THE ANTITRICHOMONAL ACTIVITY OF AMIDO-NITROTHIAZOLES

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Received August 24, 1954

Waletzky, Brandt, Bliznick, and Hughes[1] have shown 2-amino-5-nitrothiazole to be effective in the treatment of experimental infections of turkeys with *Hisomonas meleagridis*, and Carmichael and Maclay[2], McGreggor[3], and Swales[4] found it effective for the treatment of the natural disease. Because of this success Stabler and Mellentin[5] treated pigeons infected with *Trichomonas gallinae* with it and found it to be very effective.

During the past few years we have been investigating, in collaboration with our colleague Dr. R. A. Neal, the antiprotozoal properties of a number of nitropyridines and when the activity of aminonitrothiazole was first reported we extended our investigations to include the nitrothiazoles. We were interested in drugs suitable for the treatment of vaginitis due to trichomonas in women but aminonitrothiazole is brightly coloured and stains skin and clothing an intense yellow—a most undesirable property for a drug which is to be used in the local treatment of this disease. Our researches with the nitrothiazoles have therefore been for an analogue free from this disadvantage and preferably with higher activity. Since the intensity of the colour is related to basicity, we have been almost completely restricted to the synthesis and examination of the 2-acylamido-5-nitrothiazoles.

Experimental

Cultures of Trichomonas vaginalis. The strains were from women with vaginitis and were grown in serum-glucose-nutrient broth enriched with an extract of ox liver. The medium was prepared by extracting 1 lb. of minced fresh liver with 1 l. of nutrient broth at 56° C. for 60 minutes, and then precipitating the coagulable proteins of the extract by raising its temperature to 80° C. for 5 minutes. After filtration through filter paper, glucose 2 g./100 ml., and calcium pantothenate 0·5 mg./100 ml. were added and the *p*H adjusted to 6·1. The medium was sterilised by heating at 100° C. for 20 minutes on 3 consecutive days, and at the time of use sterile 20 per cent. horse serum was added. The primary cultures were freed of bacteria by low concentrations of appropriate antibiotics, usually penicillin and polymyxin, and by incubating the subcultures anærobically.

Trichomonacidal and Trichomonastatic Tests. 2-day old cultures containing approximately 2000 actively motile *T. vaginalis* per cu.mm. were diluted with fresh medium to contain 400 per cu.mm. Solutions or suspensions of the test substances containing 20 mg./ml. were 3-fold diluted in the medium, after being heated at 56° C. for 30 minutes.

In the trichomonacidal tests 1 ml. of the diluted culture was added to 1 ml. of each of the dilutions. After 2 hours, 6 hours and 24 hours incubation at 37° C. in a McIntosh and Fildes anærobic jar the cultures were

TABLE I

Number	 Substitution	Colour	Minimum effective concentration—μg./ml.			
			Trichomonacidal activity			Trichomonastatic activity
			2 hr.	6 hr.	24 hr.	48 hrs.
454 C 50	2-NH_2	None	>1000	>1000	>1000	>1000
190 C 54	2-NH·CHO 5-H	None	>1000	>1000	>1000	1000
426 C 50	2-NH_2 5-NO_2	Yellow	1000	300	300	3
291 C 51	2-NH·CHO 5-NO_2	Cream	300	100	10	1
107 C 51	2-NH·$COCH_3$ 5-NO_2	Cream	300	100	30	1
292 C 51	2-NH·CO·C_2H_5 5-NO_2	Cream	300	100	30	1
38 C 53	2·NH·CO·C_3H_7 5-NO_2	Cream	1000	100	10	3
39 C 53	2-NH·CO·C_4H_9 5-NO_2	Cream	1000	100	10	3
40 C 53	2-NH·CO·C_5H_{11} 5-NO_2	Cream	1000	300	30	3
41 C 53	2-NH·CO·C_6H_{13} 5-NO_2	Cream	>1000	300	10	3
42 C 53	2-NH·CO·C_7H_{15} 5-NO_2	Cream	>1000	1000	30	10
109 C 51	2-NH·CO·$CHCl_2$	Cream	300	300	30	1
243 C 51	2-NH·CO·$CHCl_2$ 5-Br	White	>1000	>1000	>1000	100
277 C 53	2-NMe·CHO 5-NO_2	Cream	>1000	300	100	3
43 C 53	2-NMe·CO·CH_3 5-NO_2	Cream	>1000	100	30	3
276 C 53	2-NMe·CO·C_5H_{11} 5-NO_2	Cream	>1000	1000	100	10
34 C 53	2-OH 5-NO_2	Yellow	300	100	100	100
35 C 53	2-NH·CO·CH_3 4-CH_3 5-NO_2	Cream	>1000	>1000	300	100
189 C 54	2-NH·CO·$(CH_2)_4COOH$ 5-NO_2	Cream	>1000	>1000	300	300
	Acetarsol	White	>1000	>1000	>1000	1000
	Aureomycin	Yellow	>1000	>1000	1000	1000

