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SYNTHETIC AMOEBICIDES: PART I. ANTHRAPYRIMIDINES

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Of the wide range of drugs used in the treatment of amoebiasis, emetine, conessine, the iodohydroxyquinolines, the arsenicals, and certain antibiotics are perhaps the best known. None is entirely satisfactory when used alone, and treatment is usually by a combination of the different types. The lack of any certainly successful course of treatment has stimulated several attempts to discover more effective drugs, but the chemical modification of known active types has so far failed to produce any significant improvement.

In view of this, and because of the lack of knowledge of the physiology and nutritional requirements of *E. histolytica*, our approach to the problem was necessarily empirical. A wide variety of compounds was selected for examination merely on account of chemical novelty or activity in other biological spheres. It was hoped that the methodical examination of different chemical types might provide leads for more detailed study.

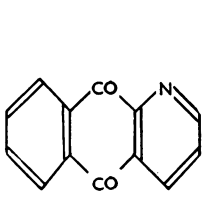
EXPERIMENTAL WORK

The main screening test used was that involving the experimental amoebic infection of young rats (Jones, 1946). Before this test was adequately standardized, numerous compounds were screened for amoebicidal action *in vitro* against a culture of *E. histolytica* containing a mixed unidentified bacterial flora. Tenfold dilutions of drug (1: 1,000 to 1: 1,000,000) were tested for their capacity to inhibit the growth of *E. histolytica* in a liquid medium (Jones, 1946) incubated for 48 hours at 37° C. The results of the *in vitro* tests were not in agreement with those of the *in vivo* tests. Of 132 compounds examined by both tests, 20 were active *in vitro* at concentrations of 1: 100,000 or less, 38 at 1: 10,000—1: 100,000, and the remaining 74 were not active at 1: 10,000. In these three groups 30 per cent, 23.7 per cent, and 13.5 per cent respectively were active *in vivo*. Thus it is not possible to select compounds likely to be active *in vivo* on the basis of their activity *in vitro*.

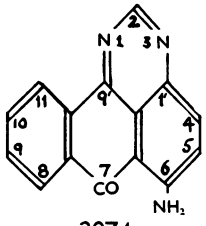
Nevertheless, the *in vitro* test was of value in directing attention to 1-azanthraquinone (Ref. No. 1916) which showed moderate activity both *in vitro* and *in vivo*, and on the basis of this observation numerous nitrogenous anthraquinone derivatives and similar polycyclic compounds provided by colleagues engaged in dyestuffs research were examined. Of these, 6-aminoanthrapyrimidine* (Ref. No. 3074) was

* 4-Aminopyrimidino-4′: 5′: 6′-1: 13: 9-anthrone. Anthrapyrimidine is the name commonly applied to this compound in the literature, apart from the occasional use of the name 1: 3-diazabenzanthrone. Other alternatives are given in the Ring Index (No. 2676).

found to be slightly active *in vitro* and strikingly active over a wide range of doses *in vivo*.



1916



3074

Because of its low solubility in water, 3074 was administered to rats as a dispersion made by ball-milling with a dispersing agent (1 per cent Dispersol OG, I.C.I.). Its LD50 after a single oral dose was found to be greater than 1,000 mg./kg., and complete therapeutic effect on rats was found at doses down to 30 mg./kg. (given twice daily for two days after infection). Unfortunately, an undesirable photodynamic effect occurred even after low doses.

Albino rats dosed orally with 3074 and then exposed to sunlight rapidly developed inflammation and oedema in the exposed parts, particularly the ears and snout. If exposure was adequate, irreversible damage occurred with necrosis of the ears (Jones, in press). The therapeutic use of 3074 appeared to be hazardous, and efforts were made to synthesize active compounds in which this toxic effect was reduced or eliminated, e.g., by reducing the strong fluorescence shown by 3074 and related substances.

In the past, anthrapyrimidines have been investigated as dyestuffs and published information concerning them is confined almost entirely to patent specifications, and is generally unsupported by analytical data or other criteria of purity. We have therefore included analytical figures at the end of Table III, except for compounds not prepared by us, and references to any published descriptions are also given.

