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Oxaline and Neoxaline

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Oxaline (**1**) has been isolated from *Penicillium* species Fg-234 and the structure of the compound (**2**) obtained by treatment of **1** with hydrochloric acid is deduced on the basis of spectral data. Neoxaline (**9**) isolated from *Aspergillus japonicus* Fg-551 has a structural framework similar to that of **1**.

Keywords——alkaloids; α-carbolines; structure elucidation; nuclear magnetic resonance (^{1}H and ^{13}C); carbon-13-proton selective proton-decoupling

In the course of our studies on alkaloids from microorganisms, the alkaloids (**1**) and (**9**) having unique structure were isolated from culture broths of *Penicillium* species Fg-234 and *Aspergillus japonicus* Fg-551, respectively. The alkaloid (**1**) was biologically inactive and has been established as oxaline. The alkaloid (**9**) was found to stimulate the central nervous system and was named neoxaline by one of us (S. Ō.).[2] This paper describes the structure elucidation of these alkaloids and related compounds.

Oxaline (1)

The alkaloid (**1**), $C_{24}H_{25}N_5O_4$, possesses several interesting structural features, *e.g.*, an N–OMe group, a dehydrohistidine unit, a reversed isopentenyl group and a single carbon atom having three nitrogen functionalities. The physical and spectral properties are in accord with those of oxaline isolated from *Penicillium oxalicum* M-555.[3] Also, **1** exhibited unusual chemical characteristics, as reported in the literature.[3]

Treatment of **1** with hydrochloric acid gave the compound (**2**), $C_{18}H_{14}ClN_5O_3$, which was resulted from the formal addition of hydrogen chloride and the loss of the isopentenyl group and methanol. The structure of **2** has not yet been decided.[3b] We attempted to elucidate its structure as follows. Examinations of optical rotatory dispersion and circular dichroism (CD) showed that **2** was optically inactive, *e.g.*, C-2 and -3 were not asymmetric. The ultraviolet (UV) spectrum revealed that **2** had a conjugated system different from that in **1** (see "Experimental"). The proton magnetic resonance (^{1}H NMR) spectrum of **2** provided an ABC system due to a 1,2,4-trisubstituted benzene [δ 7.04 (dd, J 9 and 2 Hz, 7-H), 7.01 (d, J 2 Hz, 5-H) and 6.70 (d, J 9 Hz, 8-H)], suggesting the presence of a chlorine atom in the benzene ring (C-6). Two one-proton singlets at δ 9.09 and 6.71 (2″- and 4-H's), a two-proton singlet at δ 7.91 (3′- and 5′-H's) and a three-proton singlet at δ 3.71 (3-OMe) were also observed in addition to two two-proton singlets (broad) at δ *ca.* 7.05 and 6.02 (4×NH), exchangeable with deuterium oxide. The presence of one methyl, seven methine and ten quaternary carbons in **2** was confirmed by the proton-noise-decoupled and off-resonance proton-decoupled ^{13}C NMR spectra. Each carbon was assigned by gated and carbon-13-proton selective proton-decouplings (Table III). One quaternary carbon resonating at δ 128.1 and two methine carbons at δ 138.2 and 133.1 were not observed clearly in the gated proton-decoupled spectrum. However, since nine quaternary carbons were reasonably assigned, the remaining one (δ 128.1)

