Pteridine Studies. Part 46.[1] 2-Alkylpteridin-4-ones from 2-Aminopyrazine-3-carboxamide and its Derivatives

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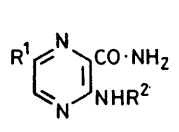
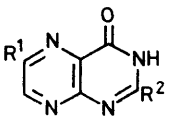
2-Aminopyrazine-3-carboxamide (1a) and its 5-bromo-derivative (1b) were converted, variously, into pteridin-4-one (2a) and its 2-methyl-, 2-trifluoromethyl-, 2-phenyl-, 6-bromo-2-methyl-, 6-bromo-2-phenyl-, and 6-ethoxy- (and 6-butoxy-)-2-trifluoromethyl-derivatives; also into 4-mercapto-2-trifluoromethylpteridine. The following methods were compared and evaluated: heating with ortho esters, with amides (alone or alkoxide-catalysed), with acid anhydrides, or with amidines; also formation of a 2,*N*-acyl derivative prior to ring-closure with aqueous alkali. ^{1}H N.m.r. and i.r. spectra were measured and discussed.

In Part 1 of this series, it was shown that 2-aminopyrazine-3-carboxamide (1a), when heated with triethyl orthoformate, furnished pteridin-4-one (2a) (75%).[2] Although, in the intervening years, this reaction was extended to the 6-methyl derivative of pyrazine (1a),[3] there has been no systematic attempt to produce pteridin-4-ones bearing an alkyl-substituent in the 2-position. Interest in such 2-alkyl derivatives stems from knowledge that the 2-amino-group of folic acid (3) has been



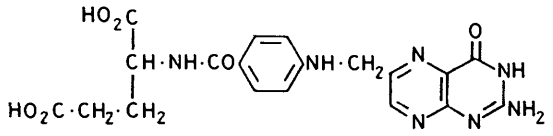
(1)

	R^1	R^2
a;	H	H
b;	Br	H
c;	H	Ac
d;	H	Bz
e;	Br	Ac
f;	Br	Bz
g;	H	CHO
h;	H	$CO\cdot CF_3$



(2)

	R^1	R^2
a;	H	H
b;	H	Me
c;	H	Ph
d;	Br	Me
e;	Br	Ph
f;	H	CF_3
g;	OEt	CF_3
h;	OBu	CF_3



(3)
Folic acid

found unnecessary [4] when forming biologically antagonistic analogues by small, stepwise alterations of the molecule. The present paper explores possible routes to 2-alkylpteridin-4-ones from 2-aminopyrazine-3-carboxamide (1a) and the 5-bromo-derivative (1b) into which bromine converts it.[5] This project is a step in the search for drugs active against methotrexate-resistant malignancies which are still a major unsolved clinical problem in antifolate chemotherapy.

Orthoesters other than orthoformates are usually much less reactive with amines than the latter.[6] However triethyl orthoacetate proved here to be nearly as effective as orthoformate with the pyrazine (1a) by giving 2-methylpteridin-4-one (2b) in 62% yield. The same conditions were found to be optimal as for the orthoformate, namely 2 h at 150 °C and the need for acetic anhydride. Ethoxymethylene-aminopyrazines of type (4) are presumed intermediates whose stabilization, by conjugation, furthers the reaction. Although it seemed that this approach with ortho esters would be general, it was set aside to explore possibilities offered by commoner and less expensive reagents.

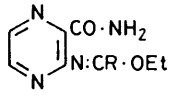
It was then found that *N*-acyl derivatives of pyrazine (1a) undergo ring-closure in dilute aqueous potassium hydroxide. Thus the pyrazines (1c—f) gave excellent yields of, respectively, 2-methyl- (2b),[7] 2-phenyl-(2c), 6-bromo-2-methyl- (2d), and 6-bromo-2-phenyl- (2e) pteridin-4-one. The end of the reaction, signalled by dissolution of the insoluble amide, was usually reached quickly at room temperature. However, the reaction leading to 6-bromo-2-phenylpteridin-4-one had to be conducted at 100 °C because, at 20 °C, a poorly soluble salt of the product coated the reacting particles. It is notable that this bromopteridinone is resistant to boiling alkali whereas 6-chloropteridine is rapidly hydrolysed in cold 2M-sodium carbonate.[8] Evidently the CO·NH group is exerting a protective action.[9]

2-Phenylpteridin-4-one has been made also by the condensation of 4,5-diamino-2-phenylpyrimidin-6-one with glyoxal; one preparation [10] melted at 264 °C, the other [11] above 310 °C. The material obtained in the present work melted sharply at 277 °C and had the same ^{1}H n.m.r. and u.v. spectra as those published by Clark, *et al.*[12] for material (m.p. not quoted) prepared by oxidizing 2-phenylpteridine with hydrogen peroxide. In yet another synthesis, to be described in what follows, the m.p. of 277 °C was re-established.

