the thiolactone **27**, mp 192–193°. The analytical sample was recrystallized from benzene, mp 194–195°.

Anal. Calcd for $C_{12}H_8OS$: C, 71.97; H, 4.02; S, 16.01. Found: C, 71.93; H, 4.09; S, 15.83.

1,3-Dihydronaphtho[2,3-*c*]thiophen-1-one (24).—A mixture of 0.100 g (1.00 mmol) of 1,3-dihydronaphtho[2,3-*c*]furan-1-thione (**27**) and 1.8 ml of pyridine was heated under nitrogen in a sealed tube at 190° for 8 hr. The reaction mixture was diluted with chloroform, washed with dilute aqueous hydrochloric acid, dried over magnesium sulfate, and evaporated to dryness *in vacuo*. Recrystallization of the residue from benzene gave 0.072 g (72%) of the product, mp 174–175°, obtained in two crops.

Anal. Calcd for $C_{12}H_8OS$: C, 71.97; H, 4.02; S, 16.01. Found: C, 71.85; H, 4.30; S, 15.84.

The product was also obtained when quinoline was used as the solvent but in lesser yield and poorer quality.

Registry No.—**1**, 3533-72-0; **2**, 31739-49-8; **3**, 270-82-6; **4**, 232-81-5; **8**, 13129-12-9; **9**, 13129-13-0; **11**, 31736-38-6; **12**, 31790-98-4; **16**, 13129-15-2; **17**, 13129-16-3; **19**, 28238-02-0; **21**, 31736-40-0; **22**, 31736-41-1; **23**, 31739-52-3; **24**, 31739-53-4; **26**, 4711-50-6; **27**, 31739-55-6.

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The Synthesis, Properties, and Base-Catalyzed Interactions of 8-Substituted 6,7-Dimethyllumazines[1]

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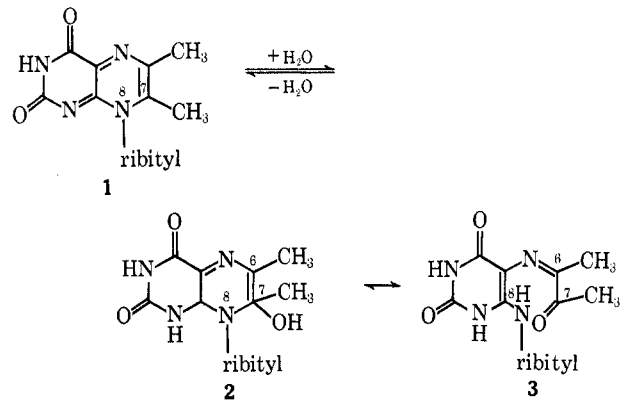
Alkaline solutions of 6,7-dimethyllumazines substituted at position 8 with groups bearing a 2′-hydroxyl group exhibit no long wavelength absorption; analogs without a 2′-hydroxyl group show absorption in the visible range. In H_2O, at alkaline pH, analogs with a 2′-hydroxyl substituent show nmr absorption of the 7-methyl group at −1.37 ppm, while the 6-methyl group exhibits singlets at −2.17 and −2.07 ppm. Analogs lacking the 2′-hydroxyl group do not absorb at −1.37 ppm but exhibit two resonance peaks between −3.90 and −4.30 ppm and a single absorption peak of the 6-methyl group at −2.07 ppm. These data suggest that 8-substituted 6,7-dimethyllumazines which bear a 2′-hydroxyl group form an equilibrium mixture in alkaline solutions containing primarily an intramolecular ether formed between the 2′-hydroxyl group of the side chain at position 8 and carbon 7 of the pyrazine ring (7-methyl group at −1.37 ppm, 6-methyl group at −2.17 ppm) and a minor amount of the 7-exo methylene form (6-methyl group at −2.07 ppm). Without a 2′-hydroxyl on the group at position 8, the intramolecular ether cannot form and the 7-exo methylene form predominates in basic media. The synthesis and properties of eight new 6,7-dimethyllumazine derivatives bearing D- and L-erythrityl, D- and L-threityl, 2′-deoxy-D-ribityl, DL-glycerityl, and 3′-hydroxypropyl substituents at position 8 and their corresponding 4-(1′-alditylamino)-5-nitroso-2,6-dihydroxypyrimidine precursors are reported. The preparation and characterization of the oximes of D- and L-erythrose, D- and L-threose, 2-deoxy-D-ribose, 3-deoxy-D-ribose, and the corresponding amines formed by reduction are described. These syrupy amines are characterized as their crystalline salicylidene derivatives.

The mechanism for conversion of 6,7-dimethyl-8-ribityllumazine to riboflavin chemically[3–7] and enzymically[8,9] has been studied in some detail over the past decade. It was thought originally that the conversion occurred by an aldol condensation involving an α-methyl ketone resulting from hydration and ring opening of the pyrazine ring.[3,5]

More recent work strongly suggests a 7-exo methylene intermediate **7** described below[6–9] rather than the α-methyl ketone **3**. Pfleiderer[10] has interpreted the spectra of alkaline solutions of various lumazines as evidence of hydration and the ring-opening reaction sequence. This report presents nuclear magnetic resonance data substantiating the presence of the 7-exo methylene (**7**) structure and the absence of the open ring (**3**) form in basic solution.



Nuclear magnetic resonance spectra of a number of selected and newly synthesized 6,7-dimethyllumazines substituted at position 8 with various groups indicate that, if the substituent at position 8 bears a 2′-hydroxyl group, an equilibrium mixture results. This is predominantly an intramolecular ether resulting from the base-catalyzed interaction of the 2′-hydroxy group and carbon 7 of the pyrazine ring, with a minor amount of 7-exo methylene form which may result from either the addition of hydroxide ion from the solvent to carbon 7

(1) A preliminary report was presented at the 106th National Meeting of the American Chemical Society, Chicago, Ill., Sept 13–18, 1970. Supported in part by a grant from the National Institute of Arthritis and Metabolic Diseases (AM10501 and AM15404), U. S. Public Health Service.

(2) The data presented in this publication were derived from a Ph.D. Thesis by R. L. Beach submitted to Rutgers University, 1970. Summaries of more detailed results can be obtained from this author upon request.

(3) R. M. Cresswell and H. C. S. Wood, *J. Chem. Soc.*, 4768 (1960).

