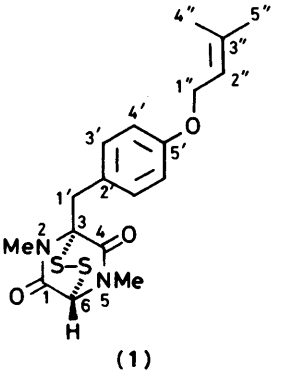
Studies on Fungal Products. Part 13.[1] Isolation and Structures of Dithiosilvatin and Silvathione, Novel Dioxopiperazine Derivatives from *Aspergillus silvaticus*

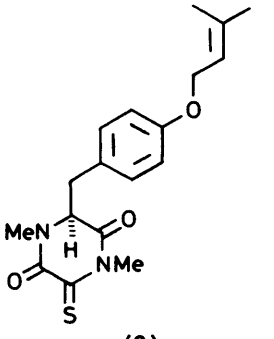
Nobuo Kawahara, Koohei Nozawa, Shoichi Nakajima, and Ken-ichi Kawai*
Faculty of Pharmaceutical Sciences, Hoshi University, Ebara 2-4-41, Shinagawa-ku, Tokyo 142, Japan

Dithiosilvatin (**1**), an epidithiodioxopiperazine, and silvathione (**2**), a dioxopiperazinethione, were isolated from *Aspergillus silvaticus*. Their structures were established on the basis of chemical reactions and spectroscopic evidence. Dithiosilvatin (**1**) is a very rare example of naturally occurring epidithiodioxopiperazines, a class of compounds which appear to be biosynthesized originally from glycine, and phenylalanine or tyrosine. Silvathione (**2**) is the second example of a natural dioxopiperazinethione and is biogenetically a possible key intermediate in the formation of trioxopiperazine from epidithiodioxopiperazine.

Aspergillus silvaticus Fennell & Raper (IFO 8173), a fungus which develops an abundance of hülle cell masses[2] even in Czapek-Dox medium, belongs to the *Aspergillus versicolor* group. The atrovenetin-like naphthalic anhydride (**3**)[3] and the nitrogen-containing 'seco anthraquinone,' silvaticamide (**4**),[4] were isolated from the above fungus. Recently we reported the isolation of three phthalides, silvaticol, nidulol, and *O*-methylsilvaticol.[5] In the course of searching for related compounds of the above metabolites, two other types of compound designated dithiosilvatin (**1**) and silvathione (**2**) were isolated from the mycelial chloroform extract and from the methylene dichloride extract of the culture filtrate, respectively. The structure elucidation and the biogenetical importance of the above compounds (**1**) and (**2**) are reported in this paper.



(**1**)



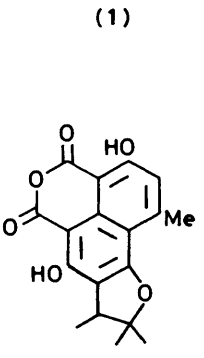
(**2**)

Results and Discussion

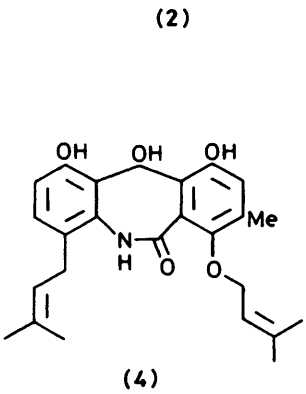
Dithiosilvatin (**1**), m.p. 100—102 °C, $[\alpha]_{435}$ +35.9° (chloroform), gave a molecular ion at *m/z* 378 by electron impact (e.i.) mass spectrometry, and elemental analysis confirmed the molecular formula as $C_{18}H_{22}N_2O_3S_2$. A positive silver nitrate test (dark brown)[6] and the ion at *m/z* 314 $[(M - S_2)^+]$ in the e.i.m.s suggested the presence of a dithio bond in (**1**). Absorption at 1 690 and 1 660 cm^{-1} in the i.r. spectrum and the ^{13}C n.m.r. signals at δ_C 164.89 and 165.51 (Table 1) indicated the presence of two amide entities. The ^1H n.m.r. signals at δ_H 2.97 and 3.17 were assigned to two methyl groups attached to the nitrogen atoms of the amides. Thus, it was proposed that the partial structure of dithiosilvatin contained an epidithiodioxopiperazine moiety with two methylated nitrogen atoms.

