Pteridines XCVIII* Synthesis and Properties of 1-Methylpterins

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Summary

A series of 1-methyl-pterin derivatives (**6-13**) have been synthesized by condensation and methylation reactions. The compounds have been characterized by elemental analyses, pK_a determinations and UV spectra. Comparisons of the physical data of the corresponding N-1 and N-3 methyl-pterins indicate why the normal pterins exist in aqueous solution preferentially in the N³-H tautomeric configuration. The thermodynamical stability of the lactam group forces the acidic H-atom to adapt the nitrogen adjacent to the carbonyl function but no other vinylogous position. A lactam group does not participate in heteroaromatic cyclic resonance, in general.

Key words: 1-methylpterins, Elemental analyses, pK_a determinations, UV spectra

Introduction

Studies of the fine structure of pterin (**1**), the basic molecule of most naturally occurring pteridines (**2**), have revealed that the predominant tautomeric form in aqueous solution is represented by the 2-amino-pteridin-4 (3H)-one (**1**). This result is in agreement with the fact that in the series of the common nitrogen heterocycles amino groups exist always as such whereas hydroxy groups adjacent to a ring nitrogen atom adapt the thermodynamically more stable lactam (**1**) in preference to the lactim or iminol form (**2**) (**3**).

It is interesting to note that the acidic proton of the lactam configuration is always bound at the adjacent ring nitrogen atom even if there are, from valency considerations, other vinylogous positions available as, for example, the N-1 (**3**) and N-8 atoms (**4**), respectively, in the pteridine nucleus. The amide function can therefore be regarded as a prototype of a strongly resonance stabilized tautomeric configuration which does not participate in interactions known as cyclic heteroaromatic stabilization or resonance (**4**).

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In order to study the physical properties of the N1-H tautomers in comparison to the corresponding pterins a series of 1-methyl-pterins have been synthesized.

Results and Discussion

The synthesis of 1-methylpterin derivatives is commonly performed by a so-called *Gabriel-Isay* reaction (**5**) condensing 2,5,6-triamino-1-methylpyrimidin-4-one (**5**) with the appropriate 1,2-dicarbonyl compound. The starting compound **5** was applied in form of its dihydrochloride salt which resulted from reflux of 2,6-diamino-5-formylamino-1-methyl-pyrimidin-4-one in methanolic HCl. The latter product

was prepared from 2,6-diamino-1-methyl-5-nitrosopyrimidin-4-one to which Roth et al. (6) have assigned the 3-methyl instead of the correct 1-methyl structure. 1-Methyl-(6) (1), 1,6,7-trimethyl-(7) (6) and 1-methyl-6,7-diphenyl-pterin (8) (7, 8) have been obtained by condensation reactions with glyoxal, diacetyl and benzil, respectively, but 7 and 8 resulted also during alkylation of 6,7-dimethyl- and 6,7-diphenyl-pterin in a mixture with its 3-methyl isomer (8, 9). Acetylation of 1-methylpterin (6) by heating in acetic anhydride afforded N²-acetyl-1-methylpterin (9) in 77% yield.

The synthesis of 1-methylpterin-6-carboxylic acid (10) is the result of a methylation reaction of pterin-6-carboxylic acid leading to a mixture of the 1,3- and 8-methyl derivatives (10).

1-Methyl- (11) and 1,6-dimethyl-isoxanthopterins (12) were achieved by condensation reactions of 1 with ethyl glyoxylate-ethylhemiacetal and ethyl pyruvate, respectively, giving moderate yields. Finally the 1-methylleucopterins (13) resulted from a fusion reaction between 1 and oxalic acid at a temperature up to 200°C. Purification of this material could only be achieved by reprecipitation dropping an alkaline solution of 13 into dilute AcOH.

The newly synthesized compounds have been characterized by elemental analyses and their UV spectra of the various molecular forms (Table 1) based upon the spectrophotometrically determined pK_a -values (11) whereas the ¹H-NMR spectra turned out to be not very informative and helpful in the discussion of structural features.