In the Tables I–III the minimal effective therapeutic doses are recorded. Normally drugs were administered orally twice daily on the two days succeeding the day of infection. With some drugs a fifth dose was given on the third day, and with others a single dose only was given on the day after the day of infection. Assessment of therapeutic effect was made at necropsy on the fifth day after the day of infection.

TABLE I

THE ANTI-AMOEBIC ACTION OF ANTHRAPYRIMIDINE AND ITS AMINO DERIVATIVES IN RATS

In Tables I–III, " activity " is given as +, ±, or −. + = P <0.01; ± = P 0.01 to 0.20; − = P >0.20. References (*a*) to (*l*) and all analyses are given at the end of Table III

Ref. No.	Compound	M. p. °C.	Dose (mg./kg.)	Activity
7147	Anthrapyrimidine (*b,h*)		500×4	−
5667	2-Aminoanthrapyrimidine (*c,d,i*)		1,000×3	+
5620	4-Aminoanthrapyrimidine (*d,l*)		1,000×1	−
3074	6-Aminoanthrapyrimidine (*a,l*)	275	30×4	+
4867	8-Aminoanthrapyrimidine (*c,l*)		1,000×1	−
6262	Leuco derivative of 3074 (*k*)	>300	50×5	±

TABLE II

THE ANTI-AMOEBIC ACTION OF SUBSTITUTED 6-AMINOANTHRAPYRIMIDINES IN RATS

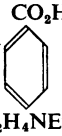
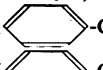
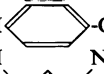
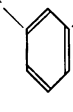
Ref. No.	Substituent	Formula	M.p. °C.	Dose (mg./kg.)	Activity
2-Substituted 6-aminoanthrapyrimidines					
6508	Me (*e*)	$C_{16}H_{11}ON_3$	267	100×5	+
				20×5	−
6939	Et	$C_{17}H_{13}ON_3$	237	50×4	+
				25×4	±
6866	Pr[α]	$C_{18}H_{15}ON_3$	223	50×4	−
6940	Ph.CH_2	$C_{22}H_{15}ON_3$	221	250×4	−
6378	Ph (*c*)	$C_{21}H_{13}ON_3$	278	500×5	±
7320	OH (*a*)	$C_{15}H_9O_2N_3$,0.5H_2O	—	500×4	+
				100×4	−
6567	NH_2 (*d*)	$C_{15}H_{10}ON_4$	336	100×5	+
6974	NMe_2 (*d*)	$C_{17}H_{14}ON_4$	271	50×4	−
6865	N<$(CH_2)_5$	$C_{20}H_{18}ON_4$	245	50×4	−
5915	NHPh	$C_{21}H_{14}ON_4$		400×1	−
4- and 5-Substituted 6-aminoanthrapyrimidines					
6941	4-Br (*h*)	$C_{15}H_8ON_3Br$		250×4	−
7231	4-NH_2	$C_{15}H_{10}ON_4$	283	100×4	−
7479	4-NEt_2	$C_{19}H_{18}ON_4$	230	50×4	−
7133	4-N<$(CH_2)_5$	$C_{20}H_{18}ON_4$	228	100×4	−
7446	4-N<$[C_2H_4]_2$>O	$C_{19}H_{16}O_2N_4$	250	100×4	−
7113	4-NH$[CH_2]_3$N<$(CH_2)_5$	$C_{23}H_{25}ON_5,H_2O$	146	100×4	−
6942		$C_{22}H_{16}ON_4$		250×4	−
7282	4-SNa	$C_{15}H_8ON_3SNa$	—	100×4	+
7226	4-SCH_2CO_2H	$C_{17}H_{11}O_3N_3S$,HCl	273d	100×4	−
7313		$C_{22}H_{13}O_3N_3S$	320	100×4	−
8023	4-$SC_2H_4NEt_2$	$C_{21}H_{22}ON_4S,H_2O$	128	25×4	+
				10×4	−
6951	5-Me (*c*)	$C_{16}H_{11}ON_3$		250×4	−
7823	4-OMe	$C_{16}H_{11}O_2N_3$	300d	500×4	−
7895	5-OMe (*l*)	$C_{16}H_{11}O_2N_3$	310d	500×4	−
6-Substituted anthrapyrimidines					
8832	-NH.NH_2 (*k*)	$C_{15}H_{10}ON_4$	215	50×4	+
6742	-NHEt (leuco) (*k*)	$C_{17}H_{15}ON_3$	148	50×4	±
7005	-NHC_2H_4OH (*k*)	$C_{17}H_{13}O_2N_3$	232	50×4	+
10371	(-NH.CH_2.CH_2-)$_2$	$C_{36}H_{28}O_2N_6$,0.5H_2O	273	4×50	±
7270	-N$HCH_2CH_2SO_3H$	$C_{17}H_{13}O_4N_3S$	294	100×4	±
5914	-NHPh (*j.k*)	$C_{21}H_{13}ON_3$		1,000×5	−
7002		$C_{21}H_{12}ON_3Cl$	266	250×4	−
7886	 (*k*) (leuco)	$C_{21}H_{15}O_2N_3$	294	500×4	−
7004	 (*k*)	$C_{21}H_{14}ON_4$,0.5H_2O	232	100×4	−

