The Synthesis of Compounds with Potential Anti-folic Acid Activity. Part I. 7-Amino- and 7-Hydroxy-pteridines.

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The unequivocal synthesis is reported of a number of 7-amino- and 7-hydroxy-pteridines by the reaction of 4-amino-5-nitrosopyrimidines with phenylacetonitriles and phenylacetyl chlorides respectively. Sodium alkoxides were effective catalysts for the reaction with the acetonitriles. Reaction of 2 : 4 : 6-triamino-5-nitrosopyrimidine with arylacetyl chlorides was best in the absence of a solvent. 2 : 4-Diamino-7-hydroxy-6-phenylpteridine thus obtained was also formed by treating phenylglyoxylic acid with 2 : 4 : 5 : 6-tetra-aminopyrimidine hydrochloride at pH 5: in more acid solution the isomeric 7-phenylpteridine was a minor product.

Hydroxy-groups are introduced into the 2-position by hydrolysis of 2-methylthiopteridines and into the 4-position by hydrolysis of 2 : 4 : 7-triamino-6-phenylpteridine.

Some of the 7-amino-6-arylpteridines show anti-folic acid activity but in no case of a high order.

THE first simple derivatives of pteridine to show marked anti-folic acid activity were synthesised by Mallette, Taylor, and Cain (*J. Amer. Chem. Soc.*, 1947, **69**, 1814), and Daniel *et al.* (*J. Biol. Chem.*, 1947, **169**, 689; **170**, 747) found that, in general, the replacement of hydroxy-groups in the 2- and the 4-position by the amino-group successively led to greatly enhanced activity. Maximum activity in antagonising the folic acid required for growth by certain micro-organisms was shown by 2 : 4-diamino-6 : 7-diarylpteridines and in the hope of further enhancing activity we wished to replace one of the aryl groups by an amino- and also by a hydroxy-group.

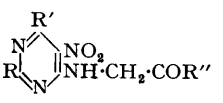
Hitherto (for summaries, see *Ann. Reports*, 1946, **43**, 250; 1948, **45**, 226; Gates, *Chem. Reviews*, 1947, **41**, 63; Albert, *Quart. Reviews*, 1952, **6**, 197) three general methods have been used for the synthesis of pteridines. Condensation of 4 : 5-diaminopyrimidines with α-diketones and related substances is the most frequently used method, but suffers from the major drawback that from an unsymmetrical dicarbonyl compound two isomers can be formed of necessarily undetermined constitution and in some cases difficultly separable. Ring closure of certain substituted pyrazines has yielded pteridines, *e.g.*, 2 : 4-dihydroxypteridine (Gabriel and Sohn, *Ber.*, 1907, **40**, 4857) and 4-hydroxy- and 4-mercapto-pteridines (Albert, Brown, and Cheeseman, *J.*, 1951, 474) but the scope of the method is severely limited. Pteridines of known structure have been synthesised by Boon, Jones, and Ramage (*J.*, 1951, 96), Boon and Jones (*ibid.*, 591), by Polonovski and Jérome (*Compt. rend.*, 1950, **230**, 392), and by Polonovski, Pesson, and Puister (*ibid.*, 2205) by reduction of α-(5-nitro-4-pyrimidylamino)acetic esters (I; R″ = OEt) and α-(5-nitro-4-pyrimidylamino)-ketones (I; R″ = alkyl or aryl), followed by ring closure; Boon and Leigh (*J.*, 1951, 1497) later developed this method by substituting a phenylazo- for the nitro-group in the pyrimidine moiety.

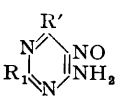
None of these methods could conveniently, if at all, lead to the required 6- or 7-aminopteridines but a convenient route was found in an extension of the reaction between 4-amino-5-nitrosopyrimidines and ketomethylene compounds, which, by the loss of water between the nitroso- and methylene and the amino- and keto-groups, yields pteridines of unequivocal structure (Timmis, *Nature*, 1949, **164**, 139). This extension involves the condensation of a 4-amino-5-nitrosopyrimidine (II) with an arylacetonitrile with the loss of one mol. of water between the methylene and the nitroso-group. By using an arylacetyl chloride instead of the nitrile corresponding 7-hydroxy-pteridines were obtained which were required for biological comparison.

The 4-amino-5-nitrosopyrimidines required for these condensations were prepared by

the conventional treatment of the appropriate 4-aminopyrimidine with nitrous acid. Since this reaction does not take place unless there are polar groups in the 4- and the 6-position of the pyrimidine ring, the scope of the new pteridine synthesis would be somewhat restricted but for the fact that it has already been shown (Bayer and Co., D.R.-P. 206,453; *Zentr.*, 1909, I, 806) that cyano(hydroxyimino)acetic ester can react with, *e.g.*, guanidine to yield 2 : 4-diamino-6-hydroxy-5-nitrosopyrimidine, and it seems likely that 4-amino-5-nitrosopyrimidines unsubstituted in the 6-position, or substituted by alkyl groups, could be analogously prepared.

