Synthesis of 6,7-Bis(trifluoromethyl)-8-substituted Pteridine-2,4(1*H*,3*H*)-dione (Lumazine) Hydrates from 4,5-Diaminouracil Hydrochlorides and Perfluorobutane-2,3-dione. Stabilization of the Transmolecular Covalent Hydrates of 8-Substituted Pteridinediones by Trifluoromethyl Groups

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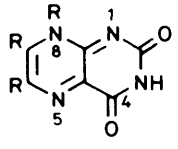
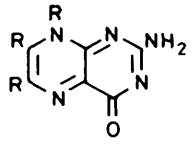
The reaction of perfluorobutane-2,3-dione (PFBD) with substituted 4,5-diaminouracil hydrochlorides (**15**) and (**16**) has been investigated in an attempt to determine the extent to which trifluoromethyl groups placed in the 6 and 7 positions of the 8-substituted lumazine products stabilize the transmolecular covalent hydrate forms. The compounds prepared in this manner were found to exist as stable hydrates (**17**) and (**18**).

The cross-conjugated π-electron systems found in the 8-substituted lumazines [pteridine-2,4(1*H*,3*H*)-diones] (**1**), pterins (**2**), and pteridin-2(8*H*)-ones (**3**) are responsible for distinct and complex chemistry not found in the simpler aromatic pteridines. Although much controversy evident in the early literature concerning these substances can be traced to misinterpretation of spectral data and the fact that differently substituted molecules in these series behave differently in aqueous solution, the hypsochromic shift in the u.v. spectrum as well as the anomalous 'acidity' observed when 3,8-dimethyl-lumazine (**4**) is dissolved in aqueous base clearly demonstrated the formation of the unstable, covalently hydrated species (**5**) and the corresponding anion (**6**). Rapid reaction techniques revealed that



(1)



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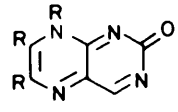

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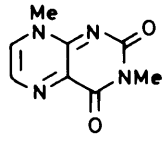

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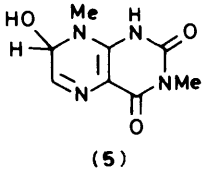
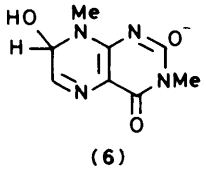
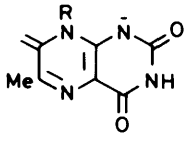


(5)



(6)

at equilibrium, the ratio of the anhydrous, neutral species (**4**) to the hydrated neutral species (**5**) is 5 360:1.[1] The 8-alkyl-6,7-dimethyl-lumazines, on the other hand, undergo proton abstraction from the 7-methyl group in alkaline solution, resulting in the formation of the 7-*exo* methylene form (**7**).[2-5] If the substituent in the 8-position of a 6,7-dimethyl-lumazine contains a 2'-hydroxy group, however, a mixture of the cyclic ether (**8**) and the 7-*exo* methylene form (**7**) results, with little if any of the covalent hydrate present at equilibrium.[2] Bulky substituents in the 7- and 8-positions favour the covalent hydrate forms to the extent that they may actually be isolated as stable, neutral molecules. For example, 3,8-dimethyl-6,7-diphenylpterin has been isolated as the covalent hydrate (**9**),[6] and the related 6,7-di-isopropyl and 6,7-diphenyl compounds (**10**), (**11**), and (**12**) have also been isolated in the 2,8-dihydropteridine series.[7] In connection with the latter three substances, it may also be pointed out that placement of isopropyl groups in the 6- and 7-positions has allowed the isolation of the cyclic ether (**13**) in neutral form.[1,8,9]



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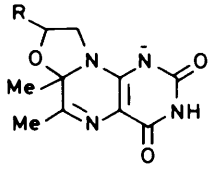

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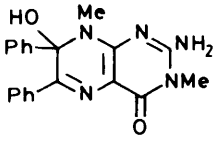

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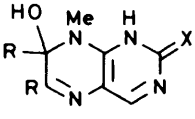
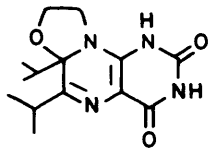


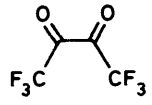
(10) R = $CHMe_2$, X = O
(11) R = $CHMe_2$, X = NMe
(12) R = Ph, X = O

