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A Convenient Synthesis of Toxoflavins, Toxoflavin 4-Oxides and 1-Demethyltoxoflavins

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A convenient synthesis of toxoflavins and toxoflavin 4-oxides, which consists of the nitrosative cyclization of the aldehyde hydrazones of 3-methyl-6-(1-methylhydrazino)uracil, is described. Both toxoflavins and toxoflavin 4-oxides gave the corresponding 1-demethyltoxoflavins (8-demethylfervenulins) by treatment with nucleophiles such as dimethylformamide, dimethylacetamide and acetic acid. The preparation of some 4,8-dihydrotoxoflavin derivatives is described.

The structure of toxoflavin, an antibiotic isolated from *Pseudomonas cocovenenans*, was determined by total synthesis in 1961.[2] The antibiotic xanthothricin[3] from a member of the genus *Streptomyces* was confirmed as being identical with toxoflavin. The present paper describes a new convenient synthesis of toxoflavins, toxoflavin 4-oxides and 1-demethyltoxoflavins.[4]

The direct cyclization of 6-anilinopyrimidines by treatment with various nitrosating agents was discovered by Goldner, *et al.*[5] as a new route to alloxazines and their 5-oxides. The nitrosation of this type has been shown to be effective for the cyclization of the aldehyde hydrazones of 1,3-dimethyl-6-hydrazinouracil to fervenulin and analogs.[6] We have extended this nitrosative cyclization to the preparation of toxoflavins and toxoflavin 4-oxides.

The key intermediates, the aldehyde hydrazones (IIa—n) of 3-methyl-6-(1-methylhydrazino)uracil (I),[2] were prepared by treatment of I with aliphatic as well as aromatic or heterocyclic aldehyde in ethanol at room temperature (Table I).

The hydrazones (IIb—n) thus obtained were treated with saturated aqueous solution of a slight excess of sodium nitrite in acetic acid at 5°. Crystals which separated were collected by filtration to give usually the corresponding toxoflavin 4-oxides (IVb—n). Dilution of the filtrate with water or ether gave the corresponding toxoflavins (IIIb—n). It should be noted that the above order of the precipitation may be oposite in some cases depending upon circumstances such as the substituent of 3-position and the experimental scale. Sometimes either toxoflavins (III) or toxoflavin 4-oxides (IV) were exclusively obtained. If products which precipitate are a mixture of III and IV, their separation could be easily carried out by fractional recrystallization from ethanol or by preparative thin-layer chromatography. Toxoflavin itself (IIIa) and toxoflavin 4-oxide (IVa) were synthesized without isolation of the corresponding formaldehyde hydrazone (IIa), because of its instability. Thus, stirring of I in acetic acid with an equimolar amount of 37% aqueous formaldehyde solution at 5° for *ca.* 15 minutes gave the hydrazone (IIa), which was *in situ* treated with saturated aqueous solution containing

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[1] Location: *Oe-honmachi, Kumamoto Kumamoto 862, Japan.*

[2] G.D. Daves, R.K. Robins, and C.C. Cheng, *J. Am. Chem. Soc.*, **83**, 3904 (1961).

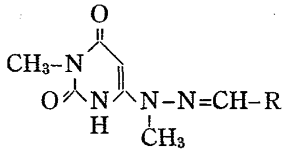
[3] R.A. Machlowitz, W.P. Fisher, B.S. McKay, A.A. Tytell, and J. Charney, *Antibiot. Chemotherapy*, **4**, 259 (1954).

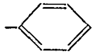
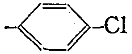
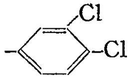
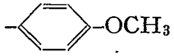
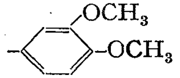
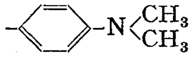
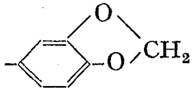
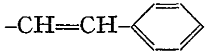
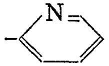
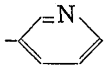
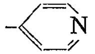
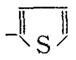
[4] A part of this paper has been reported in preliminary form. *a*) F. Yoneda, K. Shinomura, and S. Nishigaki, *Tetrahedron Letters*, **1971**, 851; *b*) F. Yoneda and T. Nagamatsu, *Tetrahedron Letters*, **1973**, 1577.

