-*nitrophenyl*)-5(4)-*nitroimidazole.* (*a*) 4(5)-*p*-Chlorophenyl-5(4)-nitroimidazole (10 g) (Ellis & others, 1964b) was nitrated by method B to give the colourless *dinitro-compound* (10 g), m.p. 223° (from acetone).

(*b*) Nitration of 4(5)-(4-chloro-3-nitrophenyl)imidazole (0·7 g) by method B gave the *dinitro-compound* (0·6 g), m.p. and mixed m.p. with sample from (*a*), 222° (from acetone).

4-(4-*Chloro*-3-*nitrophenyl*)-1-*methyl*-5-*nitroimidazole.* (*a*) 4-*p*-Chlorophenyl-1-methyl-5-nitroimidazole (11·9 g) on nitration by method B gave

very pale yellow needles (12 g), m.p. 122–125°, of the *dinitro-methylimidazole*; this, on oxidation with aqueous alkaline potassium permanganate, yielded 4-chloro-3-nitrobenzoic acid which did not depress the m.p. of an authentic sample.

(*b*) 4(5)-(4-Chloro-3-nitrophenyl)-5(4)-nitroimidazole (8·4 g) was heated at 100° for 30 min with dimethyl sulphate (3 ml). The reaction mixture was treated with aqueous sodium carbonate solution and extracted with chloroform (2 × 100 ml). The extract was dried and evaporated to quarter bulk. Addition of light petroleum (b.p. 40–60°) (200 ml) precipitated the *dinitro-methylimidazole* (4 g), m.p. and mixed m.p. with the product obtained in (*a*), 122–123° (from ethanol).

4(5)-(4-*Chloro*-2-*nitrophenyl*)-5(4)-*nitroimidazole.* 4(5)-(4-Chloro-2-nitrophenyl)imidazole (20 g) was nitrated by method A to give the yellow *dinitro-compound* (16·2 g), m.p. 241–243° (from ethanol). Oxidation of a sample with alkaline potassium permanganate gave 4-chloro-2-nitrobenzoic acid, which did not depress the m.p. of an authentic sample.

4(5)-(4,5-*Dichloro*-2-*nitrophenyl*)-5(4)-*nitroimidazole.* (*a*) 4(5)-(4,5-Dichloro-2-nitrophenyl)imidazole (1·8 g) gave, on nitration by method A, the *dinitro-compound* as yellow crystals (1·5 g), m.p. 220–221° (from aqueous ethanol).

(*b*) 4(5)-(3,4-Dichlorophenyl)-5(4)-nitroimidazole (6·8 g) by similar treatment yielded the *dinitro-compound* (6 g), m.p. and mixed m.p. with the product from (*a*), 219°.

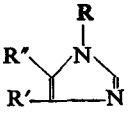
4-(4,5-*Dichloro*-2-*nitrophenyl*)-1-*methyl*-5-*nitroimidazole.* (*a*) 4(5)-(4,5-Dichlorophenyl)-1-methylimidazole (9·6 g), when nitrated with a large excess of sodium nitrate (7·5 g) by method B, gave the *dinitro-methylimidazole* (3·7 g) as almost colourless crystals, m.p. 148° (from ethanol).

(*b*) 4(5)-(4,5-Dichloro-2-nitrophenyl)-5(4)-nitroimidazole (7·7 g) was heated at 100° for 0·5 hr with dimethyl sulphate (2·4 ml). Hot sodium carbonate solution was added to the reaction mixture, which was then extracted with chloroform (2 × 100 ml). The extract was dried, evaporated to 50 ml and light petroleum (b.p. 40–60°) (200 ml) was added, whereupon an oil separated. When this was treated with hot ethanol (30 ml) and cooled, crystals of the *dinitro-methylimidazole* (2 g), m.p. and mixed m.p. with sample from (*a*), 148° (from ethanol), were deposited.

(*c*) 4-(3,4-Dichlorophenyl)-1-methyl-5-nitroimidazole (4·1 g) was nitrated by method B to give the *dinitro-methylimidazole* (2 g), m.p. and mixed m.p. with sample from (*a*), 148°.