Initial attempts to prepare the pteridines (III; R = R′ = NH_2, R″ = Ar) from 2 : 4 : 6-triamino-5-nitrosopyrimidine (II; R = R′ = NH_2) and arylacetonitriles in acetic acid in the presence of anhydrous sodium acetate were only successful when *p*-nitrophenylacetonitrile was used, the compound (III; R = R′ = NH_2, R″ = *p*-NO_2·C_6H_4) being obtained; from experiments with phenylacetonitrile and *p*-methoxyphenylacetonitrile no pteridine was isolated. In the absence of the nitro- or other strongly electron-attracting group in the *para*-position of the phenyl group, it was thought that more strongly basic


(I)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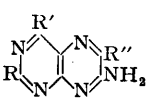
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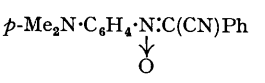
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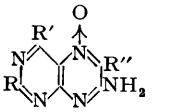
(V)

catalysts might be effective and this was proved to be the case by the ready synthesis of a series of 7-aminopteridines when the reaction was catalysed by sodium ethoxide in boiling ethanol, sodium 2-ethoxyethoxide in boiling 2-ethoxyethanol, or sodium 2-hydroxyethoxide in boiling ethylene glycol. Ethylene glycol or 2-ethoxyethanol were necessary solvents when the less soluble nitrosopyrimidines were used. Examples of the series are: 2 : 4 : 7-triamino-6-phenyl (III; R = R′ = NH_2, R″ = Ph), 2 : 4 : 7-triamino-6-α-thienyl-, 2 : 4 : 7-triamino-6-α-naphthyl-, 7-amino-2 : 6-diphenyl-, 4-hydroxy-, 7-amino-4-hydroxy-2-methylthio-6-phenyl-, 7-amino-4-hydroxy-2-methyl-6-phenyl-, and 2 : 7-diamino-4-hydroxy-6-phenyl-pteridine.

Evidence for an ortho-effect of the reaction is suggested by the cases where the *o*- and *p*-isomers of chlorophenylacetonitrile, ethoxyphenylacetonitrile, and nitrophenylacetonitrile were condensed with 2 : 4 : 6-triamino-5-nitrosopyrimidine (II; R = R′ = NH_2). In the first two cases, a considerably slower reaction was observed for the *ortho*- than for the *para*-substituted nitriles and in the third case condensation of the *ortho*-isomer was inappreciable even in boiling 2-ethoxyethanol.

Since Barrow and Thorneycroft (*J.*, 1939, 769) isolated the oxide (IV) as a by-product of the reaction of *p*-nitrosodimethylaniline with phenylacetonitrile we have looked, but without success, for pteridine *N*-oxides (V) as by-products of our reaction, since these would have had considerable potential biological interest.

The reaction of 4 : 6-diamino-5-nitroso-2-thiopyrimidine (II; R = SH, R′ = NH_2) with phenylacetonitrile in ethanol containing sodium ethoxide yielded in addition to 4 : 7-diamino-6-phenyl-2-mercaptopteridine (III; R = SH, R′ = NH_2, R″ = Ph) a product which gave correct analytical results for 4 : 7-diamino-2-ethoxy-6-phenylpteridine (III; R = OEt, R′ = NH_2, R″ = Ph). This structure is supported by the close similarity of its ultra-violet absorption spectrum with those of 2 : 4 : 7-triamino-, 4 : 7-diamino-, and 4 : 7-diamino-2-methylthio-6-phenylpteridine.

The 2 : 4 : 7-triamino-6-arylpteridines were conveniently characterised as their triacetyl and the 4 : 7-diamino-2-methylthio- and -2-ethylthio-6-phenylpteridines as their diacetyl derivatives. The pteridines were further characterised by means of their ultra-violet spectra which were also used as criteria of purity where a reliable melting point was not observed.

6-Aryl-7-hydroxypteridines have been obtained by condensation of 4-amino-5-nitrosopyrimidines with arylacetyl chlorides; *e.g.*, phenylacetyl chloride and 2 : 4 : 6-triamino-5-nitrosopyrimidine at 140° gave 2 : 4-diamino-7-hydroxy-6-phenylpteridine, which was also

the major product from phenylglyoxylic acid and a salt of 2 : 4 : 5 : 6-tetra-aminopyrimidine in water at pH 5. When this reaction was carried out in 10% hydrochloric acid solution about 60% of the total product was the 7- and 40% the 6-hydroxy-isomer. The latter compound is readily soluble in dilute ammonia solution, whereas the former is not, indicating that the hydroxyl group in the 6-position is more acidic than that in the 7-position. The ultra-violet absorption spectra of the two compounds are markedly different. Elion, Hitchings, and Russell (*J. Amer. Chem. Soc.*, 1950, **72**, 78) have found that, generally, the 6-alkyl-7-hydroxy-isomer is the major product when a similar reaction with aliphatic keto-acids is carried out at pH 5, and a greater proportion of the 6-hydroxy-isomer is formed in strongly acid solution.

p-Nitrophenylacetyl chloride and *p*-methoxyphenylacetyl chloride with 2 : 4 : 6-triamino-5-nitrosopyrimidine also gave the corresponding pteridines.

As the scope of this reaction appears to be limited by the drastic conditions required and the consequent formation of by-products, attempts were made to condense ethyl phenylacetate and phenylacetamide with 2 : 4 : 6-triamino-5-nitrosopyrimidine, but in no case was the required 2 : 4-diamino-7-hydroxy-6-phenylpteridine obtained.

Conversion of 2-alkylthiopyrimidines into hydroxy-compounds is well known (Lythgoe, *Quart. Reviews*, 1949, **3**, 197; Johnson and Hahn, *Chem. Reviews*, 1933, **13**, 193) and we have now obtained 2-hydroxypteridines by the removal of the 2-methylthio-group from 2-methylthiopteridines on mild acid hydrolysis. Thus, 4 : 7-diamino- and 7-amino-4-hydroxy-2-methylthio-6-phenylpteridine in boiling 10% hydrochloric acid afforded 4 : 7-diamino-2-hydroxy- and 7-amino-2 : 4-dihydroxy-6-phenylpteridine respectively. This method provides the best route to 2-hydroxypteridines, since condensation of 4-amino-5-nitrosopyrimidines and arylacetonitriles does not occur readily when a 2-hydroxy-group is present.

