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A New Synthetic Approach to 8-Chloroflavins and Their Conversion into 8-(Substituted-amino)flavins

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Treatment of 5-nitro-6-(N-substituted-anilino)uracils (I) with the Vilsmeier reagent (dimethylformamide–phosphorus oxychloride) gave the corresponding 8-chloroisoalloxazines (8-chloroflavins) (II) in a single step. The conversion of I into II probably proceeds *via* the intermediate formation of the corresponding isoalloxazine 5-oxides (flavin 5-oxides). In fact, treatment of flavin 5-oxides with the Vilsmeier reagent also gave the 8-chloroflavins. Under the same conditions, 7-bromoflavin 5-oxides did not give the corresponding 7-bromo-8-chloroflavins, but the deoxygenated 7-bromoflavins were obtained. In this case, the Vilsmeier reagent acted as a reducing agent as well as a dehydrating and chlorinating agent. The 8-chloroflavins were converted into 8-(substituted-amino)flavins by treatment with appropriate amines.

Keywords——flavin 5-oxide; 8-chloroflavin; 7-bromoflavin; flavin 5-oxide; Vilsmeier reagent; 8-(substituted-amino)flavin; N-oxide

The prosthetic groups of a number of flavoenzymes which function as oxido-reductive catalysts in biological systems are usually flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). In recent years, flavin coenzymes which are covalently attached to the enzyme at the 8α-carbon through a linkage to the nitrogen of a histidine imidazolyl group, or to the sulfur of a cysteine residue, have been found.[2] In microorganisms, dimethylamino[3] and hydroxy[4] groups were found at the 8-position of the flavin moiety of FMN and FAD. Since the catalytic entities are isoalloxazine (flavin) nuclei themselves, simple flavin derivatives possessing these 8-substituents would be useful as model compounds to provide mechanistic insight. For the preparation of such 8-substituted flavins, 8-chloroisoalloxazines (8-chloroflavins) are attractive potential intermediates, because the 8-chloro group is expected to be active in nucleophilic substitutions. However, 8-chloroflavin derivatives have not been widely investigated. The known synthetic methods for the preparation of 8-chloroflavins have involved the conventional condensation of alloxan or its equivalents with chlorinated phenylenediamines[5] and the condensation of 6-alkylaminouracils and chlorinated nitrosobenzene.[6] The present paper describes a new convenient synthetic approach to 8-chloroflavins which is in principle widely applicable. The route consists of treatment of 5-nitro-6-(N-substituted-anilino)uraciles with the Vilsmeire reagent (dimethylformamide–phosphorus oxychloride) and also treatment of isoalloxazine 5-oxides (flavin 5-oxides) with the Vilsmeier reagent.[7] We also report a synthesis of 8-(substituted-amino)flavins by the treatment of

1) Location: *a) Oe-honmachi, Kumamoto 862, Japan; b) Maidashi, Higashi-ku, Fukuoka 812, Japan.*
2) D.E. Edmondson and T.P. Singer, *FEBS Lett.*, **64**, 255 (1976).
3) R. Miura, K. Matsui, K. Hirotsu, A. Shimada, M. Takatsu, and S. Otani, *J. Chem. Soc. Chem. Comm.*, **1973**, 703.
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6) F. Yoneda, Y. Sakuma, M. Ichiba, and K. Shinomura, *J. Am. Chem. Soc.*, **98**, 830 (1976).
7) Preliminary report; F. Yoneda, Y. Sakuma, and K. Shinozuka, *J. Chem. Soc. Chem. Comm.*, **1977**, 681.