The ^1H n.m.r. signals at δ_H 6.84 (2 H, d, *J* 9 Hz) and 7.24 (2 H, d, *J* 9 Hz) and the ^{13}C n.m.r. signals at δ_C 158.24, 130.53 (2 C), 125.83, and 114.78 (2 C) confirmed the presence of a 1,4-disubstituted benzene moiety with an oxygen function. The ^1H n.m.r. signals at δ_H 1.74 (3 H), 1.79 (3 H), 5.45 (1 H), and 4.48 (2 H) were shown to couple with five carbons at δ_C 18.17 (Qm), 25.76 (Qm), 137.98 (m), 119.76 (Dm), and 64.80 (T), which suggested the presence of a 3-methylbut-2-enyl group in the molecule of (**1**). These results support the structure of dithiosilvatin as proposed in structure (**1**). The assignments of the ^{13}C n.m.r. signals of (**1**) are summarized in Table 1.

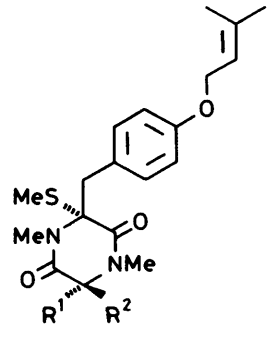
Reductive methylation[7] of dithiosilvatin (**1**) gave *cis*-bis(methylthio)silvatin (**5**), as a viscous oil, $[\alpha]_D$ −43.5° (chloroform), $C_{20}H_{28}N_2O_3S_2$, and *trans*- bis(methylthio)-silvatin (**6**), m.p. 130—132 °C, $[\alpha]_D$ +19.9° (chloroform), $C_{20}H_{28}N_2O_3S_2$. Both these compounds have two methylthio groups in the molecule, confirmed by the presence of ^1H n.m.r. signals at δ_H 2.16 and 2.29 in (**5**) and at δ_H 1.58 and 1.96 in (**6**). The similarity of the ^1H n.m.r. spectra of (**5**) and (**6**) suggests



(**3**)



(**4**)



(**5**) R^1 = SMe, R^2 = H

(**6**) R^1 = H, R^2 = SMe

Table 1. ^{13}C N.m.r. chemical shifts of dithiosilvatin (**1**) and silvathione (**2**) in $CDCl_3$

Carbon	(1)	(2)[a]
1	164.89 (m)[b]	153.02 (q)
2-Me	28.13 (Q)	33.64 (Q)[b]
3	77.14 (m)	64.41 (Dq)
4	165.51 (m)[b]	166.15 (m)
5-Me	32.26 (Qd)	33.70 (Q)[b]
6	67.14 (Dq)	187.02 (q)
1′	36.07 (Tdd)	38.05 (Tddd)
2′	125.83 (m)	124.30 (m)
3′	130.53 (Dm)	130.49 (Dddd)
4′	114.78 (Dd)	115.43 (Dd)
5′	158.24 (brdd)	159.38 (m)
1″	64.80 (T)	65.04 (T)
2″	119.76 (Dm)	119.57 (Dm)
3″	137.98 (m)	138.16 (m)
4″	18.17 (Qm)	18.72 (Qm)
5″	25.76 (Qm)	25.76 (Qm)

[a] Numbering of (**2**) corresponds to that of (**1**). [b] The assignments may be reversed.