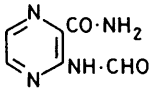
Preparation of these acyl compounds needs fairly vigorous conditions, *e.g.* the acetylation [13] (1a)→(1c) requires the use of acetic anhydride at 100 °C for 10 h. In the Experimental section, procedures are given for preparing three other acyl derivatives (1d—f). 2-Benzamidopyrazine-3-carboxamide has been made also by the successive action of benzoyl chloride and ammonia

on methyl 2-aminopyrazine-3-carboxylate,[14] an approach which did not go well in this work.

Acylating agents derived from stronger acids catalysed ring-closure. Attempted acylation with boiling formic acid, and also with trifluoroacetic acid and anhydride, gave (on t.l.c. and n.m.r. evidence) little of the acyl-derivatives (1g, h) but mainly the pteridin-4-one, even while much of the starting material (1a) was still unchanged. In such cases, it was advantageous to use forcing conditions in order to complete *both* reactions. Thus heating the pyrazine (1a) with formic acid and acetic anhydride at 120 °C gave an 80% yield of pteridin-4-one. Again autoclaving the pyrazine (1a) with trifluoroacetic acid and anhydride at 110 °C for 36 h furnished a 90% yield of the pteridinone (1f). Less successful were attempts to acylate with trichloroacetyl chloride or ethyl trichloroacetate, under various standard conditions [15] which either left the starting material unchanged or decomposed it profoundly with much charring.



(4)
(R=H, or Me)



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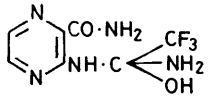

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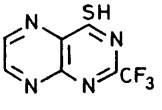

(7)

In a fresh approach to the synthesis of pteridin-4-ones, the pyrazine (1a) was heated with amides. This reaction has precedents in other series, *e.g.* the preparation of 9-benzyl-8-azapurin-6-one from 4-amino-3-benzyl-1,2,3-triazole-5-carboxamide in boiling formamide.[16] Here, the pyrazine (1a) gave an 84% yield of pteridin-4-one (2a) when heated at 195 °C with formamide for an hour. Higher temperatures were deleterious and there was no reaction at 175 °C. Longer times of heating at 195 °C lowered the yield. In none of these trials could the presence of an intermediate be detected.

The pyrazine (1a) did not react with acetamide at 195 °C and was destroyed by it at higher temperatures. Trichloroacetamide did not react at 150 °C, but decomposed the pyrazine, with much charring, at 160 °C. Trifluoroacetamide and the pyrazine (1a), heated under reflux (b.p. 162 °C), gave a 21% yield of 2-trifluoromethylpteridin-4-one (2f) in an hour, but longer heating proved destructive. The greater ease of reaction shown by formamide may signify a different intermediate, such as (5), whereas the other amides would be expected to form a tetrahedral intermediate such as (6) which should deaminate to an acyl-derivative such as (1h). Because (as reported in the foregoing) several acyl derivatives were readily cyclized by alkali, it was decided to try to facilitate the action of amides by adding a base.

Accordingly formamide and the pyrazine (1a), heated under reflux in butanolic sodium butoxide for 2 h, gave pteridinone (2a) (75%), and benzamide furnished 2-phenylpteridin-4-one (68%). However, the yield of 2-methylpteridin-4-one from acetamide was low (28%) because the alkoxide destroyed the product. The less severe conditions offered by boiling ethanolic sodium ethoxide did not initiate this condensation, but proved ideal for condensing trifluoroacetamide with the pyrazine (1a) [giving an 81% yield of the pteridinone (2f)] and with 2-aminopyrazine-3-thiocarboxamide [2] [giving 4-mercapto-2-trifluoromethylpteridine (7) (64%)]. However, the attempted condensation of 2-amino-5-bromopyrazine-3-carboxamide (1b) with trifluoroacetamide produced only 6-ethoxy- (and 6-butoxy-) 2-trifluoromethylpteridin-4-one because of reaction with the condensing agents. No product could be obtained from trichloroacetamide because the alkoxides lysed the amide faster than it could condense.