TABLE II—*continued*

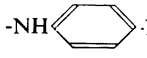
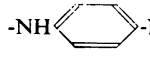
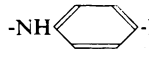
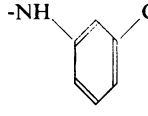
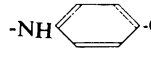
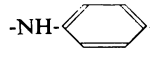
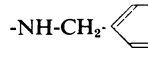
Ref. No.	Substituent	Formula	M.p. °C.	Dose (mg./kg.)	Activity
6843	 (*k*)	$C_{21}H_{14}ON_4,0.5H_2O$	286	50×4	—
7003		$C_{23}H_{16}O_2N_4$	276	250×4	—
6852		$C_{25}H_{22}ON_4$	192	100×4	±
7232		$C_{22}H_{13}O_3N_3$	—	50×4	—
7188		$C_{22}H_{13}O_3N_3$	356–8	100×4	±
7884		$C_{21}H_{14}O_3N_4S$	326	500×4	—
7647		$C_{22}H_{16}O_3N_4S$	264	50×4	—
6628	$-NH[CH_2]_2NEt_2$	$C_{21}H_{22}ON_4$	98	50×4	+
7997	$-NH[CH_2]_2N<(CH_2)_5$ (leuco)	$C_{22}H_{24}ON_4$	127	50×4	+
7998	$-NH[CH_2]_2N<[C_2H_4]_2>O$	$C_{21}H_{20}O_2N_4$	174	50×4	±
6746	$-NH[CH_2]_3NEt_2$	$C_{22}H_{24}ON_4,2HBr$	220	50×4	+
6837	$-NH[CH_2]_3N<(CH_2)_5$	$C_{23}H_{24}ON_4$	113	25×4	+
7268	$-NH[CH_2]_3N<[C_2H_4]_2>O$ (leuco)	$C_{22}H_{24}O_2N_4$	143	50×4	+
7044	$-NHCH_2CH=CHNEt_2$	$C_{22}H_{22}ON_4$	245	50×4	—
7999	$-NH[CH_2]_4NEt_2$	$C_{23}H_{26}ON_4,2HCl,0.5H_2O$	178	50×4	+
7046	$-NH[CH_2]_4NBu^a_2$	$C_{27}H_{34}ON_4$	84	50×4	+
8000	$-NH[CH_2]_4N<(CH_2)_5$ (leuco)	$C_{24}H_{28}ON_4,H_2O$	109	50×4	+
8001	$-NH[CH_2]_4N<[C_2H_4]_2>O$	$C_{23}H_{24}O_2N_4$	140	50×4	+
7045	$-NHCHMe[CH_2]_3NEt_2$	$C_{24}H_{28}ON_4$	(deliquescent)	50×4	±
7648	$-NH[CH_2]_5NEt_2$	$C_{24}H_{28}ON_4,H_2O$	93	50×4 25×4	+ ±
7649	$-NH[CH_2]_5N<(CH_2)_5$ (leuco)	$C_{25}H_{30}ON_4,H_2O$	111–2	50×4	±
7650	$-NH[CH_2]_5N<[C_2H_4]_2>O$	$C_{24}H_{26}O_2N_4$	118	50×4	—
8002	$-NH[CH_2]_6NEt_2$	$C_{25}H_{30}ON_4,2HCl$	(deliquescent)	50×4	+
8003	$-NH[CH_2]_6N<(CH_2)_5$	$C_{26}H_{30}ON_4$	116–7	50×4	+
8004	$-NH[CH_2]_6N<[C_2H_4]_2>O$	$C_{25}H_{28}O_2N_4$	142	50×4	±
7042	$-NHCH_2COOEt$	$C_{19}H_{15}O_3N_3$	182	500×4	+
7043	$-NHCH_2CO_2H$	$C_{17}H_{11}O_3N_3$	287d	50×4	—
7652	$-NHCH_2CON<(CH_2)_5$	$C_{22}H_{20}O_2N_4$	233–5	50×4	—
7653	$-NHCH_2CON<[C_2H_4]_2>O$	$C_{21}H_{18}O_3N_4$	272	50×4	—
7887	$-NHCH_2CONEt_2$	$C_{21}H_{20}O_2N_4$	203–4	100×4	—
7888	$-NHCH_2CONH[CH_2]_3NEt_2$	$C_{24}H_{27}O_2N_5,0.5H_2O$	189	50×4	—