Comparisons of the pK values and UV spectra of the 1- and 3-methylpterin analogs reveal very interesting information regarding fine-structural features of these molecules. It was found in all cases that

the 1-methylpterins are always somewhat stronger bases than their 3-methyl counterparts. These findings are in excellent agreement with the fact that those species are commonly preferred in tautomeric equilibria which show the least change on pH-variations. 3-Methylpterin, for example, reveals a basic pK_a of 2.18 whereas the 1-methyl isomer is by 0.6 pK units more basic. Pterin itself (1), therefore, adopts the N³-H form which offers a greater thermodynamical stability in a broader pH range. Another consequence of this stabilization is also reflected in the red-shifted long wavelength absorption band of the N³-over the N¹-tautomer. It is furthermore noteworthy that both cation species show very similar UV spectra indicating that the 1-methylpterin is protonated at the N-3- and 3-methylpterin at the N-1-position (1). The same behaviour is shown by the corresponding pairs of 6,7-dimethyl and 6,7-diphenyl derivatives. In the latter series besides the 1-(8) and 3-methyl-6,7-diphenylpterins also the 1,3-dimethyl-2-immino-6,7-diphenylpteridin-4-one (7) is available showing expectedly a more basic pK_a at 5.76 but forming the same type of a cation by protonation at the imino function (Fig. 1).

Similar comparisons are also valid for 1-(10) and 3-methylpterin-6-carboxylic acid (14) (12). The cations coincide here also regarding the main absorption bands at 320, 260-266 and 234-239 nm but 14 reveals an additional long wavelength absorption at 385 nm analogous to the cation of 3,6,7-trimethylpterin. These absorptions are derived from the fact that these molecules are not only protonated at N-1 but to some extent also at N-8 giving rise to a much longer chromophoric system. The cation of 14 is therefore a mixture of two cation species according to the following equilibria:

The 1-(11) and 3-methyl-isoxanthopterins (13) as well as their 6-methyl derivatives are characterized by similar spectral features since their cations reveal very closely related spectra. In this series the cations of 3-methyl- and 3,6-dimethyl-isoxanthopterins again consist of a mixture of two species, the main component protonated at N-1 whereas the long wavelength band with low extinction is due to a small concentration of the N-5 cation. The spectra of the neutral molecular forms are quite different (Fig. 3) and also the formation of their monoanions is associated with characteristic shifts of the long wave-

Table 1. Physical Data of 1- and 3-Methylpterin Derivatives.

