[CONTRIBUTION FROM THE LABORATORY FOR THE STUDY OF HEREDITARY AND METABOLIC DISORDERS AND THE DEPARTMENTS OF BIOLOGICAL CHEMISTRY AND MEDICINE, UNIVERSITY OF UTAH]

Synthesis and Properties of Certain Substituted Lumazines[1,2]

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The syntheses and properties of five new 6,7-dimethyl-8-(1′-alditol)lumazines, eight 8-(1′-D-ribityl)lumazines containing various substituents at positions 6 and 7, two substituted 8-methyllumazines, 6,7-diethyl-8-(β-hydroxyethyl)lumazine, and 5,6,7,8-tetrahydro-9-(1′-D-ribityl)isoalloxazine are described. The effect of the variation of the substituents at positions 6, 7, and 8 on the light absorption spectra in acidic and basic solution is shown. The corresponding 5-nitroso-2,6-dihydroxypyrimidines bearing an appropriately substituted amino group at position 4 served as intermediates in the preparation of these lumazine derivatives. In contrast to 6,7-dimethyl-8-(1′-D-ribityl)lumazine, none of the lumazine derivatives prepared could be converted enzymatically to flavines.

Reports from several laboratories have shown that 6,7-dimethyl-8-(1′-D-ribityl)lumazine can be converted to riboflavine by enzyme preparations from a number of microorganisms.[4-7] The changes occurring in the transformation of the lumazine to the flavine involve the heterocyclic ring system[8] and not the ribityl group. Hence, it became of interest to know how this enzyme activity would be affected by changes in substituents at position 8 of 6,7-dimethyllumazines. A number of such compounds have been tested, and it has been found that 6,7-dimethyllumazine and 6,7,8-trimethyllumazine were not converted to the corresponding flavine derivatives by extracts from several microorganisms[9] or by purified enzyme preparations from *E. coli* and *Ashbya gossypii*.[10] However, it has been reported that the transformation of 6,7-dimethyl-8-(β-hydroxyethyl)lumazine to 6,7-dimethyl-9-(β-hydroxyethyl)isoalloxazine was carried out by intact cells of *Clostridium acetobutylicum* and by cell-free extracts of *Aerobacter aerogenes* and *Eremothecium ashbyii*[11]; although the rate of the enzymic conversion was considerably slower than that of the ribityllumazine compound to riboflavine.

The side chains at position 8 of the compounds above differ markedly from that of 6,7-dimethyl-8-(1′-D-ribityl)lumazine. It became desirable to know whether the riboflavine synthetase could distinguish between 6,7-dimethyl-8-(1′-D-ribityl)lumazine and compounds with only minor modifications of the ribityl moiety. Therefore, a number of analogs of 6,7-dimethyl-8-(1′-D-ribityl)lumazine were prepared differing only in the configuration of the hydroxyl groups of the pentyl side chain or by substitution of a hexityl for the ribityl group at position 8 of the lumazine.

6-Methyl-7-hydroxy-8-(1′-D-ribityl)lumazine has been isolated from *A. gossypii*[12] and *E. ashbyii*[13] in addition to 6,7-dimethyl-8-(1′-D-ribityl)lumazine, but attempts to convert it to riboflavine with enzyme preparations from *A. gossypii* have yielded negative results.[12] It was, however, of interest to explore further the specificity of riboflavine synthetase with lumazine derivatives bearing substituents at 6 and 7, more closely related to the two methyl groups in the active compound. A series of five 8-(1′-D-ribityl)lumazines was prepared in which one of the substituents was a methyl group and the other varied in size from ethyl through *n*-propyl, *n*-butyl, *n*-pentyl, and phenyl. The 6,7-diethyl, di-*n*-propyl, and diphenyl derivatives and 5,6,7,8-tetrahydro-9-(1′-D-ribityl)isoalloxazine were also synthesized. In view of the reported enzymic conversion of 6,7-dimethyl-8-(β-hydroxyethyl)lumazine to 6,7-dimethyl-9-(β-hydroxyethyl)isoalloxazine,[11] we prepared the diethyl analog. Completing the list of compounds employed in this study are the 6,7-diethyl-, di-*i*-propyl-, and diphenyl-8-methyllumazines.

The procedure for the synthesis of 6,7-dimethyl-8-(1′-D-ribityl)lumazine[14] was adapted for use in the preparation of the 8-(1′-alditol)lumazine derivatives. The 4-(1′-alditylamino)-2,6-dihydroxypyrimidines (II) were prepared by condensation of the appropriate glycamine with 4-chloro-2,6-dihydroxypyrimidine (I). The glycamine was obtained by catalytic hydrogenation of the sugar oxime.

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(2) We wish to thank Mr. LaVell R. Johnson for his assistance in the preparation of a number of 6,7-dimethyl-8-(1′-alditol)lumazines.

(3) Senior Research Fellow (SF261) United States Public Health Service.

(4) S. Kuwada, T. Masuda, T. Kishi, and M. Asai, *Chem. & Pharm. Bull. (Tokyo)*, **6**, 618 (1958).

(5) H. Katagiri, I. Takeda, and K. Imai, *J. Vitaminol. (Osaka)*, **4**, 211 (1958).

(6) F. Korte and H. V. Aldag, *Ann.*, **628**, 144 (1959).

(7) G. F. Maley and G. W. E. Plaut, *J. Am. Chem. Soc.*, **81**, 2025 (1959).

(8) G. W. E. Plaut, *J. Biol. Chem.*, **235**, PC 41 (1960).

(9) H. Katagiri, I. Takeda, and K. Imai, *J. Vitaminol. (Osaka)*, **4**, 278 (1958).