(4) T. Rowan and H. C. S. Wood, *ibid.*, 21 (1963).

(5) T. Rowan and H. C. S. Wood, *J. Chem. Soc. C*, 452 (1968).

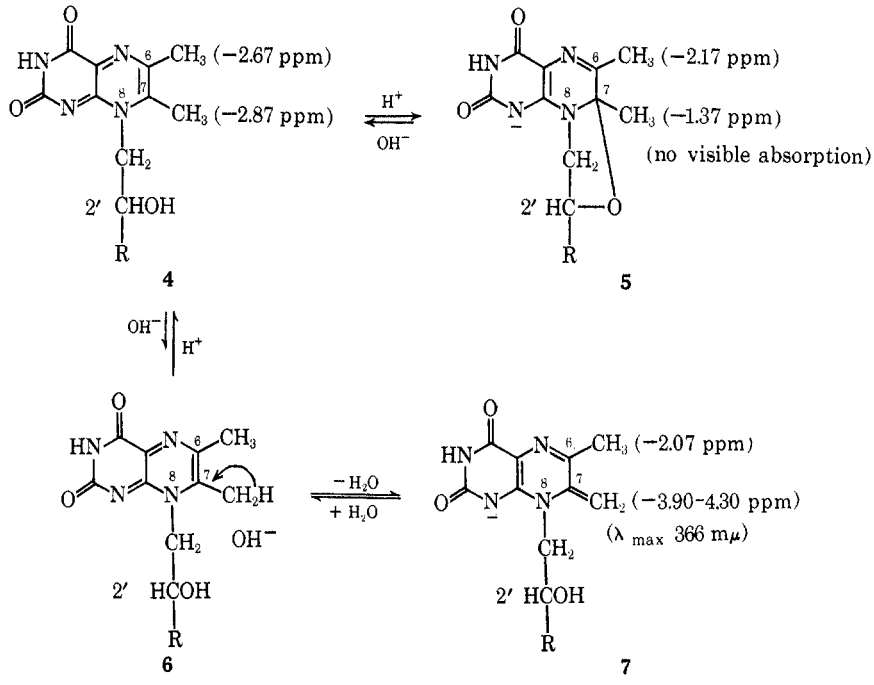
(6) T. Paterson and H. C. S. Wood, *Chem. Commun.*, 290 (1969).

(7) R. L. Beach and G. W. E. Plaut, *Tetrahedron Lett.*, 3489 (1969).

(8) G. W. E. Plaut, R. L. Beach, and T. Aogaichi, *Biochemistry*, **9**, 771 (1970).

(9) R. L. Beach and G. W. E. Plaut, *J. Amer. Chem. Soc.*, **92**, 2913 (1970).

(10) W. Pfleiderer, J. W. Bunting, D. D. Perrin, and G. Nübel, *Ber.*, **99**, 3503 (1966).



of the pyrazine ring (**2**), followed by an elimination reaction, or by direct proton abstraction.

If the substituent at position 8 lacks the 2′-hydroxyl group, the predominant form is that of the 7-exo methylene (**7**) since the intramolecular ether can *only* be formed through interaction of the 2′-hydroxyl group and carbon 7 of the pyrazine ring.

The phenomena outlined above explain the apparent confusion in the literature with respect to the absorption spectra in basic solution of 6,7-dimethyl-8-substituted lumazines. It has been shown that 6,7,8-trimethyllumazine,[11] 6,7-dimethyl-8-(2′-hydroxyethyl)-lumazine,[12] and 6,7-dimethyl-8-ethyllumazine[12] exhibit long wavelength absorption in basic solution, while the 6,7-dimethyllumazines bearing alditol substituents[13] at position 8 do not exhibit long wavelength absorption. The long wavelength absorption can now be attributed to the presence of the 7-exo methylene form (**7**); the absence of long wavelength absorption indicates the intramolecular ether form (**5**) predominant when the alditol substituent bears the 2′-hydroxyl group. A similar lack of long wavelength absorption has been shown by Hemmerich and Wood[14] when various nucleophiles are added to the 7-carbon of the lumazine nucleus.

Experimental Section

Materials.—The following materials were purchased from the suppliers indicated: 2,4-dimethoxy-6-chloropyrimidine, 2-deoxy-D-ribose, 3-amino-1-propanol, 3-amino-1,2-propanediol (Aldrich); 2,3-butanedione (Fisher Scientific); Dowex AG-1 X 10 (minus 400 mesh), Dowex AG-50W X 12 (200–400 mesh) (Bio-Rad); platinum oxide (American Platinum Works of Newark); deuterium oxide (99.7%) (Mallinckrodt); deuterium oxide with 1% DSS[15] (Merck Sharp and Dohme). All other compounds were of reagent quality.

The following compounds were prepared by the methods indicated: **2,4-Dihydroxy-6-chloropyrimidine,**[11] mp 300–302° (lit. 301–302°); **D-erythrose,**[16] $[\alpha]^{25}D$ −25° (lit. −32°); **D-threose,**[16] $[\alpha]^{25}D$ +10° (lit.[17] +12°), mp 24–28° (lit. 24–30°); **L-erythrose,**[17] $[\alpha]^{25}D$ +22° (lit. +39°); **1,3-*O*-benzylidene-L-arabinitol;**[18] **2,4-*O*-benzylidine-L-threose hemihydrate,**[19] mp 115–119° (lit. 119–120°); **L-threose,**[19] $[\alpha]^{25}D$ −11°; **methyl 3-deoxy-β-D-ribofuranoside;**[20] **6,7-dimethyl-8-(1′-D-ribityl)lumazine,**[11] λ_{max} 407 mμ (ε 10,500) [lit. λ_{max} 407 mμ (ε 10,300) in 0.1 *N* H_2SO_4]; **6,7-dimethyl-8-(2′-hydroxyethyl)lumazine,**[21] λ_{max} 407, 225 mμ, λ_{min} 275 mμ (lit. λ_{max} 407, 256 mμ, λ_{min} 270 mμ in 0.1 *N* H_2SO_4); **6,7,8-trimethyllumazine,**[22] λ_{max} 407, 275, 256 mμ (lit. λ_{max} 408, 274, 256 mμ in 0.1 *N* H_2SO_4).