[5] H. Goldner, G. Dietz, and E. Carstens, *Ann.*, **694**, 142 (1966).

[6] G. Blankenform and W. Pfleiderer, *Chem. Ber.*, **105**, 3334 (1972).

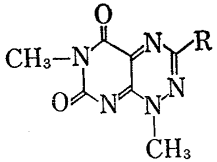
Table I.

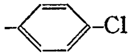
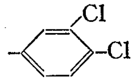
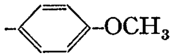
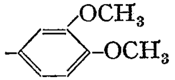
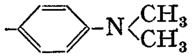
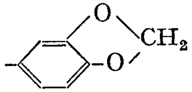
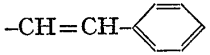
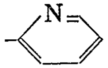
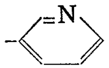
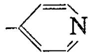
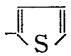


Compd. No.	R	mp (°C)	Yield (%)	Appearance[a)]	Formula	Analysis (%)					
						Calcd.			Found		
						C	H	N	C	H	N
IIa	-H	not isolated									
IIb	$-CH_3$	202	92	pale yellow powder	$C_8H_{12}O_2N_4$	48.97	6.17	28.56	48.91	6.19	28.50
IIc		245[b)]	96	pale yellow powder	$C_{13}H_{14}O_2N_4$	—	—	—	—	—	—
IId		194[b)]	97	colorless needles	$C_{13}H_{13}O_2N_4Cl$	—	—	—	—	—	—
IIe		261[b)]	71	colorless needles	$C_{13}H_{12}O_2N_4Cl_2$	—	—	—	—	—	—
IIf		223[b)]	92	colorless needles	$C_{14}H_{16}O_3N_4$	—	—	—	—	—	—
IIg		212	87	pale yellow needles	$C_{15}H_{18}O_4N_4$	56.59	5.70	17.60	56.61	5.79	17.65
IIh		244[b)]	88	colorless prisms	$C_{15}H_{19}O_2N_5$	—	—	—	—	—	—
IIi		273	95	colorless needles	$C_{14}H_{14}O_4N_4$	55.62	4.67	18.54	55.59	4.65	18.51
IIj		251	88	pale yellow powder	$C_{15}H_{16}O_2N_4$	63.36	5.67	19.71	63.31	5.61	19.51
IIk		249	89	pale yellow powder	$C_{12}H_{13}O_2N_5$	55.59	5.05	27.02	55.57	5.00	27.00
IIl		228	85	colorless needles	$C_{12}H_{13}O_2N_5$	55.59	5.05	27.02	55.61	5.11	27.21
IIm		270	90	pale yellow powder	$C_{12}H_{13}O_2N_5$	55.59	5.05	27.02	55.60	5.02	27.30
IIn		230	74	pale yellow powder	$C_{11}H_{12}O_2N_4S$	49.98	4.58	21.20	49.95	4.57	21.18

a) All compounds were recrystallized from EtOH.
b) F. Yoneda and T. Nagamatsu, *Synthesis*, **1973**, 300

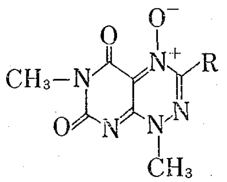
TABLE II.

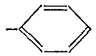
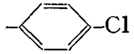
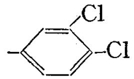
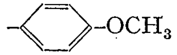
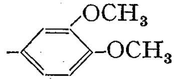
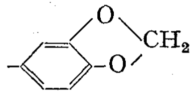
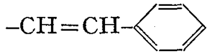
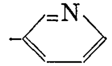
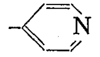