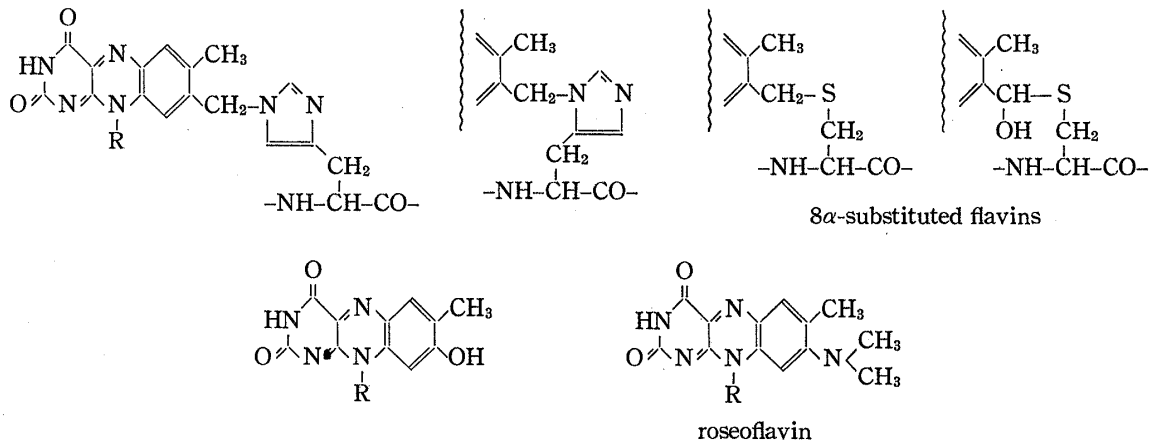


Chart 1

8-chloroflavins with appropriate amines.

Heating of 3-methyl-6-(N-methylanilino)-5-nitrouracil (Ia)[8] with the Vilsmeier reagent, followed by dilution with water, caused the separation of 8-chloro-3,10-dimethylisoalloxazine (IIa) exclusively and in a high state of purity. This product was identical with an authentic sample prepared by the condensation of 3-methyl-6-methylaminouracil with 4-chloronitrosobenzene in acetic anhydride.[6] Similarly, heating other 5-nitrouracils (Ib—e[8] and k) with the Vilsmeier reagent led to the formation of the corresponding 8-chloroflavins (IIb—e and k) (Table I). The structures of compounds II were supported by satisfactory NMR and mass spectra as well as microanalytical data.

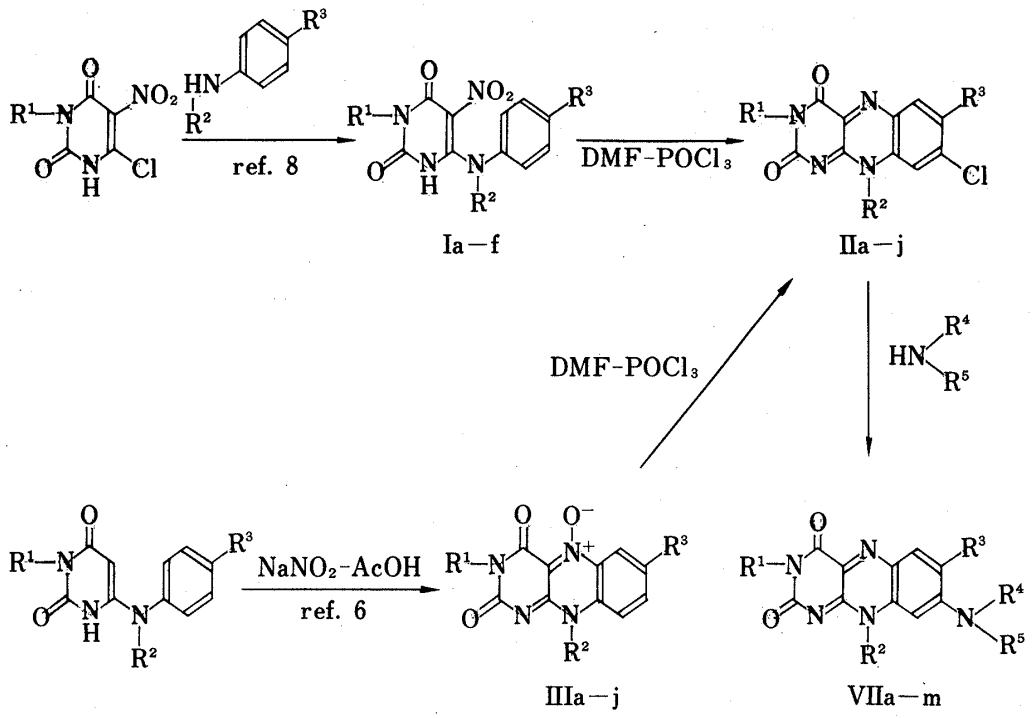


Chart 2

The conversion of I to II probably involves the initial formation of the flavin 5-oxides (III)[6] by dehydrative cyclization of I, followed by subsequent chlorination at the 8-position and loss of the N-oxide group (Chart 3).

