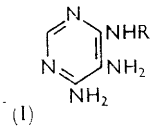
976. *The Synthesis of 9-Glycitylpurines, 3-Glycityl-[1,2,3]-triazolo[d]-pyrimidines, 8-Glycitylpteridines, and 10-Glycitylbenzo[g]pteridines, including Riboflavin and Riboflavin 2-Imine.*

By J. Davoll and D. D. Evans.

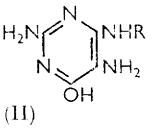
5-Amino-4-glycitylaminopyrimidines, prepared from appropriate 5-nitro- or 5-nitroso-derivatives, have been converted by ring closure into the compounds named in the title.

The structural similarity between purine nucleosides, pteridines, and riboflavin has been noted by several authors,[1,2] who have also suggested that 8-glycosyl- or 8-glycitylpteridines may be involved as biosynthetic intermediates. Recent work [3] has shown that adenine and guanine can serve as precursors of the pyrimidine portion of riboflavin, and that 8-D-ribitylpteridines are also involved in its biosynthesis. The aim of the present work was the preparation of compounds which might be intermediates in these biosynthetic reactions or might act as antimetabolites to the compounds actually involved.

Since all the required compounds (I—III) contain the carbon–nitrogen skeleton of a 5-amino-4-glycitylaminopyrimidine as part of their structures, the preparation of this class of compound was investigated. Suitable precursors were readily obtained from appropriate chloropyrimidines and glycitylamines in boiling aqueous or aqueous-ethanolic solution; thus, 6-amino-4-chloro-5-nitropyrimidine and D-ribitylamine (2 mols.) gave 6-amino-5-nitro-4-D-ribitylaminopyrimidine (83% yield), which on catalytic hydrogenation afforded the triamine (Ia); the sorbitylamino-compound (Ib) was similarly prepared by starting from D-glucitylamine.



(I)



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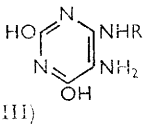


(III)

a: R = D-ribityl. b: R = D-sorbityl.

4-Alkylamino-2,5-diamino-6-hydroxypyrimidines (II; R = alkyl) have been prepared by heating 2-amino-4-chloro-6-hydroxypyrimidine with an excess of alkylamines at 120—140° and then introducing a 5-amino-group by nitrosation [1] or by azo-coupling [11] followed by reduction. Alternatively, 2-amino-4-chloro-6-hydroxy-5-phenylazopyrimidine

[1] Forrest, Hull, Rodda, and Todd, *J.*, 1951, 3.
[2] Elion, Ciba Foundation Symposium on Chemistry and Biology of Pteridines, J. and A. Churchill, London, 1954, p. 49.
[3] Stadtman, "The Biosynthesis and Degradation of Riboflavin," Symposium No. XI, IVth Internat. Congr. of Biochemistry, Vienna, 1958, Pergamon Press, London, 1960; see also refs. 4—10 and other references given therein.

has been used as starting material,[12] the phenylazo-group providing a potential 5-amino-group and also activating the chlorine atom. In the present work a reactive halogenopyrimidine was obtained by nitration of 2-amino-4-chloro-6-hydroxypyrimidine; the resulting 5-nitro-derivative reacted readily with glycitylamines in boiling water to give, after reduction, the 5-amino-compounds (IIa and b).

A simple preparation of 4-chlorouracil (from 2,4,6-trichloropyrimidine) and its conversion into 4-alkylaminouracils has been reported.[13] Similarly, 4-chlorouracil was condensed with glycitylamines in boiling water, and the products were converted, by nitrosation and reduction, into the 5-amino-compounds (IIIa and b); preparation of the ribitylamine (IIIa) by similar methods [4-6,9] and also by way of 4-chloro-5-nitrouracil [14] has been described since the present work was carried out.

Purines and [1,2,3]-*Triazolo*[d]*pyrimidines.*—9-D-Ribityl- and 9-D-sorbityl-adenine were prepared by cyclisation of the N^5-formyl derivatives of the amines (Ia and b) in dilute alkali; [15] on deamination with nitrous acid they afforded the corresponding 9-glycitylhypoxanthines. The corresponding 9-glycitylguanines were obtained by heating the sulphates of compounds (IIa and b) in formamide [16] and converted into 9-glycitylxanthines by deamination.

The 8-aza-analogues of the above compounds (*i.e.*, 3-glycityl-[1,2,3]-triazolo[*d*]-pyrimidines) were prepared by similar reactions, except that cyclisation of the bases (I) and (II) was carried out with nitrous acid. The yields of 5,7-dihydroxy-3-glycityl-[1,2,3]-triazolo[*d*]pyrimidines obtained by deamination of the 5-amino-7-hydroxy-compounds were low, however, and the dihydroxy-compounds were better prepared from the compounds (IIIa and b) with nitrous acid.

Pteridines.—Four types of pteridine derivative were prepared, with the general formulæ (IV—VII).

2,8-Dihydro-4-hydroxy-6,7-dimethyl-2-oxo-8-D-ribitylpteridine (IV; R = D-ribityl, X = O) has been isolated from *E. ashbyii* and from *A. gossypii*, and has been named Compound G from its green fluorescence in solution.[3] It can be converted into riboflavin enzymically or by reaction with biacetyl, and has recently been synthesised [4,6,8,14] by methods similar to that described here. We found that the pyrimidine (IIIa) reacted with biacetyl in aqueous solution, to give compound (IV; R = D-ribityl, X = O) in good yield; and the sorbityl analogue (IV; R = D-sorbityl, X = O) from (IIIb), the 2-imino-compound (IV; R = D-ribityl, X = NH) from (IIa), and the 6,7-diethyl analogue from (IIIa) and hexane-3,4-dione, were similarly prepared.

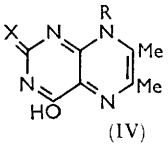
Another pteridine [3] isolated from *E. ashbyii* and from *A. gossypii* has been named Compound V (or Compound A), and has been shown by synthesis [5,7,8,9] to have the structure (V; R = D-ribityl, R′ = OH). It is apparently not directly involved in riboflavin biosynthesis.[3] We prepared analogous 2-amino-compounds (V; R = D-ribityl and D-sorbityl, R′ = NH_2) from pyrimidines (IIa and b) by condensation with pyruvic acid.