Compd. No.	R	mp (°C)	Yield (%)	Appearance[a]	Formula	Analysis (%)					
						Calcd.			Found		
						C	H	N	C	H	N
IIIa	-H	172[2]	40	yellow plates	$C_7H_7O_2N_5$	43.52	3.65	36.26	43.38	3.61	36.02
IIIb	-CH_3	181[2]	63	yellow needles	$C_8H_9O_2N_5$	46.37	4.38	33.80	46.42	4.42	33.53
IIIc		228 (dec.)	68	yellow needles	$C_{13}H_{11}O_2N_5$	57.98	4.12	26.01	58.06	4.07	26.20
IIId		227	35	yellow needles	$C_{13}H_{10}O_2N_5Cl$	51.41	3.32	23.06	51.30	3.29	23.11
IIIe		231	30	yellow needles	$C_{13}H_9O_2N_5Cl_2$	46.17	2.68	20.71	46.06	2.66	20.75
IIIf		244 (dec.)	71	orange needles	$C_{14}H_{13}O_3N_5$	56.18	4.38	23.40	56.33	4.37	23.22
IIIg		229	67	orange needles	$C_{15}H_{15}O_4N_5$	54.71	4.59	21.27	54.63	4.53	20.97
IIIh		270 (dec.)	87	dark yellow needles	$C_{15}H_{16}O_2N_6$	57.68	5.16	26.91	57.51	4.81	26.65
IIIi		264 (dec.)	45	orange needles	$C_{14}H_{11}O_4N_5$	53.67	3.54	22.36	53.72	3.58	22.31
IIIj		213	67	yellow needles	$C_{15}H_{13}O_2N_5$	61.01	4.44	23.72	60.79	4.20	24.01
IIIk		210	74	yellow needles	$C_{12}H_{10}O_2N_6$	53.33	3.73	31.10	53.15	3.55	30.88
IIIl		205 (dec.)	59	yellow needles	$C_{12}H_{10}O_2N_6$	53.33	3.73	31.10	53.13	3.60	31.11
IIIm		209 (dec.)	35	yellow needles	$C_{12}H_{10}O_2N_6$	53.33	3.73	31.10	53.45	3.71	30.83
IIIn		233	67	yellow needles	$C_{11}H_9O_2N_5S$	47.99	3.30	25.44	48.13	3.33	25.35

[a] All products were recrystallized from EtOH.

TABLE III.

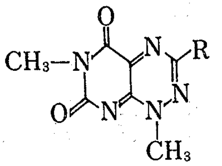


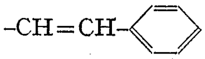
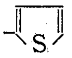
Compd. No.	R	mp (°C)	Yield (%)	Appearance[a)]	Formula	Analysis (%) Calcd. C	H	N	Found C	H	N
IVa	-H	189 (dec.)	35	yellow powder	$C_7H_7O_3N_5$	40.19	3.37	33.48	39.95	3.34	33.28
IVc		204 (dec.)	22	yellow powder	$C_{13}H_{11}O_3N_5$	54.78	3.89	24.55	54.64	3.86	24.63
IVd		221 (dec.)	60	yellow powder	$C_{13}H_{10}O_3N_5Cl$	48.84	3.15	21.91	48.55	3.14	21.95
IVe		207 (dec.)	62	yellow powder	$C_{13}H_9O_3N_5Cl_2$	44.09	2.56	19.78	43.91	2.49	19.80
IVf		230 (dec.)	28	yellow powder	$C_{14}H_{13}O_4N_5$	53.33	4.16	22.22	53.07	4.08	22.14
IVg		191 (dec.)	30	yellow powder	$C_{15}H_{15}O_5N_5$	52.17	4.38	20.28	52.25	4.30	20.16
IVi		233 (dec.)	42	orange powder	$C_{14}H_{11}O_5N_5$	51.06	3.37	21.27	50.79	3.45	20.97
IVj		209 (dec.)	20	yellow podwer	$C_{15}H_{13}O_3N_5$	57.87	4.21	22.50	57.93	4.48	22.23
IVl		200 (dec.)	35	yellow powder	$C_{12}H_{10}O_3N_6$	50.35	3.52	29.36	50.48	3.52	29.07
IVm		215 (dec.)	40	yellow powder	$C_{12}H_{10}O_3N_6$	50.35	3.52	29.36	50.65	3.70	29.29

a) All products were recrystallized from EtOH or dioxane.