8) F. Yoneda, Y. Sakuma, and K. Shinozuka, *J. Chem. Soc. Perkin I*, **1978**, 348.

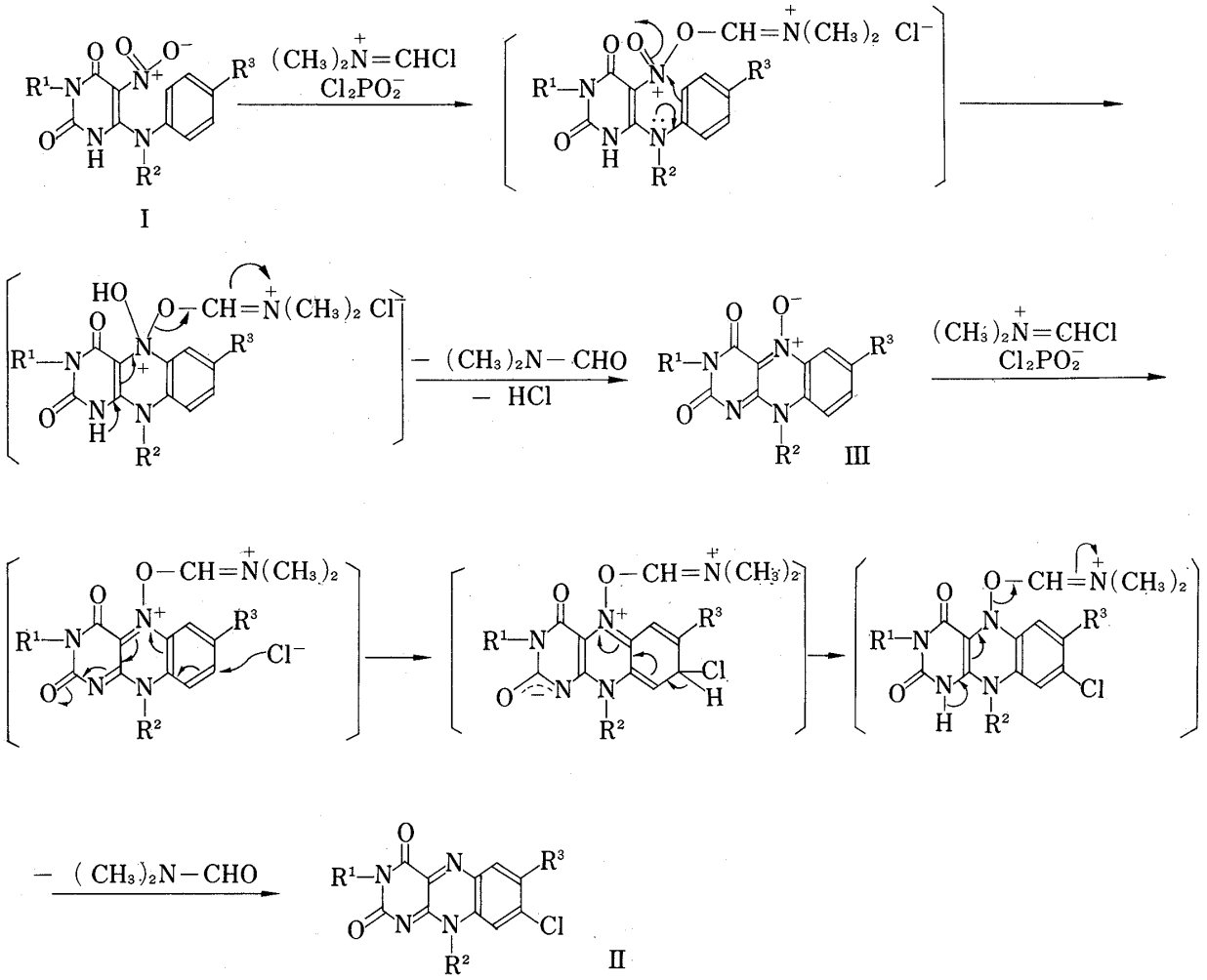


Chart 3

In fact, the treatment of 3,10-dimethylisoalloxazine 5-oxide (IIIa), prepared by nitrosative cyclization of 6-(N-methylanilino)-3-methyluracil,[6] with the Vilsmeier reagent gave the corresponding 8-chloroflavin (IIa) in good yield. Similarly, treatment of other flavin 5-oxides (IIIb—j) with the Vilsmeier reagent led to the formation of corresponding 8-chloroflavins (IIb—j) (Table I). However, the flavins themselves did not react with the Vilsmeier reagent, and the starting materials were recovered. This implies that the chloro group introduction at the 8-position is attributable to the N-oxide functionalization. Nucleophilic chlorination of aromatic N-oxides with loss of the N-oxide group has been reviewed;[9] many heterocyclic N-oxides have been converted into the corresponding