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Further Studies on Synthesis and Antimicrobial Activity of Thioformin Analogues[1]

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N-Substituted N-thioformylhydroxylamine derivatives represented by the general formula R–N(OH)–CHS (I) were synthesized and examined for antimicrobial activities. The synthesis was performed by the replacement of the carbonyl in an appropriate N-substituted N-formylhydroxylamine with sulfur. These compounds were analogues of an antibiotic thioformin (II), N-methyl N-thioformylhydroxylamine, which was isolated as cupric complex (antibiotic YC-73 (III)) from a culture filtrate of *Pseudomonas* sp. The derivatives synthesized showed broad antimicrobial activities *in vitro* similar to that of the original antibiotic, particularly against gram-positive bacteria.

The new copper containing antibiotic designated YC-73 (III) was isolated from the culture broth of *Pseudomonas fluorecens* MCRL-10107 in our laboratory.[3] The structure of thioformin (II), which was obtained by removing cupric ion from the antibiotic (III), was assumed and finely determined by synthesis as N-methyl N-thioformylhydroxylamine.[4] Thioformin was later isolated as a numerous metallic salt by K. Shirahata, *et al.*[5] under the name of Fluopsin-F (Fe-salt), -C (Cu-salt) and -N (Ni-salt), which were produced by *Pseudomonas fluorecens* KY 4932. YC-73 itself was also isolated from *Pseudomonas reptilivora* by L.A.Del Rio[6] under the name of Antibiotic Substance B_1, from *Streptomyces* sp. ATCC 21775 by S. Miyamura,[7] and *Streptomyces* sp. 4601 by H. Otsuka.[8]

$$(CH_3\text{–}N(O^-)\text{–}CHS)_2Cu^{2+} \underset{Cu^{2+}}{\overset{H_2S}{\rightleftharpoons}} CH_3\text{–}N(OH)\text{–}CHS$$

III II

Chart 1

To obtain the compound possessing an improved antimicrobial activity, syntheses of some thioformin analogues were attempted.[1]

This paper deals with the further syntheses of thioformin analogues represented by the general formula R–N(OH)–CHS (I) and the antimicrobial activities of intermediary N-substituted N-formylhydroxylamines (V) and N-substituted N-thioformylhydroxylamine ferric complexes.

Results and Discussion

Chemistry

Thioformin analogues (N-substituted N-thioformylhydroxylamines) were synthesized by the route shown in the Chart 2.

1) This forms Part IV of "Antibiotic YC-73 of *Pseudomonas* Origin." Part III: Y. Ito, K. Umino, T. Sekiguchi, T. Miyagishima, and Y. Egawa, *J. Antibiotics*, **24**, 131 (1971).
2) Location: *2-2-50 Kawagishi, Toda-shi, Saitama.*
3) Y. Egawa, K. Umino, S. Awataguchi, Y. Kawano, and T. Okuda, *J. Antibiotics*, **23**, 267 (1970).
4) Y. Egawa, K. Umino, Y. Ito, and T. Okuda, *J. Antibiotics*, **24**, 124 (1971).
5) K. Shirahata, T. Deguchi, T. Hayashi, I. Matsubara, and T. Suzuki, *J. Antibiotics*, **23**, 546 (1970).
6) L.A. Del Rio, J. Olivares, M.C. Blesa, and F. Mayor, *Antimicrob. Ag. Chemother.*, **2**, 186 (1972); L.A. Del Rio, J.L. Gorge, L. Olivares, and F. Mayor, *Antimicrob. Ag. Chemother.*, **2**, 189 (1972).
7) S. Miyamura, Japan Patent 87087 (1973) [*C.A.*, **80**, 942585 (1974)].
8) H. Otsuka, S. Niwayama, H. Tanaka, T. Take, T. Uchiyama, H. Nakazawa, K. Abe, and K. Koizumi, *J. Antibiotics*, **25**, 369 (1972).