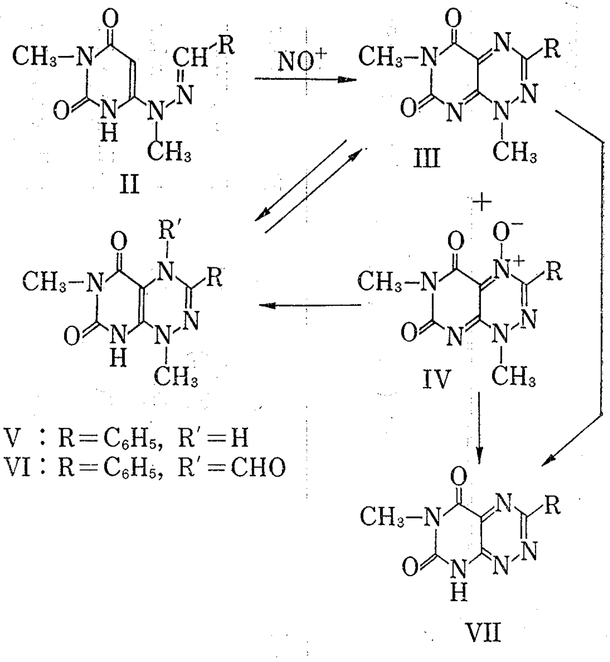
an equimolar amount of sodium nitrite for 20 minutes. Dilution of the reaction mixture with ether gave a mixture of IIIa and IVa. IIIa was identical in all respects with an authentic sample.[2] The structure of other 3-substituted toxoflavins (IIIb—n) were determined by elemental analyses and molecular weight determination by mass spectrometry. Moreover, their infrared (IR) spectra (see Fig. 1) showed quite similarity with the general pattern of IR spectrum of toxoflavin itself (IIIa). The structure of IVa and other toxoflavin 4-oxides (IVb—n) were assigned by elemental analyses, satisfactory spectral data, especially the presence of their strong parent ions and remarkable M-16 ions in their mass spectra, and furthermore a mode of the formation. The assigned structures of IV were confirmed with the formation of the corresponding toxoflavins by their mild reduction in methanol or ethanol using benzenethiol at room temperature, the latter being converted into phenyl disulfide. It is noted that these toxoflavin 4-oxides (IV) are the first representatives of the azapteridine N-oxide system.

TABLE IV.



Compd. No.	R	Procedure	Yield (%)
IIIa	–H	isoamyl nitrite in EtOH–AcOH (3 : 2) 20°, 30 min	35
IIIj		isoamyl nitrite in EtOH 80°, 30 min	67
IIIn		isoamyl nitrite in EtOH–trace of HCl 45°, 30 min	57

Some toxoflavins could be also obtained in moderate yields by treatment of the aldehyde hydrazones in ethanol with an equimolar amount of isoamyl nitrite. In these cases, however, the corresponding toxoflavin 4-oxides could not be obtained (Table IV).

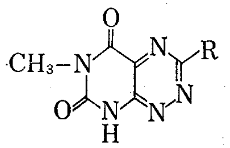


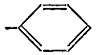
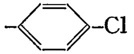
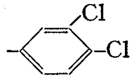
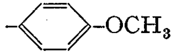
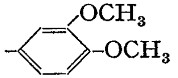
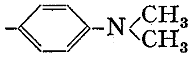
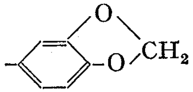
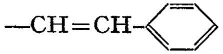
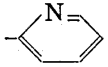
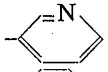
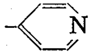
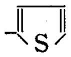
V : R=C_6H_5, R'=H
VI : R=C_6H_5, R'=CHO

Chart 1

Treatment of both III and IV with sodium dithionite in water at room temperature gave the corresponding 4,8-dihydrotoxoflavins. These dihydrotoxoflavins were oxidized to the original toxoflavins themselves, when they were allowed to stand in air for several days. For instance, 3-phenyl-4,8-dihydrotoxoflavin (V) thus obtained showed two NH absorptions at 3220 and 3070 cm^{-1} and a strong parent ion (*m/e* 271) in its mass spectrum. Refluxing of IIIc or IVc in formic acid in the presence of excess sodium dithionite gave 4-formyl-3-phenyl-4,8-dihydrotoxoflavin (VI), a fixed type of dihydrotoxoflavin. The structure of VI was based on microanalysis, infrared spectrum (the presence of a NH absorption band at 3230 cm^{-1}), molecular weight determination (*m/e* 299 (M$^+$)) by mass

TABLE V.