chloro-heterocycles, mainly by using phosphorus oxychloride. However, the conversion of I or III into II required the Vilsmeier reagent; phosphorus oxychloride alone was not effective even under more drastic conditions, the starting materials being recovered.

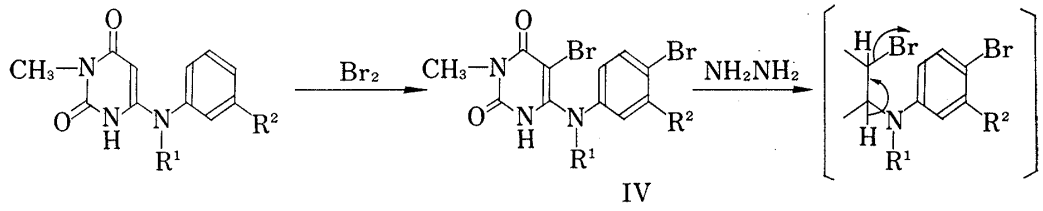
When flavin 5-oxides possess a bromo group at the 7-position, the chlorination at the 8-position was prevented, presumably because of steric hindrance and the electron-withdrawing effect of the bromine, and only loss of the N-oxide group took place. For example, treatment of 7-bromo-3,10-dimethylisoalloxazine 5-oxide (IIIl) with the Vilsmeier reagent under the same conditions gave 7-bromo-3,10-dimethylisoalloxazine (VIa). Furthermore, treatment

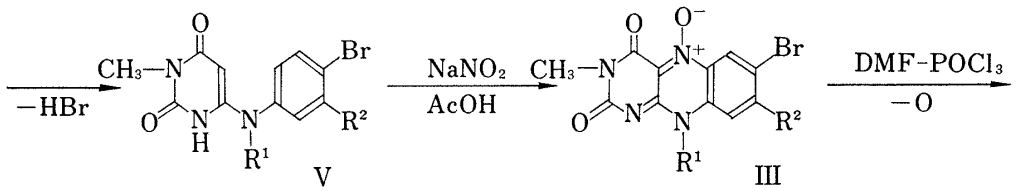
9) A.R. Katritzky and J.M. Lagowski, "Chemistry of Heterocyclic N-Oxides," Academic Press, 1971, pp. 259—270.

Table I. Syntheses of 8-Chloroflavins (II)