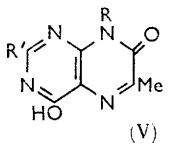
Derivatives of type (VI) were prepared from the glycitylaminopyrimidines (IIa and b) and (IIIa) by condensation with ethyl glyoxylate hemiacetal. The first products of the reaction were the azomethines (VIII), which cyclised to pteridines in hot sodium hydrogen

[4] Masuda, Kishi, Asai, and Kuwada, *Chem. and Pharm. Bull.* (*Japan*), 1959, **7**, 361.
[5] Masuda, Kishi, Asai, and Kuwada, *Chem. and Pharm. Bull.* (*Japan*), 1959, **7**, 366.
[6] Maley and Plaut, *Fed. Proc.*, 1958, **17**, 168; *J. Biol. Chem.*, 1959, **234**, 641.
[7] Plaut and Maley, *Arch. Biochem. Biophys.*, 1959, **80**, 219.
[8] McNutt, *Fed. Proc.*, 1959, **18**, 286.
[9] McNutt, *J. Amer. Chem. Soc.*, 1960, **82**, 217.
[10] Korte and Aldag, *Annalen*, 1959, **628**, 144.
[11] Elion and Hitchings, *J. Amer. Chem. Soc.*, 1953, **75**, 4311.
[12] (*a*) Boon and Leigh, *J.*, 1951, 1497; (*b*) Taylor and Loux, *J. Amer. Chem. Soc.*, 1959, **81**, 2474; (*c*) Koppel, O'Brien, and Robins, *ibid.*, p. 3046.
[13] Langley and Walpole, Seventh Internat. Cancer Congr., London, 1958.
[14] Cresswell and Wood, *Proc. Chem. Soc.*, 1959, 387.
[15] Baker, Joseph, and Schaub, *J. Org. Chem.*, 1954, **19**, 631.
[16] Robins, Dille, Willits, and Christensen, *J. Amer. Chem. Soc.*, 1953, **75**, 263.

carbonate solution.[17] The amino-compounds (VI; R′ = NH_2) are derivatives of isoxanthopterin; their alkyl analogues (VI; R = alkyl, R′ = NH_2) have previously been obtained by decarboxylation of their 6-carboxylic acids.[11]

The 8-glycityl-leucopterins (VII; R = D-ribityl and D-sorbityl) were obtained by boiling aqueous solutions of the pyrimidines (IIa and b) with ethyl oxalate; more drastic conditions have previously been used for this type of pteridine synthesis.[18]


(IV)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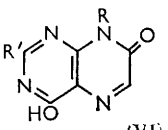
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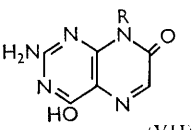
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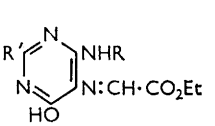
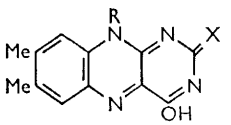

(VIII)

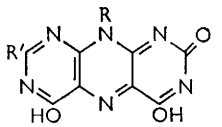
10-*Ribitylbenzo*[g]*pteridines* (*Isoalloxazines*).—Previous syntheses of riboflavin (IX; R = D-ribityl, X = O) have started from an *N*-D-ribitylaniline derivative, with the exception of a very recent synthesis [14] from the pyrimidine (IIIa) and di- or tri-meric forms of biacetyl. The use of 4,5-dimethyl-1,2-benzoquinone dimer for the synthesis of benzo[g]-pteridines [19] makes possible another approach to 10-substituted derivatives, and, in fact, " lumiflavin 2-imine " (IX; R = Me, X = NH) has been synthesised from an appropriate methylaminopyrimidine and the dimeric quinone.[20] Condensation of the pyrimidine (IIIa) with the dimeric quinone in boiling aqueous ethanol afforded a 25% yield of riboflavin, identified by mixed melting point, ultraviolet spectrum, and microbiological assay.

Application of the same reaction to the ribitylaminopyrimidine (IIa) afforded " riboflavin 2-imine " (IX; R = ribityl, X = NH) in similar yield; the compound was recently obtained as an orange powder from methyl 3,4-dihydro-6,7-dimethyl-3-oxo-4-D-ribityl-quinoxaline-2-carboxylate and guanidine.[20] We originally obtained the compound as an amorphous powder, but after purification by the method given by Cresswell *et al.*[20] it formed orange needles, agreeing in ultraviolet maxima with the reported values.[20]

Pyrimidopteridines.—The glycitylaminopyrimidines (IIa), (IIIa), and (IIIb) condensed readily with alloxan in boiling dilute acetic acid to give yellow products with an intense blue fluorescence in dilute solution. The structure (X) for these compounds is preferred to the possible alternative (XI), by analogy with similar compounds.[12b,21]

Ultraviolet Spectra.—The ultraviolet spectra of the 9-glycitylpurines and 3-glycityl-[1,2,3]-triazolo[*d*]pyrimidines are closely similar to those of the corresponding glycosyl


(IX)


(X)


(XI)

derivatives,[22] and details have been omitted. A minor exception is that the 9-glycityl-guanines at pH 1 show two peaks (at 254 and 278 mμ; ε 11,700 and 7800 respectively), rather than a peak (at 255 mμ) and a shoulder (at 280 mμ) as shown by guanosine. The

[17] Pfleiderer, *Chem. Ber.*, 1957, **90**, 2588.
[18] Albert, Brown, and Wood, *J.*, 1956, 2066.
[19] Bardos, Olsen, and Enkoji, *J. Amer. Chem. Soc.*, 1957, **79**, 4704.
[20] Cresswell, Hill, and Wood, *J.*, 1959, 698.
[21] Taylor, Cain, and Loux, *J. Amer. Chem. Soc.*, 1954, **76**, 1874; Taylor, Loux, Falco, and Hitchings, *ibid.*, 1955, **77**, 2243.
[22] Chargaff and Davidson (ed.), " The Nucleic Acids," Academic Press New York, 1955, Vol. I, p. 493; Davoll, *J.*, 1958, 1593.

Table gives the spectral characteristics of other ribityl compounds prepared; those of the corresponding sorbityl derivatives are virtually identical.