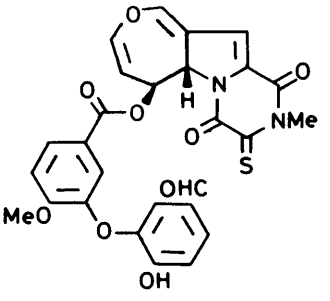
that these compounds are stereoisomers. Compound (**5**) was found to be identical, including absolute stereochemistry, with the metabolite recently isolated from the culture filtrate of *Gliocladium deliquescens* Sopp,[8] and it was thus shown that compound (**6**) was the *trans* isomer of (**5**). Comparison of the c.d. curve of dithiosilvatin (**1**) [227 (negative), 258 (positive), and 328 nm (negative)] with that of emestrin (**8**) [233 (negative), 266 (positive), and 338 nm (negative)] suggested that these two compounds had the same configuration about the epidithiodioxopiperazine ring. Thus the structure of dithiosilvatin, including the absolute stereochemistry, was confirmed as depicted in (**1**).

Silvathione (**2**),[9] m.p. 155—157 °C, $[\alpha]_{435}$ −19.7° (chloroform), gave a molecular ion at *m/z* 346 by e.i. and field desorption (f.d.) mass spectrometry, and elemental analysis, including sulphur, confirmed the molecular formula as $C_{18}H_{22}N_2O_3S$. The ^1H n.m.r. spectrum of (**2**) was similar to that of (**1**), except that no proton signal was observed at δ_H 5.33 (1 H, s) in (**1**) and that a new proton signal at δ_H 4.529 (1 H, t, *J* 3.9 Hz) coupled with the proton signals at δ_H 3.188 (2 H, d, *J* 3.9 Hz), which were assigned as $-CH_2-\underset{|}{C}H-$ of tyrosyl residue, was observed. These results suggest that compound (**2**) has a (3-methylbut-2-enyloxy)phenyl group and a dioxopiperazine moiety. The ^{13}C n.m.r. signals of (**2**) were also comparable to those of (**1**) (Table 1). The carbon signal at δ_C 67.14 (Dq) in (**1**) was observed further downfield at δ_C 187.02 (q) in (**2**), and was assigned to the carbon thioamide similar to the one at δ_C 187.05 in aurantioemestrin (**7**).[1,9] The ^{13}C n.m.r. signals at δ_C 153.02 and 166.15, and 187.02 were assigned to the two carbonyl and one thiocarbonyl carbons of the dioxopiperazinethione moiety respectively, by comparison with compound (**7**).

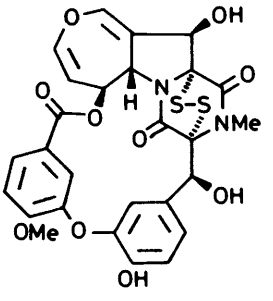
In order to determine the assignments of the carbon signals, long-range proton selective decoupling experiments at *N*-methyl groups in the ^{13}C n.m.r. spectrum of (**2**) were performed. The carbon signals at δ_C 64.41 (Dq) and 153.02 (q) were observed to be changed into a simple doublet and singlet, respectively, by selective irradiation of the protons at δ_H 3.199. These results confirmed that the one *N*-methyl group referred to the tyrosyl residue and to a carbonyl group. The carbon signals at δ_C 166.15 (m) and 187.02 (q) were observed to be changed into a double-doublet and a singlet, respectively, by selective irradiation of the signals at δ_H 3.276. These results confirmed that the other *N*-methyl group was attached in the position neighbouring the tyrosyl carbonyl and a thiocarbonyl group. The carbonyl carbon at δ_C 153.02 was observed at about 10 p.p.m. upfield from the usual amide carbon because of the conjugation with thioamide, by comparison with (**7**). Thus the structure of silvathione was confirmed as depicted in (**2**).

Dithiosilvatin (**1**) is a very rare example of naturally occurring epidithiodioxopiperazines which appear to be biosynthesized from glycine, and tyrosine or phenylalanine. In the case of *Emericella striata*, aurantioemestrin (**7**) seems to be biodegraded from emestrin (**8**) by C–C bond cleavage. Compound (**1**) may possibly be synthesized biogenetically from a compound composed from tyrosine and alanine (or serine) as in the synthesis of gliotoxin, or from tyrosine and another amino acid other than glycine.

