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Thio-analogues of 6-Substituted 1,3-Dimethyl-7-azalumazine (Fervenulin) and 7-Substituted 1,3-Dimethyl-6-azalumazine (Isofervenulin)

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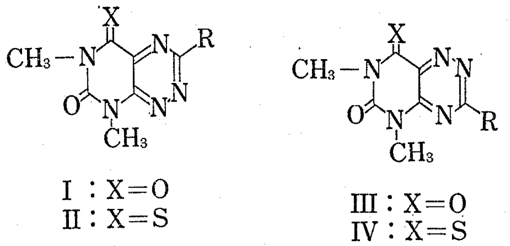
1,3-Dimethyl-4-thio-7-azalumazine derivatives (5-thiofervenulins) and 1,3-dimethyl-4-thio-6-azalumazine derivatives (5-thioisofervenulins) were synthesized by the thiation of 1,3-dimethyl-7-azalumazine derivatives (fervenulins) and 1,3-dimethyl-6-azalumazine derivatives (isofervenulins). The 1,3-dimethyl-4-thio-6-azalumazines were converted into theophyllines by treatment with sodium dithionite in formic acid. These thio-azalumazines displayed low antibacterial activities *in vitro*.

For the past decade there has been considerable interest in the synthesis and chemistry of 7-aza- and 6-aza-pteridines, which have recently been reviewd.[2] This review covers the literatures until 1969, and since those data there have been already more than 10 reports.[3–7] However, the sulfur-containing azapteridines are little known except pyrimido[5,4-*e*]-*as*-triazine-5(6H)-thiones[4,8] and 7-alkylmercapto-6-azalumazines.[9] It should be noted that the latter compounds exhibited antiviral activities.[10]



I : X=O
II : X=S

III : X=O
IV : X=S

Chart 1

This paper is concerned with the syntheses and properties of biologically interesting 4-thio-analogues of 6-substituted 1,3-dimethyl-7-azalumazine (fervenulin) and 7-substituted 1,3-dimethyl-6-azalumazine (isofervenulin). The starting materials, 1,3-dimethyl-7-azalumazine derivatives (I), were prepared by the condensation of 6-amino-1,3-dimethyl-5-nitrosouracil and aldehyde hydrazones.[6a] These 1,3-dimethyl-7-azalumazine derivatives were converted into 1,3-dimethyl-4-thio-7-azalumazine derivatives (5-thiofervenulins) (II) by treatment with phosphorus pentasulfide in pyridine. The characteristic two carbonyl bands of I at 1689 and 1720 cm^{-1} region disappeared and a new carbonyl

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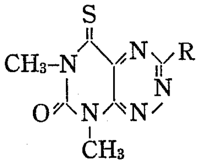
1) Location: *a) Oe-honmachi, Kumamoto*; *b) Shinanomachi, Shinjuku-ku, Tokyo.*
2) F. Yoneda, *Kagaku-no Ryoiki*, **24**, 1077 (1970).
3) *a*) D.J. Brown and T. Sugimoto, *J. Chem. Soc.* (*C*), **1972**, 237; *b*) *Idem*, *ibid.*, **1971**, 2616; *c*) *Idem*, *Austral. J. Chem.*, **24**, 633 (1971); *d*) *Idem*, *J. Chem. Soc.* (*C*), **1970**, 2661; *e*) M.E.C. Biffin, D.J. Brown, and T. Sugimoto, *ibid.*, **1970**, 139.
4) *a*) C. Temple, C.L. Kussner, and J.A. Montgomery, *J. Org. Chem.*, **36**, 3502 (1971); *b*) *Idem*, *ibid.*, **36**, 2974 (1971).
5) E.C. Taylor and S.F. Martin, *J. Org. Chem.*, **35**, 3792 (1970).
6) *a*) F. Yoneda, M. Kanahori, K. Ogiwara, and S. Nishigaki, *J. Heterocyclic Chem.*, **7**, 1443 (1970); *b*) F. Yoneda, M. Kanahori, and S. Nishigaki, *ibid.*, **8**, 523 (1971); *c*) F. Yoneda, K. Shinomura, and S. Nishigaki, *Tetrahedron Letters*, **1971**, 851; *d*) F. Yoneda, S. Nishigaki, and K. Shinomura, *Chem. Pharm. Bull.* (Tokyo), **19**, 2647 (1971).
7) M. Brugger, H. Wamhoff, and F. Korte, *Ann.*, **758**, 173 (1972).
8) C. Temple, C.L. Kussner, and J.A. Montgomery, *J. Org. Chem.*, **34**, 3161 (1969).
9) L. Heinisch, W. Ozegowski, and M. Mühlstadt, *Chem. Ber.*, **97**, 5 (1964).
10) C. Küchler, W. Küchler, and L. Heinishi, *Arzneimittel-Forsch.*, **16**, 1122 (1966).