Ultraviolet absorption maxima (mμ) ($10^{-3}\varepsilon$ *in parentheses*).

Compound (R = D-ribityl)	In HCl	At pH 6·8	In NaOH
(IV; X = NH) ...	257 (14·1), 285 (12·4) 400 (13·2)	230 (15·1), 268 (14·8) 318 (5·0), 410 (7·4)	233 (18·2), 271 (8·4) 282 (8·4), 312 (8·1)
(IV; X = O)	257 (14·2), 275 (sh) (9·7), 406 (10·5)	258 (15·0), 275 (10·5), 404 (11·5)	229 (22·4), 280 (12·7), 313 (9·1)
(V; R′ = NH_2) ...	218 (32·4), 294 (11·4), 339 (13·0)	220 (30·4), 294 (10·8), 340 (12·9)	258 (11·8), 281 (5·0), 353 (13·8)
(VI; R′ = NH_2) ...	220 (32·8), 257 (4·1), 219 (9·5), 344 (13·8)	219 (34·1), 256 (4·5), 290 (8·8), 342 (13·3)	259 (11·9), 280 (sh) (3·6), 358 (15·1)
(VI; R′ = OH) ...	278 (11·2), 332 (10·8),	260 (5·2), 287 (9·1), 350 (12·8)	260 (9·1), 280 (5·0), 362 (13·0)
(VII; R′ = NH_2)	230 (17·0), 304 (13·8), 331 (7·9)	228 (17·6), 303 (13·4), 332 (8·1)	233 (14·3), 300 (9·2), 350 (10·8)
(VIII; R′ = NH_2)	218 (17·3), 270 (11·4)	260 (9·1), 275 (sh) (7·2), 370 (5·8)	250 (9·7), 275 (sh) (5·8), 363 (4·9)
(IX; X = NH) ...	225 (27·2), 270 (31·9), 387 (12·3), 441 (14·0)		
Riboflavin 2-imine [20]	224 (27·0), 270 (32·1), 386 (12·3), 442 (14·1)		
(IX; X = O)	223 (30·6), 266 (33·4) 371 (10·6), 440 (11·8)		
Riboflavin [20]	223 (35·5), 267 (35·5), 376 (10·7), 445 (11·5)		
(X; R = NH_2) ...	246 (42·4), 293 (10·9), 427 (36·2)	243 (37·1), 291 (12·8), 431 (37·3)	242 (33·5), 275 (14·8), 437 (38·5)
(X; R = OH)	233 (22·8), 241 (23·1) 280 (10·9), 426 (26·5)	241 (36·6), 290 (9·7), 426 (32·3)	230 (28·9), 272 (12·2) 435 (32·3)

Biological Properties.—Most of the above compounds were tested against a number of pathogenic and non-pathogenic micro-organisms, and several against sarcoma 180 in mice, but no significant effects were observed.

Experimental

Except where otherwise stated, samples were dried for analysis in a high vacuum at 100°.

4-*Glycitylaminopyrimidines.*

D-*Ribitylamine.*—This was prepared initially by reductive amination of D-ribose [23] and used as a crude syrup, but the following modification of the method of Kagan *et al.*[24] gave more reproducible results and a cleaner product.

Benzylamine (43 g., 0·4 mole) was added to a suspension of D-ribose (60 g., 0·4 mole) in methanol (400 c.c.). After 20 hr., the clear solution was hydrogenated over platinum oxide (2 g.), 8·5 l. of hydrogen being absorbed in 24 hr. After filtration and evaporation to 200 c.c., ethyl acetate (600 c.c.) was added, giving *N*-benzyl-D-ribitylamine (55·3 g., 57%), m. p. 99—102° (lit., 46%, m. p. 102—103°). On hydrogenation in methanol (500 c.c.) over palladised charcoal, this (48 g.) absorbed 4·7 l. in 7 hr. After removal of catalyst and most of the solvent, the residue was evaporated with water to remove toluene, then used directly as an aqueous solution containing a calculated 30 g. of D-ribitylamine.

D-*Glucamine* [D-*Sorbitylamine*].—This was prepared either directly from glucose [25] or through the *N*-benzyl derivative.[24]

2-*Amino*-4-*chloro*-6-*hydroxy*-5-*nitropyrimidine.*—2-Amino-4-chloro-6-hydroxypyrimidine [1] (13 g.) was added all at once with vigorous stirring to a mixture of fuming nitric acid (90 c.c.) and concentrated sulphuric acid (90 c.c.) at 25°; a clear yellow solution was formed and the temperature rose to 38°. After 30 min. the mixture was added to crushed ice (*ca.* 500 g.), and the yellow solid (14·8 g., 87%) collected and washed with water. It could not be recrystallised

[23] Holly, Peel, Cahill, Koniuszy, and Folkers, *J. Amer. Chem. Soc.*, 1952, **74**, 4047.
[24] Kagan, Rebenstorf, and Heinzelman, *J. Amer. Chem. Soc.*, 1957, **79**, 3541.
[25] Holly, Peel, Mozingo, and Folkers, *J. Amer. Chem. Soc.*, 1950, **72**, 5416.

but was sufficiently pure for further use (Found: C, 25·1; H, 2·4; N, 28·3; Cl, 17·6. Calc. for $C_4H_3ClN_4O_3$: C, 25·2; H, 1·6; N, 29·4; Cl, 18·6%). The dried compound is fairly stable, but a sample kept for a year appeared to have been partly hydrolysed by atmospheric moisture.

6-*Chlorouracil*.[13]—2,4,6-Trichloropyrimidine (74·5 g., 0·41 mole) and a solution of sodium hydroxide (65 g., 1·64 moles) in water (650 c.c.) were stirred under reflux on the steam-bath for 1 hr. To the hot suspension of crystalline material was added concentrated hydrochloric acid (160 c.c.), giving a clear solution from which 6-chlorouracil (48·5 g., 82%), m. p. 292—296° (decomp.), separated on cooling [lit., m. p. 300° (decomp.)] (Found: N, 18·5. Calc. for $C_4H_3ClN_2O_2$: N, 19·1%).

