## 501. *Pteridine Studies. Part XIX.*[1] *Covalent Hydration of 2-Aminopteridines.*

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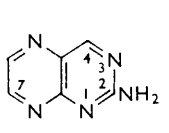
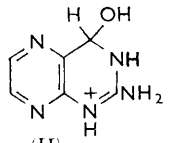
Physical measurements in aqueous acid show that 2-aminopteridine (I) forms mainly a cation (II) in which water has added across the 3,4-double bond. The insertion of a methyl group, particularly in the 4-position, displaces the equilibrium of hydration in favour of the anhydrous cation *e.g.*, the cation of 2-amino-4,7-dimethylpteridine is predominantly anhydrous. The anhydrous cation of 2-aminopteridine can be examined in acidic solvents of low water content. The relevant electronic effects are discussed.

This study was initiated on observing that the yellow aqueous solution of 2-aminopteridine (I) was decolorised on acidification, whereas that of 2-amino-4-methylpteridine remained yellow. The latter behaviour appeared normal, because 2-aminopyrimidine [2] has $\lambda_{max.}$ at 292 and its cation at 302 mμ. It was thought that the abnormality of 2-aminopteridine might consist of a covalent hydration of the cation in the 3,4-position to give the resonance-stabilised ion (II), because the cations of quinazoline [3] and pteridine,[4] and

[1] Part XVIII, Albert and Matsuura, *J.*, 1962, 2162.
[2] Brown, Hoerger, and Mason, *J.*, 1955, 4035.
[3] Albert, Armarego, and Spinner, *J.*, 1961, 5267.
[4] Perrin, *J.*, 1962, 645.

the neutral molecule of 2-hydroxypteridine,[5] are all hydrated in this manner, and because a 4-methyl group greatly reduces the tendency to hydration in each case.[3-5]


(I)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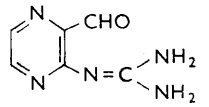
(III)

Because covalent hydration had not previously been encountered in an aminopteridine, it was decided to examine the ultraviolet spectra and ionisation constants of all the other *C*-methyl derivatives of 2-aminopteridine. These were prepared by condensing the appropriate 2,4,5-triaminopyrimidine with methylglyoxal or diacetyl, or (specifically for 6-methyl derivatives) by oxidation of the dihydropteridine (obtained from the corresponding 4-acetonylamino-2-amino-5-nitropyrimidine by reduction and ring-closure).[6] The above use of methylglyoxal, which could lead to both 6- and 7-methyl derivatives, was made to favour the 7-derivative by the customary use of a neutral medium and the avoidance of aldehyde-binding reagents. Although, as has been found for similar pairs of substances,[5] the 6- and the 7-methyl isomer were not distinguishable by paper chromatography, each had a characteristic infrared spectrum.

Some hitherto unrecorded properties of 2-aminopteridine [7] are noted on p. 2599.

*Ionisation Constants.*—pH Readings obtained in potentiometric titration with acid, and back-titration with alkali, of 2-aminopteridine produce a hysteresis loop. This can be exactly retraced several times. During the acid titration there is a slight drift to lower potentials: p$K_a$ 4·29 $\pm$ 0·03, obtained from seven well-spaced parts of this curve, represents the equilibrium between the anhydrous neutral species and the predominantly hydrated cation (see below). Higher p$K_a$ values (4·5—5·3), fleetingly revealed during back-titration, point to a more basic, but highly unstable, hydrated neutral species.

Table 1 illustrates the following broader aspects of this anomaly. 2-Aminopteridine, paradoxically, is a stronger base than any of its methyl derivatives; each dimethyl derivative is a weaker base than either of its parent monomethyl derivatives. Thus the base strengths decrease in the order in which they are expected to increase. Only the 4,6,7-trimethyl derivative is more basic (by 0·4 p$K$ unit) than the 4,7-dimethyl derivative. The decrease in basic strength also follows successive methylations of the primary amino-group in 2-aminopteridine, an extraordinary departure from the normal increase exemplified by 2-aminopyrimidine and 4-aminopteridine.