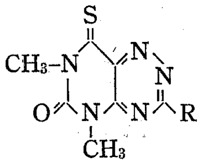
band came out at 1680 cm^{-1} region. Similarly, 1,3-dimethyl-4-thio-6-azalumazine derivatives (5-thioisofervenulins) (IV) were obtained by the thiation of 1,3-dimethyl-6-azalumazines (III) which were prepared by the reaction of 1,3-dimethyl-6-hydroxylaminouracil with acid hydrazides.[6b] The characteristic two carbonyl bands of III at 1670 and 1720 cm^{-1} region disappeared and a new carbonyl band came out at 1690 cm^{-1} region.

Table I. Preparation of 6-Substituted 1,3-Dimethyl-4-thio-7-azalumazines (5-Thiofervenulins)



No.	R	Reaction time (hr) (180°)	Recrystn. solvent	mp (°C)	Yield (%)	Formula	Analysis (%)					
							Calcd.			Found		
							C	H	N	C	H	N
IIa	phenyl	16	$CHCl_3$+ MeOH	269	56.6	$C_{13}H_{11}ON_5S$	54.73	3.89	24.55	54.66	3.91	24.29
IIb	*p*-chlorophenyl	15	$CHCl_3$+ MeOH	273	53.1	$C_{13}H_{10}ON_5SCl$	48.83	3.15	21.90	48.68	3.04	21.71
IIc	3,4-methylenedioxyphenyl	32	benzene	290	32.2	$C_{14}H_{11}O_3N_5S$	51.06	3.37	21.27	50.87	3.32	21.15
IId	2-pyridyl	15	$CHCl_3$+ MeOH	252	49.6	$C_{12}H_{10}ON_6S$	50.35	3.52	29.36	50.17	3.42	29.30
IIe	3-pyridyl	18	$CHCl_3$+ MeOH	210	48.6	$C_{12}H_{10}ON_6S$	50.35	3.52	29.36	50.24	3.51	29.09
IIf	4-pyridyl	21	$CHCl_3$+ MeOH	250	39.1	$C_{12}H_{10}ON_6S$	50.35	3.52	29.36	50.16	3.43	29.12

Table II. Preparation of 7-Substituted 1,3-Dimethyl-4-thio-6-azalumazines (5-Thioisofervenulins)



No.	R	Reaction time (hr) (180—200°)	Recrystn. solvent	mp (°C)	Yield (%)	Formula	Analysis (%)					
							Calcd.			Found		
							C	H	N	C	H	N
IVa	2-hydroxyphenyl	18	benzene	253	33.8	$C_{13}H_{11}O_2N_5S$	51.83	3.68	23.25	51.77	3.54	23.04
IVb	3-pyridyl	17	$CHCl_3$+ MeOH	244	42.0	$C_{12}H_{10}ON_6S$	50.35	3.52	29.36	50.28	3.50	29.31
IVc	4-pyridyl	20	$CHCl_3$+ MeOH	240	43.5	$C_{12}H_{10}ON_6S$	50.35	3.52	29.36	50.19	3.46	29.22
IVd	2-thienyl	20	$CHCl_3$+ MeOH	228	32.1	$C_{11}H_9ON_5S_2$	45.36	3.12	24.05	45.23	3.07	24.21