(10) G. W. E. Plaut, unpublished observations.

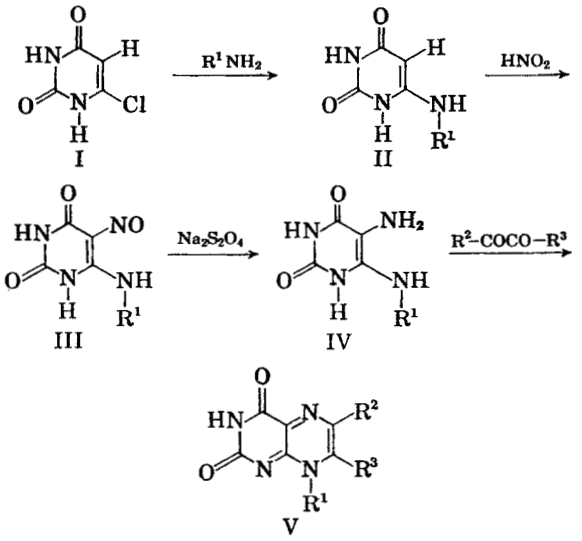
(11) H. Katagiri, I. Takeda, and K. Imai, *J. Vitaminol. (Osaka)*, **5**, 81 (1959).

(12) G. W. E. Plaut and G. F. Maley, *J. Biol. Chem.*, **234**, 3010 (1959).

(13) T. Masuda, *Pharm. Bull. Tokyo*, **4**, 72 (1956).

(14) G. F. Maley and G. W. E. Plaut, *J. Biol. Chem.*, **234**, 641 (1959).

The amine was purified by adsorption on a column of a cationic exchange resin, Dowex 50 W (hydrogen form), followed by displacement with dilute ammonium hydroxide. The use of a cationic instead of an anionic exchange resin[14] produced a cleaner amine and was more convenient, since it could be converted to the hydrogen form more easily than the anionic exchanger (Dowex 1) in the hydroxyl form. After condensation of the glycamine and the 4-chloropyrimidine (I), the 4-(1′-alditylamino)pyrimidine (II) was nitrosated without prior isolation from the reaction mixture. The resulting 4-(1′-alditylamino)-5-nitroso-2,6-dihydroxypyrimidine



(III) was separated from other components in the aqueous solution by ion exchange chromatography.[14] 4-(1′-Alditylamino)-5-nitroso-2,6-dihydroxypyrimidine was reduced to 4-(1′-alditylamino)-5-amino-2,6-dihydroxypyrimidine (IV) with sodium hydrosulfite and condensed immediately with the appropriate α-diketone to form an 8-(1′-alditylumazine (V). The lumazine derivative was purified by chromatography on a column of acid-washed alumina, followed by a benzyl alcohol extraction step and crystallization from a suitable solvent. Inclusion of the chromatographic step in the purification procedure eliminated organic impurities that could not be removed by recrystallization and markedly reduced the formation of emulsions in the extraction of the lumazines into benzyl alcohol.

The absorption spectra of 4-(1′-D-arabitylamino)-5-nitroso-2,6-dihydroxypyrimidine at three *p*H values, representative of other 4-(1′-alditylamino)-5-nitroso-2,6-dihydroxypyrimidines, are shown in Fig. 1. These values are in reasonable agreement with those given by McNutt[15] for 4-(β-hydroxyethylamino) - 5 - nitroso - 2,6 - dihydroxypyrimidine; however, McNutt did not report the extinction in the visible range of the spectrum. The molar

(15) W. S. McNutt, *J. Am. Chem. Soc.*, 82, 217 (1960).

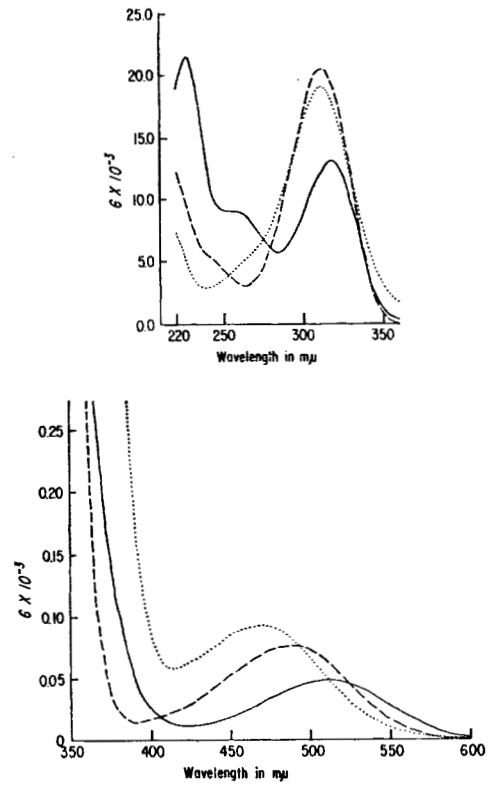


Fig. 1. Absorption spectra of 4-(1′-D-arabitylamino)-5-nitroso-2,6-dihydroxypyrimidine. ———, 0.1*N* H_2SO_4; – – –, 0.1*M* phosphate at *p*H 6.8; . . . . . , 0.1*N* NaOH

absorbancies of these pyrimidines are collected in Table I. Other data obtained for the compounds are summarized in Table II.