6,7-Dimethyl-8-ribityl-lumazine has been identified as an important intermediate in the biosynthesis of riboflavin in a variety of micro-organisms.[10] The mechanism of this biosynthetic conversion is thought to involve an unstable cyclic ether related to structure (**8**) or the corresponding covalent hydrate form.[10] We have recently been interested in the possibility that the covalently hydrated or cyclic ether forms of the 8-substituted lumazines and related pyrazine-containing nitrogen heterocyclic systems might be significantly stabilized

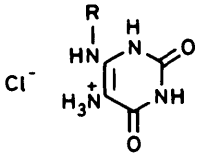
relative to the dehydrated species by the placement of trifluoromethyl groups in the 6- and 7-positions, since the attachment of electronegative substituents to sp^2 hybridized carbon atoms is destabilizing.[11] These considerations have led to an investigation of the reaction of perfluorobutane-2,3-dione (PFBD) (**14**)[12] with 5-amino-4-benzylaminouracil hydrochloride (**15**) and 5-amino-4-(2′-hydroxyethyl)aminouracil hydrochloride (**16**).[13]



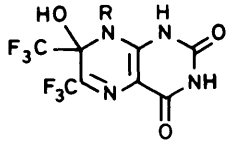
(**13**)



(**14**)



(**15**) R = $PhCH_2$
(**16**) R = CH_2CH_2OH

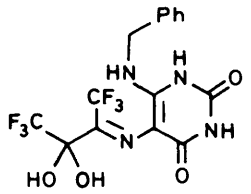


(**17**) R = CH_2Ph
(**18**) R = CH_2CH_2OH

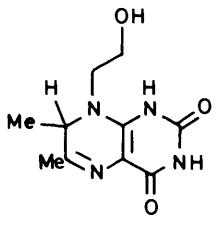
The reaction of PFBD with the hydrochloride salt (**15**) in DMF followed by evaporation of the solvent and hydrolysis of the residue led to the precipitation of the covalent hydrate (**17**) as a stable solid material. The ^{19}F n.m.r. spectrum of compound (**17**) displayed two quartets (J 6 Hz) at 14.33 and −4.70 (δ CF_3CO_2H = 0), which are assigned to the 6- and 7-trifluoromethyl groups, respectively. Although the electron impact mass spectrum of (**17**) showed a 'molecular ion' at m/z 390 corresponding to a dehydrated species, the FAB mass spectrum clearly supported the assigned covalent hydrate structure by exhibiting a prominent peak at m/z 409 ($M^+ + 1$). The intense band located at 1 190 cm^{-1} in its i.r. spectrum is attributed to an asymmetric CF_3 stretching mode and has been observed previously in the spectra of a variety of other bis(trifluoromethylated) pyrazine-containing heterocyclic systems.[11,14] An alternative structure (**19**) was considered and ruled out since the ^1H n.m.r. spectrum of the product did not show coupling between the 4-NH and benzylic protons characteristic of 4-benzylaminouracils [*e.g.* as seen in the starting material (**15**), J 6 Hz].

The u.v. spectrum of the product (**17**) exhibited an absorption maximum in aqueous acid (pH 1) at 340 nm, which is at a longer wavelength than expected on the basis of simple comparison with similar dihydrolumazines. For example, compound (**20**) produces a u.v. maximum at 310 nm in aqueous solution (pH 5).[1] The 6,7-dimethyl-8-substituted lumazines themselves display a u.v. maximum at 407 nm.[2] The bathochromic shift seen in compound (**17**) relative to compound (**20**) may be related to a greater hyperconjugation of the C-6 trifluoromethyl group with the diene system as expressed in the resonance structure (**21**).

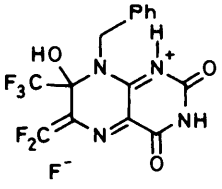
As noted above, complete dehydration of the covalent hydrate (**17**) was observed during electron impact mass spectrometry. In addition, a vigorous bubbling and decomposition were noted during melting point determination. The possible thermal dehydration of compound (**17**) to the corresponding 6,7,8-trisubstituted lumazine system (**1**) was therefore investigated. When (**17**) was heated neat at 210 °C, a product was isolated which displayed a u.v. absorption maximum 335 nm.



