Pteridine Studies. Part XLV.[1] Addition of Sodium Hydrogen Sulphite and Other Nucleophilic Agents to Pteridin-4-one and Related Compounds

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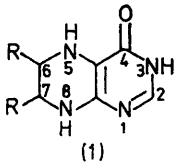
Although pteridin-4-one, unlike its 2- and 6-oxo-isomers, does not form stable adducts with weak nucleophiles such as water (and is less reactive than pteridin-7-one with the moderately strong nucleophile, acetylacetone), it reacts easily with more strongly nucleophilic reagents. Thus sodium hydrogen sulphite, in water at 25°, gives the isolable 2:1 adduct, disodium 3,4,5,6,7,8-hexahydro-4-oxopteridine-6,7-disulphonate (1b); and 5,5-dimethylcyclohexane-1,3-dione (dimedone), barbituric acid, and 2-thiobarbituric acid give similar 2:1 adducts. Addition of sodium hydrogen sulphite occurs across the 5,6- and 7,8-double bonds of pteridine-2,4-dione (lumazine) and 4-aminopteridine, and across the 7,8-double bond of 2-aminopteridine-4,6-dione (xanthopterin).

PTERIDIN-2-ONE AND -6-ONE give isolable adducts [2] with the weak nucleophiles ethanol, methanol, and water, which are added across the 3,4-double bond. However pteridin-4-one does not undergo covalent addition of water.[3] We now report that treatment of this pteridinone with primary alcohols, under conditions effective for other pteridines,[2,4] yielded only unchanged starting material. However because pteridin-7-one, which combines neither with water nor alcohols, reacts smoothly with stronger nucleophiles (such as sodium hydrogen sulphite and Michael reagents),[5] it was of interest to determine if pteridin-4-one would do so.

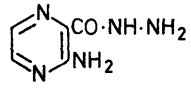
We found that pteridin-4-one formed isolable adducts with appropriate nucleophilic agents, even under mild conditions (aqueous solution, 25°, no added catalyst). For example, with sodium hydrogen sulphite it rapidly gave an adduct which contained two molecules of sodium bisulphite per molecule of pteridine; this adduct reverted to pteridin-4-one in dilute acid or alkali, but was reasonably stable in neutral solution. The close resemblance of its u.v. absorption ($\lambda_{max.}$ 293 and 221 nm in water) to that of 5,6,7,8-tetrahydropteridin-4-one [6] (1a) ($\lambda_{max.}$ 289 and 220 nm) suggested that addition had taken place across both double bonds in the pyrazine portion of the molecule. ^{1}H N.m.r. data confirmed the formulation (1b) [τ (D_2O) 2·25 (1H, sharp s, H-2) and 4·9 and 5·0 (2H, ABq, H-6 and H-7)]. This pattern of signals is consistent with that reported for several 5,6,7,8-tetrahydropteridines [3b,7,8] and related 1,2,3,4-tetrahydropolyazanaphthalenes.[9] The observed reactivity of the pyrazine portion towards sodium hydrogen sulphite but not towards water is paralleled by that of quinoxaline, which readily forms a 2 : 1 adduct with the former reagent [10] but does not react detectably with water.[11]

That addition of sodium hydrogen sulphite occurred by nucleophilic attack at positions 6 and 7 was indicated also by the observation that the 6-methyl, 7-methyl, and 5,7-dimethyl derivatives of pteridin-4-one did not form stable adducts with this reagent under conditions in which pteridin-4-one itself was converted rapidly into the adduct, nor was u.v. evidence of adduct formation obtained when 10^{-4}M-solutions of 6- and 7-methylpteridin-4-one were mixed with sodium hydrogen sulphite (10^{-2}M)



(1)

a, R = H
b; R = $SO_2 \cdot ONa$
c, R = —CH·CO·CH_2·CMe_2·CH_2·CO
d, R = —CH·CO·NH·CO·NH·CO



(2)

under conditions (25°) in which immediate adduct formation was demonstrable with pteridin-4-one. On the other hand, 2-methylpteridin-4-one reacted with sodium hydrogen sulphite as readily as did pteridin-4-one, and a 2 : 1 adduct was isolated. This previously encountered [12] hindrance to addition exerted, both sterically and electronically, by a methyl group in the pyrazine ring was partly overcome, for 6- and 7-methyl-4-pteridinone, by mass action forcing conditions (see Experimental section), under which ^{1}H n.m.r. signals appropriate for the adducts were obtained in solution (D_2O).