The purity of the lumazines prepared was determined by paper chromatography in three solvent systems. Because of the ability of these compounds to chelate metals, it was found desirable to use acid-washed paper to avoid the appearance of secondary spots caused by binding with trace metals in the paper. Each of the lumazines exhibited a single fluorescent component that was green with all but those carrying a phenyl group. These showed yellow-green fluorescence. The movement on paper as a single spot was of considerable interest in the cases of the lumazines assymetrically substituted at positions 6 and 7 where one would expect the formation of two isomers. Similarly, no separation could be observed with these preparations upon column chromatography on acid alumina. The bulk of the ribityl group could direct the condensation in favor of placing the smaller group in position 7. However, the high yield (94%) obtained in the preparation of 6,7-di-*n*-propyl-8-(1′-D-ribityl)lumazine shows that a group of this size is not seriously hindered by the ribityl moiety. However, 6,7-di-*i*-propyl-8-(1′-D-ribityl)lumazine could not be prepared by condensation of 2,5-dimethyl-3,4-hexanedione with 4-(1′-D-ribitylamino)-5-amino-2,6-dihydroxypyrimidine at *p*H 4.4 or at *p*H 9, or in the presence of boric acid, a

TABLE I

MOLAR EXTINCTIONS OF 5-NITROSO-2,6-DIHYDROXYPYRIMIDINES SUBSTITUTED AT POSITION 4 WITH D-XYLITYLAMINE, D-LYXITYLAMINE, D- AND L-ARABITYLAMINE, D-RIBITYLAMINE, AND D-GALACTITYLAMINE

0.1N H_2SO_4				0.1M Phosphate Buffer, pH 6.8				0.1N NaOH			
λ_{max}	Log ϵ	λ_{min}	Log ϵ	λ_{max}	Log ϵ	λ_{min}	Log ϵ	λ_{max}	Log ϵ	λ_{min}	Log ϵ
510	1.65–1.72	420	1.10–1.30	490	1.83–1.91	390	1.20–1.38	465	1.89–1.98	415	1.73–1.83
318	3.93–4.12	283	3.55–3.77	312	4.24–4.31	263	3.41–3.60	312	4.25–4.36	238	3.45–3.54
226	4.14–4.33										

TABLE II

4-(1′-ALDITYLAMINO)-5-NITROSO-2,6-DIHYDROXYPYRIMIDINES

Substituent	Yield, %	Decomp. Point, °	Formula	Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
D-Xylityl	36	152	$C_9H_{14}O_7N_4 \cdot 2H_2O$	35.1	5.2	18.2	35.6	5.1	18.2
D-Lyxityl	75	212–213	$C_9H_{14}O_7N_4$	37.2	4.9	19.3	37.4	5.1	19.2
D-Arabityl	43	236	$C_9H_{14}O_7N_4$	37.2	4.9	19.3	37.3	4.9	19.6
L-Arabityl	52	236	$C_9H_{14}O_7N_4$	37.2	4.9	19.3	37.2	5.0	19.3
D-Galactityl	46	178–181	$C_{10}H_{16}O_8N_4$	37.5	5.1	17.5	37.0	5.4	18.8

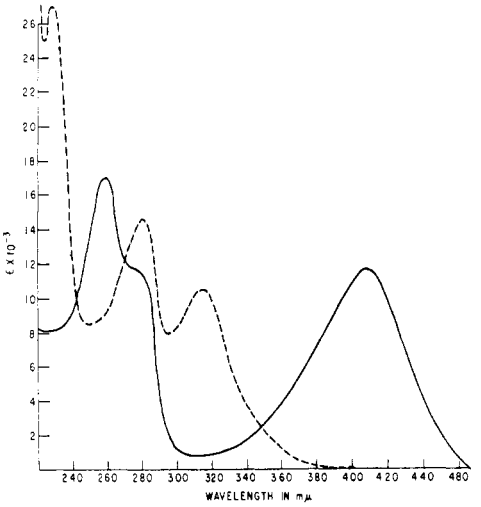


Fig. 2. Absorption spectra of 6,7-(methyl-n-butyl)-8-(1′-D-ribityl)lumazine. ———, 0.1N H_2SO_4; – – –, 0.1N NaOH

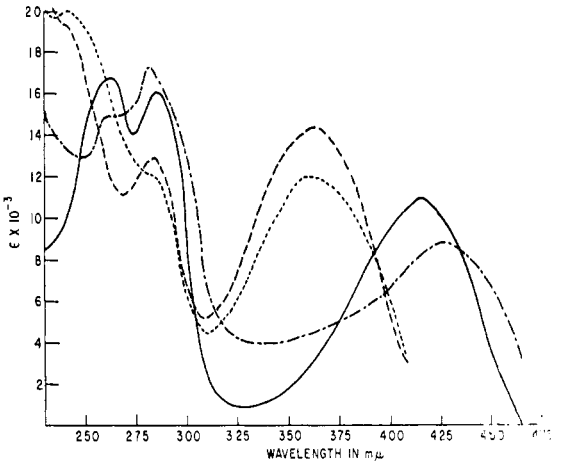


Fig. 3. Absorption spectra of 6,7-(phenyl, methyl)-8-(1′-D-ribityl)lumazine in 0.1N H_2SO_4, ———; in 0.1N NaOH, – – –; and of 6,7-diphenyl-8-(1′-D-ribityl)lumazine in 0.1N H_2SO_4, –·–·–; in 0.1N NaOH, ...

substance reported to facilitate such condensations.[16] Since 6,7-di-i-propyl-8-methyllumazine was prepared in 94% yield, it is assumed that steric hindrance prevented the ring from closing in the first case.

