406. *Pteridine Studies. Part VIII.* The Degradation of Pteridine. Methylation of the Hydroxypteridines and Degradation of the Products.*

By Adrien Albert, D. J. Brown, and H. C. S. Wood.

Pteridine (I) was found to be a weak acid of pK 12·2. Acid degradation led to 2-amino-3-formylpyrazine (II), and alkaline breakdown in the presence of hydroxylamine gave the oxime of the amidine (III; R = CH:N·OH). The acid degradation of 4-methylpteridine also stopped at the amidine stage.

Attempted quaternization of pteridine was unsuccessful. Methylation of 2-, 6-, and 7-hydroxypteridine gave in each case a single N-methyl derivative (*e.g.*, V), and 2 : 4- and 6 : 7-dihydroxypteridine gave each an NN'-dimethyl derivative. However, 4-hydroxypteridine, depending on conditions, gave either two isomeric N-methyl derivatives or one of these plus 4-methoxypteridine. In most cases the products of methylation were synthesized from (and also degraded to) known pyrazines or pyrimidines. The further methylation of 8-methyl-7-pteridone (X; R = H) was shown to occur at position 6, and the mechanism of this is discussed.

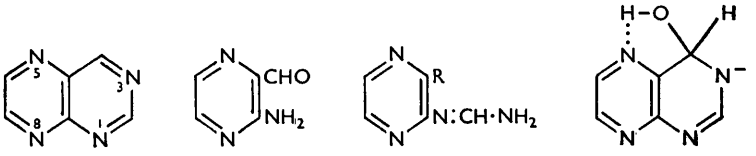
In Part VII,* degradations of 4-, 6-, and 7-hydroxypteridine by acid and alkali were described. Degradation of the 2-isomer is more complex, but a preliminary account has been given.[1] The present studies deal with the degradation of pteridine, its attempted methylation, the methylation of the hydroxypteridines, and the degradation of the methylated products.

Pteridine.—When pteridine (I) was slowly titrated with one equivalent of hydrochloric acid, each pH reading drifted to more alkaline values, signifying decomposition. Under

* Part VII, *J.*, 1955. 2690.

[1] Albert, Ciba Symposium on Chemistry and Biology of Pteridines, Churchill, London, 1954, pp. 207—209.

severer conditions 2-amino-3-formylpyrazine (II) was isolated and identified by oxidation to the known 3-aminopyrazine-2-carboxylic acid. 4-Methylpteridine, similarly treated gave the highly basic *N'*-(3-acetyl-2-pyrazinyl)formamidine (III; R = Ac), and this suggests that the alkaline drift during the titration of pteridine is due to formation of a similar amidine (III; R = CHO).



(I) (II) (III) (IV)

Pteridine proved to be more stable to alkali, giving a pK_a of 12·20 * when titrated with carbonate-free potassium hydroxide; back-titration showed that no decomposition had occurred. This weak acidic character of pteridine explains the spectroscopic shift in alkaline solution.[2] The anion is formulated as the resonance-stabilized hydrate (IV).[3]

In severer alkaline conditions pteridine decomposed, *e.g.*, in 2N-sodium carbonate it formed a basic, asphalt-like mass. This appears to be a self-condensation product from the amidine (III; R = CHO) because, when hydroxylamine was also present, the aldoxime (III; R = CH:N·OH) was formed in good yield. This oxime was hydrolysed by acid or alkali to the very stable oxime of the amino-aldehyde (II).

This tendency for the pteridine nucleus to lose the pyrimidine (rather than the pyrazine) ring on acid and alkaline hydrolyses is consonant with electron-density diagrams [4] which show the pyrimidine ring to be the more polar and hence more readily attacked. Thus, too, in the 4-, 6-, 7-monohydroxypteridines [5] the ring opened is that bearing the hydroxy-group. The analogous (acid) hydrolysis [6] of quinazoline to 2-aminobenzaldehyde needs more vigorous conditions than that of pteridine, as would be expected from the lower N : C ratio.[4]

It was previously reported [7] that butanol–acetic acid is unsatisfactory for paper chromatography of pteridine because the latter is photo-decomposed, but re-investigation showed that it was only being hydrolysed by the acid; aqueous ammonium chloride has now been found satisfactory (see Table).

Pteridine could not be quaternized with methyl iodide in ether (10 days at 20°) and decomposed to a pitch when excess of methyl iodide was used without solvent.

Monohydroxypteridines can be methylated on oxygen, nitrogen, or carbon, the last only under forcing conditions. All the monomethoxypteridines are known and are recognizable by their melting points and by their rapid hydrolysis to hydroxypteridines. With this knowledge, it soon became apparent that methylation usually favoured the nitrogen atom.

Methylation of 2-Hydroxypteridine.—Methanolic diazomethane did not react with 2-hydroxypteridine, and methyl sulphate profoundly decomposed it in aqueous suspension at pH 8. However, methyl iodide in methanolic sodium methoxide readily gave a product $C_7H_6ON_4,H_2O$ (not 2-methoxypteridine).[8,9] The ultraviolet spectrum of the neutral molecule resembled that of 2-hydroxypteridine,[8] and hence the choice lay between 1-methyl-2-pteridone † (V) and the 3-methyl isomer. The remaining isomer (VI) is excluded because

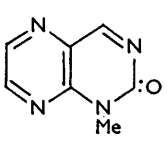
* Thermodynamic; spread = ± 0·01 (0·02M).

† We prefer this name to 1 : 2-dihydro-1-methyl-2-oxopteridine which the Editor states is correct practice for this *Journal*; and similarly for analogous compounds. It is hoped that a ruling will shortly be given by I.U.P.A.C.