3-Deoxy-D-ribose.—Methyl 3-deoxy-β-D-ribofuranoside (2.4 g) in 60 ml of 1.0 *N* H_2SO_4 was heated on a steam bath for 1 hr. The solution was cooled, neutralized with exchange resin AG-1 X 10 (bicarbonate form), and filtered. The resin was washed with two 25-ml portions of water, the washings were combined with the main solution, and the solvent was removed under reduced pressure at 25°, yielding 2.1 g of 3-deoxy-D-ribose as a pale yellow syrup.

Oximes. D- and L-erythrose, D- and L-threose, 2-deoxy-D-ribose, and **3-deoxy-D-ribose** were converted to their respective oximes by the method of Winestock and Plaut.[13] Only 2-deoxy-D-ribose oxime was obtained crystalline from absolute ethanol, mp 95–96°. *Anal.* Calcd for $C_5H_{11}NO_4$: C, 40.26; H, 7.44. Found: C, 40.36; H, 7.50. The nmr spectra of aldose oximes are reported elsewhere.[2]

Alditylamines.—Various aldose oximes were suspended in glacial acetic acid and reduced at room temperature over PtO_2 in a Parr hydrogenation apparatus at an initial hydrogen pressure of 50 lb/in^2. The amines were purified on columns of AG-50W X 12 as described by Winestock and Plaut.[13] Nmr spectra of individual amines are reported elsewhere.[2]

Salicylidene Derivatives of Alditylamines.—The alditylamines, isolated as syrups, were characterized further as crystalline salicylidene derivatives as follows. Salicylaldehyde (0.5 ml) was added to each alditylamine (2 mmol) dissolved in 50 ml of absolute ethanol. The resulting yellow solution was heated for 30 min at 80° and left overnight at room temperature. The solvent was removed under reduced pressure at 35°, and the yellow residue was triturated with two 5-ml portions of diethyl ether

(11) G. F. Maley and G. W. E. Plaut, *J. Biol. Chem.*, **234**, 641 (1959).
(12) W. Pfleiderer and G. Nübel, *Ber.*, **93**, 1406 (1960).
(13) C. Winestock and G. W. E. Plaut, *J. Org. Chem.*, **26**, 4456 (1961).
(14) P. Hemmerich and H. C. S. Wood, *Proc. Chem. Soc.*, 260 (1961).
(15) DSS, sodium 2,2-dimethyl-2-silapentane-5-sulfonate.
(16) A. S. Perlin and C. Brice, *Can. J. Chem.*, **33**, 1216 (1955).
(17) J. N. Baxter and A. S. Perlin, *ibid.*, **38**, 2217 (1960).
(18) W. T. Haskins, R. M. Hahn, and C. S. Hudson, *J. Amer. Chem. Soc.*, **65**, 1663 (1943).
(19) K. Gätze and T. Reichstein, *Helv. Chim. Acta*, **21**, 195 (1938).
(20) E. Walton, R. F. Nutt, S. R. Jenkins, and F. W. Holly, *J. Amer. Chem. Soc.*, **86**, 2952 (1964).
(21) R. M. Cresswell, T. Neilson, and H. C. S. Wood, *J. Chem. Soc.*, 4776 (1960).
(22) T. Masuda, T. Kiski, M. Asai, and S. Kuwada, *Chem. Pharm. Bull.*, **7**, 366 (1959); **6**, 291 (1958).

TABLE I

LIGHT ABSORPTION SPECTRA OF 6,7-DIMETHYL-8-(1′-ALDITYL)LUMAZINES

8-(1′-Alditol) groups	Registry no.	0.1 N H_2SO_4 λ_{max}	ϵ	λ_{min}	ϵ	0.1 N NaOH λ_{max}	ϵ	λ_{min}	ϵ
D-Threityl	31735-28-1	407	1.01×10^4	300	8.40×10^2	313	9.55×10^3	292	6.55×10^3
		256	1.48×10^4	225	8.06×10^3	279	1.20×10^4	255	7.91×10^3
						277	2.22×10^4		
L-Threityl	31735-29-2	407	1.04×10^4	300	8.62×10^2	313	1.05×10^4	292	7.47×10^3
		256	1.50×10^4	225	8.21×10^3	279	1.27×10^4	255	9.11×10^3
						227	2.38×10^4		
D-Erythrityl	31735-30-5	407	9.99×10^3	300	8.11×10^2	313	6.91×10^3	292	5.08×10^3
		256	1.18×10^4	225	7.72×10^3	279	9.89×10^3	255	6.39×10^3
						227	1.84×10^4		
L-Erythrityl	31735-31-6	407	9.95×10^3	300	4.68×10^2	313	6.66×10^3	292	4.85×10^3
		256	1.37×10^4	225	6.34×10^3	279	9.48×10^3	255	5.69×10^3
						227	1.72×10^4		
2′-Deoxy-D-ribityl	31735-32-7	407	1.05×10^4	300	2.24×10^3	366	3.48×10^3	290	7.78×10^3
		256	1.24×10^4	225	6.66×10^3	313	1.63×10^4	262	7.09×10^3
						282	8.33×10^3		
						235	1.77×10^4		
3′-Deoxy-D-ribityl	31735-33-8	407	1.07×10^4	300	8.81×10^2	313	9.63×10^3	292	7.41×10^3
		256	1.45×10^4	225	8.13×10^3	280	1.21×10^4	255	8.89×10^3
						228	2.25×10^4		
3′-Hydroxypropyl	31735-34-9	407	1.07×10^4	300	3.72×10^2	365	4.76×10^3	335	4.40×10^3
		275	9.80×10^3	270	9.53×10^3	313	1.90×10^4	275	6.67×10^3
		256	1.34×10^4	225	6.70×10^3	265	6.74×10^3	262	6.63×10^3
						235	1.50×10^4		
DL-Glyceryl	31790-90-6	407	1.06×10^4	300	3.43×10^2	313	1.00×10^4	292	7.11×10^3
		256	1.39×10^4	225	6.54×10^3	279	1.12×10^4	255	7.41×10^3
						227	2.01×10^4		

leaving a thick yellow syrup. The residue was dissolved in 10 ml of hot absolute ethanol and hexane was added until the solution became cloudy. The solution was allowed to cool to room temperature, yielding a yellow crystalline solid which may be recrystallized from benzene. The melting points of the derivatives were as follows: D-threitylamine, 74–76°; D-erythritylamine, 84–86°; L-erythritylamine, 86–87°; 2-deoxy-D-ribitylamine, 76–77°; D-ribitylamine, 121–123°. The derivatives had the following absorption characteristics: in 0.1 *N* HCl, maxima at 274–279 and 345 mμ and minima at 238–240 and 303 mμ; in 0.1 *N* NaOH, maxima were at 223–227, 263–265, 375 mμ, and minima at 248–250 and 295 mμ. Molar absorbancies at these wavelengths have been recorded.[2]

