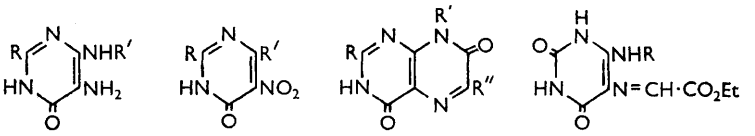
82. *Synthesis of 8-Substituted Glucosylpteridines Related to Known Naturally Occurring Materials.*

By Rolf Lohrmann and H. S. Forrest.

Some 8-glucosylpteridines, with substituents in the pyrimidine portion of the ring system identical with those in the corresponding positions of guanine or xanthine, have been synthesized, and their properties are described.

Glycosyl-purines and -pyrimidines are well-known naturally occurring compounds. In the closely related pteridine series, however, there is no well-authenticated occurrence of a glycosyl derivative in Nature. Tschesche, Korte, and Reichle [1] prepared *N*-glucosyl derivatives of several pteridines, and showed that that of isoxanthopterin-6-carboxylic acid was a growth factor for *Streptococcus feacalis* R; Ziegler [2] has suggested that " tetrahydrobiopterin " occurs naturally as a ribosyl or ribosyl phosphate derivative which is presumably substituted on a ring-nitrogen atom. The structures of these compounds, however, have not been well defined. On the other hand, it is now well established that pteridines with a sugar alcohol at position 8 (*e.g.*, 8-ribitylpteridines) are precursors or products in riboflavin metabolism.[3] These sugar-alcohol derivatives could conceivably arise by reduction of the corresponding glycosylpteridines although there is no evidence for this.



* To avoid considerable duplication, R = OH is used in formulæ (I—III) although in fact (and as is indicated in the naming of these compounds in the text) a —CO·NH— structure at this part of the molecules is more appropriate.

The possibility that glycosylpteridines might occur in Nature has led various investigators to attempt to synthesize them in the expectation that examination of their properties might aid in their isolation from natural sources and/or that they might act as biological antagonists to the purine nucleosides or the flavins. For instance, Forrest, Hull, Rodda, and Todd [4] developed methods for the synthesis of 8-substituted pteridines and synthesized one glycosylpteridine—4-amino-8-D-glucosyl-5,6,7,8-tetrahydro-6,7-dioxo-2-methylthiopteridine. The structure of this compound is, however, only remotely related to pteridine derivatives which might be expected to occur naturally. More recently Pfleiderer and his co-workers [5] have attempted to prepare glycosylpteridines (*e.g.*, the 8-glycoside of the naturally occurring isoxanthopterin) which would be much more analogous to the purine ribonucleosides (*e.g.*, guanosine), but, apparently, their method yielded *O*-glycosides and not *N*-glycosides.

While attempting to synthesize unambiguously 3- and 9-glycosyluric acids, the 5,6-diaminopyrimidine (I; R = OH, R′ = 2,3,4,6-tetra-*O*-acetyl-D-glucosyl) was prepared (for subsequent conversion into 9-D-glucosyluric acid),[6] and, since diamines of this type readily undergo the Isay reaction to give pteridines,[7] its conversion into substituted pteridines was explored. The diamine reacted with ethyl pyruvate and with ethyl

[1] Tschesche, Korte, and Reichle, *Z. Naturforsch.*, 1955, **10**b, 346.
[2] Ziegler, *Z. Naturforsch.*, 1960, **15**b, 460; Ziegler, *Biochem. Z.*, 1963, **337**, 62.
[3] Cf. Plaut, *Ann. Rev. Biochem.*, 1961, **30**, 409.
[4] Forrest, Hull, Rodda, and Todd, *J.*, 1951, 3.
[5] Pfleiderer and Lohrmann, *Chem. Ber.*, 1962, **95**, 738; Pfleiderer and Reisser, *ibid.*, p. 1621.
[6] Lohrmann, Lagowski and Forrest, preceding paper.
[7] Cf. Albert, *Quart. Rev.*, 1952, **6**, 197.