-Pterin	pK in H ₂ O	UV-Absorptionsspektren								pH	Molec- ular Form						
		$\lambda_{\max}$ (nm)				l g ϵ											
3-Methyl-pterin	2.18	230		318		4.17		3.88		0.0	+						
			240	274	353		4.17	4.07	3.79	5.0	○						
1-Methyl-pterin (6)	2.86	228	[248]		314		4.09	[3.94]	3.89	0.0	+						
	12.74		240	[285]	327			4.21	[3.63]	3.93	5.0	○					
N ¹ -Acetyl-1-methyl- pterin (9)		227		273	333		4.06		4.30	3.98	MeOH	○					
3,6,7-Trimethyl-pterin	2.60	219	248		321	389	4.22	3.87		3.96	2.61	0.0	+				
		[224]	241	275		351	[4.10]	4.14	4.07		3.82	7.0	○				
3,6,7-Trimethyl-pterin (7)	3.25	215	253		320	[330]	4.25	3.93		3.98	[3.91]	0.0	+				
	13.24		240	277	329	[337]		4.21	3.47	4.01	[3.98]	7.0	○				
3-Methyl-6,7-diphenyl- pterin	2.29	228		279		362	4.45		4.13		4.18	0.0	+				
		223	[244]	292		378	4.42	[4.29]	4.35		4.10	5.0	○				
1-Methyl-6,7-diphenyl- pterin (8)	2.95	221		279		361	4.26		3.98		4.00	0.0	+				
	12.85	[223]	264			361	[4.23]	4.13			4.05	7.0	○				
1,3-Dimethyl-2-imino- 6,7-diphenyl-pteridin- 4-one	5.76	229		277		362	4.45		4.15		4.18	0.0	+				
		[218]	266	[283]		378	[4.43]	4.27	[4.24]		4.14	8.0	○				
3-Methyl-pterin-6- carboxylic acid	1.29	239	[260]		322	[330]	385	4.13	[3.99]		3.97	[3.90]	2.83	-1.0	+		
	3.30	[218]	245	297		359		[3.94]	4.11	4.12		3.88		2.2	○		
		217	242	289		356	4.03	4.14	4.18		3.89		6.0	-			
1-Methyl-pterin-6-carb- oxylic acid (10)	1.73	234	266		319	[331]		4.08	4.09		4.02	[3.94]		0.0	+		
	2.85	[234]	257		320	[328]	[4.01]	4.08			4.00	[3.97]		2.25	○		
			250		330	[338]		4.33			4.01	[3.97]		5.0	-		
3-Methyl-isoxantho- pterin	-0.89	[224]	267	[276]		318		[380]	[4.13]	3.80	[3.77]		4.05		[3.03]	-2.0	+
	7.45	221	[252]	286		342		4.55	[3.62]	3.95		4.15				○	
		228	258	282	332	[342]		4.60	3.95	3.86	4.11	[4.06]		10.0	-		
1-Methyl-isoxantho- pterin (11)	2.22	205	264		314	[326]		4.42	3.81		4.12	[4.04]		0.0	+		
	5.25	216		281	325	[337]		4.39		3.95	4.15	[4.07]		4.0	○		
	14.08	209	247	275	330	[342]		4.45	4.15	3.71	4.22	[4.12]		7.0	-		
			236	[254]	354				4.11	[4.01]	4.20			14.70	-	-	
3,6-Dimethyl-isoxantho- pterin	-0.02	212	269	[278]	327		[372]	4.35	3.92	[3.89]	3.94		[3.75]	-2.0	+		
	8.00	226		288		338		4.09		4.07		4.18		5.8	○		
		227	256	284	332			4.58	4.04	3.94	4.14			10.6	-		
1,6-Dimethyl-isoxantho- pterin (12)	2.40	218	266		317	[328]		4.22	3.86		4.09	[4.00]		0.0	+		
	5.03	217		285	324	[337]		4.32		3.94	4.21	[4.02]		3.7	○		
	14.30	204	239	278	330	[342]		4.38	4.11	3.66	4.21	[4.09]		8.0	-		
			241	280	348	[359]								15.0	-	-	
1-Methyl-leucopterin (13)	1.29	[237]	284	284	323	[338]		[3.86]	3.97	3.96	3.95	[3.89]		-1.0	+		
	4.65	234	[286]	294	328	342	359	4.14	[3.88]	3.90	4.13	4.17	3.92	3.0	○		
	11.73	232	277	286	330	346	363	4.19	3.74	3.74	4.12	4.15	3.91	7.0	-		
		232		288	[331]	342	357	4.26		3.80	[4.16]	4.24	4.08	14.0	-	-	
3-Methyl-leucopterin	-0.53		248	289	319	[336]			3.72	4.06	3.99	[3.88]		-3.0	+		
	7.60	226		298		337		4.14		4.05		3.82		5.0	○		
	10.95	217	246	295	334	[344]		4.23	4.19	3.95	3.97	[3.96]		9.2	-		
		220	241	299		342		4.12	4.32	3.97		4.09		14.0	-	-	

[] = Shoulder; + = cation; ○ = neutral form; - = monoanion; - - = dianion.

length band towards shorter wavelengths in the 3-methyl- and longer wavelengths in the 1-methyl-isoxanthopterins (Fig. 4).

It should also be mentioned that 1-methyl-isoxanthopterine (11) is a much stronger acid than its 3-methyl isomer despite the fact that the basicities of these two compounds show the reverse order of magnitude. This fact is due to an acidifying effect of

the peri-located 1-methyl group which influences the acidic amide function from steric reason as recognized earlier in the lumazine series (14, 15). 1-Methyl- (11) and 1,6-dimethyl-isoxanthopterine (12) offer in the normal pH range even a second acidic pK_a of 14.08 and 14.30, respectively, indicating that the 2-amino group possesses the character of a vinylogous amide function explaining the somewhat high acid-

Figure 1. UV-Absorption spectra of the cations of 1-(8) (---) and 3-methyl-6,7-diphenyl-pterin (—) and 1,3-dimethyl-2-imino-6,7-diphenylpteridin-4-one (-·-) at pH 0.

Figure 2. UV-Absorption spectra of the neutral species of 1-(8) (--- pH 7) and 3-methyl-6,7-diphenyl-pterin (— pH 5) and 1,3-dimethyl-2-imino-6,7-diphenylpteridin-4-one (-·- pH 8).

ity which is also seen in 1-methyl-(6)-, 1,6,7-trimethyl-(7) and 1-methyl-6,7-diphenyl-pterin (8).

