Further Synthetic Approaches to Flavines (Isoalloxazines). A New Synthesis of Riboflavine

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Dehydrative cyclization of 5-nitro-6-(*N*-substituted anilino)uracils (2) with concentrated sulphuric acid gave the corresponding flavine 5-oxides, which were converted into flavines. Treatment of 5-amino-6-(*N*-substituted anilino)uracils (5) [prepared by hydrogenation of the nitro-derivatives (2)] with diethyl azodiformate (DAD) or nitrosobenzene in indirect sunlight or under irradiation with a sun-lamp gave the corresponding flavines. The reaction mechanism has been demonstrated by the isolation of the intermediate 5-imino-6-(*N*-substituted anilino)-uracils. Autoxidation of the amino-derivatives (5) also gave flavines; by this method riboflavine and related compounds were obtained. Treatment of the nitro-compounds (2) with DAD in the presence of bases such as triphenylphosphine and pyridine gave flavines; in this case DAD becomes the source of N-5 of the flavine ring system.

PREVIOUS papers have described convenient synthetic routes to flavine derivatives involving cyclizations of 6-(*N*-substituted anilino)uracils with nitrogen sources such as sodium nitrite,[1] potassium nitrate,[1] and diethyl azodiformate.[2] We now report several new synthetic approaches to flavine derivatives from the readily available 5-nitro-6-(*N*-substituted anilino)uracils.

The 5-nitro-6-(*N*-substituted anilino)uracils (2a—n) were prepared by condensation of 6-chloro-5-nitrouracil (1a)[3] and 6-chloro-3-methyl-5-nitrouracil (1b)[4] with *N*-substituted anilines (Table 4).†

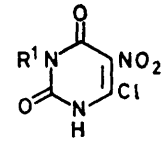
*Dehydrative Cyclization of 5-Nitro-6-(*N*-substituted anilino)uracils with Sulphuric Acid.*[5]—Treatment of compounds (2a and g—j) with an excess of concentrated sulphuric acid at 60—80 °C afforded the corresponding flavine 5-oxides (isoalloxazine 5-oxides) (3a and g—j)[1] (Table 1). Deoxygenation of (3a and g—j) was effected with sodium dithionite in water to give the corresponding flavines (4a and g—j). The deoxygenation was also accomplished thermally by refluxing in dimethylformamide. Furthermore, deoxygenation of (3) was also effected by exposure to indirect sunlight or by irradiation in acetic acid with a sun-lamp.

TABLE 1

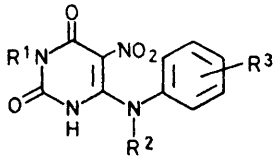
Flavine 5-oxide formation by reaction of 5-nitro-6-(*N*-substituted anilino)uracils with sulphuric acid

Starting material	Temp. (°C)	Time (min)	Product	M.p. (°C)	Yield (%)
(2a)	80	60	(3a)[1]	>360	75
(2g)	60	30	(3g)[1]	322	83
(2h)	60	30	(3h)[1]	267	80
(2i)	80	60	(3i)[1]	319	79
(2j)	80	60	(3j)[1]	308	85

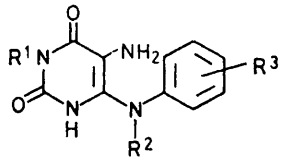
*Oxidative Cyclization of 5-Amino-6-(*N*-substituted anilino)uracils.*[5,6]—Compounds (2a—l) were hydrogenated in ethanol–acetic acid over palladium–charcoal to give the 5-amino-6-(*N*-substituted anilino)uracils (5a—l) (Table 5).† Solutions of (5a and g—j) in ethanol kept in the indirect sunlight or irradiated with a sun-lamp afforded the corresponding flavines (4a and g—j) in 20—40% yields. The yields were affected markedly by slight changes in reaction conditions.