*Ultraviolet Spectra.*—The spectra of the neutral species of 2-aminopteridine and its methyl derivatives are all very similar (cf. Table 1 and Fig. 1) but the cationic spectra (cf. Fig. 2) are not. The long-wavelength band is found near 350 mμ for the ions of the 4,6- and 4,7-dimethyl and the 4,6,7-trimethyl derivative, but near 305 mμ for the unsubstituted ion and its 6- and 7-methyl and 6,7-dimethyl derivatives. The abnormally large hypsochromic shift on cation formation in the latter (60—70 mμ) suggests the loss of a double bond by addition of water. The 4-methyl derivative represents an intermediate case in showing both cation bands (Fig. 2).

To show that water is involved in the spectrally anomalous cases, we determined the spectra of these ions in dichloroacetic acid (Hammett $H_0$ value,[8] −0·9), in which quinazoline forms a normal anhydrous monocation.[8] In this solvent (see Table 2) 2-aminopteridine shows a prominent band at 355 mμ (cf. 370 mμ for the neutral species). Thus, in dichloroacetic acid, 2-aminopteridine has a spectrum very like those of its 4-methyl derivatives;

[5] Albert and Howell, *J.*, 1962, 1591.
[6] Boon and Jones, *J.*, 1951, 591.
[7] Albert, Brown, and Cheeseman, *J.*, 1951, 474.
[8] Albert, Armarego, and Spinner, *J.*, 1961, 2689.

TABLE 1.

Physical properties of aminopteridines in water at 20°.

| Pteridine | p$K_a$ | Spread Concn. (±) | (M) | $\lambda_{max.}$ (mμ) ** | log ε | pH |
|---|---|---|---|---|---|---|
| 2-Amino ..... | — | — | — | 225, 259,‡ 370 | 4·39, 3·81, 3·82 | 7·0 |
| cation ..... | 4·29 * | 0·03 | 0·01 | 232, 302 | 3·92, 3·87 | 2·0 |
| 2-Amino-4-methyl ... | — | — | — | 225, 260,‡ 367 | 4·44, 3·83, 3·85 | 7·0 |
| cation ..... | 2·82 | 0·04 | 0·0025 | 217, 307, 339 + 346 | 4·27, 3·72, 3·71 ± 3·71 | 0·5 |
| 2-Amino-6-methyl ... | — | — | — | 224, *256*, 376 | 4·42, 3·92, 3·84 | 7·0 |
| cation ..... | 4·05 | 0·03 | 0·005 | 238, 309 | 3·98, 3·91 | 1·0 |
| 2-Amino-7-methyl ... | — | — | — | 228, *255*, 367 | 4·35, 3·83, 3·89 | 7·0 |
| cation ..... | 3·76 | 0·02 | 0·01 | 237, 306 | 3·74, 3·98 | 1·0 |
| 2-Amino-4,6-dimethyl | — | — | — | 226, 258, 373 | 4·46, 3·94, 3·85 | 7·0 |
| cation ..... | 2·70 † | 0·03 | 0·0001 ¶ | 219, 355 | 4·40, 3·77 | −0·5 |
| 2-Amino-4,7-dimethyl | — | — | — | 228, 257,‡ 363 | 4·39, 3·83, 3·92 | 7·0 |
| cation ..... | 2·63 † | 0·05 | 0·0001 ¶ | 218, 347 | 4·27, 4·02 | 0·0 |
| 2-Amino-6,7-dimethyl | — | — | — | 227, *257*, 367 | 4·36, 3·87, 3·88 | 7·0 |
| cation ..... | 3·41 | 0·01 | 0·0025 | 240, 310 | 3·93, 3·98 | 1·0 |
| 2-Amino-4,6,7-trimethyl ..... | — | — | — | 227, 366 | 4·41, 3·91 | 7·0 |
| cation ..... | 3·03 †† | 0·05 | 0·00005 | 219, 350 | 4·42, 4·05 | 1·0 |
| 4-Amino- ..... | — | — | — | 244, 335 * | 4·20, 3·82 | 7·3 |
| cation ..... | 3·56 * | — | — | 229, 324 | 4·10, 3·99 | 1·1 |
| 4-Amino-2-methyl- ... | — | — | — | 219, 245, 336 | 3·97, 4·23, 3·81 | 7·0 |
| cation ..... | 4·30 | 0·03 | 0·005 | 232, 322 | 4·14, 3·97 | 2·0 |
| 2-Methylamino..... | — | — | — | 229, 273, 388 | 4·36, 3·96, 3·82 | 6·5 |
| cation ..... | 3·62 § | — | — | 235, 304, *363* | 4·09, 3·93, 2·07 | 1·0 |
| 2-Dimethylamino ... | — | — | — | 236, 281, 410 * | 4·37, 4·02, 3·82 | 7·1 |
| cation ..... | 3·03 * | — | — | 238, 305, *370* | 4·17, 3·91, 2·59 | 1·0 |

