Polyazanaphthalenes. Part II. Attempted Synthesis of Some Analogues of Pteroic Acid.*

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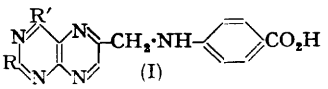
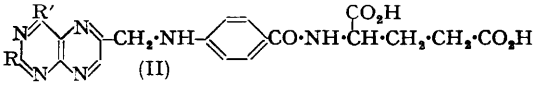
[Reprint Order No. 5930.]

Attempts to prepare a series of analogues of pteroic acid were frustrated by the failure of the standard synthetic methods when applied to 4 : 5-diaminopyrimidines other than 2 : 4 : 5-triamino-6-hydroxy- and 2 : 4 : 5 : 6-tetra-amino-pyrimidine. Unsuccessful attempts were also made to prepare the required analogues from 4-amino-5-nitrosopyrimidines. A number of 7-hydroxy-6-methylpteridines were prepared by condensing 4 : 5-diaminopyrimidines with ethyl oxaloacetate and two of these were converted into 7-hydroxy-derivatives of the required pteroic acid analogues; attempts to remove the 7-hydroxy-group failed. Condensation of 2 : 4 : 5-triamino-6-hydroxypyrimidine with hydroxyiminoacetone in sodium sulphite solution afforded 2 : 6-diamino-4-hydroxy-7-methylpteridine.

THE work described in this paper had as its objective the synthesis of analogues of pteroic acid (I; R = NH_2, R′ = OH) and folic acid (II; R = NH_2, R′ = OH), in which R and R′ were varied; † these two growth factors are over-provided with possible points of attachment to enzyme molecules and it was hoped that correlation of biological activity with variations in R and R′ would yield information as to the participation of the amino- and hydroxy-groups normally present in these positions in attachment of the growth factors to enzymes.



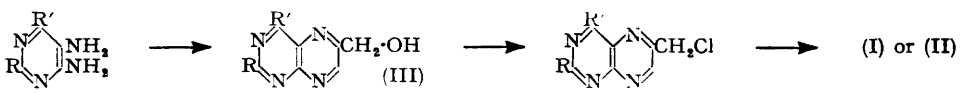
(I)



(II)

No great difficulty was expected in preparing the required analogues by the obvious modification of the method (condensation of 2 : 4 : 5-triamino-6-hydroxypyrimidine with αβ-dibromopropaldehyde and *p*-aminobenzoic or *p*-aminobenzoylglutamic acid) used originally for the synthesis of pteric acid and folic acid (Waller *et al.*, *J. Amer. Chem. Soc.*, **1948**, **70**, 19), since aminopterin (II; R = R′ = NH_2) is satisfactorily prepared by applying this method to 2 : 4 : 5 : 6-tetra-aminopyrimidine (Seeger, Cosulich, Smith, and Hultquist, *ibid.*, 1949, **71**, 1753). However, no pteridine could be isolated from the products obtained by applying this reaction to 4 : 5-diamino-6-hydroxy-, 4 : 5-diamino-6-hydroxy-2-methyl-, 4 : 5 : 6-triamino-2-hydroxy-, and 4 : 5 : 6-triamino-6-mercapto-pyrimidine, even in the presence of sodium dichromate, which has been shown (Boothe *et al.*, *ibid.*, p. 2304) to increase the yield. In the first two cases mentioned, light-absorption data indicated the presence of a small amount of pteridine in the crude product.

Condensation of 2 : 4 : 5-triamino-6-hydroxypyrimidine with dihydroxyacetone in the presence of hydrazine (Forrest and Walker, *J.*, 1949, 79, 2077; Weygand, Wacker, and Schmied-Kowarzik, *Chem. Ber.*, 1949, **82**, 25; Karrer and Schwyzer, *Helv. Chim. Acta*, **1949**, **32**, 423, 1041) leads to the 6-hydroxymethyl compound (III; R = NH_2, R′ = OH), which can be converted into pteroic and folic acid, thus :