TABLE III

THE ANTI-AMOEBIC ACTION OF MISCELLANEOUS ANTHRAPYRIMIDINE DERIVATIVES IN RATS

Ref. No.	Substituent	Formula	M.p. °C.	Dose (mg./kg.)	Activity
5869	6-NHAc	$C_{17}H_{11}O_2N_3$	262	100×4	−
5868	6-NHBz (*c*)	$C_{22}H_{13}O_2N_3$	258	1,000×5	±
6434	2-$NH_2$6-NHBz (*c,f,i*)	$C_{22}H_{14}O_2N_4$	298	100×4	−
7059	2-N<$(CH_2)_5$6-NHBz	$C_{27}H_{22}O_2N_4$	258	200×4	−
7334	6-$NH_2$3-(?) methochloride (*g*)	$C_{16}H_{12}ON_3Cl,H_2O$	242	100×4 50×4	+ −
7604	6-NHPh3-(?) Metho-p-toluene sulphonate	$C_{22}H_{16}ON_3,C_7H_7O_3S$	261	250×4	−
9082	6-NH[CH_2]$_3$N<$(CH_2)_5$ Methiodide	$C_{24}H_{27}ON_4I$	262	30×4	±
6307	6-OH (leuco) (*k*)	$C_{15}H_{10}O_2N_2$	167	250×5	−
7084	2: 7-Bis-dimethylamino-1: 3: 6: 8-tetraza-9: 10-benzpyrene	$C_{20}H_{18}N_6$	244	100×4	−

(*a*) Bayer, B.P. 5998/1909. (*b*) I. G. Farbenind., B.P. 296386. (*c*) Idem, B.P. 385295. (*d*) Idem, B.P. 401731. (*e*) Idem, B.P. 412005. (*f*) Idem, G.P. 633564. (*g*) Idem, B.P. 438122. (*h*) Idem, B.P. 449537. (*i*) Battegay, Conr. intern. quim. pura aplicata, 1937, **4**, 337 (*Chem. Zentr.*, 1936, **2**, 3299). (*j*) I. G. Farbenind., B.P. 461883. (*k*) Idem, B.P. 465040. (*l*) Idem, B.P. 523892.

ANALYSES

Table I.—**3074**. Found: C, 72.6; H, 3.5; N, 17.2. Calc. for $C_{15}H_9ON_3$: C, 72.8; H, 3.6; N, 17.0%. **6262**. Found: N, 16.85. Calc. for $C_{15}H_{11}ON_3$: N, 16.9%.