* Ref. 7. † Analytical wavelength for spectrometric determinations of p$K$, † 310, †† 265 mμ; in other cases, p$K$ was obtained potentiometrically. ¶ Stable at pH 2 for 1 hr. ‡ Shallow peaks. ** 1 and 4 cm. cells; shoulders in italics. § Albert, Brown, and Cheeseman, *J.*, 1952, 4219.

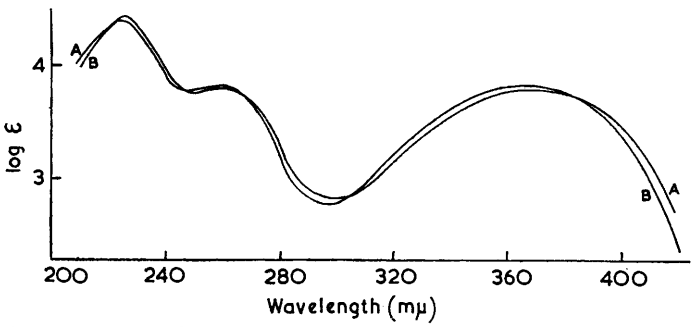


FIG. 1. Ultraviolet spectra of neutral species in water (pH 7).
A, 2-Aminopteridine. B, 2-Amino-4-methylpteridine.

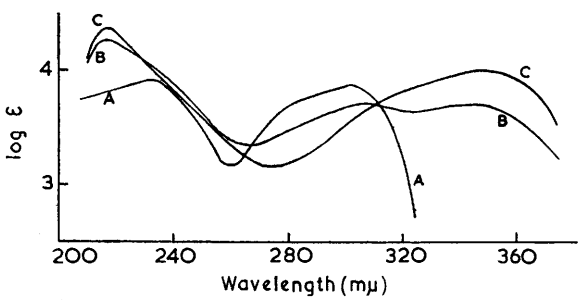


FIG. 2. Ultraviolet spectra of cations in water (pH 0·5).
A, 2-Aminopteridine. B, 2-Amino-4-methylpteridine. C, 2-Amino-4,7-dimethylpteridine.

for the latter the position of this band is almost the same in aqueous acid and in dichloroacetic acid.

It is concluded that in dichloroacetic acid the spectra of the normal, anhydrous cations are observed. However, 2-aminopteridine, its 6- and its 2-*N*-methyl, and possibly its 7-methyl and 6,7-dimethyl, derivatives show also a band in the range 308—318 mμ, attributed to the presence of a trace of moisture in the dichloroacetic acid which causes some hydration.* The intensity of this band, relative to one near 350 mμ, and hence the tendency for water addition, decreased in the above order.

TABLE 2.