(1) a; R^1 = H
b; R^1 = Me



(2)



(5)

	M.p. (°C); yield (%) of (2)	M.p. (°C); yield (%) of (5)
a; $R^1 = R^3 = H$, $R^2 = Me$	271; 82	>360; 75
b; $R^1 = R^3 = H$, $R^2 = Et$	279; 85	197; 75
c; $R^1 = R^3 = H$, $R^2 = Pr^n$	276; 84	245; 78
d; $R^1 = R^3 = H$, $R^2 = Bu^n$	261; 87	211; 80
e; $R^1 = H$, $R^2 = Et$, $R^3 = 3$-Me	273; 82	265; 82
f; $R^1 = H$, $R^2 = Et$, $R^3 = 4$-Me	263; 84	269; 72
g; $R^1 = R^3 = Me$, $R^3 = H$	236; 90	164; 70
h; $R^1 = Me$, $R^2 = Et$, $R^3 = H$	234; 92	181; 73
i; $R^1 = Me$, $R^2 = Pr^n$, $R^3 = H$	178; 89	171; 75
j; $R^1 = Me$, $R^2 = Bu^n$, $R^3 = H$	157; 95	118; 82
k; $R^1 = Me$, $R^2 = Et$, $R^3 = 3$-Me	222; 87	149; 83
l; $R^1 = Me$, $R^2 = Et$, $R^3 = 4$-Me	182; 91	191; 80
m; $R^1 = Me$, $R^2 = Et$, $R^3 = 3,4$-Me_2	267; 86	(not isolated)
n; $R^1 = Me$, $R^2 =$ D-ribityl, $R^3 = 3,4$-Me_2	168; 79	(not isolated)
o; $R^1 = H$, $R^2 =$ D-ribityl, $R^3 = 3,4$-Me_2	(not isolated)	(not isolated)

Better results were obtained by using DAD (as oxidizing agent) in this oxidative cyclization. Thus a slight

† Tables 4—6 [analytical data for compounds (2), (5), and (4)] are available as Supplementary Publication No. SUP 22172 (4 pp.). For details of Supplementary Publications see Notice to Authors No. 7, *J.C.S. Perkin I*, 1976, Index issue.

[1] F. Yoneda, Y. Sakuma, M. Ichiba, and K. Shinomura, *J. Amer. Chem. Soc.*, 1976, **98**, 830.

[2] F. Yoneda, Y. Sakuma, T. Nagamatsu, and S. Mizumoto, *J.C.S. Perkin I*, 1976, 2398.

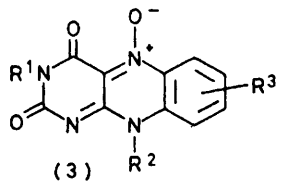
[3] K.-Y. Zee-Cheng and C. C. Cheng, *J. Medicin. Chem.*, 1968, **11**, 1107.

[4] G. D. Daves, R. K. Robins, and C. C. Cheng, *J. Amer. Chem. Soc.*, 1962, **84**, 1724.

[5] Preliminary report, Y. Sakuma, T. Nagamatsu, and F. Yoneda, *J.C.S. Chem. Comm.*, 1975, 977.

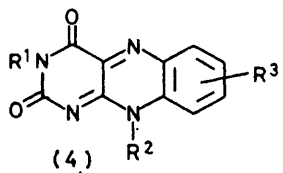
[6] Preliminary report, F. Yoneda and Y. Sakuma, *Heterocycles*, 1977, **6**, 25.

excess of DAD was added to the solution of the amino-uracil (5a—l) in ethanol or acetic acid, and the mixtures were set aside in indirect sunlight or irradiated with a



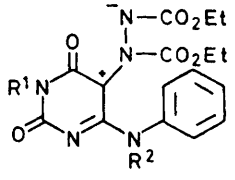
(3)

a; $R^1 = R^3 = H$, $R^2 = Me$
g; $R^1 = R^2 = Me$, $R^3 = H$
h; $R^1 = Me$, $R^2 = Et$, $R^3 = H$
i; $R^1 = Me$, $R^2 = Pr^n$, $R^3 = H$
j; $R^1 = Me$, $R^2 = Bu^n$, $R^3 = H$