In a preliminary investigation of the reaction of amidines with the pyrazine (1a), formamidine acetate furnished pteridin-4-one (64%) when boiled in butanol for 48 h. It was necessary to replace the formamidine as it was destroyed by heat. 4-Amino-1,2,3-triazole-5-carboxamide, and its *N*-alkyl derivatives, react much faster with amidines,[17] but the pyrazine nucleus is known to be more electron-deficient than that of the triazole.[18]

^{1}H *N.m.r. Spectra.*—The results in Table 1 indicate

TABLE 1

^{1}H N.m.r. spectra [*ca.* 25 °C; solvent $(CD_3)_2SO$]

Compound	τ Values [a]
Pteridin-4-one	
(Unsubstituted)	1.03 (1 H, 6-CH), 1.18 (1 H, 7-CH), 1.67 (1 H, 2-CH)
2-Methyl	1.02, 1.21, 7.61 (3 H, Me)
2-Phenyl	−3.17 [b] br (1 H, NH), +0.92 (1 H, 6-CH), 1.10 (1 H, 7-CH), 1.75 (2 H, m) and 2.32 (3 H, m; in all 5 H, Ph)
6-Bromo-2-methyl	0.93 (1 H, 7-CH), 7.62 (3 H, Me)
6-Bromo-2-phenyl	0.78 (1 H, 7-CH), 1.72 (2H) and 2.34 (3 H, m; in all 5 H, Ph)
Other	
4-Mercapto-2-trifluoromethylpteridine	0.90 (6-CH), 1.04 (7-CH), 5.11 [b] (1 H, SH)
2-Acetamidopyrazine-3-carboxamide	1.46 (1 H, d, 6-CH), 1.56 [b] sl br (NH), 1.68 (1 H, d, 5-CH), 1.98 [b] sl br (NH), 7.72 (3 H, Me)
5-Bromo-deriv. of lastnamed	1.32 (1 H, s, 6-CH), 1.72 [b] sl br (NH), 200 [b] sl br (NH), 7.84 (3 H, Me)

[a] Tetramethylsilane as internal standard. [b] Vanishes when D_2O is added.

that there is little difference between the spectrum of a pteridin-4-one and that of the 2-aminopyrazine-3-carboxamide from which it was derived. The NH signals are the most diagnostic. Those of the pteridinones lie far downfield (see 2-phenylpteridin-4-one in the Table, and other examples in ref. 19), whereas in such pyrazines as (1a) they occur near to 2 on the τ scale. The C–H allocation for the pyrazines were made as in ref. 20.

The signal for a mobile hydrogen atom associated with sulphur can be used to diagnose the predominant tautomeric state of the equilibrium S=C–NH ⇌ HS.C=N. On the τ scale, the mobile hydrogen of methanethiol generates a signal at 8.8, and that of 4-mercaptotoluene at 6.73, whereas '2-mercaptopyridine'[21] exhibits the signal at −3.48 and '2-mercaptopyrimidine'[22] at −2.06 (both heterocycles are known from other evidence to exist mainly in the S=C–NH form). The presence of halogens in an *N*-heteroaromatic nucleus favours the SH form, as in 2,3,6-trichloro-4-mercaptopyridine[23] where the mobile hydrogen generates a signal at +5.7. These data support the allocation of the mercapto-form to compound (7).

TABLE 2

I.r. spectra (Nujol)

Compound	$\nu_{max.}$/cm^{-1}
2-Acetamido-5-bromo-pyrazine-3-carboxamide	3 460w (NH), 1 725m (amide I), 1 665m, 1 555br m (amide II), 1 300s, 1 220m (amide III), 1 135m, 815m, 600br,m (C–Br), 550m, 520m, and 440m
Pterid-4-one	
2-Methyl	3 400w (NH), 1 715br s (amide I), 1 595m, 1 580m, 1 545s (amide II), 1 320m, 1 270 (amide III), 1 170m, 895m, and 885m
2-Trifluoromethyl	3 660m (NH), 1 710s (amide I), 1 685m, 1 580m (amide II), 1 360br,m (C–F bend, sym), 1 225s (amide III), and 1 150br, ms (C–F bend, asym)
6-Butoxy-2-trifluoromethyl	3 350w (NH), 1 685s (amide I), 1 580m (amide II), 1 490m, 1 350m, 1 335ms (C–F bend, sym), 1 310m, 1 225m (amide III),[a] 1 200m (ether),[a] 1 165s, and 1 110s (C–F bend, asym)

[a] These assignments may be reversed.

The i.r. spectra in Table 2 show that the amide bands of a typical pyrazinecarboxamide and of several pteridinones are too similar for use in monitoring ring-closure. Characteristic bands for fluorine and bromine substituents are allocated.