A representative of II, 6-(*p*-chlorophenyl)-1,3-dimethyl-4-thio-7-azalumazine (IIb), was hydrolyzed by alcoholic sodium hydroxide at room temperature to give 3-(*p*-chlorophenyl)-6-methylamino-5-methylaminothiocarbonyl-*as*-triazine (V). Its structure was ascertained by satisfactory elemental analysis and the presence of two NH absorption bands at 3000 and 3200 cm^{-1} and disappearance of the characteristic carbonyl band at 1680 cm^{-1} and the molecular weight determination by mass spectrometry. Similarly, treatment of a representative of IV, 1,3-dimethyl-7-(3-pyridyl)-4-thio-6-azalumazine (IVb), with ethanolic sodium hydroxide at room temperature gave the 5-methylamino-6-methylaminothiocarbonyl-3-(3-pyridyl)-*as*-triazine (VI). This compound also gave satisfactory analytical and spectral data. The facts described above show that the thiation took place at the 4-position of the azalumazines.

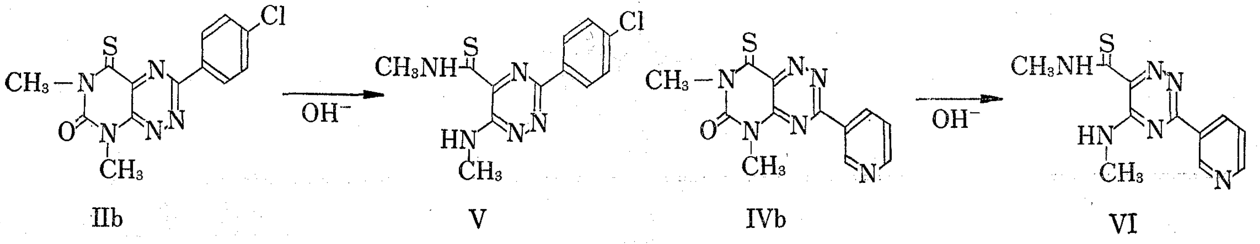


Chart 2

The reduction of IV with excess sodium dithionite in formic acid gave the respective 8-substituted theophyllines (VIII), whose structures were established by comparison with authentic samples by the known route.[11] This reaction proceeds without doubt *via* the dethiation of IV to III, because the initial reaction mixture includes only the intermediate 6-azalumazine. Furthermore, treatment of 4-thio-6-azalumazine derivatives with aniline gave also 6-azalumazine derivatives. These facts show that the 4-thione of IV can be easily changed to the 4-one. Therefore, the ring contraction observed in the conversion of IV to 8-substituted theophyllines (VIII) is best rationalized by assuming the reductive nitrogen-nitrogen bond cleavage of the initially formed 6-azalumazine derivative into a 5-amino-6-amidinouracil derivative (VII), followed by intramolecular cyclization with elimination of ammonia.

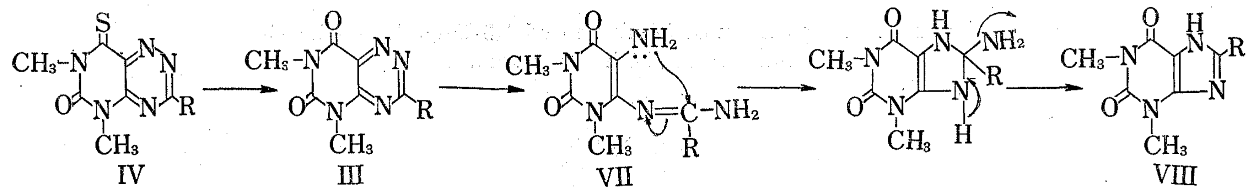


Chart 3

TABLE III. Conversion of 6-Substituted 1,3-Dimethyl-4-thio-6-azalumazines into 8-Substituted Theophyllines