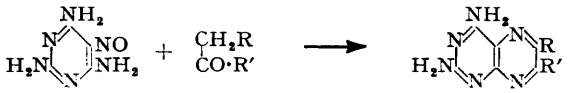
The preparation of pteroic acid by this route is described in B.P. 624,394 and we have used it to prepare pure pteroic acid in excellent yield. In spite of its success with 2 : 4 : 5-triamino-6-hydroxypyrimidine, however, this method failed completely with 4 : 5-diamino-6-hydroxy-, 4 : 5-diamino-6-hydroxy-2-methyl-, 2 : 4 : 5-triamino-, 2 : 4 : 5-triamino-6-methyl-, and 4 : 5 : 6-triamino-2-hydroxy-pyrimidine, none of which yielded any pteridine.

* Part I, Leese and Rydon, *J.*, 1955, 303.

† Since our work was completed, Brown (*J.*, 1953, 1644) has described the synthesis of deaminofolic acid (II; R = H, R′ = OH).

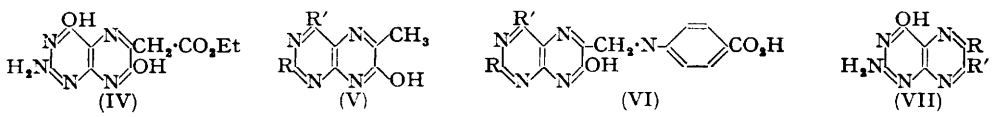
In view of the ready availability of 4-amino-5-nitrosopyrimidines (Landauer and Rydon, *J.*, 1953, 3721) we next investigated two methods requiring these compounds as starting materials. Condensation of dihydroxyacetone with 2 : 4-diamino-6-hydroxy-5-nitrosopyrimidine has been reported (U.S.P. 2,461,503; Swiss P. 253,475) to give pure 2-amino-4-hydroxy-6-hydroxymethylpteridine (III; R = NH_2, R′ = OH); under the conditions we employed, however, the main product was the isomeric 2-amino-4-hydroxy-7-hydroxymethylpteridine and this route was not investigated further.

Timmis (*Nature*, 1949, **164**, 139) synthesised a number of pteridines by condensing 4-amino-5-nitrosopyrimidines with ketones, *e.g.* :



This route is attractive, owing to its apparent lack of ambiguity, and we sought to adapt it to our purposes. However, no pteridine could be isolated from the reaction products of 2 : 4-diamino-6-hydroxy-5-nitrosopyrimidine and propaldehyde diethyl acetal, β-chloropropaldehyde diethyl acetal, or the condensation product of the latter with *p*-aminobenzoic acid; nor was any pteridine produced when 2 : 4-diamino-6-hydroxy-5-nitrosopyrimidine was heated with ethyl sodioacetoacetate, despite the success of the analogous reaction for the preparation of 1 : 4 : 5-triazanaphthalenes (Leese and Rydon, *J.*, 1955, 303).

Tschesche, Köhncke and Korta (*Z. Naturforsch.*, 1950, **5***b*, 132; *Chem. Ber.*, 1951, **84**, 485) condensed 2 : 4 : 5-triamino-6-hydroxypyrimidine with ethyl oxaloacetate and obtained a good yield of the ester (IV), which underwent ready hydrolysis and decarboxylation to 2-amino-4 : 7-dihydroxy-6-methylpteridine (V; R = NH_2, R′ = OH); side-chain bromination of (V), followed by condensation with *p*-aminobenzoylglutamic acid, yielded



7-hydroxyfolic acid. This reaction we have found to be widely applicable to 4 : 5-diaminopyrimidines and we have used it to prepare 4 : 7-dihydroxy- (V; R = H, R′ = OH), 2-amino-7-hydroxy- (V; R = NH_2, R′ = H), 4-amino-7-hydroxy- (V; R = H, R′ = NH_2), 4 : 7-dihydroxy-2-methyl (V; R = Me, R′ = OH), 2-amino-4-methyl- (V; R = NH_2, R′ = Me), 4-amino-2 : 7-dihydroxy- (V; R = OH, R′ = NH_2), and 4-amino-7-hydroxy-2-mercapto- (V; R = SH, R′ = NH_2) -6-methylpteridine. Bromination of the first two of these compounds, followed by condensation with *p*-aminobenzoic acid, afforded " 7-hydroxydeamino-pteroic acid " (VI; R = H, R′ = OH) and " 7-hydroxydeoxy-pteroic acid " (VI; R = NH_2, R′ = H), respectively, but attempts to remove the 7-hydroxy-group from these compounds by reduction or by way of the derived 7-halogeno-compounds were unsuccessful.