examined microscopically for motile organisms. Viability tests were made on the tubes showing no motile organisms by transferring one standard loopful (3 mm.) into 1 ml. of fresh medium and incubating for four days.

In the trichomonastatic tests the diluted culture was diluted a further

10 times before an equal volume was added to the medium containing the test substances. The cultures were incubated for 48 hours and examined microscopically for evidence of multiplication.

In Vivo *Test.* This test was used as a means of measuring the systemic possibilities of the drugs and not for comparing topical activities. Groups of mice were injected intraperitoneally with 0·5 ml. of a suspension containing approximately 1000 actively motile *T. vaginalis* per c.mm. The

TABLE II

NEW THIAZOLES 

Substituents		M.pt. °C.	Solvent for crystallisation	Formula	Found per cent.			Required per cent.		
2	5				C	H	N	C	H	N
NH·CHO	NO_2	194	Ethanol	$C_4H_3O_3N_3S$	27·9	1·8	—	27·75	1·75	—
NH·CO·C_2H_5	NO_2	193	Methanol	$C_6H_7O_3N_3S$	36·0	3·5	—	35·8	3·5	—
NH·CO·C_3H_7	NO_2	177	Ethanol	$C_7H_9O_3N_3S$	39·2	4·1	19·3	39·1	4·2	19·5
NH·CO·C_4H_9	NO_2	157	Methanol	$C_8H_{11}O_3N_3S$	42·2	4·9	18·3	41·9	4·8	18·3
NH·CO·C_5H_{11}	NO_2	172 to 173	Ethanol	$C_9H_{13}O_3N_3S$	44·6	5·2	17·3	44·4	5·4	17·2
NH·CO·C_6H_{13}	NO_2	148 to 149	Methanol	$C_{10}H_{15}O_3N_3S$	46·7	5·6	16·15	46·7	5·9	16·3
NH·CO·C_7H_{15}	NO_2	138 to 139	Methanol	$C_{11}H_{17}O_3N_3S$	49·05	6·1	—	48·7	6·3	—
NH·CO·$CHCl_2$	NO_2	148 to 149	Benzene	$C_5H_3O_3N_3Cl_2S$	—	—	16·3	—	—	16·4
NH·CO·$CHCl_2$	Br	197	Benzene	$C_5H_3ON_3Cl_2BrS$	21·1	1·2	9·55	20·7	1·0	9·7
NMe.CHO	NO_2	152·5	Ethanol	$C_5H_5O_3N_3S$	32·3	2·9	22·6	32·1	2·7	22·5
NMe·CO·CH_3	NO_2	143 to 144	Ethanol	$C_6H_7O_3N_3S$	35·4	3·4	—	35·8	3·5	—
NMe·CO·C_6H_{11}	NO_2	54 to 55	Ethanol	$C_{10}H_{15}O_3N_3S$	46·6	5·7	16·3	46·7	5·9	16·3
NH·CO·$(CH_2)_2$·CO_2H	NO_2	241	Ethoxyethanol	$C_7H_7O_6N_3S$	34·0	2·8	—	34·3	2·9	—

maximum tolerated dose of the test drug suspended in 10 per cent. gum acacia was then given subcutaneously twice daily for 5 days. 10 to 15 days later the animals were killed and examined for trichomonads. When present, these were also invariably found in abscesses of variable size in the omentum and the mesentery. In successful experiments 80 per cent. of the control mice were found to be infected.

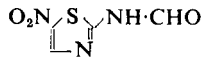
Results. The results of the *in vitro* trichomonastatic and trichomonacidal tests are shown in Table I. The inhibitory figures are the average result of 3 tests. Acetarsol was chosen as the standard as it is probably the most widely used therapeutic drug for this infection.