Compd. No.	R	mp (°C)	Yield (%)	Appearance[a]	Formula	Analysis (%) Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
VIIa	-H	210[7] (dec.)	85	yellow powder	$C_6H_5O_2N_5$	40.23	2.81	39.10	40.16	2.85	38.99
VIIb	$-CH_3$	247[7] (dec.)	89	yellow powder	$C_7H_7O_2N_5$	43.52	3.65	36.26	43.29	3.63	36.21
VIIc		>300	93	yellow powder	$C_{12}H_9O_2N_5$	56.47	3.55	27.44	56.23	3.62	27.31
VIId		>300	88	yellow powder	$C_{12}H_8O_2N_5Cl$	49.75	2.78	24.18	49.69	2.77	24.02
VIIe		>300	95	yellow powder	$C_{12}H_7O_2N_5Cl_2$	44.46	2.18	21.61	44.38	2.19	21.40
VIIf		>300	87	yellow powder	$C_{13}H_{11}O_3N_5$	54.73	3.89	24.55	54.49	3.73	24.24
VIIg		>300	95	yellow powder	$C_{14}H_{13}O_4N_5$	53.33	4.16	22.22	53.30	4.08	22.08
VIIh		>300	72	brown powder	$C_{14}H_{14}O_2N_6$	56.37	4.73	28.18	56.22	4.65	28.23
VIIi		>300	90	dark yellow powder	$C_{13}H_9O_4N_5$	52.18	3.03	23.41	52.36	3.29	23.26
VIIj		>300	95	yellow powder	$C_{14}H_{11}O_2N_5$	59.78	3.94	24.90	59.81	3.94	24.77
VIIk		>300	96	yellow powder	$C_{11}H_8O_2N_6$	51.56	3.15	32.80	51.47	3.13	32.66
VIIl		>300	97	yellow prisms	$C_{11}H_8O_2N_6$	51.56	3.15	32.80	51.50	3.19	32.61
VIIm		>300	80	yellow powder	$C_{11}H_8O_3N_6$	51.56	3.15	32.80	51.27	3.14	32.74
VIIn		>300	88	yellow powder	$C_{10}H_7O_2N_5S$	45.97	2.70	26.81	45.78	2.61	26.56

[a] All products were recrystallized from EtOH or DMF.

spectrometry and nuclear magnetic resonance (NMR) spectrum (the presence of a formyl proton at 8.61 ppm).

Next, it was found that refluxing both III and IV in dimethylformamide or dimethylacetamide for 30 minutes or heating them in acetic acid at 90—100° for 2—3 hr caused the demethylation of 1-position of III and IV and further deoxygenation in the cases of IV to give the corresponding 1-demethyltoxoflavins (8-demethylfervenulins) (VIIa—n) in excellent yields. 1-Demethyltoxoflavins (VII) thus obtained were identical in all respects with authentic samples prepared by an alternative route.[7] As some of VII have been prepared before in multiple steps,[7] this procedure offers a convenient synthetic method for 1-demethyltoxoflavins (VII). Fig. 1 shows IR spectra of IIIc, IVc and VIIc as typical examples of toxoflavins, toxoflavin 4-oxides and 1-demethyltoxoflavins.

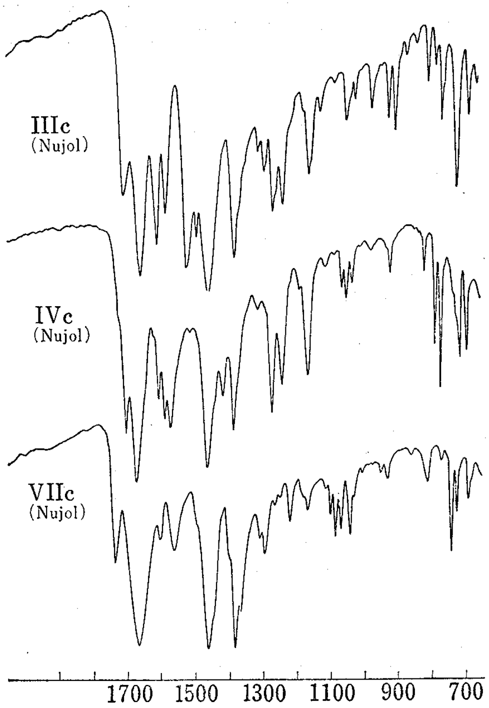


Fig. 1. IR Spectra of IIIc, IVc and VIIc (in Nujol)