4-(1′-Alditylamino)-5-nitroso-2,6-dihydroxypyrimidine.—These compounds were prepared according to the method of Plaut.[23] The absorption properties of these compounds are similar to those of analogous substances reported previously;[13] yields, analytical data, and decomposition points are recorded elsewhere.[2]

6,7-Dimethyl-8-(1′-alditol)lumazines.—Reduction of specific 4-(1′-alditylamino)-5-nitroso-2,6-dihydroxypyrimidines with sodium hydrosulfite and condensation of the resulting diamine with 2,3-butanedione according to the procedure of Winestock and Plaut[13] yielded the corresponding 6,7-dimethyllumazines selectively substituted with various alditol groups at position 8.

The light absorption spectra of the compounds are summarized in Table I and Figures 1 and 2. Nmr spectra in neutral solutions were similar to analogous compounds described previously.[9] Details of nmr spectra, decomposition points, yields, and analytical data have been recorded elsewhere.[2]

Methods.—Melting or decomposition points were determined in a Thomas-Hoover capillary melting point apparatus and are uncorrected. Ultraviolet and visible spectra were recorded in a Cary Model No. 14 spectrophotometer. Microanalysis was done by Mr. George I. Robertson of Florham Park, N. J. Nuclear magnetic resonance spectra were measured in a Varian A-60A spectrometer using 1.5% solutions of each compound in D_2O (99.7%) containing 0.25% DSS as an internal standard absorbing at 0.00 ppm. All chemical shifts are reported in parts per million (ppm) shifted downfield from the internal standard assigned a chemical shift of 0.00 ppm.

Nmr Spectra (Figures 3 and 5).—The following conditions were used to determine the spectra shown. Figure 5: (a) A solution of each compound (7.5 mg) in 0.45 ml of D_2O, (b) was made alkaline with 0.05 ml of 1.0 *M* NaOD, (c) followed by neutralization with 0.05 ml of 1.0 *M* DCl. The conditions described in Figure 3 (A, B, and C) were identical with those under Figure 5 (a–c) except that the solvent was H_2O. An internal standard of 0.25% DSS was present in all solutions.

(23) G. W. E. Plaut, *J. Biol. Chem.*, **238**, 2225 (1963).

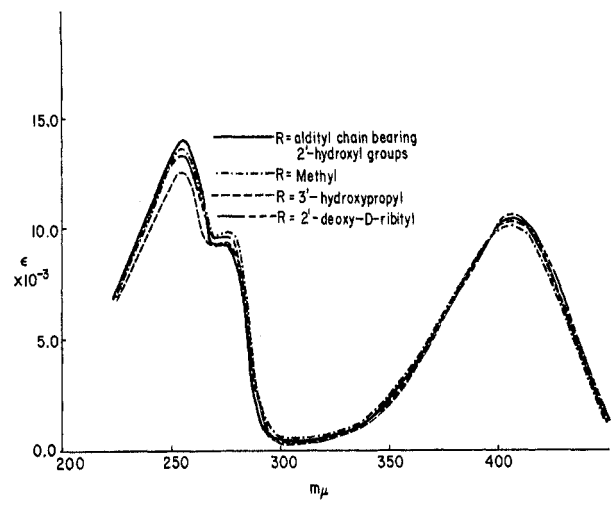


Figure 1.—Absorption spectra in 0.1 *N* H_2SO_4 of 6,7-dimethyllumazines with varying substituents at position 8.

Results and Discussion

Spectroscopy.—Winestock and Plaut[13] have noted that a number of 6,7-dialkyl-8-substituted lumazines show very similar ultraviolet and visible light absorption in *acid media*, typically exhibiting maxima at 407 and 256 mμ and minima at 300 and 225 mμ. Similar characteristics of light absorption have been obtained with the newly synthesized lumazines (Table I and Figure 1).

It has been observed previously[10,11,13,21] that in alkaline media a number of 6,7-alkyl-8-substituted

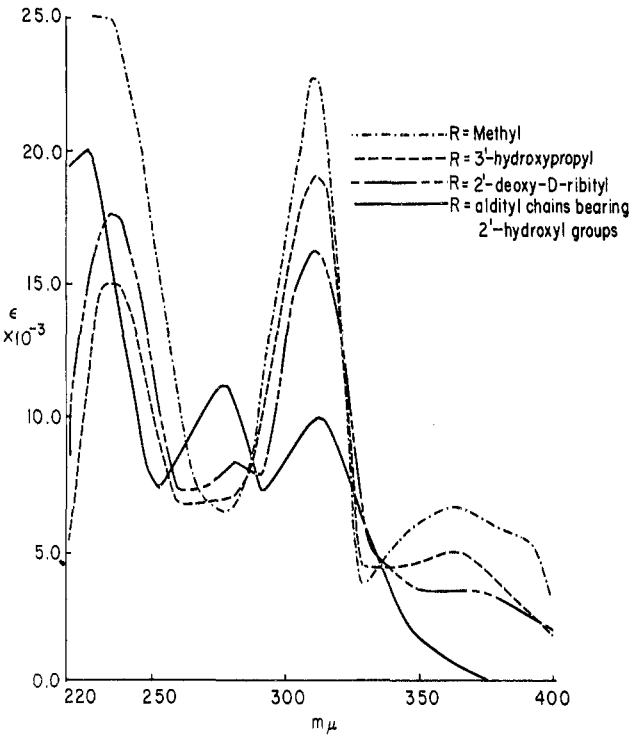


Figure 2.—Absorption spectra in 0.1 *N* NaOH of 6,7-dimethyllumazines with varying substituents at position 8.

lumazines (*e.g.*, compounds bearing tetrahydroxypentyl and pentahydroxyhexyl groups at position 8) and the new compounds (Table I) excepting 6,7-dimethyl-8-[1′-(2′-deoxy-D-ribityl)]lumazine and 6,7-dimethyl-[1′-(3′-hydroxypropyl)]lumazine exhibit no absorption in the visible region but do absorb in the ultraviolet region with maxima at 313, 279, and 227 mμ (Figure 2). Under alkaline conditions a number of other lumazine derivatives (*e.g.*, 6,7,8-trimethyllumazine,[13] 6,7-dimethyl-8-(2′-hydroxyethyl)lumazine,[12] and the new compounds, 6,7-dimethyl-8-[1′-(2′-deoxy-D-ribityl)]lumazine and 6,7-dimethyl-8-[1′-(3′-hydroxypropyl)]lumazine, Table I and Figure 2) retain absorption in the visible range (360–400 mμ) with a maximum in the vicinity of 366 mμ. The only structural difference between compounds with visible absorption in alkaline solution and those without is the absence and presence, respectively, of a 2′-hydroxyl group on the substituent at position 8. 6,7-Dimethyl-8-[1′-(2′-hydroxyethyl)]lumazine deviates from this pattern, and an explanation is offered later for its apparent aberrant spectral properties.