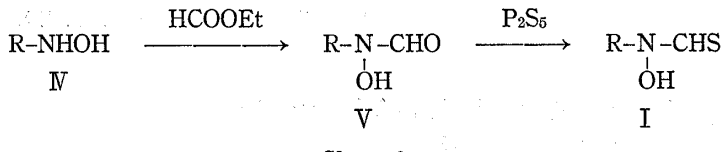


Chart 2

As shown in Chart 2, N-substituted N-formylhydroxylamines (V) were first obtained by treatment of an appropriate hydroxylamine (IV) with ethyl formate, and the carbonyl oxygen in an intermediary (V) was readily converted to sulfur atom by stirring (V) with phosphorus pentasulfide in a solvent such as dioxane, pyridine, carbon disulfide, benzene or toluene. Among these solvents tested, dioxane was proved to be best in the respect of yield and easiness for the isolation of the product. The reactions were generally conducted by stirring at room temperature for 5—10 hr in dioxane. The pure products could be obtained by isolating from the reaction mixtures using silica gel column chromatography as stable metal complexes. The average yield in replacement of oxygen to sulfur was about 60%. Since the N-substituted N-thioformylhydroxylamines thus obtained were usually unstable as free acids, physical properties were analyzed as metal complexes. Physical properties of (V) and N-substituted N-thioformylhydroxylamine metal complexes were listed in Table I and II, respectively.

Antimicrobial Activity

The antimicrobial activities of these derivatives were examined as stable metal complexes by the agar dilution method. Antibacterial activities of N-substituted N-thioformylhydroxylamine ferric complexes were shown in Table III.

Table I. Physical Properties of N-Substituted N-Formylhydroxylamines

R–N(OH)–CHO

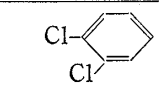
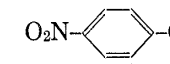
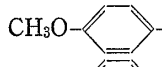
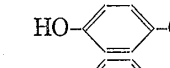
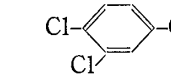
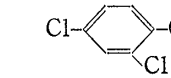
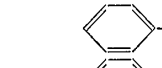
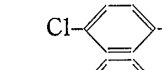
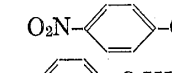
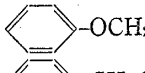
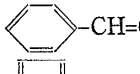
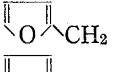
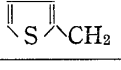
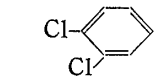
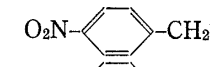
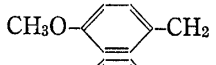
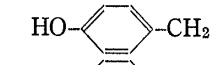
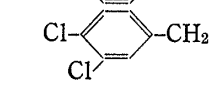
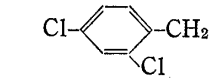
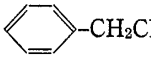
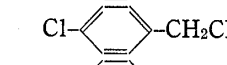
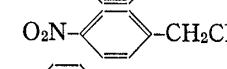
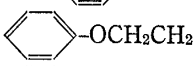
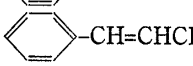
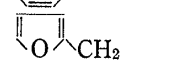
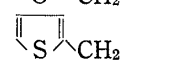
Compd. No.	R	Yield (%)	mp (°C)	Appearance	Formula	Analysis (%) Calcd. (Found) C	H	N	IR ν_{max}^{Nujol} cm^{-1} (CO)
1		45	85—87	colorless needles	$C_7H_5O_2NCl_2$	40.81 (40.99)	2.45 (2.56)	6.80 (6.89)	1693
2		72	121	colorless prisms	$C_8H_8O_4N_2$	48.98 (48.82)	4.11 (4.21)	14.28 (14.49)	1692
3		64	83	colorless prisms	$C_9H_{11}O_3N$	59.66 (59.79)	6.21 (6.25)	7.73 (7.81)	1678
4		72	161	colorless plates	$C_8H_9O_3N$	57.48 (57.42)	5.43 (5.47)	8.38 (8.11)	1660
5		57	84	colorless prisms	$C_8H_7O_2NCl_2$	43.66 (43.75)	3.21 (3.19)	6.37 (6.41)	1680
6		60	129	colorless prisms	$C_8H_7O_2NCl_2$	43.66 (43.72)	3.21 (3.29)	6.37 (6.51)	1677
7		70		yellow oil	$C_9H_{11}O_2N$	65.44 (65.52)	6.71 (6.81)	8.48 (8.63)	1665
8		83	113	colorless prisms	$C_9H_{10}O_2NCl$	54.13 (53.81)	5.11 (4.99)	7.01 (6.83)	1658
9		72	130	colorless prisms	$C_9H_{10}O_4N_2$	51.42 (51.47)	4.80 (4.77)	13.33 (13.36)	1655
10		75	100	colorless plates	$C_9H_{11}O_3N$	59.66 (59.50)	6.12 (6.10)	7.73 (7.70)	1668
11		65	105	colorless prisms	$C_{10}H_{11}O_2N$	67.78 (67.93)	6.26 (6.31)	7.91 (7.77)	1675
12		73		yellow oil	$C_6H_7O_3N$	51.06 (51.21)	5.00 (5.11)	9.93 (9.78)	1650
13		70		yellow oil	$C_6H_7O_2NS$	45.85 (45.99)	4.49 (4.57)	8.91 (8.75)	1665

