398. *The Use of Nitro- and Halogeno-ketones in the Synthesis of Pteridines, including Pteroic Acid, from 2 : 4 : 5-Triamino-6-hydroxypyrimidine.*

By F. E. KING and P. C. SPENSLEY.

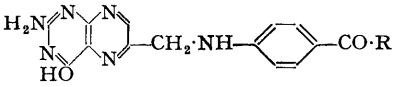
2 : 4 : 5-Triamino-6-hydroxypyrimidine (I) combines with both ω-nitroacetophenone and phenylglyoxal to give 2-amino-4-hydroxy-7-phenylpteridine, and forms the isomeric 6-phenylpteridine with ωω-dichloroacetophenone. The products were differentiated by alkaline hydrolysis to the respective 2-hydroxy-6-phenyl- and 2-hydroxy-5-phenyl-pyrazine-3-carboxylic acids.

Details are given of the synthesis, already briefly reported (*Nature*, 1949, **164**, 574), of pteroic acid from the pyrimidine (I), *p*-aminobenzoic acid, and 3-bromo-1 : 1-dichloroacetone, and of its extension to pteroyl-L-glutamic acid (cf. Hultquist and Dreisbach, *Chem. Abs.*, 1949, **42**, 7944, and *Ann. Reports*, 1950, **47**, 243, for other references). An attempt to prepare pteroic acid from the pyrimidine (I) and *p*-(3-hydroxy-2-nitroallylideneamino)benzoic acid, resulted in the extrusion of *p*-aminobenzoic acid and the formation of an analogous pyrimidine Schiff's base.

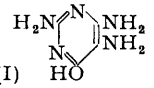
IN connexion with a projected synthesis of pteroic acid from 2 : 4 : 5-triamino-6-hydroxypyrimidine (I), *p*-aminobenzoic acid, and nitromalondialdehyde (II; R = NO_2), analogous to that in which the required three-carbon intermediate is reductone (II; R = OH) (Angier *et al.*, *J. Amer. Chem. Soc.*, 1948, **70**, 25; Forrest and Walker, *J.*, 1949, 2002), we have investigated the condensation of 4 : 5-diaminopyrimidines with α-nitro-carbonyl compounds. As potentially the simplest member of the series, methazonic acid, $NO_2{\cdot}CH_2{\cdot}CH{:}N{\cdot}OH$, was at first the subject of these experiments, and its readily available sodium salt was observed to undergo an easy reaction with the pyrimidine (I) " bisulphite " described by Cain, Mallette, and Taylor (*J. Amer. Chem. Soc.*, 1946, **68**, 1996). The resulting compound was shown by analysis and its rapid hydrolysis with acid to be a Schiff's base, and, in view also of the greater electron-availability at the pyrimidine 5-amino-group under the conditions of pH pertaining to the condensation, the product was at first regarded as 2 : 4-diamino-6-hydroxy-5-2′-nitroethylideneaminopyrimidine. However, its positive response to the Folin–Denis test for 5-aminopyrimidines (*J. Biol. Chem.*, 1912, **12**, 239; Johnson and Johns, *J. Amer. Chem. Soc.*, 1914, **36**, 970) appears to exclude the pyrimidine-5-anil structure and to favour the alternatives (III; R = H) or that in which the pyrimidine 2-position is involved. Attempts to complete ring-closure by reduction methods on the assumption that (III; R = H) correctly represents the constitution of the condensation product invariably gave intractable products, but the strong blue fluorescence of their alkaline solutions implied at least partial formation of the pteridine ring.

The investigation was therefore transferred from methazonic acid to the more stable ω-nitroacetophenone which readily combined with the pyrimidine (I) bisulphite in aqueous

alcoholic solution to give a product of pteridine nature. Of the two isomers theoretically obtainable in this reaction, namely (IV) and (V), evidence is adduced (below, and forthcoming publication, F. E. King and B. K. Martin) which identifies the new compound as 2-amino-4-hydroxy-7-phenylpteridine (IV). Pteridine formation also occurred on com-

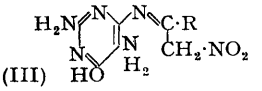


[Pteroic acid; R = OH]
[Pteroylglutamic acid; R = HN·CH(CO$_2$H)·CH$_2$·CH$_2$·CO$_2$H]

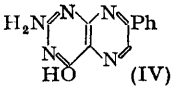


(I)

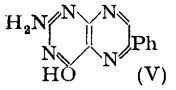
(II) HO·CH:CR·CHO



(III)



(IV)



(V)

bination of the two reagents in aqueous acetic acid, but the reaction failed in aqueous-alcoholic hydrochloric acid. This result is connected with the participation of the sulphite radical, as was apparent when a solution of ω-nitroacetophenone and the pyrimidine (I) dihydrochloride in aqueous alcohol and buffered with sodium acetate gave, not the pteridine, but a Schiff's base. The formation of 2-amino-4-hydroxy-7-phenylpteridine on the addition of sodium dithionite to a suspension of the anil in boiling aqueous ethanol, and its colour with the Folin–Denis reagent, leave little doubt that the intermediate is 2 : 5-diamino-6-hydroxy-4-(2-nitro-1-phenylethylideneamino)pyrimidine (III; R = Ph).

The dependence of pteridine formation on the presence of sulphite or dithionite implies that the mechanism of ring-closure involves reduction of the nitro-group, possibly to oximino or amino, followed by the elimination of hydroxylamine or ammonia with the pyrimidine 5-substituent, and thereby suggests the possible application of α-oximino- or α-amino-ketones to pteridine synthesis. 2 : 4 : 5-Triamino-6-hydroxypyrimidine dihydrochloride, first prepared during the course of this research from the bisulphite by treatment with concentrated hydrochloric acid, has the advantage of greater water solubility than either the bisulphite or the sulphate of the pyrimidine.