Loss of absorption in the visible range of the spectrum was demonstrated by Hemmerich and Wood[14] when nucleophiles were added at position 7 of lumazine derivatives. Hydroxylation at carbon 7 similarly results in the loss of absorption in the visible range of the spectrum.[10] The absorption at long wavelength of 6,7,8-trimethyllumazine and 6,7-dimethyl-8-(2′-hydroxyethyl)lumazine in 0.1 *N* NaOH[10,11,13] (Figure 2) disappears upon reduction leading to formation of 1,7-dihydro-6,7,8-trimethyllumazine[24] and 7,8-dihydro-6,7-dimethyl-8-(2′-hydroxyethyl)lumazine.[14] This suggests that covalent bond formation between carbon 7 and another group, be it a nucleophile or hydrogen, causes loss of visible absorption.

(24) J. M. McAndless and R. Stewart, *Can. J. Chem.*, **48**, 263 (1970).

In basic solution the 2′-hydroxyl group, therefore, appears to act as the preferred nucleophile and covalently bonds with carbon 7 of the pteridine ring, forming an intramolecular ether **5** and resulting in abolition of visible absorption. With analogs lacking a 2′-hydroxyl group, hydroxide ion from solution may add to carbon 7 of the pteridine ring, as suggested by Cresswell and Wood,[3] Rowan and Wood,[4,5] and Pfleiderer,[10] and depicted as structure **2**. If this intermediate is present, it must be in low concentration since its formation would also result in the abolition of visible absorption. Since visible absorption is observed, an alternate mechanism (**4**–**7**) involving the direct abstraction of proton from the 7-methyl group by solvent hydroxide ion (**6**) is suggested, leading directly to the 7-exo methylene intermediate **7** which absorbs in the visible region of the spectrum. It will be shown that the nmr data in Figures 3–5 are consistent with a direct elimination rather than hydration followed by elimination.

Nuclear Magnetic Resonance Spectroscopy. Analog with a 2′-Hydroxyl Group at the Substituent at Position 8.—6,7-Dimethyllumazines which are substituted at position 8 with groups bearing a 2′-hydroxyl group (Figure 3, I and II) as well as the D-ribityl,[25] D- and L-erythrityl, and D- and L-threityl analogs (spectra not included since they are virtually indistinguishable from those in Figures 3, I and II) exhibit significant absorption at −1.37 ppm in basic H_2O. The loss of long wavelength (visible) absorption in 0.1 *N* NaOH is attributable to covalent bond formation between a nucleophilic group and carbon 7. It appears that the chemical shift to −1.37 ppm for the 7-methyl group similarly arises because of the change in the electronic environment in the vicinity of the 7-methyl group caused by the formation of a covalent bond at carbon 7.

The chemical shifts of 6,7-dimethyl-8-ribityllumazine and 6-deuteriomethyl-7-methyl-8-ribityllumazine in neutral and basic solution have been previously reported by the authors.[25] The assignments of the chemical shifts were based upon the fact that the 7-methyl group exhibits hydrogen–deuterium exchange, slowly in neutral D_2O and very rapidly in basic D_2O. Furthermore, absorption in the vicinity of −2.10 ppm in alkaline solution is due to the 6-methyl group, since absorption in this region is observed with 6,7-dimethyl-8-ribityllumazine but not with 6-deuteriomethyl-7-methyl-8-ribityllumazine.[25] The chemical shift assignments and the exchange phenomena are in accord with those found concurrently by Paterson and Wood[6] and later by McAndless and Stewart.[25] The chemical shift of −1.37 ppm is assigned to the 7-methyl group of the intramolecular ether form **5** in which the 2′-hydroxyl group is acting as the nucleophile covalently bound to carbon 7 of the pteridine ring. The compounds [V (276) and V (344)] below reported in the Varian NMR Spectra Catalog[26] exhibit methyl groups in a similar electronic environment and exhibit equivalent chemical shifts.

Further proof that the absorption at −1.37 ppm is properly assigned to the 7-methyl group is obtained by neutralizing the basic H_2O solutions with 1 equiv of

(25) R. L. Beach and G. W. E. Plaut, *Biochemistry*, **9**, 760 (1970).

(26) "NMR Spectra Catalog," Varian Associates, Vol. 1 and 2, 1962–1963.