Compd. No.	R^1	R^2	R^3	Yield (%) Method A[a]	Yield (%) Method B[b]	mp (°C)[c]	Formula	Analysis (%) Calcd (Found) C	H	N
IIa	CH_3	CH_3	H	73	82	344	$C_{12}H_9ClN_4O_2$	52.09 (52.37	3.26 3.30	20.29 20.21)
IIb	CH_3	C_2H_5	H	71	92	321	$C_{13}H_{11}ClN_4O_2$	53.79 (53.81	3.79 3.69	19.31 19.40)
IIc	CH_3	n-C_3H_7	H	72	80	279	$C_{14}H_{13}ClN_4O_2$	55.26 (55.36	4.28 4.10	18.42 18.52)
IId	CH_3	n-C_4H_9	H	65	84	267	$C_{15}H_{15}ClN_4O_2$	56.60 (56.30	4.72 4.52	17.61 17.30)
IIe	CH_3	C_2H_5	CH_3	82	85	296	$C_{14}H_{13}ClN_4O_2$	55.26 (55.31	4.28 4.23	18.42 18.20)
IIf	H	CH_3	H		78	331 (dec.)	$C_{11}H_7ClN_4O_2$	50.30 (50.58	2.69 2.43	21.33 21.57)
IIg	H	C_2H_5	H		77	354 (dec.)	$C_{12}H_9ClN_4O_2$	52.09 (51.97	3.28 3.06	20.25 20.49)
IIh	H	n-C_3H_7	H		75	321 (dec.)	$C_{13}H_{11}ClN_4O_2$	53.71 (53.59	3.81 3.89	19.27 19.14)
IIi	H	n-C_4H_9	H		78	322 (dec.)	$C_{14}H_{13}ClN_4O_2$	55.18 (55.08	4.30 4.48	18.39 18.12)
IIj	H	C_2H_5	CH_3		75	340 (dec.)	$C_{13}H_{11}ClN_4O_2$	53.71 (53.49	3.81 3.55	19.27 19.23)
IIk	CH_3	C_6H_5	H	88		>360	$C_{17}H_{11}ClN_4O_2$	60.27 (60.37	3.27 3.31	16.54 16.27)

a) One-step synthesis of 8-chloroflavins (II) by the reaction of 5-nitro-6-(N-substituted-anilino)uracils (I) with the Vilsmeier reagent (dimethylformamide–phosphorus oxychloride).
b) 8-Chloroflavins (II) formation by the reaction of flavin 5-oxides (III) with the Vilsmeier reagent.
c) Compounds IIa—j were recrystallized from ethanol and compound IIk was recrystallized from acetic acid.





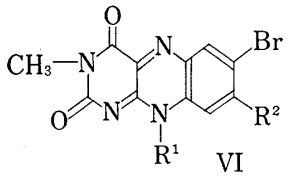


Chart 4

Table II. 6-(N-Alkyl-4-bromoanilino)-5-bromo-3-methyluracils (IV)

Compd. No.	R^1	R^2	Yield (%)	mp[a] (°C)	Formula	Analysis (%) Calcd C	H	N	Found C	H	N
IVa	CH_3	H	87	176	$C_{12}H_{11}Br_2N_3O_2$	37.05	2.85	10.80	36.90	3.10	10.69
IVb	C_2H_5	H	89	175	$C_{13}H_{13}Br_2N_3O_2$	38.74	3.26	10.42	38.49	3.18	10.34
IVc	C_2H_5	CH_3	78	176	$C_{14}H_{15}Br_2N_3O_2$	40.31	3.62	10.07	40.07	3.87	10.00
IVd	n-C_4H_9	H	72	172	$C_{15}H_{17}Br_2N_3O_2$	41.79	3.97	9.75	41.51	4.05	9.67

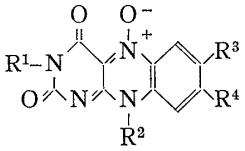
a) All products were recrystallized from ethanol.

Table III. 6-(N-Alkyl-4-bromoanilino)-3-methyluracils (V)

Compd. No.	R^1	R^2	Yield (%)	mp[a] (°C)	Formula	Analysis (%) Calcd C	H	N	Found C	H	N
Va	CH_3	H	90	269	$C_{12}H_{12}BrN_3O_2$	46.47	3.90	13.55	46.18	4.07	13.49
Vb	C_2H_5	H	92	226	$C_{13}H_{14}BrN_3O_2$	48.17	4.35	12.96	48.01	4.59	12.81
Vc	C_2H_5	CH_3	91	190	$C_{14}H_{16}BrN_3O_2$	49.72	4.77	12.42	49.49	5.01	12.37
Vd	n-C_4H_9	H	83	211	$C_{15}H_{18}BrN_3O_2$	51.15	5.15	11.93	50.90	5.26	11.87

a) All products were recrystallized from ethanol.