Table II. Physical Properties of N-Substituted N-Thioformylhydroxylamine Metal Complexes

$(R\text{-}\underset{\underset{O^-}{|}}{N}\text{-}CHS)_nM^{n+}$

Compd. No.	R	Yield (%)	Metal	mp (°C)	Appearance	Formula	Analysis (%) Calcd. (Found) C	H	N
14		55	Fe^{3+}	132	black prisms	$C_{21}H_{12}O_3N_3S_3Cl_6Fe$	35.08 (35.34)	1.68 (1.97)	5.84 (5.77)
15		68	Fe^{3+}	120—122	black needles	$C_{24}H_{21}O_9N_6S_3Fe$	41.81 (41.95)	3.07 (3.27)	12.19 (12.14)
16		65	Fe^{3+}	149	black needles	$C_{27}H_{30}O_6N_3S_3Fe$	50.31 (50.03)	4.69 (4.84)	6.52 (6.92)
17		57	Fe^{3+}	186	black prisms	$C_{24}H_{24}O_6N_3S_3Fe$	47.85 (47.58)	4.02 (4.05)	6.97 (6.89)
18		63	Fe^{3+}	158	black prisms	$C_{24}H_{18}O_3N_3S_3Cl_6Fe$	37.88 (37.77)	2.33 (2.59)	5.52 (5.55)
19		55	Fe^{3+}	184	black prisms	$C_{24}H_{18}O_3N_3S_3Cl_6Fe$	37.88 (37.87)	2.33 (2.52)	5.52 (5.54)
20		47	Cu^{2+}	143	brown prisms	$C_{18}H_{20}O_2N_2S_2Cu$	51.11 (50.53)	4.77 (4.48)	6.62 (6.46)
21		55	Cu^{2+}	163	brown prisms	$C_{18}H_{18}O_2N_2S_2Cl_2Cu$	43.87 (43.46)	3.67 (3.68)	5.68 (5.38)
22		62	Cu^{2+}	154	yellow prisms	$C_{18}H_{18}O_6N_4S_2Cu$	41.90 (41.95)	3.91 (3.82)	10.85 (10.73)
23		67	Fe^{3+}		black oil	$C_{27}H_{30}O_6N_3S_3Fe$	50.34 (50.23)	4.69 (4.55)	6.52 (6.45)
24		60	Fe^{3+}	166	black prisms	$C_{30}H_{30}O_3N_3S_3Fe$	59.96 (59.54)	4.77 (4.75)	6.64 (6.54)
25		68	Fe^{3+}	155	black needles	$C_{18}H_{18}O_6N_3S_3Fe$	42.04 (41.83)	3.52 (3.44)	8.17 (7.93)
26		57	Fe^{3+}	146	black needles	$C_{18}H_{18}O_3N_3S_6Fe$	37.76 (37.90)	3.17 (3.28)	7.34 (7.41)

The N-substituted N-thioformylhydroxylamine ferric complexes synthesized exhibited similar orders of activity to the parent thioformin ferric complex against gram-positive bacteria, while they were less effective against gram-negative bacteria including *Pseudomonas aeruginosa* A_3. N-Substituted N-formylhydroxylamines were found to be almost ineffective against all of the tested bacteria at the concentration of 100 mcg per ml.

As to antifungal activities of intermediary N-substituted N-formylhydroxylamines and N-substituted N-thioformylhydroxylamine ferric complexes (Table IV), N-substituted N-thioformylhydroxylamines were found to be strongly active against *Candida albicans* Eiken, *Saccharomyces cereviciae* S-100 and *Trichophyton asteroides* 429 IMCF 07, but were ineffective against *Aspergillus fumigatus* IAM 2612 and *Penicillium chrysogenum* 49—132. It is interesting that the derivatives having phenethyl or thenyl substituent showed higher activities than the parent thioformin ferric complexes to some fungi. Intermediary N-formylhydroxylamines were inactive for all the fungi tested.