[2] Lister, Ramage, and Coates, *J.*, 1954, 4109.
[3] Brown and Mason, unpublished work.
[4] Albert, *Quart. Rev.*, 1952, **6**, 213, 211.
[5] Albert, *J.*, 1955, 2690.
[6] Gabriel, *Ber.*, 1903, **36**, 809.
[7] Albert, Brown, and Cheeseman, *J.*, 1952, 1620.
[8] *Idem, ibid.*, p. 4219.
[9] Mason, *J.*, 1955, 2336.

the transannular structure would absorb at a much longer wavelength.[3] No unambiguous synthesis of (V) could be devised, but the 3-isomer was prepared from 4 : 5-diamino-1-methyl-2-pyrimidone [10] which was cyclized with glyoxal. This 3-methyl-2-pteridone was different from the methylation product, which must be 1-methyl-2-pteridone. The bound water in these isomers could not be removed by heat without decomposing them, but that in the 1-methyl isomer was readily exchanged for ethanol (2-hydroxypteridine monohydrate is dehydrated at 180°, and re-forms the original material on addition of water).

Attempted degradation of the 1- and the 3-isomer with boiling N-acid or -alkali gave a complex mixture from which nothing was isolated.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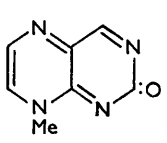
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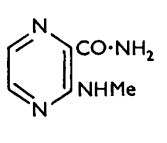
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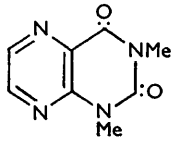

(VIII)

Methylation of 4-Hydroxy- and 2 : 4-Dihydroxy-pteridine.—Diazomethane converted 4-hydroxypteridine into a mixture of 4-methoxypteridine [11] and 3-methyl-4-pteridone (no isomer was detectable) in the ratio of 1 : 3. This methylpteridone has previously been obtained from 2-aminopyrazine-3-carboxymethylamide and formic acid.[11] Methyl sulphate converted 4-hydroxypteridine into 3-methyl- and 1-methyl-4-pteridone (2 : 1). A paper chromatogram (developed in butanol–acetic acid) of the crude methylation mixture showed spots for only these two substances; in particular, 4-methoxypteridine and 2-methylaminopyrazine-3-carboxyamide (VII) were absent. The 1-methyl-4-pteridone was difficult to isolate because the solubilities resembled those of sodium methyl sulphate : however it was precipitated as the reineckate and regenerated; it was also synthesized by our general method [12] from 2-methylaminopyrazine-3-carboxyamide, prepared from 2-hydroxypyrazine-3-carboxylic acid through methyl 2-chloropyrazine-3-carboxylate. When the methyl sulphate reaction was carried out at pH 7 (where only 10% of the hydroxypteridine is anionic) instead of pH 8, very little methylation occurred, even at 50°.

3-Methyl-4-pteridone was readily decomposed by hot alkali, giving 2-aminopyrazine-3-carboxymethylamide (32%) and 2-aminopyrazine-3-carboxylic acid (51%). 3-Methyl-4-pteridone was unchanged by cold, but profoundly decomposed by boiling, N-hydrochloric acid. 1-Methyl-4-pteridone was unstable in cold alkali, 2-methylaminopyrazine-3-carboxyamide being formed to the extent of 90% in 5 minutes at pH 12, 70% in 1 hour at pH 11, and none in 1 hour at pH 10.

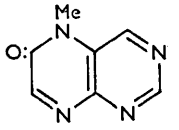
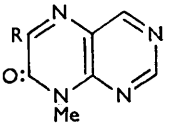
Methyl sulphate similarly methylated 2 : 4-dihydroxypteridine to 1 : 2 : 3 : 4-tetrahydro-1 : 3-dimethyl-2 : 4-dioxopteridine (VIII) which was unchanged by refluxing 10N-hydrochloric acid for 30 minutes. It was quantitatively converted in one minute by boiling N-sodium hydroxide into 2-methylaminopyrazine-3-carboxymethylamide, which was synthesized from methyl 2-chloropyrazine-3-carboxylate. The dione has also been prepared by condensation [13] of glyoxal with 5 : 6-diamino-1 : 2 : 3 : 4-tetrahydro-1 : 3-dimethyl-2 : 4-dioxopyrimidine (30% yield).

Methylation of 6-Hydroxypteridine.—5-Methyl-6-pteridone (IX) was formed by the action of methyl sulphate on 6-hydroxypteridine in methanolic sodium methoxide (14% yield). It formed complexes with unchanged 6-hydroxypteridine which were difficult to decompose. No other product of methylation was found. The constitution of 5-methyl-6-pteridone was confirmed by synthesis from 4-amino-5-methylaminopyrimidine.[14] 6-Hydroxypteridine was largely unchanged by diazomethane (acting on freshly precipitated material suspended in methanol at −10°), by methyl sulphate in water at pH 10·5 (self-buffered,

[10] Brown, Hoerger, and Mason, *J.*, 1955, 211.
[11] Albert, Brown, and Cheeseman, *J.*, 1952, 4219.
[12] *Idem*, *J.*, 1951, 474.
[13] Blicke and Godt, *J. Amer. Chem. Soc.*, 1954, **76**, 2798.
[14] Brown, *J. Appl. Chem.*, 1955, **5**, 358.

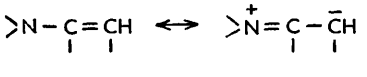
20°), or by methyl iodide and aqueous potassium hydroxide.[15] It was destroyed when the silver salt was shaken with methyl iodide in methanol at 70°.

Similar attempts to methylate 7 : 8-dihydro-6-hydroxypteridine produced the iodide of a highly basic ($pK_a = 11{\cdot}4$) trimethyl derivative. The analytical figures suggest the constitution 5 : 6 : 7 : 8-tetrahydro-5 : 8 : *x*-trimethyl-6-oxopteridinium iodide, where *x* is

(IX)   (X)

probably 3 because of resonance stabilization of the cation when the charge is shared between $N_{(3)}$ and $N_{(8)}$.