glyoxalate hemiacetal to give, after deacetylation, the 8-glucosylpteridines (III; R = OH, R′ = D-glucosyl, R″ = Me and H, respectively), in good yield. The ultraviolet absorption spectra of the different molecular forms of the 8-glucosyl-6-methyl compound and those of the 6,8-dimethyl homologue prepared by Pfleiderer [8] are essentially identical at pH 1 (neutral molecule) and are very similar at pH 7·8 (monoanion) with, however, a bathochromic shift in the spectrum of the glycosylpteridine presumably due to interaction between the glucosyl residue and the oxygen atom in the 7-position. [This is probably also reflected in the differing pK_a values between the glucosyl derivative (**4·40**) and the dimethyl compound (**4·26**)]. On the other hand, the spectra of both these compounds differ from those of the analogous *O*-glucosyl compounds.[5] Further, both our *N*-glucosylpteridines are moderately stable to acid (complete hydrolysis with 2N-hydrochloric acid at 100° only after several hours), whereas the *O*-glycosylpteridines are very labile to both acid and alkali. All these results, then, are consistent with an 8-glycosyl formulation.

TABLE 1.

Chromatographic behaviour of 8-substituted pteridines.*

2-Amino-3,4,7,8-tetrahydro-4,7-dioxopteridine derivative	Pr^{n}OH–1% aq. NH_3 (2 : 1)	Bu^{n}OH–30% aq. AcOH (2 : 1)	3% NH_4Cl	4% Na citrate
6-Carboxy-8-methyl	0·17, B, BB	0·20, B, B	0·58, B, BB	0·64, B, BB
6-Methyl-8-phenyl	0·65, B, BB	0·67, B, B	0·58, B, BB	0·57, B, B
8-2′-Hydroxyethyl-6-methyl	0·50, B, BB	0·43, B, BB	0·53, B, BB	0·53, B, BB
6-Carboxy-8-2′-hydroxyethyl	0·19, B, BB	0·19, B, BB	0·63, B, BB	0·68, B, BB
6-Carboxy-8-2′-hydroxyethyl ethyl ester	0·64, B, BB	0·50, B, BB	0·62, B, BB	0·55, B, BB
8-D-Glucosyl	0·37, BB, BB	0·09, BB, BB	0·73, B, BB	0·74, B, BB
8-D-Glucosyl-6-methyl	0·43, BB, BB	0·15, BB, BB	0·74, BB, BB	0·74, BB, BB
6-Carboxy-8-D-glucosyl	0·17, B, B	0·08, B, B	0·80, B, B	0·82, B, B
1,2,3,4,7,8-Hexahydro-2,4,7-trioxopteridine derivative				
8-D-Glucosyl	0·40, B, BB	0·08, B, BB	0·74, B, BB	0·64, B, BB
8-D-Glucosyl-6-methyl	0·45, LB, LB	0·15, LB, LB	0·76, LB, LB	0·68, LB, LB

* R_F values (1st entry) and colour of fluorescence as observed with 254 mμ ultraviolet light (2nd entry) and with 365 mμ ultraviolet light (3rd entry). B, blue; BB, bright blue; LB, light blue.

TABLE 2.

Ultraviolet absorption spectra and pK_a values.

2-Amino-3,4,7,8-tetrahydro-4,7-dioxopteridine derivative	pH of solvent	$\lambda_{max.}$ (mμ)	$10^{-3}\varepsilon$	pK_a in water *
8-D-Glucosyl	5·2	350, 292	12·3, 9·1	7·96 ± 0·05
	11·1	366, 281sh, 262	12·9, 3·6, 10·2	
8-2′-Hydroxyethyl-6-methyl	5·2	340, 294	13·8, 9·1	8·43 ± 0·05 †
	11·1	354, 283, 258	14·1, 4·2, 11·2	
8-D-Glucosyl-6-methyl	5·2	346, 295	10·2, 9·1	8·35 ± 0·04
	11·1	359, 280sh, 259	10·5, 4·1, 8·9	
6-Carboxy-8-2′-hydroxyethyl	1·0	383, 292, 267, 222	23·4, 6·9, 7·6, 33·9	3·53 ± 0·15
	6·2	355, 294, 257sh	15·5, 9·3, 4·4	8·38 ± 0·10
	11·6	368, 283sh, 263	17·0, 3·8, 12·0	
6-Carboxy-8-D-glucosyl	1·0	386, 294, 265, 216	20·4, 6·8, 6·3, 28·2	3·21 ± 0·04
	6·2	359, 294, 230sh	13·8, 8·9, 11·0	8·14 ± 0·07
	11·6	375, 264	14·1, 11·5	
1,2,3,4,7,8-Hexahydro-2,4,7-trioxopteridine derivative				
8-D-Glucosyl-6-methyl	1·0	328, 282	10·2, 11·8	4·40 ± 0·06
	7·8	353, 292	11·5, 10·5	
6,8-Dimethyl [8]	2·0	327, 282	12·6, 11·7	4·26 ± 0·02
	6·5	344, 288	14·5, 11·5	