Polonovski, Vieillefosse, and Pesson (*Bull. Soc. chim.*, 1945, **12**, 78) converted 4-hydroxy- and 6 : 7-diphenyl-4-hydroxy-2-mercaptopteridine into the corresponding 2-ethylthio-derivatives by treatment with ethyl bromide in alcoholic alkali. Similarly we have converted 4 : 7-diamino-6-phenyl-2-mercaptopteridine by ethyl bromide into 4 : 7-diamino-2-ethylthio-6-phenylpteridine which was identical with the synthetic material.

Hydrolysis of 2 : 4 : 7-triamino-6-phenylpteridine with 5N-hydrochloric acid yielded a mixture from which 2 : 4-diamino-7-hydroxy-6-phenylpteridine and a product having the spectrum of 2 : 7-diamino-4-hydroxy-6-phenylpteridine was isolated. The triaminopteridine was recovered unchanged on treatment with nitrous acid and thus behaved similarly to the 2 : 4-diaminopteridines (Taylor and Cain, *J. Amer. Chem. Soc.*, 1949, **71**,

Absorption spectra of 2 : 4 : 7-*triaminopteridines* (III; R = R′ = NH_2) *in* 4·5% *formic acid.*

R″	Maxima λ (mμ)	Maxima ε	Minima λ (mμ)	Minima ε	Shoulder λ (mμ)	Shoulder ε
Ph	356	21,000	306	3250	281	7050
	257	15,250				
o-EtO·C_6H_4	352	21,400	301	3450	276	8850
	256	16,900				
m-EtO·C_6H_4	358	21,400	307	3750	286	7200
	259	15,650				
p-EtO·C_6H_4	364	21,400	313	4400	287	8000
	250	19,700				
p-NO_2·C_6H_4	368	22,470	300	6800		
	258	21,150				
α-Thienyl	377	18,900	324	3800		
	302	8,050	287	7600		
	260	15,700				
o-Cl·C_6H_4	351	20,550	301	2470	280	6350
	259	15,100				
m-Cl·C_6H_4	358	21,400	309	3250	285	7200
	254	15,250				
p-Cl·C_6H_4	359	21,700	309	3200	285	7550
	251	16,800				
4 : 7-Diamino-2-ethoxy(?)-6-phenylpteridine	354	19,400	306	3800	280	7200
	258	14,500				

Absorption spectra of 7-*aminopteridines* (III; R″ = Ph) *in acid and alkaline solution.*

		4·5% HCO_2H solution				0·1N-NaOH solution			
		Maxima		Minima		Maxima		Minima	
R	R′	λ (mμ)	ε	λ (mμ)	ε	λ (mμ)	ε	(λ (mμ)	ε
MeS	NH_2	363	22,900	311	3,400				
		267	21,500						
EtS	NH_2	364	23,100	311	3,600				
		266	20,700						
NH_2	OH	347	16,300	307	5,500	365	18,000	312	2,600
		287	9,900	260	3,950	267	12,800	253	10,800
						228	35,600		
Me	OH					352	15,800	295	3,100
						263	13,500	254	11,800
						231	24,300		
H	NH_2	356	16,200	309	3,850				
		290	5,150	286	5,100				
		262	16,650						
H	OH					352	14,200	290	3,000
						262	12,400	255	11,700
						230	23,050		
SH	NH_2	375	24,400	333	8,600	383	20,450	328	4,100
		311	15,200	290	11,200	283	20,750	263	12,800
		271	21,900	259	16,300	239	26,120	222	16,200
		249	18,750						
OH	NH_2	355	19,400	312	5,400	369	17,100	314	2,800
		292	10,300	265	5,000	273	13,400	255	10,400
						288	33,900		
MeS	OH					357	15,600	305	3,000
						264	13,100	251	10,500
						288	25,700		

Absorption spectra of 2 : 4-*diamino*-7-*hydroxypteridines* (VIII; R = R′ = NH_2).

	4·5% HCO_2H solution				0·1N-NaOH solution			
	Maxima		Minima		Maxima		Minima	
R″	λ (mμ)	ε	λ (mμ)	ε	λ (mμ)	ε	λ (mμ)	ε
Ph	362	19,650	310	4,000	362	19,900	324	9,400
	267	12,100	256	11,000	306	14,000	271	3,950
	229	43,150			217	34,800		
p-$MeO{\cdot}C_6H_4$	367	20,700	314	4,500				
	272	13,400	257	11,850				
	228	44,700						
p-$NO_2{\cdot}C_6H_4$	394	19,300	294	6,350				
	249	20,700 *						
	224	37,700						

* Shoulder.

2538) in which the 4-amino-group was removed by hydrolysis with 5N-hydrochloric acid but neither group was affected by nitrous acid.

The results of the biological tests were disappointing since none of the anti-folic acid activities approached that found for 2 : 4-diamino-6 : 7-diphenylpteridine (Daniel *et al.*, *loc. cit.*). The two most active compounds 2 : 4 : 7-triamino-6-*p*-chlorophenyl- and -6-α-thienyl-pteridine had approximately one hundredth of the activity of the diphenylpteridine (H. Proom, personal communication).

Ultra-violet absorption spectra, which have been determined (where possible) in acid and alkaline solution, are shown in the Tables. Owing to the insolubility of most of the compounds in dilute hydrochloric acid, solutions in 4·5% formic acid were used which were prepared by dissolving the substance in 90% formic acid and diluting it with water.

