CHEMOTHERAPY OF MALARIA

Part VIII. Synthesis of Uracils, Thiouracils, Pteridines and Thiopteridines

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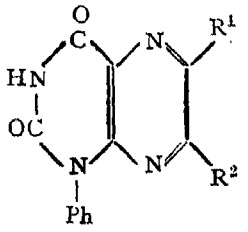
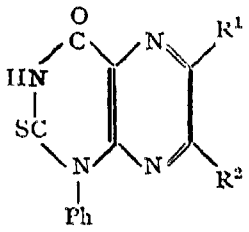
In recent years, the heterocyclic compounds are proving to be fruitful sources of chemotherapeutic agents and of these, derivatives of pyrimidine and pteridine are of special interest. Sulphadiazine and its methyl derivatives are not only potent bacteriostatic agents but show antimalarial properties as well. A number of guanidylpyrimidine derivatives have been discovered by the British workers[1] to show significant antimalarial properties. Recently, outstanding anti-malarial activity has been met with in some 2: 4-diamino-pyrimidines.[2] These point to a need for search for anti-malarials among the pyrimidines. The pteridine derivatives because of their possible interference with folic acid metabolism are also of interest both as bacteriostatic and antimalarial agents. A number of pteridine derivatives have been synthesised and studied[3] by many workers and some have shown antibacterial properties.[4] It has been shown[5] that 2: 4-diamino-6: 7-diphenylpteridine suppresses parasitæmia in chicks infected with *P. gallinaceum*. In view of these, we undertook to synthesise pteridine derivatives of formula (I) and (II), so that these, as well as the intermediate uracil and thiouracil derivatives, could also be tested for their antimalarial as well as their antibacterial properties. The grouping *p*-chlorophenyl and also other substituted phenyl radicals were introduced because this is present in the anti-malarial paludrine.



(I)

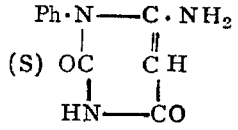


(II)

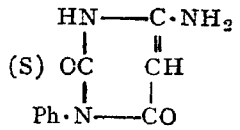
R^1—H, Me, Ph
R^2—H, Me, Ph

A number of methods of synthesis of the pteridines have now been examined and of these we have chosen the well-known one consisting of the condensation of the diketones with the 4: 5-diaminouracils and thiouracils, because we are also interested in these intermediates. Suitable phenylurea and phenylthiourea derivatives were condensed with cyanacetic ester according to the method of Traube[6] as improved by Bogert and Davidson.[7] The

reaction proceeded when the reactants were heated in butyl alcohol furnishing the 4-aminouracil and 4-aminothiouracil derivatives respectively. This condensation can lead to two isomers (III) and (IV). Since the condensation of the N-alkyl substituted ureas with cyanacetic ester has been proved to lead to the 3-substituted uracils,[8] we assign the structure (III) to the uracils and thiouracils formed.

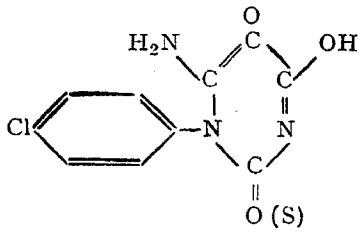


(III)

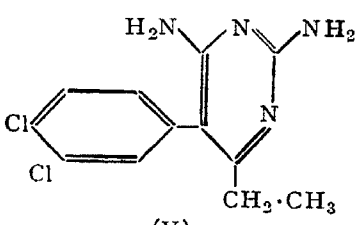


(IV)

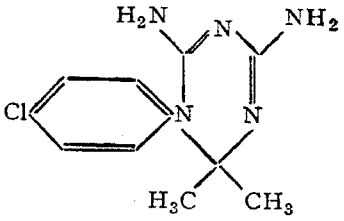
The close formal resemblance between the uracils and thiouracils, and the diaminopyrimidine[2] (V), and the triazine derivative[9] (VI) both of which have been shown to possess outstanding antimalarial activity, as far as the peripheral groups about the ring are concerned, should be noted. Further work done in search of antimalarials of the above type will be reported later on.