* Calc. from ultraviolet absorption spectra by Mattoo's method, *Trans. Faraday Soc.*, 1958, **54**, 19.)
† Ref. 5 gives 7·80 ± 0·1.

The parent pyrimidine (I; R = OH, R′ = 2,3,4,6-tetra-*O*-acetyl-D-glucosyl) for the synthesis of these glucosylpteridines was obtained in two ways: (1) by condensation of

[8] Pfleiderer, *Chem. Ber.*, 1957, **90**, 2588.

6-chloro-1,2,3,4-tetrahydro-2,4-dioxo-5-phenylazopyrimidine (6-chloro-5-phenylazouracil) with 2,3,4,6-tetra-*O*-acetyl-D-glucosylamine,[6] followed by reduction; and (2) by nitration of 6-chloro-1,2,3,4-tetrahydro-2,4-dioxopyrimidine (6-chlorouracil)[9] and condensation of the nitropyrimidine with the same glucosylamine, followed by reduction of the nitro-group. A second series of 8-substituted pteridines, and in particular, 2-amino-3,4-dihydro-8-D-glucosyl-4-oxopteridines, which are even more closely related to the pteridines commonly occurring in Nature, was prepared by the latter route. For instance, nitration of 2-amino-6-chloro-3,4-dihydro-4-oxopyrimidine (6-chloroisocytosine)[10] gave the nitropyrimidine (II; R = NH_2, R′ = Cl), which, although less reactive than the corresponding uracil derivative, condensed with methylamine, aniline, and 2-hydroxyethylamine, giving compounds (II; R = NH_2, R′ = NHMe, NHPh, and NH·CH_2·CH_2·OH, respectively). These nitro-compounds were reduced catalytically or with sodium dithionite, and the resulting diamines condensed with Isay reagents. For example, the methylaminopyrimidine and diethyl mesoxalate gave 2-amino-3,4,7,8-tetrahydro-8-methyl-4,7-dioxopteridine-6-carboxylic acid, and the anilinopyrimidine with ethyl pyruvate gave 2-amino-3,4,7,8-tetrahydro-6-methyl-4,7-dioxo-8-phenylpteridine. Similarly the 2-hydroxyethylaminopyrimidine (I; R = NH_2, R′ = CH_2·CH_2·OH) with ethyl pyruvate gave a compound (III; R = NH_2, R′ = CH_2·CH_2·OH, R″ = Me) and with ethyl mesoxalate gave the acid (III; R = NH_2, R′ = CH_2·CH_2·OH, R″ = CO_2H). Finally, the nitropyrimidine (II; R = NH_2, R′ = Cl) reacted with 2,3,4,6-tetra-*O*-acetyl-D-glucosylamine, affording a 6-glucosylamino-derivative (I; R = NH_2, R′ = 2,3,4,6-tetra-*O*-acetyl-D-glucosyl) which was reduced; the diamine was then condensed with an Isay reagent, and the product deacetylated. Thus glucosylpteridines were obtained with the following reagents: (III; R = NH_2, R′ = D-glucosyl, R″ = Me) with ethyl pyruvate; (III; R = NH_2, R′ = D-glucosyl, R″ = CO_2H) with ethyl mesoxalate (after hydrolysis); and (III; R = NH_2, R′ = D-glucosyl, R″ = H) with ethyl glyoxylate hemiacetal. In the last case, treatment with alkali was necessary to effect the condensation.

