1-Phenyl-3-(3-chloro-4-thiocyanatophenyl)urea (XVII)——To a solution of 18 g (0.1 mole) of 3-chloro-4-thiocyanatoaniline in 200 ml of dry benzene was added a solution of 14 g (0.12 mole) of phenyl isocyanate in 200 ml of dry benzene, and the mixture was refluxed for 1 hr. After benzene was evaporated under reduced pressure, the residue was recrystallized from EtOH to give 23 g (76%) of XVII as colorless prisms, mp 187—188°. 1-(4-Nitrophenyl)-3-(3-chloro-4-thiocyanatophenyl)urea (XVIII) was prepared in the same way as that for XVII.

N-(4-Chlorobenzylidene)-3-chloro-4-thiocyanatoaniline (XIX)——To a solution of 18 g (0.1 mole) of 3-chloro-4-thiocyanatoaniline in 200 ml of dry benzene was added a solution of 17 g (0.13 mole) of 4-chlorobenzaldehyde in 200 ml of dry benzene at 5°, the mixture was evaporated to dryness under reduced pressure and the residue was recrystallized from EtOH to give 21 g (70%) of XIX as colorless needles, mp 135—136°.

Other N-benzylidene-3-chloro-4-thiocyanatoanilines (XX to XXII) were prepared in the same way as that for XIX.

2-Trichloroacetamido-6-chlorobenzothiazole (XXIII)——This compound was prepared from compound (B) and trichloroacetyl chloride by the same procedure as that for I.

Alkyl N-[2-(6-Chlorobenzothiazoyl)]carbamate (XXIV to XXVI)——These compounds were prepared from compound (B) and alkyl chloroformates in the same way as that for IX.

2-(3-Arylureido)-6-chlorobenzothiazoles (XXVII and XXVIII)——These compounds were prepared from compound (B) and phenyl isocyanates in the same way as that for XVII.

N-(4-Chlorobenzylidene)-2-amino-6-chlorobenzothiazole (XXIX)——This compound was prepared from compound (B) and 4-chlorobenzaldehyde by the same method as that for XIX.

Acknowledgement The authors express their deep gratitude to Professor Haruo Saikachi of the Kobe Gakuin University for his encouragement throughout the course of this work. Thanks are also due to the Analytical Center of Kyoto University for elemental analyses.

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Synthesis of New Antimicrobials. IV.[1] Synthesis of Alkylenebis-(thiourea) Derivatives and Their Related Compounds

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In order to examine the antimicrobial activity, alkylenebis(thiourea) derivatives and their related compounds were synthesized. 3,3′-Substituted 1,1′-alkylene-bis-(thiourea) derivatives were prepared from alkylenebis(isothiocyanates) and amines. Also 3,3′-alkylene-bis[2-thio-2,4(1H, 3H)-quinazolinediones] were prepared by the reaction of alkylenebis(isothiocyanates) and anthranilic acid, or alkylene diamines and ethyl *o*-isothiocyanatobenzoate respectively.

Antibacterial and anticandida's activities of thiocyanatobenzene derivatives have been of great interest and the present authors reported the synthesis of thiocyanatoaniline derivatives in previous papers.[1,3] In 1953, Kerk, *et al.*[4] reported the potent antimicrobial activities of isothiocyanates. In general, the potent antimicrobial activity has been found in many compounds which have a thiocyanate or thiourea group. Some of them have been

1) Part III: T. Yabuuchi, M. Hisaki, and R. Kimura, *Chem. Pharm. Bull.* (Tokyo), **23**, 659 (1975).
2) Location: *15-Morimoto-cho, Shimogamo, Sakyo-ku, Kyoto.*
3) R. Kimura, T. Yabuuchi, M. Hisaki, H. Sugimoto, A. Ohyama, and K. Mochida, *Chem. Pharm. Bull.* (Tokyo), **10**, 1226 (1962).
4) G.T.M. van der Kerk, H.C. van Os, G. de Vries, and A.K. Sijpestein, *Mededel. Landbouwhogeschool Opzoekingssta. Staat Gent.*, **18**, 402 (1953).

used as antituberculosis agents, such as 1,3-di(4′-isoamyloxyphenyl)thiourea.[5] Davies, *et al.*[6] reported that Chlorhexidine [1,6-di(4′-chlorophenyldiguanido)hexane] had a most potent antimicrobial activity in disubstituted alkylene guanidine derivatives, as a result of investigation the relationship between the activity and number of alkylene groups in the main chain.

As part of an effort to develop new antimicrobial agents, 1,1′-alkylenebis(3,3′-substituted thiourea) derivatives were prepared from alkylenebis(isothiocyanates) in our laboratory. Alkylenebis(isothiocyanates) were generally synthesized by thermal decomposition[7] of sodium alkylenebis(dithiocarbamate) from alkylenediamines as in Chart 1.

On the other hand, 1,4-bis(isothiocyanatomethyl)benzene was synthesized by Tariton[8] by refluxing 1,4-bis(chloromethyl)benzene with sodium thiocyanate and sodium iodide in dimethylformamide (DMF) solution. We prepared alkylenebis(isothiocyanates) (I to VI) in 40—70% yield from alkylene dihalides by Tariton's method, which are summarized in Table I.

