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PTERIDINES, LXXXVI[1]

Structure and Synthesis of Leucettidine

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Dedicated to my old friend Prof. E.C. Taylor

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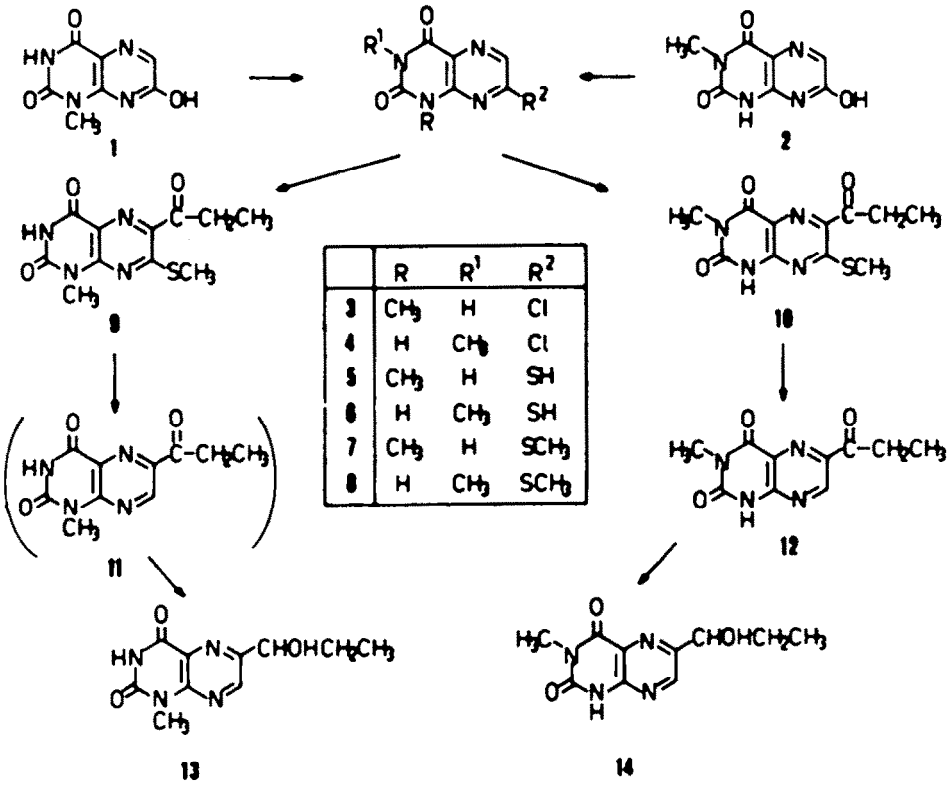
Abstract - The structure of leucettidine (13), a new naturally occurring pteridine derivative in Leucetta microraphis, has been revised to 6-(1-hydroxypropyl)-1-methyllumazine. Unambiguous syntheses of 13 and its 3-methyl isomer 14 prove the correct structure, which can also be depicted from comparisons of pK_a values, UV- and NMR-data.

Introduction. - In 1981 Cardellina and Meinwald[2] isolated a minor metabolite from leucetta microraphis, a Calcareous sponge common in Bermudan waters, and called it leucettidine. Its structure was deduced from various spectral data including high resolution mass spectrometry to determine the empirical formula $C_{10}H_{12}N_4O_3$, NMR-spectra to elucidate the nature of the substituents and IR- and UV-spectra to find the lumazine (pteridine-2,4-dione) chromophor as the basic nucleus of this novel pteridine derivative. Comparisons of the physical data of leucettidine (13) with those of various model substances from literature led to the assignment of the 6-(1-hydroxypropyl)-3-methyl-pteridine-2,4(1H)-dione structure (14). The location of the methyl group at N-3 was derived only from comparisons of chemical shift data, whereas the more precise structural distinctions on the basis of the UV-spectral properties have been completely overlooked. It can be seen easily from the reported UV-data of leucettidine that the methyl group cannot be located at N-3 but at N-1, as reported earlier[3], since anion formation of an N-1 substituted lumazine is always associated with a small bathochromic shift of the long wavelength absorption band, whereas substitution at N-3 causes a much stronger shift due to a more pronounced resonance stabilization of this anion species[4]. In order to prove the new structural assignment[3,5] of leucettidine we synthesized the two isomeric 6-(1-hydroxypropyl)-1-(13) and 3-methyl lumazine (14) in form of their racemates by unambiguous routes.