EXPERIMENTAL

^{1}H N.m.r. spectra were obtained with a JEOL Minimar-200 instrument. The i.r. spectra of the pteridinones were made with a Perkin-Elmer 257 (grating) spectrophotometer calibrated with polystyrene at 1 603 cm^{-1}, and of the bromopyrazine with a P-E 225 instrument. Elemental analyses were done by this University's Analytical Chemistry Services Unit, with the results given in Table 3. Purifications were monitored by ascending chromatography on two Whatman No. 1 papers developed in (*a*) 3% aqueous NH_4Cl and (*b*) butanol–5N-acetic acid (7 : 3).

2-Aminopyrazine-3-carboxamide (1a) was usually prepared[2,13] from commercial 2-aminopyrazine-3-carboxylic acid, but one batch was made[24] equally conveniently, from aminomalonamideamidine and glyoxal. The following details of new preparations underpin detailed information given in Table 3.

Use of Ortho Esters.—2-Aminopyrazine-3-carboxamide (1a) (0.138 g, 0.001 mol), triethyl orthoacetate (2 ml), and acetic anhydride (2 ml) were heated under reflux (air condenser). The solution was taken to dryness at 105 °C/25 mmHg. The powdery residue was suspended in water, the pH adjusted to 5, and the 2-methylpteridin-4-one[7] filtered off and recrystallized from 30 parts of water. It chars at *ca.* 300 °C (an amendment of ref. 7).

Ring-closure of Acylamido-compounds by Aqueous Alkali.—2-Acetamido-pyrazine-3-carboxamide (1c)[13] (0.180 g, 0.001 mol) in N-potassium hydroxide (2 ml), after instant dissolution, was set aside for 1 h at 22 °C and then acidified to pH 4.5 (acetic acid); this precipitated 2-methylpterid-4-one (2b). Pteridones (2c and d) were prepared similarly, the latter at 40 °C for solubility reasons. 2-Benzamido-5-bromopyrazine-3-carboxamide (0.160 g, 0.000 5 mol) and 0.1N-sodium hydroxide (7.5 ml) were heated under reflux for 20 min, cooled, and acidified to pH 2.0 (H_2SO_4). The 6-*bromo*-2-*phenylpterid*-4-*one* (2e) was filtered off and dried at 22 °C.

Preparation of Acylamido-compounds such as (1d).—2-Aminopyrazine-3-carboxamide (0.276 g, 0.002 mol) was refluxed for 5 min with dried (NaOH) pyridine (4 ml). To this suspension, removed from the source of heat, was added, dropwise, benzoyl chloride (0.420 g, 1.5 equiv.) in pyridine (2 ml). The whole was then heated under reflux, in a bath at 130 °C, for 2 h. The solution was refrigerated at −10 °C and then filtered to give the acylamide (1d). 2-

TABLE 3

Properties of pyrazines and pteridines

Product	Yield[a] (%)	Recrystallization: Solvent	Parts	M.p. (°C)	U.v. colour[b]	Found (%): C	H	N	Hal	Formula	Required (%): C	H	N	Hal
(1d)[c]	48	Ethanol	170	235[e]	D	59.45	4.3	23.1		$C_{12}H_{10}N_4O_2$	59.5	4.2	23.1	
(1e)	89	Ethanol	140	216[d]	Y	32.7	2.95	21.7	30.9	$C_7H_7BrN_4O_2$	32.45	2.7	21.6	30.8
(1f)	91	DMF	15	*f*	Y	44.8	2.7	17.6	25.0	$C_{12}H_9BrN_4O_2$	44.9	2.8	17.45	24.9
(2b)[c]	81	Water	30	*d*	D→B									
(2c)[c]	91	Ethanol–water (1 : 3)	120		D→Y	64.0	3.7	24.9		$C_{12}H_8N_4O$	64.3	3.6	25.0	
(2d)	83	Ethanol	50	*f*	Y	35.3	2.15	23.0	33.3	$C_7H_5BrN_5O$	34.9	2.1	23.25	33.2
(2e)	80	Ethanol	190	277[d]	Y	47.8	2.55	18.2	26.3	$C_{12}H_7BrN_4O$	47.55	2.3	18.5	26.4
(2f)	81	Ethanol	25	214	D→B	39.0	1.5	26.15	26.2	$C_7H_3F_3N_4O$	38.9	1.4	25.9	26.4
(2g)	60	Benzene	115	256	B	41.4	2.95	21.6	21.6	$C_9H_7F_3N_4O_2$	41.5	2.7	21.5	21.9
(2h)[g]	39	Benzene	44	229	B	45.8	4.2	19.5	19.6	$C_{11}H_{11}F_3N_4O_2$	45.8	3.9	19.4	19.8
(7)	64	Trichloroethylene	250	*f*	D	36.4	1.5	24.5		$C_7H_3F_3N_4S$	36.2	1.3	24.1	