All the glucosylpteridines described in this paper (except the 6-carboxylic acid, which was sufficiently insoluble in water to be purified by crystallization from it) were purified by paper chromatography followed by ion-exchange chromatography. They were isolated as yellow or white solids and exhibited strong blue fluorescence in solution. Their purity was established by paper chromatography in four solvent systems (Table 1). The ultraviolet absorption spectra of the different molecular forms of 2-amino-8-D-glucosyl-3,4,7,8-tetrahydro-6-methyl-4,7-dioxopteridine can be compared with the previously prepared and strictly analogous 2-amino-3,4,7,8-tetrahydro-8-2′-hydroxyethyl-6-methyl-4,7-dioxopteridine[5] (Table 2). As in the case of the 2-oxo-compounds described above, the spectra of the neutral molecules (measured at pH 5) and of the monoanions (measured at pH 11) are almost identical, except for the same bathochromic shift of 5—6 mμ in the long-wavelength band ascribed to interaction between the glucose residue and the oxygen at position 7. The spectra of the neutral molecule and anion of analogous *O*-glycosyl derivatives,[5] on the other hand, are different. Again, all of the *N*-glucosyl derivatives in this series are reasonably stable to acid hydrolysis (2N-hydrochloric acid for several hours at 100° for complete hydrolysis) in contrast to the *O*-glucosyl compounds. Thus both the method of synthesis and the physical and chemical properties of the compounds leave no doubt that they are true 8-glycosyl derivatives.

Experimental

5-Nitro-6-(2,3,4,6-tetra-O-acetyl-D-glucosylamino)uracil (II; R = OH, R′ = 2,3,4,6-tetra-*O*-acetyl-D-glucosylamino).—To a suspension of 4-chloro-5-nitrouracil[9] (dried over P_2O_5 *in vacuo*; 4 g.) in hot ethyl acetate (50 ml.) was added a solution of freshly prepared 2,3,4,6-tetra-*O*-acetyl-D-glucosylamine (14·5 g.) in ethyl acetate (100 ml.). The resulting clear yellow

[9] Cresswell and Wood, *J.*, 1960, 4768.
[10] Davoll and Evans, *J.*, 1960, 5041; Nielson and Wood, *J.*, 1962, 44.

solution was heated at 60—70° for 2 hr. and the white precipitate which formed was separated by filtration (hot) and washed with hot ethyl acetate. The combined filtrates were evaporated to dryness *in vacuo*, and the residue was dissolved in hot ethanol. Addition of ether precipitated the *uracil derivative* (8·65 g., 82%) which, recrystallized from ethanol, had m. p. 157° (Found: C, 42·8; H, 4·4; N, 10·6. $C_{18}H_{22}N_4O_{13}$ requires C, 43·0; H, 4·4; N, 11·1%).

8-D-*Glucosyl*-1,2,3,4,7,8-*hexahydro*-6-*methyl*-2,4,7-*trioxopteridine* (III; R = OH, R′ = D-glucosyl, R″ = Me).—5-Nitro-6-(2,3,4,6-tetra-*O*-acetyl-D-glucosylamino)uracil (4 g.) was hydrogenated in methanol (70 ml.) over Raney nickel. After removal of the catalyst, the solution was treated with ethyl pyruvate (4 ml.), and the whole refluxed under nitrogen for 2 hr. The resulting suspension was filtered, the filtrate evaporated to dryness, and the residue triturated with ether. The yellow product (4 g., 96%) was deacetylated by dissolution in hot ethanol (80 ml.) and gradual addition of ethanolic sodium ethoxide [from sodium (1 g.) in ethanol (40 ml.)] until the solution became turbid. Additional sodium ethoxide solution was added as required to keep the mixture alkaline during 10 min. at 60—70°. After cooling, the precipitate (2·4 g., 95%) was collected by centrifugation and washed with alcohol and ether. It was further purified by paper chromatography [0·5 g. per sheet of Whatman's No. 17, 46 × 57 cm.; Pr^nOH–1% aq. NH_3 (2 : 1)]. The band containing the product appeared as a strongly blue-fluorescent band under ultraviolet light (360 mμ); it was cut out and the material eluted from it with water. The yellow eluate was placed on a Dowex 2 ion-exchange column (OAc^-). The column was washed with water and the fluorescent material eluted with 0·25N-acetic acid. The eluate was concentrated *in vacuo*, passed through a small column of Dowex 50 ion-exchange resin (H^+), and evaporated to dryness *in vacuo*. The residue crystallized from water–acetone to give the *glucosylpteridine*, m. p. >190° (decomp.), rapid heating 194—198° (decomp.) (Found: C, 42·5; H, 5·0; N, 14·9. $C_{13}H_{16}N_4O_8,H_2O$ requires C, 41·7; H, 4·9; N, 15·0%).