Syntheses. - Regioselective syntheses of 6- and 7-substituted pteridines respectively via the Gabriel-Isay condensation[6] between a 5,6-diaminopyrimidine and an appropriate 1,2-dicarbonyl component is still a difficult task and leads in most cases to isomeric mixtures difficult to be separated by chromatographical means. The π-electron deficiency of the pyrazine moiety on the other hand allows the direct introduction of carbon side chains by a radical nucleophilic substitution according to the fundamental investigations of Minisci et al.[7,8] with various nitrogen heterocycles. We have shown[9] for the first time that the lumazine system is also prone to easy radical acylations which proceed selectively at C-7 as the most π-electron deficient center in the molecule. In order to direct the incoming radical nucleophile to C-6 the adjacent position has to be blocked by a substituent. This reaction behaviour of the lumazine moiety in mind prompted us to apply 1- (7) and 3-methyl-7-methylthiolumazine (8) to radical acylations. These compounds were obtained from 1- (1) and 3-methyl-7-hydroxylumazine (2)[10] respectively in three steps including first treatment with $POCl_3$/KCl at 80-90° to achieve a selective conversion to 7-chloro-1-methyl- (3) and 7-chloro-3-methyllumazine (4)[11], second with sodium hydrogensulfide to form the corresponding 7-mercapto derivatives 5 and

6[11] and finally by methylation with methyl iodide in dilute sodium hydrogencarbonate to give 7 and 8 in good yields. Introduction of the propionyl side chain was performed by the system propionaldehyde/Fe^{++}/tert.butylhydroperoxide in aqueous acetic acid generating the propionyl radical and leading to 1-methyl- (9) and 3-methyl-7-methylthio-6-propionyllumazine (10) respectively in 78 % yield each.



	R	R^1	R^2
3	CH_3	H	Cl
4	H	CH_3	Cl
5	CH_3	H	SH
6	H	CH_3	SH
7	CH_3	H	SCH_3
8	H	CH_3	SCH_3

During desulfurization experiments to remove the methylthio group it became evident that the usual Raney nickel treatment is not successful due to the known difficulties[12] encountered with thiopteridines in general. Minor success was achieved by a strongly deactivated Raney nickel in a less polar solvent and much improvement resulted from the use of aluminum-copper alloy in alkaline medium. Under these conditions 9 was converted directly into 6-(1-hydroxypropyl)-1-methyllumazine (13) obviously via 11, whereas the isomer 10 reacted cleanly with desulfurization to 3-methyl-6-propionyllumazine (12). Sodium borohydride reduction transformed 12 in 55 % yield into 6-(1-hydroxypropyl)-3-methyllumazine (14).

Structures and Physical Data. - The newly synthesized compounds have been characterized in their structures by elemental analysis, pK_a determinations and their UV- and NMR-spectra (Tab. 1). Comparisons of various physical data of the corresponding 1- and 3-methyllumazine derivatives indicate common molecular features, which allow a structural differentiation between both series of compounds. The 1-methyllumazines are always the weaker acids by about 0.5 pK_a units in comparison to the 3-methyl analog. From the UV-spectra can be seen that the N-1 substitution causes a small bathochromic shift of the long wavelength absorption band over the N-3 methyl isomer, and the NMR spectra locate the chemical shift of the 1-methyl always at lower field than the 3-methyl group despite the fact that the latter is adjacent to two carbonyl functions. The best differentiation between 1- and 3-methyllumazine derivatives, however, will be noticed from the UV-spectral