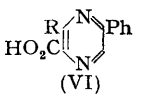
A further preparation was then carried out from the pyrimidine (I) and phenylglyoxal which by analogy with the synthesis of 2-amino-4-hydroxy-7-methylpteridine from methylglyoxal (Mowat *et al.*, *J. Amer. Chem. Soc.*, 1948, **70**, 14; Forrest and Walker, *J.*, 1949, 79) may be expected to offer an alternative route to the 7-phenylpteridine (IV). A crystalline sodium salt and sulphate were prepared and similar products were obtained from the pteridine derived from ω-nitroacetophenone but the properties of these derivatives were insufficiently defined for the pteridine from the two sources to be identified with complete certainty. The orientation of 6- and 7-substituted pteridines may sometimes be determined by oxidation to the respective carboxylic acids, each of which exhibits in alkaline solution a characteristic fluorescence (Forrest and Walker, *Nature*, 1948, **161**, 308, and *loc. cit.*), but for the phenylpteridines this method was precluded by the comparative stability of the foregoing products to oxidation. The complete identity of the pteridines from the two sources was demonstrated by their degradation to the same series of substituted pyrazinecarboxylic acids by the method of Weijlard, Tishler, and Erickson (*J. Amer. Chem. Soc.*, 1945, **67**, 802). The phenylpteridines proved resistant to sulphuric acid, but with aqueous sodium hydroxide at 170° gave a mixture of 2-amino- and 2-hydroxy-6-phenylpyrazine-3-carboxylic acid (VI; R = NH_2) and (VI; R = OH), the latter being characterised by an ethyl ester, m. p. 112°. None of these products has yet been obtained by synthesis and their constitution rests upon the structure (IV) ascribed to the parent pteridine. Decarboxylation of the aminopyrazinecarboxylic acid (VI; R = NH_2) in hot sulphuric acid gave 2-amino-6-phenylpyrazine. It resembles the compound obtained by Weijlard, Tishler, and Erickson (*loc. cit.*) from 2 : 4-dihydroxy-6(or 7)-phenylpteridine which being derived from phenylglyoxal is also, therefore, most probably the 7-phenylpteridine.

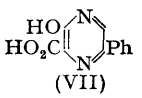
By means of yet a third method of synthesis, the results of which have already been briefly described (King and Spensley, *Nature*, 1949, **164**, 574), the isomeric 2-amino-4-

hydroxy-6-phenylpteridine (V) has been prepared. It consists in the condensation of 4 : 5-diaminopyrimidines with αα-dihalogeno-ketones, thus eliminating the intermediate dihydropteridine stage inherent in the employment of a bromopropaldehyde with only one α-halogen atom, a method introduced by Waller *et al.* (*J. Amer. Chem. Soc.*, 1948, **70**, 19) for the earliest syntheses of the folic acid factors. At the time our experiments were in progress (1947—1948), apart from Purrmann's somewhat analogous synthesis of xanthopterin from the pyrimidine (I) and dichloroacetic acid (*Annalen*, 1940, **546**, 98), no data concerning this newer method were available (cf. King and Spensley, *loc. cit.*), but it has since been reported from several laboratories (see *Ann. Reports*, 1950, **47**, 243). It is exemplified in the condensation of 1 : 1-dichloroacetone with the pyrimidine (I) which at room temperature afforded 53% of 2-amino-4-hydroxy-6-methylpteridine, in marked contrast to the 6% yield of xanthopterin under the drastic conditions of Purrmann's synthesis. The 6-methylpteridine has been prepared *via* the pteridine-6-acetic acid by Mowat *et al.* (*loc. cit.*); the orientation of the methyl substituent was ascertained by oxidation to the corresponding pteridinecarboxylic acid, which was recognised as the 6-carboxylic acid by its characteristic fluorescence.

The condensation of the pyrimidine (I) with ωω-dichloroacetophenone, although less rapid, was equally successful and gave what is now known to be 2-amino-4-hydroxy-6-phenylpteridine (V). Superficially there is little to distinguish it from the 7-phenyl isomer, but alkaline degradation, which gave only the 2-hydroxy-5-phenylpyrazine-3-carboxylic acid (VII) (ethyl ester, m. p. 158—159°) decisively established the individuality of the new pteridine. Marked differences in the fluorescence of the isomeric hydroxy-phenylpyrazinecarboxylic acids (VI; R = OH) and (VII) have been observed.

The action of ωω-dichloroacetophenone on 2 : 4 : 5 : 6-tetra-aminopyrimidine similarly resulted in a pteridine which by analogy may be regarded as 2 : 4-diamino-6-phenylpteridine. With phenylglyoxal, the tetra-aminopyrimidine gave the isomeric 2 : 4-diamino-7-phenylpteridine, and in this case it was possible to distinguish between the two isomers by determinations of melting point. On the other hand, ω-nitroacetophenone, from which 2-amino-4-hydroxy-7-phenylpteridine (IV) had earlier been obtained, gave in its reaction with the tetra-amino-base a product consisting, apparently, of a mixture of the 2 : 4-diamino-6- and -7-phenylpteridines.


(VI)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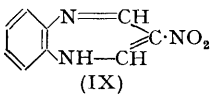
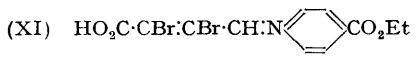
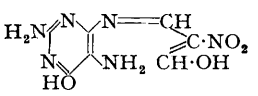

(IX)


(VIII) HO·CH:CR·CH:N—C₆H₄—CO₂R′


(XI) HO₂C·CBr:CBr·CH:N—C₆H₄—CO₂Et


(X)

The experience gained in the preparation of pteridines from ω-nitroacetophenone was then applied to the problem of substituting nitromalondialdehyde (II; R = NO_2) for reductone (II; R = OH) in the pteroic acid synthesis of Angier *et al.* and of Forrest and Walker. The appropriate intermediate (VIII; R = NO_2, R′ = H) was readily prepared from nitromalondialdehyde and *p*-aminobenzoic acid, and was then treated in aqueous solution with the pyrimidine (I). From an examination of the very sparingly soluble bright yellow compound which thereupon separated it was found that the *p*-aminobenzoic acid residue had been extruded during the condensation, and the identical product was obtained by the action of (I) on nitromalondialdehyde alone. This unexpected result appeared to denote the preferential formation of a new type of heterocyclic compound, *i.e.*, a pyrimidinodiazepine, and the ready synthesis of the analogous 6-nitro-2 : 3-benzo-1 : 4-diazepine (IX) both from the ester (VIII; R = NO_2, R′ = Et), again with the loss of the *p*-aminobenzoic moiety, and from nitromalondialdehyde itself, appeared to support this conclusion. Careful analysis of the intensively dried material and other evidence (King and Martin, forthcoming publication), however, are in agreement with a Schiff's base structure and it is probable that the compound is the pyrimidine-4-anil (X).

