140. *Pteridine Derivatives. Part VII.* The Synthesis of Riboflavin 2-Imine and Related* iso*Alloxazine 2-Imines.*

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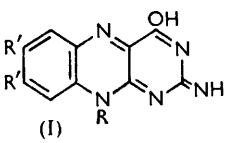
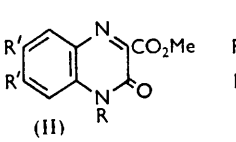
A new synthesis of *iso*alloxazine 2-imines (I) is described which starts from methyl 4-alkyl-3 : 4-dihydro-3-oxoquinoxaline-2-carboxylates (II) and guanidine. The 2-imine-analogue of riboflavin was synthesised by this method. Intermediate compounds formed in the reaction have been identified as octahydroglyoxaline-4-*spiro*-2′-quinoxalines (III). A second synthesis of an *iso*alloxazine 2-imino (I; R = R′ = Me) from 2 : 5-diamino-4-hydroxy-6-methylaminopyrimidine and the dimer of 3 : 4-dimethyl-*o*-benzoquinone is also described. The formation of 2-aminoalloxazines (IV) on treatment of the *iso*alloxazine 2-imines (I) with dilute alkali is discussed in relation to the fine structure of the *iso*alloxazine 2-imines.

CERTAIN of the naturally occurring purines have been shown to stimulate the production of riboflavin by the mould *Eremothecium ashbyii* without at the same time affecting the growth of the organism.[1,2,3] In particular, experiments with labelled adenine indicate that the purines contribute an intact pyrimidine ring to the riboflavin molecule,[2] after loss of $C_{(8)}$. Recent views on the biosynthesis of pteridines suggest that these are also formed from purines *via* an intermediate 4 : 5-diaminopyrimidine derivative.[4] There is thus the possibility that riboflavin and the pteridines arise from some common intermediate, and it seemed to us desirable to investigate the synthesis and properties of the 2-imine of riboflavin (I; R = D-ribityl, R′ = Me) which contains the 2-amino-4-hydroxypteridine structure characteristic of most naturally occurring pteridines.



(I)



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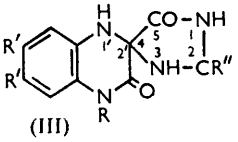


(III)

Recently two syntheses of an *iso*alloxazine 2-imine (I; R = R′ = Me) have been reported,[5,6] but in view of the low yields in these reactions together with the observation [7] that many unsuccessful attempts had been made to prepare riboflavin 2-imine, it appeared unlikely that standard *iso*alloxazine syntheses would be successful. Modification of a recent pteridine synthesis [8] seemed feasible.

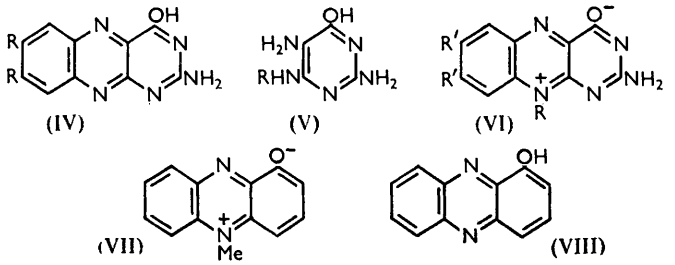
* Part VI, *J.*, 1958, 3730.

[1] McNutt, *J. Biol. Chem.*, 1954, **210**, 511; Goodwin and Pendlington, *Biochem. J.*, 1954, **57**, 631.
[2] McNutt, *J. Biol. Chem.*, 1956, **219**, 365.
[3] Brown, Goodwin, and Jones, *Biochem. J.*, 1958, **68**, 40.
[4] Albert, *ibid.*, 1957, **65**, 124; Ziegler-Günder, Simon, and Wacker, *Z. Naturforsch.*, 1956, **11**b, 82.
[5] Hemmerich, Fallab, and Erlenmeyer, *Helv. Chim. Acta*, 1956, **39**, 1242.
[6] Hemmerich, *ibid.*, 1958, **41**, 514.
[7] Hitchings, Ciba Symposium on the "Chemistry and Biology of Pteridines," Churchill, London, 1954, p. 121.
[8] Dick, Fidler, and Wood, *Chem. and Ind.*, 1956, 1424.

Condensing methyl 3 : 4-dihydro-4-methyl-3-oxoquinoxaline-2-carboxylate (II; R = Me, R′ = H), prepared by *N*-methylation of the corresponding hydroxy-ester, with guanidine gave a variety of products depending on the conditions. Fusion with guanidine carbonate, conditions which were successful for the synthesis of a pteridine derivative,[8] gave a mixture including a large proportion of guanidine 3 : 4-dihydro-4-methyl-3-oxoquinoxaline-2-carboxylate, and this was also obtained from the same components in refluxing aqueous methanol.