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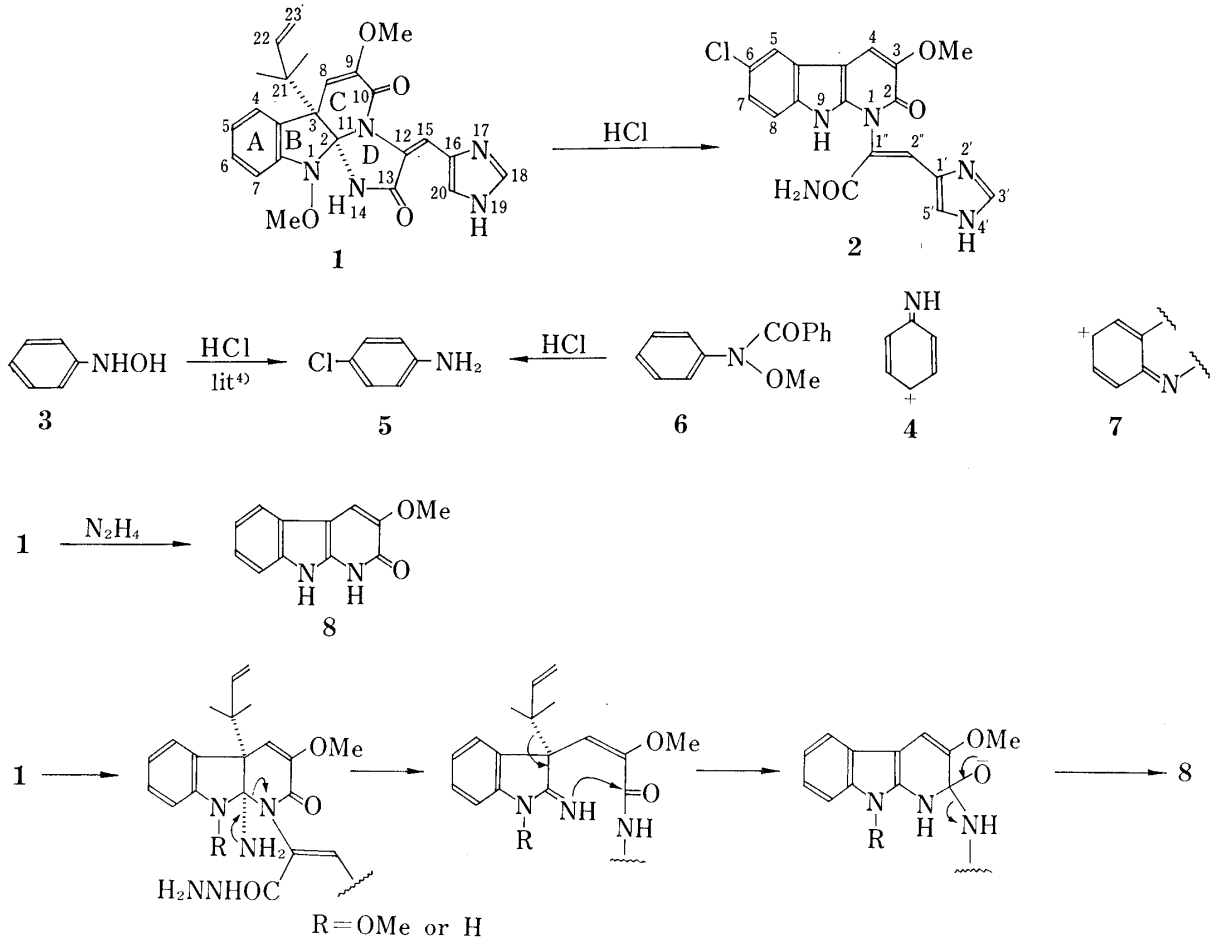

Chart 1

Table I. ^{1}H NMR Data for **1** and **9**

H	**1**[a]	**1**[a,b]	**9**[c]
4	7.57, d(8)[d]	7.60	7.58, dd (8 and 2)
5	7.06, t(8)	7.09	7.02, dt (2 and 8)
6	7.27, t(8)	7.23	7.27, dt (2 and 8)
7	6.95, d(8)	6.92	6.91, dd (8 and 2)
8	5.12, s	5.14	2.44, t (12)
			2.23, dd (12 and 6)
9			4.60, dd (12 and 6)
15	8.32, s	8.33	8.29, s
18	7.42, s	7.44	7.79, s
20	7.02, s	7.02	7.31, s
22	6.09, dd (18 and 10)	6.10, dd (18 and 10)	6.12, dd (18 and 10)
23	5.06, d(18)	5.05, d(10)	5.11, d(18)
	5.02, d(10)	4.95, d(18)	*ca.* 4.97
1-OMe	3.70, s	3.72	3.73, s
9-OMe	3.62, s	3.64	
21-Me_2	1.29, s	1.32	1.31, s
	1.25, s	1.28	1.31, s
NH[e]	12.76, s	12.72	8.98, br s
	9.59, s	9.70	2.32, s
OH[e]			*ca.* 1.31

a) $CDCl_3$. *b*) Lit.[3b] The coupling constants of the aromatic protons were not described.
c) Me_2CO-d_6. *d*) The figures in parentheses are the coupling constants (Hz).
e) On addition of D_2O, this signal disappeared.