Ultraviolet spectra of the cations of 2-aminopteridines in dichloroacetic acid.*

| Pteridine | $\lambda_{max.}$ † | log ε |
|---|---|---|
| 2-Amino | 308, 355, *370* | 3·80, 3·52, 3·38 |
| 2-Amino-4-methyl | — 347, *367* | — 3·91, 3·76 |
| 2-Amino-6-methyl | 317, 361, *379* | 3·75, 3·69, 3·59 |
| 2-Amino-7-methyl ‡ | *318*,§ 351, *369* | 3·77, 3·90, 3·79 |
| 2-Amino-4,6-dimethyl | — 355, *370* | — 3·98, 3·98 |
| 2-Amino-4,7-dimethyl | — 348, *364* | — 4·04, 3·94 |
| 2-Amino-6,7-dimethyl ‡ | *318*,§ 356, *372* | 3·65, 3·85, 3·77 |
| 2-Amino-4,6,7-trimethyl | — 353, *366* | — 4·04, 3·99 |
| 2-Methylamino | 312, 365, — | 3·75, 3·89, — |
| 2-Dimethylamino | — 375, — | — 3·97, — |

* Lower transmission limit 290 mμ. This acid was purified by fractional distillation and fractional freezing, but removal of the last traces of water was not attempted. † Inflections in italics. ‡ These two substances were attacked by the acid and hence the measurements may be less accurate. § Doubtful; these inflections are very weak.

For the 4,7-dimethyl and the 4,6,7-trimethyl, but not for the 4,6-dimethyl, derivative, the intensity of the 350 mμ band is as high in aqueous acid as it is in dichloroacetic acid, showing that the first two ions are essentially "anhydrous" even in water. Similar comparisons indicate that the ion of the 4-methyl derivative is about 50% hydrated in aqueous acid. A broad end-absorption in spectra of the ions of 2-methylamino- and 2-dimethylamino-pteridine (Table 1) suggests the presence of about 1% and 4%, respectively, of the anhydrous cation in aqueous solution.

*The Effect of Methyl Substituent on the Hydration Equilibrium in the 2-Aminopteridine Cation.*—The spectra show that the extent of water addition decreases [from very much (>99%) to very little] in the order: unsubstituted > 6-Me > *N*-Me ≈ 7-Me > 6,7-$Me_2$ > *NN*-$Me_2$ > 4-Me > 4,6-$Me_2$ > 4,7-$Me_2$. The decrease in the observed (equilibrium) p$K_a$ values, in the same order, is in agreement with this.

If the cation of 2-aminopteridine did not add water, its p$K_a$ value would be about 1·6; this figure is obtained by subtracting from 2·6 (the p$K_a$ value of the 4,7-dimethyl derivative, the cation of which is now known to be essentially anhydrous) 0·3 for the 7-methyl, and 0·7 for the 4-methyl group (cf. 2-aminopyrimidine, p$K_a$ 3·5, and 2-amino-4-methylpyrimidine, p$K_a$ 4·2). That the observed value is 4·29 and not ~1·6 suggests that the ratio of hydrated to anhydrous cation [9] is about 500 : 1 (the p$K_a$ difference is large enough to offset uncertainties due to the method of calculation). The base-weakening effect produced when a methyl-group lowers this ratio materially can outweigh the normal base-strengthening effect.

The mechanism by which a methyl group affects the hydration equilibrium may vary somewhat with its position. Bryson's observation,[10] that a nitro-group in every available position of the naphthalene ring depresses the basic strength of 1- and 2-aminonaphthalene by at least 0·8 unit, shows that an inductive effect can be transmitted from one ring to

* When 2-aminopteridine was dissolved in dichloroacetic acid that had been mixed with a little acetic anhydride and fractionated, a strongly enhanced band at 356 mμ was found and only a very weak one at 313 mμ.

[9] Perrin and Inoue, *Proc. Chem. Soc.*, 1960, 342.