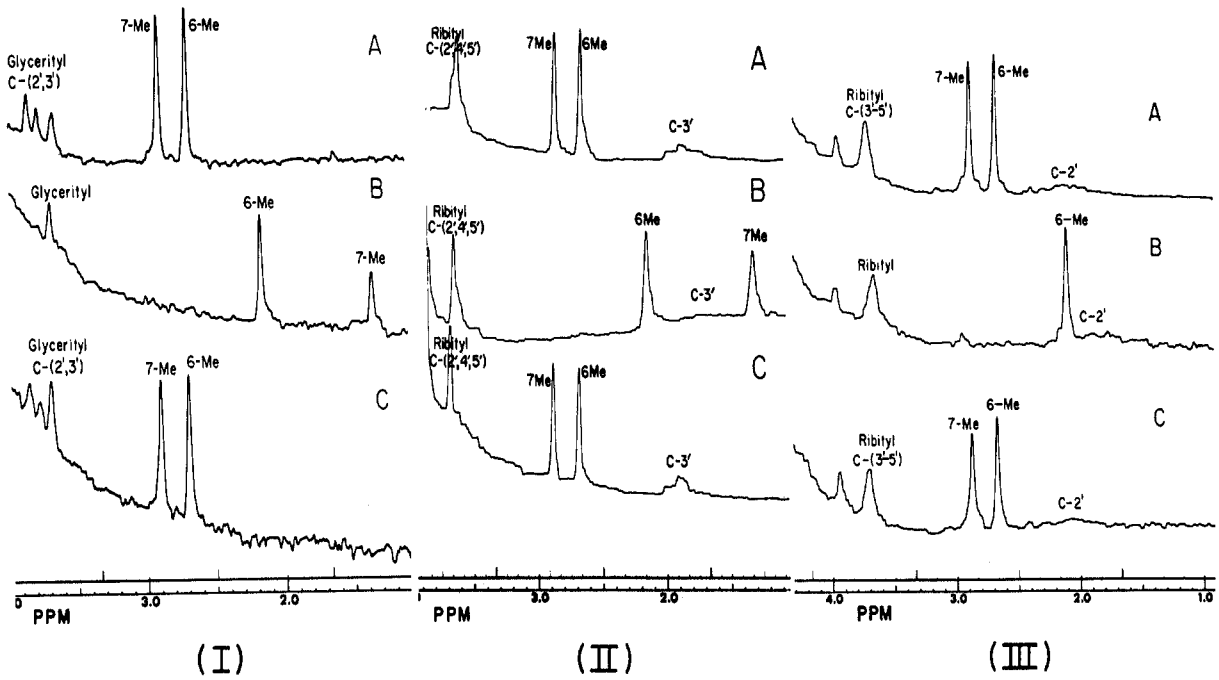
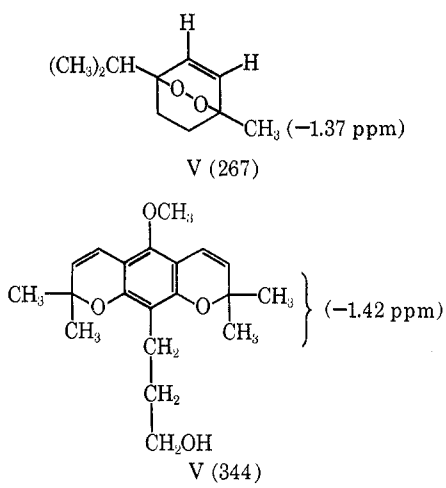


Figure 3.—Nmr spectra of 6,7-dimethyl-8-[1′-(DL-glyceryl)]lumazine (I), 6,7-dimethyl-8-[1′-(3-deoxy-D-ribityl)]lumazine (II), and 6,7-dimethyl-8-[1′-(2′-deoxy-D-ribityl)]lumazine (III) in H_2O: A, compounds in water; B, in 0.1 *N* NaOH; C, B brought to neutrality with 0.1 *N* HCl. The spectra of I and II were recorded at 37°; III was determined at 7°. Other conditions are described under Methods.



HCl (Figure 3C, I and II). The original spectra of the lumazine analogs in neutral solution (*cf.* Figure 3A, I and II) reappear and absorption at −2.87 ppm (7-methyl group[25]) is present. When observations of nmr spectra of 6,7-dimethyllumazines containing the 2′-hydroxyl group (*e.g.*, as shown in Figure 3, I and II) were done in D_2O under conditions where hydrogen–deuterium exchange equilibrium occurred, absorption peaks attributable to the 7-methyl group disappeared.[2,25]

The fact that the 7-methyl group is capable of rapid hydrogen–deuterium exchange in basic D_2O suggests that there is an equilibrium between the intramolecular ether **5** and the 7-exo methylene forms **7**. If the molecules were totally in the intramolecular ether form **5**, hydrogen–deuterium exchange could not occur. Two observations support the occurrence of these two forms, **5** and **7**, in alkaline solution. First, the absorption intensity at −1.37 ppm (7-methyl group of the ether form) is only about 75% of that in the vicinity of −2.10 ppm (6-methyl group)[25] indicating that only 75% of the molecules are in the ether form. Secondly, the 6-methyl group should exhibit two different chemical shifts since the electronic environment of the 6-methyl group in the ether form **5** is different from that in the 7-exo methylene form **7**.

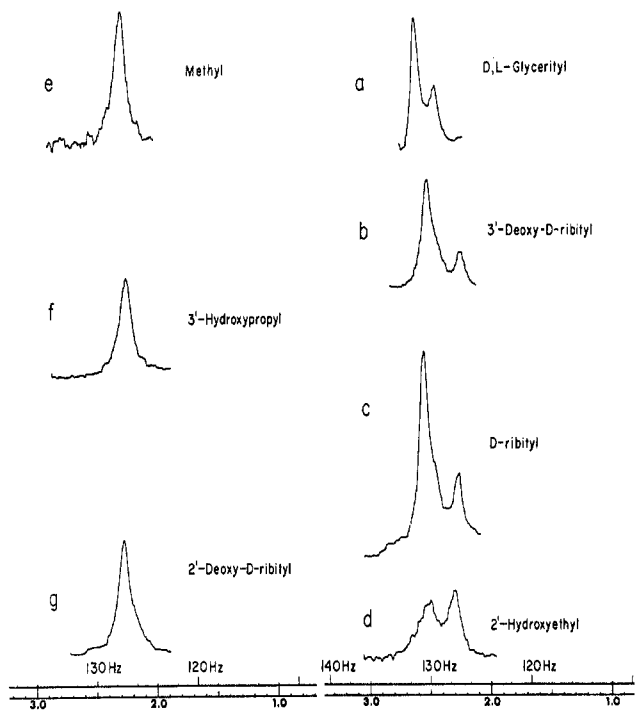
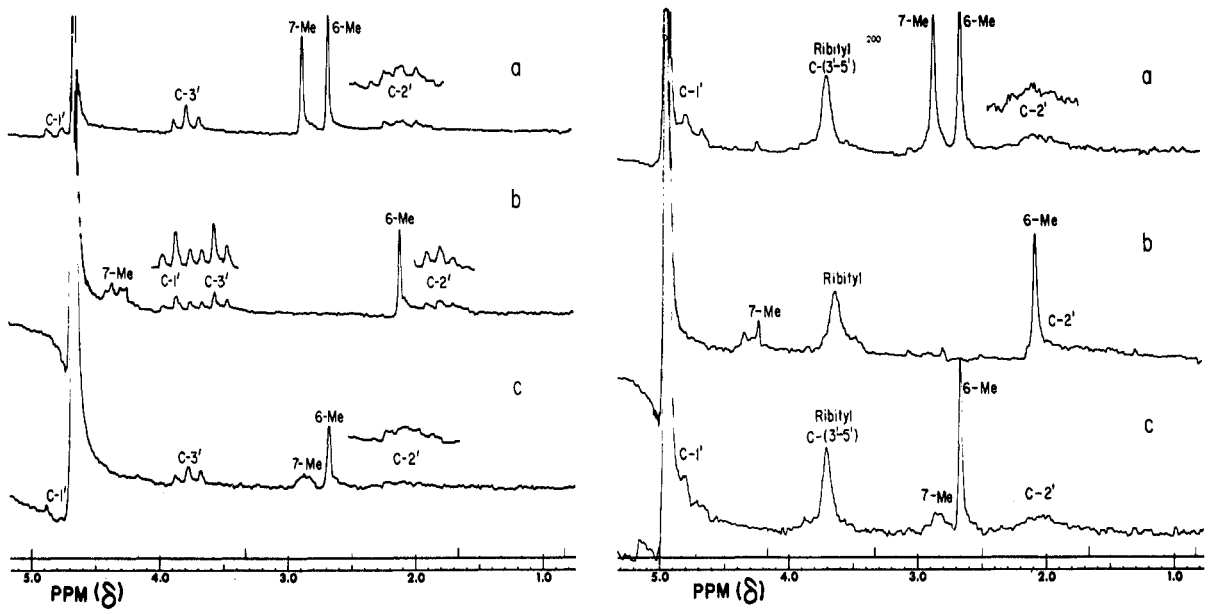