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TABLE II. ^{13}C NMR Data for **1** and **9** ($CDCl_3$)

C	**1**	**1**[a]	**9**[b]	C	**1**	**1**[a]	**9**[b]
2	101.7, s	101.6, s	100.6, s	13	166.3, s	166.1, s	165.6, s
3	52.5, s	52.6, s	53.3, s	15	109.6, d	109.7, d	110.3, d
3a	126.1, s[c]	146.5, s	128.2, s	16	126.1, s[c]	126.2, s	125.8, s
4	124.7, d	124.7, d	124.8, d	18	136.4, d	136.4, d	136.8, d
5	123.2, d	123.3, d	123.6, d	20	133.8, d	133.8, d	134.4, d
6	128.5, d	128.4, d	128.9, d	21	42.5, s	42.5, s	43.5, s
7	112.0, d	112.0, d	111.5, d	22	142.8, d	142.8, d	144.5, d
7a	146.8, s[c]	146.6, s	145.7, s	23	113.9, t	113.9, t	114.1, t
8	106.9, d	107.0, d	40.1, t	1-OMe	65.2, q	65.2, q	65.2, q
9	146.4, s[c]	126.0, s	66.4, d	9-OMe	55.7, q	55.7, q	
10	157.5, s	157.3, s	171.6, s	21-Me_2	24.1, q	24.1, q	24.7, q[d]
12	123.2, s	123.1, s	122.3, s		23.7, q	23.7, q	24.5, q[d]

a) Lit.[3b]
b) These assignments were based on the data for **1**.
c) See "Experimental."
d) This signal was not observed clearly. This value was taken in Me_2SO-d_6 at 130°.

TABLE III. ^{13}C NMR Data for **2** (Me_2SO-d_6)

C	δ (ppm)[a]	$^1J_{CH}$(Hz)	$^{>1}J_{CH}$(Hz)	Irradiated[b] H	Resultant splitting
2	154.7, S, d		8	4-H	s
3	143.4, S, m			3-OMe	d (3 Hz)
				4-H	q (4 Hz)
4	116.1, D, d[c]	160		4-H (8-H)	s
4a	94.5, S, br s			4-H	Sharpened s
4b	119.7, S, dd		4	4-H	s
			7	8-H	
5	128.1, D, dd	164	7	5-H	s
				7-H	
6	132.4, S, d[d]		7	8-H (4-H)	s
7	129.9, D, dd	164	7	5-H	s
				7-H	
8	117.3, D, d[c]	160		8-H (4-H)	s
8a	145.6, S, t		7	5-H	s
				7-H	
9a	118.6, S, d[d]		7	4-H (8-H)	s
1′	128.1, s[e]				
3′	138.2, D[e]				
5′	133.1, D[e]				
1″	124.1, S, d		6	2″-H	s
2″	115.8, D, dd	160	4	2″-H	br s
3-OMe	56.1, Q[f]				
1″-$CONH_2$	162.6, S, d		10	2″-H	s

a) Capital and small letters refer to the splittings observed in the off-resonance and gated proton-decoupled spectra, respectively.
b) 4-H (δ 6.71), 8-H (δ 6.70), 5-H (δ 7.01), 7-H (δ 7.04). Power level: 48 dB for one-bond coupling; 36 dB for two- and three-bond couplings.
c,d) Assignments may be reversed.
e) This signal was not observed clearly in the gated proton-decoupled spectrum.
f) This signal was not measured in the gated proton-decoupled spectrum.

should be C-1′. Assignments of the two methine carbons resonating at δ 138.2 and 133.1 to C-3′ and -5′, respectively, were made by comparison with the chemical shifts of the corresponding carbons in **1**. Although two methine carbons resonating at δ 129.9 and 128.1 had the same splitting, assignments to C-5 (δ 128.1) and -7 (δ 129.9) were based on the chemical shifts of related compounds. The formation of a double bond at C-2 and -3 is presumably a result of the elimination of the isopentenyl group and the fission of the C(2)–N(14) bond in **1** by a one-step or two-step process. It is known that phenylhydroxylamine (**3**) is converted into *p*-chloroaniline (**5**) *via* the ion (**4**) by the action of hydrochloric acid.[4] Treatment of N-methoxybenzanilide (**6**)[5] with hydrochloric acid also afforded **5**. Oxaline (**1**) would give **2** *via* the ion (**7**) before or after the formation of the double bond at C-2 and -3.

