Interference between *peri*-Substituents at Positions 3 and 9 in Purines and Positions 1 and 8 in Pteridines, shown by Nuclear Magnetic Resonance Spectroscopy. Proposal of a Steric Model

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In purines, including uric acids, steric interference between 3- and 9-methyl substituents leads to a marked downfield shift of their n.m.r. signals, of 0.20—0.40 p.p.m. In 1,8-dimethylpteridine-2,4,7-triones, the displacement of the methyl bands is less marked (0.12—0.18 p.p.m.). Two models are discussed to explain these downfield shifts: either moving the methyl groups out-of-plane, or spreading them apart within the plane of the heterocyclic structure. Calculations show that only the latter model is in accordance with the measured values. The smaller change of the δ-values in pteridine-2,4,7-triones can be explained by the observation that in 1- or 8-monomethyl derivatives the 7, 8- or 1,2-lactam group, respectively, is partially lactimised.

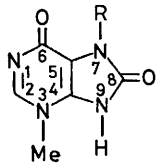
In the purine series, steric interference between substituents at positions 3 and 9 has been established by two methods.

(1) *Acid Dissociation Constants.*—If in purine anions, formed by dissociation of a 3-NH group, a 9-methyl sustituent is present, recombination of the anions with a proton is sterically hindered. Therefore the pK of such a derivative will be lower than the value of the corresponding non-methylated homologue. In fact, a 3- or 9-NH group dissociates at a markedly lower pK, if a 9- or 3-methyl substituent, respectively, is present (Table 1). Thus the pK of the 3-NH group in 9-methylxanthine is 2.5 units below that of the 7-methyl isomer.[1] For the corresponding pair of 6-thioxanthines, ΔpK is 1.9.[2] Similarly, the pK of the 9-NH group in 3-methylpurine-6,8-dione (I; R = H) is 5.5, while in the 7-methyl isomer (II; R = H) the 9-NH group dissociates with a pK of 7.8.[3] Furthermore the pK of the 9-NH group in (I; R = Me) is 6.5, but for (II; R = Me) it is 8.5.[3] Analogous differences have been found for the corresponding 6-thioxopurin-8-ones (see Table 1).[4] In the series of uric acids, ionisation of either 3- or 9-NH leads to pK values between 5.0 and 6.2. On the other hand, the dissociation constants of 3,9-dimethyl derivatives, involving the 1- or 7-NH groups, are all in the range of 9.0—9.4.[5]

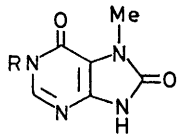
TABLE 1

pK Values for dissociation of 3- and 9-NH groups in various purines

Compound	pK for anion formation: Group involved	pK	ΔpK	Ref.
7-Methylxanthine	3-NH	8.4	2.5	1
9-Methylxanthine	3-NH	5.9		
7-Methyl-6-thioxanthine	3-NH	6.8	1.9	2
9-Methyl-6-thioxanthine	3-NH	4.9		
7-Methylpurine-6,8-dione (II; R = H)	9-NH	7.8	2.3	3
3-Methylpurine-6,8-dione (I; R = H)	9-NH	5.5		
1,7-Dimethylpurine-6,8-dione (II; R = Me)	9-NH	8.5	2.5	3
3,7-Dimethylpurine-6,8-dione (I; R = Me)	9-NH	6.0		
1,7-Dimethyl-6-thioxopurin-8-one	9-NH	8.2	3.4	4
3,7-Dimethyl-6-thioxopurin-8-one	9-NH	4.8		



(I)



(II)

(2) *N.m.r. Spectra.*—If 3- and 9-methyl groups are present together, their n.m.r. signals are shifted downfield by 0.2—0.4 p.p.m., relative to the purines of the same structure, but bearing only one of these methyl substituents. This has been shown for the case that one or both these *N*-methyl groups are adjacent to a carbonyl (Table 2),[1-8] but no example is known for the situation where both 3- and 9-methyl are neighbouring

TABLE 2

Mutual deshielding of *N*-methyl groups in 3,9-dimethyl derivatives of various purines

Compound type	δ (neutral form) for: 3-Me	9-Me	3,9-Dimethyl: 3-Me	9-Me	Δδ [a]: 3-Me	9-Me	Reference
Xanthines	3.45	3.68	3.65	3.96	0.20	0.28	1
6-Thioxanthines	3.45	3.73	3.67	3.94	0.22	0.21	2
Purine-6,8-diones	3.99	3.44	4.21	3.79	0.22	0.35	3
6-Thioxopurine-8-ones	3.97	3.50	4.40	3.76	0.43	0.36	4
6-Methylthiopurin-8-ones (zwitterions)	4.03	3.52	4.38	3.80	0.35	0.28	6
6-Methylsulphonylpurin-8-ones (zwitterions)	4.08	3.57	4.48	3.93	0.40	0.36	7
Uric acids	3.69	3.60	3.95	3.86	0.26	0.26	5

[a] Δδ = Difference between the chemical shift of a methyl group in the 3,9-dimethyl derivative and the corresponding methyl signal in the monomethyl homologue.

a CH-group. So far, all attempts to prepare 3,9-dimethylhypoxanthine or one of its derivatives have failed.