The apparent instability of the nitromalondialdehyde anil (VIII; R = NO_2, R′ = H) in presence of the pyrimidine is in contrast to the properties of the analogous compounds from reductone (Angier, *loc. cit.*), and it appears to preclude the formation of the dianil necessary for the synthesis of pteroic acid. Experiments with derivatives of bromomalondialdehyde (II; R = Br) have given results similar to those from the nitro-aldehyde. In this case, however, the alternative was adopted of introducing the pyrimidine fraction first, using mucobromic acid (αβ-dibromo-β-formylacrylic acid) as the source of the bromoaldehyde. The action of the pyrimidine (I) on mucobromic acid readily gave a Schiff's base but it failed to undergo the expected reaction with ethyl *p*-aminobenzoate to the required bromomalondianil. The monoanil (VIII; R = Br, R′ = Et), which was prepared from ethyl *p*-aminobenzoate and mucobromic acid, without isolation of the intermediate anil (XI), gave with the pyrimidine (I) an indefinite product from which the *p*-aminobenzoate residue had evidently been eliminated.

Finally the αα-dichloro-ketone method was applied to the synthesis of pteroic acid, as already reported (King and Spensley, *loc. cit.*), a suitable trihalogeno-ketone being obtained by the bromination of 1 : 1-dichloroacetone. A liquid obtained in this way by Cloez (*Ann. Chim. Phys.*, 1886, [vi], **9**, 176) has been described as 3-bromo-1 : 1-dichloroacetone, but not analysed. The authentic compound is a crystalline solid and has been characterised by its semicarbazone. Its constitution has been proved by a synthesis from dichloroacetyl bromide and diazomethane, the intermediate diazo-ketone then being treated with hydrogen bromide. The hydrolysis with 30% sulphuric acid of ethyl γ-bromo-αα-dichloroacetoacetate, prepared both by the bromination of the 1 : 1-dichloro-ester or chlorination of the 3-bromo-ester also afforded some bromodichloroacetone, but it is not a preparative method.

The isolation of a condensation product of ethyl *p*-aminobenzoate and the bromodichloroacetone could not be realised, but the alkali-hydrolysed product obtained after the addition of the pyrimidine (I) showed appreciable biological activity for *Streptococcus fæcalis* R. Better results were obtained when all three components were combined simultaneously, as in the standard procedure for the synthesis of pteroic acid, and the pH maintained at 4—4·3, the yield of pteroic acid determined by biological assay being 9·9%. By substituting *p*-aminobenzoyl-L-glutamic acid for *p*-aminobenzoic acid and operating at a pH of approximately 3·4, a 14% yield of pteroylglutamic acid (assayed with *Lactobacillus casei*) was obtained which compares with the results obtained by others, for example, Weygand and Schmied-Kowarzik (*Ber.*, 1949, **82**, 333), who estimated the yield of folic acid from 1 : 1 : 3-tribromoacetone to be 14%.

We are indebted to Dr. R. H. Nimmo-Smith for these biological determinations.

Experimental

2 : 4 : 5-*Triamino*-6-*hydroxypyrimidine Dihydrochloride*.—The pyrimidine bisulphite described by Cain, Mallette, and Taylor (*loc. cit.*) (9 g.) was dissolved in hot water (35 c.c.) and treated with concentrated hydrochloric acid (15 c.c.). The solution was filtered from the precipitate of sulphur, and a further quantity of concentrated acid added, the mixture then being left to crystallise at 0° for an hour. The *dihydrochloride* (4·9 g.) was recrystallised by precipitation from an aqueous solution with concentrated hydrochloric acid, and it separated in clusters of colourless prisms, darkening at 260°, m. p. >300° (Found: Cl, 33·1. $C_4H_7ON_5,2HCl$ requires Cl, 33·2%).

2(or 4) : 5-*Diamino*-4(or 2)-6-*hydroxy*-2′-*nitroethylideneaminopyrimidine*.—A filtered solution of 2 : 4 : 5-triamino-6-hydroxypyrimidine bisulphite (6 g., 1 mol.) in hot water (25 c.c.), treated with the sodium salt of methazonic acid (3 g., 1 mol.), became orange-coloured, and when the solution was warmed to 50° an orange-brown solid separated. The mixture was heated for 30 minutes, and the *product* (4·2 g., 61%) was then collected, washed with hot water, and dried at 100° under low pressure for analysis (Found: C, 31·3; H, 4·4; N, 36·9. $C_6H_8O_3N_6,H_2O$ requires C, 31·3; H, 4·4; N, 36·5%. Found, after drying at 150° in a high vacuum: C, 33·4; H, 4·2. $C_6H_8O_3N_6$ requires C, 34·0; H, 3·8%). The compound, which was difficult to recrystallise, had m. p. >300°, and in ammoniacal solution gave a dark bottle-green colour with the Folin–Denis reagent. It dissolved in dilute sodium hydroxide solution but the solution showed no fluorescence in ultra-violet light. A solution in warm 2N-sulphuric acid on cooling

deposited 2 : 4 : 5-triamino-6-hydroxypyrimidine sulphate (Found: C, 20·5; H, 3·6. Calc. for $C_4H_7ON_5,H_2SO_4$: C, 20·1; H, 3·8%).

The Schiff's base was heated under reflux with aqueous alkalis, with dilute hydrochloric acid containing urea, and in water to which portions of sodium dithionite (hydrosulphite) were added; an alkaline solution was also hydrogenated over palladised strontium carbonate for 15 hours, absorbing 6 equivalents. The products, liberated where necessary by acid, exhibited in dilute alkaline solution a sky-blue fluorescence in ultra-violet light, but the expected 2-amino-4-hydroxypteridine could not be isolated.