Table II.—**6508**. Found: N, 16.0. Calc. for $C_{16}H_{11}ON_3$: N, 16.1%. **6939**. Found: N, 15.3. $C_{17}H_{13}ON_3$ requires N, 15.3%. **6866**. Found: N, 14.3. $C_{18}H_{15}ON_3$ requires N, 14.5%. **6940**. Found: N, 12.0. $C_{22}H_{15}ON_3$ requires N, 12.45%. **6378**. Found: C, 77.1; H, 4.1; N, 13.0. Calc. for $C_{21}H_{13}ON_3$: C, 78.0; H, 4.0; N, 13.0%. **7320**. Found: C, 66.1; H, 4.0; N, 15.4. Calc. for $C_{15}H_9O_2N_3,0.5H_2O$: C, 66.1; H, 3.7; N, 15.4%. **6567**. Found: C, 68.2; H, 4.0; N, 21.1. Calc. for $C_{15}H_{10}ON_4$: C, 68.6; H, 3.8; N, 21.4%. **6974**. Found: N, 19.3. Calc. for $C_{17}H_{14}ON_4$: N, 19.3%. **6865**. Found: C, 73.0; H, 5.45; N, 17.3. $C_{20}H_{18}ON_4$ requires C, 72.7; H, 5.45; N, 17.0%. **7231**. Found: N, 20.9. $C_{15}H_{10}ON_4$ requires N, 21.4%. **7479**. Found: N, 17.3. $C_{19}H_{18}ON_4$ requires N, 17.6%. **7133**. Found: N, 16.6. $C_{20}H_{18}ON_4$ requires N, 17.0%. **7446**. Found: N, 16.8. $C_{19}H_{16}O_2N_4$ requires N, 16.85%. **7113**. Found: C, 67.9; H, 6.5; N, 17.5. $C_{23}H_{25}ON_5,H_2O$ requires C, 68.1; H, 6.65; N, 17.3%. **7282**. Found: N:S::3.3:1. **7226**. Found: S, 8.4. $C_{17}H_{11}O_3N_3S,HCl$ requires S, 8.55%. **7313**. Found: N, 9.95; S, 6.7. $C_{22}H_{13}O_3N_3S$ requires N, 10.5; S, 8.0%. **8023**. Found: N, 14.3; S, 8.0. $C_{21}H_{22}ON_4S,H_2O$ requires N, 14.15; S, 8.1%. **7823**. Found: N, 15.05. $C_{16}H_{11}O_2N_3$ requires N, 15.15%. **7895**. Found: N, 15.6. Calc. for $C_{16}H_{11}O_2N_3$: N, 15.15%. **8832**. Found: C, 68.3; H, 3.9; N, 20.9. Calc. for $C_{15}H_{10}ON_4$: C, 68.7; H, 3.9; N, 21.3%. **6742**. Found: C, 73.55; H, 5.15; N, 15.8. Calc. for $C_{17}H_{15}ON_3$: C, 73.6; H, 5.5; N, 15.2%. **7005**. Found: C, 68.7; H, 4.9; N, 14.4. Calc. for $C_{17}H_{13}O_2N_3$: C, 68.7; H, 4.5; N, 14.4%. **10371**. Found: C, 73.4; H, 5.2; N, 13.95. $C_{36}H_{28}O_2N_6,0.5H_2O$ requires C, 73.8; H, 5.0; N, 14.3%. **7270**. Found: C, 56.9; H, 3.6; N, 11.5. $C_{17}H_{13}O_4N_3S$ requires C, 57.4; H, 3.7; N, 11.8%. **7002**. Found: C, 70.2; H, 3.6; N, 11.4. $C_{21}H_{12}ON_3Cl$ requires C, 70.5; H, 3.6; N, 11.7%. **7886**. Found: C, 73.5; H, 4.2; N, 12.2. Calc. for $C_{21}H_{15}O_2N_3$: C, 73.9; H, 4.4; N, 12.3%. **7004**. Found: C, 73.1; H, 4.2; N, 15.5. Calc. for $C_{21}H_{14}ON_4,0.5H_2O$: C, 72.6; H, 4.4; N, 16.1%. **6843**. Found: C, 73.1; H, 4.4; N, 15.8. Calc. for $C_{21}H_{14}ON_4,0.5H_2O$: C, 72.6; H, 4.4; N, 16.1%. **7003**. Found: C, 72.8; H, 4.5; N, 13.5. $C_{23}H_{16}O_2N_4$ requires C, 72.6; H, 4.5; N, 14.6%. **6852**. Found: C, 75.8; H, 5.2; N, 14.2. $C_{25}H_{22}ON_4$ requires C, 76.1; H, 5.6; N, 14.2%. **7232**. Found: N, 11.6. $C_{22}H_{13}O_3N_3$ requires N, 11.45%. **7188**. Found: N, 11.7. $C_{22}H_{13}O_3N_3$ requires N, 11.45%. **7884**. Found: C, 62.4, H, 3.8; N, 13.1. $C_{21}H_{14}O_3N_4S$ requires C, 62.6; H, 3.5; N, 13.9%. **7647**. Found: C, 63.2; H, 3.8; N, 13.4. $C_{22}H_{16}O_3N_4S$ requires C, 63.5; H, 3.9; N, 13.5%. **6628**. Found: C, 73.1; H, 6.0; N, 15.5. $C_{21}H_{22}ON_4$ requires C, 72.8; H, 6.4; N, 16.2%. **7997**. Found: C, 73.0; H, 6.75; N, 15.5. $C_{22}H_{24}ON_4$ requires C, 73.3; H, 6.6; N, 15.5%. **7998**. Found: C, 69.9; H, 5.4; N, 15.3. $C_{21}H_{20}O_2N_4$ requires C, 70.0; H, 5.5; N, 15.5%. **6746**. Found: C, 50.9; H, 5.5; N, 10.7. $C_{22}H_{14}ON_4,2HBr$ requires C, 50.5; H, 5.0; N, 10.7%. **6837**. Found: C, 74.3; H, 6.3; N, 15.4. $C_{23}H_{24}ON_4$ requires C, 74.2; H, 6.5;