(III)

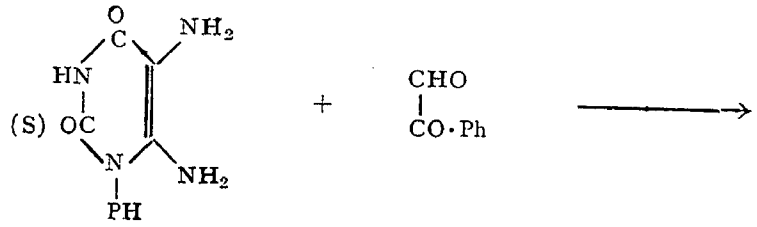


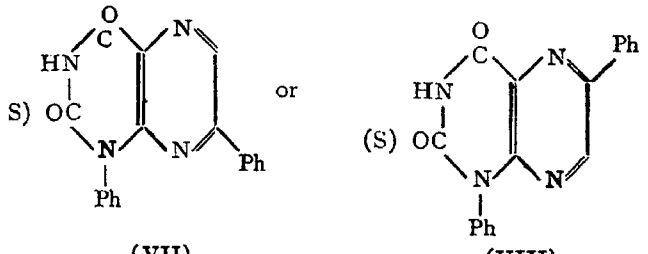
(V)



(VI)

The 4-aminouracils and 4-aminothiouracils were converted into the 4: 5-diamino derivatives through the nitroso derivatives and the pteridines and thiopteridines were prepared by condensing the diamino compounds with glyoxal, diacetyl, phenylglyoxal and benzil individually. In the case of phenylglyoxal, there is the possibility of formation of two isomers (VII) and (VIII).





(VII)

(VIII)

On the basis of analogy[10] with previous work, we ascribe the structure (VII) to the compounds obtained. The pteridines and thiopteridines prepared are listed in Tables III and IV.

Of the compounds prepared, a number of representatives are being tested for their antimalarial properties against *P. berghii* infections in mice.

EXPERIMENTAL

Urea and Thiourea Derivatives

Phenyl-, *p*-chlorophenyl-, *m*-chlorophenyl-, *p*-tolyl-, *p*-anisyl-, *o*-anisyl-ureas were prepared by the action of potassium cyanate on the corresponding amine hydrochloride. Phenyl-, *o*-chlorophenyl-, *m*-chlorophenyl-, *p*-chlorophenyl-, *p*-bromophenyl-, *o*-tolyl-, *p*-tolyl-, *o*-anisyl-, and *p*-anisyl- thioureas were prepared by the action of ammonium thiocyanate on the respective amine hydrochlorides.

4-*Aminouracils*.—The compounds listed in Table I were prepared according to the following general method which differs from the standard procedure[6, 7] in the use of butyl alcohol instead of ethyl alcohol.

Sodium (3·5 g.) was dissolved in absolute butanol (125 c.c.), the solution was cooled and to this was added ethyl cyanoacetate (12·5 g.) whereby a bulky white precipitate of the sodium salt separated. After about 30 minutes, *p*-tolylurea (15 g.) was added to this and the mixture gently refluxed for about 30 minutes. The solid went into solution and immediately the condensation product separated. The heating was stopped and the reaction mixture was allowed to stand overnight. Next day it was refluxed for about 30 minutes, the solvent distilled off under reduced pressure, the sodium salt remaining dissolved in water and the uracil obtained by carefully acidifying the clarified solution. The product was separated and washed with water and ether (yield, 16 g.). The product as such could be used in the next stage. A sample was crystallised from dilute alcohol for analysis.

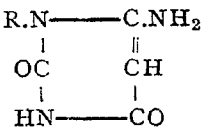
The uracils are soluble in alcohol, acetone, pyridine and dioxane but insoluble in ether and benzene. They dissolve in both dilute hydrochloric acid and alkali.