6-*Amino*-5-*nitro*-4-D-*ribitylaminopyrimidine*.—4-Amino-6-chloro-5-nitropyrimidine [26] (17·5 g., 0·1 mole), D-ribitylamine (30·2 g., 0·2 mole), water (150 c.c.), and ethanol (950 c.c.) were boiled under reflux for 3 hr. Water (500 c.c.) was then added and the mixture refluxed for a further 2½ hr. and filtered hot, with charcoal. The *ribitylamino-compound* (24 g., 83%) separated on cooling as needles, m. p. 212—214°, raised to 214—216° by recrystallisation from water (Found: C, 37·1; H, 5·5; N, 24·0. $C_9H_{15}N_5O_6$ requires C, 37·4; H, 5·2; N, 24·2%).

6-*Amino*-5-*nitro*-4-D-*sorbitylaminopyrimidine*.—Prepared in a similar manner, from D-sorbitylamine, the *sorbitylamino-compound* (88% yield) formed plates (from water), m. p. 211—212° (Found: C, 37·6; H, 5·3; N, 22·0. $C_{10}H_{17}N_5O_7$ requires C, 37·6; H, 5·4; N, 21·9%).

5,6-*Diamino*-4-D-*ribitylamino- and* 5,6-*Diamino*-4-D-*sorbitylamino-pyrimidine* (Ia *and* b).—These compounds were not isolated, but were prepared by hydrogenation of the above nitro-compounds over palladised charcoal, either in 90% formic acid for the preparation of purines, or in 50% acetic acid for the preparation of [1,2,3]-triazolo[*d*]pyrimidines.

2-*Amino*-6-*hydroxy*-5-*nitro*-4-D-*ribitylaminopyrimidine*.—2-Amino-4-chloro-6-hydroxy-5-nitropyrimidine (5 g.), D-ribitylamine (7·9 g.), and water (30 c.c.) were boiled together under reflux for 4 hr. The solution was filtered hot (charcoal), giving the *ribitylamino-compound* (5·9 g., 74%) as very pale yellow needles, m. p. 245—246° (decomp.; sinters above 230°) (Found: C, 35·9; H, 5·5; N 22·7. $C_9H_{15}N_5O_7$ requires C, 35·4; H, 4·95; N, 23·0%). In some preparations the product was partly gelatinous, and was best collected and washed by centrifugation. These preparations had lower m. p.s (*ca.* 224°) but could be used without purification.

2-*Amino*-6-*hydroxy*-5-*nitro*-4-D-*sorbitylaminopyrimidine*.—Prepared similarly from D-sorbitylamine, the *sorbitylamino-compound* (71% yield) formed needles (from water), m. p. 220—223° (decomp.) (Found: C, 35·8; H, 5·6; N, 20·4. $C_{10}H_{17}N_5O_8$ requires C, 35·8; H, 5·1; N, 20·9%). Like the ribityl compound, the material sometimes separated in gelatinous form.

2,5-*Diamino*-6-*hydroxy*-4-D-*ribitylamino- and* 2,5-*Diamino*-6-*hydroxy*-4-D-*sorbitylamino-pyrimidine* (IIa and b).—These were prepared by hydrogenation of the above nitro-compounds with palladised charcoal, either in 50% acetic acid at room temperature or in water at 45—50°; in both cases the compounds dissolved as reduction proceeded. The products were used directly without isolation, any necessary evaporation being carried out at 15 mm. in nitrogen.

Barium Salt of 2,6-*Dihydroxy*-5-*nitroso*-4-D-*ribitylaminopyrimidine*.—6-Chlorouracil (13·9 g.), D-sorbitylamine (28·5 g.), and water (90 c.c.) were boiled under reflux for 11 hr., then evaporated to half-volume and treated with ethanol (300 c.c.). The mixture was kept overnight at 0°, the supernatant liquid decanted, and the residual gum washed with ethanol, dried, and dissolved in hot water (135 c.c.). The solution was cooled to 5°, and barium nitrite monohydrate (26·6 g.) added. When the barium nitrite had dissolved, glacial acetic acid (40 c.c.) was added; the temperature rose to 35—40°, some gas was evolved, and solid began to separate. Collected after 18 hr. at 0°, washed with water, and dried at room temperature, the *barium salt* (22·5 g., 58%) was obtained as a hexahydrate [Found: N, 13·3; Ba, 16·4. $(C_9H_{13}N_4O_7)_2Ba,6H_2O$ requires N, 13·6; Ba, 16·7%].

5-*Amino*-2,6-*dihydroxy*-4-D-*ribitylaminopyrimidine* (IIIa).—To a solution of the above barium salt in hot water (12 c.c./g.) was added the calculated quantity of N-sulphuric acid. After removal of barium sulphate, the filtrate was hydrogenated with palladised charcoal to give an aqueous solution of the 5-amino-compound. Any necessary evaporation was carried out at 15 mm. under nitrogen.

2,6-*Dihydroxy*-4-D-*sorbitylaminopyrimidine*.—6-Chlorouracil (9·3 g.), D-sorbitylamine (23 g.), and water (50 c.c.) were boiled under reflux for 6 hr., diluted to 90 c.c., and filtered hot (charcoal). On cooling, the *sorbitylamino-compound* (12·8 g., 69%) separated as a crystalline powder, m. p.

[26] Boon, Jones, and Ramage, *J.*, 1951, 96.

236—237° (decomp.), unchanged by recrystallisation (Found: C, 41·1; H, 6·0; N, 13·8. $C_{10}H_{17}N_3O_7$ requires C, 41·2; H, 5·9; N, 14·4%).

5-*Amino*-2,6-*dihydroxy*-4-D-*sorbitylaminopyrimidine* (IIIb).—A solution of the above compound (1·45 g., 5 mmoles) and barium nitrite monohydrate (0·62 g.) in hot water (25 c.c.) was cooled rapidly to 25° and treated with acetic acid (1·7 c.c.). After 18 hr. the gelatinous solid was dissolved by heating the mixture to 70° and N-sulphuric acid (5 c.c.) was added. After filtration, the solution was hydrogenated with palladised charcoal and used, after removal of catalyst, without isolation of the product. Any necessary evaporation was carried out at 15 mm. under nitrogen.