Methylation of 7-Hydroxypteridine and 6 : 7-Dihydroxypteridine.—We have previously [7] described the methylation of 7-hydroxypteridine with diazomethane to give a mono- and a di-methylated product of unknown orientation. It is now found that the former is obtained



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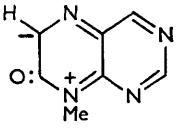

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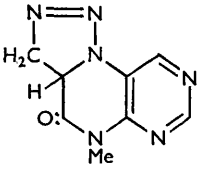

(XIV)

in better yield by the use of methyl sulphate in water at pH 8, and its synthesis from 5-amino-4-methylaminopyrimidine [16] proves it to be 8-methyl-7-pteridone (X; R = H). No 7-methoxypteridine [15] was detected, but an excess of methyl sulphate produced a little of the dimethylated product. This was shown, by its synthesis from the above pyrimidine and ethyl pyruvate, to be the *CN*-dimethyl derivative (X; R = Me). Both substances were hydrolysed to 5-amino-4-methylaminopyrimidine, quantitatively determined by paper chromatography.[5]

Methylation on a carbon atom bearing a highly negative charge is well known, *e.g.*, in the pyrrole, indole, and pyrrocoline series, but electron diagrams show that in pteridine all the carbon atoms are positive.[4] The above example is best explained along the lines of *C*-methylation of ethyl β-diethylaminocrotonate [17] and the 1 : 2-dihydro*iso*quinolines,[18] *viz.*, that resonance between the three-atom systems (XI) and (XII) produces a hybrid with a negatively charged carbon atom which is attacked by a methyl cation and, finally, a proton is eliminated.[19] The equivalent of (XII) in the present case is (XIII) which contains a five-atom system vinylogous with (XII) and demonstrable by proceeding anticlockwise from the 8-position. This accounts for the *C*-methylation by methyl sulphate, but that by diazomethane is more likely to involve addition across the 5 : 6-double bond to give, at first, the 1 : 2 : 3-triazoline (XIV), just as in the reaction of benzylideneaniline with diazomethane.[20]

Oxidation of 8-methyl-7-pteridone with nitric acid gave 6-hydroxy-8-methyl-7-pteridone (X; R = OH), just as 7-hydroxypteridine gave 6 : 7-dihydroxypteridine.[7] The structure was proved by synthesis from 5-amino-4-methylaminopyrimidine and dimethyl oxalate.

Methylation of 6 : 7-dihydroxypteridine gave a *NN*-dimethyl derivative, shown to be 5 : 6 : 7 : 8-tetrahydro-5 : 8-dimethyl-6 : 7-dioxopteridine by (i) alkaline degradation to

[15] Albert, Brown, and Wood, *J.*, 1954, 3832.
[16] Brown, *J. Appl. Chem.*, 1954, **4**, 72.
[17] Robinson, *J.*, 1916, **109**, 1038; Lauer and Lones, *J. Amer. Chem. Soc.*, 1937, **59**, 232.
[18] Freund and Fleischer, *Annalen*, 1915, **409**, 188.
[19] Gensler in Elderfield's "Heterocyclic Compounds," Wiley, New York, 1952, Vol. IV, p. 394.
[20] Buckley, *J.*, 1954, 1850.

4 : 5-bismethylaminopyrimidine, and (ii) synthesis (methylation) from the above 6-hydroxy-8-methyl-7-pteridone (from considerations of valency, the entering group can go only in the 5-position).

Discussion.—It is known that diazomethane does not attack weak acids,[21] and hence it is not surprising that 2- and 6-hydroxypteridine are unaffected whereas the stronger 4- and 7-isomers are methylated. Fischer [22] pointed out that purines become more subject to hydrolysis by alkali upon *N*-methylation. The two *N*-methyl-4-pteridones exemplify this effect in the pteridine series, the time of hydrolysis of 4-hydroxypteridine being reduced from several hours [5] to less than a minute. Where the product proved to be sensitive to cold aqueous alkali, the methylation was carried out in methanol. Like the *N*-methylpurines, the *N*-methylpteridones are less sensitive to acid than to alkali (cf. the 1 : 3-dimethyl-2 : 4-dione, above).

Experimental

The analyses are by Mr. P. R. W. Baker (Wellcome Research Laboratories, Beckenham). Chromatography was performed on No. 1 paper by the ascending method (authentic material was included as a control). Substances were dried in air at 110° unless otherwise specified.

Acid Hydrolysis of Pteridine.—(*a*) Pteridine [12] (2 g.) and N-sulphuric acid (40 ml.) were refluxed (bath at 120°) for 5 min. The cooled solution was adjusted to pH 7, treated with carbon (0·5 g.), refluxed for 10 min., and filtered through kieselguhr to remove tar. Hydroxylamine hydrochloride and hydrated sodium acetate (5 g. of each) were added to the yellow filtrate, giving *2-amino-3-formylpyrazine oxime* (85%) (a second crop obtained on concentration), recrystallizing from water (30 parts) as needles, m. p. 201—202° (Found: C, 43·4; H, 4·1; N, 40·2. $C_5H_6ON_4$ requires C, 43·5; H, 4·4; N, 40·6%). The oxime is unchanged by boiling N-acetic acid during 1 hr. or N-sodium hydroxide during 24 hr. at 20°.

(*b*) The above yellow filtrate was evaporated *in vacuo* at 20°. The powdered residue was sublimed at 65—70°/0·01 mm., giving *2-amino-3-formylpyrazine* (55%), crystallizing from benzene and resubliming as pale yellow needles, m. p. 119—120°, soluble in 5 parts of water at 100° and volatile in steam (Found: C, 49·1; H, 3·8; N, 34·2. $C_5H_5ON_3$ requires C, 48·8; H, 4·1; N, 34·1%). When hydrolysis was for 15 min., no aldehyde was obtained. It formed the above oxime (97%), m. p. and mixed m. p. 200—201°. The aldehyde could not be obtained by the action of Raney nickel on 2-aminopyrazine-3-carboxythioamide, or of sodium amalgam at −5° under slightly acid conditions on ethyl 2-aminopyrazine-3-carboxylate. Neither the aldehyde nor the oxime was converted into 2-hydroxypteridine by urea or urethane at 150°, urea and formic acid at 125°, or aqueous urea or urethane and a trace of copper acetate; nor into 2-aminopteridine by guanidine carbonate at 200°. Pteridine was not re-formed from the aldehyde by formamide at 125°.