Only 3 of the drugs were examined *in vivo* and none of these was active. These were 291 C 51, 107 C 51 and 109 C 51.

DISCUSSION

In comparing the activity of these nitrothiazoles we attach more value to trichomonacidal than trichomonastatic activity, and although in practice it may not be important whether at a given concentration a drug takes 1 or 24 hours to kill we have regarded the compound with the more rapid action as being the more active. On this basis aminonitrothiazole (426 C 50) is decidedly more active than acetarsol and some of the analogues examined are even more active. The most active analogue is 2-formamido-5-nitrothiazole (291 C 51) and although the immediate lower homologues of the acylamido-derivatives are only slightly less active, further lengthening the chain very definitely reduces activity. Introduc-

tion of a carboxyl group apparently lessens activity as shown by the lower activity of the succinamido derivative (189 C 54) as compared with that of 292 C 50. The importance of the nitro-group is shown by the low activities of 454 C 50, 243 C 51 and 190 C 54, and the difference in activity between 107 C 54 and 35 C 53 suggests that further substitution of the thiazole ring will diminish activity.

Although 2-formamido-5-nitrothiazole (291 C 51)  is feebly coloured, its staining properties are very much less than aminonitrothiazole and any discolouration produced by it is removed from the skin by washing with soap and water. Therefore it is not only more active *in vitro* but more suitable than aminonitrothiazole for the treatment of trichomonal vaginitis; Busby, Catterall and Wiliamson[6] have used it in the treatment of 96 women with this disease comparing the results with those obtained in 49 women treated with acetarsol. Toxicity tests in animals made before the trial showed 291 C 51 to be of low toxicity; 5 mg. produced no reaction when placed on the conjunctiva of rabbits once daily on 3 consecutive days, and the insertion twice daily for 15 days of 100 mg. pessaries of the drug into the vaginæ of two dogs caused no irritation.

In this clinical trial the protozoa rapidly disappeared and the symptoms quickly subsided during treatment with both drugs. However, as judged by microscopic and physical evidence, the final results were rather disappointing since although 291 C 51 was more active than acetarsol in our *in vitro* tests, the relapse rates in the two groups were not significantly different.

Bushby *et al.*[6] suggest that as 291 C 51 is some hundred times more active *in vitro* than acetarsol, the relapses are probably not due to poor antitrichomonas activity of the drug, but rather to some of the flagellates being in inaccessible sites or to re-infection. It is unknown whether a systemic drug would be more effective through reaching inaccessible organisms as there is as yet no suitable drug. Chlortetracycline is a highly efficient drug for bacterial infections but its antitrichomonas activity is too low for it to be effective except when applied locally; the antibiotic, trichomycin has antitrichomonal properties *in vivo* when given to mice with peritoneal infections[7], but as it has only been used locally in women[7,8] information about its systemic use is awaited with interest. Bushby *et al.*[6] explored the possibility of using 291 C 51 systemically since it has low oral toxicity, but neither the serum nor urine of rabbits (200 mg./kg.), monkey (100 mg./kg.) or man (10 mg./kg.) possessed demonstrable antitrichomonas activity after oral administration, and chromatographic analysis of the blood or urine showed that the drug was excreted in an altered form. Our *in vivo* experiments in mice also showed that the drug is not active when given orally or subcutaneously.

If at present the resistant character of trichomonal vaginitis, due either to failure to eradicate the infection or to unavoidable re-infection, necessitates prolonged and suppressive local treatment, then 291 C 51 has the advantage of being non-arsenical and non-toxic and in the opinion

of Bushby *et al.*[6] it is as effective as any non-arsenical drug at present available.

Chemical Methods

Hitherto 2-acylamido-5-nitrothiazoles have usually been prepared by nitration of the appropriate 2-acylamido-5-nitrothiazole according to the method of Ganapathi and Venkataraman[9] but it was more convenient for our purpose to react 2-amino-5-nitrothiazole with the appropriate range of acylating agents. Contrary to the claims of Hubbard, Groves and Steahly[10], nitration of 2-formamidothiazole led directly to 2-amino-5-nitrothiazole rather than its *N*-formyl derivative. The other starting material, 2-methylamino-5-nitrothiazole, was obtained by condensation of methylamine and 2-chloro-5-nitrothiazole under acid conditions but the yield was very poor.