A base-catalysed condensation would formally resemble the familiar pyrimidine synthesis [9] from guanidine and β-keto-esters. However, the *N*-methyl ester with guanidine in the presence of sodium methoxide gave a high yield of 2 : 3 : 4 : 5 : 1′ : 2′ : 3′ : 4′-octahydro-2-imino-4′-methyl-3′ : 5-dioxoglyoxaline-4-*spiro*-2′-quinoxaline (III; R = Me, R′ = H, R″ = NH). This compound had an ultraviolet absorption spectrum (Table) analogous to that of the tetrahydroquinoxaline*spiro*hydantoins described by Clark-Lewis.[10] The *N*-methyl ester with urea gave an analogous product (III; R = Me, R′ = H, R″ = O) which on methylation (methyl iodide–acetone–potassium carbonate) gave a trimethyl derivative identical with a sample prepared from 3-hydroxyquinoxaline-2-carboxyureide by Clark-Lewis's method.[10]

Ring closure to the alloxazine ring system was finally achieved by prolonged reaction of the *N*-methyl ester (II; R = Me, R′ = H) with guanidine. Unexpectedly, the product proved to be 2-amino-4-hydroxybenzo[*g*]pteridine (IV; R = H), identical with the compound prepared under similar conditions from methyl 3-hydroxyquinoxaline-2-carboxylate. The identity of these products was confirmed by comparison of the infrared spectra of the compounds and of their dimethyl and diethyl derivatives (which were prepared by the technique outlined above). Prolonged treatment of thes *piro*-compound (III; R = Me, R′ = H, R″ = NH) with sodium methoxide gave a small yield of the same benzopteridine (IV; R = H). The loss of a methyl group under these conditions is unusual, and it is shown below that this is characteristic of *iso*alloxazine 2-imines (I) when treated with alkali in the presence of air. Repetition of the reaction in an atmosphere of nitrogen gave a mixture of 2 : 10-dihydro-4-hydroxy-2-imino-10-methylbenzo[*g*]-pteridine (I; R = Me, R′ = H), and its decomposition product, the alloxazine derivative (IV; R = H). Separation of these compounds was achieved readily by paper chromatography, but the extreme instability of the *iso*alloxazine 2-imine (I; R = Me, R′ = H) made its isolation in good yield very difficult.



In the hope that riboflavin 2-imine (I; R = D-ribityl, R′ = Me) would be somewhat more stable, an analogous synthesis was carried out starting from methyl 3 : 4-dihydro-6 : 7-dimethyl-3-oxo-4-D-ribitylquinoxaline-2-carboxylate (II; R = D-ribityl, R′ = Me). This compound was prepared from the corresponding acid which is readily obtained from riboflavin by treatment with dilute sodium hydroxide.[11] This ester with guanidine in the presence of sodium propoxide gave a high yield of 2 : 3 : 4 : 5 : 1′ : 2′ : 3′ : 4′-octahydro-2-imino-6′ : 7′-dimethyl-3′ : 5-dioxo-4′-D-ribitylglyoxaline-4-*spiro*-2′-quinoxaline (III;

[9] Jaeger, *Annalen*, 1891, **262**, 365; Wheeler and Johnson, *Amer. Chem. J.*, 1903, **29**, 496.
[10] Clark-Lewis, *J.*, 1957, 422.
[11] Surrey and Nachod, *J. Amer. Chem. Soc.*, 1951, **73**, 2337.

R = D-ribityl, R′ = Me, R″ = NH) which was isolated as the crystalline hydrochloride. More prolonged reaction in the presence of air gave 2-amino-4-hydroxy-7 : 8-dimethylbenzo[*g*]pteridine (IV; R = Me; " 2-aminolumichrome "), apparently identical with the material prepared by Bardos, Olsen, and Enkoji.[12] A similar reaction carried out under nitrogen gave a mixture of products including a large proportion of 2 : 10-dihydro-4-hydroxy-2-imino-7 : 8-dimethyl-10-D-ribitylbenzo[*g*]pteridine (I; R = D-ribityl, R′ = Me; " 2-iminoriboflavin "). Isolation of pure riboflavin 2-imine was achieved, with difficulty, by chromatography on " Florisil " columns, or alternatively by repeated precipitation from acid. These experiments were carried out in the dark as riboflavin 2-imine readily underwent photolysis.