Tab. 1 - Physical Data of Lumazine Derivatives

-lumazine	pK_s in H_2O	UV - Absorption Spectra λ_{max} (nm)	lg ε	pH	Molecular Form	NMR - Spectra in $CDCl_3$ N-CH_3	7-Subst./6-Subst.
7-Chloro-1-methyl- (3)		203 237 335	4.30 4.08 3.99	MeOH	o	3.65s	- 8.54s
7-Chloro-3-methyl- (4)		202 236 333	4.24 4.12 4.00	MeOH	o	3.51s	- 8.58s
1-Methyl-7-thio- (5)	1.51 10.16	222 256 352 400 223 [282] 300 380 228 256 [272] 380	4.30 4.08 4.18 3.65 4.30 [3.76] 3.79 4.31 4.38 4.00 [3.92] 4.33	-1.0 4.0 13.0	o - --	3.84s*	8.48s*
3-Methyl-7-thio- (6)	2.08 9.58	220 307 360 396 220 278 300 378 230 255 275 383	4.27 3.93 3.94 4.12 4.37 3.72 3.82 4.28 4.28 4.32 3.90 4.26	0.0 5.0 12.0	o - --	3.60*	8.68s*
1-Methyl-7-methyl-thio- (7)	9.21	225 270 358 228 258 361	4.30 3.87 4.31 4.31 4.07 4.31	5.0 13.0	o -	3.68s	2.68s 8.40s
3-Methyl-7-methyl-thio- (8)	8.18	220 265 355 235 277 371	4.39 3.80 4.31 4.37 4.12 4.17	3.0 11.0	o -	3.49s	2.62s 8.40s
1-Methyl-7-methyl-thio-6-propionyl- (9)	8.45	233 259 310 365 257 296 369	4.07 4.31 4.09 4.19 4.44 4.08 4.25	4.0 11.0	o -	3.70s	2.60s 3.33(q, CH_2)
3-Methyl-7-methyl-thio-6-propionyl-(10)	7.52	245 307 363 270 308 382	4.35 4.07 4.18 4.38 4.11 4.26	5.0 11.0	o -	3.50s	2.53s 3.28(q, CH_2)
3-Methyl-6-propionyl- (12)	7.05	247 270 326 256 309 369	4.11 4.00 4.03 4.09 4.18 4.02	4.0 10.0	o -	3.55s	9.27s 3.33(q, CH_2) 1.24(t, CH_3)
6-(1-Hydroxypropyl)-1-methyl- (13)	8.82	237 [248] 334 234 [248] 335 245 285 343	4.12 [4.08] 3.85 4.14 [4.08] 3.88 4.29 3.44 3.92	MeOH 5.0 13.0	o o -	3.66s	8.78s 4.91(dd, CHOH) 1.86(m, CH_2) 0.99(t, CH_3)
6-(1-Hydroxypropyl)-3-methyl- (14)	8.22	235 331 234 330 247 273 366	4.21 3.86 4.22 3.92 4.28 4.12 3.82	MeOH 5.0 13.0	o -	3.49s	8.70s 4.88(dd, CHOH) 1.86(m, CH_2) 0.98(t, CH_3)
Natural Leucettidine		238 334 246 292 344	3.93 3.62 4.15 3.48 3.66	MeOH MeOH MeOH	o -	3.63s	8.76s 4.84(dd, CHOH) 1.85(m, CH_2) 0.97(t, CH_3)

[ ] = Shoulder. o = Neutral form; - = Monoanion, -- = Dianion.
s = Singlet; dd = double dublet, t = triplet; q = quadruplet; m = multiplet.
* = in CF_3COOH.

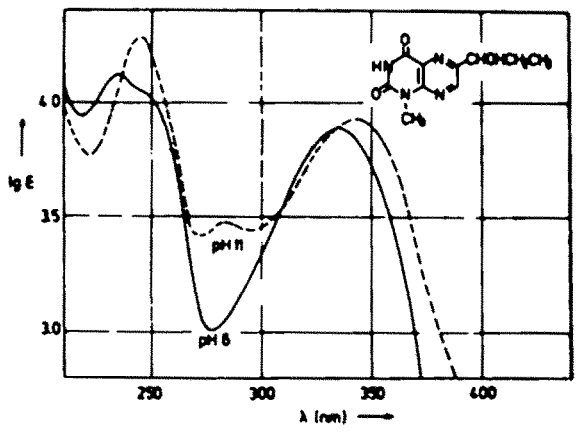


Fig. 1 - UV-Absorption spectra of the neutral and monoanion form of synthetic leucettidine (13).

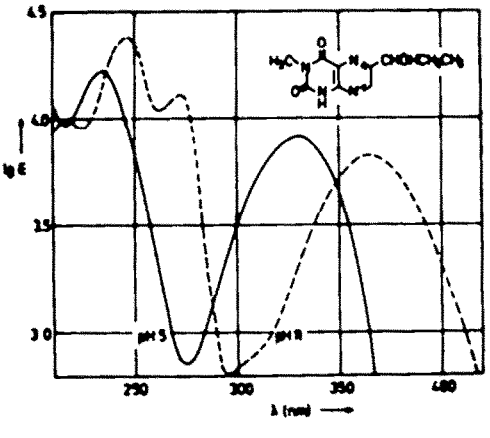


Fig. 2 - UV-Absorption spectra of the neutral and monoanion form of 6-(1-hydroxypropyl)-3-methyllumazine (14).

shifts on monoanion formation. The 1-methyl series shows a small bathochromic shift of the long wavelength absorption band, whereas the deprotonation from N-1 in the 3-methyl series is associated with a much more distinct red shift due to a more pronounced resonance stabilization in the monoanion form. The typical UV-spectral changes are reproduced in figure 1 and 2.