8-(1′-Alditiyl)lumazines with various alkyl substituents at positions 6 and 7 show very similar light absorption (Fig. 2). They markedly differ in this respect from those substituted with one or two aryl groups (Fig. 3) and from 5,6,7,8-tetrahydro-9-(1′-D-ribityl)isoalloxazine (Fig. 4). Substitution of 6,7-dialkyllumazines with a methyl, ethyl, β-hydroxyethyl, or 1′-alditiyl group at position 8 has little effect on the contours of their light absorption spectra in acid solution (Fig. 5A). However, in basic solution considerable differences among compounds with these groups are noticed (Fig. 5B). Especially striking are the changes in

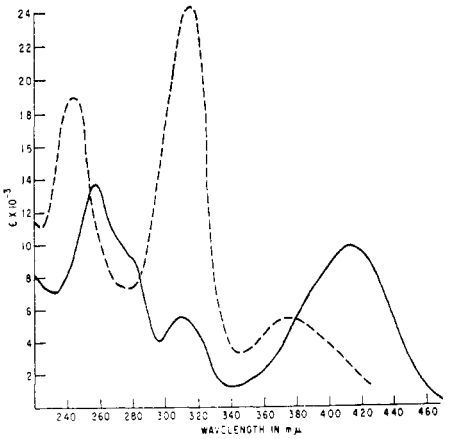


Fig. 4. Absorption spectra of 5,6,7,8-tetrahydro-9-(1′-D-ribityl)isoalloxazine in 0.1N H_2SO_4, ———; in 0.1N NaOH, – – –

(16) R. Kuhn and R. Ströbele, *Ber.*, 70, 750 (1937).

TABLE III

SUBSTITUTED LUMAZINE DERIVATIVES

Compound	Method	Yield, %	Formula	Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N	Ash	Crystallization Solvent
8-(1′-D-RIBITYL)LUMAZINES											
Ethyl, methyl[a]		35–86[b]	$C_{14}H_{20}O_6N_4$	49.4	5.9	16.5	49.6	6.1	16.4		W
n-Propyl, methyl	B	44[b]	$C_{15}H_{22}O_6N_4$	50.8	6.3	15.8	50.2	6.3	15.3	1.0[c]	W
n-Butyl, methyl	B	88[b]	$C_{16}H_{24}O_6N_4$	52.2	6.6	15.2	52.3	6.3	15.6		E-W
n-Pentyl, methyl	B	79[b]	$C_{17}H_{26}O_6N_4$	53.4	6.8	14.6	53.0	7.0	14.7		E-W
Phenyl, methyl	B	46	$C_{18}H_{20}O_6N_4 \cdot 2\,H_2O$[d]	50.9	5.7	13.2	50.5	5.5	14.0		W
Diethyl[e]	B	103	$C_{15}H_{18}O_6N_4 \cdot {}^1/_2\,H_2O$[d]	49.6	6.4	15.4	49.6	7.0	15.5	0.8	E
Di-*n*-propyl	B	94[b]	$C_{17}H_{26}O_6N_4 \cdot 1{}^1/_2\,H_2O$[d]	49.9	7.1	13.7	49.5	7.1	13.7		E-W
Diphenyl	B	42	$C_{23}H_{22}O_6N_4 \cdot H_2O$	59.0	5.2	12.0	59.2	4.5	11.7		D
6,7-DIMETHYL-8-(1′-ALDITYL)LUMAZINES											
D-Xylityl	A	59	$C_{13}H_{18}O_6N_4 \cdot 2H_2O$	43.1	6.1	15.5	43.7	6.2	14.9	0.6	E-W
D-Lyxityl	A	36	$C_{13}H_{18}O_6N_4 \cdot 2H_2O$	43.1	6.1	15.5	43.0	6.3	15.8		E-W
D-Arabityl	A	52	$C_{13}H_{18}O_6N_4 \cdot H_2O$	45.3	5.9	16.3	45.0	6.0	16.9		E-W
L-Arabityl	A	26	$C_{13}H_{18}O_6N_4 \cdot H_2O$	45.3	5.9	16.3	44.6	6.3	16.5		E-W
D-Galactityl	A	40	$C_{14}H_{20}O_7N_4$	47.2	5.7	15.7	47.2	6.2	17.2		E-W
OTHER DERIVATIVES											
6,7-Diethyl-8-methyllumazine		45	$C_{11}H_{14}O_2N_4$	56.4	6.0	23.9	56.4	6.2	23.8		W
6,7-Di-*i*-propyl-8-methyllumazine		94[b]	$C_{13}H_{18}O_2N_4 \cdot H_2O$[d]	55.7	7.2	20.0	55.8	6.7	20.4	0.6	W
6,7-Diphenyl-8-methyllumazine		75	$C_{19}H_{14}O_2N_4 \cdot {}^1/_2\,H_2O$[d]	67.2	4.5	16.5	66.8	4.5	16.6		E-D
6,7-Diethyl-8-(β-hydroxyethyl)-lumazine		32	$C_{12}H_{16}O_3N_4 \cdot H_2O$	51.8	6.5	20.1	51.4	6.7	19.3		W

W = water, E = ethanol, and D = dioxane-ether.

[a] Prepared by Mrs. Janice Rogers. [b] The yield was determined spectrophotometrically at 407 mμ after condensation. [c] Probably a heavy metal oxide. [d] Moisture determinations supported these assignments. [e] This compound has also been prepared by J. Davoll and D. D. Evans, *J. Chem. Soc.*, 5041 (1960).