Azomethine from 5-*Amino*-6-(2,3,4,6-*tetra*-O-*acetyl*-D-*glucosylamino*)*uracil* (IV; R = 2,3,4,6-tetra-*O*-acetyl-D-glucosyl).—5-Nitro-6-(2,3,4,6-tetra-*O*-acetyl-D-glucosyl)uracil (2·8 g.) was hydrogenated in methanol (60 ml.) over Raney nickel, the catalyst removed, ethyl glyoxylate hemiacetal (3 ml.) added to the filtrate, and the whole was refluxed under nitrogen for 2 hr. After concentration *in vacuo* and cooling, the solution (10 ml.) deposited light yellow crystals (1·3 g.) which were recrystallized from methanol (charcoal) to give the white, crystalline *azomethine*, m. p. 198—200° (Found: N, 9·9. $C_{22}H_{28}N_4O_{13}$ requires N, 10·1%).

8-D-*Glucosyl*-1,2,3,4,7,8-*hexahydro*-2,4,7-*trioxopteridine*.—The above compound (0·9 g.) in methanol was treated carefully with methanolic sodium methoxide to turbidity and heated gently for 15 min., additional sodium methoxide solution being added as required to keep the mixture just alkaline. The yellow precipitate (0·55 g., 83%) was collected and washed with alcohol. A further quantity (1·1 g.) of the same material was obtained from the mother-liquors of the azomethine preparation by the same treatment. These crude products were combined and purified by the procedure used for the glucosides described above except that elution from the Dowex 2 column (OAc^-) could not be effected with acetic acid; elution was accomplished, however, with 0·05—0·1N-hydrochloric acid containing a small amount of methanol. Evaporation of the eluate *in vacuo* below 50° yielded a residue which was crystallized from water–acetone to give the *glucosylpteridine*, m. p. >200° (decomp.) (Found: C, 41·0; H, 4·3; N, 14·5. $C_{12}H_{14}N_4O_8$ requires C, 41·0; H, 4·3; N, 16·0%).

2-*Amino*-6-*chloro*-3,4-*dihydro*-5-*nitro*-4-*oxopyrimidine*.[10]—Finely powdered 2-amino-6-chloro-3,4-dihydro-4-oxopyrimidine (5 g.) was dissolved in concentrated sulphuric acid (18 ml.) below 15°. The temperature was then lowered to −5° and concentrated nitric acid (5·6 ml.; *d* 1·5) was added while the temperature was maintained at 0°. The mixture was then allowed to warm to 25° and this temperature maintained for 25 min. with external cooling. The clear solution was poured on ice (30 g.); the crystalline nitro-compound (6 g., 92%) which separated was collected, washed with ice–water and dried; it darkened at >250° and melted at >350° (Found: C, 26·5; H, 2·0; Cl, 17·7. Calc. for $C_4H_3ClN_4O_3$: C, 25·2; H, 1·6; Cl, 18·6%).

2-*Amino*-3,4-*dihydro*-6-*methylamino*-5-*nitro*-4-*oxopyrimidine* (II; R = NH_2, R′ = NHMe).—A suspension of the chloronitropyrimidine (0·5 g.) in a small amount of warm methanol was treated with an excess of 40% aqueous methylamine, and the resulting thick slurry was heated at 100° for 10—30 min. Water was then added to dissolve the precipitate; acidification of the hot solution with 50% acetic acid gave a cream-coloured precipitate which was collected after cooling. The *nitropyrimidine* (0·37 g., 75%) was purified by dissolution in

7% aqueous ammonia (charcoal) and reprecipitation from the hot solution with acetic acid; it then had m. p. >350° (Found: C, 32·3; H, 3·6; N, 37·4. $C_5H_7N_5O_3$ requires C, 32·4; H, 3·8; N, 37·8%).