Figure 4.—Nmr spectra of the 6-methyl group of various 6,7-dimethyl-8-substituted lumazines in 0.1 *N* NaOH at 37°.

Scale expansion (Figure 4a–d) shows that the absorption at −2.10 ppm is composed of two singlets, one at −2.17 ppm and the other at −2.07 ppm. The intensities of absorption at −1.37 ppm (7-methyl



(I) (II)

Figure 5.—Nmr spectra of 6,7-dimethyl-8-substituted lumazines lacking a 2′-hydroxyl group: I, 6,7-dimethyl-8-[1′-(3′-hydroxypropyl)]lumazine, and II, 6,7-dimethyl-8-[1′-(2′-deoxy-D-ribityl)]lumazine. All readings were taken in D_2O at 7°. Compounds were dissolved in (a) D_2O, (b) adjusted to 0.1 *N* NaOD, and (c) neutralized with DCl as described under Methods.

group) and at −2.17 ppm (6-methyl group) are about equal (Figure 3B, I and II). Consequently, the peaks at −2.17 and −2.07 ppm are assigned to the 6-methyl groups of the intramolecular ether **5** and the 7-exo methylene forms **7**, respectively.

Analogs Lacking the 2′-Hydroxyl Group.—Compounds exhibit differences in their visible absorption spectra (Figure 2) as well as their nmr spectra, depending upon the presence (Figure 3B, I and II) or absence (Figure 3B, III and Figure 5b) of a 2′-hydroxyl group. The substances without the 2′-hydroxyl group do not absorb at −1.37 ppm, but exhibit peaks in the vicinity of −3.90 to −4.30 ppm (Figure 5b).

The absence of absorption at −1.37 ppm suggests that the intramolecular ether does not exist and consequently *can only be formed between the 2′-hydroxyl group and carbon 7 of the pteridine ring.* This point is best illustrated by the 3′-hydroxypropyl analog (Figure 5, I) and the 2′-deoxy-D-ribityl derivative (Figure 3, III, and Figure 5, II) which lack the 2′-hydroxyl group but have hydroxyl groups elsewhere on the 8 substituent. These hydroxyl groups (including OH at the 3′ position), however, appear incapable of interacting with the pteridine ring to form the intramolecular ether. Consequently, the long wavelength absorption is retained; lacking covalent bonds at carbon 7, the absorption at −1.37 ppm is not observed.

The lack of any high-field absorption (*e.g.*, Figure 3B, III) further suggests that a hydrated intermediate such as structure **2** is absent or present in very low concentration, since the 7-methyl group of the molecule would be expected to absorb in the vicinity of −1.20 to −1.50 ppm characteristic of other methyl carbinols. The absence of this absorption peak suggests that the 7-exo methylene intermediate may be formed by a direct elimination reaction (**4**, **6**, **7**) rather than following an initial hydration reaction (**1** to **2**).

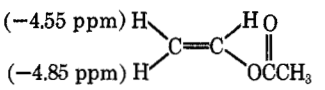
In H_2O the absorption by OH masks absorptions in the range of −3.80 to −4.60 ppm. This problem can be alleviated by the use of D_2O as the solvent. However, under such conditions it becomes necessary to slow the rate of hydrogen–deuterium exchange at the 7-methyl group. The spectra of the 3′-hydroxypropyl (Figure 5, I) and the 2′-deoxyribityl derivatives (Figure 5, II) were, therefore, recorded at 7° resulting in retention of sufficient protium at the 7-exo methylene group to show absorption in the vicinity of −3.90 and −4.30 ppm (Figure 5b). The 7-exo methylene group of 6,7,8-trimethyllumazine at 7° also exhibits absorption in this range,[2] shifted downfield from the −3.65-ppm value previously reported[25] at 37°.

Analogs lacking the 2′-hydroxyl group (Figure 5b) all exhibit two singlets in the vicinity of −3.90 to −4.30 ppm, attributed to the two nonequivalent hydrogens of the 7-exo methylene group of the molecule. The 7-exo methylene group should be coplanar with the pteridine ring resulting in a cis and trans orientation of the hydrogens of the group with respect to the pteridine ring. These hydrogens should therefore be nonequivalent and exhibit different chemical shifts, as was observed. The chemical shift of the 7-exo methylene group of the lumazine derivatives compares favorably with that of a number of methylene analogs [V (65), V (111), and V (596)] reported in the Varian Catalog[26] and 5-*H*-tetrahydrofavin cation.[27]

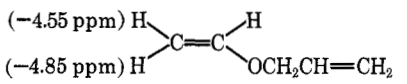
Confirmation that the two peaks in the vicinity of −3.90 to −4.30 ppm (Figure 5b) arise from the 7-methyl group is obtained upon neutra zation of the basic solution with 1.0 *N* DCl (Figure 5c). Residual absorption at −2.85 ppm due to the partially exchanged 7-methyl group (*cf.* Figure 5a) is observed.