Ethylation of 4(5)-(4,5-*dichloro*-2-*nitrophenyl*)-5(4)-*nitroimidazole.* The title compound (10 g) was refluxed in acetone (250 ml) for 20 hr with ethyl iodide (5·1 g) and potassium carbonate (2·2 g). The reaction mixture was cooled, filtered and evaporated to dryness. Fractional crystallization of the residue from ethyl acetate gave 4-(4,5-dichloro-2-nitrophenyl)-1-ethyl-5-nitroimidazole (2·2 g), m.p. and mixed m.p. with product of the

TABLE 1. NITRO(NITROARYL)IMIDAZOLES



Compound No.	R	R′	R″	m.p. °C	Formula	Analysis							
						Found				Required			
						C	H	Cl	N	C	H	Cl	N
1	H[a]	NO_2	4-Chloro-2-nitrophenyl	241–243	$C_9H_5ClN_4O_4$	40·3	1·9	13·4	21·1	40·2	1·9	13·2	20·8
2	H[a]	NO_2	4-Chloro-2-nitrophenyl	225	$C_9H_5ClN_4O_4$	40·4	2·0	13·4	20·9	40·2	1·9	13·2	20·8
3	H[a]	NO_2	4,5-Dichloro-2-nitrophenyl	221	$C_9H_4Cl_2N_4O_4$	36·0	1·5	24·0	19·0	35·6	1·3	23·4	18·5
4	H[a]	NO_2	3,4,6-Trichloro-2-nitrophenyl	224–225	$C_9H_3Cl_3N_4O_4$	32·3	1·0	32·2	—	32·0	0·9	31·6	—
5	H[a]	NO_2	2,3,4-Trichloro-6-nitrophenyl	168–169	$C_9H_3Cl_3N_4O_4$	32·4	0·9	32·3	—	32·0	0·9	31·6	—
6[b]	Me	4-Nitrophenyl	NO_2	215–216	$C_{10}H_8N_4O_4$	48·2	3·4	—	22·5	48·4	3·2	—	22·6
7	Me	4-Chloro-3-nitrophenyl	NO_2	122–125	$C_{10}H_7ClN_4O_4$	42·5	2·6	12·4	19·3	42·5	2·5	12·5	19·8
8	Me	4,5-Dichloro-2-nitrophenyl	NO_2	148	$C_{10}H_6Cl_2N_4O_4$	38·0	1·8	—	17·4	37·8	1·9	—	17·6
9	Et	NO_2	4,5-Dichloro-2-nitrophenyl	188–189	$C_{11}H_8Cl_2N_4O_4$	40·3	3·0	—	17·4	39·9	2·4	—	16·9
10	Et	4,5-Dichloro-2-nitrophenyl	NO_2	158–159	$C_{11}H_8Cl_2N_4O_4$	40·6	2·5	—	16·8	39·9	2·4	—	16·9
11	Pr	4,5-Dichloro-2-nitrophenyl	NO_2	121–123	$C_{12}H_{10}Cl_2N_4O_4$	41·8	3·0	—	16·4	41·7	2·9	—	16·2
12	Bu	4,5-Dichloro-2-nitrophenyl	NO_2	96	$C_{13}H_{12}Cl_2N_4O_4$	43·8	3·2	—	15·4	43·5	3·3	—	15·6
13	$MeCO{\cdot}CH_2{\cdot}CH_2$	NO_2	4,5-Dichloro-2-nitrophenyl	163–164	$C_{13}H_{10}Cl_2N_4O_5$	41·0	2·9	—	15·0	41·8	2·7	—	15·0
14	2-(pyrid-2-yl)-ethyl	NO_2	4,5-Dichloro-2-nitrophenyl	143	$C_{16}H_{11}Cl_2N_5O_4$	47·8	2·6	—	16·9	47·0	2·7	—	17·1
15	$CN{\cdot}CH_2{\cdot}CH_2$	NO_2	4,5-Dichloro-2-nitrophenyl	241–242	$C_{12}H_7Cl_2N_5O_4$	40·4	2·0	—	19·8	40·4	2·0	—	19·7
16	$CO_2H{\cdot}CH_2$	4,5-Dichloro-2-nitrophenyl	NO_2	283–284	$C_{11}H_6Cl_2N_4O_6$	36·6	1·8	—	15·1	36·6	1·7	—	15·5

a Tautomeric compounds. *b* See Hazeldine, Pyman & Winchester (1924).

nitration (method B) of 4-(3,4-dichlorophenyl)-1-ethylimidazole, 158–159°, and 5-(4,5-dichloro-2-nitrophenyl)-1-ethyl-4-nitroimidazole (2·0 g) m.p. 188–189°.