The spectra of 2 : 4-diamino-7-hydroxy-6-phenylpteridine and 2 : 4-diamino-6-hydroxy-7-phenylpteridine in both acid and alkaline media resemble those of the corresponding methylpteridines (Elion, Hitchings, and Russell, *J. Amer. Chem. Soc.*, 1950, **72**, 78). The presence of a 6- or 7-phenyl group has, as expected (because of the extra conjugation of the aryl group with the pteridine nucleus), increased the intensity of the bands, which are also shifted to longer wave-lengths. However a close analogy between the methyl and the

phenyl isomers is shown in the changes brought about by using alkaline instead of acid solutions (see Table).

2 : 4-Diaminopteridine	0·1N-Acid λ (mμ)	0·1N-Acid ε	0·1N-Alkali λ (mμ)	0·1N-Alkali ε
7-Hydroxy-6-methyl	333	15,000	340	15,000
7-Hydroxy-6-phenyl	362	19,800	362	19,600
6-Hydroxy-7-methyl	350	7,100	385	6,100
6-Hydroxy-7-phenyl	380	17,400	409	11,200

The long wave-length band undergoes little change in the series of 7-aminopteridines, with a change of substituents in the 2-, 4-, or 6-position but the introduction of a 2-hydroxy- or 2-thio-group causes a marked change in the short wave-length band. It is also the short wave-length band which is most affected by a change of solvent: the long wave-length band is only shifted to a longer wave-length in changing from acid to alkaline solution but is relatively unchanged in intensity.

The spectrum of the alkali-insoluble sulphur-free material from the condensation of 4 : 6-diamino-5-nitroso-2-thiopyrimidine and phenylacetonitrile, in acid solution more closely resembles the spectra of 4 : 7-diamino-6-phenylpteridines with amino-, hydrogen, or 2-methylthio-substituents in the 2 position than those with a hydroxyl or thio-group in this position. Combined with the analytical figures the spectral data given in the first Table indicate that this compound is 4 : 7-diamino-2-ethoxy-6-phenylpteridine.

Experimental

M. p.s were determined in an electrically heated copper block. Absorption spectra were measured with a Beckman spectrophotometer. Fluorescence was observed in ultra-violet light from a Hanovia lamp. Analyses of compounds marked * are tabulated below. Analytical samples were dried *in vacuo* at temperatures stated in parentheses.

4-Chlorobenzyl Bromide.—*p*-Chlorotoluene (31·6 g.) was dissolved in dry carbon tetrachloride (150 c.c.), finely powdered *N*-bromosuccinimide (53·5 g.) and benzoyl peroxide (0·1 g.) were added, and the mixture was refluxed for 5—6 hr. The succinimide was filtered off and washed

2 : 4 : 7-*Triamino*-6-*arylpteridines* (III; R = R′ = NH_2).

R″	Cryst. form and solvent *		M. p.†	Formula	Found (%)‡ C	Found (%)‡ H	Found (%)‡ N	Required (%) C	Required (%) H	Required (%) N
Ph	Yellow plates	B	316°	$C_{12}H_{11}N_7$	57·2	4·7	39·5	56·9	4·4	38·7
p-NO_2·C_6H_4	Orange prisms	F	356—358	$C_{12}H_{10}O_2N_8$	48·3	3·8	37·2	48·3	3·4	37·6
p-MeO·C_6H_4	Yellow plates	A	328	$C_{13}H_{13}ON_7$	55·6	4·9	34·6	55·1	4·6	34·6
p-EtO·C_6H_4	Yellow needles	A	348—350	$C_{14}H_{15}ON_7$	56·4	4·9	32·65	56·6	5·09	33·0
m-MeO·C_6H_4	Yellow needles	F	330	$C_{13}H_{13}ON_7$	55·4	5·0	35·1	55·1	4·6	34·6
m-EtO·C_6H_4	Yellow needles	A	310—312	$C_{14}H_{15}ON_7$	56·3	4·9	32·9	56·6	5·1	33·0
o-MeO·C_6H_4	Orange-yellow rods	P	334	$C_{13}H_{13}ON_7$	54·4	4·3	36·0	55·1	4·6	34·6
o-EtO·C_6H_4	Yellow prisms	P	308	$C_{14}H_{15}ON_7$	56·7	5·3	33·8	56·6	5·1	33·0
p-Cl·C_6H_4	Lemon-yellow prisms	A	378—380	$C_{12}H_{10}N_7Cl$	50·2	3·5	34·0	50·5	3·5	34·1
m-Cl·C_6H_4	Yellow prisms	A	353	$C_{12}H_{10}N_7Cl$	50·3	4·1	34·3	50·5	3·5	34·1
o-Cl·C_6H_4	Pale yellow rods	DA	342	$C_{12}H_{10}N_7Cl$	50·1	4·2	34·1	50·5	3·5	34·1
p-F·C_6H_4	Yellow needles	A	362	$C_{12}H_{10}N_7F$	53·2	4·5	34·9	53·1	3·7	36·2
m-NO_2·C_6H_4	Yellow needles	F	360	$C_{12}H_{10}O_2N_8$	48·5	5·0	37·75	48·3	3·4	37·6
α-Thienyl	Deep yellow prisms	A	356—358	$C_{10}H_9N_7S$	46·7	3·8	37·8	46·3	3·5	37·8
α-Naphthyl	Yellow needles	A	384	$C_{16}H_{13}N_7$	63·7	4·5	32·0	63·4	4·3	32·3

* Solvents were: B, *n*-butanol; A, glacial acetic acid; DA, aqueous dimethylformamide; F, 80% formic acid; P, precipitation from a hot dilute acid solution with dilute ammonia; D, dimethyl formamide; E, ethanol.