No.	Substituent	Reaction Time (hr)	Reaction Temp. (°C)	Recrystn. solvent	mp (°C)	Yield (%)	Formula	Analysis (%) Calcd. C	H	N	Found C	H	N
VIIIa	3-pyridyl	18	185	DMF	>320	53	$C_{12}H_{11}O_2N_5$	56.02	4.31	27.23	56.13	4.33	27.09
VIIIb	4-pyridyl	20	185	DMF	>320	60	$C_{12}H_{11}O_2N_5$	56.02	4.31	27.23	56.20	4.27	27.34
VIIIc	2-thienyl	20	160	DMF	>320	40	$C_{11}H_{10}O_2N_4S$	50.38	3.84	21.37	50.22	3.78	21.26

The reduction of 4-thio-7-azalumazine derivatives with sodium dithionite in formic acid under the same conditions did not give the corresponding purines, but only the dethiation products, 7-azalumazine derivatives.

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11) F. Yoneda, K. Ogiwara, M. Kanahori, and S. Nishigaki, *Chem. Commun.*, **1970**, 1068.

Aa can be seen from Table IV and V, these compounds demonstrated weak activity *in vitro* against *Staphylococcus aureus* and *Trichophyton mentagrophytes*. These compounds did not exhibited activity against gram-negative organisms. However, it is interesting to note that only compound IIe exhibited activity *in vitro* against *Mycobacterium tuberculosis* H37 Rv at 3.13—6.25 μg/ml.

TABLE IV. Antibacterial Activity of Thioazaluamzines[a)]
Min. Inhib. Concn., μg/ml

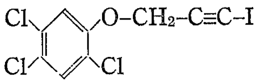
Compound	*Staphylococcus aureus*	*Trichophyton mentagrophytes*
IIa	100	50
IIc	100	[b)]
IId	50	6.25
IIe	50	6.25—12.5
IVa	50	[b)]
IVb	12.5	[b)]
IVc	50	[b)]
IVd	100	[b)]
Sulfamonomethoxine[c)]	6.3	—
Bithinol[d)]	—	0.5

a) None of these compounds inhibited growth of gram-negative organisms.
b) See Table V.
c) N^1-(6-methoxy-4-pyrimidinyl)sulfanilamide
d) bis(2-hydroxy-3,5-dichlorophenyl)sulfide

TABLE V. Antifungal Activity Inhibitory Zone (mm)

Compound	*Trichophyton mentagrophytes*	
	NS	S
IIc	—	—
IVa	12.5	—
IVb	17.5	—
IVc	—	—
IVd	12.0	—
Haloprogine	26.0	25.0

NS: non-synthetic medium (glucose, peptone, yeast extracts, agar)
S: synthetic medium (glucose, Na-glutamate, biotin, thiamine-HCl, inositol, KH_2PO_4, $MgSO_4$-$7H_2O$, Agar)
—: no inhibition
haloprogine:



Experimental[12)]

General Procedure for Synthesis of 6-Substituted 1,3-Dimethyl-4-thio-7-azalumazines (IIa—f)——A mixture of 0.54 g (0.002 mole) of 1,3-dimethyl-6-phenyl-7-azalumazine and 4.4 g (0.02 mole) of P_2S_5 in 50 ml of pyridine was refluxed for 20 hr at 180°. After cooling, the reaction mixture was diluted with 50 ml of H_2O and allowed to stand at room temperature overnight. The precipitated crystals were collected by filtration, dried and extracted with 100 ml of hot AcOH. After the AcOH extracts were evaporated under reduced pressure, the residue was diluted with MeOH to separate yellow crystals, which were filtered off and purified by recrystallization.

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12) Melting points are uncorrected. Infrared spectra were determined on a Japan Spectroscopic Co., Ltd. spectrophotometer, Model IR-E, from samples mulled in Nujol.