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Pteridin-4-one and dimedone, in water, in dilute sulphuric acid (pH 2·5), or in phosphate buffer (pH 7·0), gave a 2 : 1 adduct, the ^{1}H n.m.r. properties of which were consistent with formulation as the 6,7-disubstituted derivative (1c) of 5,6,7,8-tetrahydropteridin-4-one. For example, the signals given by pteridin-4-one (in NaOD) at τ 1·25 and 1·35 (H-6, H-7) and 1·6 (H-2) were shifted upfield in the dimedone adduct, to give a singlet at τ 1·97 (H-2) and two doublets centred at 4·6 and 4·9 (H-6, H-7). The spectrum of this adduct was similar when determined in deuterium chloride solution. The adduct slowly reverted to pteridin-4-one and dimedone in dilute aqueous solution.

Barbituric acid and its 2-thio-analogue, which have been found to add readily across activated double bonds of pteridines [4,5] and 8-azapurines,[13] also reacted with pteridin-4-one to produce 2 : 1 adducts in good yield. ^{1}H N.m.r. spectra of these adducts indicated that they may be formulated as tetrahydro-derivatives. The barbituric acid adduct (1d) was unstable under the strongly alkaline conditions used for n.m.r. analysis; the H-2 singlet at τ 2·3 and the characteristic quartet observed near the HOD peak for the adduct gradually disappeared during 1 h and the singlet and AB quartet of pteridin-4-one appeared concomitantly. The failure of pteridin-4-one to form an adduct with barbital (5,5-diethylbarbituric acid) demonstrated that barbituric acid reacted by virtue of the potentially carbanionic centre at position 5. 6,7-Dimethylpteridin-4-one did not react with barbituric acid, an observation expected from the studies of hydrogen sulphite addition described above.

Although pteridin-7-one, when treated with acetylacetone in water at 25°, reacts readily to give a 1 : 1 adduct,[5] pteridin-4-one did not react under such conditions. Reagents, such as diethyl malonate, ethyl cyanoacetate, and ethyl acetoacetate, which form adducts readily with 2-, 6-, and 7-oxopteridines,[2,5] did not react with pteridin-4-one under a variety of conditions, indicating that pteridin-4-one is the pteridinone least susceptible to attack by nucleophilic reagents.

Treatment of pteridin-4-one with hydrazine at 80°, in boiling ethanol, gave 2-aminopyrazine-3-carbohydrazide (2) by ring scission, presumably initiated by attack of hydrazine at position 4 in the pyrimidine ring. Refluxing hydrazine hydrate attacks (mainly) the pyrazine ring, to yield 4,5-diaminopyrimidin-6-one.[14]

Attempts to form 1 : 1 adducts by treatment of pteridin-4-one with equimolar (or smaller) amounts of nucleophilic agent were unsuccessful, formation of 2 : 1 adducts being observed in each instance. This shows that the energy required for the second nucleophilic addition is not greater than that for the first. This conclusion is not surprising in view of the extreme susceptibility of 2-amino-7,8-dihydropteridin-4-one to nucleophilic addition reactions.[15] Although folic acid (a 6-substituted 2-aminopteridin-4-one) forms a 1 : 1 adduct when treated with sodium hydrogen sulphite,[16] the failure to observe a 2 : 1 adduct may be attributable to the steric influence of the large substituent on position 6.

Pteridine-2,4-dione (lumazine) reacted with sodium hydrogen sulphite in aqueous solution to form a 2 : 1 adduct, which on the basis of u.v. and n.m.r. spectroscopy is considered to be a derivative of 5,6,7,8-tetrahydropteridine-2,4-dione. This adduct reverted rapidly to lumazine in dilute aqueous solution whereas that of pteridin-4-one was considerably more stable. It is relevant that 5,6,7,8-tetrahydropteridine-2,4-dione is extremely susceptible to oxidation in air whereas 5,6,7,8-tetrahydropteridin-4-one is relatively stable.[6]

4-Aminopteridine yielded a 2 : 1 adduct with sodium hydrogen sulphite; the spectroscopic properties of the product indicated that it was a 5,6,7,8-tetrahydropteridine. As in the pteridin-4-one series, the presence of methyl groups in the pyrazine portion of the molecule strongly suppressed adduct formation. 2-Aminopteridine-4,6-dione (xanthopterin) yielded, when treated with sodium hydrogen sulphite, a 1 : 1 adduct which was assigned the 7,8-dihydro-structure on the basis of the qualitative similarity of its u.v. spectrum ($\lambda_{max.}$ 312 and 283 nm in water) to that of 7,8-dihydroxanthopterin.[17] Isoxanthopterin, the 7-oxo-isomer of xanthopterin, did not react with hydrogen sulphite under our reaction conditions.