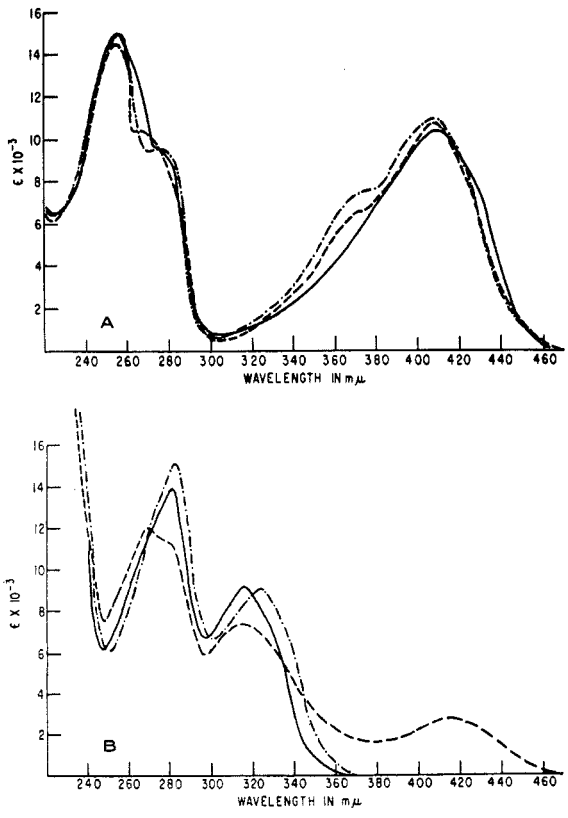


Fig. 5. Absorption spectra in 0.1*N* H_2SO_4 (*A*) and 0.1*N* NAOH (*B*) of 6,7-diethyl-8-(β-hydroxyethyl)lumazine, ———; of 6,7-diethyl-8-methyllumazine, – – –; and of 6,7-di-*i*-propyl-8-methyllumazine, -·-·-.

the spectra in the range from 360 mμ to 460 mμ. Alkaline solutions of the 6,7-dialkyl-8-(1′-alditol)-lumazines (Fig. 2), 6,7-diethyl-8-(β-hydroxyethyl)-lumazine, and 6,7-di-*i*-propyl-8-methyllumazine (Fig. 5B) show no absorbance in this region. However, under the same conditions, solutions of 6,7,8-trimethyllumazine,[14] 6,7-dimethyl-8-ethyl-lumazine,[17] and 6,7-dimethyl-8-(β-hydroxyethyl)-lumazine[17] show absorption maxima between 360 mμ and 400 mμ; the maximal absorbance of 6,7-diethyl-8-methyllumazine is at 415 mμ (Fig. 5B). 6,7,8-Trimethyllumazine exhibits broad absorption in this area with a maximum at 365 mμ and a minimum at 334 mμ.[14] 6,7-Dimethyl-8-ethyllumazine is reported to have an absorbance maximum at 370 mμ and a minimum at 335 mμ; 6,7-dimethyl-8-(β-hydroxyethyl)lumazine shows a broad shoulder at 360–375 mμ.[17] 6,7-Diethyl-8-methyllumazine possesses an absorption maximum at a higher wave length (415 mμ) than any of the lumazines studied. It is unlikely that these characteristics of light absorption can be attributed to an impurity in the sample of 6,7-diethyl-8-methyllumazine, since the preparation exhibited only one fluorescent component upon chromatography on columns of alumina and in three solvent systems of paper chromatography. The intensity of absorption in the range 310 mμ to 315 mμ in basic solutions of these pteridines is greatest with 6,7,8-trimethyl-lumazine and lowest with the 6,7-dialkyl-8-(1′-aldi-

(17) W. Pfleiderer and G. Nübel, *Ber.*, 93, 1406 (1960).

TABLE IV

SUBSTITUTED LUMAZINE DERIVATIVES

Compound	Decomp. Point, °	Absorption Spectra: 0.1N H_2SO_4 λ_{max}	Log ϵ	λ_{min}	Log ϵ	0.1N NaOH λ_{max}	Log ϵ	λ_{min}	Log ϵ
8-(1′-D-RIBITYL)LUMAZINES									
Ethyl, methyl	285–290	407	3.98	300	2.75	313	3.92	292	3.81
		256	4.15	225	3.81	279	4.08	248	3.81
						227	4.33	223	4.32
n-Propyl, methyl	267–268	407	4.00	300	3.03	313	4.04	292	3.94
		256	4.20	225	3.91	279	4.18	248	3.99
						227	4.46		
n-Butyl, methyl	269–272	407	4.08	300	3.03	313	4.03	292	3.89
		256	4.23	230	3.91	279	4.16	248	3.93
						227	4.43	223	4.40
n-Pentyl, methyl	175–179	407	3.81	300	3.03	313	3.84	292	3.72
		256	4.08	225	3.53	279	4.03	248	3.86
Phenyl, methyl	254–260	415	4.06	325	2.94	363	4.18	308	3.72
		285	4.23	274	4.17	284	4.14	269	4.07
		263	4.24	230	3.94				
Diethyl	Amorph.	407	3.83	300	3.21	313	3.87	292	3.76
	129–145	256	4.09	225	3.77	279	4.09	248	3.82
						227	4.33	223	4.30
Di-*n*-propyl	Amorph.	407	3.97	300	3.12	313	3.94	292	3.80
	130–137	256	4.11	225	3.87	279	4.14	248	3.85
						227	4.40	223	4.35
Diphenyl	212–218	426	3.97	335	3.61	365	4.09	310	3.67
		282	4.25	248	4.12	242	4.31	234	4.30
6,7-DIMETHYL-8-(1′-ALDITYL)LUMAZINES									
D-Xylityl	135–140	407	4.04	300	2.80	313	4.02	292	3.86
		256	4.17	220	3.81	279	4.09	257	3.92
						227	4.36		
D-Lyxityl	132–140	407	4.04	300	2.71	313	3.96	292	3.83
		256	4.18	220	3.80	279	4.12	257	3.91
						227	4.38		
D-Arabityl	134–150	407	4.00	300	3.09	313	3.99	292	3.81
		256	4.17	220	3.87	279	4.05	257	3.86
						227	4.29		
L-Arabityl	220–224	407	4.02	300	3.03	313	4.00	292	3.83
		256	4.17	220	3.84	279	4.06	257	3.87
						227	4.32		
D-Galactityl	244–247	407	3.97	300	2.76	313	3.94	292	3.78
		256	4.11	220	3.93	279	4.01	257	3.82
						227	4.28		
D-Ribityl[a]	270–274	407	4.01	300	3.22	313	4.00	292	3.92
		256	4.20	220	3.93	279	4.15	257	4.01
OTHER DERIVATIVES									
6,7-Diethyl-8-methyl-lumazine	273–282	407	4.03	300	2.74	415	3.46	377	3.25
		275	3.98	270	3.93	315	3.87	296	3.77
		256	4.16			270	4.08	249	3.87
6,7-Di-*i*-propyl-8-methyl-lumazine	272–275	404	4.02	300	2.75	323	3.93	299	3.81
		256	4.17	225	3.81	282	4.17	250	3.79
						230	4.44	222	4.36
6,7-Diphenyl-8-methyl-lumazine	288–292	424	4.00	336	3.81	350	3.88	310	3.59
		290	4.10	244	3.87	243	4.20	234	4.11
		265	4.03						
6,7-Diethyl-8-(β-hydroxy-ethyl)lumazine	254–260	407	4.00	300	2.91	315	3.93	296	3.83
		256	4.14	225	3.79	280	4.12	247	3.79