Treatment of **1** with hydrazine afforded the compound (**8**), $C_{12}H_{10}N_2O_2$, which was thought to result from the loss of the D ring with the dehydrohistidine unit, the isopentenyl and methoxyl groups, accompanied by reduction. Its spectral data are in accord with those for the structure shown for **8** (see "Experimental"). The compound (**8**) may arise *via* a path including reductive cleavage of the N(1)-OMe bond with hydrazine (in air) at some stage (Chart 1).

Neoxaline (**9**)

Neoxaline (**9**), $C_{23}H_{25}N_5O_4$, showed absorptions at 330 and 237 nm associated with an extended imidazole moiety in the UV spectrum. Its infrared (IR) spectrum exhibited bands at 3500 (OH), 3425 (CONH), 3200 (NH, imidazole), 1710 and 1685 (NC=O). The ^{1}H and ^{13}C NMR spectra of **9** are very similar to those of **1** except in the following respects. The presence of an ABX system consisting of resonances at δ 4.60 (dd, J 12 and 6 Hz), 2.44 (t, J 12 Hz) and 2.23 (dd, J 12 and 6 Hz), corresponding to 9-H and 8-H_2, respectively, was observed in the ^{1}H NMR spectrum. The ^{13}C NMR spectrum of **9** showed signals due to C-8, -9 and -10 at δ 40.1, 66.4 and 171.6, respectively.

Oxidative acetylation of **9** with dimethyl sulfoxide and acetic anhydride afforded the compounds (**10**) and (**11**). The compound (**10**) showed a band at 1770 cm^{-1} (OC=O) in the IR spectrum, and a one-proton singlet (vinylic H) at δ 5.97 and a three-proton singlet (Me) at δ 2.26 in the ^{1}H NMR spectrum. These spectral data support the presence of an enol acetate function in **10**. The compound (**11**), a minor product, has a methylthiomethyl group in the molecule [^{1}H NMR: δ 4.88 (2H, s) and 2.11 (3H, s)].

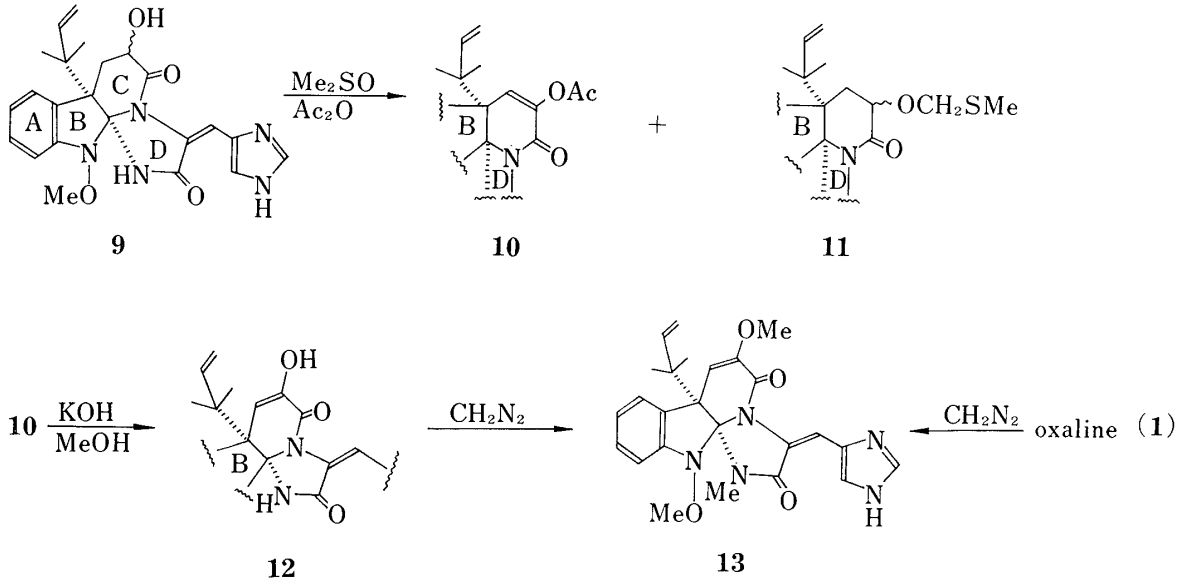

Chart 2

5) E. Bamberger, *Chem. Ber.*, **52**, 1111 (1919).