4-*Aminothiouracils*.—The compounds listed in Table II were prepared essentially as described above, using the thiourea derivatives instead of the urea derivatives.

4: 5-*Diaminouracils and* 4: 5-*Diaminothiouracils*.—The compounds were prepared according to the following method,[7] found to be the best after

TABLE I

4-Aminouracils

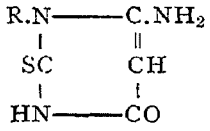


(All the compounds do not melt below 285°)

Serial No.	R	Molecular Formula		Nitrogen % Req.	Found
1	H-	$C_4H_5N_3O_2$		..	..
2	*p*-Chlorophenyl-	$C_{10}H_8N_3O_2Cl$		17·68	17·25
3	*m*-Chlorophenyl-	$C_{10}H_8N_3O_2Cl$		17·68	17·49
4	*p*-Tolyl-	$C_{11}H_{11}N_3O_2$		19·35	19·56
5	*p*-Anisyl-	$C_{11}H_{11}N_3O_3$		18·02	18·16
6	*o*-Anisyl-	$C_{11}H_{11}N_3O_3$		18·02	17·70
7	Phenyl-	$C_{10}H_9N_3O_2$		20·68	20·22
			C %	59·11	59·06
			H %	4·43	4·28

TABLE II

4-Amino-2-thiouracils

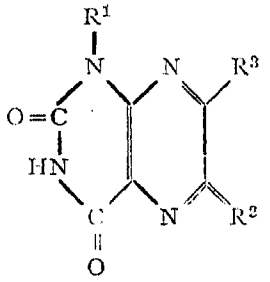


Serial No.	R	Molecular Formula	M.P./°C.		Nitrogen % Req.	Found
1	H-	$C_4H_5N_3OS$	above 295		..	..
2	Phenyl-	$C_{10}H_9N_3OS$	239–240		19·18	19·04
3	*p*-Chlorophenyl-	$C_{10}H_8N_3OSCl$	241–242		16·56	16·37
4	*m*-Chlorophenyl-	$C_{10}H_8N_3OSCl$	218–219		16·56	16·16
5	*o*-Chlorophenyl-	$C_{10}H_8N_3OSCl$	228–229		16·56	16·30
6	*d*-Tolyl-	$C_{11}H_{11}N_3OS$	234–235		18·02	18·32
7	*o*-Tolyl-	$C_{11}H_{11}N_3OS$	226–227		18·02	17·46
8	*o*-Anisyl-	$C_{11}H_{11}N_3O_2S$	222–223		16·86	16·70
9	*p*-Bromophenyl-	$C_{10}H_8N_3OSBr$	242–243		14·09	14·17
10	*p*-Anisyl-	$C_{11}H_{11}N_3O_2S$	212–213		16·86	16·56
				C %	53·01	53·09
				H %	4·41	4·45

many trials. N^3-*p*-Tolyl-4-aminouracil (16·0 g.) was dissolved in a solution of sodium hydroxide (6·4 g. in 160 c.c. water), a solution of sodium nitrite (16·0 g.) in water (80 c.c.) was added, and maintaining at 2 to 5° C., sulphuric acid (100 c.c. of 20 per cent.) was added dropwise under good stirring. The rose-red nitroso compound that separated was collected, filtered, suspended in water (100 c.c.) and made strongly alkaline with ammonia. The nitroso