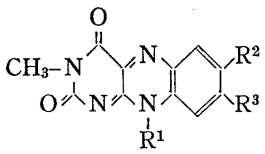
Table IV. Flavin 5-Oxides Formation by the Nitrosative Cyclization of 6-(N-Alkylanilino)uracils



Compd. No.	R^1	R^2	R^3	R^4	Yield (%)	mp[a] (°C)	Formula	Analysis (%) Calcd (Found) C	H	N
IIIe	CH_3	C_2H_5	CH_3	H	89	289	$C_{14}H_{14}N_4O_3$	58.73 (58.92	4.93 4.93	19.57 19.41)
IIIj	H	C_2H_5	CH_3	H	86	>360	$C_{13}H_{12}N_4O_3$	57.35 (57.09	4.44 4.42	20.08 20.30)
IIIl	CH_3	CH_3	Br	H	93	293	$C_{12}H_9BrN_4O_3$	42.75 (43.05	2.69 2.71	16.62 16.73)
IIIm	CH_3	C_2H_5	Br	H	87	275	$C_{13}H_{11}BrN_4O_3$	44.46 (44.71	3.16 3.09	15.95 15.71)
IIIn	CH_3	C_2H_5	Br	CH_3	90	255	$C_{14}H_{13}BrN_4O_3$	46.05 (45.79	3.59 3.60	15.34 15.41)
IIIo	CH_3	n-C_4H_9	Br	H	78	264	$C_{15}H_{15}BrN_4O_3$	47.51 (47.55	3.99 4.13	14.77 15.17)
IIIp	CH_3	C_2H_5	H	CH_3	92	292	$C_{14}H_{14}N_4O_3$	58.73 (59.02	4.93 5.04	19.57 19.41)

a) All products were obtained as orange or red needles and recrystallized from ethanol.

Table V. Formation of Flavins by the Reduction of Flavin 5-Oxides with the Vilsmeier Reagent



Compd. No.	R^1	R^2	R^3	Yield (%)	mp[a] (°C)	Formula	Analysis (%) Calcd C	Calcd H	Calcd N	Found C	Found H	Found N
VIa	CH_3	Br	H	91	314	$C_{12}H_9BrN_4O_2$	44.88	2.82	24.88	44.76	2.81	24.64
VIb	C_2H_5	Br	H	80	288	$C_{13}H_{11}BrN_4O_2$	46.59	3.31	16.72	46.63	3.29	16.89
VIc	C_2H_5	Br	CH_3	89	287	$C_{14}H_{13}BrN_4O_2$	48.16	3.75	16.04	47.97	3.77	16.17
VId	n-C_4H_9	Br	H	73	297	$C_{15}H_{15}BrN_4O_2$	49.60	4.16	15.43	49.40	4.12	15.26
VIe[8]	C_2H_5	H	CH_3	89	314	$C_{14}H_{14}N_4O_2$	62.21	5.22	20.73	62.03	5.22	20.71

a) All products were recrystallized from ethanol.

Table VI. 8-(Substituted-amino)flavins (VII)

Compd. No.	R^1	R^2	R^3	R^4	R^5	Yield (%)	mp[a] (°C)	Formula	Analysis (%) Calcd (Found) C	H	N
VIIa	CH_3	C_2H_5	CH_3	H	CH_3	90	>360	$C_{15}H_{17}N_5O_2$	60.19 (59.89)	5.72 6.24	23.40 23.10)
VIIb	CH_3	C_2H_5	CH_3	CH_3	CH_3	81	263	$C_{16}H_{19}N_5O_2$	61.32 (60.83)	6.11 6.27	22.35 21.70)
VIIc	CH_3	C_2H_5	CH_3	C_2H_5	C_2H_5	76	230	$C_{18}H_{23}N_5O_2$	63.32 (63.12)	6.79 6.29	20.52 20.82)
VIId	CH_3	C_2H_5	CH_3	$-(CH_2)_4-$		64	315	$C_{18}H_{21}N_5O_2$	63.70 (63.67)	6.24 6.12	20.64 20.20)
VIIe	CH_3	C_2H_5	CH_3	$-(CH_2)_5-$		58	293	$C_{19}H_{23}N_5O_2$	64.57 (64.20)	6.56 6.52	19.82 19.30)
VIIf	CH_3	C_2H_5	CH_3	$-CH_2CH_2-O-CH_2CH_2-$		55	319	$C_{18}H_{21}N_5O_3$	60.83 (60.44)	5.96 6.19	19.71 19.52)
VIIg	CH_3	C_2H_5	CH_3	H	$CH_2C_6H_5$	64	215	$C_{21}H_{21}N_5O_2$	67.18 (66.97)	5.64 5.75	18.66 18.43)
VIIh	CH_3	C_2H_5	H	H	CH_3	93	345	$C_{14}H_{15}N_5O_2$	58.93 (58.90)	5.30 5.03	24.55 24.30)
VIIi	CH_3	C_2H_5	H	H	C_2H_5	86	346	$C_{15}H_{17}N_5O_2$	60.19 (60.09)	5.72 5.59	23.40 23.29)
VIIj	CH_3	C_2H_5	H	H	n-C_3H_7	88	343	$C_{16}H_{19}N_5O_2$	61.32 (61.42)	6.11 6.32	22.35 22.05)
VIIk	CH_3	C_2H_5	H	H	n-C_4H_9	83	340	$C_{17}H_{21}N_5O_2$	62.36 (62.46)	6.47 6.35	21.39 21.59)
VIIl	H	CH_3	H	CH_3	CH_3	68	>360	$C_{13}H_{13}N_5O_2$	57.56 (57.47)	4.83 4.97	25.82 25.22)
VIIm	H	n-C_4H_9	H	CH_3	CH_3	59	320	$C_{16}H_{19}N_5O_2$	61.32 (61.58)	6.11 5.97	22.35 22.06)