As described above, the conversion of a formyl into a thioformyl group exerted a remarkable effect on antimicrobial activity. All N-substituted N-thioformylhydroxylamine derivatives synthesized exhibited a few hundred times stronger antimicrobial activities than N-substituted N-formylhydroxylamines, therefore it is likely to conclude that $-\underset{\underset{OH}{|}}{N}\text{-}CHS$ group plays an important role with regard to antimicrobial activity. Besides, as we expected, N-substituents influence effectiveness. The derivatives having aralkyl or heteroaralkyl sub-

Table III. Antibacterial Activity (MIC: mcg/ml) of N-Substituted N-Thioformylhydroxylamine Ferric Complexes

$$(R\text{–}\underset{\underset{O^-}{|}}{N}\text{–}CHS)_3Fe^{3+}$$

Compound No.	Test organisms									
	A	B	C	D	E	F	G	H	I	J
14	25	25	25	25	12.5	100	100	50	100	50
15	6.25	6.25	6.25	25	3.12	25	25	25	100	25
16	3.12	3.12	3.12	25	3.12	12.5	25	12.5	100	12.5
17	3.12	3.12	6.25	6.25	1.56	25	50	12.5	>100	6.25
18	50	50	50	100	25	>100	>100	>100	>100	100
19	25	25	25	>100	25	>100	>100	>50	>100	25
20′	6.25	6.25	12.5	3.12	3.12	25	12.5	12.5	>100	6.25
21′	12.5	12.5	6.25	12.5	6.25	50	50	50	>100	25
22′	6.25	12.5	1.56	6.25	6.25	50	50	50	>100	25
23	6.25	6.25	6.25	6.25	6.25	50	25	25	>100	25
24	12.5	12.5	25	50	3.12	50	50	50	>100	50
25	1.56	3.12	6.25	12.5	1.56	12.5	12.5	12.5	>100	25
26	3.12	1.56	6.25	6.25	1.56	12.5	12.5	25	100	25
27[a)]	1.56	1.56	3.12	3.12	1.56	3.12	6.25	6.25	50	6.25

abbreviation of test organism; A: *Staphylococcus aureus* 209P, B: *S. aureus* B-20, C: *S. epidermidis* 10131, D: *Streptococcus faecalis*, E: *Bacillus subtilis* PCI-219, F: *Escherichia coli* NIHJ, G: *Salmonella typhimurium*, H: *Klebsiella pneumoniae*, I: *Pseudomonas aeruginosa* A_3, J: *Proteus vulgaris*

a) R=CH_3 (thioformin)

Table IV. Antifungal Activity (MIC: mcg/ml) of N-Substituted N-Thioformylhydroxylamine Ferric Complexes

$$(R\text{–}\underset{\underset{O^-}{|}}{N}\text{–}CHS)_3Fe^{3+}$$

Compound No.	Test organisms					
	K	L	M	N	O	P
14	100	25	6.25	>100	100	50
15	100	1.56	25	>100	100	6.25
16	50	0.19	0.19	>100	50	3.12
17	50	0.19	0.19	>100	50	3.12
18	>100	50	25	>100	>100	50
19	>100	100	12.5	>100	>100	50
20′	3.12	0.39	0.19	100	100	0.78
21′	50	6.25	6.25	>100	100	6.25
22′	100	3.12	12.5	>100	>100	50
23	6.25	0.19	1.56	6.25	3.12	3.12
24	25	50	6.25	50	25	12.5
25	50	3.12	50	>100	>100	25
26	3.12	0.39	0.39	100	25	3.12
27[a)]	25	0.19	1.56	25	25	6.25

abbreviation of test organism: K: *Candida albicans* IAM 4888, L: *C. albicans* Eiken, M: *Saccharomyces cereviciae* S-100, N: *Aspergillus fumigatus* IAM 2612, O: *Penicillium chrysogenum* 49-132, P: *Trichophyton asteroides* 429 (IMCF 07)

a) R=CH_3 (thioformin)

stituents seemed to have higher and broader activities against bacteria tested. The introduction of dichloro function in the phenyl substituent decreased the antimicrobial activity. Sub stitution of CH_3 group of thioformin with more complex substituents tends to weeken the activity against Gram-positive bacteria.