TABLE III

Pterins



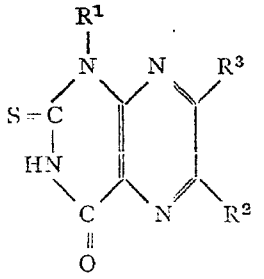
Serial No.	R^1	R^2	R^3	Molecular Formula	M.P./°C.	Nitrogen % Req.	Found
1	H-	CH_3	CH_3	$C_8H_8N_4O_2$	..	..	..
2	Phenyl-	CH_3	CH_3	$C_{14}H_{12}N_4O_2$	above 280	20·89	20·41
3	*p*-Chlorophenyl-	CH_3	CH_3	$C_{14}H_{11}N_4O_2Cl$	above 285	18·51	18·96
4	*m*-Chlorophenyl-	CH_3	CH_3	$C_{14}H_{11}N_4O_2Cl$	above 280	18·51	18·36
5	*p*-Tolyl-	CH_3	CH_3	$C_{15}H_{14}N_4O_2$	above 280	19·85	19·46
6	*p*-Anisyl-	CH_3	CH_3	$C_{15}H_{14}N_4O_3$	289–290	18·79	18·61
7	*o*-Anisyl-	CH_3	CH_3	$C_{15}H_{14}N_4O_3$	257–258	18·79	18·63
					C%	60·40	59·98
					H%	4·69	4·23
8	H-	C_6H_5	C_6H_5	$C_{18}H_{12}N_4O_2$	..	..	..
9	Phenyl-	C_6H_5	C_6H_5	$C_{24}H_{16}N_4O_2$	above 280	14·28	14·58
10	*p*-Chlorophenyl-	C_6H_5	C_6H_5	$C_{24}H_{15}N_4O_2Cl$	above 290	13·13	13·43
11	*m*-Chlorophenyl-	C_6H_5	C_6H_5	$C_{24}H_{15}N_4O_2Cl$	281–282	13·13	13·20
12	*p*-Tolyl-	C_6H_5	C_6H_5	$C_{25}H_{18}N_4O_2$	above 285	13·79	13·74
13	*p*-Anisyl-	C_6H_5	C_6H_5	$C_{25}H_{18}N_4O_3$	above 285	13·27	13·38
14	*o*-Anisyl-	C_6H_5	C_6H_5	$C_{25}H_{18}N_4O_3$	above 290	13·27	12·93
15	*p*-Chlorophenyl-	H	H	$C_{12}H_8N_4O_2Cl$	above 285	20·33	19·99
16	*m*-Chlorophenyl-	H	H	$C_{12}H_8N_4O_2Cl$	242–243	20·33	20·37
17	*p*-Tolyl-	H	H	$C_{13}H_{11}N_4O_2$	above 290	22·04	21·81
18	*o*-Anisyl-	H	H	$C_{13}H_{11}N_4O_3$	276–277	20·74	20·11
19	H-	H	C_6H_5	$C_{12}H_8N_4O_2$	..	..	..
20	Phenyl-	H	C_6H_5	$C_{18}H_{12}N_4O_2$	above 290	17·72	17·88
21	*p*-Chlorophenyl-	H	C_6H_5	$C_{18}H_{11}N_4O_2Cl$	above 295	15·97	15·35
22	*m*-Chlorophenyl-	H	C_6H_5	$C_{18}H_{11}N_4O_2Cl$	above 280	15·97	15·57
23	*p*-Tolyl-	H	C_6H_5	$C_{19}H_{14}N_4O_2$	above 290	16·96	16·73
24	*p*-Anisyl-	H	C_6H_5	$C_{19}H_{14}N_4O_3$	above 295	16·18	16·37
25	*o*-Anisyl-	H	C_6H_5	$C_{19}H_{14}N_4O_3$	above 270	16·18	16·23

compound was filtered, washed well with water. The colour of the nitroso compound in many cases ranged from crimson red to green.

The nitroso compound (13 g.) was suspended in water (100 c.c.) and under efficient mechanical stirring, sodium hydrosulphite (40 g.) was added slowly. While the addition continued, the colour of the product changed