Our present isolation of silvathione (**2**) is the second case of the isolation of a dioxopiperazinethione along with an epidithiodioxopiperazine, dithiosilvatin (**1**), from the same



(**7**)



(**8**)

fungus, *A. silvaticus* [the first example being aurantioemestrin (**7**) and emestrin (**8**) from *E. striata*[8]]. Concurrent isolation of the compound (**2**) seems to clearly indicate that the dioxopiperazinethiones are the important key intermediates in the biosynthesis of trioxopiperazines from epidithiodioxopiperazines. It is interesting to note that dioxopiperazinethione and epidithiodioxopiperazine were isolated from two taxonomically related fungi: *A. silvaticus* belonging to *A. versicolor* group and *E. Striata* belonging to *A. nidulans* group.

Experimental

M.p.s were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-181 spectrometer. E.i. and f.d. mass spectra were taken with a JEOL JMS-D 300 spectrometer. U.v. spectra and i.r. spectra were recorded on a Hitachi 124 spectrophotometer and a Hitachi 215 spectrophotometer, respectively. ^1H (99.60 MHz) N.m.r. spectra were recorded on a JEOL JNM-FX 100 spectrometer, while ^1H (399.78 MHz) and ^{13}C (100.43 MHz) n.m.r. spectra were taken with a JEOL JNM-GX 400 spectrometer, using tetramethylsilane as internal standard. The coupling patterns are indicated as follows: singlet = s, doublet = D or d, triplet = T or t, quartet = Q or q, multiplet = m, and broad = br. Capital letters refer to the pattern resulting from directly bonded coupling ($^1J_{C,H}$). C.d. curves were determined on a JASCO J-40 spectrophotometer. Column chromatography was performed using Kieselgel 60 (Art. 7734; Merck). Low pressure liquid chromatography (l.p.l.c.) was performed on a Chemco Low-Prep pump 81-M-2 and glass column (200 × 10- or 20-mm) packed with silica gel CQ-3 (30—50 μ; Wako). T.l.c. was conducted on pre-coated Kieselgel 60 F_{254} (Art. 5715; Merck). Spots on t.l.c. were detected by their absorption under u.v. light, and/or by spraying aqueous silver nitrate solution followed by heating.

Isolation of Metabolites from Aspergillus silvaticus.—*A. silvaticus*, strain IFO 8173, was cultivated at 27 °C for 21 days in Czapek-Dox medium, using 340 Roux flasks containing 250 ml of the above medium in each flask. The dried mycelia (570 g) was extracted with chloroform, and the organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. The chloroform extract (37 g) was chromatographed on silica gel with chloroform–methanol (50:1, v/v) followed by the repeated purification by l.p.l.c. using benzene to obtain dithiosilvatin (**1**) (1.2 g). The culture filtrate (85 l) was extracted with dichloromethane at pH 2, and the organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. The residue (11.9 g) was chromatographed on silica gel with chloroform–methanol (50:1, v/v) followed by the repeated purification by l.p.l.c. using chloroform to give silvathione (**2**) (30 mg).

Dithiosilvatin (**1**) was obtained as plates (from benzene), m.p. 100—102 °C; $[\alpha]_{435}^{25}$ +35.9° (*c* 1.00 in $CHCl_3$) (Found: C, 57.3; H, 5.95; N, 7.5. $C_{18}H_{22}N_2O_3S_2$ requires C, 57.12; 5.86; N, 7.40%); *m/z* 378 (M^+, 1%, e.i.), 346 [$(M - S)^+$, 3], 314 [$(M - S_2)^+$, 100], 278 (14), 246 (46), 245 (36), 218 (17), 172 (30), 107 (90), and 69 [$(C_5H_9)^+$, 36]; $\lambda_{max.}$(EtOH) 227 (log ε 4.26), 260sh (3.41), 277sh (3.36), and 284sh nm (3.28); $\nu_{max.}$(KBr) 1 690 and 1 660 cm^{-1} (CON); $\delta_H(CDCl_3)$ 1.74 (3 H, s, olefinic Me), 1.79 (3 H, s, olefinic Me), 2.97 (3 H, s, NMe), 3.17 (3 H, s, NMe), 3.45 (1 H, d, *J* 15 Hz, CH_2), 3.94 (1 H, d, *J* 15 Hz, CH_2), 4.48 (2 H, d, *J* 7 Hz, OCH_2CH=), 5.33 (1 H, s, COCHN), 5.45 (1 H, br t, *J* 7 Hz, OCH_2CH=), 6.84 (2 H, d, *J* 9 Hz, ArH), and 7.24 (2 H, d, *J* 9 Hz, ArH); c.d. (*c* 9.0 × 10^{-4} in dioxane) $[\theta]_{227}$ −4.11 × 10^4, $[\theta]_{258}$ 2.22 × 10^4, and $[\theta]_{328}$ −0.08 × 10^4. ^{13}C N.m.r. signals are summarized in Table 1.