Seeger *et al.* (*loc. cit.*) showed that the condensation of pyruvic aldehyde with 2 : 4 : 5-triamino-6-hydroxy- and 2 : 4 : 5 : 6-tetra-amino-pyrimidine was sensitive to the reaction conditions, the 7-methyl compound (VII; R = H, R′ = Me) being formed in acid and the 6-methyl compound (VII; R = Me, R′ = H) in sodium sulphite solution. In an attempt to avoid contamination arising from impurities present in commercial pyruvaldehyde, we condensed 2 : 4 : 5-triamino-6-hydroxypyrimidine with hydroxyiminoacetone in saturated aqueous sodium sulphite. Analysis showed the beautifully crystalline product, obtained in excellent yield, to be, not the expected 2-amino-4-hydroxy-6-methylpteridine (VII; R = Me, R′ = H), but a diamino-hydroxy-methylpteridine, clearly (VII; R = Me, R′ = NH_2 or *vice versa*); the yield is much reduced if more dilute sodium sulphite is used and no condensation occurs in glacial acetic acid. Deamination by nitrous acid afforded a trihydroxy-methyl-pteridine; this is not identical with 2 : 4 : 7-trihydroxy-6-methylpteridine, obtained by similar deamination of 2-amino-4 : 7-dihydroxy-6-methylpteridine (VII; R = Me, R′ = OH) prepared by the method of Tschesche *et al.* (*loc. cit.*;

cf. Elion, Hitchings, and Russell, *J. Amer. Chem. Soc.*, 1950, **72**, 78); it is thus clearly 2 : 4 : 6-trihydroxy-7-methylpteridine. The condensation product from 2 : 4 : 5-triamino-6-hydroxypyrimidine and hydroxyiminoacetone is thus clearly 2 : 6-diamino-4-hydroxy-7-methylpteridine (VII; R = NH_2, R′ = Me); the condensation, which fails with 2 : 4-diamino-6-hydroxypyrimidine, possibly involves the initial formation of 2-amino-4-hydroxy-7-methyl-6-hydroxyaminopteridine (VII; R = NH·OH, R′ = Me), which is then reduced.

Modified methods for the preparation of 2 : 4 : 5-triamino- and 2 : 4 : 5-triamino-6-methyl-pyrimidine are described below.

Experimental

Light-absorption data are wave-lengths in mμ for maxima (inflections are italicised) and are followed by ε (in parentheses). All pteridines were dried *in vacuo* at 100—120° before analysis.

Condensation of 2 : 4-*Diamino*-6-*hydroxy*-5-*nitrosopyrimidine with Dihydroxyacetone.*—To a solution of the pyrimidine (0·75 g.) in N-sodium hydroxide (10 ml.), dihydroxyacetone dimer was added and the mixture boiled for 2 min. The product, which separated as a brown solid (0·35 g.) on acidification with acetic acid, was oxidised with alkaline permanganate (Forrest and Walker, *J.*, 1949, 2077); the oxidation product was almost entirely 2-amino-4-hydroxypteridine-7-carboxylic acid, identified by the bright green fluorescence of its sodium salt in aqueous solution; only a trace of the blue-fluorescing 6-carboxylic acid was present.