The action of methazonic acid on a solution of 2 : 4 : 5 : 6-tetraminopyrimidine bisulphite gave no precipitate.

2 : 5-*Diamino*-6-*hydroxy*-4-(2-*nitro*-1-*phenylethylideneamino*)*pyrimidine* (III; R = Ph).—To a clear solution of 2 : 4 : 5-triamino-6-hydroxypyrimidine dihydrochloride (0·5 g., 1 mol.) in water (5 c.c.), powdered sodium acetate (1 g., 3 mols.) and then ω-nitroacetophenone (0·4 g., 1 mol.) in warm 50% aqueous ethanol (20 c.c.) were added. From the reddish-orange mixture a solid (0·5 g., 70%) gradually separated in the cold and was next day collected and crystallised from 50% alcohol. The *Schiff's base*, m. p. >300°, separated in orange-coloured needles, insoluble in ethanol, and giving a very faintly fluorescent solution in aqueous alkali hydroxides. In ammoniacal solution the Folin–Denis reagent gave a bottle green colour (Found, after drying at 100° in a vacuum: C, 47·9; H, 4·9; N, 27·4. $C_{12}H_{12}O_3N_6,H_2O$ requires C, 47·1; H, 4·6; N, 27·5%).

2-*Amino*-4-*hydroxy*-7-*phenylpteridine* (IV).—(i) A suspension of 2 : 5-diamino-6-hydroxy-4-(2-nitro-1-phenylethylideneamino)pyrimidine (1·1 g.) in refluxing 25% ethanol (25 c.c.) was treated with sodium dithionite (hydrosulphite) (5 g.) added in portions during 1 hour. The suspended solid became canary-yellow, and after a further hour's heating, the mixture was allowed to cool. The product (0·95 g.) then collected contained sulphur, and 2-*amino*-4-*hydroxy*-7-*phenylpteridine* was obtained from it, by dissolving it in 2N-sodium hydroxide and acidifying the filtered solution with concentrated hydrochloric acid to pH 2, as a buff-coloured crystalline solid, m. p. >360° (Found, after drying at 100° in a vacuum: C, 56·9; H, 3·9; N, 27·3. $C_{12}H_9ON_5,H_2O$ requires C, 56·0; H, 4·3; N, 27·2%).

(ii) When a filtered solution of 2 : 4 : 5-triamino-6-hydroxypyrimidine bisulphite (2 g., 1 mol.) in hot 50% aqueous alcohol (75 c.c.) was treated with ω-nitroacetophenone (1·6 g., 1 mol.) in 50% alcohol, the yellow-orange pteridine began to separate after 5 minutes' heating on a steam-bath, and at the end of 2 hours' refluxing it was collected, washed with aqueous alcohol, and dried (yield, 0·65 g., 26%) (Found, after drying at 100° in a vacuum: C, 55·8; H, 4·1; N, 26·8%). Similar results were obtained in 50% acetic acid solution, but on repetition of the experiment with the addition of hydrochloric acid (4 mols.) the nitro-ketone was largely recovered.

(iii) When a solution of the pyrimidine hydrochloride (0·9 g., 1 mol.) in water (9 c.c.) was treated with sodium acetate (1·8 g., 3 mols.) and freshly distilled phenylglyoxal (0·6 g., 1 mol.) dissolved in 50% aqueous alcohol (5 c.c.), an immediate reaction occurred, and after a few hours the precipitate of 2-amino-4-hydroxy-7-phenylpteridine monohydrate (1·05 g., 97%) was collected. Purified as before by solution in 2N-sodium hydroxide, it was a pale yellow solid, very sparingly soluble in hot water and acetic acid (Found: C, 56·3; H, 4·5; N, 27·4%). The pteridine was not completely dehydrated even after drying at 190° in a vacuum. In 2N-hydrochloric acid solution it formed a jelly, but on cooling of its solution in hot 2N-sulphuric acid the *sulphate* was obtained as a yellow powder, m. p. >300° (Found: C, 47·8; H, 3·8; S, 4·8. $C_{12}H_9ON_5,\frac{1}{2}H_2SO_4,H_2O$ requires C, 47·1; H, 3·9; S, 5·2%. Found, after drying at 110° in a vacuum: C, 50·1; H, 3·7; N, 24·1. $C_{12}H_9ON_5,\frac{1}{2}H_2SO_4$ requires C, 50·0; H, 3·5; N, 24·3%). The pteridine *sodium* salt separated from 2N-sodium hydroxide in minute yellow aggregates which shrivelled on heating (m. p. >300°) (Found, after drying at 100° in a vacuum: C, 51·8; H, 3·4. $C_{12}H_8ON_5Na,H_2O$ requires C, 51·6; H, 3·6%). In very dilute aqueous solution its ultra-violet fluorescence was intense sky-blue.

Both alkaline and acid solutions of the pteridine treated at 90° with 0·5M-potassium permanganate for 1½ hours gave evidence of partial oxidation but on working up only unchanged phenyl compound was recovered.

2-*Amino*-6-*phenylpyrazine*-3-*carboxylic Acid* (VI; R = NH_2).—2-Amino-4-hydroxy-7-phenylpteridine was heated in 80% sulphuric acid at 200° for 15 minutes and the charred mixture poured into ice-water. The solution was basified with ammonia to liberate the expected aminophenylpyrazine, but the resulting solid was insoluble in ether and consisted largely of the impure pteridine (IV).

The pteridine (5 g.) was heated with 4N-sodium hydroxide (50 c.c.) in an autoclave at 170° for 20 hours; water (50 c.c.) was then added and the boiling solution was filtered through a sintered-glass funnel. The solid crystallising on cooling formed pearly plates and consisted of *sodium 2-amino-6-phenylpyrazine-3-carboxylate* (1 g., 20%), which when recrystallised from dilute sodium hydroxide decomposed *ca.* 295° (Found: Na, 9·5. $C_{11}H_8O_2N_3Na$ requires Na, 9·7%).

