ANTIBIOTIC YC 73 OF PSEUDOMONAS ORIGIN. III*
SYNTHESIS OF THIOFORMIN ANALOGUES

YUKIO ITO, KIMIO UMINO, TOSHIO SEKIGUCHI,**
TOSHIKAZU MIYAGISHIMA and YOSHIYUKI EGAWA

Microbial Chemistry Research Laboratory and
Organic Chemistry Research Laboratory**,
Tanabe Seiyaku Co., Ltd., Toda, Saitama, Japan

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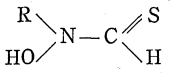
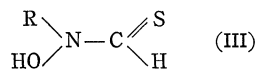
N-Substituted thioformylhydroxylamine derivatives represented by the general formula  were synthesized and examined for antimicrobial activity. These compounds were analogues of an antibiotic thioformin which was confirmed to be N-methyl-N-thioformylhydroxylamine.

In the earlier paper of this series[1], it was reported that YC 73 (I) of a *Pseudomonas* origin was a cupric complex of an acidic substance named thioformin (II), which could be synthesized by treating N-methylhydroxylamine with potassium dithioformate.

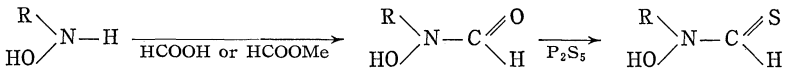
Soon after the structure determination of II, the fact that such a simple thiohydroxamic acid derivative as II possesses significant antimicrobial activity prompted us to synthesize structural analogues of II. The analogues represented in the general formula III were readily prepared by the following methods.

 (III)

Method A: Direct thioformylation of N-substituted hydroxylamines with potassium dithioformate.

An aqueous solution of N-substituted hydroxylamine was reacted with an excess of an aqueous solution of potassium dithioformate at room temperature.

Method B: Replacement of the carbonyl oxygen in N-formyl-N-substituted hydroxylamine by sulfur.



The N-substituted hydroxylamine was first formylated with an excess of methylformate or formic acid by the usual method and the hydroxamic acid derivative thus obtained was treated with phosphorus pentasulfide in a solvent such as dioxane, benzene or pyridine at 20~40°C for the replacement reaction.

Some of physicochemical and biological properties of the iron (ferric) complexes of analogues synthesized by the above methods are summarized in Table 1. Com-

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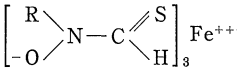
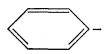
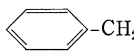
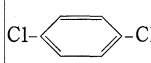
Table 1. Physicochemical and antimicrobial properties of synthetic thioformin analogues (Fe complex)



Compound	R	Formula	M. p. (°C)	Elemental analysis (%)		M. W.		Antimicrobial activity *	
				Calcd.	Found	Calcd.	Found	*S. aureus* 209 P	*E. coli* NIHJ
IV	CH_3	$C_6H_{12}N_3O_3S_3Fe$	>250 (184 sinter)	C 22.09 H 3.68 N 12.88	22.22 3.72 12.69	326	—**	1.56	12.5
V	C_2H_5	$C_9H_{18}N_3O_3S_3Fe$	141	C 29.34 H 4.89 N 11.41	29.32 4.98 11.22	368	356	0.78	6.25
VI	$(CH_3)_2CH$	$C_{12}H_{24}N_3O_3S_3Fe$	130~132	C 35.13 H 5.85 N 10.25	35.05 5.85 10.06	410	396	0.78	6.25
VII		$C_{21}H_{36}N_3O_3S_3Fe$	151	C 47.54 H 6.79 N 7.92	47.02 6.81 7.53	530	542	0.78	25
VIII		$C_{21}H_{18}N_3O_3S_3Fe$	157~159	C 49.22 H 3.52 N 8.20	48.61 3.66 8.12	512	521	25	100
IX		$C_{24}H_{24}N_3O_3S_3Fe$	151~152	C 51.99 H 4.33 N 7.58	51.26 4.34 8.12	554	544	3.12	100
X		$C_{24}H_{21}Cl_3N_3O_3S_3Fe$	183~184	C 43.77 H 3.19 N 6.38	43.36 3.46 6.10	658	642	3.12	6.25

* Determined by agar dilution method (M.I.C. : μg/ml)
** Undetermined because of low solubility in $CHCl_3$

pounds **V**, **VI** and **VII** were synthesized by both methods A and B, and **VIII**, **IX** and **X** were synthesized by method B. Molecular weights of the compounds were determined by the VPO method in chloroform. Main absorption peaks observed in the IR and UV spectra of these compounds are also listed in Table 2.