2 : 10-Dihydro-4-hydroxy-2-imino-7 : 8 : 10-trimethylbenzo[*g*]pteridine (I; R = R′ = Me; " 2-iminolumiflavin ") could not be prepared by condensing methyl 3 : 4-dihydro-4 : 6 : 7-trimethyl-3-oxoquinoxaline-2-carboxylate (II; R = R′ = Me) with guanidine, although the intermediate *spiro*-compound (III; R = R′ = Me, R″ = NH) was formed readily in good yield. Condensing 2 : 5-diamino-4-hydroxy-6-methylaminopyrimidine [13] (V; R = Me) with the dimer of 3 : 4-dimethyl-*o*-benzoquinone,[12] however, gave the *iso*alloxazine 2-imine (I; R = R′ = Me) which was isolated as the crystalline hydrochloride. This compound had ultraviolet absorption identical with that of the material prepared by Hemmerich *et al.*[5,6] and both the free bases and the hydrochlorides showed similar R_F values on paper chromatography in several solvent systems (we are indebted to Dr. Hemmerich for these observations).

The *iso*alloxazine 2-imines (I), when treated with dilute alkali in the presence of air, readily lose the 10-alkyl substituent with the formation of 2-aminoalloxazine derivatives (IV). This behaviour is in distinct contrast with that of *iso*alloxazines such as riboflavin and lumiflavin which under similar conditions undergo ring cleavage to quinoxaline derivatives.[11,14] and it may have some bearing on the biological transfer of C_1—C_5 fragments. For *iso*alloxazines and *iso*alloxazine 2-imines (I) there is the possibility of resonance involving dipolar forms such as (VI). The insolubility of the imines in chloroform and other non-polar solvents makes it likely that the contribution of such ionic forms to the resonance hybrid is much greater than with *iso*alloxazines such as lumiflavin which readily dissolve in chloroform. The existence of such contributing structures may explain the ease with which 10-alkyl substituents may be cleaved from *iso*alloxazine 2-imines (I). The bacterial pigment, pyocyanine (VII), which has an analogous dipolar structure,[15] is similarly demethylated [16] in alkaline solution to 1-hydroxyphenazine (VIII).

Irradiation of riboflavin 2-imine (I; R = D-ribityl, R′ = Me) with ultraviolet light gave products analogous to those obtained on photolysis of riboflavin.[17] Thus in acid solution 2-aminolumichrome (IV; R = Me) was produced, and in alkaline solution a mixture of lumiflavin 2-imine (I; R = R′ = Me), 2-aminolumichrome (IV; R = Me), and a third, unidentified product.

It has been shown that guanine stimulates the production of riboflavin by *Eremothecium ashbyii* more effectively than do the other naturally occurring purines.[3] There is therefore the possibility that riboflavin 2-imine (I; R = D-ribityl, R′ = Me) may be an intermediate in the guanine–riboflavin transformation, being formed *via* the pyrimidine (V; R = D-ribosyl or D-ribityl) by condensation with 3 : 4-dimethyl-*o*-benzoquinone or its equivalent.[18] Riboflavin 2-imine, however, does not stimulate riboflavin synthesis in *Eremothecium ashbyii*, neither has it any inhibitory effect. (We are indebted to Dr. T. W. Goodwin for these results.) Deamination must therefore occur at some earlier

[12] Bardos, Olsen, and Enkoji, *J. Amer. Chem. Soc.*, 1957, **79**, 4704.
[13] Fidler and Wood, *J.*, 1957, 4157.
[14] Kuhn, Rudy, and Wagner-Jauregg, *Ber.*, 1933, **66**, 1956.
[15] Jensen and Holten, *Acta Chem. Scand.*, 1949, **3**, 1446.
[16] Wrede and Strack, *Z. physiol. Chem.*, 1928, **177**, 177.
[17] Shimizu, *J. Vitaminol.* (*Japan*), 1955, **1**, 39; *Chem. Abs.*, 1955, **49**, 12,631.
[18] Cf. Birch and Moye, *J.*, 1957, 412; 1958, 2622.

stage in the guanine–riboflavin transformation, either prior to the opening of the iminazole ring or, as seems more likely, at the intermediate pyrimidine stage.