(4)

a; $R^1 = R^3 = H$, $R^2 = Me$
b; $R^1 = R^3 = H$, $R^2 = Et$
c; $R^1 = R^3 = H$, $R^3 = Pr^n$
d; $R^1 = R^3 = H$, $R^2 = Bu^n$
e; $R^1 = H$, $R^2 = Et$, $R^3 = 8\text{-Me}$
f; $R^1 = H$, $R^2 = Et$, $R^3 = 7\text{-Me}$
g; $R^1 = R^2 = Me$, $R^3 = H$
h; $R^1 = Me$, $R^2 = Et$, $R^3 = H$
i; $R^1 = Me$, $R^2 = Pr^n$, $R^3 = H$
j; $R^1 = Me$, $R^2 = Bu^n$, $R^3 = H$
k; $R^1 = Me$, $R^2 = Et$, $R^3 = 8\text{-Me}$
l; $R^1 = Me$, $R^2 = Et$, $R^3 = 7\text{-Me}$
m; $R^1 = Me$, $R^2 = Et$, $R^3 = 7,8\text{-Me}_2$
n; $R^1 = Me$, $R^2 = D\text{-ribityl}$, $R^3 = 7,8\text{-Me}_2$
o; $R^1 = H$, $R^2 = D\text{-ribityl}$, $R^3 = 7,8\text{-Me}_2$



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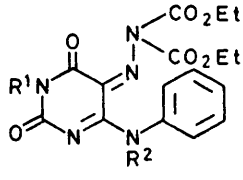

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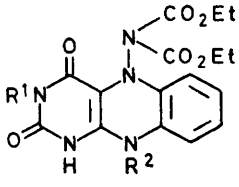


(10)

sun-lamp to precipitate the corresponding flavines (4a—l) in high yields (Tables 2 and 6).†

Oxidative cyclization of (5g—l) with an excess of nitrosobenzene in ethanol under the same conditions also gave the flavines (4g—l) in slightly lower yields than obtained by the DAD oxidation (Table 2).

The reaction mechanism was demonstrated by the isolation of the intermediate 5-imino-6-(*N*-substituted anilino)uracils (7a and b). Stirring compound (5h) and DAD in ethanol or tetrahydrofuran at room temperature in the dark, followed by dilution with ether, precipitated an unstable adduct, 5-(2,3-bisethoxycarbonyltriazano)-6-(*N*-ethylanilino)-3-methyluracil (6). Heating compound (6) in ethanol at 60 °C for 10 min in the dark, followed by dilution with ether, caused separation of 6-(*N*-ethylanilino)-5-imino-3-methyluracil (7b). Concentration of the filtrate gave diethyl hydrazodiformate in almost quantitative yield. The reaction of (5g) with DAD in tetrahydrofuran in the dark gave directly 5-imino-3-methyl-6-(*N*-methylanilino)uracil (7a). The structures of the 5-imino-compounds (7a and b) were assigned on the basis of i.r. (sharp NH= absorption at 3 180 cm^{-1}) and n.m.r. spectra, and molecular weight [*m/e* 244.110 9 ($C_{12}H_{12}N_4O_2$)].