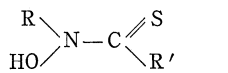
Table 2. IR and UV maxima of thioformin analogues (Fe complex)

Compound	IR : ν_{max}^{nujol} cm^{-1}	UV : λ_{max} nm
IV	1557, 1153, 920, 897, 864	254, 374^{sh}, 515 (MeOH)
V	1543, 1145, 903, 883	255, 380^{sh}, 535 (MeOH)
VI	1530, 1142, 1062, 928, 897, 843	256, 374^{sh}, 540 (MeOH)
VII	1527, 1132, 910, 895	256, 374, 540 (MeOH)
VIII	1508, 1332, 1218, 910, 855	285 ($CHCl_3$)
IX	1538, 1350, 1206, 1142, 918, 833	254, 374, 524 ($CHCl_3$)
X	1586, 1545, 1494, 1340, 1170, 1145, 1100, 1020, 922, 908, 818	370, 525, 630 ($CHCl_3$)

As briefly described in Table 1, these analogues showed similar antimicrobial activity to the parent thioformin iron complex (**IV**). Detailed examination of the biological activity of these compounds is now under progress.

Thioformin itself was first synthesized by the method A but it was also synthesized by method B (R=CH_3).

Extention of the method B may give a way to synthesize the analogues which are represented by the general formula **XI**.



(XI)

Physicochemical and biological properties of the compounds in these categories will be reported later.

Experimental

N-Ethyl-N-thioformylhydroxylamine (**V**). Method A

An aqueous solution of N-ethylhydroxylamine oxalate (10 g, 94.3 mmol) was reacted with an aqueous solution of potassium dithioformate[2] (80 ml, 209 mmol) at room temperature. The reaction mixture was adjusted to pH 1.0 with conc. hydrochloric acid. Extraction with chloroform followed by evaporation of the solvent *in vacuo* afforded a crude product (2.6 g). For purification, this was dissolved in chloroform and the chloroform solution was shaken with an excess of an aqueous solution of cupric sulfate to yield a copper complex of **V**. The organic layer was evaporated *in vacuo* to yield a residue, which was treated with ether to give black crystals of N-ethyl-N-thioformylhydroxylamine copper complex (3.28 g). The free form was then regenerated from the complex by treating with H_2S gas in chloroform. Finally, the product was purified by vacuum distillation to give pure N-ethyl-N-thioformylhydroxylamine as a colorless oil (2.28 g), b. p. 68~69°C at 5 mm; UV, λ_{max}^{MeOH} 273 nm; IR, ν_{max}^{liquid} 1550, 1145 cm^{-1}; NMR (τ values in CCl_4), 8.6 (3H, t), 6.1 (2H, q), 1.22 (1H, s) and 0.16 (1H, s). By adding an aqueous solution of ferric chloride to an ethanol solution of **V**, ferric complex of **V** was obtained.

N-Isopropyl-N-thioformylhydroxylamine (**VI**). Method A

N-Isopropylhydroxylamine oxalate (12 g, 100 mmol in 120 ml of water) was reacted with aqueous solution of potassium dithioformate (80 ml, 209 mmol). The crude product thus obtained (3.9 g) was converted into the copper complex of **VI** (5.1 g). Regeneration with H_2S and vacuum distillation gave the free form of N-isopropy-N-thioformylhydroxylamine as colorless oil (3.8 g), b. p. 66~67°C at 5 mm; UV, λ_{max}^{MeOH} 272 nm; IR, ν_{max}^{liquid} 1540, 1147 cm^{-1}; NMR (τ values in $COCl_3$), 8.55 (6H, d), 5.85 (1H, m), 1.35 (1H, s) and −0.1 (1H, s).

N-Cyclohexyl-N-thioformylhydroxylamine (**VII**). Method B

1) N-Cyclohexyl-N-formylhydroxylamine: N-Cyclohexyl hydroxylamine (5.76 g, 50 mmol) was reacted with methyl formate (350 ml, 5.75 mol) overnight. The crude product was taken up in chloroform, which was shaken with an aqueous solution of ferric chloride to form the iron complex. The crude iron complex was purified by chromatography on silica gel (Mallinckrodt SILICAR CC-7). The chloroform - methanol (95 : 5) eluates from the column were evaporated under reduced pressure to give reddish brown needles of N-cyclohexyl-N-formylhydroxylamine iron complex, m. p. 96~97°C. The free form was regenerated by treating with 1 N hydrochloric acid in chloroform solution. Recrystallization from ether-petroleum ether afforded crystals of N-cyclohexyl-N-formylhydroxylamine (3.94 g) as colorless plates, m. p. 92~94°C.