In the present study these observations have been extended to the pteridine series. Here, a similar interference between *peri*-substituents can arise only when lactam groups are present both at positions 1,2 and 7,8.

For our measurements we have selected the pteridine-2,4,7-triones, because in this series all possible *N*-methyl derivatives are known (see also Experimental section).[9,10] These compounds are comparable to uric acids.

The p*K* for monoanion formation in compounds (1)—(8) is 3.3—4.2 (Table 3), *i.e.* these are the most acidic oxopteridines known so far. Evidently the presence of NH-groups at positions 1 and 8, or of a methyl group at one of them, creates powerful strain and thus enhances the dissociation tendency most efficiently. In contrast, dissociation of the 3-NH group in compounds (9) and (10) is characterised by much higher p*K* values (see Table 3).

The interference between 1- and 8-substituents finds also an expression in lactim formation. For the HN·CO group, lactimisation has been demonstrated by comparison of the u.v. spectra of compounds (4) and (5) with those of the corresponding 7-methoxy- (12) and (13) and 8-methyl derivatives, *e.g.* (11).[9] In compounds (7) and (8), lactimisation of the (1)NH—(2)C:O group is less pronounced, but is still recognisable in the u.v. absorption spectra.[10]

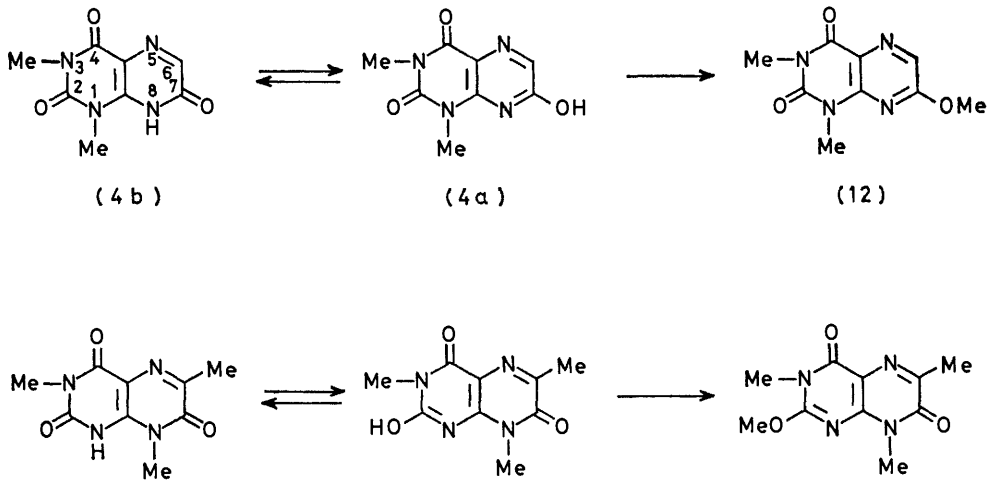
TABLE 3

p*K* Values and n.m.r. signals of pteridine-2,4,7-triones

No.	Methyl groups at positions	p*K* Values for anion formation [a]	δ for neutral molecules [b]					Δδ [c]	
			6-H	6-Me	1-Me	3-Me	8-Me	1-Me	8-Me
(1)		3.4; 9.8	7.62						
(2)	1	3.3; 10.5	7.87		3.39				
(3)	3	3.6; 10.3	7.99			3.29			
(4)	1,3	3.5	8.00		3.45	3.30			
(5)	1,3,6	3.8		2.43	3.48	3.31			
(6)	8	3.8; 11.7	7.62				3.45		
(7)	3,8	3.8	7.62			3.22	3.44		
(8)	3,6,8	4.2		2.31		3.23	3.48		
(9)	1,8 [d]	8.0	7.86		3.53		3.63	0.14	0.18
(10)	1,6,8 [d]	9.1		2.40	3.51		3.63	0.12	0.18
(11)	1,3,8		7.87		3.56	3.33	3.61	0.17	0.16

[a] First figure for formation of monoanion, second figure for di-anion. The p*K* values are taken from refs. 9 and 10. Only for the new compounds (9) and (10). the dissociation constants were measured in the present work, by plotting $\lambda_{max.}$ as function of pH. [b] Measurements in $(CD_3)_2SO$–D_2O (9 : 1 v/v) at 70 °C. [c] Δδ = Difference between these *N*-methyl signals and those in the monomethyl derivatives (2) and (6) respectively. [d] New compounds, showing $\lambda_{max.}$ for the neutral form at 336 nm.