N, 15.1%. **7268**. Found: C, 70.1; H, 6.5; N, 15.3. $C_{22}H_{24}O_2N_4$ requires C, 70.2; H, 6.4; N, 14.9%. **7044**. Found: C, 73.2; H, 5.8; N, 15.45. $C_{22}H_{22}ON_4$ requires C, 73.7; H, 6.2; N, 15.6%. **7999**. Found: C, 60.2; H, 6.45; N, 12.0; Cl, 14.8. $C_{23}H_{26}ON_4,2HCl,0.5H_2O$ requires C, 60.5; H, 6.4; N, 12.2; Cl, 15.5%. **7046**. Found: C, 74.9; H, 8.2; N, 13.3. $C_{27}H_{34}ON_4$ requires C, 75.1; H, 7.9; N, 13.0%. **8000**. Found: C, 70.45; H, 7.15; N, 14.2. $C_{24}H_{28}ON_4,H_2O$ requires C, 70.9; H, 7.4; N, 13.8%. **8001**. Found: C, 71.5; H, 6.7; N, 14.2. $C_{23}H_{24}O_2N_4$ requires C, 71.15; H, 6.2; N, 14.4%. **7045** (monopicrate). Found: C, 58.2; H, 5.2; N, 15.7. $C_{24}H_{28}ON_4,C_6H_3O_7N_3$ requires C, 58.4; H, 5.1; N, 15.8%. **7648**. Found: C, 71.1; H, 8.6; N, 14.2. $C_{24}H_{28}ON_4,H_2O$ requires C, 70.9; H, 7.5; N, 13.8%. **7649**. Found: C, 71.1; H, 7.5; N, 13.0. $C_{25}H_{30}ON_4,H_2O$ requires C, 71.4; H, 7.7; N, 13.3%. **7650**. Found: N, 13.9. $C_{24}H_{26}O_2N_4$ requires N, 13.9%. **8002** (dipicrate). Found: C, 51.5; H, 4.2; N, 16.9. $C_{25}H_{30}ON_4$, $2C_6H_3O_7N_3$ requires C, 51.6; H, 4.2; N, 16.3%. **8003**. Found: C, 75.0; H, 7.6; N, 13.5. $C_{26}H_{30}ON_4$ requires C, 75.4; H, 7.2; N, 13.5%. **8004**. Found: C, 72.0; H, 7.1; N, 13.2. $C_{25}H_{28}O_2N_4$ requires C, 72.1; H, 6.7; N, 13.5%. **7042**. Found: C, 68.1; H, 4.8; N, 11.9. $C_{19}H_{15}O_3N_3$ requires C, 68.4; H, 4.5; N, 12.6%. **7043**. Found: C, 67.1; H, 3.5; N, 13.8. $C_{17}H_{11}O_3N_3$ requires C, 66.9; H, 3.6; N, 13.8%. **7652**. Found: C, 71.5; H, 5.3; N, 15.1. $C_{22}H_{20}O_2N_4$ requires C, 71.0; H, 5.4; N, 15.0%. **7653**. Found: C, 67.4; H, 5.0; N, 14.8. $C_{21}H_{18}O_3N_4$ requires C, 67.4; H, 4.9; N, 15.0%. **7887**. Found: C, 71.3; H, 5.3; N, 15.2. $C_{21}H_{20}O_2N_4$ requires C, 71.0; H, 5.6; N, 15.6%. **7888**. Found: C, 67.5; H, 6.4; N, 16.6. $C_{24}H_{27}O_2N_5$, 0.5 H_2O requires C, 67.6; H, 6.6; N, 16.5%.