Finally, the discrepancies of the physical data of natural and synthetic leucettidine can be explained by the fact that the isolated material from natural sources was so far no analytically pure sample as seen from the lower extinction coefficients and the missing elemental analysis[2], which reveals 0.5 mol of crystal water.

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E X P E R I M E N T A L

UV-Absorption spectra were measured with a Perkin-Elmer Lambda 5 spectrometer. The determination of the pK_a values was achieved by the spectrophotometric method[13]. ^{1}H-NMR-spectra are taken at 250 MHz with a Bruker WM-250 spectrometer; as standards have the $CHCl_3$ and DMSO signals been used and the chemical shifts are recorded as δ-values in ppm. Schleicher & Schüll precoated silica gel sheets F 1500 LS 254 were used for TLC and Merck silica gel PF_{254} for separations on preparative plates (40x 20x0.2 cm). Drying of the substances was performed in a Büchi TO-50 oven under high vacuum or in a normal drying oven at 100°C. M.ps. are not corrected.

7-Mercapto-1-methyllumazine (5)[11]. - Compound 3[11] (2.4 g, 0.01 mol) was heated with 2 g of NaSH in EtOH (20 ml) and H_2O (80 ml) for 30 min to 50° with stirring. The clear solution was treated with charcoal, filtered and acidified with conc. HCl to pH 0. The precipitate is filtered off, washed with H_2O and dried at 100°C to give pure 7-mercapto-1-methyllumazine (Found: C 37.32; H 2.88; N 24.55. $C_7H_6N_4O_2S \cdot H_2O$ (228.3) requires: C 36.81; H 2.63; N 24.54 %) as a yellow microcrystalline powder; m.p. >300°; yield 1.68 g (80 %).

7-Mercapto-3-methyllumazine (6)[11]. - Compound 4[11] (2.4 g, 0.01 mol) was heated in EtOH (20 ml) and H_2O (100 ml) with 2 g NaSH for 30 min to 50° with stirring. The clear solution was treated with little charcoal, filtered and then acidified with 5 N HCl to pH 0. The precipitate is collected, washed with H_2O and dried at 100° to give pure 7-mercapto-3-methyllumazine as a yellow crystalline powder; m.p. >270° (decomp.); yield 1.79 g (85 %).

1-Methyl-7-methylmercaptolumazine (7). - Compound 5 (2.1 g, 0.01 mol) was dissolved in 400 ml of 0.1 N $NaHCO_3$, then 5 ml of CH_3I added and stirred at room temp. for 1 hour. The solution is neutralized with acetic acid, the precipitate collected and then recrystallized from 500 ml of H_2O to give pure 1-methyl-7-methylmercaptolumazine (Found: C 42.76; H 3.58; N 24.91. $C_8H_8N_4O_2S$ (224.2) requires: C 42.85; H 3.60; N 24.98 %) as yellowish crystals; m.p. 266-268°; yield 1.57 g (70 %).

3-Methyl-7-methylmercaptolumazine (8). - Compound 6 (2.1 g, 0.01 mol) was dissolved in 900 ml of 0.1 N $NaHCO_3$, then 8 ml of CH_3I added and vigorously stirred at room temp. for 1 hour. Neutralization with acetic acid formed a precipitate which was filtered off, washed with H_2O and recrystallized from 400 ml of EtOH to give

pure 3-methyl-7-methylmercaptolumazine (Found: C 42.81; H 3.53; N 24.97. $C_8H_8N_4O_2S$ (224.2) requires: C 42.85; H 3.60; N 24.98 %) as yellowish crystals; m.p. 272-280°; yield 1.68 g (75 %).