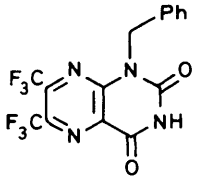
(**19**)



(**20**)



(**21**)



(**22**)

This is not consistent with a 6,7,8-trisubstituted lumazine system. It instead indicates the presence of an 8-unsubstituted lumazine containing an aromatic pyrazine ring.[11,16] The dehydration of (**17**) is evidently followed by a [1,3]-shift of the benzyl group, to yield the 1-benzyl-lumazine (**22**). Since the mass spectrum of (**22**) is identical with that of compound (**17**), the dehydration and rearrangement to form (**22**) must also be occurring prior to ionization during mass spectrometry.

The reaction of the free base derived from the 2′-hydroxyethyl substituted salt (**16**) with PFBD in DMF led to the formation of unstable substances which were converted into the dihydrate of PFBD on attempted isolation. In contrast, utilization of the hydrochloride salt (**16**)[13] smoothly afforded the covalently hydrated 8-(hydroxyethyl) substituted lumazine (**18**), whose spectral characteristics were similar to those of (**17**). The FAB mass spectrum in particular substantiated the assigned structure and excluded an alternative dehydrated cyclic ether in which the oxygen of the hydroxyethyl side chain is bonded directly to C-7 of the lumazine.

In conclusion, it may be stated that whereas the previously isolated compounds (**9**), (**10**), (**11**), and (**12**) exist in their un-ionized forms as covalent hydrates in order to relieve the steric interactions between the bulky substituents located at C-6 and C-7, the un-ionized forms of the presently reported compounds (**17**) and (**18**) occur as covalent hydrates owing to the electronegativity of the trifluoromethyl groups.

Experimental

All reactions were performed under a nitrogen atmosphere. DMF was removed from the reaction mixtures under reduced pressure at ambient temperature overnight. DMF solutions of perfluorobutane-2,3-dione were stored at 8 °C in the dark prior to use. M.p.s were determined on a Thomas-Hoover Unimelt or on a Wagner and Munz apparatus. ^1H n.m.r. spectra were recorded on Varian FT-80 80 MHz or Varian T-60 60 MHz spectrometers using $(CD_3)_2SO$ or $(CD_3)_2CO$ as the solvent. Chemical shifts are reported in p.p.m. relative to internal Me_4Si. ^{19}F N.m.r. spectra were determined on a Jeol C 60 HL spectrometer operating at 56.45 MHz or on a Perkin-Elmer instrument at 84.6 MHz using $(CD_3)_2SO$ as the solvent. Chemical shifts are reported in p.p.m. relative to external CF_3CO_2H. I.r. spectra were recorded on a Beckman IR-33 or on Perkin-Elmer 157G and 257 spectrometers. Microanalyses were performed by the Purdue and Munich Technical University Microanalytical Laboratories. The electron impact mass spectra (e.i.m.s.) were obtained on either a Finnegan 4000

or a MAT CH-5 spectrometer operating with an ionization potential of 70 eV. Chemical ionization mass spectra (c.i.m.s.) were obtained on a Finnegan 4000 instrument using methane as the reagent gas. Fast atom bombarment mass spectra (f.a.b.m.s.) were run on a Kratos MS50 spectrometer at room temperature using a glycerol matrix. U.v. spectra were determined on a Zeiss PM6 or on a Cary 17 spectrophotometer.

8-Benzyl-6,7-bis(trifluoromethyl)-7,8-dihydropteridine-2,4(1H,3H)-dione (**17**).—A solution of PFBD (**14**) (388 mg, 2 mmol) in DMF (2.35 ml) was added to a stirred suspension of the hydrochloric salt (**15**) (270 mg, 1 mmol) in DMF (2 ml) at room temperature. The reaction mixture was stirred for 7 h and then evaporated. The dark red, semi-solid residue was triturated with water (3 ml) at 90 °C for 5 min. The hygroscopic solid (232 mg, 57%) was filtered and dried over P_2O_5 (0.5 mmHg). The analytical sample was recrystallized by dissolution in a minimum volume of Me_2CO and dilution of the solution with water, m.p. 210—211 °C; $\lambda_{max.}$ (aqueous HCl; pH 1) 340 (ε 6 040) and 267 nm (15 625); $\lambda_{max.}$ (aqueous NaOH; pH 12) 345 (ε 7 500), 275 (10 210), and 250 nm (15 625); $\nu_{max.}$(KBr) 3 700—2 500, 1 710, 1 665, 1 610, 1 560, 1 530, 1 455, 1 390, 1 355, 1 330, 1 190, 1 140, 1 110, 1 085, 1 025, 950, 900, 775, and 710 cm^{-1}; $\delta_H[(CD_3)_2CO]$ 7.30 (s, 5 H) and 5.33 (s, 2 H); $\delta_H[(CD_3)_2SO]$ 7.33 (m, 5 H), 5.30 (d, 1 H, *J* 18 Hz), and 4.92 (d, 1 H, *J* 18 Hz); $\delta_F[(CD_3)_2SO]$ 14.33 (q, 3 F, *J* 6 Hz) and −4.70 (q, 3 F, *J* 6 Hz); *m/z* (e.i.m.s.; relative intensity) 390 (20), 319 (4), 318 (6), 299 (4), 92 (9), and 91 (100); *m/z* (f.a.b.m.s.; relative intensity) 409 [(M^+ + 1), 100] (Found: C, 43.35; H, 2.45; N, 13.7. Calc. for $C_{15}H_{10}F_6N_4O_3 \cdot \frac{1}{2}H_2O$: C, 43.18; H, 2.66; N, 13.43%).