The structure and activity relationship of these derivatives will be analyzed quantitatively in succeeding paper of this series.[9]

Many hydroxamic acid derivatives[10] have been reported to display antimicrobial activities. However, the syntheses and the antimicrobial activities of N-thioformylhydroxylamines have not been described yet until our discovery and synthesis of thioformin analogues. The fact that these derivatives showed strong activities against Gram-positive and -negative bacteria as well as against some fungi adds a new group of compounds in the field of antimicrobial substance.

Experimental

All melting points were uncorrected. Infrared (IR) spectra were recorded on a Hitachi EPI-5 spectrometer. As a silica gel, Mallinckrodt silic AR CC-7 was used.

General Procedure for the Preparation of N-Substituted N-Formylhydroxylamine (V)——As a typical procedure, N-formylation of N-(*p*-nitrobenzyl)hydroxylamine with ethyl formate is described. N-(*p*-nitrobenzyl)hydroxylamine (1.68 g) was dissolved in ethyl formate (15 ml). To this solution was added triethylamine (2.0 g) and the mixture was stirred for 2 hr. After standing overnight at room temperature, excess solvents were removed *in vacuo*. The residue was purified by silica gel column chromatography using the solvent system of $CHCl_3$–MeOH (9: 1). The ferric chloride coloring effluent was recrystallized from ethyl ether–petroleum ether to give 1.49 g of N-(*p*-nitrobenzyl)-N-formylhydroxylamine (**1**) as colorless needless, mp 121°. Yield, 75%. Other N-substituted N-formylhydroxylamines were obtained in a similar way.

General Procedure for the Preparation of N-Substituted N-thioformylhydroxylamine (I)——As a typical procedure, replacement of the carbonyl oxygen in N-(*p*-nitrobenzyl)-N-formylhydroxylamine (**1**) by sulfur is described. To a solution of 1.96 g of **1** in 30 ml of dioxane was added 2.22 g of phosphorus pentasulfide. The mixture was stirred at room temperature for 3 hr. To the reaction mixture was added $CHCl_3$ and the insoluble substance was filtered off. The filtrate was shaken with 5% ferric chloride solution. The blackish $CHCl_3$ layer was washed with water and dried over anhyd. Na_2SO_4. After evaporation of the solvent, the residue was purified by silica gel column chromatography using the solvent system of benzen and $CHCl_3$–MeOH (9: 1). Effluent with benzene gave sulfur. The blackish effluent with $CHCl_3$–MeOH was concentrated and the residue was recrystallized from $CHCl_3$–MeOH to give 1.56 g of N-(*p*-nitrobenzyl)-N-thioformylhydroxylamine ferric complex (**14**) as black needles, mp 120—122°. Yield, 68%. Other N-substituted N-thioformylhydroxylamines were obtained in a similar way.

Antibacterial Test——The minimum inhibitory concentration (MIC) of thioformin analogues was determined according to the two-fold serial dilution method using Heart infusion agar (HIA) (Eiken), as culture medium. The test organisms were previously cultured for 18—24 hr on Trypticase soy broth (TSB), and one loopful of a suspension containing about 10^7 viable cell units per ml of the test organisms was streaked on each assay plate. The plates were incubated at 37° and the antibacterial readings were made routinely 24 hr later. The MIC of the test compounds was defined as the prevention of the visible growth of the test organism, and tetracycline was used as a control for comparison.

Antifungal Test——As similar as the above described antibacterial test, the MIC of the test compounds against some fungi was determined according to the two-fold serial dilution method using Sabouraud agar medium. Cultures of *Candida* species and *Saccharomyces cereviciae* S-100 were incubated at 27° for 2 days, while all other cultures were at 27° for 6 days. The all test organisms except yeasts were previously cultivated at 27° for 14 days in Sabouraud medium, and preculture of yeasts at 27° for 2 days in yeast-malt medium (0.4% yeast extract, 1.0% malt extract, 0.4% glucose, pH: no-adjust.) were used. One loopful of a suspension containing about 10^6 viable units per ml of the test organisms was streaked on each assay plate.

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9) T. Miyagishima, *Chem. Pharm. Bull.* (Tokyo), **22**, 2288 (1974).
10) J.B. Neilands, *Science*, **156**, 1443 (1967).