Table III.—**5869**. Found: C, 70.3; H, 3.8; N, 14.5. $C_{17}H_{11}O_2N_3$ requires C, 70.6; H, 3.8; N, 14.5%. **6434**. Found: C, 71.7; H, 4.2; N, 15.3. Calc. for $C_{22}H_{14}O_2N_4$: C, 72.1; H, 3.8; N, 15.3%. **7059**. Found: C, 74.2; H, 4.7; N, 12.9. $C_{27}H_{22}O_2N_4$ requires C, 74.7; H, 5.1; N, 12.9%. **7334**. Found: C, 61.1; H, 4.1; N, 12.8. Calc. for $C_{16}H_{12}ON_3Cl,H_2O$: C, 60.5; H, 4.4; N, 13.3%. **7604**. Found: C, 67.9; H, 4.5; N, 8.1; S, 6.3. $C_{22}H_{16}ON_3,C_7H_7O_3S$ requires C, 68.3; H, 4.5; N, 8.25; S, 6.3%. **9082**. Found: C, 55.0; H, 5.25; N, 10.6; I, 25.6. $C_{24}H_{27}ON_4I$ requires C, 55.1; H, 5.35; N, 10.7; I, 24.2%. **6307**. Found: C, 72.0; H, 4.1. Calc. for $C_{15}H_{10}O_2N_2$: C, 72.0; H, 4.0%. **7084**. Found: C, 69.8; H, 5.0; N, 24.4. $C_{20}H_{18}N_6$ requires C, 70.1; H, 5.25; N, 24.6%.

The minimal effective therapeutic dose was that dose producing in the group of treated rats an average degree of infection significantly lower ($P<0.01$) than that of the control group (Jones, 1946). No appreciable improvement on the parent compound (3074) was made. Several compounds were equally effective in rats, but were also equally phototoxic. The parent substance (3074) 6-aminoanthrapyrimidine was selected for further study.

Action of 3074 *in amoebic dysentery of cats and monkeys*

3074 had no therapeutic effect in three dysenteric cats to which the drug was administered rectally in daily doses of 1,000 mg./kg. These cats died of the disease 3, 7, and 11 days after onset. A therapeutic effect was apparent when the drug was given orally (200–500 mg./kg. daily) to three cats. Two of the three were completely cured. The third was temporarily cured. It relapsed after 10 days, was cured a second time, then died 4 days afterwards. Four of five untreated dysenteric cats died of the disease; the fifth suffered dysentery for 21 days and subsequently made a spontaneous recovery.

A naturally infected monkey was treated with 50 mg./kg. of 3074 given orally thrice daily for 5 days, without any effect on the cysts. Another naturally infected monkey was treated with 6837 (6-γ-piperidino-propylaminoanthrapyrimidine). After thrice daily dosing with 20 mg./kg. of drug for 5 days, *E. histolytica* could still be detected in the stools. After thrice daily dosing with 50 mg./kg. for the next 5 days, *E. histolytica* disappeared, although *Entamoeba coli* remained. *E. histolytica* reappeared in the stools 14 days after the last dose.