Compound	R_F (A)	R_F (B)	$\lambda_{max.}$ (mμ) (ε in parentheses) in H_2O at pH given	
Spiran (III)				
R = Me, R′ = H, R″ = NH	0·46	—	222(34,000), 298(4600)	pH 7
			213(28,000), 236[b] (19,000), 316(6100), 392(3200)	pH 1
R = Me, R′ = H, R″ = O	—	—	223(29,800), 300(4900) [ac]	
R = Me, R′ = Me, R″ = NH	0·60	—	226(28,000), 306(4600)	pH 13
			218(31,100), 241(21,800), 340(11,300), 410(7500)	pH 1
R = D-Ribityl, R′ = Me, R″ = NH	0·24	—	230[b] (18,900), 306(4500)	pH 7
			220(31,500), 240[b] (22,700), 342(11,300), 412(7200)	pH 1
Compounds (IV)				
R = H	0·46	0·14	218(28,800), 262(44,500), 333(7000), 416(9000)	pH 13
			212(27,800), 244(23,500), 263(32,900), 345(9200), 370[b] (6200), 430[b] (2100)	pH 1
R = Me	0·54	0·03	224(23,600), 264(43,650), 344(5500), 420(7400)	pH 13
			220(23,900), 266(27,500), 362(8200) [d]	pH 1
Compounds (I)				
R = Me, R′ = H	0·23	0·36	216(24,500), 264(29,800), 358(8200), 428(11,020)	pH 1
R = Me, R′ = Me	0·34	0·23	223(21,100), 268(25,000), 384 (9100), 443(10,300)	pH 1
R = D-Ribityl, R′ = Me	0·22	0·28	224(27,000), 270(32,100), 386(12,300), 442(14,100)	pH 1
Riboflavin	0·22	0·39	223(35,500), 267(35,500), 376(10,700), 445(11,500)	pH 1

[a] In EtOH. [b] Shoulder. [c] Clark-Lewis [10] gives $\lambda_{max.}$ 219 mμ (ε 21,300), and 300 mμ (4400). [d] Bardos *et al.*[12] give $\lambda_{max.}$ 266 (ε 38,900) and 362 mμ (10,720).

Experimental

Yields of substances that have no definite m. p. refer to the stage when they appeared homogeneous in paper chromatography. Chromatograms were developed by the ascending technique, solvents being (A) butan-1-ol–5N-acetic acid (7 : 3), and (B) 3% aqueous ammonium chloride, and were viewed in ultraviolet light of wavelengths 254 and 365 mμ. Infrared spectra were determined for Nujol mulls.

Methyl 3-Hydroxyquinoxaline-2-carboxylate.—3-Hydroxyquinoxaline-2-carboxylic acid [19] (1·6 g.) suspended in dry methanol (100 c.c.) saturated with dry hydrogen chloride was refluxed for 2 hr. Refrigeration gave the *methyl ester* (1·48 g., 86%) which recrystallised from methanol as prisms, m. p. 222° (Found: C, 58·8; H, 3·5; N, 14·1. $C_{10}H_8O_3N_2$ requires C, 58·8; H, 4·0; N, 13·7%).

Methyl 3 : 4-Dihydro-4-methyl-3-oxoquinoxaline-2-carboxylate (II; R = Me, R′ = H).—Methyl 3-hydroxyquinoxaline-2-carboxylate (12 g.), methyl iodide (20 g.), and anhydrous potassium carbonate (60 g.) were refluxed in dry acetone (180 c.c.) for 24 hr. The mixture was then cooled, water was added, and the whole extracted with chloroform. Evaporation of the dried chloroform extract, and recrystallisation of the residue from methanol gave the *N*-methyl ester (10 g., 78%) as rosettes of needles, m. p. 126 (lit.,[19] 126°).

Guanidine 3 : 4-Dihydro-4-methyl-3-oxoquinoxaline-2-carboxylate.—(*a*) The above *N*-methyl ester (1·44 g.) and guanidine carbonate (0·6 g.) in 75% aqueous methanol (30 c.c.) were refluxed for 5 hr. On cooling, the *guanidine salt* (1·46 g., 84%) separated as yellow prisms, m. p. 248—249°. Recrystallisation from 75% aqueous methanol raised the m. p. to 252·5° (Found: C, 50·4; H, 4·6; N, 26·8. $C_{11}H_{13}O_3N_5$ requires C, 50·2; H, 5·0; N, 26·6%).

(*b*) The *N*-methyl ester (1 g.) and guanidine carbonate (3 g.) were ground and heated at 160—170° for 30 min., cooled, and dissolved in warm water (20 c.c.), and acetic acid was added to give pH 7. The solution was extracted with ether and chloroform (extracts rejected) and taken to dryness *in vacuo*. The yellow residue, recrystallised from aqueous methanol, gave the guanidine salt, m. p. and mixed m. p. 251°. The residual mother-liquors contained fluorescent substances which were not identified.

(*c*) An authentic sample of the guanidine salt was prepared by mixing the *N*-methyl acid (100 mg.) (prepared by hydrolysis of the methyl ester) and guanidine carbonate (45 mg.) in

[19] King and Clark-Lewis, *J.*, 1951, 3379.

warm 75% aqueous methanol (3 c.c.); yellow prisms (82 mg.) separated almost immediately, having m. p. 251°.