5-(4,5-*Dichloro*-2-*nitrophenyl*)-4-*nitro*-1-[2-(*pyrid*-2-*yl*)*ethyl*]*imidazole.* 4(5)-(4,5-Dichloro-2-nitrophenyl)-5(4)-nitroimidazole (3 g) was warmed with 2-vinylpyridine (5 ml) at 100° for 1 hr, treated with Triton B solution (5 drops) and warmed for a further hour. Trituration with ethanol gave needles (2·5 g) of the *pyridylethyl compound*, m.p. 143° (from ethanol).

5-(4,5-*Dichloro*-2-*nitrophenyl*)-4-*nitro*-1-(3-*oxobutyl*)-*imidazole.* 4(5)-(4,5-Dichloro-2-nitrophenyl)-5(4)-nitroimidazole (10 g) was warmed on a steam-bath with methyl vinyl ketone (25 ml). Triton B (1 ml) was added and after an hour on the steam-bath the volatile materials were removed, leaving a thick oil which solidified on trituration with ether. The *methyl ketone*, m.p. 163° (from ethyl acetate), crystallized as yellow needles (6 g).

BIOLOGICAL METHODS

The compounds were assayed *in vitro* against the following organisms obtained from the sources stated: *Trichomonas vaginalis* (T 70), Liverpool Public Health Laboratory; *T. foetus* (T 69, Belfast strain), Agricultural Research Council, Weybridge; *Histomonas meleagridis* (Joyner strain, Agricultural Research Council, M.A.F.F., Weybridge; *Entamoeba histolytica* (Strain DC), Liverpool School of Tropical Medicine. The methods used were identical with those described previously (Ellis & others, 1964a,b).

Results

Table 2 lists the activity of those compounds which possess anti-trichomonal action at a concentration of less than 3 μg/ml. The dinitro-derivatives showed no *in vitro* antifungal activity but several compounds inhibited the growth of trichomonads (see Table 2) at very low concentrations. Compounds 7, 8 and 10 showed good activity against the other two protozoa.

TABLE 2. MICROBIOLOGICAL ACTIVITY OF IMIDAZOLE DERIVATIVES

Compound No.	Minimum cidal concentration, μg/ml			M.I.C., μg/ml
	Trichomonas	Histomonas	Entamoeba	Trichophyton
2	2			50
3	2			50
5	1·5			50
6	0·1			100
7	0·1	1·5	2·5	50
8	2·5	2·5		50
10	0·25	2	2·5	50
11	1			20
Metronidazole	0·3	2·5	2·5	
Acinitrazole	1	1		
*Nithiazide	1	3		
†Dimetridazole	0·2	0·5	2	

* 1-Ethyl-3-(5-nitrothiazol-2-yl)urea.
† 1,2-Dimethyl-5-nitroimidazole.

Discussion

Comparison of the *in vitro* antimicrobial activity of un-nitrated aryl-imidazoles with that of their mono- and di-nitro-derivatives shows a decrease in antifungal activity and an increase in antiprotozoal properties, although the presence of more than one nitro-group has little effect on the latter type of activity. In our earlier paper (1964b) a significant difference in trichomonacidal potency was found to exist between a 4- and a 5-nitro-1-substituted imidazole, the 5-nitro-compound being several times more active than its isomer. A fivefold difference in activity was also found between the isomeric dinitro-compounds (compounds 9 and 10). Moreover, alkylation of the ring nitrogen atom in the dinitro-compounds as in the mononitro-compounds, improves activity only if the alkyl group is a small one.

The majority of the compounds synthesized in this work have contained one or more halogen atoms in the benzene ring but it is significant that high *in vitro* antitrichomonal activity has also been shown by mono- and dinitro-compounds which contained no halogen atom, for example, compound 6 in Table 1 and 4-*p*-acetamidophenyl-1-methyl-5-nitroimidazole (Ellis & others, 1946b). Fungicidal potency, on the other hand appears to require two or more halogen atoms in the aryl group.

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References

Ellis, G. P.,'Epstein, C., Fitzmaurice, C., Golberg, L. & Lord, G. H. (1964a). *J. Pharm., Pharmac.*, **16**, 400–407.
Ellis, G. P., Epstein, C., Fitzmaurice, C., Golberg, L. & Lord, G. H. (1946b). *Ibid.* **16**, 801–809.
Grant, R. L. & Pyman, F. L. (1921). *J. chem. Soc.*, **119**, 1893–1903.
Hazeldine, C. E., Pyman, F. L. & Winchester, J. (1924). *Ibid.*, 1431–1441.
Keneford, J. R. & Simpson, J. C. E. (1947). *Ibid.*, 227–232.