[a] Ref. 14.

tyl)lumazines. With all alkyl substituted lumazines excepting 6,7,8-trimethyllumazine, 6,7-dimethyl-8-ethyllumazine, and 6,7-diethyl-8-methyllumazine a maximum occurs at 279 mμ. The first two exceptions show minimum absorbance at this wave length, while the last shows a shoulder at 279 mμ with a new maximum appearing at 270 mμ. 6,7-Dimethyl-8-ethyllumazine exhibits a shoulder at 270 mμ and a maximum at 245 mμ. 6,7,8-Trimethyllumazine also possesses a shoulder at 268 mμ. Too few compounds have been prepared so far to explain and predict fully the effect of the charac-

ter of the groups in positions 6, 7, and 8 on the light absorption spectra of lumazines.

The physical properties of the lumazines prepared are summarized in Tables II, IV, and V.

TABLE V

OPTICAL ROTATION OF 6,7-DIMETHYL-8-(1′-ALDITYL)-LUMAZINES

D-Xylityl	$[\alpha]_D^{24}$	-204 ± 3	(c, 0.497 in water)[a]
D-Lyxityl	$[\alpha]_D^{24}$	$+130 \pm 3$	(c, 0.486 in water)[a]
D-Arabityl	$[\alpha]_D^{24}$	$+203 \pm 4$	(c, 0.498 in water)[a]
L-Arabityl	$[\alpha]_D^{24}$	-238 ± 1	(c, 0.480 in water)[a]
D-Ribityl[b]	$[\alpha]_D^{22}$	-180 ± 4	(c, 0.517 in water)
D-Galactityl	$[\alpha]_D^{24}$	-214 ± 3	(c, 0.527 in water)[a]

[a] The concentration of lumazine in the solution was determined spectrophotometrically at 407 mμ in 0.1N sulfuric acid. [b] Footnote 17.

In contrast to 6,7-dimethyl-8-(1′-D-ribityl)lumazine, none of the substituted lumazines described here could be converted to flavines by a purified enzyme preparation[3] from *A. gossypii*. A detailed description of the biological properties of these substances will be given elsewhere.

EXPERIMENTAL[18]

D-Lyxose oxime. Powdered hydroxylamine hydrochloride (11.8 g.) was suspended in 60 ml. of ethanol containing 2 drops of 1% phenolphthalein. A freshly prepared solution of 6.48 g. of commercial sodium methoxide in 60 ml. of ethanol was added slowly to the stirred suspension of hydroxylamine hydrochloride until the pink indicator color persisted for 1 min. An excess of sodium methoxide should be avoided, but it can be removed by the addition of a small amount of solid hydroxylamine hydrochloride. The hydroxylamine solution was filtered, the filtrate warmed to 70°, and 10.9 g. of D-lyxose added in small portions to the stirred solution. When the sugar had dissolved, the reaction mixture was allowed to cool to room temperature; 10.4 g. (87%) of colorless crystalline D-lyxose oxime was collected; m.p. 101–102°.

This method was also used for the preparation of the following compounds:

D-Arabinose oxime, m.p. 136–140° (lit.,[19] m.p., 138–139°).

L-Arabinose oxime, m.p. 137–139.5° (lit.,[19] m.p., 138–139°).

D-Xylose oxime was obtained as a sirup.

D-Galactose oxime was prepared by the method of Rischbieth.[20]

D-Lyxitylamine. A suspension of 10.0 g. of D-lyxose oxime and 0.104 g. of platinum oxide in 56 ml. of glacial acetic acid was treated with hydrogen (40 p.s.i.) for 18 hr. at room temperature. The oxime was reduced when all white solids had disappeared. The catalyst was removed by filtration and the liquid concentrated to a sirup in a vacuum. The residue was taken up in 250 ml. of water and passed through a column of Dowex 50 W X-8 hydrogen form (2 cm. diameter × 20 cm. height). The column was washed with 200 ml. of water, and the glycamine was eluted with 200 ml. of 3N ammonium hydroxide. The alkaline eluate was collected and concentrated to a sirup in a vacuum to remove ammonia. The yield (74%) of D-lyxitylamine was determined by titration with standard acid.