2 : 3 : 4 : 5 : 1′ : 2′ : 3′ : 4′-Octahydro-2-imino-4′-methyl-3′ : 5-dioxoglyoxaline-4-spiro-2′-quinoxaline (III; R = Me, R′ = H, R″ = NH).—Methyl 3 : 4-dihydro-4-methyl-3-oxoquinoxaline-2-carboxylate (3 g.) in dry methanol (70 c.c.) was added to a solution from sodium (0·75 g.) in dry methanol (70 c.c.). Dry powdered guanidine hydrochloride (1·35 g.) was added, and the mixture was refluxed for 30 min. The solution was concentrated *in vacuo* to *ca.* 30 c.c., cooled in ice-water, and diluted by addition of distilled water (40 c.c.). 2N-Hydrochloric acid was added dropwise to give pH 6·8, and the resulting precipitate was collected, washed with methanol (30 c.c.), and dried at 70° (yield, 2·92 g., 87%). Recrystallisation from water (200 c.c.) (charcoal) gave the spiro-*compound* as colourless needles, m. p. 229—230° (decomp.) (Found: C, 54·1; H, 4·2; N, 28·8. $C_{11}H_{11}O_2N_5$ requires C, 53·9; H, 4·5; N, 28·6%).

A solution of the *spiro*-compound (0·34 g.) in dry methanol (25 c.c.) saturated with dry hydrogen chloride was refluxed for 1 hr. The intense yellow precipitate which was formed gradually was collected and recrystallised from dry methanol, to give the *hydrochloride* (0·18 g., 46%) of the *spiro*-compound as yellow needles, m. p. 265—266° (Found: C, 47·1; H, 4·1; N, 24·8; Cl, 12·6. $C_{11}H_{11}O_2N_5$,HCl requires C, 46·9; H, 4·3; N, 24·9; Cl, 12·6%).

2 : 3 : 4 : 5 : 1′ : 2′ : 3′ : 4′-Octahydro-4′-methyl-2 : 3′ : 5-trioxoglyoxaline-4-spiro-2′-quinoxaline (III; R = Me, R′ = H; R″ = O).—A similar condensation of the *N*-methyl ester (II; R = Me R′ = H) (1 g.) with sodium (0·24 g.) and urea (0·28 g.) in dry methanol (20 c.c.) gave an analogous *spiro*-compound (0·88 g., 78%). Recrystallisation from aqueous methanol gave colourless needles, m. p. 224—226° (decomp.) (Kuhling and Kazelitz [20] give m. p. 224° for the compound described as "methylaminophenyliminoalloxansaure;" Clark-Lewis [10] gives m. p. 238°) (Found: C, 53·4; H, 3·7. Calc. for $C_{11}H_{10}O_3N_4$: C, 53·7; H, 4·1%).

2 : 3 : 4 : 5 : 1′ : 2′ : 3′ : 4′-Octahydro-1 : 3 : 4′-trimethyl-2 : 3′ : 5-trioxoglyoxaline-4-spiro-2′-quinoxaline.—The preceding spiran (0·425 g.), methyl iodide (2 c.c.), and anhydrous potassium carbonate (3 g.) were refluxed in dry acetone (25 c.c.) for 20 hr. The mixture was cooled, water was added, and the whole was extracted with chloroform (3 × 50 c.c.). The dried chloroform extracts were evaporated to dryness, and the residue recrystallised from ethanol, to give the trimethyl compound (0·32 g., 68%), m. p. 194° undepressed when mixed with an authentic sample prepared from 3-hydroxyquinoxaline-2-carboxyureide by Clark-Lewis's method.[10]

2-Amino-4-hydroxybenzo[g]*pteridine* (IV; R = H).—(*a*) A solution from sodium (0·347 g.) in dry propan-1-ol (40 c.c.) was added to a warm solution of dry guanidine hydrochloride (1·49 g.) in dry propan-1-ol (50 c.c.). The mixture was shaken, added to a hot solution of the *N*-methyl ester (II; R = Me, R′ = H) (1 g.) in dry propan-1-ol (50 c.c.), and refluxed for 90 hr. The resulting suspension was evaporated to dryness *in vacuo*, the residue was dissolved in water, and 2N-hydrochloric acid was added to give pH 6. The yellow gelatinous precipitate which separated was collected by centrifugation, washed with water, and dried. Recrystallisation from dimethylformamide (850 c.c.) gave *2-amino-4-hydroxybenzo*[g]*pteridine* (0·55 g., 56%) as small prismatic needles, m. p. >360° (Found: C, 56·2; H, 3·3; N, 33·1. $C_{10}H_7ON_5$ requires C, 56·3; H, 3·3; N, 32·9%).