A possible reaction mechanism has been proposed for this novel demethylation of III by several nucleophiles.[8] The conversion of toxoflavin 4-oxides (IV) to 1-demethyltoxoflavins (VII) by nucleophiles such as dimethylformamide, dimethylacetamide or acetic acid may be initiated by the demethylation of 1-position of IV to give transient 1-demethyltoxoflavin 4-oxides (VIII), which could not however be detected under any conditions. Subsequent deoxygenation of VIII could yield the 1-demethyltoxoflavins (VII) (Chart 2). This process is supported by following facts. If the deoxygenation of IV takes place in the first place, the corresponding toxoflavins (III) should be obtained and they must form stable toxoflavin radical anions with dimethylformamide as stated in a previous paper.[8] But any radical spieces including toxoflavin 4-oxide redical anions ($IV^{\overline{\cdot}}$) anticipated in Chart 2 could not be observed during the conversion of IV to VII by usual electron spin resonance (ESR)

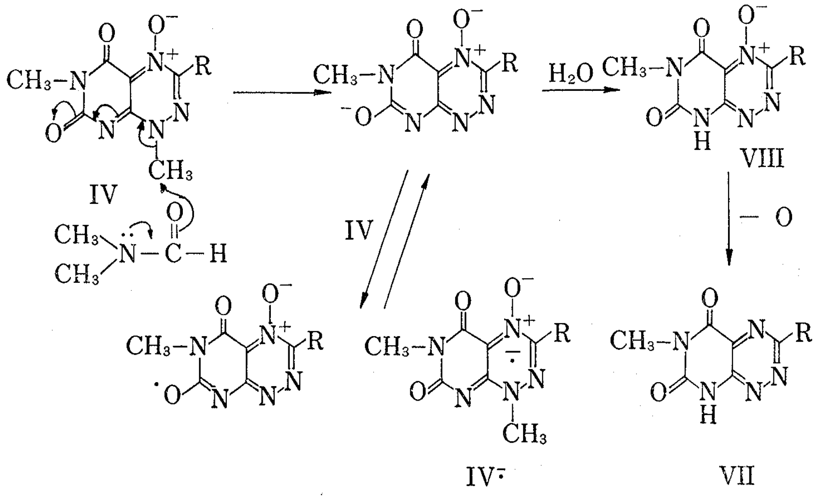


Chart 2

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[7] T.K. Liao, F. Baiocchi, and C.C. Cheng, *J. Org. Chem.*, **31**, 900 (1966).
[8] F. Yoneda and T. Nagamatsu, *J. Am. Chem. Soc.*, **95**, 5735 (1973).

techniques. In connection with this coversion, the reaction of IV with potassium *t*-butoxide in dimethylformamide gave the corresponding toxoflavin 4-oxide radical anions (IV$^{\bar{\cdot}}$), which were much shorter-lived than toxoflavin radical anions.[8] Fig. 2 shows the ESR spectrum of 3-phenyl-toxoflavin 4-oxide radical anion (IVc$^{\bar{\cdot}}$), whose hyperfine structure[9] consists of 10 lines with approximate intensity ratios 1:6:18:35:48:48:35:18:6:1. This spectrum can be explained by the synthesis based on the following coupling constants: $a_{N_1}=a_{N_4}=a_{H(N_1-CH_3)}=5.02$ G, $a_{N_2}=4.50$ G (Fig. 2).

It is interesting to note that the ultraviolet absorption maxima of IV showed blue shifts of about 3 nm compared with those of III (see Fig. 5). Furthermore, the NMR data in trifluoroacetic acid showed that the N-methyl protons of IV are at higher field than those of III. For instance, IVc showed two singlets of N-methyl protons at 3.48 and 4.40 ppm, while IIIc showed them at 3.64 and 4.63 ppm respectively.