Acidification of an aqueous solution of the salt to pH 3 with hydrochloric acid precipitated *2-amino-6-phenylpyrazine-3-carboxylic acid*, which crystallised from a fairly large volume of 50% alcohol in small pale yellow prisms, m. p. 225° (decomp.) (Found: C, 61·8; H, 4·1; N, 19·3. $C_{11}H_9O_2N_3$ requires C, 61·4; H, 4·2; N, 19·5%). An acid solution of the pyrazine exhibited a purple-blue fluorescence in daylight and mid-blue in ultra-violet light which was largely quenched by alkali.

2-Hydroxy-6-phenylpyrazine-3-carboxylic Acid (VI; R = OH).—When the filtrate from the preparation of sodium 2-amino-6-phenylpyrazine-3-carboxylate was brought to pH 2 with hydrochloric acid, the corresponding *2-hydroxy-acid* (2·1 g., 50%) separated, which was first colourless and then became yellow. Crystallisation from 50% alcohol gave buff-coloured needles, m. p. 208—209° (decomp.) (Found: C, 60·8; H, 4·1; N, 13·0. $C_{11}H_8O_3N_2$ requires C, 61·1; H, 3·7; N, 13·0%). The dilute acid solution had a mid-blue ultra-violet fluorescence, considerably suppressed by alkali.

Ethyl 2-Hydroxy-6-phenylpyrazine-3-carboxylate.—The pyrazinecarboxylic acid (0·5 g.) was heated under reflux in ethanol (5 c.c.) containing sulphuric acid (0·3 c.c.) for 2 hours and, on cooling, the *ester* (0·44 g.) was obtained as pale yellow prisms which, on recrystallisation from alcohol, had m. p. 112° (Found: C, 63·5; H, 4·8; N, 11·4. $C_{13}H_{12}O_3N_2$ requires C, 63·9; H, 4·9; N, 11·5%).

2-Amino-6-phenylpyrazine.—Sodium 2-amino-6-phenylpyrazine-3-carboxylate (0·8 g.) was mixed with 80% sulphuric acid (12 c.c.) and, after being heated at 200° for 15 minutes, the solution was cooled and poured on ice. After being made alkaline with 10N-ammonia it was extracted thrice with ether, and evaporation of the dried (Na_2SO_4) ethereal extracts gave *2-amino-6-phenylpyrazine* (0·41 g., 70%) which, by one crystallisation from water, was obtained as colourless pearly plates, m. p. 125—126° (Found: C, 70·0; H, 5·2; N, 24·5. $C_{10}H_9N_3$ requires C, 70·1; H, 5·3; N, 24·6%). Weijlard, Tishler, and Erickson, *loc. cit.*, give m. p. 130—131° for 2-amino-5(or 6)-phenylpyrazine.

2-Amino-4-hydroxy-6-methylpteridine.—To a solution of 2 : 4 : 5-triamino-6-hydroxypyrimidine dihydrochloride (0·6 g., 1 mol.) in water (6 c.c.), sodium acetate (1·15 g., 3 mols.) and 1 : 1-dichloroacetone (0·3 c.c., 1 mol.) were added. The orange-coloured solid (0·25 g., 53%) which immediately began to form was collected after an hour and dissolved in 2N-sodium hydroxide (12 c.c.). The introduction of 10N-alkali (10 c.c.) then slowly caused crystallisation of the pteridine sodium salt in yellow needles, and, after repetition of the process, a portion of the product was dissolved in water and acidified (pH 2). The precipitated 2-amino-4-hydroxy-6-methylpteridine was dried in a vacuum at 100° (Found: C, 47·1; H, 4·0; N, 39·4. Calc. for $C_7H_7ON_5$: C, 47·5; H, 4·0; N, 39·5%). Oxidation of the remaining sodium salt with alkaline permanganate, in the manner described by Mowat *et al.* (*J. Amer. Chem. Soc.*, 1948, **70**, 18) for the 7-methyl compound, gave 2-amino-4-hydroxypteridine-6-carboxylic acid, identified by its sky-blue ultra-violet fluorescence and absorption maximum, 263 mμ, in N/10-sodium hydroxide (Found: C, 40·5; H, 3·1. Calc. for $C_7H_5O_3N_5$: C, 40·6; H, 2·4%).

2-Amino-4-hydroxy-6-phenylpteridine (V).—The triaminohydroxypyrimidine dihydrochloride (4·5 g., 1 mol.) in 50% aqueous alcohol (80 c.c.) was treated with sodium acetate (13·5 g., 5 mol.) followed by ωω-dichloroacetophenone (3·8 g., 1 mol.), and since the reaction occurred very slowly the mixture was heated under reflux for 1½ hours. *2-Amino-4-hydroxy-6-phenylpteridine* separated as a deep orange solid (3·1 g., 60%), and this was purified by dissolving it in hot 2N-sodium hydroxide and collecting the sodium salt which crystallised on cooling. The pteridine, m. p. >360°, was then obtained by dissolving the salt in water and acidifying the solution to pH 2 (Found, after drying at 100° in a vacuum: C, 55·6; H, 3·9; N, 27·7. $C_{12}H_9ON_5,H_2O$ requires C, 56·0; H, 4·3; N, 27·2%). From a solution of the pteridine in 2N-sulphuric acid the *sulphate* crystallised in minute pale yellow clusters of prisms, m. p. >360° (Found: C, 47·6; H, 3·6; S, 4·9. $C_{12}H_9ON_5,\frac{1}{2}H_2SO_4,H_2O$ requires C, 47·1; H, 3·9; S, 5·2%).