(*b*) Similar condensation of methyl 3-hydroxyquinoxaline-2-carboxylate (1 g.) with guanidine hydrochloride (1·49 g.) and sodium (0·345 g.) in propanol (150 c.c.) gave, after 17 hours' refluxing, the benzopteridine (0·56 g., 54%). The ultraviolet and infrared spectra of this material were identical with those of the specimen prepared as above.

(*c*) The *spiro*-compound (III; R = Me, R′ = H, R″ = NH) (0·4 g.), suspended in dry methanol (15 c.c.), was added to a solution from sodium (0·08 g.) in dry methanol (10 c.c.). The mixture was refluxed for 44 hr. and worked up as above, to give the benzopteridine (43 mg., 12%), identical with the material prepared as in (*a*) and (*b*).

Methylation of 2-Amino-4-hydroxybenzo[g]*pteridine* (IV; R = H).—The benzopteridine (126 mg.), methyl iodide (1 c.c.), and anhydrous potassium carbonate (2 g.) were refluxed in dry acetone (25 c.c.) for 24 hr. The mixture was shaken with water and chloroform (3 × 50 c.c.), and the chloroform extracts were dried and evaporated to dryness. Recrystallisation from methanol (charcoal) gave the *dimethyl derivative* (56 mg., 40%) of the benzopteridine as pale yellow needles, m. p. 350° (Found: C, 59·5; H, 4·5; N, 29·4. $C_{12}H_{11}ON_5$ requires C, 59·7; H, 4·6; N, 29·0%). Starting materials obtained by methods (*a*) and (*b*) gave identical products.

[20] Kuhling and Kazelitz, *Ber.*, 1906, **39**, 1314.

Ethylation of 2-Amino-4-hydroxybenzo[g]*pteridine* (IV; R = H).—Treatment of the benzopteridine prepared by methods (*a*) and (*b*) above with ethyl iodide in the presence of anhydrous potassium carbonate gave the *diethyl derivative* as pale yellow needles, m. p. and mixed m. p. 307—308° (decomp.) (Found: C, 62·4; H, 5·4; N, 25·7. $C_{14}H_{15}ON_5$ requires C, 62·4; H, 5·6; N, 26·0%), having identical ultraviolet and infrared spectra.

2 : 10-*Dihydro-4-hydroxy-2-imino-10-methylbenzo*[g]*pteridine* (I; R = Me, R′ = H).—Methyl 3 : 4-dihydro-4-methyl-3-oxoquinoxaline-2-carboxylate (1 g.) was condensed with guanidine (3 equivs.) as in the preparation of the alloxazine derivative (IV; R = H) above, except that the reaction was carried out in the dark and in oxygen-free nitrogen. The cooled reaction mixture yielded a bright orange precipitate (1·6 g.) readily separated by paper chromatography into the aminoalloxazine (IV; R = H) and the *iso*alloxazine 2-imine (I; R = Me, R′ = H). Chromatography of a larger sample (0·3 g.) on a column of cellulose powder (Whatman No. 1; 70 g.) with butan-1-ol–water–acetic acid (63 : 27 : 10) as solvent did not, however, separate the two components completely. The remainder of the crude product (1·3 g.) was dissolved in the minimum quantity of hot 5N-hydrochloric acid. The hydrochloride of the aminoalloxazine (IV; R = H) which separated on cooling was filtered off, and the filtrate was diluted with ethanol and evaporated to dryness *in vacuo*. The residue, when shaken with dry ethanol (100 c.c.), gave a bright orange powder (100 mg.). Recrystallisation from ethanol gave the bright yellow *isoalloxazine 2-imine* (I; R = Me, R′ = H), m. p. >350° (Found: C, 53·3; H, 4·0. $C_{11}H_9ON_5,H_2O$ requires C, 53·9; H, 4·5%).

Methyl 3 : 4-*Dihydro*-6 : 7-*dimethyl*-3-*oxo*-4-D-*ribitylquinoxaline*-2-*carboxylate* (II; R = D-ribityl, R′ = Me).—To a solution of 3 : 4-dihydro-6 : 7-dimethyl-3-oxo-4-D-ribitylquinoxaline-2-carboxylic acid [11] (4·6 g.) in the minimum quantity of methanol was added ethereal diazomethane (8 equivs.). After 30 min., the solution was evaporated to dryness *in vacuo* and the residue recrystallised from methanol, to give the *methyl ester* (4·25 g., 89%) as pale yellow needles, m. p. 185—186° (Found: C, 55·9; H, 6·3; N, 7·6. $C_{17}H_{22}O_7N_2$ requires C, 55·7; H, 6·1; N, 7·7%).