Alkaline Hydrolysis of Pteridine.—Pteridine (0·66 g.) was rapidly dissolved at 20° in 2N-sodium carbonate (7·5. ml., 3 equiv.). Hydroxylamine hydrochloride (0·35 g., 1 equiv.) was added at once, whereupon some *3-formyl-2-pyrazinylformamidine oxime* (III; R = CH:N·OH) was deposited. After 24 hr. in the dark at 25° (pH remained at 9·5), the mixture was filtered and the washed crystals were boiled with water (10 ml.) for 2 min. (filtrate rejected), giving 60% of white product, decomp. slightly at 165°, and almost insoluble in boiling water and organic solvents (Found: C, 44·1; H, 4·2; N, 42·95; O, 9·8. $C_6H_7ON_5$ requires C, 43·6; H, 4·3; N, 42·4; O, 9·7%). $\lambda_{max.}$ = 303 mμ, log ε = 3·90, in 0·01N-acetic acid; no other peak. This amidine (0·16 g.) and N-acetic acid (5 ml.) were refluxed for an hour, adjusted to pH 5, and refrigerated, giving 60% of 2-amino-3-formylpyrazine oxime, m. p. and mixed m. p. 199—200° (identity checked by chromatography).

Oxidation of 2-Amino-3-formylpyrazine.—0·1M-Potassium permanganate (4·4 ml.) was slowly added to the aldehyde (0·08 g.) in water (4 ml.) at 20°. The mixture was boiled and filtered. From the sodium salt which crystallized, 2-aminopyrazine-3-carboxylic acid was liberated at pH 2·5 in 70% yield, having m. p. 199—200° (mixed m. p. and R_F).[12]

Hydrolysis of 4-Methylpteridine.—0·5N-Sulphuric acid (10 ml., 1·2 equiv.) and 4-methylpteridine (0·6 g.) were refluxed for exactly 5 min. (pH was 1·5 before and after heating), then adjusted to pH 6·5 with sodium hydrogen carbonate. The crystals were dried at 20° and recrystallized from water, giving 50% of pale crystals of *N'*-(3-acetyl-2-pyrazinyl)formamidine as

[21] Arndt and Martius, *Annalen*, 1932, **499**, 247.
[22] Fischer, *Ber.*, 1898, **31**, 3266.

sulphate, decomp. 110° (Found, for material dried at 20°/0·01 mm.: C, 39·9; H, 5·4; N, 26·6; S, 3·9. $C_7H_8ON_4,0{\cdot}25H_2SO_4,1{\cdot}25H_2O$ requires C, 39·8; H, 5·3; N, 26·5; S, 3·8%). Chromatography, and a negative benzidine test for aldehydes showed the absence of 4-methylpteridine and 4-amino-5-glyoxylidenepyrimidine respectively, which were not excluded by the analytical figures. The sulphate was made alkaline with sodium carbonate, giving the *base*, which recrystallized from alcohol. It is readily soluble in boiling water, but insoluble in benzene and decomposes at 200° and on storage at 20° (Found, for material dried at 110°/0·01 mm.: C, 49·3; H, 5·1; N, 33·05. $C_7H_8ON_4,0{\cdot}33H_2O$ requires C, 49·4; H, 5·1; N, 32·95%).

Reaction of Pteridine with Methyl Iodide.—Pteridine (0·12 g.), dissolved in methyl iodide (5 ml.), was set aside in the dark at 20° for 14 days. The supernatant liquid (which contained a trace of pteridine) was decanted from the black pitch (0·23 g.) which was extracted with boiling water (8 × 15 ml.) in which it did not visibly dissolve. The extract was titrated with alkali to pH 10, which revealed that 1·05 equiv. of acid had been formed. The iodide ion in this solution was precipitated as silver iodide (0·25 g.), revealing an iodine : pteridine ratio of 1·17 : 1.

Paper chromatography : [a] R_F, *and colour of spot in ultraviolet light.*

Substance	Developed in butanol–5N-acetic acid (7 : 3) [b] R_F	254 mμ	365 mμ	Developed in 3% aqueous ammonium chloride [h] R_F	254 mμ	365 mμ
Pteridines						
Pteridine	—[c]	—	—	70	B	B
4-Methylpteridine	60[d]	DB	W	80	D	X
2-Hydroxypteridine	55—70[e]	W	X	70	W	X
O-Me (m. p. 151°)	75	B	B	70	B	B
1-Me (dec. 150°)	80	B	X	80	B	X
3-Me (dec. 210°)	75	W	X	75	W	X
4-Hydroxypteridine	25	D[f,g]	X	70	D[f]	X
O-Me (m. p. 195°)	65	B	B	70	D	X
1-Me (m. p. 224°)	35	DB	B	80	DB	DB
3-Me (m. p. 286°)	40	D	X	75	D	X
6-Hydroxypteridine	25—75[e]	D	X	60[i]	D	X
O-Me (m. p. 125°)	75	D	X	—[i,c]	—	—
5-Me (dec. 190°)	25—75[e]	D	X	70[i]	D	X
7-Hydroxypteridine	50	D[f]	X	65	D[f]	X
O-Me (m. p. 130°)	70	D[f]	X	75	D[f]	X
8-Me (m. p. 125°)	65	D[f]	X	75	D[f]	X
6 : 8-Me_2 (m. p. 146—147°)	75	D[f]	X	80	D[f]	X
6-OH-8-Me	40	B	B	65	B	X
Pyrazines						
2-NH_2-3-CHO	70	B	B	60	B	B
2-NH_2-3-CH:N·OH	70	B	B	45	B	B
2-(N:CH·NH_2)-3-CH:N·OH	5	D	X	55	D	X
2-(N:CH·NH_2)-3-COMe	0—35[e]	D	X	55	D	X
2-NH_2-3-CO_2H	40	B	B	65	B	B
2-NH_2-3-CO·NHMe (m. p. 134°)	75	B	B	60	B	B
2-NHMe-3-CO·NH_2 (m. p. 199°)	80	G	DB	60	G	DB

[a] Ascending method. [b] pH 2·7. [c] Decomp. (see text). [d] *I.e.*, 60% of distance from starting-point to front. [e] Elongated. [f] Irradiation at 254 mμ quickly produces a blue fluorescence, visible at 365 mμ. [g] As this substance forms chelates, batches of paper contaminated with heavy metals need a trace of sodium sulphide in the developing solution. [h] pH 5·5. [i] Applied to paper in 0·1N-HCl and chromatographed at 0° (at least 60 cm. ascent is necessary).