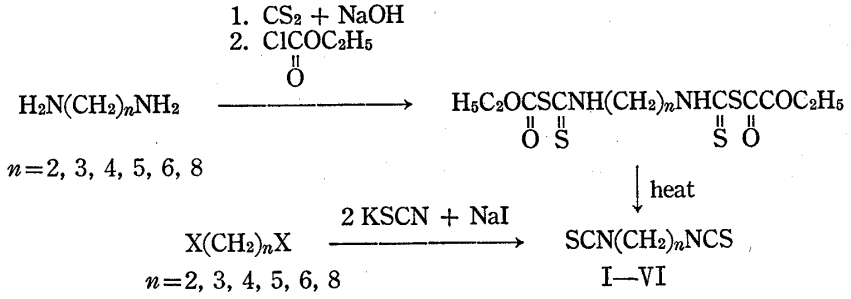


Chart 1

TABLE I. Alkylenebis(isothiocyanate)

Compd. No.	Structure of formula	bp (°C/mm Hg)	Appearance	Yield (%)
I	$SCN(CH_2)_2NCS$	135—140/10	colorless oil	60
II	$SCN(CH_2)_3NCS$	134/4	colorless oil	70
III	$SCN(CH_2)_4NCS$	144—150/3	colorless oil	65
IV	$SCN(CH_2)_5NCS$	150—156/5	colorless oil	50
V	$SCN(CH_2)_6NCS$	160—180/3	colorless oil	70
VI	$SCN(CH_2)_8NCS$	175—180/3	colorless oil	40

3,3′-Disubstituted 1,1′-alkylene-bis(thioureas) (VIII to XXVI) were obtained by the reaction of alkylenebis(isothiocyanates) (I, II, III, V) and two equivalents of amines at room temperature. The reaction of propylenebis(isothiocyanate) and aniline at −10° gave 1-(3-isothiocyanatopropyl)-3-phenylthiourea (VII) which was changed to 3,3′-diphenyl-1,1′-propylene-bis(thiourea) (XIII) by the addition of another mole of aniline at room temperature. The above-mentioned reactions are shown in Chart 2, and these new compounds are listed in Table II.

In the reaction of equimolar amount of alkylenebis(isothiocyanates) (II, III, V) and anthranilic acid or its ester at −20°, 3,3′-alkylene-bis[2-thio-2,4(1H, 3H)-quinazolinediones] (XXVIII, XXIX) were obtained except in the case of 3-(4-isothiocyanatobutyl)-2-thio-2,4-(1H, 3H)-quinazolinedione (XXX), which afforded 3,3′-tetramethylene-bis[2-thio-2,4(1H, 3H)-

5) L. Doub, L.M. Richardson, D.R. Herbst, M.L. Black, O.L. Stevenson, L.L. Bambas, G.P. Youmans, and H.S. Youmans, *J. Am. Chem. Soc.*, **80**, 2205 (1658).
6) G.E. Davies, J. Francis, A.R. Martin, F.L. Rose, and G. Swain, *Brit. J. Pharmacol.*, **9**, 192 (1954).
7) H.L. Klopping and G.J.M. van der Kerk, *Rec. Trav. Chim.*, **70**, 949 (1951).
8) E.J. Tariton and A.F. Mackay, Ger. Patent, 1148540 (1963) [*C.A.*, **60**, 2825h (1964)].

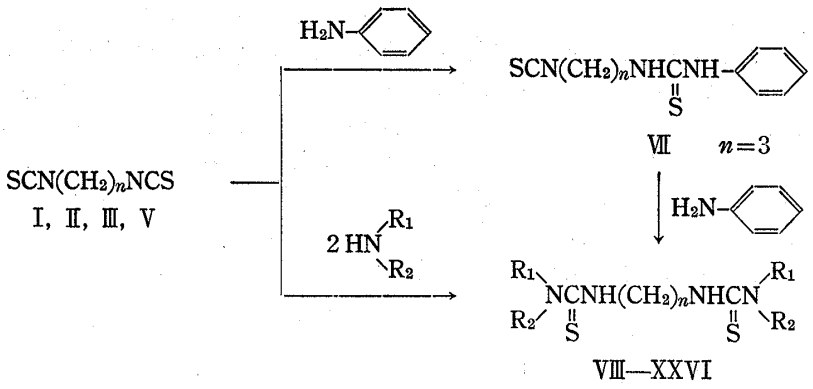
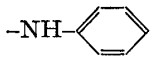
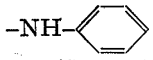
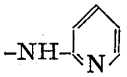
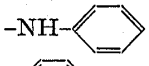


Chart 2

TABLE II. 3,3′-Disubstituted 1,1′-Alkylene-bis (thioureas)
R-CNH(CH$_2$)$_n$NHC-R
S S