TABLE 2

Flavine formation by oxidative cyclization of 5-amino-6-(*N*-substituted anilino)uracils with DAD or nitrosobenzene

Product (flavine)	Solvent	Yield (%) with DAD	Yield (%) with nitrosobenzene	M.p. (C°)
(4a) [1]	AcOH	85		>360
(4b) [1]	AcOH	82		347
(4c) [1]	AcOH	79		349
(4d) [1]	AcOH	77		335
(4e)	AcOH	74		363
(4f)	AcOH	69		>360
(4g) [1]	EtOH	92	84	334
(4h) [1]	EtOH	90	80	299
(4i) [1]	EtOH	85	78	232
(4j) [1]	EtOH	87	76	315
(4k)	EtOH	87	75	314
(4l)	EtOH	82	70	276

An ethanolic solution of (7a or b) set aside at room temperature in indirect sunlight (or irradiated with a sun-lamp) deposited the flavine (4g or h), formed *via* intramolecular cyclization followed by aerial oxidation.

Treatment of (5g) with nitrosobenzene in ethanol at room temperature in the dark gave directly the same 5-imino-intermediate (7a). Use of 30% hydrogen peroxide and nitric acid was also effective for the oxidative cyclization; however the yields were much lower.

A New Synthesis of Riboflavine and Related Compounds.—The principle of this synthesis is the same as described above. However in these cases, light and oxidizing agent were not necessary for the cyclization of the 5-aminouracils to the corresponding flavines; the presence of light and/or oxidizing agent did not improve the yields.

Thus, 5-nitro-6-(*N*-D-ribityl-3,4-xylidino)uracil (2o), prepared *in situ* from (1a) and *N*-D-ribityl-3,4-xylidine, was hydrogenated over palladium–charcoal. After consumption of hydrogen had stopped, the filtrate was set aside in the dark at room temperature to precipitate riboflavine (4o). Similarly, 3-methylriboflavine (4n) was obtained by autoxidation of 5-amino-3-methyl-6-(*N*-D-ribityl-3,4-xylidino)uracil (5n) [prepared *in situ* by hydrogenation of (2n)]. However, 5-amino-6-(*N*-ethyl-3,4-xylidino)-3-methyluracil (5m) [prepared *in situ* by hydrogenation of (2m)] did not cyclize without light; formation of 10-ethyl-3,7,8-trimethylisoalloxazine (4m) required indirect sunlight.

Reaction of 5-Nitro-6-(N-substituted anilino)uracils with Diethyl Azodiformate (DAD) in the Presence of Base.[7]—Recently we developed a new route to flavines which involves oxidative cyclization of the Michael-type

† Same footnote as on page 348.

[7] Preliminary report, F. Yoneda and Y. Sakuma, *Heterocycles*, 1977, **6**, 431.

adducts from 6-(*N*-substituted anilino)uracils and DAD.[2] A reasonable intermediate in this reaction is the 1,5- or 1,3-dipolar compound (8), which undergoes intramolecular rearrangement to the *NN*-bisethoxycarbonylhydrazone (9). Compound (9) could then cyclize thermally or photochemically to the dihydroflavine (10), which could lose diethyl iminodiformate to give the corresponding flavine (4).

We considered that the elimination of nitrous acid from the adducts formed by reaction of compounds (2) with DAD would give directly the 1,3-dipolar intermediates (8) which give rise to the flavines. Thus, 6-(*N*-methylanilino)-3-methyl-5-nitrouracil (2g) was heated with an excess of DAD in the presence of triphenylphosphine to give the corresponding flavine (4g). The reaction of (2g) with DAD in the presence of pyridine under the same conditions gave the same flavine in slightly lower yield. Neither heating (2g) with triphenylphosphine or pyridine alone nor heating (2g) in the absence of the base yielded (4g); the starting material was recovered.

Similarly, treatment of (2h—j) with DAD in the presence of triphenylphosphine or pyridine gave the corresponding flavines (4h—j) (Table 3).