[a] See text for yields in alternative syntheses of compounds (2b, c, and f). [b] Colour of spots, on paper chromatogram, when viewed by light of 254 nm: B, blue; Y, yellow; D, dark (absorption spot); D→Y (*etc.*), initially D but changed by irradiation to Y. [c] Known compound. [d] Decomposes. [e] Varies with rate of heating. [f] Darkens progressively when heated, but no m.p. observed [g] [M^+] 288.

Amino-5-bromopyrazine-3-carboxamide [5] (1.736 g, 0.008 mol), acetic anhydride (1.630 g, 2 equiv.), and acetic acid (16 ml) were heated under reflux for 3 h. Volatile material was then removed at 90 °C/25 mmHg. The cooled residue was triturated with water (8 ml) and the pH raised to 4 with 3M-sodium acetate. Filtration gave *2-acetamido-5-bromopyrazine-3-carboxamide* (1e). To a suspension of 2-amino-5-bromopyrazine-3-carboxamide (0.434 g, 0.002 mol) in dried pyridine (4 ml) was slowly added at 22 °C a solution of benzoyl chloride (0.420 g) in pyridine (2 ml). The mixture was stirred at 22 °C for 10 h and then chilled at −10 °C and filtered to give *2-benzamido-5-bromopyrazine-3-carboxamide* (1f), which was found to be poorly soluble in common solvents.

Combined Acylation and Ring-closure.—2-Aminopyrazine-3-carboxamide (0.138 g), trifluoroacetic acid (1.5 ml), and trifluoroacetic anhydride (0.420 g, 2 equiv.) were heated, enclosed in a Teflon-lined autoclave, in an oven at 110 °C for 36 h. The cooled mixture was stripped of volatiles at 40 °C/25 mmHg, homogenized with water (2 ml), and the pH raised to 3 with 4M-sodium formate. The *2-trifluoromethylpteridin-4-one* (2f) was filtered off and dried. The pyrazine (1a) (0.138 g), acetic anhydride (2 ml), and formic acid (2 ml) were heated under reflux in a bath (120 °C) for 2 h. The mixture was taken to dryness at 90 °C/25 mmHg, and the residue recrystallized from water (33 parts), to give pteridin-4-one.

Uncatalysed Condensation with Amides.—The pyrazine (1a) (0.138 g) and formamide (1 ml) were heated as described, and the excess of formamide removed at 150 °C/25 mmHg. The residue, homogenized with water and filtered, yielded chromatographically pure pteridin-4-one.

Sodium Alkoxide-catalysed Condensation with Amides.—Ethanol (10 ml) was added, down a condenser, to sodium hydride (0.120 g, 5 equiv.). When effervescence had ceased (*ca.* 1 min), 2-aminopyrazine-3-carboxamide (0.138 g), and trifluoroacetamide (0.4 g, 3.5 equiv.) were added, and the mixture heated under reflux for 4 h. The ethanol was removed at 40 °C/25 mmHg. The residue was dissolved in ice–water (4 ml). The solution (pH 12) was clarified by filtration and the pH adjusted to 2.0 with sulphuric acid. The 2-trifluoromethylpteridin-4-one (2f) was filtered off and dried at 22 °C. *2-Trifluoromethyl-4-mercaptopteridine* (7) made similarly (but required 8 h of refluxing), formed dark products when boiled with ethanol or water, but was unchanged by chlorinated hydrocarbons. Sodium butoxide solution, required for making two of the pteridinones (2a and c), was prepared from sodium hydride and butanol.

Condensation with Amidines.—The pyrazine (1a) (0.414 g, 0.003 mol), formamidine acetate (0.620 g, 2 equiv.), and butanol (12 ml) were heated under reflux, and extra formamidine acetate (0.620 g) was added at 12 h intervals. After heating for 48 h, the solution was taken to dryness at 90 °C/25 mmHg. The cooled residue was homogenized in N-potassium hydroxide (4.5 ml) and filtered free from unchanged starting material (26% recovery). The pH of the filtrate was adjusted to 5 with acetic acid, and the precipitated pteridin-4-one (2a) recrystallized from 33 parts of water.

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