1-Benzyl-6,7-bis(trifluoromethyl)pteridine-2,4(1H,3H)-dione (**22**).—The solid (**17**) (200 mg, 0.48 mmol) was heated *in vacuo* at 210 °C (0.5 mmHg) for 4 min. The residue was dissolved in boiling Me_2CO (3 ml), a small amount of insoluble material was removed by filtration, and the filtrate was diluted with water (3 ml). The solid (68 mg, 36%) was filtered and dried over P_2O_5 overnight. The analytical sample was recrystallized once from aqueous Me_2CO and once from EtOH, m.p. 246—247 °C; $\lambda_{max.}$(MeOH) 335 (ε 5 170) and 255 nm (13 120); $\nu_{max.}$(KBr) 3 700—2 800, 1 740, 1 705, 1 585, 1 495, 1 455, 1 390, 1 370, 1 335, 1 285, 1 265, 1 225, 1 175, 1 095, 1 075, 1 035, 950, 740, and 700 cm^{-1}; $\delta_H[(CD_3)_2CO]$ 7.67—7.23 (m, 5 H), 5.47 (s, 2 H), and 2.80 (br s, 2 H, exchangeable with D_2O); $\delta_F[(CD_3)_2CO]$ 11.70 (q, 3 F, *J* 12 Hz) and 10.33 (q, 3 F, *J* 12 Hz); *m/z* (e.i.m.s.) essentially identical to that of (**17**) above (Found: C, 46.35; H, 2.05; N, 14.3. Calc. for $C_{15}H_8F_6N_4O_2$: C, 46.17; H, 2.07; N, 14.36%).

8-(2-Hydroxyethyl)-6,7-bis(trifluoromethyl)-7,8-dihydropteridine-2,4(1H,3H)-dione (**18**).—A solution of PFBD (194 mg, 1 mmol) in DMF (1.94 ml) was added to a stirred suspension of the hydrochloride salt (**18**) (111 mg, 0.5 mmol) in DMF (2 ml) at room temperature. The reaction mixture was stirred at room temperature for 5 h before it was filtered and the filtrate evaporated. Water (1 ml) was added to the purple, oily residue and the solution was heated at 90 °C for 3 min. The solution was stored at 8 °C overnight before the solid product was filtered off (79 mg, 44%). The analytical sample was recrystallized from water, m.p. 285—286.5 °C (decomp.); $\lambda_{max.}$ (aqueous HCl; pH 1) 340 (ε 6 525), 267 (13 214), and 225 nm (9 950); $\lambda_{max.}$ (aqueous NaOH; pH 13) 345 (ε 7 015), 275 (7 340), and 250 nm (12 400); $\nu_{max.}$(KBr) 3 600—2 700, 1 750, 1 640, 1 610, 1 570, 1 525, 1 465, 1 395, 1 355, 1 330, 1 250, 1 200, 1 150, 1 055, 970, and 925 cm^{-1}; $\delta_H(CF_3CO_2D)$ 4.08 (m, 4 H); δ_F 15.10 (q, 3 F, *J* 7 Hz) and −4.50 (q, 3 F, *J* 7 Hz); *m/z* (e.i.m.s.; relative intensity) 344 (15), 275 (100), 232 (63), 204 (18), 70 (14), and 69 (26); *m/z* (f.a.b.m.s.; relative intensity) 363 [(M^+ + 1), 100] (Found: C, 33.35; H, 2.15; N, 15.35. Calc. for $C_{10}H_8F_6N_4O_4$: C, 33.16; H, 2.23; N, 15.47%).

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