TABLE IV

Thiopterins



Serial No.	R^1	R^2	R^3	Molecular Formula	M.P./°C.	Nitrogen % Req.	Found
1	H-	CH_3	CH_3	$C_8H_8N_4OS$	..	..	..
2	Phenyl-	CH_3	CH_3	$C_{14}H_{12}N_4OS$	280–281	19·71	19·10
3	*p*-Chlorophenyl-	CH_3	CH_3	$C_{14}H_{11}N_4OSCl$	above 270	17·58	17·10
4	*p*-Tolyl-	CH_3	CH_3	$C_{15}H_{14}N_4OS$	above 300	18·79	18·56
5	*p*-Anisyl-	CH_3	CH_3	$C_{15}H_{14}N_4O_2S$	above 280	17·83	17·87
6	*o*-Anisyl-	CH_3	CH_3	$C_{15}H_{14}N_4O_2S$	282–283	17·83	17·53
7	*p*-Bromophenyl-	CH_3	CH_3	$C_{14}H_{11}N_4OSBr$	291–292	15·42	14·92
8	*m*-Chlorophenyl-	CH_3	CH_3	$C_{14}H_{11}N_4OSCl$	266–267	17·58	17·54
					C%	52·74	53·20
					H%	3·45	2·91
9	H-	C_6H_5	C_6H_5	$C_{18}H_{12}N_4OS$	..	..	..
10	Phenyl-	C_6H_5	C_6H_5	$C_{24}H_{16}N_4OS$	274–275	13·72	13·63
11	*p*-Chlorophenyl-	C_6H_5	C_6H_5	$C_{24}H_{15}N_4OSCl$	above 280	12·65	12·84
12	*m*-Chlorophenyl-	C_6H_5	C_6H_5	$C_{24}H_{15}N_4OSCl$	257–258	12·65	12·73
13	*p*-Anisyl-	C_6H_5	C_6H_5	$C_{25}H_{18}N_4O_2S$	297–298	12·78	12·73
14	*o*-Anisyl-	C_6H_5	C_6H_5	$C_{25}H_{18}N_4O_2S$	285–286	12·78	12·68
15	*p*-Bromophenyl-	C_6H_5	C_6H_5	$C_{24}H_{15}N_4OSBr$	above 280	11·49	11·27
16	*p*-Tolyl-	C_6H_5	C_6H_5	$C_{25}H_{18}N_4OS$	above 300	13·27	13·38
					C%	71·09	71·68
					H%	4·26	4·10
17	*m*-Chlorophenyl-	H	H	$C_{12}H_8N_4OSCl$	264–265	19·28	19·44
18	H-	H	C_6H_5	$C_{12}H_8N_4OS$	..	..	..
19	Phenyl-	H	C_6H_5	$C_{18}H_{12}N_4OS$	above 280	16·86	16·41
20	*p*-Chlorophenyl-	H	C_6H_5	$C_{18}H_{11}N_4OSCl$	above 280	15·28	15·39
21	*m*-Chlorophenyl-	H	C_6H_5	$C_{18}H_{11}N_4OSCl$	above 280	15·28	15·36
22	*p*-Tolyl-	H	C_6H_5	$C_{19}H_{14}N_4OS$	above 285	16·18	16·02
23	*p*-Anisyl-	H	C_6H_5	$C_{19}H_{14}N_4O_2S$	above 280	15·46	15·63
24	*o*-Anisyl-	H	C_6H_5	$C_{19}H_{14}N_4O_2S$	above 290	15·46	15·08

from rose red to yellowish white. The mixture was heated on the water-bath for 30 minutes, then cooled and the product that separated collected by filtration, washed with water and finally with alcohol (yield, 10 g.).

The product could not be recrystallised. So it was used as such in the next step. The free bases get oxidised on standing in contact with air.

Pteridines and Thiopteridines

These compounds described in Tables III and IV, were prepared according to the general methods described below:

1-(N^1-*p-Chlorophenyl*)-2: 4-*dioxy*-6: 7-*dimethylpteridine*.—To 3-(N-*p*-chlorophenyl)-4: 5-diaminouracil sulphate (1·6 g.) dissolved in a mixture of glacial acetic acid (30 c.c.) and alcohol (20 c.c.), was added diacetyl (1 c.c.) and the mixture refluxed for 12 hours. The solution was treated with a little charcoal, filtered, the filtrate evaporated to a syrupy mass and diluted with a little water. The product that separated (1·1 g.) was crystallised from a mixture of alcohol and benzene. In 4 per cent. alcoholic alkali the compound showed a bright blue fluorescence in the ultra-violet light.