The 6-methyl group, upon scale expansion (Figure 4e–g), appears as a single peak at −2.07 ppm. The

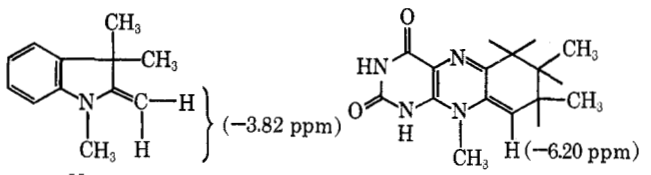
(27) (a) C. Heizmann, P. Hemmerich, R. Mengel, and W. Pfleiderer in "Chemistry and Biology of Pteridines," K. Iwai, M. Akino, M. Goto, and Y. Iwanami, Ed., International Academic Printing Co., Tokyo, Japan, 1970, p 105 (b) We wish to thank Dr. P. Hemmerich, Universität Konstanz (Konstanz, Germany), for communicating this information to us before publication.



V (65)



V (III)



V (596) 27a, b

peak at −2.17 ppm assigned to the 6-methyl group of the intramolecular ether (Figure 4a–d) is absent.

Absorption at −3.90 to −4.30 ppm does not agree with the proposed α-methyl ketone **3** which would arise by ring opening,[2,4,5,10] since methyl ketones typically exhibit chemical shifts between −2.00 and −2.40 ppm but never downfield in the range of −4.00 ppm. Furthermore, the monooxime of 2,3-butanedione, a model compound to which the hypothetical α-methyl ketone **3** could be compared, exhibits an enhanced rate of hydrogen–deuterium exchange in alkaline solution, but absorption at −2.45 ppm disappears in less than 1 hr with only minor changes in its chemical shift (no change for the methyl α to the carbonyl and a shift to −1.82 ppm for the methyl β to the carbonyl). With the pteridine analogs, shifts from −2.85 ppm (**4**) to −3.90 to −4.30 ppm (**7**) were obtained.

Preliminary rate studies with the 3′-hydroxypropyl analog indicate that the first hydrogen of the 7-methyl group is removed instantly; the remaining two hydrogens exchange at a rapid but calculable rate. When 6,7-dimethyl-8-[1′-(3′-hydroxypropyl)]lumazine was dissolved in D_2O, made basic with NaOD, and immediately neutralized with DCl, only 1.7 of the 3 original hydrogens remained unexchanged. These data indicate that the first hydrogen is removed during the elimination reaction while the remaining hydrogens are exchanged with deuterium at a relatively slow rate, further suggesting that a hydrated intermediate **2** is not involved.

Occurrence of 7-Exo Methylene in 2′-Hydroxyl Group Bearing Compounds.—The position of equilibrium between the inner ether **5** and the 7-exo methylene **7** forms of compounds containing a 2′-hydroxyl group is difficult to establish by measurement at −3.90 to −4.30 ppm because of the technical restrictions imposed by OH absorption when H_2O is the solvent or by the hydrogen–deuterium exchange at the 7-methyl group which occurs when the determinations are made in D_2O. A more promising approach in determining the abundance of the 7-exo methylene form is by measuring the areas under the peaks of the absorption of the 6-methyl group at −2.07 and −2.17 ppm. In all of the compounds tested in 0.1 *N* NaOH, except one, less than 25% of the equilibrium mixture is due to the 7-exo methylene form (*e.g.*, Figure 4a–c). Although present in minor amounts, it should be emphasized that evidence for the existence of the 7-exo methylene group *has* been adduced from measurements at −2.10 to −2.20 ppm (Figure 4a–d) and at −3.90 to −4.30 ppm.[2] These results are, therefore, in accord with an equilibrium between compounds **7**, **4**, and **5**, indicated by the hydrogen–deuterium exchange which occurs with all of these forms of 2′-hydroxyl-substituted 6,7-dimethyllumazines.[2,25]

6,7-Dimethyl-8-[1′-(2′-hydroxyethyl)]lumazine is the only analog bearing a 2′-hydroxyl group which retains substantial visible absorption in alkaline solution (Figure 2). It seemed possible that with this compound the exo methylene form **7** is in higher concentration than the intramolecular ether form **5** in the equilibrium mixture. This appears to be confirmed by the nmr spectrum which shows in 0.1 *N* NaOH a relatively minor absorption at −1.37 ppm, *i.e.*, one-fourth that of the absorption between −2.20 and −2.10 ppm (6-methyl group).[2] Furthermore, scale expansion of the 6-methyl group (Figure 4d) exhibits two singlets; the singlet at −2.07 ppm (6-methyl group of the 7-exo methylene form **7**) is considerably larger than the absorption at −2.17 ppm (6-methyl group of the intramolecular ether form).

The shift in equilibrium, favoring the 7-exo methylene form and visible absorption, probably arises since the 2′-hydroxyl group of 6,7-dimethyl-8-[1′-(2′-hydroxyethyl)]lumazine is not confined to a rigid conformation as are the 2′-hydroxy groups of the higher homologs. Consequently, removal of a proton from the 7-methyl group by hydroxide ion can result in sufficient electrostatic repulsion to force the relatively unhindered 2′-hydroxyl group of the 2′-hydroxyethyl analog away from the 7-carbon of the pteridine ring as the electron pair is accommodated establishing a π bond with carbon 7. Steric and electrostatic effects of the neighboring hydroxyl groups of the higher homologs resist this electrostatic repulsion and thus remain primarily in the intramolecular ether form.

Registry No.—2-Deoxy-D-ribose oxime, 31735-35-0; D-threitylamine, salicylidine derivative, 31735-36-1; D-erythritylamine, salicylidine derivative, 31735-37-2; L-erythritylamine, salicylidine derivative, 31735-38-3; 2-deoxy-D-ribitylamine, salicylidine derivative, 31735-39-4; D-ribitylamine, salicylidine derivative, 31735-40-7.

Acknowledgment.—We wish to thank Dr. F. W. Holly of Merck and Co. for a gift of methyl 2,3-dehydro-D-ribofuranoside. We thank Dr. Peter Hemmerich for extensive correspondence and exchange of information dealing with nmr spectra of various lumazine derivatives.