Hydrolysis of **10** with methanolic potassium hydroxide gave the compound (**12**), in which the presence of an enol function was confirmed on the basis of the ^{1}H NMR spectrum showing a one-proton singlet (vinylic H) at δ 5.50 and no methyl signal of the acetoxy group.

Methylation of **12** with diazomethane yielded 14-methyloxaline (**13**), which was identical with an authentic sample derived from **1** by comparison of spectral properties and mixed melting point determination.

Thus, from these results, it is clear that **9** has a structure similar to that of **1** except as regards the substituent at C-9, and the double bond at C-8 and -9. The 9-OH conformation in **9** is assigned to be equatorial, judging from the coupling constant of 9-H (*vide supra*) in the ^{1}H NMR spectrum. X-Ray analysis of **1** showed the C(2)–N(14) and C(3)–C(21) bonds to be equatorial and axial, respectively, with respect to the C ring.[3a] If the *cis* B/C ring fusion in **1** and **9** is assumed to be conformationally the same on the basis of the similarity of ^{13}C NMR data, and if the C ring has a chair form, the 9-OH group in **9** is *trans* to the 3-isopentenyl group. Further investigation of this point is required.

Experimental

Melting points were determined on a micro hot-stage apparatus and are uncorrected. UV spectra were recorded on a Hitachi EPS-2U spectrometer. Optical rotations were measured with a JASCO DPI-181 polarimeter. CD curves were taken on a JASCO J-20 spectrometer. IR spectra were measured with a JASCO IR-G spectrometer in chloroform unless otherwise noted. ^{1}H and ^{13}C NMR spectra were taken on a JEOL JNM PS-100 spectrometer at 100 and 25.1 MHz, respectively. Mass spectra (MS) were measured with a JEOL JMS-OIS spectrometer.

Oxaline (1)——The culture of *Penicillium sp.* Fg-234 was maintained on potato-dextrose-agar or as freeze-dried stock.

The fermentation was performed at 27° for three days using a 100 liter tank fermenter containing a medium (70 l) composed of glucose (2.0%), pentose (0.5%), NaCl (0.2%), K_2HPO_4 (0.1%) and $CaCO_3$ (0.3%).

The culture broth (68 l) containing mycelia was adjusted to pH 10 with aqueous ammonia and extracted with butyl acetate (10 l). The extracts were concentrated *in vacuo* to one-tenth of the initial volume, and the precipitate was removed by filtration. The filtrate was dried over Na_2SO_4, and concentrated *in vacuo* to a small volume to yield pale brownish crystals (2.0 g). Its recrystallization from acetone afforded **1** (1.5 g) as colorless prisms of mp 230—232° (dec.) (lit.[3b]: mp 220—221°) with $[\alpha]_D^{22}$ −45° (c=0.3, MeOH). UV λ_{max}^{MeOH} nm (ε): 345 (25200), 228 (21300). CD (c=0.001, MeOH) $[\theta]^{21}$ (nm): −24400 (344) (negative maximum), +38200 (273) (positive maximum), +34300 (260) (negative maximum), +42000 (247) (positive maximum), −98300 (223) (negative maximum). IR ν_{max} cm^{-1}: 3420 (CONH), 3180 (NH), 1710 and 1680 (NC=O). ^{1}H NMR (Table I). ^{13}C NMR (Table II).[6] *Anal.* Calcd for $C_{24}H_{25}N_5O_4$: C, 64.68; H, 5.73; N, 15.33. Found: C, 64.59; H, 5.68; N, 15.33. MS *m/e*: M$^+$, 447.189 (M, 447.190).