† With decomp., except the first. ‡ Dried at 170° *in vacuo*.

with a little dry carbon tetrachloride. The solvent was evaporated from the combined filtrate and washings, and the residue was distilled *in vacuo*, 4-chlorobenzyl bromide (37·25 g.), b. p. 122—126°/20 mm., being obtained.

3-Chlorobenzyl bromide, b. p. 115—119°/20 mm., and 3-nitrobenzyl bromide, m. p. 55°, were obtained in good yield by the same method.

2 : 4 : 7-*Triamino*-6-*phenylpteridine.*—Sodium (0·45 g.) was dissolved in hot dry 2-ethoxyethanol (180 c.c.), and 2 : 4 : 6-triamino-5-nitrosopyrimidine (2·25 g.) was added, followed by redistilled phenylacetonitrile (2·1 g.). After 2 hours' refluxing the dark brown solution, which showed an intense blue fluorescence in ultra-violet light, was evaporated to dryness *in vacuo*, the

residue dissolved in hot 10% hydrochloric acid, and the solution treated with charcoal and filtered. The yellow filtrate was neutralised with 10% aqueous ammonia and allowed to cool, and the yellow base collected. Several crystallisations from *n*-butanol gave 2 : 4 : 7-triamino-6-phenylpteridine * (2 g., 55%) as yellow plates, m. p. 316°.

2 : 4 : 7-*Triamino*-6-p-*nitrophenylpteridine*.—2 : 4 : 6-Triamino-5-nitrosopyrimidine (0·75 g.), freshly fused sodium acetate (0·5 g.), and *p*-nitrophenylacetonitrile (0·9 g.) were refluxed in glacial acetic acid (40 c.c.) for 20 hr. The solution was filtered hot and, on cooling, gave a mixture apparently containing unchanged nitroso-compound. The product, on trituration with 10% hydrochloric acid, left a yellow solid, which was extracted with 10% ammonia solution. Crystallisation from 80% formic acid gave 2 : 4 : 7-triamino-6-*p*-nitrophenylpteridine * (0·85 g.) as orange prisms, m. p. 356—358° (decomp.).

Other 2 : 4 : 7-*Triamino*-6-*arylpteridines* (III; R = R′ = NH_2).—Sodium (0·01 mol.) was dissolved in dry 2-ethoxyethanol (60 c.c.), and 2 : 4 : 6-triamino-5-nitrosopyrimidine (1·50 g.) and the appropriate arylacetonitrile (0·012 mol.) were added to the hot solution which was then refluxed for 1—2 hr. At this stage the pteridine was usually precipitated and the mixture was cooled and filtered, and the product recrystallised from the appropriate solvent. The properties and analyses of these compounds are tabulated (p. 2891).

2 : 4 : 7-*Triacetamido*-6-*arylpteridines*.—These derivatives were prepared by refluxing the base with acetic anhydride and are described in the annexed Table.

Acetyl derivatives.

	Cryst. form and solvent *	M. p.	Formula	Found (%) N	Required (%) N
2 : 4 : 7-*Triacetamidopteridine derivative*					
6-Phenyl	Lemon-yellow needles A	282—284°	$C_{18}H_{17}O_3N_7$	26·1	25·85
6-*p*-Nitrophenyl	Yellow needles D	315—316	$C_{18}H_{16}O_5N_8$	26·6	26·4
6-*p*-Methoxyphenyl	Yellow needles D	272	$C_{19}H_{19}O_4N_7$	24·3	23·95
6-*p*-Ethoxyphenyl	Yellow needles D	230	$C_{20}H_{21}O_4N_7$	23·7	23·2
6-*p*-Chlorophenyl	Yellow prisms D	350	$C_{18}H_{16}O_3N_7Cl$	24·0	23·7
4 : 7-*Diacetamido*-6-*phenylpteridines*					
2-Methylthio-6-phenyl	Yellow needles E	230	$C_{17}H_{16}O_2N_6S$	22·7	22·8
2-Ethylthio-6-phenyl	Yellow needles E	209—210	$C_{18}H_{18}O_2N_6S$	21·2	22·0

* Footnote as preceding Table.

4 : 7-*Diamino*-2-*methylthio*-6-*phenylpteridine*.—4 : 6-Diamino-2-methylthio-5-nitrosopyrimidine (0·5 g.) and phenylacetonitrile (0·35 g.) were added to a solution of sodium (0·1 g.) in dry ethanol (60 c.c.), and the mixture was refluxed for 1 hr. When separation of the crystalline solid was complete the mixture was cooled, the solid collected, dissolved in cold 5% hydrochloric acid, and the solution treated with charcoal and neutralised with 2N-ammonia. The dried precipitate after several crystallisations from *n*-butanol gave pale yellow plates of 4 : 7-*diamino*-2-*methylthio*-6-*phenylpteridine*, m. p. 306° (pale yellow needles were obtained if the solution cooled slowly) [Found (135°): C, 55·2; H, 4·16; N, 29·6. $C_{13}H_{12}N_6S$ requires C, 55·0; H, 4·23; N, 29·5%].