Clinical trial in man

A clinical trial was carried out by Dr. A. R. D. Adams and Dr. G. T. Stewart. Seven patients were treated; six had amoebic dysentery and one was a chronic cyst-passer. None of the patients showed any symptomatic response, nor was there even a temporary diminution in the numbers of parasites. Large doses were given; the maximum amount received by any one patient was 150 mg./kg. No definite toxic effects were observed.

Discussion

The anti-amoebic action of the anthrapyrimidines appeared to be confined rather narrowly to derivatives of 2- or 6-aminoanthrapyrimidine. Activity was readily lost by the introduction of substituents. With two exceptions (7282 and 8023, Table II), 6-aminoanthrapyrimidines carrying substituents in the 4- or 5-position or large substituents in the 2-position were inactive. Certain substituents are readily removed from the 4-position of 6-aminoanthrapyrimidine, e.g., by reduction, and this may account for the activity of 7282 and 8023. Acylation or substitution of the amino group of 3074 usually reduced activity unless the substituent carried a basic group. The 6-aminoalkylaminoanthrapyrimidines (Table II) formed water-soluble hydrochlorides and other salts, and were generally highly active, the nature of the side chain having little influence. The anthrapyrimidine nucleus was active both in the oxidized and the reduced (leuco) states. The methochloride of 3074 (7334, Table III) was water-soluble and fairly active.

Studies of the metabolism of 3074 in several animal species by one of us (Senior, unpublished) showed that the drug was rapidly absorbed and gave measurable blood concentrations. The major portion of the drug was metabolized, a conjugated derivative of the leuco form being excreted in the urine. The unchanged drug appeared in both urine and faeces.

Phototoxicity was probably due to the action of light on the drug in the tissues of the exposed parts, and thus afforded evidence of absorption. Since no anthrapyrimidine derivative devoid of photodynamic action showed any appreciable anti-amoebic action it is probable that the anti-amoebic effect of these compounds was also dependent on their absorption into the blood stream.

It is by no means certain that the therapeutic effect of the drug was due to its direct action on *E. histolytica*. Furthermore, an action on secondary bacterial infection could be ruled out, as tests *in vitro* against *B. coli* showed the compound to have no action. It is more likely that the action was primarily on the host, for in experiments carried out in rats a remarkable feature of the action of the drug was the manner in which the infection was eradicated. Thus when treatment of infected rats was delayed to the fifth morning after infection, and rats so treated were examined about six hours later, it was found that the process of tissue repair had advanced to an astonishing degree, although amoebae could still be detected microscopically in considerable numbers. This suggested that the drug in some way assisted in the process of tissue repair, and disappearance of the amoebae followed later, not directly as a result of drug action.

In experimentally infected cats there was some evidence of a similar mode of action. Amoebic dysentery of cats is notoriously difficult to cure, and if left untreated

is almost invariably fatal. The action of 3074 was possibly an indirect one, for although amoebae disappeared slowly from the stools, clinical improvement was more prompt. The effect of the drug on one of the cats was striking in this respect. Clinical improvement was noticed before parasites disappeared from the stools, and although dosing was continued for five days, the amoebae subsequently reappeared eight days after the last dose. A single dose of drug was sufficient to control the symptoms for a further eleven days, but then the relapse lasted for seven days in spite of treatment. Thus at no time during the forty-three days of the experiment to the final cure could the animal have been entirely free from amoebae. In spite of this, there were considerable periods when the cat was apparently in normal health and passing normally formed stools.

The rate of metabolism of 3074 appears to be much greater in man than in other species, and this may account for the unfavourable result of the clinical trial. Although several related compounds, notably 6-γ-piperidino-propylaminoanthrapyrimidine (6837), showed equal therapeutic effect in rats and superior pharmacological properties, the activity of 6837 in an infected monkey was not great enough to justify hopes of clinical success with these substances.

Summary

1. 2- or 6-Aminoanthrapyrimidines and their derivatives were found to have therapeutic action when administered to rats experimentally infected with *E. histolytica*. The therapeutic effect was associated with an undesirable photodynamic action.

2. 6-Aminoanthrapyrimidine (3074) showed a therapeutic effect against experimental amoebic dysentery in cats, but had no action on the infection in monkeys and men.

Reference

Jones, W. R. (1946). *Ann. trop. Med. Parasit.*, **40**, 130.