Anal. Calcd. for $C_7H_{13}NO_2$: C 58.72, H 9.15, N 9.78 %.
Found: C 58.68, H 8.79, N 9.62 %.

2) Replacement reaction: A dioxane solution (40 ml) of N-cyclohexyl-N-formylhydroxylamine (1.43 g, 10 mmol) was reacted with P_2S_5 (2.23 g, 10 mmol) for 10 hours at 35~40°C. The crude product was taken up in chloroform and converted into its iron complex, which was purified by chromatography on silica gel by the manner described above (solvent; $CHCl_3$). Recrystallization from methanol gave crystals of N-cyclohexyl-N-thioformylhydroxylamine ferric complex (0.78 g) as black prisms. When the ferric complex (0.78 g) was treated with conc. hydrochloric acid in chloroform solution, N-cyclohexyl-N-thioformylhydroxylamine was regenerated in the solution and it was then purified by vacuum distillation to give a yellow oil (0.63 g), b. p. 98~99°C at 1 mm. It solidified to prism crystals upon cooling, m. p. 35°C. UV, λ_{max}^{MeOH} 273 nm; IR, $\nu_{max}^{CHCl_3}$ 1125, 1545 cm^{-1}; NMR (τ values in $CDCl_3$), 7.7~8.9 (10H, m), 6.25 (1H, m), 1.28 (1H, s) and 0.3~1.0 (1H, broad).

N-Phenyl-N-thioformylhydroxylamine (**VIII**). Method B

1) N-Phenyl-N-formylhydroxylamine: The compound was synthesized by a slightly modified method of DESTRAZ[3]. Copper complex: green plates, m. p. 228~230°C (dec.). Free form: white plates, m. p. 70~71°C (Lit. 70~71°C[3]).

2) Replacement reaction: N-Phenyl-N-formylhydroxylamine (0.41 g, 3 mmol in 10 ml of dioxane) was reacted with P_2S_5 (0.57 g, 3 mmol) at 35°C for 5 hours. The product was converted into the ferric complex and recrystallized from $CHCl_3$-MeOH as black prisms of N-phenyl-N-thioformylhydroxylamine ferric complex (77 mg).

N-Benzyl-N-thioformylhydroxylamine (**IX**). Method B

1) N-Benzyl-N-formylhydroxylamine: Synthesis of this compound followed the method of JONES[4] with slight modification. Copper complex: m. p. 184~187°C (dec.). Free form: m. p. 49~50°C (Lit. 49~50°C[4]).

2) Replacement reaction: N-Benzyl-N-formylhydroxylamine (1.51 g, 10 mmol in 30 ml of dioxane) was reacted with P_2S_5 (2.22 g, 10 mmol) at 35~40°C for 6 hours. The product was converted into ferric complex. Recrystallization from MeOH-$CHCl_3$ gave black needles of ferric complex (0.15 g).

N-(*p*-Chlorobenzyl)-N-thioformylhydroxylamine (**X**). Method B

1) N-(*p*-Chlorobenzyl)-N-formylhydroxylamine: N-(*p*-Chlorobenzyl)-hydroxylamine (15.7 g, 100 mmol) was formylated with methyl formate (100 ml, 1.65 mol) overnight. The crude product was converted into the iron complex, which was recrystallized from MeOH-$CHCl_3$ to yield red brown prisms of N-(*p*-chlorobenzyl)-N-formylhydroxylamine (13.21 g), m. p. 210~212°C. Regeneration and recrystallization from ether-petroleum ether afforded colorless plates of N-(*p*-chlorobenzyl)-N-formylhydroxylamine (11.11 g), m. p. 99~100°C.

Anal. Calcd. for $C_8H_8ClNO_2$: C 51.77, H 4.34, N 7.54%.
Found: C 51.49, H 4.19, N 7.48%.

UV, λ_{max}^{MeOH} 220.5, 248, 294 nm; IR, ν_{max}^{Nujol} 1655, 1619, 1556, 1496, 1398, 1350, 1100, 1024, 820 and 755 cm^{-1}.

2) Replacement reaction: N-(*p*-Chlorobenzyl)-N-formylhydroxylamine (1.85 g, 10 mmol in 40 ml of dioxane) was reacted with P_2S_5 (2.22 g, 10 mmol) at room temperature for 10 hours. Recrystallization from MeOH-$CHCl_3$ afforded black prisms of N-(*p*-chlorobenzyl)-N-thioformylhydroxylamine ferric complex (1.4 g).

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References

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