The foregoing studies have interdisciplinary interest in view of recent reports [18] of the interaction of hydrogen sulphite and sulphite anions with other biologically important nitrogen heterocycles.

EXPERIMENTAL

Elemental analyses were carried out by the Analytical Section of the Department of Medical Chemistry, Australian National University (under Dr J. E. Fildes) and by Schwarzkopf Microanalytical Laboratory, Woodside, New York, U.S.A. ^{1}H N.m.r. spectra were determined on either a Perkin-Elmer R10 or a Varian A-60 instrument, and sodium 3-trimethylsilylpropane-1-sulphonate was used as internal standard. U.v. spectroscopy was performed using Perkin-Elmer 4000 and 202 recording spectrophotometers. I.r. spectra were obtained for Nujol mulls using a Unicam SP 200 or Perkin-Elmer 137 instrument. All compounds were dried at 25° and 15 mmHg over P_2O_5 for analyses, unless otherwise indicated.

Reactions of Pteridin-4-one.—(a) *Condensation with sodium hydrogen sulphite.* Pteridin-4-one (0·20 g) was added to sodium hydrogen sulphite (0·50 g) dissolved in water (5 ml). The mixture was shaken vigorously, set aside for 30 min, then clarified by filtration, and the filtrate was treated with ethanol (5 ml) and chilled. The precipitate was collected,

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washed twice with cold 60% ethanol and then with successive portions of absolute ethanol and ether to give 52% of a white solid, *disodium* 3,4,5,6,7,8-*hexahydro*-4-*oxopteridine*-6,7-*disulphonate*, decomp. 254—255° (Found: C, 18·6; H, 2·7; N, 13·9. $C_6H_6N_4Na_2O_7S_2,2H_2O$ requires C, 18·4; H, 2·7; N, 13·9%), τ (D_2O) 2·25 (H-2) and 4·9 and 5·0 (H-6, H-7).

When 6- and 7-methylpteridin-4-one (0·02 g) were, separately, stirred with sodium hydrogen sulphite (0·14 g) in deuterium oxide (0·5 ml), a pattern of n.m.r. signals was obtained which clearly indicated formation of an adduct; τ 2·1 and 5·03 (each 1H) and 7·87 (3H) (for the 7-methyl derivative); τ 2·0 and 4·85 (each 1H) and 7·90 (3H) (for the 6-methyl derivative).

(b) *Condensation with dimedone.* Pteridin-4-one (0·20 g) and dimedone (0·40 g), suspended in water (20 ml), were heated on a steam-bath for 1 h; the mixture was filtered, the filtrate was set aside at 25° overnight, and the yellow solid was isolated. This solid was suspended in water (15 ml), dissolved by careful addition of N-potassium hydroxide, and acidified (to pH 4) with acetic acid (anhydrous). The deposited 6,7-*bis*-(4,4-*dimethyl*-2,6-*dioxocyclohexyl*)-5,6,7,8-*tetrahydropteridin*-4-*one* (48%; softens and gradually melts above 180°) was filtered off, and washed with water (Found: C, 60·0; H, 6·6; N, 12·5. $C_{22}H_{28}N_4O_5,0·5H_2O$ requires C, 60·3; H, 6·6; N, 12·8%), τ (0·3N-DCl) 1·95 (H-2) and 4·60 and 4·90 (H-6, H-7).

(c) *Condensation with* 2-*thiobarbituric acid.* 2-Thiobarbituric acid (0·30 g) and water (30 ml) were shaken vigorously for 5 min. The mixture was clarified by filtration, pteridin-4-one (0·10 g) was added, and the product was shaken at 25° for 24 h. The resulting yellow precipitate, filtered off, suspended in water, and dissolved by dropwise addition of 6N-potassium hydroxide gave 50% of 6,7-*bis*-(4,6-*dioxo*-2-*thioxohexahydropyrimidin*-5-*yl*)-5,6,7,8-*tetrahydropteridin*-4-*one* (chars slowly without melting above 180°) on acidification (pH 2) [Found (material dried at 105°): C, 36·65; H, 3·2; N, 24·7. $C_{14}H_{12}N_8O_5S_2,H_2O$ requires C, 36·9; H, 3·1; N, 24·6%], τ (N-NaOD) 2·33 (H-2) and 5·25 and 5·55 (H-6, H-7).