7-*Hydroxypteridines and* 7-*Hydroxypteroic Acids.*—(a) 4-*Hydroxy-compounds.* 4 : 5-Diamino-6-hydroxypyrimidine (2·5 g.), ethyl sodio-oxaloacetate (4·5 g.), and acetic acid (20 ml.) were heated on a boiling water-bath for 1 hr. Water (20 ml.) was then added and the heating continued for a further 30 min. The mustard-yellow solid (3·1 g., 70%) was filtered off and a portion purified by dissolution in hot dilute sodium hydroxide solution and acidification after boiling with charcoal; 4 : 7-*dihydroxy*-6-*methylpteridine hydrate* (V; R = H, R′ = OH) forms peach-coloured needles, m. p. 345° (decomp.) (Found: C, 43·5; H, 4·15; N, 27·8. $C_7H_6O_2N_4,H_2O$ requires C, 42·85; H, 4·1; N, 28·6%). Light absorption in 0·1N-NaOH: 227 (23,140), 327 (11,200).

Bromine (0·15 ml.) was added dropwise to a solution of this pteridine (0·49 g.) in formic acid (20 ml.), and the mixture kept overnight at room temperature. After removal of hydrogen bromide by partial evaporation under reduced pressure in a stream of nitrogen, *p*-aminobenzoic acid (0·7 g.) in formic acid (10 ml.) was added, and the mixture kept for a further 48 hr. Filtration, followed by dilution with water (to 400 ml.), led to precipitation of the pteroic acid (0·7 g.). This was dissolved in the minimum amount of aqueous ammonia (*d* 0·880), diluted with boiling water to 1200 ml., decolorised with charcoal, and precipitated from the hot solution by acidification (2N-hydrochloric acid) to pH 5. p-N-(4 : 7-*Dihydroxy*-6-*pteridylmethyl*)*aminobenzoic acid* ("7-*hydroxy-deamino-pteroic acid*") (VI; R = H, R′ = OH) (0·2 g., 25%), so obtained was a yellow crystalline solid, m. p. >360° (Found: N, 21·6. $C_{14}H_{11}O_4N_5$ requires N, 22·4%). Light absorption in 0·1N-NaOH: 251 (16,270), 257 (16,270), 280 (19,270), 290 (19,270), 324 (17,210).

(b) 2-*Amino-compounds.* Similar condensation of 2 : 4 : 5-triaminopyrimidine sulphate (1·0 g.) and ethyl sodio-oxaloacetate (1·2 g.) in acetic acid (8 ml.) afforded 2-*amino*-7-*hydroxy*-6-*methylpteridine* (0·5 g., 62%) (V; R = NH_2, R′ = H), buff-coloured crystals, m. p. >360° (Found: C, 47·6; H, 4·2; N, 39·8. $C_7H_7ON_5$ requires C, 47·5; H, 3·95; N, 39·5%). Light absorption in 0·1N-NaOH: 227 (20,180), 342 (18,580). Bromination and condensation with *p*-aminobenzoic acid, as for the 4-hydroxy-compound, yielded p-N-(2-*amino*-7-*hydroxy*-6-*pteridylmethyl*)*aminobenzoic acid* ("7-*hydroxy-deoxy-pteroic acid*") (VI; R = NH_2, R′ = H), as a brown powder, m. p. >360° (Found: N, 25·3. $C_{14}H_{12}O_3N_6$ requires N, 26·9%). Light absorption in 0·1N-NaOH: 228 (27,770), 251 (10,290), 268 (13,730), 280 (13,730), 346 (17,780).

(c) 4-*Amino*-7-*hydroxy*-6-*methylpteridine* (with Maxwell Gordon). Similar condensation of 4 : 5 : 6-triaminopyrimidine sulphate (4·35 g.) with ethyl sodio-oxaloacetate (5·0 g.) in acetic acid (60 ml.) afforded the *pteridine* (V; R = H, R′ = NH_2) (2·5 g., 70%) as pale pink crystals, m. p. >360° (Found: C, 46·7; H, 4·6. $C_7H_7ON_5$ requires C, 47·5; H, 3·95%). Light absorption, at pH 2: 292 (7370), 327 (7530). At pH 7: *282—291*, 336 (7940)).