2-*Methylamino-5-nitrothiazole.* Methylamine acetate (5 g.) was added slowly to a solution of 2-chlor-5-nitrothiazole (3·3 g.) and anhydrous sodium acetate (1·6 g.) in glacial acetic acid (5 ml.). The mixture was heated on a steam bath for 60 minutes and cooled, water (15 ml.) was added to give a thick brown precipitate, which was collected, washed with water and dissolved in dilute hydrochloric acid. The solution was treated with charcoal, filtered, and carefully neutralised at *p*H 6·7 with ammonia, when a yellow solid separated. This was collected and recrystallised from a mixture of ethanol and water (2:1), m.pt. 223·4° to 224·5° C. (950 mg.). Found: C, 30·6; H, 3·0; $C_4H_5O_2N_3S$ requires C, 30·2; H, 3·2 per cent.

2-*Acylamido-5-nitrothiazoles.* Descriptive and analytical data are summarised in Table II. The methods used for their preparation are illustrated by the following two examples.

(1) 2-Amino-5-nitrothiazole (6·5 g.) was suspended in anhydrous formic acid (12 ml.) at 60° C. and the mixture stirred whilst acetic anhydride (10 g.) was added over a period of 10 minutes. The mixture was then stirred at 60° C. for a further 30 minutes. A clear solution first formed which subsequently crystallised. After cooling, the product, 2-formamido-5-nitrothiazole, was collected and crystallised from ethanol to give pale, straw-coloured needles, m.pt. 194° C. (5·0 g.). Evaporation of the mother liquors gave an unidentified by-product m.pt. 173° to 176° C. 2-*N*-Methylformamido-5-nitrothiazole was similarly prepared.

(2) A suspension of 2-amino-5-nitrothiazole (10 g.) in dry acetone (25 ml.) and dry pyridine (8 ml.) was stirred and maintained by external cooling at 15° to 20° C. during the gradual addition of caproyl chloride (11 g.) over a period of 30 minutes. After standing for a further hour, the mixture was heated to reflux for 15 minutes, cooled and poured into water. The separated oil rapidly crystallised and formed cream-coloured needles on crystallisation from ethanol, m.pt. 172° to 173° C.

2-Formamido-, 2-propionamido- and 2-butyramido-5-nitrothiazole are mentioned but not described by Hubbard, Groves and Steahly.[10] The estimation of 5-nitro-2-succinamidothiazole is described by Ballard and Spice.[11]

Summary

1. Aminonitrothiazole is more active than acetarsol *in vitro* against *T. vaginalis*, but it is highly coloured and unsuitable for the local treatment of trichomonal vaginitis of women.

2. The amidonitrothiazoles are less coloured and some, especially 2-formamido-5-nitrothiazole, are more active than is aminonitrothiazole.

3. Three of the most active were tested as systemic drugs in mice infected intraperitoneally, but none was active.

4. Chemical data are presented.

We wish to thank Miss Joyce Peacock, A.I.M.L.T., for technical assistance with the biological tests and Mr. A. R. Elphick for chemical assistance.

References

1. Waletzky, Brandt, Bliznick and Hughes, *J. Parasit.*, 1949, **35** (6–Sect. 2), 16.
2. Carmichael and Maclay, *Vet. Rec.*, 1952, **64**, 54.
3. McGreggor, *Amer. J. Vet. Res.*, 1952, **13**, 108.
4. Swales, *Can. J. Comp. Med. Vet. Sci.*, 1952, **16**, 57.
5. Stabler and Mellentin, *J. Parasit.*, 1953, **39**, 637.
6. Bushby, Catterall and Williamson, *Brit. med. J.*, 1955, **1**, 78.
7. Hosoya, Soeda, Komatsu, Okada and Watanabe, *J. Antibiot.*, 1953, **6**, 92.
8. Magara, Yokouti and Amino, *Antibiot. and Chemother.*, 1954, **6**, 433.
9. Ganapathi and Venkataraman, *Proc. Indian Acad. Sci.*, 1945, **22A**, 343.
10. Hubbard, Groves and Steahly (Monsanto Chemical Company), U.S.Pat. 2,617,809.
11. Ballard and Spice, *J. Pharm. Pharmacol.*, 1952, **4**, 1067.