[10] Bryson, *Trans. Faraday Soc.*, 1949, **45**, 258; 1951, **47**, 528.

another and can influence ionisation regardless of its position. Thus the influence of the 6-methyl group can be characterised as inductive ($+I$), but that of the 7-methyl group may be conjugative ($+M$) as well (cf. the $+M$ effect of groups in the 7-position on the 3,4-hydration of quinazoline [11]). A 4-methyl group may, in addition, exert steric hindrance to hydration, as it does in 4-methylquinazoline.[8]

The possibility that 2-aminopteridine may break down to the aldehyde (III), analogously to the behaviour of pteridine [4] at pH 2, was examined. Solutions (0·03M) of 2-aminopteridine in N- and 0·1N-hydrochloric acid, and in glycine and citrate buffers, covering the pH range 0·4—3·8 were set aside overnight at 20°. Next day, each solution was mixed with 0·05M-aqueous *p*-nitrophenylhydrazine at the same pH. No precipitation occurred. Because 0·03M-benzaldehyde, pyridine-2-, -3-, and -4-aldehyde, and 2-aminopyrazine-3-aldehyde all gave voluminous precipitates when similarly treated, it is concluded that cold acidic hydrolysis does not convert 2-aminopteridine into an aldehyde. Dr. Jaro Komenda, at the University of Brno, examined our specimen polarographically and found a step corresponding to hydration, but the further step found for pteridine was absent.[12]

The *p*-nitrophenylhydrazine test, applied to 1,4,6-triazanaphthalene, gave no evidence of the supposed aldehyde formation,[13] so that the abnormalities found in potentiometric titration must be hydration phenomena.[9]

## Experimental

Elementary analyses were carried out by the Analytical Section of the Department, under Dr. J. E. Fildes. Ionisation constants were measured by Mr. F. V. Robinson using standard methods.[14] The ultraviolet spectra were obtained with a Hilger "Uvispek" spectrophotometer. For the method of calculating yields, and the use of paper chromatography, see ref. 5.

2-Aminopteridine [7] is stable to 10N-sodium hydroxide at 20° and to boiling 2N-potassium carbonate for 3 hr., but boiling N-sodium hydroxide instantly decomposes it (blue colour). One equivalent of potassium dichromate in boiling 0·5N-sulphuric acid produces a 25% yield of 2-amino-4-hydroxypteridine in 20 min.

2-Aminopteridine (0·37 g.) in N-sulphuric acid (5 ml., 2 equiv.) deposited colourless crystals of a hydrated *sulphate* [Found, for material dried at 20°/20 mm.: C, 33·8; H, 3·7; N, 32·6; S, 7·6. $(C_6H_5N_5,H_2O)_2,H_2SO_4$ requires C, 33·6; H, 3·8; N, 32·7; S, 7·5%. Loss in weight at 110°/0·01 mm., 0·6 (slight charring); $1H_2O$ requires 8·4%]. The *C*-methyl homologues decomposed to violet products when the preparation of salts was attempted.

2-Amino-4-methylpteridine [15] was prepared from 2,4,5-triamino-6-methylpyrimidine [16] and glyoxal hydrate (Found, for material dried at 20°/15 mm.: C, 52·35; H, 4·5; N, 43·8. Calc. for $C_7H_7N_5$: C, 52·2; H, 4·4; N, 43·5%).

2-Amino-6-methylpteridine [6] was prepared by oxidising the 7,8-dihydro-derivative (obtained from 4-acetonylamino-2-amino-5-nitropyrimidine). It was chromatographed in 1·5N-acetic acid on a cellulose column; selected fractions were concentrated under reduced pressure and adjusted to pH 7. The product recrystallised from 50 parts of water and was dried at 80°/0·01 mm. (Found: C, 52·1; H, 4·4; N, 43·0%).

*2-Amino-7-methylpteridine.*—Commercial methylglyoxal solution (1·1 equiv.) was added to a solution of 2,4,5-triaminopyrimidine [17] (0·25 g.) in 0·2M-phosphate buffer (pH 7; 5 ml.). After 2 hr. at 20° in the dark, the precipitate was collected and, recrystallised from 70 parts of water, gave bright yellow crystals of *2-amino-7-methylpteridine* (87%), $\nu_{max.}$ (KBr disc) 1277 and 787 cm.$^{-1}$ [comparison with the corresponding bands (1214 and 775 cm.$^{-1}$) of 2-amino-6-methylpteridine showed that not more than 5% of the latter could have been present] (Found: C, 52·1; H, 4·4; N, 43·45%).