1-[α-Carbamoyl-β-(imidazol-4-yl)ethenyl]-6-chloro-1,2-dihydro-3-methoxy-2-oxo-α-carboline (2)——A solution of **1** (60 mg) in 20% HCl (2 ml) was stirred at room temperature for 24 hr. The precipitate was collected by filtration, washed with acetone and poured into aqueous ammonia. After filtration, and washing with water (0.4 ml) and acetone (0.4 ml), the solid was recrystallized from acetone/methanol to yield **2** (31 mg) as orange granules of mp 295° (dec.). UV λ_{max}^{MeOH} nm (ε): 450 (13100), 374 (22900), 340 (27900), 336 (15400), 320 (26800), 234 (24100). IR ν_{max}^{KBr} cm^{-1}: 3450—3050 (NH), 1700 and 1680 (NC=O). ^{1}H NMR (Me_2SO-d_6) δ: 9.09 (1H, s, 2″-H), 7.91 (2H, s, 3′- and 5′-H's), 7.04 (1H, dd, J 9 and 2 Hz, 7-H), 7.01 (1H, d, J 2 Hz, 5-H), 6.71 (1H, s, 4-H), 6.70 (1H, d, J 9 Hz, 8-H), 3.71 (3H, s, 3-OMe). ^{13}C NMR (Table III). MS *m/e*: M$^+$, 385.075 and 383.078. Calcd for $C_{18}H_{14}Cl^{37}N_5O_3$ and $C_{18}H_{14}Cl^{35}N_5O_3$: M, 385.075 and 383.078.

6) Four quaternary carbons resonating at δ 146.7, 146.4 and 126.1 (2) were assigned on the basis of gated and long-range selective proton-decoupling experiments in the ^{13}C NMR spectrum. The gated proton-decoupled spectrum showed a triplet (J 7 Hz) at δ 146.7 and two multiplets at δ 146.4 and 126.1. Irradiation of the 9-OMe (δ 3.62) converted the multiplet at δ 146.4 into a broad singlet and left the multiplet at δ 126.1 unchanged. On irradiation of 8-H (δ 5.12), the multiplets at δ 146.4 and 126.1 changed to a quartet (J 5 Hz) and a sharpened splitting, respectively. Irradiation of 6-H (δ 7.27) changed the triplet at δ 146.7 to a singlet, the multiplet at δ 126.1 to a broad singlet and left the multiplet at δ 146.4 unchanged. Irradiation at δ 7.27 is thought to saturate more-or-less all protons in the benzene and imidazole rings. These data suggest the following signal assignments: δ 146.7 to C-7a, δ 146.4 to C-9 and δ 126.1 (2) to C-3a and -16. The assignments to C-3a and -9 are different from those reported in the literature.[3b]

Reaction of N-Methoxybenzanilide (6) with Hydrochloric Acid——A mixture of **6** (50 mg) in 20% HCl (2 ml) was stirred at 55° for 6 hr. Benzoic acid (17 mg) formed was removed by filtration, and the filtrate was made alkaline with 20% aq. NaOH, then extracted with benzene. Work-up gave **5** (7 mg) as colorless plates of mp 73—74° (from hexane) which was identical with an authentic sample of *p*-chloroaniline by comparison of the IR spectrum and mixed melting point determination. IR ν_{max}^{KBr} cm^{-1}: 3475 and 3385 (NH).

1,2-Dihydro-3-methoxy-2-oxo-α-carboline (8)——A solution of **1** (20 mg) in anhyd. N_2H_4 (1 ml) was stirred at 110° for 20 hr. The reaction mixture was then concentrated *in vacuo* and extracted with chloroform. The residue obtained from the chloroform solution was purified by prep. TLC[7] (silica gel; chloroform/methanol=10/1, v/v) to afford **8** (4 mg) as colorless granules of mp 245—247° (from chloroform/hexane), *Rf* 0.33. UV λ_{max}^{MeOH} nm (ε): 363 (9310), 320 (4580), 280 (5530), 242 (18600). IR ν_{max} cm^{-1}: 3250 (NH), 1645 (NC=O). ^1H NMR (Me_2SO-d_6) δ: 11.18 (1H, s, NH),[8] 7.83 (1H, dd, *J* 8 and 2 Hz, 5-H), 7.76 (1H, s, 4-H), 7.37 (1H, dd, *J* 8 and 2 Hz, 8-H), 7.17 (1H, dt, *J* 2 and 8 Hz, 7-H), 7.05 (1H, dt, *J* 2 and 8 Hz, 6-H), 4.14 (1H, br s, NH),[8] 3.80 (3H, s, 3-OMe). ^{13}C NMR (Me_2SO-d_6) δ: 154.9 (s, C-2), 140.2 and 139.6 (s each, C-3 and -8a), 136.5 (s, C-9a), 122.5 (d, C-7), 121.8 (s, C-4b), 118.9 (d, C-6), 118.3 (d, C-5), 112.3 and 111.0 (d each, C-4 and -8), 102.7 (s, C-4a), 56.2 (q, 3-OMe). MS *m/e*: M^+, 214.075. Calcd for $C_{12}H_{10}N_2O_2$: M, 214.074.