2-Hydroxy-5-phenylpyrazine-3-carboxylic Acid (VII).—2-Amino-4-hydroxy-6-phenylpteridine (3·1 g.) was hydrolysed by heating it in an autoclave with 4N-sodium hydroxide (32 c.c.) at 170° for 24 hours, and the product isolated by diluting the solution with water (32 c.c.), heating it to boiling, and acidifying the filtered solution to pH 2. The initially pale precipitate became

yellow and consisted of *2-hydroxy-5-phenylpyrazine-3-carboxylic acid* (1·5 g., 57%). It was soluble in hot 50% alcohol containing a small quantity of hydrochloric acid and separated, when rubbed, in bright yellow needles. Slow crystallisation under dust-free conditions gave minute clusters of yellow prisms, both varieties having m. p. 200° (decomp.), the former sintering <100° and evidently being solvated. In dilute acid solution a brilliant pale green ultra-violet fluorescence was observed, quite distinct from that of the isomeric 6-phenylpyrazine (VI; R = OH); the mixed m. p. with the latter was 189° (Found, after drying at 100° in a vacuum: C, 61·0; H, 3·7; N, 13·0. $C_{11}H_8O_3N_2$ requires C, 61·1; H, 3·7; N, 13·0%).

When heated under reflux in ethanol (5 c.c.) containing sulphuric acid (0·3 c.c.) for 2 hours, the pteridine (0·5 g.) gave *ethyl 2-hydroxy-5-phenylpyrazine-3-carboxylate* which separated after a while at 0° in pale yellow plates, m. p., after recrystallisation from 50% alcohol, 158—159° (Found: C, 63·8; H, 4·9; N, 11·6. $C_{13}H_{12}O_3N_2$ requires C, 63·9; H, 4·9; N, 11·5%).

2 : 4-*Diamino-6-phenylpteridine*.—A solution of 2 : 4 : 5 : 6-tetra-aminopyrimidine trihydrochloride (1 g., 1 mol.) in water (5 c.c.) containing sodium acetate (2·8 g., 5 mol.) was treated with ωω-dichloroacetophenone (0·75 g., 1 mol.) dissolved in alcohol (5 c.c.). After refluxing for 6 hours the mixture was cooled and the solid (0·3 g.) collected and dissolved in hot 2N-sulphuric acid. The pteridine *sulphate* which separated on cooling was recrystallised from 2N-sulphuric acid, forming minute clusters of yellow needles, m. p. >300° (Found, after drying at 100° in a vacuum: C, 47·3; H, 4·3; N, 26·8. $C_{12}H_{10}N_6,\frac{1}{2}H_2SO_4,H_2O$ requires C, 47·2; H, 4·3; N, 27·5%). When an aqueous acid solution of the sulphate was basified 2 : 4-*diamino-6-phenylpteridine* separated in minute yellow needles, m. p. 285—286° unchanged by further crystallisation from water (Found, after drying at 100° or 140° in a vacuum: C, 58·6; H, 4·2; N, 34·5. $C_{12}H_{10}O_6,\frac{1}{2}H_2O$ requires C, 58·3; H, 4·45; N, 34·0%). The ultra-violet fluorescence of a saturated solution of the pteridine in water is brilliant light blue-green.

2 : 4-*Diamino-7-phenylpteridine*.—Solutions of 2 : 4 : 5 : 6-tetra-aminopyrimidine bisulphite (2 g., 1 mol.) and of phenylglyoxal (1·3 g., 1 mol.) in 50% ethanol (30 c.c. and 50 c.c. respectively) became yellow when mixed and, the mixture, after refluxing for 15 minutes and cooling, deposited a pale yellow solid. This was best purified by dissolving it in hot 2N-sulphuric acid, whereupon the *pteridine sulphate* separated on cooling as pale yellow needles, m. p. >300° (Found: C, 47·6; H, 4·6; N, 27·2. $C_{12}H_{10}N_6,\frac{1}{2}H_2SO_4,H_2O$ requires C, 47·2; H, 4·3; N, 27·5%). 2 : 4-*Diamino-7-phenylpteridine* was precipitated by alkali from a dilute acid solution of the sulphate, and when recrystallised from a large volume of water formed pale yellow needles, m. p. 290—291° (decomp.) and 277° when mixed with the isomeric 6-phenylpteridine of m. p. 285—286° (Found, after drying at 110° in a vacuum: C, 60·8; H, 4·6. $C_{12}H_{10}N_6$ requires C, 60·5; H, 4·2%). In aqueous solution the fluorescence of the 7-phenylpteridine cannot be distinguished from that of the 6-isomer.

A solution of the tetra-aminopyrimidine trihydrochloride (1 g.) in water (5 c.c.) was mixed with ω-nitroacetophenone (0·7 g.) in alcohol (8 c.c.), and sodium dithionite (4 g.) added. After 2 hours under reflux the solution was cooled and basified, and the solid (0·07 g.) collected. When purified through the sulphate and crystallised from water the product consisted of minute pale yellow needles, m. p. 280—281° after contracting at *ca.* 270°, not depressed by either the 6- or the 7-methylpteridine. A comparable experiment with the pyrimidine bisulphite in 50% alcohol also failed to give a pure compound.

p-(3-*Hydroxy-2-nitroallylideneamino*)*benzoic Acid* (VIII; R = NO_2, R′ = H).—The mixing of solutions (10 c.c.) of *p*-aminobenzoic acid (1·75 g., 1 mol.) and of sodionitromalondialdehyde (1·4 g., 1 mol.) in water caused the immediate precipitation of p-(3-*hydroxy-2-nitroallylideneamino*)*benzoic acid* (2·1 g., 88%). It was collected and crystallised from 50% ethanol (500 c.c.), and separated in minute yellow prisms, m. p. 254° (decomp.), darkening from 250° (Found: C, 51·0; H, 3·4; N, 11·6. $C_{10}H_8O_5N_2$ requires C, 50·8; H, 3·4; N, 11·8%).