4 : 7-*Diamino*-2-*ethylthio*-6-*phenylpteridine*.—4 : 6-Diamino-2-ethylthio-5-nitrosopyrimidine (0·5 g.) and phenylacetonitrile (0·35 g.) were condensed in ethanol (60 c.c.) containing dissolved sodium (0·1 g.) as above, and the product, after purification by dissolution in 5% hydrochloric acid and precipitation with dilute ammonia solution, crystallised from *n*-butanol in lemon-yellow plates, m. p. 272—274° [Found (135°): C, 56·7; H, 4·8; N, 27·85. $C_{14}H_{14}N_6S$ requires C, 56·4; H, 4·7; N, 28·2%].

2 : 7-*Diamino*-4-*hydroxy*-6-*phenylpteridine*.—To a solution from sodium (0·5 g.) in dry ethylene glycol (45 c.c.) were added 2 : 4-diamino-6-hydroxy-5-nitrosopyrimidine (0·75 g.) and phenylacetonitrile (0·7 g.), and the mixture was refluxed for 7 hr. The solvent was removed *in vacuo*, the residue dissolved in hot water, and the solution treated with charcoal and neutralised with acetic acid, yielding a brown amorphous precipitate. Crystallised from 10% hydrochloric acid (charcoal), this gave the *hydrochloride* (0·3 g.). Two further crystallisations from 2% hydrochloric acid gave a pure specimen as light buff needles [Found (170°): C, 49·3; H, 4·35; N, 29·0. $C_{12}H_{10}ON_6$,HCl requires C, 49·6; H, 3·8; N, 29·0%].

The *base*, prepared from the pure hydrochloride by treatment with hot dilute ammonia

solution, was purified by precipitation from hot 0·1N-sodium hydroxide with N-acetic acid as a buff solid which decomposed above 350° [Found (170°): C, 53·5; H, 5·1; N, 31·0. $C_{12}H_{10}ON_6,H_2O$ requires C, 52·9; H, 4·45; N, 30·8%]. Longer drying then 3 hr. gave variable analytical results indicating 0—1 mol. of water of crystallisation.

7-Amino-4-hydroxy-2-methyl-6-phenylpteridine.—When 4-amino-6-hydroxy-2-methyl-5-nitrosopyrimidine and phenylacetonitrile were condensed in 2-ethoxyethanol containing sodium 2-ethoxyethoxide, a colourless solid was obtained. Crystallisation from 2% hydrochloric acid gave needles of the *hydrochloride* [Found (100°): C, 53·3; H, 4·4; N, 23·9. $C_{13}H_{11}ON_5,HCl$ requires C, 53·9; H, 4·18; N, 24·2%].

The *base*, prepared by dissolving the hydrochloride in hot 0·1N-sodium hydroxide and adding the theoretical amount of N-acetic acid, separated as colourless plates, which decomposed above 350° [Found (135°): C, 61·6; H, 4·4; N, 27·9. $C_{15}H_{11}ON_5$ requires C, 61·6; H, 4·4; N, 27·7%].

4 : 7-Diamino-6-phenylpteridine.—The crude product from the reaction of 4 : 6-diamino-5-nitrosopyrimidine with phenylacetonitrile was purified by dissolution in acid and precipitation with alkali. 4 : 7-*Diamino*-6-*phenylpteridine* crystallised from *n*-butanol in pale yellow prisms, m. p. 340° (decomp.) [Found (135°): C, 60·5; H, 4·4; N, 35·1. $C_{12}H_{10}N_6$ requires C, 60·5; H, 4·25; N, 36·3%].

7-Amino-4-hydroxy-6-phenylpteridine, similarly prepared from 4-amino-6-hydroxy-5-nitrosopyrimidine and purified by dissolving it in 0·1N-sodium hydroxide and adding the theoretical quantity of 0·1N-acetic acid to the boiling solution, separated as buff plates which decomposed without melting above 350° [Found (170°): C, 60·2; H, 4·1; N, 30·4. $C_{12}H_9ON_5$ requires C, 60·2; H, 3·8; N, 29·3%].

7-Amino-4-hydroxy-2 : 6-diphenylpteridine.—4-Amino-6-hydroxy-5-nitroso-2-phenylpyrimidine (0·54 g.) and phenylacetonitrile (0·35 g.) were condensed in 2-ethoxyethanol (60 c.c.) containing dissolved sodium (0·13 g.). The solid (0·35 g.) obtained by evaporation to dryness *in vacuo* and trituration with 10% acetic acid was dissolved in 5% potassium hydroxide solution and neutralised with dilute acetic acid, whereupon 7-*amino*-4-*hydroxy*-2 : 6-*diphenylpteridine* separated as a pale yellow solid which rapidly decomposed above 400° [Found (170° over P_2O_5): C, 67·2; H, 4·25; N, 22·1. $C_{18}H_{13}ON_5$ requires C, 67·3; H, 4·1; N, 21·8%].

4 : 7-Diamino-2-mercapto-6-phenylpteridine.—A solution of 4 : 6-diamino-5-nitroso-2-thiopyrimidine (0·84 g.) and phenylacetonitrile (0·7 g.) in ethanol (150 c.c.) in which sodium (0·23 g.) had been dissolved was refluxed for 4 hr., evaporated to small bulk, diluted with one volume of water, and cooled. The precipitated alkali-insoluble material A (0·2 g.) was purified by dissolution in hot 10% hydrochloric acid solution, treatment with charcoal, and neutralisation of the hot filtrate with ammonia, followed by several crystallisations from *n*-butanol from which it separated as pale yellow prisms, m. p. 290° (decomp.) [Found (135°): C, 59·3; H, 4·6; N, 30·2. 4 : 7-*Diamino*-2-*ethoxy*-6-*phenylpteridine*, $C_{14}H_{14}ON_6$, requires C, 59·6; H, 5·0; N, 29·8%]. Solutions of this substance had an intense blue fluorescence in ultra-violet light.