Purines and [1,2,3]-*Triazolo*[d]*pyrimidines.*

9-D-*Ribityladenine*.—A solution of compound (Ia) prepared from the nitro-compound (2·9 g.) in 90% formic acid (40 c.c.) was boiled for 1 hr. under reflux, then evaporated to dryness, and the residue was evaporated three times with water and dried. The resulting solid was heated under reflux for 15 min. with N-sodium hydroxide (50 c.c.) and ethanol (50 c.c.), then ethanol (100 c.c.) was added and the solution filtered and cooled, to give 9-D-*ribityladenine* (1·5 g., 55%), m. p. 190—195°, raised to 209—210° by recrystallisation from water (Found: C, 44·8; H, 5·7; N, 25·8. $C_{10}H_{15}N_5O_4$ requires C, 44·6; H, 5·6; N, 26·0%).

9-D-*Sorbityladenine*.—This was similarly prepared from compound (Ib) (from 4·75 g. of nitro-compound), except that the alkaline solution was evaporated to dryness, the residue dissolved in water (10 c.c.), and the product (3·6 g.) precipitated with ethanol (100 c.c.). Recrystallisation from water gave 9-D-*sorbityladenine* (2·45 g., 55%) as needles, m. p. 198—200° (after drying at 100°; undried samples also sintered at 92—100°) (Found: C, 42·3; H, 5·7; N, 22·8. $C_{11}H_{17}N_5O_5,\frac{1}{2}H_2O$ requires C, 42·8; H, 5·9; N, 22·7%).

9-D-*Ribitylhypoxanthine*.—To a solution of 9-D-ribityladenine (3·2 g.) in 10% acetic acid (85 c.c.) was added sodium nitrite (8·3 g.) in water (20 c.c.). After 20 hr. the product was isolated by precipitation with excess of aqueous lead acetate and ammonia, treatment of the lead salt in 20% acetic acid with hydrogen sulphide, and evaporation of the filtrate. Trituration of the product with aqueous ethanol gave 9-D-ribitylhypoxanthine (2·1 g., 65%) as needles, m. p. 207—209°, raised to 209—210° by recrystallisation from 85% ethanol (Found: C, 44·0; H, 5·1; N, 20·4. $C_{10}H_{14}N_4O_5$ requires C, 44·4; H, 5·2; N, 20·7%).

9-D-*Sorbitylhypoxanthine*.—Similarly prepared (50% yield), the *sorbityl compound* formed needles (from water), m. p. 214—216° (Found: C, 44·4; H, 5·9; N, 18·4. $C_{11}H_{16}N_4O_6$ requires C, 44·0; H, 5·4; N, 18·7%).

9-D-*Ribitylguanine*.—To a solution of the compound (IIa) (3 mmoles) in 50% acetic acid (30 c.c.) was added N-sulphuric acid (3 c.c.). After removal of solvent the dried residue was boiled under reflux for 15 min. with formamide (7 c.c.). The cooled solution was diluted with water, and the product isolated through the lead salt. 9-D-*Ribitylguanine* (0·32 g., 37%) formed a crystalline powder, m. p. 279—280° (decomp.), after recrystallisation from water (Found: C, 41·8; H, 5·8; N, 24·3. $C_{10}H_{15}N_5O_5$ requires C, 42·1; H, 5·3; N, 24·6%).

9-D-*Sorbitylguanine*.—Similarly prepared, 9-D-*sorbitylguanine* (41% yield) formed leaflets (from water), m. p. 254—255° (decomp.) (Found: C, 40·3; H, 6·2; N, 21·1. $C_{11}H_{17}N_5O_6,\frac{1}{2}H_2O$ requires C, 40·7; H, 5·6; N, 21·6%).

9-D-*Ribitylxanthine*.—A solution of 9-D-ribitylguanine (0·72 g.) and barium nitrite monohydrate (2·5 g.) in hot water (20 c.c.) was cooled rapidly to 45° and treated with acetic acid (2·5 c.c.). After 5 hr. N-sulphuric acid (20·2 ml.) was added and after a further 18 hr. the mixture was filtered and evaporated to dryness. A solution of the residue in hot 70% ethanol (10 c.c.) deposited 9-D-*ribitylxanthine* (0·33 g., 46%) as a white powder, m. p. 150° (decomp.; sinters above 142°) after recrystallisation from 75% ethanol (Found: C, 38·3; H, 5·5; N, 18·6. $C_{10}H_{14}N_4O_6,1\frac{1}{2}H_2O$ requires C, 38·3; H, 5·5; N, 17·9%).

9-D-*Sorbitylxanthine*.—A solution of 9-D-sorbitylguanine (1·85 g.) and sodium nitrite (4·2 g.) in hot water (50 c.c.) was cooled rapidly to 45° and treated with acetic acid (4·2 c.c.). After 18 hr. the product was isolated through the lead salt; 9-D-*sorbitylxanthine* (0·84 g., 46%) separated from 50% ethanol (14 c.c.) as an amorphous powder, m. p. 160—162° (decomp.; sinters above 155°) (Found: C, 41·7; H, 5·5; N, 16·8. $C_{11}H_{16}N_4O_7$ requires C, 41·8; H, 5·1; N, 17·7%).

7-*Amino*-3-D-*ribityl*-[1,2,3]-*triazolo*[d]*pyrimidine*.—To a solution of the amine (Ia) (from 14·5 g. of nitro-compound) in 50% acetic acid (200 c.c.) was added sodium nitrite (3·6 g.) in

water (20 c.c.). Collected after 2 hr., the *compound* (8·4 g., 62%) formed needles, m. p. 237—238°, raised to 239—240° by recrystallisation from water (Found: C, 40·2; H, 5·3; N, 31·1. $C_9H_{14}N_6O_4$ requires C, 40·0; H, 5·2; N, 31·1%).

7-*Amino*-3-D-*sorbityl*-[1,2,3]-*triazolo*[d]*pyrimidine*.—Similarly prepared (72% yield), the *compound* had m. p. 240—241° (Found: C, 40·5; H, 5·7; N, 28·0. $C_{10}H_{16}N_6O_5$ requires C, 40·0; H, 5·4; N, 28·0%).