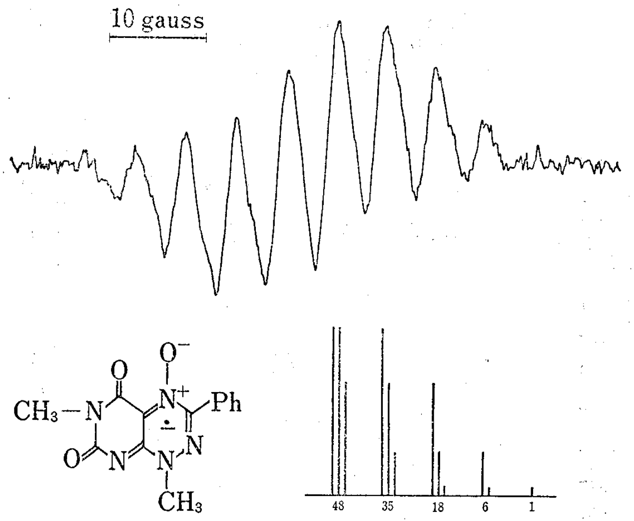

Fig. 2. ESR Spectra of 3-Phenyltoxoflavin 4-Oxide Radical Anion in DMF in the Presence of *t*-BuOK and Reconstruction based on the Following Constants [$a_{N_1}=a_{N_4}=a_{H(N_1-CH_3)}=5.02$ G, $a_{N_2}=4.50$ G]

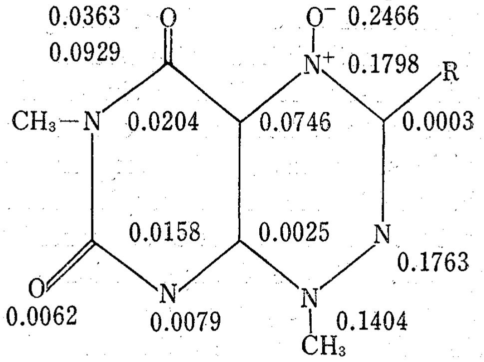

Fig. 3. Spin Densities of Toxoflavin N-Oxide Radical Anion Calculated Hückel MO Method

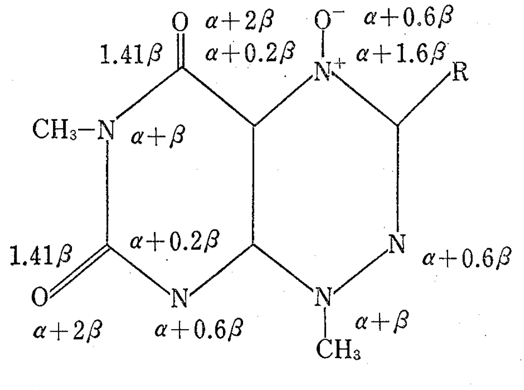

Fig. 4. The Parameters used in Calculation

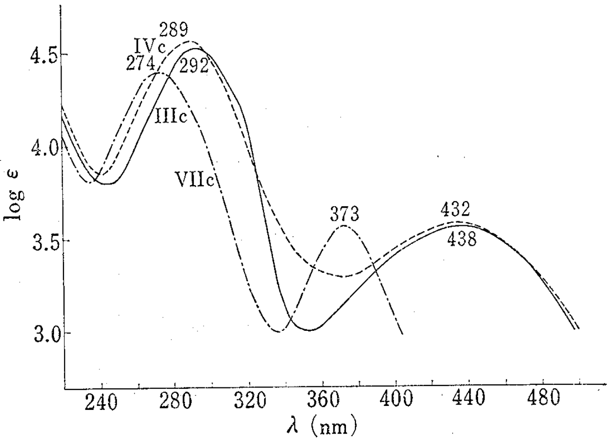

Fig. 5. Ultraviolet Spectra of IIIc, IVc and VIIc (EtOH)

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[9] The Hückel LCAO–MO calculation on the toxoflavin 4-oxide radical anion is consistent on the whole with the observed ESR spectrum.

Experimental[10]

Aldehyde Hydrazones (IIb—n) of 3-Methyl-6-(1-methylhydrazino)uracil (I)——Genral Procedure: To a stirred solution of I (0.01 mole) in EtOH (50—100 ml) was added the respective aldehydes (0.015 mole) at room temperature and the mixture was stirred for about 1 hr. The crystals which separated were collected and recrystallized from EtOH.

Toxoflavin Derivatives (IIIb—n) and Their 4-Oxides (IVb—n)——General Procedure: To a stirred solution of the aldehyde hydrazones (IIb—n) in acetic acid (50 ml) was added saturated aqueous solution of sodium nitrite (0.012 mole) drop by drop under cooling at 5°. The reaction solution was then stirred at room temperature for 2 hr, during which time yellow crystals were gradually separated. The crystals were collected by filtration to give IV or III. The filtrate was diluted with H_2O or ether to separate III or IV. All products were recrystallized from EtOH. If these products were mixtures of III and IV, their separation could be carried out by fractional recrystallization from EtOH or by preparative thin-layer chromatography [Silica Gel G acc. to Stahl, $CHCl_3$–MeOH (9: 1)].