2-*Amino*-3,4-*dihydro*-5-*nitro*-4-*oxo*-6-*anilinopyrimidine* (II; R = NH_2, R′ = NHPh).—Prepared in the same way as the preceding compound from the chloronitropyrimidine (0·5 g.) and aniline (2 ml.) in ethanol (15 ml.), the *anilino-compound* (0·5 g.; 77%) was also purified by dissolution in aqueous 7% ammonia and precipitation from the hot solution with hydrochloric acid; it had m. p. 345—348° (decomp.) (Found: N, 27·9. $C_{10}H_9N_5O_3$ requires N, 28·3%).

2-*Amino*-3,4-*dihydro*-6-2′-*hydroxyethylamino*-5-*nitro*-4-*oxopyrimidine* (II; R = NH_2, R′ = $NH{\cdot}CH_2{\cdot}CH_2{\cdot}OH$).—In a similar preparation the chloronitropyrimidine (1 g.) in hot ethanol (20 ml.) treated with ethanolamine (4 ml.) yielded, on acidification and cooling, the *hydroxyethylamino-derivative* (0·93 g., 82%), m. p. 297—299° (decomp.) (from water) (Found: N, 32·0. $C_6H_9N_5O_4$ requires N, 32·5%).

2-*Amino*-3,4-*dihydro*-5-*nitro*-4-*oxo*-6-(2,3,4,6-*tetra*-O-*acetyl*-D-*glucosylamino*)*pyrimidine* (II; R = NH_2, R′ = 2,3,4,6-tetra-*O*-acetyl-D-glucosylamino).—Treatment of the above chloronitropyrimidine (10 g.) in dioxan (100 ml.) and ethanol (70 ml.) with 2,3,4,6-tetra-*O*-acetyl-D-glucosylamine (36 g.) at 100° for 2½ hr. gave a clear, then turbid, solution. The suspension was poured into water, and the solution was adjusted to pH 2—3 with 2N-hydrochloric acid and extracted with chloroform. The chloroform extract was treated with a small amount of dimethylformamide and dried (Na_2SO_4). The residue obtained on evaporation of the solution to dryness was dissolved in a small amount of chloroform, and ethanol was added. The precipitate which separated on cooling was filtered off and the filtrate treated with light petroleum (b. p. 40—60°). The resulting precipitate (21·5 g., 81%) was collected, dried, and recrystallized from dimethylformamide, to give the *glucosylpyrimidine*, m. p. 295—300° (decomp.) (Found: C, 42·8; H, 4·8; N, 14·0. $C_{18}H_{23}N_5O_{12}$ requires C, 43·1; H, 4·8; N, 14·0%).

2-*Amino*-3,4,7,8-*tetrahydro*-8-*methyl*-4,7-*dioxopteridine*-6-*carboxylic Acid* (III; R = NH_2, R′ = Me, R″ = CO_2H).—2-Amino-3,4-dihydro-6-methylamino-5-nitro-4-oxopyrimidine (0·5 g.) in hot 3·5% aqueous ammonia was reduced with a small amount of dithionite. The clear colourless solution was adjusted to pH 6 with acetic acid, treated with diethyl mesoxalate (0·5 ml.), and heated at 100° for 5 min. The resulting yellow precipitate was collected, dissolved in 0·5N-sodium hydroxide, heated for a short time, and filtered into hot dilute hydrochloric acid. The *pteridine* was precipitated as yellow crystals (200 mg., 31%), m. p. >350° (Found: C, 39·7; H, 3·3; N, 28·4. $C_8H_7N_5O_4,\frac{1}{2}H_2O$ requires C, 39·1; H, 3·3; N, 28·5%).