7-*Hydroxy*-3-D-*ribityl*-[1,2,3]-*triazolo*[d]*pyrimidine*.—To a solution of the 7-amino-compound (1·04 g.) in N-nitric acid (40 c.c.) was added sodium nitrite (2·9 g.) in water (10 c.c.). After 5 hr., isolation through the lead salt, trituration with aqueous ethanol, and crystallisation from 85% ethanol (45 c.c.) gave the *hydroxy-compound* (0·6 g., 58%) as needles, m. p. 153—154° (after drying at 100°) (Found: C, 37·8; H, 5·1; N, 24·5. $C_9H_{13}N_5O_5,H_2O$ requires C, 37·4; H, 5·2; N, 24·2%).

7-*Hydroxy*-3-D-*sorbityl*-[1,2,3]-*triazolo*[d]*pyrimidine*.—Sodium nitrite (3 g.) was added to a solution of the 7-amino-compound (1·3 g.) in N-nitric acid (45 c.c.). Collected after 18 hr., the *hydroxy-compound* (1·04 g., 80%) formed needles (from water), m. p. 210—212° (Found: C, 38·0; H, 5·3; N, 22·3. $C_{10}H_{15}N_5O_6,H_2O$ requires C, 37·6; H, 5·4; N, 21·9%).

5-*Amino*-7-*hydroxy*-3-D-*ribityl*-[1,2,3]-*triazolo*[d]*pyrimidine*.—To a solution of the amine (IIa) (from 6·1 g. of nitro-compound) in 50% acetic acid (200 c.c.) was added sodium nitrite (1·40 g.) in water (10 c.c.). The separated material (4·11 g.) was collected after 5 hr. at 0°, a further crop being obtained by evaporation of the filtrate, giving a total of 4·91 g. (86%) of the *ribityl compound* as needles (from water), m. p. 258—259° (decomp.) (Found: C, 37·7; H, 5·2; N, 29·5. $C_9H_{14}N_6O_5$ requires C, 37·8; H, 4·9; N, 29·4%).

5-*Amino*-7-*hydroxy*-3-D-*sorbityl*-[1,2,3]-*triazolo*[d]*pyrimidine*.—Similarly prepared (67% yield), the *sorbityl compound* formed needles (from water), m. p. 246—247° (decomp.) (Found: C, 37·8; H, 5·3; N, 27·1. $C_{10}H_{16}N_6O_6$ requires C, 38·0; H, 5·1; N, 26·6%).

5,7-*Dihydroxy*-3-D-*ribityl*-[1,2,3]-*triazolo*[d]*pyrimidine*.—To a solution of the amine (IIIa) (3 mmoles) in water (20 c.c.) was added glacial acetic acid (3 c.c.) followed by barium nitrite monohydrate (0·41 g.) in a little water. After 1½ hr., barium was removed with N-sulphuric acid (3·3 ml.). Removal of solvent and crystallisation from 75% ethanol gave the *dihydroxy-compound* (0·51 g., 59%) as rods or needles, m. p. 208—209° (decomp.) after recrystallisation (Found: C, 37·6; H, 4·7; N, 24·0. $C_9H_{13}N_5O_6$ requires C, 37·6; H, 4·6; N, 24·4%).

Prepared from the 5-amino-7-hydroxy-compound by the method used for 9-D-ribityl-xanthine (26% yield), the compound had m. p. 198—200° (decomp.) undepressed by the above material (Found: C, 37·7; H, 5·0; N, 24·7%). The ultraviolet absorption spectra of the two samples were identical.

5,7-*Dihydroxy*-3-D-*sorbityl*-[1,2,3]-*triazolo*[d]*pyrimidine*.—Similarly prepared from the sorbitylamine (IIIb) (81% yield), the *dihydroxy-compound* formed needles (from 60% ethanol), m. p. 229—230° (decomp.) (Found: C, 38·0; H, 4·5; N, 22·3. $C_{10}H_{15}N_5O_7$ requires C, 37·9; H, 4·8; N, 22·1%); prepared from the 5-amino-7-hydroxy-compound (40% yield) the material had m. p. 227—229° (decomp.) alone or in admixture with the first sample (Found: C, 37·5; H, 5·1; N, 22·3%).

Pteridines.

2,8-*Dihydro*-4-*hydroxy*-2-*imino*-6,7-*dimethyl*-8-D-*ribitylpteridine* (IV; R = D-ribityl, X = NH).—To a solution of the ribitylamine (IIa) (3 mmoles) in water (10 c.c.) at 60° was added biacetyl (0·31 g.). The mixture was kept for 30 min. at 80—90° in a stoppered flask, then evaporated to 1—2 c.c.; the *pteridine* (0·5 g., 51%) slowly separated as an amorphous orange-yellow powder, m. p. *ca.* 190° (decomp.), unchanged by " recrystallisation " from 70% ethanol (Found: C, 46·3; H, 6·4; N, 20·8. $C_{13}H_{19}N_5O_5,\frac{1}{2}H_2O$ requires C, 46·7; H, 6·0; N, 21·0%).

2,8-*Dihydro*-4-*hydroxy*-6,7-*dimethyl*-2-*oxo*-8-D-*ribitylpteridine* (" *Compound G* ") (IV; R = D-ribityl, X = O).—To a solution of the pteridine (IIIa) (6 mmoles) in water (20 c.c.) at 60° was added biacetyl (0·62 g.). The mixture was kept for 30 min. at 80—90° in a stoppered flask, then cooled, and the *pteridine* (1·17 g., 60%) collected as yellow needles, m. p. 274—275° (decomp.) unchanged by recrystallisation from water, $[\alpha]_D^{22}$ −183° (*c* 1·1% in H_2O) (Found: C, 48·1; H, 5·8; N, 17·0. $C_{13}H_{18}N_4O_6$ requires C, 47·9; H, 5·6; N, 17·2%).

6,7-*Diethyl*-2,8-*dihydro*-4-*hydroxy*-2-*oxo*-8-D-*ribitylpteridine*.—A similar condensation was carried out between the amine (IIIa) (0·01 mole) and hexane-3,4-dione (1·4 g.). After evaporation to dryness, the residue in water (5 c.c.) was treated with ethanol (50 c.c.) and ether (100

c.c.), and the crude product thus obtained again precipitated from ethanol (50 c.c.) with ether (200 c.c.) and crystallised, with difficulty, from 96% ethanol (12 c.c.). The *diethyl compound* (2·06 g., 58%) was a yellow, crystalline powder, m. p. 229—231° (decomp.) (Found: C, 50·8; H, 6·9; N, 15·2. $C_{15}H_{22}N_4O_6$ requires C, 50·8; H, 6·3; N, 15·8%).