The filtrate from A was treated with charcoal when hot, and neutralised with dilute acetic acid. The yellow precipitate was dissolved in hot 0·2N-sodium hydroxide solution; the solution was filtered and neutralised with the theoretical amount of dilute acetic acid. After cooling, the yellow precipitate was dissolved in hot 10% hydrochloric acid and this was neutralised with dilute ammonia solution, to yield 4 : 7-*diamino*-2-*mercapto*-6-*phenylpteridine* (0·65 g.) as deep yellow prisms, which decomposed above 310° [Found (170° over P_2O_5): C, 53·5; H, 4·0; N, 31·1. $C_{12}H_{10}N_6S$ requires C, 53·3; H, 3·7; N, 31·1%]. This substance has a dull blue fluorescence in both acid and alkaline solution under ultra-violet light.

4 : 7-Diamino-2-hydroxy-6-phenylpteridine.—To a solution of sodium (0·23 g.) in ethylene glycol (60 c.c.) was added 4 : 6-diamino-2-hydroxy-5-nitrosopyrimidine and phenylacetonitrile (0·7 g.), the mixture was refluxed for 1 hr. and the solvent removed *in vacuo*. The residue was dissolved and purified by alkali, charcoal, and acid as usual, then dissolved in a little 100% formic acid, diluted with one volume of water, treated with charcoal, and neutralised with dilute ammonia solution when still hot. The precipitated solid was further purified by dissolution in 0·1N-sodium hydroxide and reprecipitation with the theoretical quantity of dilute acetic acid solution, yielding 4 : 7-*diamino*-2-*hydroxy*-6-*phenylpteridine* as a yellow solid [Found (170° over P_2O_5): N, 32·4. $C_{12}H_{10}ON_6$ requires N, 33·1%]. The ultra-violet absorption spectrum of this material showed its identity with the pteridine obtained by the dilute acid hydrolysis of 4 : 7-diamino-2-methylthio-6-phenylpteridine.

2 : 4-Diamino-7-hydroxy-6-phenylpteridine.—2 : 4 : 6-Triamino-5-nitrosopyrimidine (1 g.) was stirred with phenylacetyl chloride (10 c.c.) and heated to 140°, reaction being complete

when no more hydrogen chloride was evolved (about 10 min.). The dark brown solution, cooled and diluted with ether, yielded a light brown precipitate which, after being washed with ether, was stirred with hot dilute ammonia solution to liberate the free base. This after several crystallisations from glacial acetic acid gave yellow prisms of 2 : 4-*diamino*-7-*hydroxy*-6-*phenylpteridine* (1 g.), m. p. 406—408° (decomp.) [Found (170° over P_2O_5) : C, 56·5; H, 3·8; N, 33·2. $C_{12}H_{10}ON_6$ requires C, 56·7; H, 4·0; N, 33·1%]. This substance had an intense blue fluorescence under ultra-violet light in both acid and alkaline solution.

2 : 4-*Diamino*-7-*hydroxy*-6-p-*nitrophenylpteridine*.—2 : 4 : 6-Triamino-5-nitrosopyrimidine was intimately ground with *p*-nitrophenylacetyl chloride (5 g.) and slowly heated to 140° as above. After similar treatment the crude base (1·3 g.) was obtained as an orange-brown solid. The solid was repeatedly extracted with hot dilute ammonia solution, the orange solution evaporated to small bulk *in vacuo*, and the orange precipitate crystallised from 80% formic acid, to yield 2 : 4-*diamino*-7-*hydroxy*-6-p-*nitrophenylpteridine* as orange-yellow needles which became deep red at 170° *in vacuo*. The substance slowly decomposed above 400° (Found : C, 47·9; H, 3·4; N, 32·5. $C_{12}H_9O_3N_7$ requires C, 48·2; H, 3·8; N, 32·8%). Solutions of this material in acid or alkali had only a weak green fluorescence in ultra-violet light.

2 : 4-*Diamino*-7-*hydroxy*-6-p-*methoxyphenylpteridine*.—Reaction of 2 : 4 : 6-triamino-5-nitrosopyrimidine (1 g.) and *p*-methoxyphenylacetyl chloride (7 c.c.) at 140° gave 1·3 g. of crude product, purified by extraction with 1 l. of 10% potassium hydroxide solution, acidification of the extract with glacial acetic acid, and several crystallisations from dimethylformamide. 2 : 4-*Diamino*-7-*hydroxy*-6-p-*methoxyphenylpteridine* crystallised as yellow prisms which decomposed above 350° [Found (170° over P_2O_5) : C, 55·3; H, 4·7; N, 29·4. $C_{13}H_{12}O_2N_6$ requires C, 54·9; H, 4·3; N, 29·6%].

Condensation of 2 : 4 : 5 : 6-*Tetra-aminopyrimidine Hydrochloride with Phenylglyoxylic Acid*.—(a) *At* pH 5. 2 : 4 : 5 : 6-Tetra-aminopyrimidine hydrochloride (0·19 g.), sodium acetate (0·45 g.), and phenylglyoxylic acid (0·15 g.), dissolved in warm water (10 c.c.), were refluxed for 10 min. and yielded a precipitate which crystallised from acetic acid as yellow prisms, (0·18 g.), m. p. 406—408° alone or admixed with 2 : 4-diamino-7-hydroxy-6-phenylpteridine obtained by the condensation of 2 : 4 : 6-triamino-5-nitrosopyrimidine and phenylacetyl chloride. The ultra-violet absorption spectra of the samples were identical.