Neoxaline (9)[2]——Colorless needles of mp 202° (dec.) (from benzene) with $[\alpha]_D^{24}$ −16.3° (*c*=1.0, $CHCl_3$). UV λ_{max}^{MeOH} nm (ε): 330 (29560), 237 (17620). CD (*c*=0.0009, EtOH) $[\theta]^{23}$ (nm): −430 (372) (negative maximum), +1200 (339) (positive maximum), +800 (335.5) (negative maximum), +1200 (328) (positive maximum), +800 (311) (negative maximum), +4800 (290) (positive maximum), +4750 (285) (negative maximum), +8970 (267) (positive maximum), −11200 (245.5) (negative maximum), +5600 (223) (positive maximum). ^1H NMR (Table I). ^{13}C NMR (Table II). *Anal.* Calcd for $C_{23}H_{25}N_5O_4$: C, 63.43; H, 5.79; N, 16.08. Found: C, 63.23; H, 5.70; N, 15.90. MS *m/e*: M^+, 435.191 (M, 435.190).

8,9-Dehydroneoxaline O-Acetate (10) and Neoxaline Methylthiomethyl Ether (11)——A solution of **9** (15 mg) in dimethyl sulfoxide (0.1 ml) and acetic anhydride (0.2 ml) was stirred at room temperature for 20 hr, and then ethanol (5 ml) was added to destroy excess acetic anhydride. After concentration *in vacuo*, the residue was made alkaline with 5% aqueous ammonia and extracted with chloroform. The residue obtained from the chloroform solution was purified by prep. TLC (silica gel; chloroform/methanol=30/1, v/v). The zone with *Rf* 0.20 gave **10** (12 mg) as colorless granules of mp 176—178° (from benzene/hexane). IR ν_{max} cm^{-1}: 3425 and 3200 (NH), 1770 (OC=O), 1710 and 1685 (NC=O). ^1H NMR ($CDCl_3$) δ: 12.70 and 8.60 (1H each, NH's),[8] 8.31 (1H, s, 15-H), 7.58 (1H, dd, *J* 8 and 1 Hz, 4-H), 7.55 (1H, s, 18-H), 7.32 (1H, dt, *J* 1 and 8 Hz, 6-H), 7.16 (1H, s, 20-H), 7.08 (1H, dt, *J* 1 and 8 Hz, 5-H), 6.98 (1H, dd, *J* 8 and 1 Hz, 7-H), 6.12 (1H, dd, *J* 18 and 11 Hz, 22-H), 5.97 (1H, s, 8-H), 5.13 (1H, d, *J* 18 Hz, 23-H), 5.06 (1H, d, *J* 11 Hz, 23-H), 3.77 (3H, s, 1-OMe), 2.26 (3H, s, 9-OCOMe), 1.33 and 1.25 (3H each ,s, 24- and 25-Me's). MS *m/e*: M^+, 475.187. Calcd for $C_{25}H_{25}N_5O_5$: M, 475.185. The zone with *Rf* 0.24 afforded **11** (3 mg) as colorless granules of mp 200—201° (from benzene/hexane). IR ν_{max} cm^{-1}: 3425 and 3200 (NH), 1710 and 1685 (NC=O). ^1H NMR ($CDCl_3$) δ: 8.35 (1H, s, 15-H), 7.64 (1H, s, 18-H), 7.53 (1H, dd, *J* 8 and 1 Hz, 4-H), 7.33 (1H, dt, *J* 1 and 8 Hz, 6-H), 7.25 (1H, s, 20-H), 7.08 (1H, dt, *J* 1 and 8 Hz, 5-H), 6.95 (1H, dd, *J* 8 and 1 Hz, 7-H), 6.21—5.92 (1H, m, 22-H), 5.14 (1H, dd, *J* 18 and 1 Hz, 23-H), 5.07 (1H, dd, *J* 11 and 1 Hz, 23-H), 4.88 (2H, s, 9-OCH_2-SMe), 4.73 (1H, dd, *J* 12 and 6 Hz, 9-H), 3.75 (3H, s, 1-OMe), 2.49 (1H, t, *J* 12 Hz, 8-H), *ca.* 2.21 (1H, 8-H, overlapping with SMe), 2.11 (3H, s, 9-OCH_2SMe), 1.29 and 1.25 (3H each, s, 24- and 25-Me's). MS *m/e*: M^+, 495.195. Calcd for $C_{25}H_{29}N_5O_4S$: M, 495.193.