Steric interference may also provide an explanation for the preference of *O*-alkylation over *N*-alkylation in certain cases. Thus both the neutral form and the anion of 1,3-dimethylpteridine-2,4,7-trione (4) yield the 7-methoxy-derivative (12) (see Scheme).[9] Similarly, 3,6,8-trimethylpteridine-2,4,7-trione (8) is converted into the corresponding 2-methoxy-derivative (14) (see Scheme).[10]



SCHEME

The n.m.r. spectra of the pteridine-2,4,7-triones are summarised in Table 3. The signal of the 1-methyl substituent shifts 0.14 ± 0.03 p.p.m. downfield when an 8-methyl group is introduced [compare compounds (9)—(11) with compound (2)]. Likewise the 8-methyl group in compounds (9)—(11) is deshielded by 0.17 ± 0.01 p.p.m., when a 1-methyl substituent is also present.

Thus the displacements of these signals are considerably smaller than the shifts observed for the corresponding derivatives of uric acid (Table 2).

The n.m.r. spectrum of compound (4) is close to that of 7-methoxy-1,3-dimethylpteridine-2,4-dione (12) (Table 4). A similar relation is apparent for the δ-values of (5) and of 7-methoxy-1,3,6-trimethylpteridine-2,4-dione (13). The resemblance of chemical shifts is less marked for the pair (8) and 2-methoxy-3,6,8-trimethylpteridine-4,7-dione (14). These observations support the assumption of partial lactimisation in 1- or 8-methylpteridine-2,4,7-triones.

We have searched for a steric model that could explain the deviation of the chemical shifts of '*peri*-methyl' substituents from the values found for the corresponding mono-methyl derivatives, and likewise the differences between uric acids and pteridine-2,4,7-triones. For this purpose, we have calculated the changes ($\Delta\sigma$) to be expected for the δ-values in two different cases. *Case* (*a*). The *peri*-methyl groups are forced out of the main plane of the heterocyclic ring structure, as expressed by their torsion angle ϕ. *Case* (*b*). The *peri*-methyl groups move apart within the main molecular plane, an effect characterised by the spreading angle β.

The shielding of a proton can generally be represented as the sum of local diamagnetic effects and of long-range shielding influences. In the case of uric acids and pteridine-2,4,7-triones, the first effect results mainly from the direct electrostatic influence of the dipoles of the two neighbouring carbonyls. Variations of the shielding of the three protons in a methyl group by these dipoles can be calculated with the aid of the equation of Schweizer *et al.*,[11] as modified by Jackman and Sternhell:[12a] Here, $\Delta\sigma$ = change in the average shielding of the three methyl protons; q_i = partial charges equivalent to the bond dipole moments (2.96 D for the carbonyl group);[13] R_i (in Angströms) = the vector, giving the distance of the methyl protons from the centre of the C=O bond; θ_i = angle between a CH-bond in the methyl group and R_i.

$$\Delta\sigma = -12.5 \times 10^{-6}\left(\sum_i \frac{q_i \cos\theta_i}{R_i^2}\right) + 17.0 \times 10^{-6}\left(\sum_i \frac{q_i}{R_i^2}\right)^2$$

The equation yields the direct electrostatic contribution to the chemical shift of the methyl protons, which is caused by variations in R_i. The distance of the methyl protons from either the 2- or the 8-carbonyl group, and thus also $\Delta\sigma$, clearly depends on the magnitude of ϕ or β.

The second effect, *viz.* the long-range influence of the carbonyls, was estimated from diagrams of Jackman and Sternhell,[12b] which relate the shielding by a carbonyl group to the principal susceptibilities of the carbon and oxygen atoms.[14]

We shall first discuss straightforward applications of the above equation to uric acids.