2 : 5-*Diamino-6-hydroxy-4-*(3-*hydroxy-2-nitroallylideneamino*)*pyrimidine*.—(i) A solution of 2 : 4 : 5-triamino-6-hydroxypyrimidine dihydrochloride (0·17 g., 1 mol.) and sodium acetate (0·4 g., 3 mol.) in water (5 c.c.) was added to one of *p*-(3-hydroxy-2-nitroallylideneamino)benzoic acid in hot 50% ethanol (60 c.c.). A colour immediately developed and an orange-yellow solid began to separate, and after the mixture had been heated to boiling the *Schiff's base* (0·16 g.), m. p. 360°, was collected and washed with hot water and alcohol. It was insoluble in organic solvents and very sparingly soluble in water, and its solutions in dilute acid (pale yellow) or alkali (deep yellow) were non-fluorescent (Found, after drying at 100° and 160° in a high vacuum: C, 34·7; H, 3·5; N, 34·4. $C_7H_8O_4N_6$ requires C, 35·0; H, 3·3; N, 35·0%).

(ii) When solutions (4 c.c.) of the pyrimidine hydrochloride (8·6 g., 1 mol.) and sodium acetate (0·8 g.) and of sodionitromalondialdehyde (0·4 g., 1 mol.) in water were mixed, there was

instant precipitation of a golden-yellow voluminous solid (0·52 g.), identical with that obtained in the previous experiment (Found, after drying at 100° in a high vacuum : N, 35·0%).

Ethyl p-(3-*Hydroxy*-2-*nitroallylideneamino*)*benzoate* (VIII ; R = NO_2, R′ = Et).—A suspension of sodionitromalondialdehyde (1·4 g.) in water (5 c.c.) was added to an aqueous solution (10 c.c.) of concentrated hydrochloric acid (1 c.c.) and ethyl *p*-aminobenzoate. The precipitate of *ethyl* p-(3-*hydroxy*-2-*nitroallylideneamino*)*benzoate* (2·3 g., 95%) crystallised from ethanol in yellow needles or prisms, m. p. 158—159° (Found : C, 54·9; H, 4·7; N, 10·4. $C_{12}H_{12}O_5N_2$ requires C, 54·5; H, 4·5; N, 10·6%).

6-*Nitro*-2 : 3-*benzo*-1 : 4-*diazepine* (IX).—(i) On heating under reflux of a solution of ethyl *p*-(2-formyl-2-nitroethylideneamino)benzoate (0·88 g.) and *o*-phenylenediamine (0·36 g.) in alcohol (20 c.c.) for 1 hour a red solid (0·51 g., 81%) was precipitated, and from the residual liquid ethyl *p*-aminobenzoate (0·37 g., 61%) was recovered. The red precipitate, consisting of 6-*nitro*-2 : 3-*benzo*-1 : 4-*diazepine*, was insoluble in dilute alkalis and mineral acids, and was only very sparingly soluble in the common organic solvents. From quinoline it crystallised in deep red prisms, m. p. 360° (Found, after drying at 140° in a vacuum over phosphoric oxide : C, 57·1; H, 3·5; N, 22·2. $C_9H_7O_2N_3$ requires, C, 57·1; H, 3·8; N, 22·2%).

(ii) A mixture of sodionitromalondialdehyde (0·32 g.) and *o*-phenylenediamine (0·25 g.) in water (6 c.c.) containing concentrated hydrochloric acid (0·25 c.c.) was heated on a steam-bath and gave a yellow precipitate which rapidly became red. After 30 minutes' heating the product (0·31 g., 71%) was collected; it was identical with the foregoing nitrobenzodiazepine, and formed deep red prisms, m. p. 360°, from quinoline (Found, after drying : C, 57·4; H, 3·6%).

Experiments with Mucobromic Acid (αβ-*Dibromo*-β-*formylacrylic Acid*).—(i) A mixture of aqueous solutions (10 c.c. each) of mucobromic acid (0·65 g., 1 mol.) and of 2 : 4 : 5-triamino-6-hydroxypyrimidine hydrochloride (1·1 g., 2 mols.) buffered with sodium acetate (0·7 g.) immediately gave a bright yellow precipitate (0·85 g.) of 2(or 4) : 5-*diamino*-4(or 2)-(2 : 3-*dibromo*-3-*carboxyallylideneamino*)-6-*hydroxypyrimidine*, m. p. >360° after darkening at *ca.* 195—200° (Found : C, 25·7; H, 2·0; N, 18·3. $C_8H_7O_3N_5Br_2$ requires C, 25·2; H, 1·8; N, 18·4%). It gave only a pale green colour in ammoniacal solution with the Folin–Denis reagent. The brown solid remaining after the pyrimidine had been heated for 40 minutes with a solution of ethyl *p*-aminobenzoate in 50% alcohol was but feebly fluorescent in alkaline solution and was not further examined.

(ii) A solution of ethyl *p*-aminobenzoate (1·65 g., 1 mol.) and mucobromic acid (2·6 g., 1 mol.) in ethanol (10 c.c.) was boiled under reflux for 20 minutes, diluted with water (300 c.c.), and after the addition of sodium hydrogen carbonate (0·84 g., 1 mol.) again refluxed for 1 hour. The hot solution was filtered, and on cooling it deposited *ethyl* p-(2-*bromo*-3-*hydroxyallylideneamino*)*benzoate* (VIII ; R = Br ; R′ = Et) in very pale yellow needles (0·6 g., 20%). After recrystallisation from ethanol this had m. p. 159—160°, identical with that of the product prepared from bromomalondialdehyde (R. M. Acheson, unpublished preparation) (Found : C, 48·0; H, 3·8. $C_{12}H_{12}O_3NBr$ requires C, 48·3; H, 4·0%). Heated with the triaminohydroxypyrimidine (I) dihydrochloride in 50% alcohol buffered with sodium acetate, it gave a yellow-orange solid, having no fluorescence in alkaline solution. This was not identified but from its nitrogen content (37·3%) appeared to have lost the *p*-aminobenzoate residue.

Dichloroacetyl Bromide.—A mixture of dichloroacetic acid (34 c.c., 1 mol.) and phosphorus tribromide (37 c.c., 1 mol.) was heated under a short column at 100° for 1 hour. The temperature was then slowly increased to 190° whereupon a distillate (76 g.) was collected, which on fractionation gave *dichloroacetyl bromide* (61·5 g., 81%), b. p. 125—129° (Found : C, 12·1; H, 0·44; halogen, 78·0. C_2HOCl_2Br requires C, 12·5; H, 0·52; halogen, 79·0%).