*2-Amino-4,6-dimethylpteridine.*—2-Amino-7,8-dihydro-4,6-dimethylpteridine [18] was prepared

[11] Armarego, *J.*, 1962, 561.
[12] Komenda and Laskafeld, *Coll. Czech. Chem. Comm.*, 1962, **27**, 199.
[13] Albert and Pedersen, *J.*, 1956, 4683.
[14] Albert and Serjeant, "Ionization Constants," Methuen, London, 1962.
[15] Lister, Ramage, and Coates, *J.*, 1954, 4109.
[16] Bitterli and Erlenmeyer, *Helv. Chim. Acta*, 1951, **34**, 835.
[17] Brown, *J. Appl. Chem.*, 1957, **7**, 109.
[18] Lister and Ramage, *J.*, 1953, 2234.

from 4-acetonylamino-2-amino-6-methyl-5-nitropyrimidine in 83% yield (Found, for material dried at 110°/0·01 mm.: C, 54·1; H, 6·35; N, 39·9. Calc. for $C_8H_{11}N_5$: C, 54·2; H, 6·3; N, 39·5%). To this substance (0·46 g.) and kieselguhr (0·5 g.), in water (150 ml.), were slowly added potassium permanganate (0·28 g.) and sodium hydroxide (0·04 g.) in water (5 ml.). The mixture was agitated at 20° for 1 hr., heated to 40°, and filtered. The residue was extracted with boiling water (140 ml., in three portions). The filtrate deposited 2-*amino*-4,6-*dimethylpteridine* (62%) which recrystallised from water (1000 parts) and sublimed at 150°/0·01 mm., to give yellow needles, m. p. 312° (decomp.) (Found: C, 54·7; H, 5·25; N, 39·7. $C_8H_9N_5$ requires C, 54·8; H, 5·2; N, 40·0%).

2-*Amino*-4,7-*dimethylpteridine*.—2,4,5-Triamino-6-methylpyrimidine [16] was condensed with methylglyoxal (as for 2-amino-7-methylpteridine). The products, recrystallised from 250 parts of water, formed orange crystals, m. p. 284° (decomp.), of 2-*amino*-4,7-*dimethylpteridine* (86%) (Found, for material dried at 110°/0·01 mm.: C, 54·45; H, 5·35; N, 40·0%). Examination of the infrared spectrum for the characteristic bands, at 1084 and 928 $cm.^{-1}$, of 2-amino-4,6-dimethylpteridine showed that less than 10% of this isomer could have been present.

2,4,5-Triaminopyrimidine [17] and biacetyl similarly gave a 96% yield of yellow 2-*amino*-6,7-*dimethylpteridine*, m. p. 308° (decomp.), soluble in 2-methoxyethanol and in 350 parts of boiling water (Found, for material dried at 110°/0·01 mm.: C, 54·95; H, 5·2; N, 39·7%).

2-*Amino*-4,6,7-*trimethylpteridine* [19] was obtained in 98% yield by the action of biacetyl on 2,4,5-triamino-6-methylpyrimidine, and recrystallised from 2-methoxyethanol (Found, for material dried at 110°/0·01 mm.: C, 57·2; H, 5·7; N, 37·0. Calc. for $C_9H_{11}N_5$: C, 57·1; H, 5·9; N, 37·0%).

4-Amino-2-methylpteridine,[20] sublimed at 140°/0·01 mm., had m. p. 235° (decomp.) (Found: C, 51·9; H, 4·5; N, 43·5. Calc. for $C_7H_7N_5$: C, 52·2; H, 4·4; N, 43·5%).

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[19] Rose, *J.*, 1952, 3448.
[20] Evans, Jones, Palmer, and Stephens, *J.*, 1956, 4106.