a) All products were obtained as red needles and recrystallized from ethanol.

of 8-methylflavin 5-oxides (IIIn and p) with the Vilsmeier reagent caused only loss of the N-oxide group to give the corresponding 8-methylflavins (VIc and e) (Table V).

The 7-bromoflavin 5-oxides (IIIl—o) were prepared as follows. Bromination of 6-(N-alkylanilino)uracils[6] with bromine in ethanol readily gave 3-methyl-6-(N-alkyl-4-bromoanilino)-5-bromouracils (IVa—d) (Table II). The dibromouracils (IV) thus obtained were

treated with hydrazine hydrate in ethanol. This caused elimination of the 5-bromo group, probably through an addition–elimination reaction (Chart 4), to give the desired 6-(*p*-bromoanilino)uracils (Va—d) in high yields (Table III). Conventional nitrosation of the latter (V) gave the corresponding 7-bromoflavin 5-oxides (IIIl—o) (Table IV). Such debromination of 5-bromo group on the uracil ring by hydrazine hydrate has a precedent: treatment of 5-bromo-1,3-dimethyl-6-methylaminouracil[10] with hydrazine hydrate readily gave 1,3-dimethyl-6-methylaminouracil.

The 8-chloroflavins (II) thus obtained are very reactive to nucleophilic agents and treatment with amines led easily to the formation of the corresponding 8-(substituted-amino)flavins (VIIa—m) (Table VI). The structures of VII were supported by the satisfactory microanalytical and NMR spectral data.

Experimental[11]

8-Chloroflavins (II). General Procedure. Method A (for IIa—e and k)——Heating 6-(N-alkylanilino)-3-methyl-5-nitrouracils (Ia—e and k) (2 mmol) with dimethylformamide (30 mmol) and phosphorus oxychloride (4 mmol) at 90° for 1 hr, followed by dilution with ice-water, caused the separation of yellow crystals, which were collected by filtration and washed with water. Recrystallization from ethanol or acetic acid gave 8-chloroflavins (Table I).

Method B (for IIa—j)——Heating flavin 5-oxides (IIIa—j) (2 mmol) with dimethylformamide (30 mmol) and phosphorus oxychloride (4 mmol) at 90° for 20 min, followed by dilution with ice-water, caused the separation of yellow crystals, which were collected by filtration and washed with water. Recrystallization from ethanol gave 8-chloroflavins (Table I).

3-Methyl-5-nitro-6-(N-phenylanilino)uracil (Ik)——A mixture of 6-chloro-3-methyl-5-nitrouracil[12] (1 mmol) and diphenylamine (1 mmol) in ethanol (10 ml) was refluxed for 1 hr. After cooling, the mixture yielded crystals, which were filtered off, washed with water and recrystallized from ethanol to give pale yellow needles, mp 168°, in 70% yield. *Anal.* Calcd for $C_{17}H_{14}N_4O_4$: C, 60.35; H, 4.17; N, 16.56. Found: C, 60.34; H, 4.09; N, 16.76.