General Procedure for Synthesis of 7-Substituted 1,3-Dimethyl-4-thio-6-azalumazines (IVa—d)——A mixture of 1.0 g (0.0037 mole) of 1,3-dimethyl-6-(4-pyridyl)-6-azalumazine, and 5.0 g (0.023 mole) of P_2S_5 in 40 ml of pyridine was heated at 180—200° for 16 hr. After excess pyridine was removed under reduced pressure, the resulting syrupy residue was diluted with 50 ml of H_2O and allowed to stand overnight. The separated crystals were collected by filtration, dried and extracted with 100 ml of hot AcOH. The AcOH extracts were evaporated under reduced pressure to separate yellow crystals, which were filtered off and purified by recrystallization.

Hydrolysis of 6-(*p*-Chlorophenyl)-1,3-dimethyl-4-thio-7-azalumazine (IIb)——To a mixture of 2 ml of 2N NaOH and 2 ml of EtOH, 0.2 g (0.00062 mole) of IIb was added. After being stirred at room temperature for 4 hr, the reaction mixture was allowed to stand overnight and then diluted with 10 ml of H_2O. The separated crystals were filtered off and recrystallized from MeOH to give colorless crystals of 3-(*p*-chlorophenyl)-6-methylamino-5-methylaminothiocarbonyl-*as*-triazine (V), mp 244°. *Anal.* Calcd. for $C_{12}H_{12}N_5SCl$: C, 49.06; H, 4.12; N, 23.84. Found: C, 48.86; H, 4.05; N, 23.74. Mass Spectrum *m/e*: 293 (M^+).

Hydrolysis of 1,3-Dimethyl-7-(3-pyridyl)-4-thio-6-azalumazine (IVb)——To a mixture of 2 ml of 2N NaOH and 2 ml of EtOH, 0.2 g (0.0007 mole) of IVb was added. After being stirred at room temperature for 2 hr, the reaction mixture was allowed to stand overnight to precipitate pale yellow crystals, which were filtered off and recrystallized from MeOH to give pale yellow needles of 5-methylamino-6-methylaminothiocarbonyl-3-(3-pyridyl)-*as*-triazine (VI), mp 200°. *Anal.* Calcd. for $C_{11}H_{12}N_6S$: C, 50.76; H, 4.65; N, 32.30. Found: C, 50.68; H, 4.62; N, 32.02. Mass Spectrum *m/e*: 260 (M^+).

Conversion of 4-Thio-6-azalumazines into Theophyllines (VIIIa—c)——A mixture of 0.5 g (0.0018 mole) of IIe and 5 g (0.029 mole) of $Na_2S_2O_4$ in 30 ml of 90% HCOOH was refluxed for 18 hr at 185°. After cooling, the precipitated crystals were filtered off, washed with H_2O and recrystallized from DMF to give 8-(3-pyridyl)-theophylline (VIIIa), which was in all respects identical with the authentic sample.[11]

Antibacterial Activity——A) Two Fold Serial Dilution Method: Casamino acids medium was used for *Staphylococcus aureus*. Sabouraud's medium (1% glucose) was used for *Trichophyton mentagrophytes*. The test compounds were dissolved in the medium mentioned above and diluted by the two fold serial dilution method and inoculated with the organisms. Casamino acids medium was incubated at 37° for 48 hr. Sabouraud's medium was incubated at 28° for 7 days. Then the minimum inhibitory concentration of test samples was estimated by comparison with sulfamonomethoxine and bithionol.

B) Paper Disk Method: Each sample was dissolved in DMSO and made up to a solution of 1000 μg/ml. Paper disks, dipped into this solution and dried under reduced pressure, were used for paper disk method. After 7 days incubation at 28°, the diameter of inhibitory zone around paper disk was measured.

Acknowledgement We express our sincere gratitute to Daiichi Seiyaku Co., Ltd. for carrying out the antibacterial tests.