D-Arabitylamine, L-arabitylamine, D-xylitylamine, and D-galactitylamine. These compounds were obtained by the same procedure as used for the preparation of D-lyxitylamine. D-Galactitylamine crystallized from the aqueous solution and was obtained as a solid in 15% yield; m.p. 143–146° (lit.,[21] m.p. 143–145°). The other amines remained as sirups and were used in this form in the next step.

4-(1′-L-Arabitylamino)-5-nitroso-2,6-dihydroxypyrimidine. A solution containing 1.46 g. of 4-chloro-2,6-dihydroxypyrimidine, 7.84 ml. of 2.55M L-arabamine, and 8.15 ml. of water was heated in a stainless steel bomb for 5 hr. at 135°. The contents of the bomb were cooled, adjusted to pH 8 with a few drops of 5% sodium hydroxide, and 1.76 g. of sodium nitrite was added. The solution was adjusted to pH 4.6 by the dropwise addition of 20% acetic acid and evaporated to dryness in a vacuum. The residue was dissolved in 50 ml. of water and passed through a column of Dowex 1 X-10 formate (2 cm. diameter × 18 cm. height). The column was washed with 100 ml. of water, then with 200 ml. of 0.01M formic acid; the compound was eluted with 500 ml. of 0.1M formic acid. The red colored percolate was concentrated to dryness in a vacuum, and the residue was crystallized from 110 ml. of water. 4-(1′-L-Arabitylamino)-5-nitroso-2,6-dihydroxypyrimidine was obtained as a red solid in a yield of 1.52 g. (52%); m.p. 259–260°.

The preparation of 8-(1′alditylamino)lumazines. Reduction, condensation, and chromatography. Method A. A solution of 500 mg. of 4-(1′alditylamino)-5-nitroso-2,6-dihydroxypyrimidine in 10 ml. of water at 90–95° was adjusted to pH 5.8 with 2N potassium hydroxide and 1.0 g. of sodium hydrosulfite added. The solution was decolorized within 2 min. indicating that reduction was complete. The solution was cooled, and a 3- to 7-mole excess of α-diketone was added. The solution was brought to pH 4.6–4.4 with 2N hydrochloric acid and heated at 76–80° for 40 min. The reaction mixture was evaporated to dryness under vacuum, and the residue was dissolved in 10 ml. of water, diluted with 40 ml. of ethanol, and applied to a column of acid-washed alumina (80 g. of alumina in absolute ethanol to make a column 2 cm. diameter × 22 cm. height). The column was washed with 100 ml. of 80% (v./v.) ethanol, and the green, fluorescent compound was eluted with 50% (v./v.) ethanol. The eluate was evaporated to dryness under reduced pressure.

Method B. To a solution of 500 mg. of 4-(1′-alditylamino)-5-nitroso-2,6-dihydroxypyrimidine in 10 ml. of water at 90° was added 1.0 g. of sodium hydrosulfite. After decoloration was complete, a drop of 2N hydrochloric acid brought the cooled solution to pH 4.6, and a 3- to 7-mole excess of α-diketone was added together with 10 ml. of ethanol. The solution was refluxed for 40 min., then cooled and applied to a column of 60 g. of acid-washed alumina (prepared in absolute alcohol in a 2-cm. diameter column). The green, fluorescent material was eluted with 80% ethanol. The eluate was evaporated to dryness under reduced pressure.

Benzyl alcohol step. The material obtained after chromatography was taken up in 20 ml. of water and extracted with 25-ml. portions of water-saturated benzyl alcohol. The number of extractions necessary was determined by spectrophotometric assay of the aqueous solution for the amount of lumazine remaining. The extracts were filtered through Whatman No. 3 paper and an equal volume of ether added. The compound was extracted from this mixture with 25-ml. portions of water. The combined aqueous phase was washed with 50 ml. of ether to remove residual benzyl alcohol and evaporated to dryness under reduced pressure. The residue was then recrystallized from an appropriate solvent.

The substituted lumazines prepared were all light-sensitive and were found to decompose upon standing in solution. Consequently, all manipulations were carried out rapidly and in as little light as possible. During recrystallization

(18) Microanalyses were carried out by Drs. G. Weiler and F. B. Strauss, Oxford, England, and A. Elek, Los Angeles, Calif. Melting points are uncorrected.

(19) O. Ruff, *Ber.*, 31, 1573 (1898).

(20) P. Rischbieth, *Ber.*, 20, 2673 (1887).

(21) F. Kagan, M. A. Rebenstorf, and R. V. Heinzelman, *J. Am. Chem. Soc.*, 79, 3541 (1957).

prolonged heating is to be avoided. The compounds are difficult to free from metals and also from moisture to which they cling tenaciously especially at the temperature (52°) at which it is practical to dry them.

Attempted preparation of 6,7-di-i-propyl-8-1'-D-ribityl)lumazine. When method A was employed starting with 4-(1'-D-ribitylamino)-5-nitroso-2,6-dihydroxypyrimidine and a 3-mole excess of 2,5-dimethyl-3,4-hexanedione the yield of 6,7-di-*i*-propyl-8-(1'-D-ribityl)lumazine (determined spectrophotometrically) was less than 2%. When the condensation was attempted at *p*H 9 the yield was again less than 2%. Similar results were obtained with the boric acid method described for the preparation of 6,7-di-*i*-propyl-8-methyllumazine.