D = Dark, against fluorescent background of paper (*i.e.*, absorption without fluorescence). X = Invisible. B = Blue or violet fluorescence. DB = Intermediate between B and D. G, W = Green and white fluorescences, respectively.

1-*Methyl*-2-*pteridone* (V).—2-Hydroxypteridine monohydrate [12] (2 g.) was added to boiling methanolic sodium methoxide (from sodium, 0·4 g., and methanol, 40 ml.). As soon as the sodium salt began to crystallize, methyl iodide (4 ml.) was added, and the suspension refluxed for 1 hr., then chilled for a day. The colourless precipitated 1-*methyl*-2-*pteridone monohydrate* (70%), recrystallized from water, had m. p. about 150° (decomp.). It is soluble in 145 parts of water at 20° and 13 parts at 100°, but poorly soluble in boiling benzene (Found : for material dried at 110°/0·01 mm.: C, 46·3; H, 4·45; O, 17·75; N, 31·3. $C_7H_6ON_4,H_2O$ requires C, 46·65;

H, 4·5; O, 17·75; N, 31·1%). [pK's : acidic, 11·43 ± 0·04 (0·01M); basic, < 1. $\lambda_{max.}$ (mμ) and log ε : 240, 3·91; 311, 3·85 (pH 7·0); 236, 3·89; 312, 3·81 (pH 13). Cf. 2-hydroxypteridine: 230, 3·88; 307, 3·83 (pH 7·0).] It gives a violet colour with N-sulphuric acid, or N-sodium carbonate, at 100° (15 min.).

When recrystallized three times from ethanol (30 parts), the *ethanol solvate* was formed, colourless needles, m. p. about 155° (decomp.) (Found, for material dried over P_2O_5 at 20° : C, 52·35; H, 5·8; N, 27·15. $C_7H_6ON_4,C_2H_6O$ requires C, 51·9; H, 5·8; N, 26·9%).

3-Methyl-2-pteridone.—To 4 : 5-diamino-1 : 2-dihydro-1-methyl-2-oxopyrimidine [23,14] (2·1 g.) in boiling water (10 ml.) was added syrupy (25%) glyoxal (7 ml.; adjusted to pH 5 with ammonia). As soon as a precipitate was seen the mixture was allowed to cool, and then chilled overnight. The solid (2·25 g.) was recrystallized from water, giving colourless needles of *3-methyl-2-pteridone monohydrate*, soluble in 350 parts of water at 20° and 35 parts at 100°, but poorly soluble in organic solvents, decomp. from 210°, m. p. about 280° (decomp.) (Found, for material dried at 110°/0·01 mm.: C, 47·0; H, 4·5; N, 31·25. $C_7H_6ON_4,H_2O$ requires C, 46·65; H, 4·5; N, 31·1%). There was no loss of weight at 155°. The compound gives a violet colour with N-sulphuric acid at 100° (1 hr.). p*K*'s : acidic, 11·01 ± 0·05 (0·01M); basic, < 1. $\lambda_{max.}$ (mμ) and log ε : 230, 3·97; 309, 3·89 (pH 7·0); 236, 3·84; 271, 3·66; 313, 3·80 (pH 13).

4-Hydroxypteridine (improved preparation).—Glyoxal (23 ml. of 50% syrup) in water (100 ml.) was adjusted to pH 5 with ammonia and added to 4 : 5-diamino-6-hydroxypyrimidine (15 g.) in water (300 ml.) at 60—70°. The mixture was heated on a steam-bath until the initial precipitate dissolved and for 10 min. longer. Carbon (4 g.) was added. The chilled filtrate gave pale crystals of 4-hydroxypteridine (80%), which became colourless when recrystallized from 33 parts of water (carbon).

Methylation of 4-Hydroxypteridine.—(a) *With diazomethane.* Methanol (75 ml.) and then water (0·1 ml.) were added to a suspension of finely divided 4-hydroxypteridine (1·5 g.) in 0·2M-ethereal diazomethane (210 ml.). The mixture was stirred at 20° for 2 hr. Next day, the solid was collected and washed with methanol (5 ml.), giving 56% of 3-methyl-4-pteridone, m. p. 283—284° (mixed m. p. 284—285°). The filtrates were evaporated and the residue crystallized from acetone (10 ml.), giving 21% of 4-methoxypteridine, m. p. 183°, which recrystallized from ethanol as pale yellow needles, m. p. 192—193° (mixed m. p.). When hydrolysed with cold N-sodium hydroxide,[11] it gave 95% of 4-hydroxypteridine (R_F and spectra).

(b) *With methyl sulphate.* To a solution (adjusted to pH 8) at 35° of 4-hydroxypteridine (1·48 g., 0·01 mole) in 0·5N-sodium hydroxide (20 ml.), methyl sulphate (1 ml., 1 equiv.) was added during 45 min. and the whole was stirred for 1 hr. longer. The pH was kept at 8 throughout by means of sodium hydroxide. After refrigeration, 45% of 3-methyl-4-pteridone [11] was filtered off (m. p. and mixed m. p. 286°). The filtrate was divided into two halves. To the first, ammonium reineckate was added and the precipitate was decomposed [24] with mercuric chloride, giving 1-methyl-4-pteridone, purified by sublimation (12% yield), m. p. and mixed m. p. 218°. The other half, adjusted to pH 11—12 with sodium hydroxide, liberated colourless *2-methylaminopyrazine-3-carboxyamide* (20%), m. p. 198—199°, soluble in 100 parts of boiling water and 35 of boiling chloroform (Found : C, 47·8; H, 5·3; N, 37·4. $C_7H_6ON_4$ requires C, 47·4; H, 5·3; N, 36·8%).

