THE STRUCTURES OF FLUOPSINS C AND F

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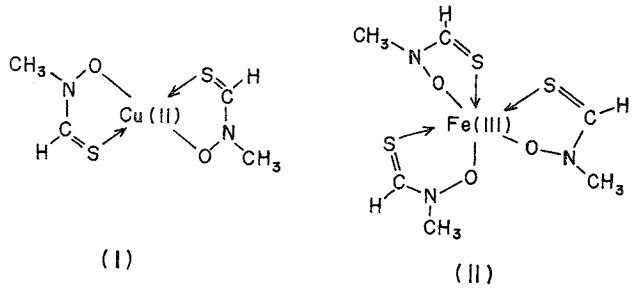
Two new antibiotics, fluopsins C and F, have been isolated from the culture broth of *Pseudomonas fluorescens* and characterized as bis(N-methyl-N-thioformylhydroxylaminato)copper(II) (I) and tris(N-methyl-N-thioformylhydroxylaminato)iron(III) (II) respectively.



Structure of fluopsins C and F.

In the course of our screening of antibiotics produced by *Ps. fluorescens* KY 4032, two new antibacterial substances, named fluopsins C and F, have been isolated*. Both are sulfur-containing compounds, and the former contains copper and the latter iron.

It has been very recently reported by Y. EGAWA *et al.*[1] that an antibiotic, YC 73, was isolated from the culture broth of fluorescent pseudomonad. The infrared spectrum (Fig. 1) and the molecular formula of fluopsin C agrees with those of YC 73. The present report deals with the full structures of fluopsins C and F.

Fluopsin C (I), dark brownish fine prisms, is soluble in strongly polar solvents such as ethyl acetate, chloroform or alcohols and decomposes gradually at about 182°C. The molecular formula was deduced as $C_4H_8N_2O_2S_2Cu$ by elementary analysis, mass spectrometry and atomic absorption analysis. Its NMR spectrum can not be measured because it contains copper. To obtain the ligand (III), hydrogen sulfide was bubbled into the solution of fluopsin C in chloroform to form a black amorphous precipitate (CuS). The reaction mixture was filterd through a short silica gel column. By addition of a methanolic solution of cupric chloride to the ligand solution obtained above, fluopsin C was reformed, *i.e.*, no skeletal change occurred during the reaction. The

* Production, isolation and antimicrobial properties of fluopsins C and F will be reported by one of the authors (T. S.) and his co-workers in near future.

Fig. 1. Infrared spectrum of fluopsin C (KBr).

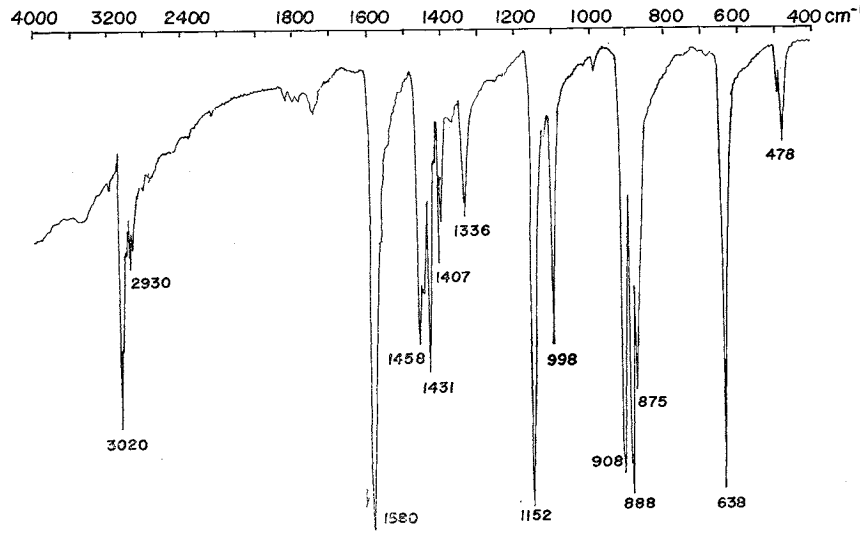


Fig. 2. Infrared spectrum of fluopsin ($CHCl_3$) (III).