Silvathione (**2**) was obtained as pale red needles (from methanol), m.p. 155—157 °C; $[\alpha]_{435}^{24}$ −19.7° (*c* 1.00 in $CHCl_3$) (Found: C, 62.4; H, 6.4; N, 8.1; S, 9.7. $C_{18}H_{22}N_2O_3S$ requires C, 62.15; H, 6.36; N, 7.87; S, 9.26%); *m/z* 346 (M^+, 2%, e.i.), 278 [$(M - C_5H_8)^+$, 18], 172 (45), 107 (100), and 69 [$(C_5H_9)^+$, 26]; *m/z* 346 (M^+, 100%, f.d.); $\lambda_{max.}$(MeOH) 228 (log ε 4.43), 274 (4.30), 284sh (3.93), and 300sh nm (3.84); $\nu_{max.}$(KBr) 1 690 and 1 650 cm^{-1} (CON); $\delta_H(CDCl_3)$ 1.731 (3 H, s, olefinic Me), 1.780 (3 H, s, olefinic Me), 3.188 (2 H, d, *J* 3.9 Hz, CH_2CH), 3.199 (3 H, s, NMe), 3.276 (3 H, s, NMe), 4.419 (2 H, d, *J* 6.6 Hz, OCH_2CH=), 4.529 (1 H, t, *J* 3.9 Hz, CH_2CH), 5.435 (1 H, br t, *J* 6.6 Hz, OCH_2CH=), and 6.734 (4 H, br s, ArH). ^{13}C N.m.r. signals are summarized in Table 1.

Reductive Methylation of Dithiosilvatin (**1**) *with Sodium Borohydride and Iodomethane.*—Methanol (2 ml) and iodomethane (1.5 ml) were added to a stirred solution of dithiosilvatin (80 mg) in dichloromethane (1 ml). After addition of sodium borohydride (30 mg), the reaction mixture was stirred at room temperature for 1 h, and the solvent evaporated under reduced pressure. The residue was dissolved with chloroform and the precipitate was filtered off. The evaporated residue was separated by l.p.l.c. using benzene to give *cis*-bis(methylthio)silvatin (**5**) (40 mg), from the former eluate, and *trans*-bis(methylthio)silvatin (**6**) (30 mg), from the slightly more polar eluate.

cis-Bis(methylthio)silvatin (**5**) was obtained as a viscous oil; $[\alpha]_D^{25}$ −43.5° (*c* 1.50 in $CHCl_3$); *m/z* 408 (M^+, 0.5%, e.i.), 361 [$(M - SMe)^+$, 36], 293 [$(M - SMe - C_5H_8)^+$, 34], 245 (45), 233 (76), 217 (14), 186 (30), 107 (100), and 69 [$(C_5H_9)^+$, 33]; $\lambda_{max.}$(MeOH) 228 (log ε 4.18), 274 (3.15), and 283sh nm (3.07); $\nu_{max.}$(NaCl) 1 665 and 1 655sh cm^{-1} (CON); $\delta_H(CDCl_3)$ 1.73 (3 H, s, olefinic Me), 1.74 (3 H, s, olefinic Me), 2.16 (3 H, s, SMe), 2.29 (3 H, s, SMe), 2.96 (3 H, s, NMe), 3.06 (1 H, d, *J* 14 Hz, CH_2), 3.24 (3 H, s, NMe), 3.54 (1 H, d, *J* 14 Hz, CH_2), 4.19 (1 H, s, $COCHN$), 4.45 (2 H, d, *J* 7 Hz, OCH_2CH=), 5.46 (1 H, br t, *J* 7 Hz, OCH_2CH=), 6.76 (2 H, d, *J* 9 Hz, ArH), and 6.97 (2 H, d, *J* 9 Hz, ArH). This compound was identical with bis-(methylthio)dioxopiperazine from *Gliocladium deliquescens* by comparison of i.r., u.v., and ^1H n.m.r. spectra.