3-Methyl-4-pteridone is soluble in about 9 parts of boiling water and 150 of boiling chloroform. It (0·5 g.), added to boiling N-sodium hydroxide (5 ml.), refluxed for 30 sec., and at once cooled in ice, gave 32% of 2-aminopyrazine-3-carboxymethylamide, m. p. 131—134° (mixed m. p. and R_F). The filtrate, adjusted to pH 2, slowly deposited 51% of pale needles of 2-aminopyrazine-3-carboxylic acid, m. p. 200° (effervescence) (mixed m. p. and R_F). The acid is not derived from the amide which is almost unaffected by 5 minutes' boiling with N-sodium hydroxide.

1-Methyl-4-pteridone.—2-Methylaminopyrazine-3-carboxyamide (0·4 g.; see below), acetic anhydride (6 ml.; redistilled), and formic acid (6 ml.) were heated for 2½ hr. under reflux at 120°. The mixture was taken to dryness at 80°/vac. The residue was recrystallized (carbon) from pentyl alcohol (10 ml.). The crystals, washed with a little ether, were recrystallized twice more from pentyl alcohol and dried at 120°, giving 70% of needles of *1-methyl-4-pteridone*, m. p. 223—224°, soluble in 2 parts of cold water, sublimes at 170°/0·001 mm. (Found : C, 51·8; H, 3·8; N, 34·5. $C_7H_6ON_4$ requires C, 51·8; H, 3·7; N, 34·5%). This pteridone (0·05 g.) in 0·1M-buffer (10 ml.; borate pH 10, or phosphate pH 11 or 12) at 20° was seeded with 2-methylaminopyrazine-3-carboxyamide and set aside (for results see p. 2068).

[23] Johns, *J. Biol. Chem.*, 1912, **11**, 73.
[24] Panouse, *Bull. Soc. chim. France*, 1949, **16**, 594.

Pyrazine Intermediates for 1-Methyl-4-pteridone.—3-Hydroxypyrazine-2-carboxylic acid was prepared by the action [25] of nitrous acid on 3-aminopyrazine-2-carboxylic acid. Dry hydrogen chloride was passed into a suspension of the hydroxy-acid (6·5 g.) in boiling, dry methanol (250 ml.) for 2½ hr. The solution was concentrated, in a vacuum, to 50 ml., chilled, and cautiously treated with ice-water (220 ml.), and the pH adjusted to 3 by sodium hydrogen carbonate. Sodium chloride (55 g.) was then added and the solution was extracted with ethyl acetate (550 ml.) for 8 hr. The extract was dried, boiled with charcoal (3 g.) for 10 min., filtered, and evaporated in a vacuum. The yellow residue was sublimed at 130° (bath)/0·05 mm., giving methyl 3-hydroxypyrazine-2-carboxylate (4·7 g., 65%), m. p. 151—152° (lit., 151°). This method is more convenient than earlier ones.[25,26] This ester (3·4 g.) and freshly distilled phosphorus oxychloride (20 ml.; catalysed by a drop of 10N-hydrochloric acid) were refluxed for 3 hr., then taken nearly to dryness at 80° in a vacuum. The residue was poured on crushed ice (140 g.), and the mixture stirred for 20 min. The pH was adjusted to 6 with ammonia, sodium chloride (68 g.) was added, and the mixture extracted with ethyl acetate (8 × 35 ml.). The dried (Na_2SO_4) extracts were evaporated under reduced pressure, to give a pale brown oil (83%; substantially pure) which on distillation (b. p. 50—52°/0·04 mm.) gave *methyl 3-chloropyrazine-2-carboxylate*, plates, m. p. 31—32° (Found: C, 41·8; H, 2·8; N, 16·6; Cl, 20·6. $C_6H_5O_2N_2Cl$ requires C, 41·75; H, 2·9; N, 16·2; Cl, 20·6%).

This ester (2·4 g.; not distilled) was swirled with aqueous ammonia (30 ml.; *d* 0·89) at 20° for 5 min., and set aside for 2 hr. The precipitate (1·83 g., 84%; m. p. 183—185°), when recrystallized from water (20 ml.; charcoal), gave the *amide* (1·6 g.), plates, m. p. 186—187° (Found: C, 38·5; H, 2·5; N, 26·7; Cl, 22·4. $C_5H_4ON_3Cl$ requires C, 38·1; H, 2·6; N, 26·7; Cl, 22·5%).

3-Methylaminopyrazine-2-carboxyamide (VII).—3-Chloropyrazine-2-carboxyamide (0·25 g.) and 33% alcoholic methylamine (5 ml.) were heated at 130°, under pressure, for 6 hr. The product was evaporated and recrystallized from water, giving 3-methylaminopyrazine-2-carboxyamide as pale yellow needles, m. p. 198—199° (80%) (Found: C, 47·1; H, 5·4; N, 36·7%). It was not obtainable from 3-aminopyrazine-2-carboxyamide by the action of formaldehyde and hydrogen over Raney nickel and sodium acetate (cf. ref. 27). Methyl 3-aminopyrazine-2-carboxylate could not be methylated with methyl iodide and sodamide in boiling benzene (cf. ref. 28). 3-Chloropyrazine-2-carboxylic acid could not be prepared by the action of phosphorus oxychloride on 3-hydroxypyrazine-2-carboxylic acid, or by the action of sodium nitrite in 10N-hydrochloric acid (with or without cuprous chloride) on the amino-acid.

Methylation of 2 : 4-Dihydroxypteridine.—A suspension of 2 : 4-dihydroxypteridine [12] (1·64 g., 0·01 mole) in water (25 ml.) was stirred in a bath at 35°, then adjusted to pH 8 by N-sodium hydroxide. Methyl sulphate (2 ml., 0·02 mole) and 10N-sodium hydroxide (to keep the pH at 8—8·5) were added in alternation, during 2 hr. The mixture was stirred (with occasional adjustment of pH) for ½ hr. more, then cooled overnight. The crystals (0·8 g.) were filtered off and the mother-liquor shaken with chloroform, which extracted 0·4 g. more (total yield, 60%). Crystallization from water gave white crystals of 1 : 2 : 3 : 4-tetrahydro-1 : 3-dimethyl-2 : 4-dioxopteridine (VIII), m. p. 200°, soluble in about 22 parts of boiling water and 150 parts at 20° (Found: C, 50·5; H, 4·2; N, 28·9. Calc. for $C_8H_8O_2N_4$: C, 50·0; H, 4·2; N, 29·2%).