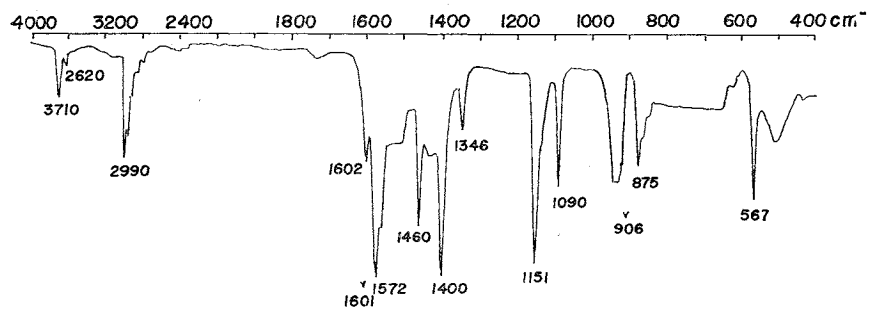


Fig. 3. NMR spectrum of fluopsin (III).

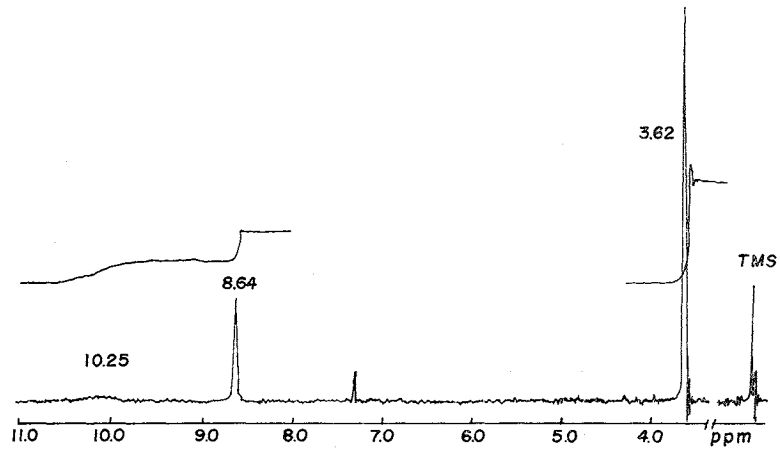


Table 1. Infrared absorption of N-methylthioformamide(IV)[4] and the ligand(III)

	Absorption at (cm^{-1})							
(IV)*	1537	1479	1443	1393	1297	1136	987	868
(III)**	1572	1460	—	1400	1346	1151	—	875

* in CCl_4, CS_2 or $CHCl_3$ ** in $CHCl_3$

infrared spectrum of this ligand solution is shown in Fig. 2. The NMR spectrum was also determined after the same treatment of fluopsin C, using deuteriochloroform instead of chloroform (Fig. 3). An attempt to obtain the solvent-free ligand (fluopsin) failed because of its volatile and unstable nature. The NMR spectrum showed three peaks at 3.62, 8.64 and 10.25 ppm (relative intensities, 3 : 1 : 1). In the case of the nickel complex, fluopsin-N, only two peaks were observed at 3.50 and 7.20 ppm (relative intensities, 3 : 1). The broad peak at 10.25 ppm of the ligand, therefore, can be assigned to an acidic proton which is exchangeable with metal ions. These NMR data demonstrate that fluopsin C has two equivalent methyl groups and two equivalent protons. On the basis of these facts and the simplicity of the infrared spectrum, it was predicted that fluopsin C is a chelate compound consisting of two similar ligands and cupric ion. The reaction of fluopsin C with hydrogen sulfide can be represented as follows:

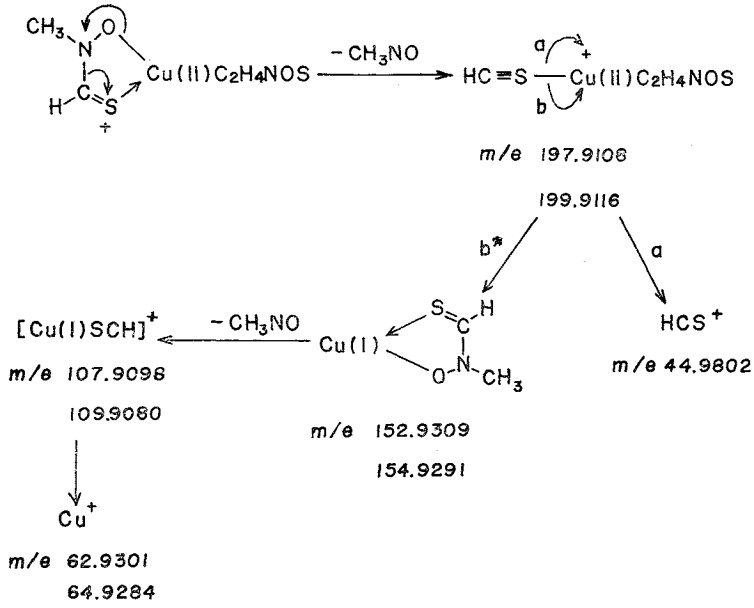
$$(C_2H_4NOS)_2Cu + H_2S \longrightarrow 2C_2H_5NOS + CuS$$

The long-range coupling (J=0.6 Hz) between the methyl group at 3.62 ppm and the proton at 8.64 ppm indicated that these are not located on the same atom. The low-field shift of the former implies that it is attached to the nitrogen or oxygen atom. Although fluopsin C has no absorption in ν_{OH} region in the infrared spectrum, the ligand has weak absorptions at 3710 and 3620 cm^{-1} attributable to a hydroxyl group which corresponds to the NMR signal at 10.25 ppm. No absorption due to N-H in the infrared spectrum of the ligand as well as fluopsin C was observed. Hence it is concluded that fluopsin C has an ionic bond between the oxygen atom and the cupric ion, and that there is an N-methyl group, the nitrogen of which is tertiary. Remaining problem is now the nature of the sulfur atom.

None of the spectra considered above showed an -SH grouping. The strong absorption at 1580 cm^{-1} of fluopsin C together with its molecular formula suggest that a thiocarbonyl group is present. In general, the infrared spectra of thioamides are so complex that the exact analyses are difficult[2], but the infrared spectrum of N-methylthioformamide (IV) has been investigated in detail[3].

As shown in Table 1, the ligand exhibits many absorptions corresponding to $\nu_{C=S}$ of N-methylthioformamide. This fact, together with the NMR spectra, reveals that the ligand is a derivative of thioformamide. The NMR spectrum of N-methylformamide was determined as a reference. Its methyl, formyl and amine protons appeared at 2.83, 8.17 and 8.4~7.2 (very broad) ppm respectively. Irradiation at the formyl proton collapsed the double doublet (J=5.0 and 0.8 Hz) due to the methyl group into a doublet (J=5.0 Hz). Such a long-range coupling through a nitrogen atom was also observed in the case of the ligand. The signal at 8.64 ppm of the ligand can be assigned to a thioformyl proton, and hence the hydroxyl group is

Scheme 1. Mass spectral fragmentation of fluopsin C.



* At this fragmentation the cupric ion is reduced to cuprous ion. Such a phenomenon is not so strange on the fragmentation of chelate compounds[4].

placed at the nitrogen atom. On the basis of the evidence already described, the structure of fluopsin C should be formulated as I.

Confirmation of I was obtained from mass spectra. The high-resolution mass spectrum of fluopsin C showed the fragments such as $[C_3H_5NOSCu]^+$, $[C_2H_4NOSCu]^+$ and $[CHSCu]^+$ formed by successive eliminations of nitrosomethane, HCS and nitrosomethane from the molecular ion $[C_4H_8N_2O_2S_2Cu]^+$ (Scheme 1). This proves the arrangements of CH_3-N-O and H-C-S.