6,7-Di-i-propyl-8-methyllumazine. To a hot solution of 393 mg. of 5-amino-4-methylamino-2,6-dihydroxypyrimidine in 20 ml. of water were added 0.7 ml. of 2,5-dimethyl-3,4-hexanedione, 23 ml. of ethanol, and 960 mg. of boric acid. The solution was heated at 80° for 45 min. The yield, determined spectrophotometrically, was 612 mg. (94%).

The solution was applied immediately to a column (2 cm. diameter) of 60 g. of acid-washed alumina prepared with absolute alcohol. The green, fluorescent pteridine was eluted with 95% ethanol. The eluate was evaporated under reduced pressure, the residue was dissolved in hot water, and upon chilling it deposited 260 mg. of yellow crystals (40%). The physical properties are summarized in Tables III and IV.

5,6,7,8-Tetrahydro-9-(1'-D-ribityl)isoalloxazine. The isoalloxazine derivative was prepared from 500 mg. of 4-(1'-D-ribitylamino)-5-nitroso-2,6-dihydroxypyrimidine and 380 mg. of 1,2-cyclohexanedione as outlined in Method B. After purification by column chromatography and transfer into and out of benzyl alcohol, the aqueous extracts were evaporated to *ca.* 2 ml. Addition of alcohol and chilling brought about the precipitation of 45 mg. (9%) of 5,6,7,8-tetrahydro-9-(1'-D-ribityl)isoalloxazine. The compound could be recrystallized from water; m.p. 261–263° dec. $\lambda_{max}^{0.1N\ H_2SO_4}$ 413 mμ (log ε 4.00), 309 mμ (3.75), 258 mμ (4.14); λ_{min} 338 mμ (3.28), 296 mμ (3.61), 233 mμ (3.85). $\lambda_{max}^{0.1N\ NaOH}$ 374 mμ (log ε 3.74), 317 mμ (4.39), 245 mμ (4.29); λ_{min} 343 mμ (3.53), 276 mμ (3.87), 225 mμ (4.05).

Anal. Calcd. for $C_{15}H_{20}O_6N_4$: C, 51.2; H, 5.7; N, 15.9. Found: C, 51.2; H, 5.7; N, 15.7.

6,7-Diphenyl-8-(1'-D-ribityl)lumazine. Method B was followed. After the material had been applied to a column of acid alumina and eluted with 50% ethanol, the eluate was evaporated to dryness. The residue was taken up in water and extracted with ether to remove excess benzil. The lumazine was then transferred into and out of benzyl alcohol. After evaporation of the water the material was dissolved in a small amount of dioxane and precipitated by addition of ether. The properties of the compound are summarized in Tables III and IV.

The preparation of 8-methyllumazines. To 4-methylamino-5-amino-2,6-dihydroxypyrimidine in water were added a 3- to 7-mole excess of α-diketone, a volume of ethanol equal to that of the water used, and a drop of 2*N* hydrochloric acid. The solution was refluxed 30–50 min., then cooled and evaporated under reduced pressure. The residue was taken up in ethanol or ethanol-water, applied to 50–70 g. of acid-washed alumina (prepared in absolute alcohol in a 2-cm. diameter column), and eluted with ethanol. The eluate was evaporated and the residue recrystallized. A summary of the properties is given in Tables III and IV.

Paper chromatography. The ascending method was used with Whatman No. 3 MM filter paper and Schleicher and Schuell No. 507, acid washed. Position of spots was determined by fluorescence and/or quenching under ultraviolet light. The red color of the nitrosopyrimidine derivatives could be detected in daylight. The composition of the solvent systems used for the development of the chromatograms were *n*-butyl alcohol:ethanol:water—50:15:36, water saturated with *t*-amyl alcohol, or *i*-butyric acid:*N* ammonia:0.1*M* ethylenediamine tetraacetate—100:60:1.6.[22]

Materials. Sugars of C. P. grade were purchased from Pfanstiehl Laboratories, Inc. The ion exchange resins Dowex AG 50 W X-8 (200–400 mesh) and Dowex AG 1 X-10 (200–400 mesh) and the acid-washed alumina were obtained from California Corp. for Biochemical Research. 3,4-Hexanedione, 4,5-octanedione, and 2,3-dimethyl-3,4-hexanedione were prepared by acyloin condensation of the appropriate esters[23] followed by oxidation of the ketol with cupric acetate.[24] The remaining diketones were purchased from Aldrich Chemical Co. and Eastman Kodak Co.

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4-Sulfanilamidopyridazines

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3,4,6-Trichloropyridazine (II) condenses in dimethylformamide solution with sulfanilamide or its acetyl derivative to give 4-sulfanilamido-3,6-dichloropyridazine (III) or its acetyl derivative (IV). The latter is dehalogenated and hydrolyzed to 4-sulfanilamidopyridazine (V). Replacement of one chlorine atom in IV gives 4-sulfanilamido-3(or 6)-hydroxy-6(or 3)-chloropyridazine (VIa or b) and 4-sulfanilamido-3(or 6)-methoxy-6(or 3)-chloropyridazine (VIIIa or b). VI (a or b) is dehalogenated to 4-sulfanilamido-3(or 6)-hydroxypyridazine (VIIa or b).

Rogers and English[1] and Druey *et al.*[2] described the synthesis of 3-sulfanilamido-6-chloropyridazine (Ia), an intermediate for the chemotherapeutically used 3-sulfanilamido-6-methoxypyridazine (Ib), from dichloropyridazine and sulfanilamide. 3-Sulfanilamidopyridazine (Ic) was obtained from Ia[3] and from 3-aminopyridazine.[4]

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