2,8-*Dihydro*-4-*hydroxy*-6,7-*dimethyl*-2-*oxo*-8-D-*sorbitylpteridine* (IV; R = D-sorbityl, X = O).—A similar condensation was carried out between the sorbitylamine (IIIb) (9 mmoles) and biacetyl (0·93 g.). After evaporation, the residue was recrystallised twice by addition of methanol or ethanol (50 c.c.) to a solution in warm water (10 c.c.), giving the *sorbityl compound* (2·2 g., 61%) as yellow needles, which melted at 60—80°, resolidified, and remelted at 229—230° (decomp.) (Found, in material dried at room temperature: C, 41·8; H, 6·3; N, 13·6. $C_{14}H_{20}N_4O_7,2\frac{1}{2}H_2O$ requires C, 41·9; H, 6·3; N, 14·0%).

2-*Amino*-7,8-*dihydro*-4-*hydroxy*-6-*methyl*-7-*oxo*-8-D-*ribitylpteridine* (V; R = D-ribityl, R′ = NH_2).—To a solution of the base (IIa) (0·011 mole) in water (45 c.c.) was added commercial 90% pyruvic acid (5 c.c.). The mixture was boiled for 1 hr. under reflux and the *ribityl compound* (2·26 g., 63%), m. p. 274—277° (decomp.), was collected after cooling. Recrystallisation from water (300 c.c.) gave yellow needles, m. p. 283—284° (decomp.) (Found: C, 43·6; H, 5·7; N, 21·9. $C_{12}H_{17}N_5O_6$ requires C, 44·0; H, 5·2; N, 21·4%).

2-*Amino*-7,8-*dihydro*-4-*hydroxy*-6-*methyl*-7-*oxo*-8-D-*sorbitylpteridine* (V; R = D-sorbityl, R′ = NH_2).—Similarly prepared from the analogue (IIb) and isolated by evaporation of the reaction mixture to small volume, the *sorbityl compound* (60% yield) formed yellow needles, m. p. 229—231° (decomp.) (Found: C, 42·5; H, 5·8; N, 18·7. $C_{13}H_{19}N_5O_7,\frac{1}{2}H_2O$ requires C, 42·6; H, 5·5; N, 19·1%).

Ethyl (2-*Amino*-6-*hydroxy*-4-D-*ribitylamino*-5-*pyrimidinylimino*)*acetate* (VIII; R = D-ribityl, R′ = NH_2).—To a solution of the ribityl compound (IIa) (3 mmoles) in water (15 c.c.) was added ethyl glyoxylate hemiacetal (0·67 g.). The mixture was boiled for 1 hr. under reflux, and the *product* (0·8 g., 69%) was collected after cooling; it formed yellow needles (from water), m. p. 232—233° (decomp.) (Found: C, 40·2; H, 6·7; N, 17·8. $C_{13}H_{21}N_5O_7,1\frac{1}{2}H_2O$ requires C, 40·4; H, 6·3; N, 18·1%).

2-*Amino*-7,8-*dihydro*-4-*hydroxy*-7-*oxo*-8-D-*ribitylpteridine* (VI; R = D-ribityl, R′ = NH_2).—(*a*) The above ester (0·39 g.) and N-sodium hydrogen carbonate (5 c.c.) were boiled for 15 min. under reflux. Glacial acetic acid (0·5 c.c.) and water (100 c.c.) were added and the mixture was boiled with a little charcoal and filtered, giving, on cooling, the *ribityl compound* (0·17 g., 54%) as a yellow microcrystalline powder, m. p. 342—343° (decomp.), unchanged by recrystallisation (Found: C, 42·3; H, 5·4; N, 22·3. $C_{11}H_{15}N_5O_6$ requires C, 42·2; H, 4·8; N, 22·4%).

(*b*) To a solution of compound (IIa) (3 mmoles) in water (15 c.c.) was added ethyl glyoxylate hemiacetal (0·67 g.). The solid which separated was collected after 30 min. and cyclised with N-sodium hydrogen carbonate as in (*a*), to give the pteridine (0·4 g., 42%) (Found: C, 42·2; H, 5·0%).

Ethyl (2-*Amino*-6-*hydroxy*-4-D-*sorbitylamino*-5-*pyrimidinylimino*)*acetate* (VIII; R = D-sorbityl, R′ = NH_2).—To a solution of the sorbityl compound (IIb) (6 mmoles) in water (30 c.c.) at 60° was added ethyl glyoxylate hemiacetal (1·34 g.). On cooling, the *product* (1·61 g., 69%) separated as yellow needles, m. p. 198—203° (decomp.), raised to 209—210° (decomp.) by recrystallisation from water (Found: C, 43·1; H, 6·3; N, 18·1. $C_{14}H_{23}N_5O_8$ requires C, 43·2; H, 6·0; N, 18·0%).

2-*Amino*-7,8-*dihydro*-4-*hydroxy*-7-*oxo*-8-D-*sorbitylpteridine* (VI; R = D-sorbityl, R′ = NH_2).—Prepared in the same way as the ribityl compound, method (*a*), the *sorbityl compound* (44% yield) was a yellow powder, m. p. 320—330° (decomp.) (Found: C, 41·5; H, 5·6; N, 19·5. $C_{12}H_{17}N_5O_7,\frac{1}{2}H_2O$ requires C, 40·9; H, 5·2; N, 19·9%).

7,8-*Dihydro*-2,4-*dihydroxy*-7-*oxo*-8-D-*ribitylpteridine* (VI; R = D-ribityl, R′ = OH).—To a solution of compound (IIIa) (0·01 mole) in water (80 c.c.) was added glacial acetic acid (2 c.c.), followed by ethyl glyoxylate hemiacetal (2·23 g.), with shaking. The solid was collected after 30 min. and boiled for 15 min. under reflux with N-sodium hydrogen carbonate. The solution was treated with acetic acid (5 c.c.), evaporated to 20 c.c., and treated with hot ethanol (35 c.c.) to give, on cooling, the crude sodium salt (4 g.) of the product. A solution of this in warm 2N-hydrochloric acid (12 c.c.) was treated with charcoal and cooled, to give the free *pteridine* (1·68 g., 54%) as buff needles, m. p. 228—229° (decomp.) after recrystallisation from water (Found: C, 42·0; H, 4·4; N, 17·9. $C_{11}H_{14}N_4O_7$ requires C, 42·0; H, 4·5; N, 17·8%).