(d) 4 : 7-*Dihydroxy*-2 : 6-*dimethylpteridine.* 4 : 5-Diamino-6-hydroxy-2-methylpyrimidine sulphate (0·6 g.) and ethyl sodio-oxaloacetate (0·6 g.) were heated at 100° for 2 hr. in acetic acid (7 ml.). Water (7 ml.) was then added and the mixture heated at 100° for a further 30 min. Concentration under reduced pressure and cooling to 0° afforded a yellow solid (0·45 g., 75%),

which was dissolved in 2N-sodium hydroxide (20 ml.); the solution was heated at 100° for 30 min. and acidified with 2N-hydrochloric acid. On cooling in ice, the *pteridine* (V; R = Me, R′ = OH) (0·23 g.) separated (Found: C, 49·4; H, 3·3; N, 29·5. $C_8H_8O_2N_4$ requires C, 50·0; H, 4·2; N, 29·2%). Light absorption in 0·1N-NaOH: 227 (41,180), 328 (13,310).

(e) 2-*Amino*-7-*hydroxy*-4: 6-*dimethylpteridine*. Condensation of 2: 4: 5-triamino-6-methylpyrimidine (0·4 g.) with ethyl sodio-oxaloacetate (0·8 g.) in acetic acid (7 ml.) yielded the *pteridine* (V; R = NH_2, R′ = Me) (0·25 g., 46%) as a cream-coloured powder (Found: C, 50·35; H, 5·0; N, 36·8. $C_8H_9ON_5$ requires C, 50·3; H, 4·7; N, 36·65%). Light absorption in 0·1N-NaOH: 264 (7640), 343 (16,230).

(f) 4-*Amino*-2: 7-*dihydroxy*-6-*methylpteridine* (with MAXWELL GORDON). Condensation of 4: 5: 6-triamino-2-hydroxypyrimidine sulphate (2·15 g.) with ethyl sodio-oxaloacetate (2·5 g.) in acetic acid (30 ml.) afforded the *pteridine* (V; R = OH, R′ = NH_2) (1·9 g., 90%) as an ivory powder (Found: C, 43·8; H, 4·5. $C_7H_7O_2N_5$ requires C, 43·5; H, 3·1%). Light absorption, at pH 2: 272 (15,400), 330 (6030). At pH 9: 272 (12,460), 336 (6830). At pH 13: 254 (8800), 339 (10,400).

(g) 4-*Amino*-7-*hydroxy*-2-*mercapto*-6-*methylpteridine* (with MAXWELL GORDON). Condensation of 4: 5: 6-triamino-2-mercaptopyrimidine hydrochloride (3·7 g.) with ethyl sodio-oxaloacetate (5·0 g.) in acetic acid (60 ml.) afforded the *pteridine* (V; R = SH, R′ = NH_2) (1·49 g., 40%) (Found: C, 40·35; H, 3·50. $C_7H_7ON_5S$ requires C, 40·2; H, 3·4%). Light absorption at pH 2: 261 (10,300), 349 (14,000).

2: 6-*Diamino*-4-*hydroxy*-7-*methylpteridine and its Derivatives*.—2: 4: 5-Triamino-6-hydroxypyrimidine sulphate (6 g., 0·025 mole) was dissolved by gentle warming in a solution of sodium sulphite (60 g.) in water (200 ml.); the solution was cooled to 30° and hydroxyiminoacetone (2·5 g.) added. A yellow precipitate separated after 15 min. and was collected by filtration (1·5 g.) after the mixture had been kept at room temperature overnight; further crops were collected at intervals over a week. The combined products were washed well with water, ethanol, and ether; the total yield was 3·4 g. (70%). 2: 6-*Diamino*-4-*hydroxy*-7-*methylpteridine* (VII; R = NH_2, R′ = Me) crystallised from boiling water in golden leaflets, m. p. >360° (Found: C, 43·8; H, 4·3; N, 43·35. $C_7H_8ON_6$ requires C, 43·75; H, 4·2; N, 43·75%). Light absorption in 0·1N-NaOH: 257 (16,130), 378 (7680).