trans-*Bis(methylthio)silvatin* (**6**) was obtained as needles (from cyclohexane), m.p. 130—132 °C; $[\alpha]_D^{25}$ +19.9° (*c* 1.00 in $CHCl_3$) (Found: C, 58.8; H, 6.9; N, 6.85. $C_{20}H_{28}N_2O_3S_2$ requires C, 58.80; H, 6.97; N, 6.74%); *m/z* 408 (M^+, 0.6%, e.i.), 361 [$(M - SMe)^+$, 36], 293 [$(M - SMe - C_5H_8)^+$, 32], 245 (52), 233 (70), 217 (18), 186 (48), 107 (100), and 69 [$(C_5H_9)^+$, 40]; $\nu_{max.}$(KBr) 1 660 and 1 650 cm^{-1}; $\delta_H(CDCl_3)$ 1.58 (3 H, s, SMe), 1.71 (3 H, s, olefinic Me), 1.77 (3 H, s, olefinic Me), 1.96 (3 H, s, SMe), 3.05 (3 H, s, NMe), 3.06 (1 H, d, *J* 14 Hz, CH_2), 3.25 (3 H, s, NMe), 3.66 (1 H, d, *J* 14 Hz, CH_2), 4.45 (2 H, d, *J* 7 Hz, OCH_2CH=), 4.62 (1 H, s, $COCHN$), 5.43 (1 H, br t, *J* 7 Hz, OCH_2CH=), 6.79 (2 H, d, *J* 9 Hz, ArH), and 7.07 (2 H, d, *J* 9 Hz, ArH).

Acknowledgements

We are grateful to Professor M. Yamazaki of the Research Institute for Chemobiodynamics, Chiba University, and to Dr. S. Udagawa of the National Institute of Hygienic Sciences, for helpful discussions. We thank Dr. M. Kubo of Tsumura Laboratory for f.d. mass measurements, and Mrs. T. Ogata of this university for elemental analyses. We are also grateful to Mrs. M. Yuyama and Miss T. Tanaka for e.i. mass and n.m.r. measurements.

References

1 Part 12, K. Kawai, K. Nozawa, H. Seya, N. Kawahara, S. Udagawa, and S. Nakajima, *Heterocycles*, 1987, **26**, 475.
2 K. B. Raper and D. I. Fennell, 'The Genus Aspergillus,' Robert E. Krieger Publishing Co., Malaba, Florida, 1965, p. 453.
3 K. Homma, K. Fukuyama, Y. Katsube, Y. Kimura, and T. Hamasaki, *Agric. Biol. Chem.*, 1980, **44**, 1333.
4 M. Yamazaki, H. Fujimoto, and Y. Ohta, *Heterocycles*, 1981, **15**, 889.
5 M. Fujita, M. Yamada, S. Nakajima, K. Kawai, and M. Nagai, *Chem. Pharm. Bull.*, 1984, **32**, 2622.
6 R. Rahman, S. Safe, and A. Taylor, *J. Chromatogr.*, 1974, **53**, 592.
7 H. Seya, K. Nozawa, S. Nakajima, K. Kawai, and S. Udagawa, *J. Chem. Soc., Perkin Trans. 1*, 1986, 109.
8 J. R. Hanson and M. A. O'Leary, *J. Chem. Soc., Perkin Trans. 1*, 1981, 218.
9 N. Kawahara, K. Nozawa, S. Nakajima, and K. Kawai, *J. Chem. Soc., Chem. Commun.*, 1986, 1495.

Received 30*th September* 1986; *Paper* 6/1930