3-Methylaminopyrazine-2-carboxymethylamide.—Methyl 3-chloropyrazine-2-carboxylate (1·35 g.) was heated with 33% alcoholic methylamine (25 ml.) for 6 hr. at 130°, then boiled with charcoal, filtered, and evaporated to dryness. The residue was recrystallized from water, sublimed at 85°/0·05 mm., and recrystallized from light petroleum (b. p. 40—60°), giving the *methylaminomethylamide* as pale yellow needles (80%), m. p. 73° alone or mixed with the sample obtained by boiling the dione for 1 min. with N-sodium hydroxide (2 equiv.) (Found: C, 50·7; H, 5·8; N, 33·8. $C_7H_{10}ON_4$ requires C, 50·6; H, 6·1; N, 33·7%). This pyrazine could not be converted into the above dione with ethyl carbonate (or ethyl chloroformate) and acetic anhydride.

Methylation of 6-Hydroxypteridine.—6-Hydroxypteridine monohydrate (1·65 g., 0·01 mole) was dissolved in a solution of sodium methoxide (from 0·46 g. of sodium in 46 ml. of methanol) at 20°. Methyl sulphate (1 ml.; 1 equiv.) was added during 30 min. and the solution set aside for 1 hr. It was acidified with 5N-acetic acid (3·3 ml.), and the methanol removed. Water (8 ml.) was added, and the mixture (pH 6·2) was chilled overnight and filtered from a residue (R). The filtrate was boiled with carbon and concentrated to 3 ml., giving 0·2 g. of material assaying spectroscopically for a 2 : 1 crystalline complex of 5-methyl-6-pteridone

[25] Spoerri and Fibel, *J. Amer. Chem. Soc.*, 1948, **70**, 3911.
[26] Macdonald and Ellingson, *ibid.*, 1947, **69**, 1034.
[27] Emerson, in "Organic Reactions," Wiley, New York, 1948, Vol. IV, p. 174.

(68·4%) and 6-hydroxypteridine (31·3%) monohydrates ($\lambda_{max.}$ 296 and 356 mμ respectively, in 0·1N-potassium hydroxide). The residue (R) was dissolved in N-hydrochloric acid (10 ml.), adjusted to pH 6·2 at 100° with 2N-sodium carbonate, and refluxed for ½ hr. The suspension was filtered at 100° from 6-hydroxypteridine (0·9 g.), and the filtrate gave, after concentration, 0·16 g. of the above 2 : 1 complex. When this complex was refluxed with insufficient methanol to dissolve it, both the solid and the filtrate maintained this composition, but when it was repeatedly recrystallized from 30 parts of water, a new proportion of 5 : 2 was established. No other solvents gave a separation. Sublimation at 140°/0·01 mm. gave a mixture containing 82% of the methylated product. The progress of this concentration was also followed by paper chromatography. Finally, chromatography on a cellulose column (1 m. long) gave pure 5-methyl-6-pteridone, identical with that described below.

5-Methyl-6-pteridone.—4-Amino-5-methylaminopyrimidine [14] (0·5 g.), ethyl glyoxylate hemiacetal (1 ml.), and 2·5N-hydrochloric acid (10 ml.) were refluxed for 1½ hr. The solution was cooled and adjusted to pH 6, and the product recrystallized from water, giving 70% of colourless *5-methyl-6-pteridone monohydrate*, decomp. about 190°, soluble in 40 parts of boiling water and 560 parts at 20°, and in about 100 parts of boiling methanol, *i.e.*, at least 10 times more soluble than 6-hydroxypteridine in these solvents (Found, for material dried at 120°/0·01 mm.: C, 46·7; H, 4·3; O, 18·1; N, 30·65. $C_7H_6ON_4,H_2O$ requires C, 46·65; H, 4·5; O, 17·8; N, 31·1%). There was no further loss at 160°. $\lambda_{max.}$ at pH 13 : 296; $\lambda_{min.}$ 244. pK_a in H_2O at 20° and 0·01M : 3·73 ± 0·01 (basic) and 10·6 (acidic). The pteridone was unaffected by N-hydrochloric acid for an hour at 100°, but in 0·1N-sodium hydroxide decomposition was detected after 15 min. at 20°.

Methylation of 7 : 8-*Dihydro-6-hydroxypteridine.*—Methanolic sodium methoxide (from 0·16 g. of sodium and 16 ml. of methanol), finely ground 7 : 8-dihydro-6-hydroxypteridine (0·8 g.), and methyl iodide (1·6 ml.) were refluxed for 30 min. and filtered. The filtrate, refrigerated for 2 days, deposited 0·5 g. of (apparently) 5 : 6 : 7 : 8-*tetrahydro*-3 : 5 : 8-*trimethyl*-7-*oxopteridinium iodide*, which crystallized from ethanol as yellow needles, softening at 220° (decomp.), very soluble in cold water (the iodine is ionic; there was no loss in weight at 150°/0·01 mm.) (Found : C, 34·2; H, 4·2; N, 17·5; I, 40·0. $C_9H_{13}ON_4I$ requires C, 34·55; H, 4·1; N, 17·5; I, 39·7%).

Methylation of 7-*Hydroxypteridine.*—7-Hydroxypteridine [7] (1·48 g., 0·01 mole) was dissolved in N-potassium hydroxide (10 ml.) and the pH lowered to 8·0. Methyl sulphate (1 ml., 1 equiv.) was added during 45 min., with stirring, the pH being kept at 8 with 10N-potassium hydroxide. The solution was stirred for 30 min. longer, and refrigerated. Next day the crystals were filtered off, and the filtrate (B) was extracted with chloroform (50 ml.) which was evaporated. The combined solids were recrystallized from light petroleum (b. p. 60—80°), giving 50% of 8-methyl-7-pteridone, m. p. 125°, identical with previous material [7] and with that described below, soluble in 3 parts of boiling chloroform, and 5 of boiling alcohol. From the aqueous filtrate (B), 10% of 7-hydroxypteridine was recovered as the sparingly soluble sodium salt; much methylamine was also present. From the petroleum mother-liquors, 1—5% of 6 : 8-dimethyl-7-pteridone, m. p. 145—146°, was recovered.