2-*Amino*-3,4,7,8-*tetrahydro*-8-2′-*hydroxyethyl*-6-*methyl*-4,7-*dioxopteridine* [5] (III; R = NH_2, R′ = $CH_2{\cdot}CH_2{\cdot}OH$, R″ = Me).—2-Amino-3,4-dihydro-6-2′-hydroxyethylamino-5-nitro-4-oxopyrimidine (2·2 g.) was reduced in water–methanol over Raney nickel, and ethyl pyruvate (2 ml.) was added to the filtered solution. After refluxing for 2 hr. and evaporation to small volume, the solution was cooled and the solid which separated was collected and recrystallized (charcoal) from water several times, to give the pale yellow *hydroxyethylpteridine* (1·45 g., 60%), m. p. 332—334° (decomp.) (Found: C, 45·9; H, 4·8; N, 29·3. $C_9H_{11}N_5O_3$ requires C, 45·6; H, 4·7; N, 29·5%).

2-*Amino*-3,4,7,8-*tetrahydro*-6-*methyl*-4,7-*dioxo*-8-*phenylpteridine* (III; R = NH_2, R′ = Ph, R″ = Me).—2-Amino-3,4-dihydro-5-nitro-4-oxo-6-phenylaminopyrimidine (0·63 g.) was reduced in aqueous ammonia (70 ml.) with sodium dithionite at 100°; the hot filtered solution was treated with ethyl pyruvate (1 ml.) and heated at 100° for 1 hr. during which time the volume was reduced by evaporation. The yellow precipitate (0·49 g., 71%) obtained on cooling of the concentrated solution was collected, dried, and purified by dissolution in aqueous ammonia (charcoal) and reprecipitation, to give the *phenylpteridine* as yellow crystals, m. p. >350° (Found: N, 25·6. $C_{13}H_{11}N_5O_2$ requires N, 26·0%).

Ethyl 2-*Amino*-3,4,7,8-*tetrahydro*-8-2′-*hydroxyethyl*-4,7-*dioxopteridine*-6-*carboxylate* (III; R = NH_2, R′ = $CH_2{\cdot}CH_2{\cdot}OH$, R″ = CO_2Et).—In a similar preparation, the appropriate nitro-compound (2·2 g.) was reduced and the reduction product treated with diethyl mesoxalate (2·2 ml.), to give the *hydroxyethylpteridine* (2·65 g., 88%), m. p. 313—315° (decomp.) (Found: N, 23·4. $C_{11}H_{13}N_5O_5$ requires N, 23·7%).

2-*Amino*-3,4,7,8-*tetrahydro*-8-2′-*hydroxyethyl*-4,7-*dioxopteridine*-6-*carboxylic Acid* (III; R = NH_2, R′ = $CH_2{\cdot}CH_2{\cdot}OH$, R″ = CO_2H).—The above ethyl ester (1·5 g.) was hydrolyzed for 1 hr. at 100° in M-sodium hydrogen carbonate (70 ml.). The filtered solution was added slowly to dilute hydrochloric acid, to yield the *carboxylic acid* (1·3 g., 96%); purification

was by reprecipitation from alkaline solution (charcoal) with dilute hydrochloric acid; the m. p. was >350° (Found: C, 40·4; H, 3·8; N, 24·9. $C_9H_9N_5O_5$ requires C, 40·4; H, 3·4; N, 26·2%).

2-Amino-8-D-glucosyl-3,4,7,8-tetrahydro-6-methyl-4,7-dioxopteridine (III; R = NH_2, R′ = D-glucosyl, R″ = Me).—2-Amino-3,4-dihydro-5-nitro-4-oxo-6-(2,3,4,6-tetra-*O*-acetyl-D-glucosylamino)pyrimidine (4 g.) in methanol (70 ml.) was reduced over Raney nickel, the filtered solution treated with ethyl pyruvate (4 ml.), and the whole refluxed for 2 hr. under nitrogen. After hot filtration, the red solution was evaporated *in vacuo* to give a syrup which was dissolved in chloroform, and the chloroform was removed *in vacuo*. After several repetitions of this procedure, the gummy product was triturated with ether to yield a powder (3·8 g., 90%) which was quickly collected and dried over P_2O_5. This material (3·3 g.) was deacetylated by sodium ethoxide, as in the procedure described for deacetylation of the 2,4,7-trioxo-compound, and the yellow product (2 g., 84%) was collected by centrifugation. The solid was purified in the same way as the 2,4,7-trioxo-analogue, by using paper chromatography and a Dowex 2 ion-exchange column from which it was eluted with 0·2N-acetic acid. The eluate was evaporated to dryness and the residue crystallized from water–acetone to give the pale yellow *glucosyl derivative*, m. p. >240° (decomp.) (Found: C, 41·6; H, 5·2; N, 17·4. $C_{13}H_{17}N_5O_7 \cdot H_2O$ requires C, 41·8; H, 5·1; N, 18·8%). The pK_a value for this compound (Table 1) is the same as that of the homologue (III; R = NH_2, R′ = $CH_2 \cdot CH_2 \cdot OH$, R″ = Me).[5] However, as expected it is 0·4—0·5 pK_a unit higher than that of the compound (III; R = NH_2, R′ = D-glycosyl, R″ = H) described below. This effect is ascribed to the hyperconjugative effect of the 6-methyl group.