TABLE 3

Flavine formation by reaction of 5-nitro-6-(*N*-substituted anilino)uracils with DAD in the presence of base

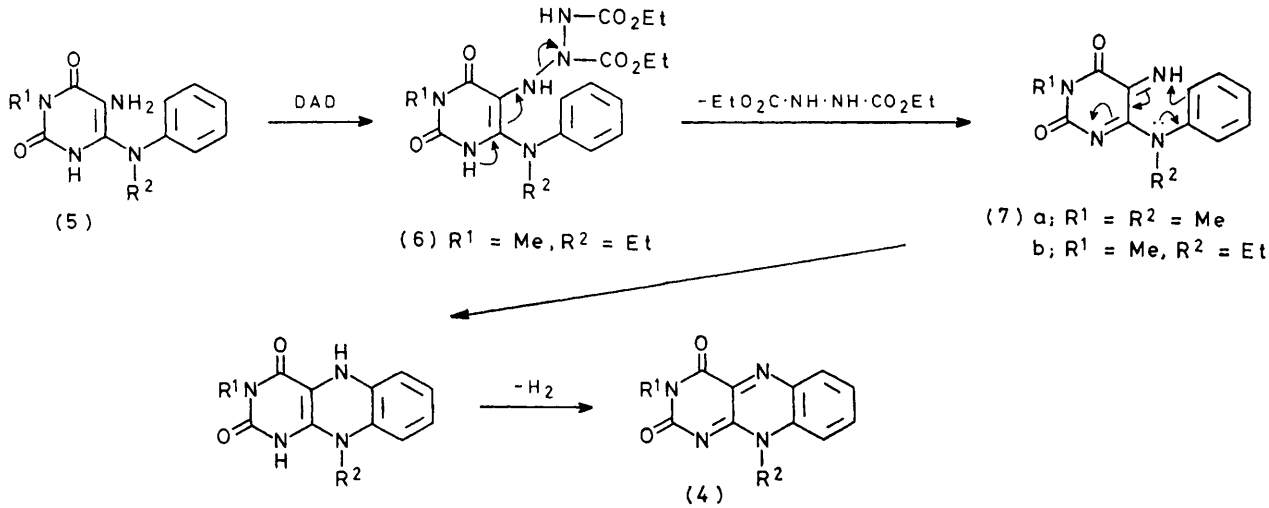
Starting material	Base	Product (flavine)	M.p. (°C)	Yield (%)
(2g)	Triphenylphosphine	(4g)	>360	74
	Pyridine	(4g)		59
(2h)	Triphenylphosphine	(4h)	347	75
(2i)	Triphenylphosphine	(4i)	349	70
(2j)	Triphenylphosphine	(4j)	335	68
	Pyridine	(4j)		48

A possible mechanism involving a flavine 5-oxide formed by dehydrative cyclization of the starting material was precluded because the flavine 5-oxide (3g)[1] remained unchanged when treated with DAD and triphenylphosphine under the same conditions.

EXPERIMENTAL

M.p.s were obtained with a Yanagimoto micro-apparatus. N.m.r. spectra were determined with a JEOL JNM 3H-60 spectrometer (tetramethylsilane as internal standard) and i.r. spectra (Nujol mulls) with a JASCO IRA-1 spectrometer.

5-Nitro-6-(N-substituted anilino)uracils (2a—m). *General Procedure.*—A 5-nitro-6-chlorouracil (1a or b) (0.01 mol) was added in portions to an *N*-substituted aniline (0.03 mol) with stirring, and the mixture was warmed at 90 °C for 1—3 h. After cooling, the precipitates were washed with ether and then water, and filtered off. Recrystallization from ethanol gave yellow *crystals* (2a—m) (Table 4).†



3-Methyl-5-nitro-6-(N-D-ribityl-3,4-xylidino)uracil (2n).—A solution of 6-chloro-3-methyl-5-nitrouracil (1b) (1.03 g, 0.005 mol) and *N*-D-ribityl-3,4-xylidine (1.28 g, 0.005 mol) in ethanol (20 ml) was stirred at room temperature for 3 h and set aside overnight. The yellow *crystals* which separated were filtered off and recrystallized from ethanol (yield 1.67 g, 79%).