2-*Amino*-7,8-*dihydro*-4,6-*dihydroxy*-7-*oxo*-8-D-*ribitylpteridine* (VII; R = D-ribityl).—A

solution of ribitylaminopyrimidine (IIa) (3 mmoles) in water (15 c.c.) was boiled under reflux for 5 hr. with ethyl oxalate (3 c.c.). After cooling, the *pteridine* (0·57 g., 58%) was collected; it formed brownish-yellow needles (from water), decomposing at 330—335° (Found: C, 39·1; H, 5·1; N, 20·2. $C_{11}H_{15}N_5O_7,\frac{1}{2}H_2O$ requires C, 39·0; H, 4·8; N, 20·7%).

2-*Amino*-7,8-*dihydro*-4,6-*dihydroxy*-7-*oxo*-8-D-*sorbitylpteridine* (VII; R = D-sorbityl).—Similarly prepared (58% yield), the *sorbityl compound* formed yellow needles, m. p. 331° (decomp.) (Found: C, 39·2; H, 5·5; N, 18·7. $C_{12}H_{17}N_5O_8,\frac{1}{2}H_2O$ requires C, 39·1; H, 4·9; N, 19·0%).

Benzo[g]*pteridines and Pyrimidopteridines.*

2,10-*Dihydro*-4-*hydroxy*-2-*imino*-7,8-*dimethyl*-10-D-*ribitylbenzo*[g]*pteridine* (" *Riboflavin* 2-*Imine* ") (IX; R = D-ribityl, X = NH).—To a solution of the pyrimidine (IIa) (5 mmoles) in water (60 c.c.) was added 4,5-dimethyl-1,2-benzoquinone dimer [19] (0·68 g.) in hot ethanol (20 c.c.). The mixture was boiled under reflux for 3 hr. in subdued light and kept for 3 days in the dark. The crude product (0·77 g., 41%) was collected, dissolved in 2N-hydrochloric acid (30 c.c.), treated with 10% hydrogen peroxide (2 c.c.) and a little charcoal, and filtered. Addition of N-sodium hydrogen carbonate (*ca.* 100 c.c.) gave the product as a powder, which after a further purification by the same method formed orange needles (0·48 g., 26%) containing a trace of inorganic material. The compound was suspended in hot water (50 c.c.) containing a few drops of acetic acid and collected after cooling; the pure isoalloxazine 2-imine (0·4 g.) decomposed above 290°, without melting (Found: C, 54·1; H, 5·6; N, 18·6. Calc. for $C_{17}H_{21}N_5O_5$: C, 54·4; H, 5·6; N, 18·7%).

Riboflavin (IX; R = D-ribityl, X = O).—To a solution of the pyrimidine (IIIa) (3 mmoles) in water (25 c.c.) was added, 4,5-dimethyl-1,2-benzoquinone dimer [19] (0·41 g.) in hot ethanol (12 c.c.). The mixture was boiled under reflux for 3 hr., then kept for 3 days at room temperature, and the solid was collected, dissolved in hot water (150 c.c.), mixed with the filtrate, and percolated through a column of Woelm alumina (acidic grade). The column was washed with water at 50°, and the combined eluates were evaporated to 25 c.c., to give riboflavin (0·28 g., 25%) as orange-yellow needles, m. p. 273—274° (decomp.), raised to 278° (decomp.) by recrystallisation from water (Found: C, 54·8; H, 5·9; N, 14·4. Calc. for $C_{17}H_{20}N_4O_6$: C, 54·3; H, 5·4; N, 14·9%). The samples did not depress the m. p. of an authentic sample, and a microbiological assay [27] showed 101% of riboflavin activity.

8-*Amino*-2,10-*dihydro*-4,6-*dihydroxy*-2-*oxo*-10-D-*ribitylpyrimido*[5,4-g]*pteridine* (X; R = D-ribityl, R′ = NH_2).—To a solution of the pyrimidine (IIa) (3 mmoles) in water (7 c.c.) was added acetic acid (2 c.c.) and alloxan monohydrate (0·53 g.). The mixture was boiled under reflux for 15 min., the initial intense purple colour disappearing and yellow needles separating. Collected after cooling, the *compound* (0·94 g., 80%) formed brownish-yellow needles (from 50% acetic acid), decomp. above 270° (Found: C, 39·9; H, 4·8; N, 24·6. $C_{13}H_{15}N_7O_7,\frac{1}{2}H_2O$ requires C, 40·0; H, 4·1; N, 25·1%).

2,10-*Dihydro*-4,6,8-*trihydroxy*-2-*oxo*-10-D-*ribitylpyrimido*[5,4-g]*pteridine* (X; R = D-ribityl, R′ = OH).—Similarly prepared (64% yield), this *compound* formed yellow needles (from water), which did not melt below 340° (Found: C, 39·9; H, 4·0; N, 21·1. $C_{13}H_{14}N_6O_8,\frac{1}{2}H_2O$ requires C, 39·9; H, 3·9; N, 21·5%).

2,10-*Dihydro*-4,6,8-*trihydroxy*-2-*oxo*-10-D-*sorbitylpyrimido*[5,4-g]*pteridine* (X; R = D-sorbityl, R′ = OH).—Similarly prepared in 86% yield, this *product* separated from water as a yellow powder, which contracted at 170—175° and charred above 290° (Found: C, 39·1; H, 4·5; N, 19·1. $C_{14}H_{16}N_6O_9,H_2O$ requires C, 39·1; H, 4·2; N, 19·5%).

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CHEMICAL RESEARCH DEPARTMENT, PARKE DAVIS & COMPANY,
STAINES ROAD, HOUNSLOW, MDDX. [*Received, April 7th*, 1960.]

[27] Barton-Wright, " Microbiological Assay of the Vitamin B Complex and Amino Acids," Pitman, London, 1952, p. 35.