(b) *In acid solution.* 2 : 4 : 5 : 6-Tetra-aminopyrimidine (0·9 g.) and phenylglyoxylic acid (0·7 g.) in 10% hydrochloric acid (25 c.c.) and ethanol (15 c.c.) were refluxed for 1 hr. The precipitate was collected and the filtrate evaporated to dryness; the combined solids (1·25 g.) were extracted with hot 10% ammonia solution (3 × 75 c.c.), to leave 2 : 4-diamino-7-hydroxy-6-phenylpteridine (0·65 g.) which crystallised in yellow prisms, m. p. 406—408°, identical in m. p. and other physical properties with the preceding product. The ammonia extract, on evaporation to small bulk, left a deep orange solid (0·5 g.) which, in contrast to 2 : 4-diamino-7-hydroxy-6-phenylpteridine, had in acid solution a deep green fluorescence in ultra-violet light. This solid, crystallised several times from water, yielded golden needles of 2 : 4-*diamino*-6-*hydroxy*-7-*phenylpteridine* which decomposed above 310° [Found (170° over P_2O_5) : C, 57·0; H, 4·2; N, 32·7. $C_{12}H_{10}ON_6$ requires C, 56·7; H, 4·0; N, 33·1%].

Hydrolysis of 4 : 7-*Diamino*-2-*methylthio*-6-*phenylpteridine*.—The 2-methylthio-compound (0·1 g.) was refluxed with 10% hydrochloric acid (15 c.c.) until no more methanethiol was evolved. The solution was cooled, and the yellow precipitate was collected, dissolved in hot N-sodium hydroxide, treated with charcoal, heated to boiling, and carefully neutralised with dilute acetic acid. 4 : 7-Diamino-2-hydroxy-6-phenylpteridine (80 mg.) separated as a pale yellow powder, decomp. > 320° [Found (170° over P_2O_5) : C, 56·6; H, 4·4; N, 32·5%], having an absorption spectrum identical with that of a synthetic specimen.

Hydrolysis of 7-*Amino*-4-*hydroxy*-2-*methylthio*-6-*phenylpteridine*.—The pteridine (0·1 g.) was boiled with 2N-hydrochloric acid (15 c.c.) until all the methanethiol had been evolved. After cooling, the precipitate was collected and dissolved in hot 0·5N-sodium hydroxide (20 c.c.), and the product purified and isolated as in the preceding experiment, to yield pale yellow rhombs of 7-*amino*-2 : 4-*dihydroxy*-6-*phenylpteridine*, decomp. > 330° [Found (170° over P_2O_5) : C, 56·6; H, 3·0; N, 26·2. $C_{12}H_9O_2N_5$ requires C, 56·6; H, 2·8; N, 27·4%].

Hydrolysis of 2 : 4 : 7-*Triamino*-6-*phenylpteridine*.—The triamine (0·2 g.) was refluxed with 5N-hydrochloric acid (30 c.c.) for 1 hr. and the solid (A) collected from the hot solution. On cooling, another solid (B) separated.

Solid (B) was dissolved in hot 0·1N-sodium hydroxide, treated with charcoal, and neutralised with dilute acetic acid, and the precipitate was extracted with boiling 10% hydrochloric acid leaving very little residue. After cooling to 30° the amorphous precipitate was filtered off and

the filtrate was treated with charcoal. During several hours at room temperature the colourless solution deposited slender needles of 2 : 7-diamino-4-hydroxy-6-phenylpteridine hydrochloride (0·13 g.), identical in ultra-violet absorption with the hydroxypteridine obtained by direct synthesis from 2-amino-4-hydroxy-5-nitrosopyrimidine.

Solid (A), after purification by precipitation from an alkaline solution with acid, was shown to be 2 : 4-diamino-7-hydroxy-6-phenylpteridine by comparison of its ultra-violet absorption spectrum in 0·1N-sodium hydroxide with that of a synthetic specimen (Found: N, 33·1. $C_{12}H_{10}ON_6$ requires N, 33·8%).

When the triamine (0·2 g.) was boiled for several hours with 10% hydrochloric acid, 0·18—0·19 g. of the starting material was recovered. No alkali-soluble material was obtained.

Ethylation of 4 : 7-*Diamino*-2-*mercapto*-6-*phenylpteridine*.—To a solution of sodium (0·05 g.) in ethanol (25 c.c.) was added the mercaptopteridine (0·1 g.), followed by ethyl bromide (0·1 c.c.). The clear yellow solution was refluxed for 2 hr.; the original dull blue fluorescence in ultra-violet light had changed to a sky-blue fluorescence. The mixture was worked up as described for the synthesis of 4 : 7-diamino-2-ethylthio-6-phenylpteridine, yellow plates, m. p. 272°, being obtained. Comparison of the ultra-violet absorption spectrum of this pteridine with that of 4 : 7-diamino-2-ethylthio-6-phenylpteridine confirmed their identity.

Oxidation of 4 : 7-*Diamino*-2-*mercapto*-6-*phenylpteridine*.—To a solution of the 2-mercapto-compound (0·1 g.) in 0·1N-sodium hydroxide (10 c.c.), was added hydrogen peroxide (5 c.c. of 20-vol.), and the mixture kept at room temperature overnight. The pale yellow solution, acidified with acetic acid, yielded a precipitate which was purified by dissolution in dilute sodium hydroxide solution and reprecipitation with acetic acid. The pale yellow microcrystalline solid showed an absorption spectrum identical with that of 4 : 7-diamino-2-hydroxy-6-phenylpteridine prepared by synthesis.

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