Dehydrative Cyclization of (2a *and* g—j) *with Sulphuric Acid. General Procedure.*—A 5-nitro-6-(*N*-substituted anilino)uracil (2a or g—j) (0.005 mol) was warmed in concentrated sulphuric acid (0.05 mol) as indicated in Table 1. The mixture was diluted with water (20 ml) and neutralized with potassium carbonate to cause separation of the corresponding *flavine 5-oxides* (3a and g—j).

Deoxygenation of Flavine 5-Oxides (3a *and* g—j) *with Dimethylformamide.*—A solution of a flavine 5-oxide (3a or g—j) (0.01 mol) in dimethylformamide (20 ml) was refluxed for 3—5 h in the dark, and the solution was evaporated *in vacuo* to dryness. The residue was recrystallized from dimethylformamide or acetic acid to give the corresponding *flavine* (4a or g—j) in 80—90% yield.

5-Amino-6-(N-substituted anilino)uracils (5a—l). *General Procedure.*—A solution of a 5-nitro-6-(*N*-substituted anilino)uracils (2a—l) (0.01 mol) in acetic acid (10 ml) and ethanol (40 ml) containing 5% palladium–charcoal (0.3 g) was hydrogenated at room temperature and atmospheric pressure. After consumption of hydrogen had stopped, the solution was filtered, the filtrate was evaporated *in vacuo* to dryness, and the residue was treated with water. The precipitate gave *crystals* from aqueous ethanol (Table 5).†

† Same footnote as on page 348.

Flavine Formation by Autoxidation of (5a *and* g—j). *General Procedure.*—A solution of a 5-amino-6-(*N*-substituted anilino)uracil (5a or g—j) (0.005 mol) in ethanol (100 ml) was set aside in indirect sunlight or irradiated with a sun-lamp (400 W; Toshiba Co.) at 50 cm range for 16—24 h at room temperature in air. The solution was concentrated to a small volume and set aside in a refrigerator. The *crystals* were filtered off and recrystallized from dimethylformamide or acetic acid. Yields of the flavines (4a and g—j) were 20—40%.

Synthesis of Flavines (4a—l) *by Oxidative Cyclization of* (5a—l). *General Procedure.*—(a) *With DAD.* To a solution of a 5-amino-6-(*N*-substituted anilino)uracil (5a—l) (0.005 mol) in ethanol (100 ml) [or in acetic acid (100 ml) for insoluble 5-aminouracils (5a—f)] was added DAD (0.005 mol), and the mixture was set aside in indirect sunlight or irradiated with a sun-lamp (400 W; Toshiba Co.) at 50 cm range for 4—8 h. The *crystals* were filtered off and recrystallized from dimethylformamide or acetic acid (Table 2).

(b) *With nitrosobenzene.* A solution of 5-amino-6-(*N*-substituted anilino)uracil (5g—l) (0.005 mol) and nitrosobenzene (0.01 mol) in ethanol (20 ml) was treated under the same conditions as above to separate *crystals* (Table 2).

5-(2,3-*Bisethoxycarbonyltriazano*)-6-(N-*ethylanilino*)-3-*methyluracil* (6).—5-Amino-6-(*N*-ethylanilino)-3-methyluracil (5h) (0.51 g, 0.002 mol) and DAD (0.52 g, 0.003 mol), dissolved in tetrahydrofuran (10 ml), were stirred for 2 h at room temperature in the dark. The mixture was diluted with ether to cause separation of the adduct (6) (0.69 g, 80%), m.p. 140°, M^+ 434, δ($CDCl_3$) 1.21 (t, *J* 7 Hz, 2 *Me*CH_2), 1.25 (t, *J* 7 Hz, *Me*CH_2), 2.20br (s, 2 NH), 3.26 (s, NMe), 3.75br (s, NH), 4.03 (q, *J* 7 Hz, 2 MeC*H*$_2$), 4.14 (q, *J* 7 Hz, MeC*H*$_2$), and 6.95—7.70 (ArH).