2 : 3 : 4 : 5 : 1′ : 2′ : 3′ : 4′-*Octahydro*-2-*imino*-6′ : 7′-*dimethyl*-3′ : 5-*dioxo*-4′-D-*ribitylglyoxaline*-4-spiro-2′-*quinoxaline* (III; R = D-*ribityl*, R′ = Me, R″ = NH).—To a solution from sodium (0·52 g.) in dry propan-1-ol (12·5 c.c.) was added dry powdered guanidine hydrochloride (0·225 g.) in propan-1-ol (12·5 c.c.). The mixture, after filtration, was added to a solution of the methyl ester (II; R = D-ribityl, R′ = Me) (0·25 g.) in dry propan-1-ol (12·5 c.c.) and kept at 25° for 20 hr. The cream-coloured precipitate was collected, washed with a little water, and dried, to give the spiro-*compound* (0·2 g., 75%), m. p. 183—186° (Found: C, 48·3; H, 6·6; N, 16·9. $C_{17}H_{23}O_6N_5,1{\cdot}5H_2O$ requires C, 48·6; H, 6·2; N, 16·7%).

A solution of the *spiro*-compound in 2N-hydrochloric acid gave the *hydrochloride*, yellow needles, m. p. 227—229° (from 2N-hydrochloric acid) (Found: C, 47·7; H, 5·9; N, 16·7. $C_{17}H_{23}O_6N_5,HCl$ requires C, 47·5; H, 5·6; N, 16·3%).

2-*Amino*-4-*hydroxy*-7 : 8-*dimethylbenzo*[g]*pteridine* (IV; R = Me).—The methyl ester (II; R = D-ribityl, R′ = Me) (1 g.) was condensed with guanidine (from 0·85 g. of the hydrochloride and 0·2 g. of sodium) in propan-1-ol as in the preparation of the analogous aminoalloxazine (IV; R = H). Recrystallisation of the crude product (0·66 g.) from dimethylformamide gave the benzopteridine (" 2-aminolumichrome ") as yellow needles, m. p. >350° (Found: C, 59·7; H, 4·2; N, 28·6. Calc. for $C_{12}H_{11}ON_5$: C, 59·7; H, 4·6; N, 29·0%).

2 : 10-*Dihydro*-4-*hydroxy*-2-*imino*-7 : 8-*dimethyl*-10-D-*ribitylbenzo*[g]*pteridine* (I; R = D-ribityl, R′ = Me).—The preceding condensation was repeated in the dark and in an atmosphere of oxygen-free nitrogen. The mixture was filtered while hot, and the crude orange-brown product (1·25 g.) was washed with ether and dried. This material, shown by paper chromatography to contain riboflavin 2-imine, was purified by one of the following methods:

(*a*) The crude material (1·25 g.) was dissolved in 50% acetic acid (300 c.c.), and the solution was neutralised with solid sodium hydrogen carbonate. The red insoluble material which separated was removed by centrifugation, and the supernatant solution was extracted with phenol (total 300 g.). Ether (1 l.) was added to the phenolic extracts which were re-extracted with water (90 c.c.). This aqueous solution deposited overnight a red product shown by paper chromatography to be largely riboflavin 2-imine. This was dissolved in the minimum of 5% acetic acid and chromatographed on Florisil (50 g.) in the dark. The column was washed with 5% acetic acid (100 c.c.), and the product finally eluted with 1 : 9 pyridine–water (150 c.c.). The eluate was taken to dryness *in vacuo* (rotatory evaporator), and the residue dissolved in

the minimum of 2N-hydrochloric acid. Addition of solid sodium hydrogen carbonate to give pH 7 yielded the iso*alloxazine 2-imine* (" 2-iminoriboflavin ") as an orange powder (0·15 g., 15%) (Found: C, 54·1; H, 5·7; N, 18·2. $C_{17}H_{21}O_5N_5$ requires C, 54·4; H, 5·6; N, 18·7%).

(*b*) The crude product (1·2 g.) was dissolved in 2N-hydrochloric acid (100 c.c.). To the filtered solution were added a few drops of hydrogen peroxide to bleach any soluble impurities, and 10% aqueous sodium hydrogen carbonate was added to give pH 7. The resulting mixture was heated at 100° for 2 hr. to coagulate the precipitate, which was collected after being kept overnight at 0°. This procedure was repeated several times and finally gave riboflavin 2-imine (0·15 g.) as an orange powder. The infrared spectrum was identical with that of the specimen prepared as above.