Fluopsin F, $C_6H_{12}N_3O_3S_3Fe$, is appreciably soluble in strongly polar solvents and the structure (II) was derived from the evidence as follows: The infrared spectrum of fluopsin F is very similar to that of fluopsin C. Addition of a methanolic solution of ferric chloride to the ligand solution, prepared as mentioned previously, afforded dark purple prisms which was identical with fluopsin F.

The syntheses of fluopsins will be reported in the near future.

Experimental

Infrared spectra were recorded on a Shimizu IR-27G spectrometer, ultraviolet spectra and rotations on a JASCO ORD/UV-5 instrument, mass spectra on a Hitachi RMU 6D spectrometer and a CEC-21-110B double focussing instrument, and NMR spectra on a Varian T-60 spectrometer for deuteriochloroform solutions with tetramethylsilane as internal standard. Nippon Jarrel-Ash Atomic Absorption Flame Emission AA-1 and Yanagimoto C.H.N. Corder MT-1 were used for atomic absorption and elementary analyses respectively.

Fluopsin C (I) Dark brownish prisms from ethanol or chloroform. Anal. found: C 19.61, H 3.48, N 10.90, Cu 27.7 (by atomic absorption analysis). Calcd. for $C_4H_8N_2O_2S_2Cu$: C 19.71, H 3.31, N 11.49, Cu 26.06. M^+=m/e 242.9324 and 244.9299. Calcd. for $C_4H_8N_2$-

O_2S_2Cu: m/e 242.9321 and 244.9309. λ_{max}^{MeOH} 230 mμ (log ε. 403), 252 (4.02), 266 (4.09), 3.18 (3.81) and 360 (shoulder, 3.45). $[\theta]_{700\sim350\ m\mu}0°$ (in methanol).

Fluopsin (III) Into the solution of I (56 mg) in chloroform (6 ml), hydrogen sulfide was bubbled for 30 minutes, followed by passage of nitrogen through the reaction mixture until the smell of hydrogen sulfide was no longer detected. The ligand solution was obtained after the reaction mixture was passed through a short silica gel column to filter off copper sulphide.

Regeneration of I To the ligand solution prepared from 6.4 mg of I, a solution of cupric chloride in methanol was added. The reaction mixture was concentrated and chromatographed on silica gel to give 3 mg of prisms, which were identified as I by infrared spectra.

Fluopsin N After the same operations as above but using nickel sulfate instead of cupric chloride, fluopsin N was obtained (60 %), which gave purple red needles from ethanol. Anal. Found: C 20.13, H 3.13, N 12.23. Calcd. for $C_4H_8N_2O_2S_2Ni$: C 20.11, H 3.37, N 11.74. IR (KBr): 3025, 1592, 1442, 1404, 1327, 1153, 1139, 904, 880, 810, 689, 643 cm^{-1}.

Fluopsin F (II) Fine, dark purple prisms from methanol. Anal. Found: C 22.19, H 3.25, N 13.25, Fe 27.7 (by atomic absorption analysis). Calcd. for $C_6H_{12}N_3O_3S_3Fe$: C 22.09, H 3.71, N 12.88, Fe 26.1. IR (KBr): 3050, 1561, 1456, 1428, 1406, 1155, 916, 893, 610, 601, 455 cm^{-1}.

Preparation of II To the ligand solution prepared from 44 mg of I, ferric chloride (53 mg) in methanol (3 ml) was added and after the pH was adjusted to 6 with 0.1 N sodium hydroxide in methanol, the reaction mixture was chromatographed on silica gel to give 13 mg (33 %) of a powder, which was dissolved in a large amount of chloroform, concentrated and collected by filtration. After these treatments were repeated, the analytical sample was obtained. Anal. Found: C 21.68, H 3.49, N 13.09. The infrared spectrum was identical with that of II.

References

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