6-(N-*Ethylanilino*)-5-*imino*-3-*methyluracil* (7b).—The adduct (6) (0.43 g, 0.001 mol) dissolved in ethanol (10 ml) was heated at 60 °C for 10 min in the dark and then diluted with ether to precipitate crystals (0.21 g, 80%), m.p. 155°, M^+ 258. The product was very unstable and could not be recrystallized.

5-*Imino*-3-*methyl*-6-(N-*methylanilino*)*uracil* (7g).—(*a*) A mixture of 5-amino-3-methyl-6-(*N*-methylanilino)uracil (5g) (0.50 g, 0.002 mol) and DAD (0.52 g, 0.003 mol) in tetrahydrofuran (10 ml) was heated at 60 °C for 10 min (or stirred at room temperature for 30 min) in the dark, then diluted with ether and set aside in a refrigerator to separate crystals (0.41 g, 85%), m.p. 145°, M^+ 244.110 9, δ[CF_3CO_2D] 3.45 (s, NMe), 4.35 (s, NMe), 5.78br (s, NH), and 7.25—7.90 (ArH).

(*b*) A solution of 5-amino-3-methyl-6-(*N*-methylanilino)-uracil (5g) (0.50 g, 0.002 mol) and nitrosobenzene (0.43 g, 0.004 mol) in ethanol (5 ml) was stirred at room temperature for 3 h in the dark, then concentrated to a small volume and diluted with ether to precipitate crystals (0.40 g, 82%), m.p. 145°.

Riboflavine (4o).—To a suspension of *N*-D-ribityl-3,4-xylidine (1.28 g, 0.005 mol) in ethanol (20 ml) cooled in ice–water was added 6-chloro-3-nitrouracil (1a) (1.03 g, 0.005 mol) in portions. The mixture was stirred for 1 h before addition of acetic acid (6 ml), and then hydrogenated over 5% palladium–charcoal (0.5 g) at room temperature and atmospheric pressure. After consumption of hydrogen had stopped, the solution was filtered and the filtrate was set aside for 3 days in the dark to separate riboflavine (0.42 g, 56%) in a high state of purity. Recrystallization from acetic acid gave yellow crystals, m.p. 280° (decomp.), identical with an authentic sample.[1]

3-*Methylriboflavine* (4n).—3-Methyl-5-nitro-6-(*N*-D-ribityl-3,4-xylidino)uracil (2n) (0.85 g, 0.002 mol) was dissolved in an ethanol–acetic acid (4 : 1; 30 ml) and hydrogenated over 5% palladium–charcoal (0.3 g). The product was treated as above to precipitate 3-methylriboflavine (0.48 g, 62%), m.p. 275° (decomp.).

10-*Ethyl*-3,7,8-*trimethylisoalloxazine* (4m).—6-(*N*-Ethyl-3,4-xylidino)-3-methyl-5-nitrouracil (2 ml) (0.64 g, 0.002 mol) was dissolved in ethanol–acetic acid (4 : 1; 30 ml) and hydrogenated over 5% palladium–charcoal (0.3 g). After consumption of hydrogen had stopped, the solution was filtered, and the filtrate was set aside in indirect sunlight for 8 h. Evaporation to dryness and recrystallization from acetic acid gave *crystals* (0.41 g, 72%), m.p. 273°.

Synthesis of Flavines by Reaction of 5-*Nitro*-6-(N-*substituted anilino*)*uracils* (2g—j) *with DAD in the Presence of Base. General Procedure.*—A 5-nitro-3-methyl-6-(*N*-substituted anilino)uracil (2g—j) (0.005 mol) was heated with DAD (0.01 mol) at 140 °C for 4 h in the presence of triphenylphosphine (0.002 5 mol) or pyridine (0.004 mol). The mixture was diluted with ether and set aside overnight to separate the corresponding *flavine* (4g—j) (Table 3).

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