This pteridine (0·40 g.) was dissolved at 0° in concentrated sulphuric acid (12 ml.) and water (2 ml.) and treated at 0° with sodium nitrite (1·2 g.) in concentrated sulphuric acid (8 ml.). The mixture was kept at room temperature for 10 min. and then poured on ice; the yellow solid, which separated with vigorous evolution of nitrogen, was filtered off after 2 hr. and washed with water, ethanol, and ether. The product (0·20 g.) was suspended in boiling water (70 ml.) and treated dropwise with 2N-sodium hydroxide until a clear solution was obtained; this was boiled with charcoal, filtered, and treated, while boiling, with 2N-hydrochloric acid until cloudy. 2: 4: 6-*Trihydroxy*-7-*methylpteridine* separated overnight as bright yellow crystals, m. p. >360° (Found: N, 28·55. $C_7H_6O_3N_4$ requires N, 28·35%). Light absorption in 0·1N-NaOH: 227 (18,430), 280 (7760), 290 (7760), 343 (10,670).

Similar deamination of 2-amino-4: 7-dihydroxy-6-methylpteridine afforded 2: 4: 7-*trihydroxy*-6-*methylpteridine* as a cream-coloured solid, m. p. >300° (Found: N, 27·9. $C_7H_6O_3N_4$ requires N, 28·35%). Light absorption in 0·1N-NaOH: 264 (6210), 330 (12,220).

2: 4: 5-*Triaminopyrimidine*.—Isay's method (*Ber.*, 1906, **39**, 250) was modified as follows (but cf. Albert, Brown, and Cheeseman, *J.*, 1951, 474): 2: 4-Dichloro-5-nitropyrimidine (3·7 g.) (Whittaker, *J.*, 1951, 1565) was heated for 3 hr. in an autoclave at 100° with ammonia (6 g.) in ethanol (30 ml.). The product was filtered off and recrystallised from boiling water; the yield of 2: 4-diamino-5-nitropyrimidine, m. p. 355°, was 2·4 g. (82%). This compound (1·3 g.), in ethanol (130 ml.), was hydrogenated at 100°/120 atm. over Raney nickel for 3 hr. Filtration and addition of the theoretical amount of ethanolic sulphuric acid yielded 2: 4: 5-triaminopyrimidine sulphate, m. p. 130° (1·2 g., 66%).

2: 4: 5-*Triamino*-6-*methylpyrimidine*.—The method of Gabriel and Colman (*Ber.*, 1901, **34**, 1234) was modified as follows: 2: 4-Dihydroxy-6-methyl-5-nitropyrimidine (11·5 g.) (Osten, *Annalen*, 1905, **343**, 137) was refluxed for 3 hr. with phosphorus oxychloride (60 ml.) and dimethylaniline (15 ml.). The resulting solution was concentrated under reduced pressure, poured on ice, and extracted with ether; distillation of the extract, after it had been washed with sodium hydrogen carbonate solution, and dried (Na_2SO_4), afforded 2: 4-dichloro-6-methyl-5-nitropyrimidine (9·4 g., 67%), b. p. 78°/0·8 mm., m. p. 51°. This was heated in an autoclave at 100° for 3 hr. with ammonia (8 g.) in ethanol (30 ml.). Filtration and recrystallisation from water (800 ml.) afforded 2: 4-diamino-6-methyl-5-nitropyrimidine (4·5 g., 60%), m. p. 232°,

which was finally hydrogenated in ethanol over Raney nickel at 100°/120 atm. to 2 : 4 : 5-triamino-6-methylpteridine, in 62% yield, m. p. 242° (lit., m. p. 243°).

We thank the Ministry of Education and the Department of Scientific and Industrial Research for Maintenance Allowances (to P. D. L.). We also thank Dr. Maxwell Gordon, in receipt of a grant from the American Cancer Society, who carried out the experiments indicated and also a number of exploratory experiments. The microanalyses were carried out in the microanalytical laboratory of this Department (Mr. F. H. Oliver); the light absorption data were determined by Mrs. A. I. Boston. The work was begun at Birkbeck College, London.

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