Methyl 3 : 4-*Dihydro*-4 : 6 : 7-*trimethyl*-3-*oxoquinoxaline*-2-*carboxylate* (II; R = R′ = Me).—3 : 4-Dihydro-4 : 6 : 7-trimethyl-3-oxoquinoxaline-2-carboxylic acid [14] (2·8 g.) was esterified with diazomethane (8 equivs.). Recrystallisation from methanol gave the *methyl ester* (II; R = R′ = Me) (1·95 g., 66%) as yellow plates, m. p. 169—170° (Found: C, 63·8; H, 5·9; N, 11·4. $C_{13}H_{14}O_3N_2$ requires C, 63·4; H, 5·7; N, 11·4%).

2 : 3 : 4 : 5 : 1′ : 2′ : 3′ : 4′-*Octahydro*-2-*imino*-4′ : 6′ : 7′-*trimethyl*-3′ : 5-*dioxoglyoxaline*-4-spiro-2′-*quinoxaline* (III; R = R′ = Me, R″ = NH).—Methyl 3 : 4-dihydro-4 : 6 : 7-trimethyl-3-oxoquinoxaline-2-carboxylate (II; R = R′ = Me) (0·25 g.) was condensed with guanidine as in the preparation of the ribityl analogue (III; R = D-ribityl, R′ = Me, R″ = NH). Recrystallisation of the crude product from 2N-hydrochloric acid gave the *hydrochloride* of the *spiro*-compound as yellow needles, m. p. 267—270° (Found: C, 50·1; H, 5·4; N, 22·3. $C_{13}H_{15}O_2N_5$,HCl requires C, 50·4; H, 5·2; N, 22·6%).

The free *base* was prepared by dissolving the hydrochloride in water and adjusting the pH to 7 by the addition of solid sodium hydrogen carbonate. The pale cream precipitate was collected, washed with hot water, and dried *in vacuo* to give a hygroscopic powder, m. p. 275° (Found: C, 57·1; H, 5·5. $C_{13}H_{15}O_2N_5$ requires C, 57·1; H, 5·5%).

2 : 10-*Dihydro*-4-*hydroxy*-2-*imino*-7 : 8 : 10-*trimethylbenzo*[g]*pteridine* (I; R = R′ = Me).—To a solution of dimeric 3 : 4-dimethyl-*o*-benzoquinone [12] (0·32 g.) in water (10 c.c.) and 10% aqueous sodium hydroxide (1 c.c.) was added a similar solution of 2 : 5-diamino-4-hydroxy-6-methylaminopyrimidine [13] (V; R = Me) (0·4 g.). The mixture was kept at room temperature for 3 days in a stoppered flask. The resulting precipitate was collected, shaken with N-sodium hydroxide (25 c.c.) in which the *iso*alloxazine 2-imine is insoluble, and finally re-filtered and washed with ether. Recrystallisation from the minimum quantity of 2N-hydrochloric acid gave the *hydrochloride* (0·06 g.) of the benzopteridine as orange needles, m. p. >360° (Found: C, 53·1; H, 4·6; N, 24·2. $C_{13}H_{13}ON_5$,HCl requires C, 53·5; H, 4·8; N, 24·0%).

Treatment of the iso*Alloxazine* 2-*Imines* (I) *with Alkali*.—The imine (2 mg.) was heated in N-sodium hydroxide (1 c.c.) at 100° for 1 hr. in the dark. The products were examined by paper chromatography and ultraviolet spectroscopy. Riboflavin 2-imine (I; R = D-ribityl, R′ = Me) and lumiflavin 2-imine (I; R = R′ = Me) were converted completelyin to 2-aminolumichrome (IV; R = Me); and the 10-methyl*iso*alloxazine 2-imine (I; R = Me, R′ = H) was converted into 2-aminoalloxazine (IV; R = H). A similar change took place more slowly at room temperature.

Photolysis of Riboflavin 2-*Imine* (I; R = D-ribityl, R′ = Me).—Riboflavin 2-imine (5 mg.) in 0·1N-hydrochloric acid (5 c.c.) was irradiated for 16 hr. with ultraviolet light (Hanovia UV.S. 500). Paper chromatography and ultraviolet spectroscopy then showed the presence of 2-aminolumichrome (IV; R = Me) and a little unchanged imine.

Similar photolysis in 0·5N-sodium hydroxide yielded lumiflavin 2-imine (I; R = R′ = Me), 2-aminolumichrome (IV; R = Me), and a product of unknown structure whose absorption spectrum (max. at 210, 270, and 370 mμ at pH 1), indicates that it is probably a quinoxaline.

The authors acknowledge helpful discussion with Dr. Peter Hemmerich of the University of Basle, and a Research Studentship from D.S.I.R. (to R. M. C.).

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[*Received, September 9th*, 1958.]