1-Methyl-7-methylmercapto-6-propionyllumazine (9). - Compound 7 (2.24 g, 0.01 mol) was dissolved in a mixture of glacial acetic acid (60 ml), trifluoroacetic acid (15 ml) and H_2O (5 ml) and then the solution cooled to 0°-5° in an ice bath, whereby separation of some starting material takes place. Propionaldehyde (9 ml) is added and followed by simultaneous addition from two dropping funnels each $FeSO_4 \cdot 7\ H_2O$ (5 g) in H_2O (12 ml) and tert-butylhydroperoxide (2.5 g) to the stirred solution. A clear yellow solution is obtained, from which after some time a colourless precipitate separates. The solid is collected after 30 min, washed with H_2O and recrystallized from EtOH (500 ml) to give pure 1-methyl-7-methylmercapto-6-propionyllumazine (Found: C 46.10; H 4.25; N 19.62. $C_{11}H_{12}N_4O_3S$ (280.4) requires: C 47.14; H 4.31; N 19.98 %) as yellowish crystals; m.p. 280°; yield 1.2 g (43 %).

3-Methyl-7-methylmercapto-6-propionyllumazine (10). - Compound 8 (2.24 g, 0.01 mol) is treated analogously to the preceding procedure to give 1.8 g crude material. Recrystallization from EtOH (500 ml) gave pure 1-methyl-7-methylmercapto-6-propionyllumazine (Found: C 46.81; H 4.16; N 19.81. $C_{11}H_{12}N_4O_3S$ (280.4) requires: C 47.14; H 4.31; N 19.98 %) as yellowish crystals; m.p. 287°; yield 1.40 g (50 %).

3-Methyl-6-propionyllumazine (12). - Compound 10 (0.28 g, 1 mmol) was dissolved in hot EtOH (100 ml), then subsequently added Raney-copper alloy (2 g) and 5 N KOH (6 ml). A vigorous reaction takes place which is stopped after 1 min by addition of glacial acetic acid. The hot solution is filtered, the filtrate evaporated to dryness and the residue dissolved in H_2O. Several extractions with $CHCl_3$ are performed, the organic layer dried over Na_2SO_4 and then concentrated to a small volume. The work-up was done by preparative TLC on silica gel plates (40x20x0.2 cm) with $CHCl_3$/MeOH (9/1). The lower moving band is cut out, eluted with $CHCl_3$ and gave on evaporation and crystallization from $CHCl_3$/n-hexane pure 3-methyl-6-propionyllumazine (Found: C 51.13; H 4.21; N 23.85. $C_{10}H_{10}N_4O_3$ (234.2) requires: C 51.28; H 4.30; N 23.92 %) as colourless crystals; m.p. 234°; yield 0.094 g (40 %).

6-(1-Hydroxypropyl)-1-methyllumazine (Leucettidine) (13). - Compound 9 (0.56 g; 2 mmol) was dissolved in EtOH (120 ml) and 5 N KOH (10 ml). To the boiling solution is added with stirring Raney-copper alloy (5 g). After 5 min was neutralized with acetic acid, evaporated to dryness and the residue again dissolved in H_2O. The solution is extracted with $CHCl_3$, the organic layer dried over Na_2SO_4 and then concentrated to a small volume. The extract is put onto preparative silica gel plates (40x20x0.2 cm) and developed with the system toluene/ethyl acetate/MeOH (5/4/1). The slow moving band is cut out, extracted with $CHCl_3$, evaporated and the residue recrystallized from $CHCl_3$/n-hexane to give 6-(1-hydroxypropyl)-1-methyllumazine (Found: C 49.20; H 5.31; N 22.73. $C_{10}H_{12}N_4O_3 \cdot 1/2\ H_2O$ (245.2) requires: C 49.32; H 5.34; N 22.84 %) as colourless crystals; m.p. 195°; yield 0.14 g (34 %).

6-(1-Hydroxypropyl)-3-methyllumazine (14). - Compound 12 (0.117 g; 0.5 mmol) was suspended in H_2O (5 ml), then $NaBH_4$ (50 mg) added and the solution stirred at room temp. for 15 min. It is neutralized by acetic acid, then extracted several times with $CHCl_3$, the organic layer dried over Na_2SO_4 and finally evaporated. The residue is recrystallized from $CHCl_3$/n-hexane to give pure 6-(1-hydroxypropyl)-3-methyllumazine (Found: C 50.67; H 5.06; N 23.46. $C_{10}H_{12}N_4O_3$ (236.2) requires: C 50.84; H 5.12; N 23.72 %) as colourless crystals; m.p. 208°; yield 65 mg (55 %).

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