8,9-Dehydroneoxaline (12)——A solution of **10** (12 mg) in 10% methanolic KOH (1 ml) was stirred at room temperature for 4 hr and then concentrated *in vacuo*. The residue was adjusted to pH *ca.* 7 with 20% aq. NH_4Cl (1 ml) and then extracted with chloroform. The residue obtained from the chloroform solution was crystallized from benzene to yield **12** (9 mg) as light yellow needles of mp 200° (dec.). IR ν_{max} cm^{-1}: 3420 and 3200 (NH and OH), 1710 (NC=O). ^1H NMR ($CDCl_3$) δ: 8.27 (1H, s, 15-H), 7.57 (1H, dd, *J* 8 and 1 Hz, 4-H), 7.56 (1H, s, 18-H), 7.27 (1H, dt, *J* 1 and 8 Hz, 6-H), 7.25 (1H, 20-H, overlapping with $CHCl_3$), 7.07 (1H, dt, *J* 1 and 8 Hz, 5-H), 6.97 (1H, dd, *J* 8 and 1 Hz, 7-H), 6.11 (1H, dd, *J* 18 and 10 Hz, 22-H), 5.50 (1H, s, 8-H), 5.11 (1H, d, *J* 18 Hz, 23-H), 5.05 (1H, d, *J* 10 Hz, 23-H), 3.73 (3H, s, 1-OMe), 1.34 and 1.27 (3H each, s, 24- and 25-Me's), 12.74, 8.07 and 7.61 (1H each, s, NH's and OH).[8] MS *m/e*: M^+, 433.174. Calcd for $C_{23}H_{23}N_5O_4$: M, 433.175.

14-Methyloxaline (13)——a) Oxaline (**1**) (10 mg) was added to an excess of ethereal diazomethane and the mixture was stirred at room temperature overnight. After concentration *in vacuo*, the residue was crystallized from hexane to yield **13** (7 mg) as colorless needles of mp 217—218° (lit.[3b]: mp 214—216°). CD (0.001, EtOH) $[\theta]^{24}$ (nm): −11500 (348) (negative maximum), +14700 (285) (positive maximum), +14900 (281) (negative maximum), +15400 (276) (positive maximum), +13000 (258) (negative maximum), +15200 (247) (positive maximum), −42000 (225.5) (negative maximum). IR ν_{max} cm^{-1}: 3200 (NH), 1690 and 1685 (NC=O). ^1H NMR ($CDCl_3$) δ: 13.08 (1H, s, NH),[8] 8.46 (1H, s, 15-H), 7.68 (1H, s, 18-H), 7.55 (1H, dd, *J* 8 and 1 Hz, 4-H), 7.36 (1H, s, 20-H), *ca.* 7.25 (1H, 6-H, overlapping with $CHCl_3$), 7.04 (1H, dt, *J* 1 and 8 Hz,

7) Preparative thin-layer chromatography.
8) On addition of D_2O, this signal disappeared.

5-H), 6.91 (1H, dd, *J* 8 and 1 Hz, 7-H), 6.12 (1H, dd, *J* 18 and 10 Hz, 22-H), 5.09 (1H, s, 8-H), 5.05 (1H, d, *J* 10 Hz, 23-H), 5.04 (1H, d, *J* 18 Hz, 23-H), 3.73 (3H, s, 1-OMe), 3.61 (3H, s, 9-OMe), 2.44 (3H, s, 14-Me), 1.31 and 1.23 (3H each, s, 24- and 25-Me's). MS *m/e*: M^+, 461.206. Calcd for $C_{25}H_{27}N_5O_4$: M, 461.206.

b) Treatment of **12** (9 mg) with an excess of ethereal diazomethane gave **13** (6 mg) as colorless needles of mp 216.5—217° (from hexane). MS *m/e*: M^+, 461.205. Calcd for $C_{25}H_{27}N_5O_4$: M, 461.206. The CD curve, and IR and ^1H NMR spectra were superimposable on those of the compound obtained above. The melting point of a mixture of both compounds was undepressed.