3-*Bromo*-1 : 1-*dichloroacetone.*—(i) Bromine (10 c.c., 1 mol.) was gradually added during 1 hour to 1 : 1-dichloroacetone (20 c.c., 1 mol.) heated on a steam-bath. The product was then washed with water and sodium hydrogen carbonate solution, dried (Na_2SO_4), and distilled. The fraction, b. p. 89—100°/25 mm. (28 g., 67%), which was solid at room temperature, was redistilled to give 3-*bromo*-1 : 1-*dichloroacetone*, b. p. 92—93°/25 mm., solidifying to needles, m. p. 30—31° (Found : C, 17·7; H, 1·5; halogen, 70·0. $C_3H_3OCl_2Br$ requires C, 17·5; H, 1·5; halogen, 72·8%). A solution of the bromodichloroacetone (0·82 g.) in ethanol (2 c.c.) and one of semicarbazide hydrochloride (0·44 g.) in water (2 c.c.) slowly deposited colourless prisms of the *semicarbazone* (0·54 g.) which were collected after 5 hours and, when twice crystallised from aqueous alcohol, had m. p. 131° (Found : C, 18·8; H, 2·6; N, 15·3; halogen, 56·4. $C_4H_6ON_3Cl_2Br$ requires C, 18·3; H, 2·3; N, 16·0; halogen, 57·4%).

(ii) A stirred solution of diazomethane (8·8 g., 2 mols.) in dry ether (500 c.c.) was treated with

dry ethereal dichloroacetyl bromide (20 g., 1 mol.; in 10 c.c.) added at room temperature in the course of 15 minutes. Three hours later the liquid was cooled to 0° and dry hydrogen bromide passed in until nitrogen evolution ceased. The ether solution was then washed with water and 5% sodium carbonate solution, dried (Na_2SO_4), and finally distilled, the fraction of b. p. 90—100° (4·9 g., 23%) being collected. The bromodichloro-ketone, m. p. *ca.* 24°, gave a semicarbazone, m. p. 130° alone or when mixed with that prepared from the bromination of dichloroacetone.

Ethyl γ-Bromo-αα-dichloroacetoacetate.—(i) Ethyl αα-dichloroacetoacetate (75 g., 1 mol.), warmed to 50°, was treated dropwise with bromine (20 c.c., 1·05 mols.). The reaction was completed during 15 minutes at 90°, and after cooling, the product was neutralised with calcium carbonate and distilled. The fraction of b. p. 120—129°/11 mm. (47 g., 45%) consisted almost entirely of *ethyl γ-bromo-αα-dichloroacetoacetate*, b. p. 127°/11 mm. (Found: C, 25·7; H, 2·5; halogen, 55·1. $C_6H_7O_3Cl_2Br$ requires C, 25·9; H, 2·5; halogen, 54·3%).

(ii) Ethyl γ-bromoacetoacetate (124 g.) was cooled in water while slowly saturated with chlorine; the bromochloro-ester was then fractionated and the portion of b. p. 125—130°/11 mm. (71 g., 43%) redistilled for analysis (Found: C, 26·0; H, 2·7; halogen, 53·0%).

When the bromodichloro-ester was heated under reflux with sulphuric acid considerable coloration occurred during the slow evolution of carbon dioxide. Fractional distillation of the ether-extracted oil gave a small quantity, b. p. 88—88°/25 mm., from which was obtained a semicarbazone, m. p. 128° undepressed when the semicarbazone was mixed with that of 3-bromo-1 : 1-dichloroacetone.

Pteroic Acid.—(i) 3-Bromo-1 : 1-dichloroacetone (2·06 g., 1 mol.), ethyl *p*-aminobenzoate (1·82 g., 1·1 mols.), and sodium hydrogen carbonate (1·26 g., 1·5 mols.) were shaken with 9% alcohol (8 c.c.) for 3 days before the addition of a solution of the triaminohydroxypyrimidine hydrochloride (2·1 g., 1 mol.) and sodium acetate (5·3 g.) in 50% alcohol (40 c.c.). The dark brown precipitate (0·9 g.) was collected next day and left in 2N-sodium hydroxide (40 c.c.) under nitrogen for 2—3 hours. Acidification of the filtered solution gave a gelatinous product (0·4 g.) having 10% pteroic acid activity for *Strep. fæcalis* R.

(ii) To a mixture of *p*-aminobenzoic acid (1 g.) in ethanol (50 c.c.) and of the pyrimidine hydrochloride (1·5 g.) in water (150 c.c.) with sodium acetate (6 g.), the bromodichloroacetone (1·5 g.) was added with stirring during 20 minutes. The dark material (0·3 g.) which had separated in 1½ hours (1% activity) was removed; when kept overnight a brown solid (0·3 g.) with 17% pteroic acid activity was deposited.

(iii) The amino-acid (1 g.) in ethanol (100 c.c.) mixed with the pyrimidine hydrochloride (1·5 g.) in water (150 c.c.) were treated with the halogeno-ketone (1·5 g.) dissolved in alcohol (50 c.c.), the solution being kept at pH 4—4·3 by sodium hydroxide and stirred by a stream of nitrogen. The brown precipitate (0·4 g.) collected after an hour had 17% activity, and subsequent paler brown products, 0·35 g. after 5 hours and 0·07 g. after 36 hours, had 36% and 50% activity, respectively; the overall yield was 9·9%.

Pteroylglutamic Acid.—After the addition of the pyrimidine hydrochloride (1·5 g.) to a solution of *p*-aminobenzoyl L-glutamic acid (1·9 g.) in 25% alcohol (200 c.c.) stirred by a current of nitrogen, the bromodichloroacetone (1·5 g.) was added in ethanol solution (100 c.c.) during 1 hour, the pH being kept at 3·25—3·55 with N-sodium hydroxide. The dark brown precipitate (0·42 g.), collected after ½ hour and assayed with *Lb. casei*, had a 34% pteroylglutamic acid activity, and the paler product (0·54 g.), deposited after a further 18 hours, 57% activity; the overall yield was 14·0%.

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