(d) *Condensation with barbituric acid.* Barbituric acid (0·30 g), pteridin-4-one (0·15 g), and a pH 7·0 phosphate buffer (35 ml) were stirred gently at 25° for 4 h. The resulting cream-coloured precipitate (85%) of *disodium* 5,6,7,8-*tetrahydro*-6,7-*bis*(2,4,6-*trioxohexahydropyrimidin*-5-*yl*)-*pteridin*-4-*one* was isolated [Found (material dried at 20°): C, 32·3; H, 3·5; N, 20·8. $C_{14}H_{10}N_8Na_2O_7,4H_2O$ requires C, 32·3; H, 3·5; N, 21·5%], τ (N-NaOD) 2·35 (H-2) and 5·25 and 5·55 (H-6, H-7).

(e) *Reaction with hydrazine.* Pteridin-4-one (0·50 g) was added to a solution of hydrazine hydrate (2·5 ml) in ethanol (22·5 ml); the mixture was heated (steam-bath) for 1 h and then chilled. The resulting yellow crystalline solid, recrystallized from ethanol, gave 70% of 2-aminopyrazine-3-carbohydrazide, m.p. 209—211° (Found: C, 39·0; H, 4·6. Calc. for $C_5H_7N_5O$: C, 39·2; H, 4·6%), identified by comparison (m.p., u.v. and i.r. spectra) with a specimen prepared from 2-aminopyrazine-3-carboxylic acid by a method [14] described for the analogous 6-methyl derivative.

Condensations of Sodium Hydrogen Sulphite with other Pteridines.—(a) *With* 2-*methylpteridin*-4-*one.* 2-Methylpteridin-4-one (0·08 g), sodium hydrogen sulphite (0·25 g), and water (3 ml) were shaken vigorously and set aside at 25° for 30 min. Ethanol (3 ml) was added, and the mixture was chilled for 60 min then treated with 70% ethanol (3 ml), and filtered. The white solid was washed twice with 70% ethanol and dried. This solid, *disodium* 3,4,5,6,7,8-*hexahydro*-2-*methyl*-4-*oxopteridine*-6,7-*disulphonate* (60%) decomposed gradually when heated above 250° (Found: C, 20·7; H, 3·05; N, 14·2. $C_7H_8N_4Na_2O_7S_2,2H_2O$ requires C, 20·75; H, 3·0; N, 13·8%).

(b) *With pteridine*-2,4-*dione.* Treatment of pteridine-2,4-dione with sodium hydrogen sulphite as in (a) yielded *disodium* 1,2,3,4,5,6,7,8-*octahydro*-2,4-*dioxopteridine*-6,7-*disulphonate* (70%; darkened above 160° but did not melt below 300°) (Found: C, 17·0; H, 2·7; N, 13·0. $C_6H_6N_4Na_2O_8S_2,3H_2O$ requires C, 17·15; H, 2·9; N, 13·3%), τ (D_2O) 4·86 and 5·04 (H-6, H-7).

(c) *With* 4-*aminopteridine.* A mixture of 4-aminopteridine (0·04 g), sodium hydrogen sulphite (0·12 g), and deuterium oxide (1·0 ml) was shaken for 10 min, during which time all the solid dissolved. Although the product was not isolated, the n.m.r. spectrum clearly indicated formation of a 5,6,7,8-tetrahydropteridine [τ (D_2O) 2·12 (H-2) and 4·9 and 5·0 (H-6, H-7)].

(d) *With* 2-*aminopteridine*-4,6-*dione* (*xanthopterin*). Xanthopterin (0·10 g) was added to a solution of sodium hydrogen sulphite (1·0 g) in water (5 ml); while the mixture was stirred vigorously for 24 h the colour changed from orange to cream. The precipitate, isolated by filtration and washed with 50% ethanol, gave 55% of *sodium* 2-*amino*-3,4,5,6,7,8-*hexahydro*-4,6-*dioxopteridine*-7-*sulphonate* (darkens gradually, without melting, above 270°) (Found: C, 23·1; H, 3·35; N, 21·4. $C_6H_6N_5NaO_5S,2H_2O$ requires C, 22·6; H, 3·1; N, 21·9%). Attempts to determine the n.m.r. spectrum of this adduct were unsuccessful owing to insolubility [even in $(D_3C)_2SO$ and $D_3C{\cdot}CO_2D$].

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