1-Demethyltoxoflavin (8-Demethylfervenulin) and Its Derivatives (VIIa—n)——General Procedure: (A) Refluxing of IIIa—n or IVa—n (0.03 mole) in DMF or dimethylacetamide (50 ml) for 30 min, followed by cooling in a refrigerator separated 1-demethyltoxoflavin and its derivatives (VIIa—n). Recrystallization from dimethylformamide (DMF) or EtOH gave pale yellow prisms.

(B) Heating of IIIa—n or IVa—n (0.03 mole) in AcOH (100—200 ml) at 90—100° for 2—3 hr, followed by evaporation of the solvent and recrystallization of the residue from DMF or EtOH gave VIIa—n.

3-Phenyl-4,8-dihydrotoxoflavin (V)——Stirring of IIIc or IVc (5 g, 0.018 mole) in H_2O (120 ml) with $Na_2S_2O_4$ (5 g, 0.028 mole) at room temperature for 2 hr gave the corresponding 3-phenyl-4,8-dihydrotoxoflavin (V). The product which separated was filtered, dried and recrystallized from EtOH to give 3.4 g (72%) of pale brown crystals, mp>219° (decomp.). UV $\lambda_{\max}^{\text{dioxane}}$ nm (log ε): 253 (undetermined because of its insolubility). NMR (CF_3COOD) ppm: 3.42 (6H, singlet, equivalent two $N\text{-}CH_3$), 7.35—7.80 (5H, C_6H_5). Mass Spectrum *m/e*: 271 (M^+). *Anal.* Calcd. for $C_{13}H_{13}O_2N_5$: C, 57.56; H, 4.83; N, 25.82. Found: C, 57.50; H, 4.79; N, 25.61.

4-Formyl-3-phenyl-4,8-dihydrotoxoflavin (VI)——A mixture of IVc (4 g, 0.014 mole) and $Na_2S_2O_4$ (12 g, 0.069 mole) in 85% formic acid (100 ml) was refluxed for 3 hr. After cooling, the precipitates were filtered off, the filtrate was evaporated to a syrup under reduced pressure and diluted with a large amount of H_2O. The crystals thus separated were collected by filtration, dried and recrystallized from DMF-dioxane to give 2.2 g (52.5%) of colorless prisms, mp 292—294°. UV $\lambda_{\max}^{\text{dioxane}}$ nm (log ε): 277 (4.38). NMR (CF_3COOD) ppm: 3.58 and 3.99 (each 3H, each singlet, two $N\text{-}CH_3$), 7.30—7.90 (5H, C_6H_5), 8.61 (1H, singlet, CHO). Mass Spectrum *m/e*: 299 (M^+). *Anal.* Calcd. for $C_{14}H_{13}O_3N_5$: C, 56.18; H, 4.38; N, 23.40. Found: C, 55.89; H, 4.33; N, 23.51.

Reduction of IV to III with Benzenethiol——General Procedure: To a solution of a toxoflavin 4-oxide (0.005 mole) in MeOH or EtOH (100 ml) was added benzenethiol (0.01 mole) and the mixture was stirred at room temperature for 8 hr. After evaporation of the solvent, the residue was recrystallized from EtOH to give the corresponding toxoflavin in about 80% yield. From the filtrate phenyl disulfide was obtained.

Measurement of the ESR Spectrum——ESR measurement was carried out at room temperature using a JEOL P-10 apparatus at the X-bands and 100 kcps field modulation.

3-Phenyltoxoflavin 4-oxide radical anion was formed by adding *t*-BuOH containing an excess of potassium *t*-butoxide to a DMF solution of 3-phenyltoxoflavin 4-oxide (IVc).

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[10] All melting points were not corrected. Infrared spectra were determined on a Japan Spectroscopic Co., Ltd. spectrophotometer, Model IR-I A, from samples mulled in Nujol. NMR spectra were determined at 60 MHz using tetramethylsilane as the internal standard.