Case (*a*). The known crystallographic structure of tetramethyluric acid[15] was used for all calculations concerning uric acid derivatives. Forcing the *peri*-methyl groups at positions 3 and 9 out-of-plane alters both the local and the long-range electrostatic effects of the dipoles of the 2- and 8-carbonyl and thus changes the overall shielding. When the *peri*-methyl groups are within the main plane of the molecule, the combined electrostatic effects of the two carbonyls cause deshielding of the methyl protons. The value of $\Delta\delta$ is considerable for the 9-methyl group (0.33 p.p.m.), but only slight for the 3-methyl substituent ($\Delta\delta = 0.05$ p.p.m.). Forcing the *peri*-methyl groups out-of-plane *reduces* the deshielding effect of the carbonyls in direct relation to the magnitude of the torsion angle ϕ. This can be seen very clearly in the diagrams of Jackman and Sternhell[12b] which picture the long-range shielding of the carbonyl as function of the distance of the hydrogens from them. Naturally the distance increases when the hydrogens are moved out of the carbonyl plane. Therefore such a process cannot explain the marked downfield shift of the 3- and 9-methyl signals in 3,9-dimethyluric acids.

Another factor that may alter local diamagnetic shielding is the van der Waals interaction. Van der Waals forces between *peri*-methyl groups cause slight deshielding of the protons. This effect *decreases* when the torsion angle is enlarged and probably cannot contribute more than a downfield shift of 0.01 p.p.m.[12c]

Finally we have to consider the long-range shielding, caused by the anisotropy of the carbonyl group. Using the appropriate values of Jackman and Sternhell,[12b] we find that moving the *peri*-methyl groups out-of-plane should reduce the deshielding influence of 2- and 8-carbonyls in uric acids.

To summarise: when the torsion angle ϕ increases, both local and long-range contributions to the deshielding of the *peri*-methyl protons are diminished. Therefore this type of deformation has to be disregarded as an explanation of the observed downfield shifts.

TABLE 4

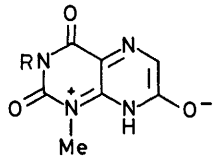
Comparison of the chemical shifts of some pteridine-2,4,7-triones and of corresponding methoxypteridines

		δ					
No.	Compound	6-H	6-Me	1-Me	3-Me	8-Me	OMe
(4)	1,3-Dimethylpteridine-2,4,7-trione	8.00		3.45	3.30		
(12)	7-Methoxy-1,3-dimethylpteridine-2,4-dione	8.20		3.54	3.30		4.08
(5)	1,3,6-Trimethylpteridine-2,4,7-trione		2.43	3.48	3.31		
(13)	7-Methoxy-1,3,6-trimethylpteridine-2,4-dione		2.47	3.56	3.34		4.11
(8)	3,6,8-Trimethylpteridine-2,4,7-trione		2.31		3.23	3.48	
(14)	2-Methoxy-3,6,8-trimethylpteridine-4,7-dione		2.35		3.34	3.54	4.12

Case (*b*). Here steric interference is relieved by enlarging the angle β between *peri*-substituents within the main plane of the molecule. Using again tetramethyluric acid as model for all uric acid derivatives, we have first calculated the local electrostatic effects of the 2- and 8-carbonyls on either of the two *peri*-methyl groups as function of β. When this angle decreases or increases, deshielding of the methyl protons does not change much. In contrast, the long-range effect of the carbonyls varies markedly with β. The overall values of these deshielding effects are very similar for the 3- and 9-methyl substituents. Thus for $\beta = +2°$, $\Delta\delta = -0.37$ p.p.m., and for $\beta = +10°$, $\Delta\delta = -0.47$ p.p.m. It now appears that a combination of local and long-range effects may explain the marked downfield shifts of the 3- and 9-methyl signals in uric acids, when these substituents are forced to move apart within the main purine plane.

Turning now to the series of pteridine-2,4,7-triones, we lack *X*-ray spectra of the crystals of suitable oxo-derivatives. Therefore, we have used a combination of the pteridine structure, as determined by Shirrell and Williams,[16] and of the *X*-ray data for tetramethyluric acid.[15] We find again an overall deshielding effect of the 2- and 7-carbonyls on the 1- and 8-methyl substituents as function of the spreading angle β; this influence is larger for the 1-methyl group. However in the derivatives (2), (4), (5)—(8), bearing either a single 1- or 8-methyl substituent, lactim groups may be present in a certain percentage. Therefore we have calculated the change in deshielding of the 1-Me, when a (8)NH—(7)C:O group is converted into (8)N—(7)C·OH, and the corresponding change in the chemical shift of an 8-methyl substituent when the (1)NH—(2)C:O group passes into the lactim form (1)N—(2)C·OH. For lactim structures, in accordance with Zürcher,[17] we may neglect anisotropy and thus have to consider only the electrostatic effects. For the dipole moment of the lactim hydroxy-group we have selected the value of 1.55 D.[13] The angle between the hydroxy-group and the C–O bond of the lactim group is assumed to be 90°. We have to take into account two possible conformations of the OH-group in the plane of the molecule, *viz.* bending towards the lactim nitrogen or away from it. Provisionally we have assumed equal contributions of either conformation. We find that replacement of the 1,2-lactam by a lactim group deshields the 8-methyl by *ca.* 0.20 p.p.m. Similarly, by passing from a 7,8-lactam to the corresponding lactim the 1-Me signal is displaced by *ca.* 0.14 p.p.m. to lower field. These effects naturally are lost in the 1,8-dimethyl derivatives (9)—(11). Therefore replacement of lactims by lactams may, at least in part, be responsible for the relatively small downfield shift of the methyl signals in 1,8-dimethylpteridine-2,4,7-triones, as compared to 3,9-dimethyluric acids, where only lactam structures have to be considered.[18]