Finally 1-(13) and 3-methyl-leucopterin (16) can be regarded as an extension of the described features of the isoxanthopterin analogs. 1-Methyl-leucopterin again is on one hand the more basic component and on the other hand reveals more acidic properties than its 3-methyl isomer from analogous reasons. The structural differences are furthermore reflected in the UV spectra of the corresponding molecular forms.

The $^1\text{H-NMR}$ spectra of the various 1- and 3-methyl-pterin derivatives are not very informative due to only a few signals of structural relevance. The $\text{N}^1\text{-CH}_3$ group resonates always at lower field than its $\text{N}^3\text{-CH}_3$ analog by a chemical shift difference of ca. 0.2 ppm measured in $\text{D}_6\text{-DMSO}$. A similar but even smaller shift of the 2-amino group to lower

Figure 3. UV-Absorption spectra of the neutral molecular forms of 1-methyl-(11) (--- pH 4) and 3-methyl-isoxanthopterin (— pH 5).

Figure 4. UV-Absorption spectra of the monoanions of 1-methyl-(11) (--- pH 7) and 3-methyl-isoxanthopterin (— pH 10).

field by about 0.05 ppm is again observed in the 1-methyl compared to the 3-methyl-pterin series. Some of the NMR data of these pterins could not been determined at all due to the insolubility of these compounds in DMSO.

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Experimental Part

General. TLC: precoated silica gel thin-layer sheets F 1500 LS 254 and cellulose thin-layer sheets F 1440 LS 254 from Schleicher & Schüll. M.p.:

Büchi apparatus, model Dr. Tottoli; no corrections. pK: the determinations were done by the spectrophotometric method (11). UV/VIS: Lambda 5 (Perkin-Elmer); $\lambda_{\max}$ (log ϵ). $^1\text{H-NMR}$: Bruker WN-250; in δ (ppm) relative to TMS.

2,6-Diamino-5-formylamino-1-methyl-pyrimidin-4-one

In 200 ml of conc. formic acid 16.9 g (0.1 mol) of 2,6-diamino-1-methyl-5-nitrosopyrimidin-4-one (6) were heated to boiling and then 20g of zinc dust added slowly in small portions with stirring. The reaction mixture is heated under reflux for 30 min till the solution has changed to slightly yellow. After cooling the zinc and zinc formate is filtered off and the filtrate evaporated in vacuum. The residue is treated with MeOH and the resulting precipitate collected. Recrystallization from H_2O with charcoal gave 14.3 g (78%) of colorless crystals of m.p. >320°. UV (MeOH): 210 (4.48), 265 (4.14). Anal. calc. for $\text{C}_7\text{H}_7\text{N}_5\text{O}_2 \cdot \text{H}_2\text{O}$ (201.2): C 35.82, H 5.51, N 34.81; found: C 35.76, H 5.46, N 34.65.

2,5,6-Triamino-1-methyl-pyrimidin-4-one dihydrochloride (5)

A suspension of 10 g (0.055 mol) of 2,6-diamino-5-formylamino-1-methylpyrimidin-4-one in 200 ml of saturated methanolic HCl was heated under reflux and simultaneous injection of HCl gas for 2 h to achieve cleavage of the formyl group. After cooling the precipitate was collected, dried in a desiccator over KOH to give 11.1 g (90%) of a colorless powder of m.p. >280° (decomp.). Anal. calc. for $\text{C}_7\text{H}_7\text{N}_5\text{O} \cdot 2\text{HCl}$ (225.1): C 26.68, H 3.58, N 31.12; found: C 26.51, H 3.64, N 30.96.

1,6,7-Trimethylpterin (7) (6)

In a boiling water bath 1.14 g (5 mmol) of 2,5,6-triamino-1-methylpyrimidin-4-one dihydrochloride (5) and 0.5 g of diacetyl were heated for 10 min with stirring. The yellow solution faded and an almost colorless precipitate separated which was collected after cooling. On recrystallization from water with charcoal 0.79 g (75%) of colorless crystals of m.p. >350° were obtained. $^1\text{H-NMR}$ ($\text{D}_6\text{-DMSO}$): 7.46 (s, 2H, NH_2); 3.57 (s, 3H, N-CH_3); 2.55 (s, 3H, C-CH_3); 2.52 (s, 3H, C-CH_3). Anal. calc. for $\text{C}_9\text{H}_{11}\text{N}_5\text{O}$ (205.2): C 52.68, H 5.40, N 34.13; found: C 52.54, H 5.36, N 34.21.