N^1-(*o-Anisyl*)-2: 4-*dioxy*-6: 7-*diphenylpteridine*.—To 3-(N-*o*-anisyl)-4: 5-diaminouracil sulphate (1·1 g.) dissolved in a mixture of acetic acid (30 c.c.) and alcohol (20 c.c.), benzil (1 g.) was added and the mixture refluxed for about 5 minutes when a solid appeared. Heating was continued for another 8 hours, the mass cooled and filtered. The colourless beautiful needles obtained (yield, 1·1 g.) were separated and recrystallised from a mixture of alcohol and benzene. The alcoholic sodium hydroxide solution of the pteridine showed bright blue fluorescence.

1-(*N-o-Anisyl*)-2: 4-*dioxy*-7-*phenylpteridine*.—To a solution of N^3-*o*-anisyl 4: 5-diaminouracil sulphate (1·1 g.) in 4N acetic acid (50 c.c.) and alcohol (10 c.c.) was added phenylglyoxal hydrate (0·6 g.). Immediately a red solution was obtained and in a few minutes a yellow solid separated. The mixture was refluxed for 30 minutes, the solid was filtered (yield, 1·2 g.) and recrystallised from benzene.

The thiopteridines do not show any fluorescence as also reported by Polonvski.[11]

SUMMARY

Twenty-two pteridines of formula (I) and twenty-one thiopteridines of formula (II) have been synthesised *via* 4-aminouracils and 4: 5-diaminouracils for assessing their antimalarial and antibacterial properties.

REFERENCES

1. Rose .. *J. Chem. Soc.*, 1951, 2785.
   Rollo .. *Nature*, 1951, **168**, 332.
   Russel and Hitchings .. *J. Amer. Chem. Soc.*, 1951, **73**, 3763.
   Falco, Goodwin, Hitchings, Rollo and Russel *Brit. J. Pharm.*, 1951, **6**, 185.
   Chase, Johnson and Walker .. *J. Chem. Soc.*, 1951, 3439.
3. Seeger, Cosulich, Smith and Hultquist *J. Amer. Chem. Soc.*, 1949, **71**, 1753.
   Campbell, Dunsmuir and Fitzgerald *J. Chem. Soc.*, 1950, 2743.
   Daniel, Norris, Scott and Heuser .. *J. Biol. Chem.*, 1947, **169**, 689.
   Daniel and Norris .. *Ibid.*, 1947, **170**, 747.
   Simpson .. *Annual Rep. Chem. Soc.*, 1946, **43**, 250.
   Gates .. *Chem. Rev.*, 1947, **41**, 63.
   Spies .. *Ann. Rev. Biochem.*, 1947, **16**, 387.
4. Collier, Campbell and Fitzgerald .. *Nature*, 1950, **165**, 1004.
5. Greenberg .. *J. Pharmacol.*, 1949, **97**, 484.
6. Traube .. *Annalen*, 1904, **331**, 64.
   Johns .. *J. Biol. Chem.*, 1911, **9**, 161.
   Lythgoe .. *Quart. Rev.*, 1949, **3**, 180.
7. Bogert and Davidson .. *J. Amer. Chem. Soc.*, 1933, **55**, 1667.
8. Conrad .. *Annalen*, **340**, 314.
   Merck .. *DRP*, 165, 562.
   Traube .. *Ber.*, 1900, **33**, 1382, 3035, 3044.
   Arch. Pharm., **244**, 13.
   Nathan and Bogert .. *J. Amer. Chem. Soc.*, 1941, **63**, 2567.
9. Carrington, Crowther, Davy, Levi and Rose *Nature*, 1951, **168**, 1080.
10. Weijlard, Tishler and Erickson .. *J. Amer. Chem. Soc.*, 1945, **67**, 802.
11. Polonvski, Guinand, Pesson and Vieillefosse *Bull. Soc. Chim.*, 1945, **12**, 924.