The inclination of pteridine-2,4,7-triones to lactimise the (8)NH—(7)C:O group is based on the mesomeric effect of a 1-methyl substituent, as shown in (III). An analogous mesomerism is not possible for an 8-Me group. Therefore lactimisation of the (1)NH—(2)C:O group, for which steric strain alone is at work, is much weaker.



(III)

The overall results of our calculations indicate that both in uric acids and in pteridine-2,4,7-triones, steric interference between *peri*-methyl groups leads to spreading of the angle between them mainly within the plane of the heterocyclic system.

EXPERIMENTAL

Most of the pteridines used in the present study have been described by Pfleiderer.[9,10] Compounds (9) and (10) are new products.

1,6,8-*Trimethylpteridine*-2,4,7-*trione* (10).—6-Methylamino-1-methyl-5-nitrosouracil[19] (1.5 g) in water (50 ml) was reduced by shaking with Raney nickel in an atmosphere of hydrogen overnight at room temperature. The catalyst was filtered off and the filtrate refluxed with ethyl pyruvate (1 ml) for 30 min. The clear solution was treated with charcoal, filtered, and concentrated under reduced pressure to half its volume. The precipitate, formed by setting the mixture aside overnight in a cold room, was removed by suction (0.75 g) and recrystallised from 65% ethanol to give colourless crystals (0.45 g, 25%), decomp. point 317 °C (Found: C, 48.5; H, 4.65; N, 24.95%. Calc. for $C_9H_{10}N_4O_3$: C, 48.65; H, 4.53; N, 25.22%).

1,8-*Dimethylpteridine*-2,4,7-*trione* (9).—6-Methylamino-1-methyl-5-nitrosouracil (3 g) in water (50 ml) was reduced with Pd–C and hydrogen at room temperature. After 5 h, the catalyst was removed by filtration and the filtrate treated with ethyl glyoxal hemiethyl acetal (3 ml). After the mixture had been stirred for 1 h, the precipitate of 6-methylamino-5-(ethoxycarbonylmethyleneamino)-1-methyluracil was collected. A small amount of this Schiff's base was recrystallised from ethanol to give colourless crystals, m.p. 211 °C (Found: C, 47.05; H, 5.5; N, 22.05%. Calc. for $C_{10}H_{14}N_4O_4$: C, 47.24; H, 5.55; N, 22.04%).

To a solution of sodium (0.18 g) in absolute ethanol (50 ml) was added crude Schiff's base (1 g); the mixture was refluxed for 24 h while being protected against moisture. The solution was then neutralised with acetic acid, evaporated to dryness, and the residue extracted with 10 ml of chloroform–methanol (10 : 1 v/v). The clear extract, after filtration, was chromatographed on a silica-gel column (40 × 3.3 cm) with the same chloroform–methanol mixture as above. Fractions of 200-ml were collected. 1,8-Dimethylpteridine-2,4,7-trione was eluted in fractions 7—10. After evaporation of the solvent, the residue was recrystallised from ethanol, with addition of charcoal, to give colourless crystals, decomp. point *ca.* 310 °C (Found: C, 46.1; H, 3.75; N, 26.85%. Calc. for $C_8H_8N_4O_3$: C, 46.15; H, 3.87; N, 26.92%).

N.m.r. spectra were measured at 70 °C on a JEOL MH-100 apparatus, using $(CD_3)_2SO$–D_2O = 9 : 1 (v/v) as solvent

and sodium 3-trimethylsilyl[2,2,3,3-$^2H_4$]propionate as internal standard.

Interatomic distances were calculated with the aid of the co-ordinate program of Dewar and Baird.[20]

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