N^2 -Acetyl-1-methylpterin (9)

In 5 ml of acetic anhydride 0.177 g (1 mmol) of 1-methylpterin (1) were heated under reflux for 30 min. On cooling colorless crystals separated which were washed with EtOH and ether and then

dried at 100° to give 0.17 g (77%) of m.p. 254°. $^1\text{H-NMR}$ ($\text{D}_6\text{-DMSO}$): 13.36 (s, 1H, N-H); 8.86 (d, 1H, H-C (7)); 8.72 (d, 1H, H-C (6)); 3.66 (s, 3H, N-CH_3); 2.19 (s, 3H, C-CH_3). Anal. calc. for $\text{C}_9\text{H}_9\text{N}_5\text{O}_2$ (219.2): C 49.31, H 4.14, N 31.95; found: C 49.22, H 4.15, N 31.84.

1-Methyl-isoxanthopterin (11)

A solution of 1.12 g (50 mmol) of 2,5,6-triamino-1-methyl-pyrimidin-4-one dihydrochloride in 25 ml of H_2O was neutralized by ammonia to pH 6, then 2.5 ml of ethyl glyoxylate-hemiethylacetal added and the mixture heated gently to 100° for 15 min. The yellow precipitate consisting of the corresponding Schiff's base, 2,6-diamino-5-ethoxycarbonylmethyleneamino-1-methyl-pyrimidin-4-one, was collected, dried (0.9 g) and then refluxed in 20 ml of 1N NaHCO_3 for 30 min. The hot solution was acidified with 2N HCl and on cooling 0.6 g (62%) of a yellowish powder of m.p. >300° were obtained. This material analysed for the monohydrochloride. Anal. calc. for $\text{C}_7\text{H}_7\text{N}_5\text{O}_2 \cdot \text{HCl}$ (229.6): C 36.62, H 3.51, N 30.51; found: C 36.78, H 3.41, N 30.48. The neutral molecule was prepared by dissolving the hydrochloride salt in dilute NaOH and dropwise addition of the hot solution into dilute AcOH. The precipitate was filtered, dried at 100° to give an almost colorless powder of m.p. >340°. Anal. calc. for $\text{C}_7\text{H}_7\text{N}_5\text{O}_2 \cdot \text{H}_2\text{O}$ (211.2): C 39.81, H 4.30, N 33.17; found: C 39.77, H 4.36, N 32.95.

1,6-Dimethyl-isoxanthopterin (12)

A mixture of 1.12 g (50 mmol) of 2,5,6-triamino-1-methyl-pyrimidin-4-one dihydrochloride and 1 ml of ethyl pyruvate were heated under reflux in 20 ml of H_2O for 20 min. On cooling a precipitate separated. The slightly yellowish crystals were collected and recrystallized from very dilute AcOH to give 0.7 g (62%) of m.p. >330°. $^1\text{H-NMR}$ (TFA): 8.50 (s, 2H, NH_2); 4.11 (s, 3H, N-CH_3); 2.90 (s, 3H, C-CH_3). Anal. calc. for $\text{C}_8\text{H}_9\text{N}_5\text{O}_2 \cdot \text{H}_2\text{O}$ (225.2): C 42.86, H 4.90, N 30.91; found: C 42.66, H 4.90, N 31.10.

1-Methyl-leucopterin (13)

A grinded mixture of 2.25 g (0.01 mol) of 2,5,6-triamino-1-methyl-pyrimidin-4-one dihydrochloride and 10 g of oxalic acid were heated in an oil-bath first to 140° to give a homogeneous melt which could be stirred magnetically. The temp. was then gradually increased to 200° and kept here for 1 h. The melt solidified partially during this procedure. After cooling the reaction mixture was heated with 50 ml of H_2O , dilute KOH added till a clear solution was obtained, charcoal added and then filtered.

The filtrate was dropped into boiling dilute AcOH to give a colorless precipitate which was difficult to filter. The colorless powder was dried at 100° and gave 1.05 g (50%) of m.p. >350°. Anal. calc. for C₇H₇N₃O₃ (209.2): C 40.19, H 3.37, N 33.48; found: C 40.20, H 3.42, N 33.33.

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