Hydrolysis of 7-*Pteridones.*—8-Methyl-7-pteridone (0·002 mole) was heated at 100° for 2 hr. with N-sodium hydroxide, or N-sulphuric acid (2 equiv.). The alkaline solution was adjusted to pH 10 and evaporated. Quantitative paper chromatography [5] showed 94% hydrolysis to 5-amino-4-methylaminopyrimidine [16] ($\lambda_{max.}$ 286 mμ). The residue was extracted with *iso*butyl methyl ketone. The extract was concentrated to 1 ml., giving 75% of pale yellow 5-amino-4-methylaminopyrimidine, m. p. and mixed m. p. 207—209°. After the acid hydrolysis, the pH was raised to 4·6. Unchanged starting material (40%) was recovered by extraction with chloroform (10 + 10 ml.). The aqueous layer was adjusted to pH 10 with sodium carbonate at 100°, evaporated, and treated as above, giving 40% of the pyrimidine, m. p. 207—209°. 6 : 8-Dimethyl-7-pteridone, similarly treated with alkali, gave the same pyrimidine (90% by chromatography, 70% isolated).

Synthesis of the 7-*Pteridones.*—5-Amino-4-methylaminopyrimidine (2·5 g.), ethyl glyoxylate hemiacetal [29] (4 ml.), and water (30 ml.) were refluxed for 1 hr. Next day, the crystals were collected and washed with water (10 ml.); concentration of filtrate and washings gave a second crop. The yield of anhydrous 8-methyl-7-pteridone (from 120 parts of light petroleum, b. p. 60—80°), m. p. 123—124°, was 85%. No isomer is structurally possible. Refractionated ethyl pyruvate [30] (2·5 g.; b. p. 84—86°/50 mm.) similarly gave 70% of 6 : 8-dimethyl-7-pteridone

[28] Tschitschibabin, *Ber.*, 1921, **54**, 814.
[29] Rigby, *J.*, 1950, 1912.
[30] Boeseken and Felix, *Ber.*, 1929, **62**, 1315.

(X; R = Me) [from 50 parts of water, then 50 parts of light petroleum (b. p. 80—100°)], m. p. 146—147°, soluble in 175 parts of water at 20°.

6-*Hydroxy*-8-*methyl*-7-*pteridone* (X; R = OH).—(*a*) Nitric acid (0·2 ml.; *d* 1·5) was added to 8-methyl-7-pteridone (0·4 g.) in sulphuric acid (0·8 ml.). After 2 hr. at 25° the mixture was poured on ice and neutralized with ammonia, giving 70% of 6-*hydroxy*-8-*methyl*-7-*pteridone*. Recrystallization from 30 parts of water gave colourless plates, m. p. 309—311° (decomp.), almost insoluble in boiling chloroform (Found: C, 47·1; H, 3·5; O, 18·0; N, 31·4. $C_7H_6O_2N_4$ requires C, 47·2; H, 3·4; O, 17·95; N, 31·45%).

(*b*) 5-Amino-4-methylaminopyrimidine [16] (1·25 g.) and dimethyl oxalate (2·5 g.) were heated at 145—150° for 1 hr. The cooled mass was triturated with alcohol (15 ml.) and filtered. The solid was recrystallized from water and gave 50% of material identical with the above. In alcohol, only the *oxalate* of the starting material was obtained, colourless hygroscopic prisms, m. p. 196—198° (from methanol) (Found, for material dried at 20°: C, 38·7; H, 5·8. $C_5H_8N_4,\frac{1}{2}C_2H_2O_4,H_2O$ requires C, 38·4; H, 5·9%). Fusion gave a little 6-hydroxy-8-methyl-7-pteridone.

Methylation of 6 : 7-*Dihydroxypteridine*.—To a stirred suspension of finely powdered 6 : 7-dihydroxypteridine (1 g.; 0·006 mole) in methanol (50 ml.) was added a solution of sodium methoxide (0·3 g. of sodium in 30 ml. of methanol) and during the next 3 min. methyl sulphate (0·62 ml., 0·006 mole). After 15 min. sodium (0·15 g. in methanol) and methyl sulphate (0·62 ml.) were added as before, and these two reagents were again added after 15 min. The mixture was stirred for 45 min., and acetic acid added until the pH of a sample (diluted 1 : 3 with water) was 7. The methanol was recovered (finally in a vacuum), water was added (3 ml.), then chloroform (50 ml.), and the whole was refluxed until no solid remained, cooled, and separated. The aqueous layer was shaken out with more chloroform (50 ml.). The non-aqueous layers were dried (Na_2SO_4), and the solvent was removed, giving 35% of colourless 5 : 6 : 7 : 8-*tetrahydro*-5 : 8-*dimethyl*-6 : 7-*dioxopteridine*, m. p. 195° (203—204°, after recrystallization from 40 parts of benzene or 14 parts of water) (Found: C, 50·6; H, 4·0; N, 28·8. $C_8H_8O_2N_4$ requires C, 50·0; H, 4·2; N, 29·2%).

6-Hydroxy-8-methyl-7-pteridone, similarly methylated, gave the same product, m. p. 202—203°.

4 : 5-*Bismethylaminopyrimidine*.—The foregoing dione (0·2 g.) and N-sodium hydroxide (3 ml., 3 equiv.) were refluxed for 90 min. (sodium oxalate crystallizes). The solution was neutralized, sodium carbonate (0·2 g.) added, and the whole taken to dryness. The residue was extracted with boiling acetone (25 ml.). The acetone was recovered and the residue recrystallized from benzene, giving 4 : 5-*bismethylaminopyrimidine*, m. p. 140—142° (Found, C, 52·5; H, 7·25; N, 40·4. $C_6H_{10}N_4$ requires C, 52·1; H, 7·3; N, 40·5%). This could not be prepared by the direct methylation of 4 : 5-diaminopyrimidine or by the reduction with lithium aluminium hydride [14] of 5-formamido-4-methylaminopyrimidine.

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