2-Amino-8-D-glucosyl-3,4,7,8-tetrahydro-4,7-dioxopteridine (III; R = NH_2, R′ = D-glucosyl, R″ = H).—2-Amino-3,4-dihydro-5-nitro-4-oxo-6-(2,3,4,6-tetra-*O*-acetyl-D-glucosylamino)pyrimidine (3·4 g.) was hydrogenated as above, the hydrogenation mixture treated with ethyl glyoxylate hemiacetal (3 ml.), and the whole refluxed under nitrogen for 30 min. Methanolic sodium methoxide was then added dropwise until the pH of the mixture was 7—8 and the whole was then refluxed for a further 30 min. To effect deacetylation, more sodium methoxide was added until a thick precipitate began to separate and the whole was heated for a further 10 min. at 100°, being kept alkaline by addition of sodium methoxide as necessary. The yellow precipitate was collected after cooling, washed with ethanol and acetone, and dried. This product (3·85 g.) was purified in the same way as the other glucosides; it was finally washed off the Dowex 2 column (OAc^-) with 0·1N-acetic acid. Evaporation of the eluate and purification of the residue by solution in aqueous ammonia (charcoal) and precipitation with 5N-acetic acid gave the *glucosylpteridine* (1·73 g., 75%) which, recrystallized from a small amount of water, decomposed at >270° but did not melt (Found: C, 41·8; H, 4·8; N, 20·8. $C_{12}H_{15}N_5O_7$ requires C, 42·2; H, 4·4; N, 20·5%).

2-Amino-8-D-glucosyl-3,4,7,8-tetrahydro-4,7-dioxopteridine-6-carboxylic Acid (III; R = NH_2, R′ = D-glucosyl, R″ = CO_2H).—2-Amino-3,4-dihydro-5-nitro-4-oxo-6-(2,3,4,6-tetra-*O*-acetyl-D-glucosylamino)pyrimidine (4 g.) was hydrogenated in methanol (70 ml.) over Raney nickel, the mixture was filtered, and the filtrate was refluxed with diethyl mesoxalate (1·5 g.) for 2 hr. under nitrogen. The red solution was filtered and then evaporated to dryness *in vacuo*. The gummy residue was triturated with ether and the yellow solid (3·1 g., 67%) was collected and dried. After deacetylation in the usual way, the yellow sodium salt of the ester (1·8 g.) was collected, washed with alcohol and acetone, and hydrolysed with 0·5M-sodium hydrogen carbonate (*ca.* 100 ml.; 2½ hr. at 100°). When the solution was adjusted to pH 6 (5N-acetic acid), the sodium salt (1·1 g.) of the desired product was precipitated. It was converted into the free acid by crystallization from 0·01N-hydrochloric acid, giving the *glucosylpteridine*, decomp. >250° (Found: C, 40·4; H, 4·4; N, 17·7. $C_{13}H_{15}N_5O_9$ requires C, 40·5; H, 3·9; N, 18·2%).

Decarboxylation of this compound by sodium amalgam in water to give the previously prepared glucoside (III; R = NH_2, R′ = D-glucosyl, R″ = H) can be demonstrated paper chromatographically.

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GENETICS FOUNDATION, THE UNIVERSITY OF TEXAS,
AUSTIN 12, TEXAS, U.S.A.

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