6-(N-Alkyl-4-bromoanilino)-5-bromo-3-methyluracils (IVa—d). General Procedure——A solution of a 6-(N-alkylanilino)uracil[6] (0.1 mol) in ethanol (30 ml) was treated with bromine (0.3 mol) drop by drop under stirring at room temperature. After being stirred for 30 min, the reaction mixture was concentrated *in vacuo* and the residue was treated with water. Crystals thus obtained were filtered off and recrystallized from ethanol to give yellow needles (Table II).

6-(N-Alkyl-4-bromoanilino)-3-methyluracils (Va—d). General Procedure——Hydrazine hydrate (0.5 mol) was added dropwise to a solution of a 6-(N-alkyl-4-bromoanilino)-5-bromo-3-methyluracil (IV) (0.1 mol) in ethanol (20 ml), and the mixture was stirred for 30 min at room temperature. The reaction mixture was diluted with water to provide colorless crystals, which were collected by filtration and recrystallized from ethanol to give colorless needles (Table III).

Flavin 5-Oxides (IIIe,j, and l—p). General Procedure——The starting materials for IIIe, j and IIIp, 6-(N-ethyltoluidino)uracils, were prepared by the conventional condensation[6] of 6-chlorouracils with N-ethyl-*p*-(or *m*-)toluidine [6-(N-ethyl-*p*-toluidino)-3-methyluracil (mp 186°, 87%), 6-(N-ethyl-*p*-toluidino)uracil (mp 280°, 91%) and 6-(N-ethyl-*m*-toluidino)-3-methyluracil (mp 145°, 74%)].

A 6-(N-alkylanilino)uracil (V and the above 6-(N-ethyltoluidino)uracils) (10 mmol) was suspended in acetic acid (30 ml) and sodium nitrite (3.5 g, 50 mmol) was added all at once to the mixture. The mixture was stirred at 0° to 5° for 20 min and then for 1 hr at room temperature. The resulting crystals were filtered off and washed with water. The filtrate was evaporated to dryness *in vacuo* and the residue was diluted with water to precipitate further crystals. The combined crystals were recrystallized from ethanol to give orange needles (Table IV).

Formation of Flavins (VIa—e) by the Deoxygenation of Flavin 5-Oxides (III) with the Vilsmeier Reagent——Treatment of a flavin 5-oxide (III) (2 mmol) with dimethylformamide (30 mmol) and phosphorus oxychloride (4 mmol) at 90° for 1 hr, followed by dilution with ice-water, gave the corresponding flavin (Table V). These compounds were identical with corresponding authentic samples prepared by the reduction of the above

10) H. Bredereck, G. Kupsch, and H. Wieland, *Chem. Ber.*, **92**, 583 (1959).
11) Melting points were taken on a Yanagimoto micro-melting point apparatus and are uncorrected. NMR spectra were determined with a JEOL-PMX 60 spectrometer (with tetramethylsilane as an internal standard). The identity of the compounds was confirmed by comparison of infrared spectra (Nujol mulls) using a JASCO IR-A1 spectrometer.
12) G.D. Daves, R.K. Robins, and C.C. Cheng, *J. Am. Chem. Soc.*, **84**, 1724 (1962).

flavin 5-oxides with sodium dithionite[6] or by thermal deoxygenation in refluxing dimethylformamide.[8]

8-(Substituted-amino)flavins (VIIa—e and g—m). General Procedure——A mixture of a 8-chloroflavin (1 mmol) and an amine (3 mmol) in dimethylformamide (3 ml) was heated at 60° for 2 hr. After cooling, the mixture yielded crystals, which were filtered off and recrystallized from ethanol to give the corresponding 8-(substituted-amino)flavins as red needles (Table VI).

10-Ethyl-3,7-dimethyl-8-morpholinoisoalloxazine (VIIIf)——A mixture of 8-chloroflavin (IIe) (1 mmol) and morpholine (3 mmol) in hexamethyl phosphoramide (HMPA) (3 ml) was heated at 100° for 3 hr. After cooling, the mixture was diluted with water to provide crystals, which were collected by filtration, washed with water and dried. Recrystallization from ethanol gave red needles (Table VI).

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