Compd. No.	n	R	mp (°C) ( ): Cryst. solvent	Appearance (colorless)	Formula	Analysis (%) Calcd. (Found) C	H	N
VIII	2	$-NHCH_2CH_2N(C_2H_5)_2$	103—104 (benzene)	prisms	$C_{16}H_{36}N_6S_2$	51.02 (51.27)	9.68 (9.64)	22.31 (22.32)
IX	2	$-NH(CH_2)_3N(CH_3)_2$	123—124 (benzene)	prisms	$C_{14}H_{32}N_6S_2$	48.23 (48.50)	9.26 (9.22)	24.11 (23.85)
X	2		214—215 (90% EtOH)	prisms	$C_{14}H_{30}N_6S_2Cl_2$	40.27 (40.05)	7.24 (7.53)	20.13 (20.13)
XI	2		184—186 (H_2O)	prisms	$C_{16}H_{32}O_2N_6S_2$	47.49 (47.47)	7.97 (8.12)	20.77 (20.90)
XII	3	$-NH-CH_2CH_3$	124—126 ($CHCl_3$)	prisms	$C_9H_{20}N_4S_2$	43.51 (43.24)	8.11 (8.06)	22.55 (22.35)
XIII	3		135—137 (EtOH)	prisms	$C_{17}H_{20}N_4S_2$	59.27 (59.09)	5.85 (5.90)	16.27 (16.31)
XIV[a)]	4		174—176 (dioxane)	prisms	$C_{18}H_{22}N_4S_2$	60.30 (60.21)	6.19 (6.30)	15.63 (15.77)
XV	6	$-NHCH_2CH=CH_2$	103—104 (EtOH)	prisms	$C_{14}H_{26}N_4S_2$	53.46 (53.21)	8.33 (8.32)	17.81 (17.85)
XVI	6	$-NHCH_2CH_2OH$	153—154 (20% MeOH)	prisms	$C_{12}H_{26}O_2N_4S_2$	44.69 (44.52)	8.12 (8.01)	17.37 (17.29)
XVII	6	$-NH(CH_2)_3OH$-*n*	159—160 (20% MeOH)	prisms	$C_{14}H_{30}O_2N_4S_2$	47.96 (48.02)	8.62 (8.63)	15.98 (15.87)
XVIII	6	$-NHCH_2CH(CH_3)OH$	136—137 (benzene)	prisms	$C_{14}H_{30}O_2N_4S_2$	47.96 (47.82)	8.62 (8.60)	15.98 (15.89)
XIX	6	$-NHC(CH_3)_2CH_2OH$	152—153 (EtOH)	prisms	$C_{16}H_{34}O_2N_4S_2$	50.75 (50.72)	9.05 (9.01)	14.75 (14.60)
XX	6	$-NHCCH_3(CH_2OH)_2$	137—138 (20% EtOH)	prisms	$C_{16}H_{34}O_4N_4S_2$	46.80 (46.57)	8.34 (8.44)	13.64 (13.72)
XXI	6	$-N(CH_2CH_2OH)_2$	135—136 (MeOH)	prisms	$C_{16}H_{34}O_2N_4S_2$	46.80 (46.79)	8.34 (8.21)	13.64 (13.62)
XXII	6		202—203 (DMF)	prisms	$C_{16}H_{24}N_6S_2$	55.58 (55.46)	6.22 (6.22)	21.62 (21.66)
XXIII	6		148—149 (MeOH)	prisms	$C_{20}H_{38}N_4S_2$	60.25 (60.31)	9.61 (9.52)	14.05 (13.97)
XXIV	6		138—139 (EtOH)	prisms	$C_{18}H_{34}N_4S_2$	58.33 (58.12)	9.24 (9.30)	15.11 (15.11)
XXV	6		124—125 (MeOH)	prisms	$C_{16}H_{32}N_4S_2$	51.03 (49.78)	8.56 (8.61)	14.87 (14.70)
XXVI	6		193—195 (dioxane)	prisms	$C_{20}H_{26}N_4S_2$	58.33 (58.40)	9.24 (9.41)	15.11 (15.10)

a) W. Lehmann and H. Rinke, Ger. Patent, 842065 (1962) (mp, no description) [*C.A.*, **52**, 10208 b (1962)].

quinazolinedione] (XXVIII) by further reaction with one equivalent of methyl anthranilate at room temperature. The reaction of alkylenebis(isothiocyanate) (II, III, V) with two equivalents of anthranilic acid ester gave 3,3′-alkylene-bis[2-thio-2,4(1H, 3H)-quinazolinedione] (XXVII to XXIX) at room temperature, and the intermediates 1,1′-bis(*o*-alkoxycarbonylphenyl)-3,3′-alkylene-bis(thioureas) were not isolated. Cherbuliez, *et al.*[9] reported that XXVII and XXIX could be synthesized by the reaction of ethyl *o*-isothiocyanatobenzoate with corresponding alkylenediamines. 3,3′-Tetramethylene-bis[2-ethylthio-2,3-dihydro-4(1H)-quinazolinone] (XXXI) was obtained by the reaction of ethyl iodide with 3,3′-

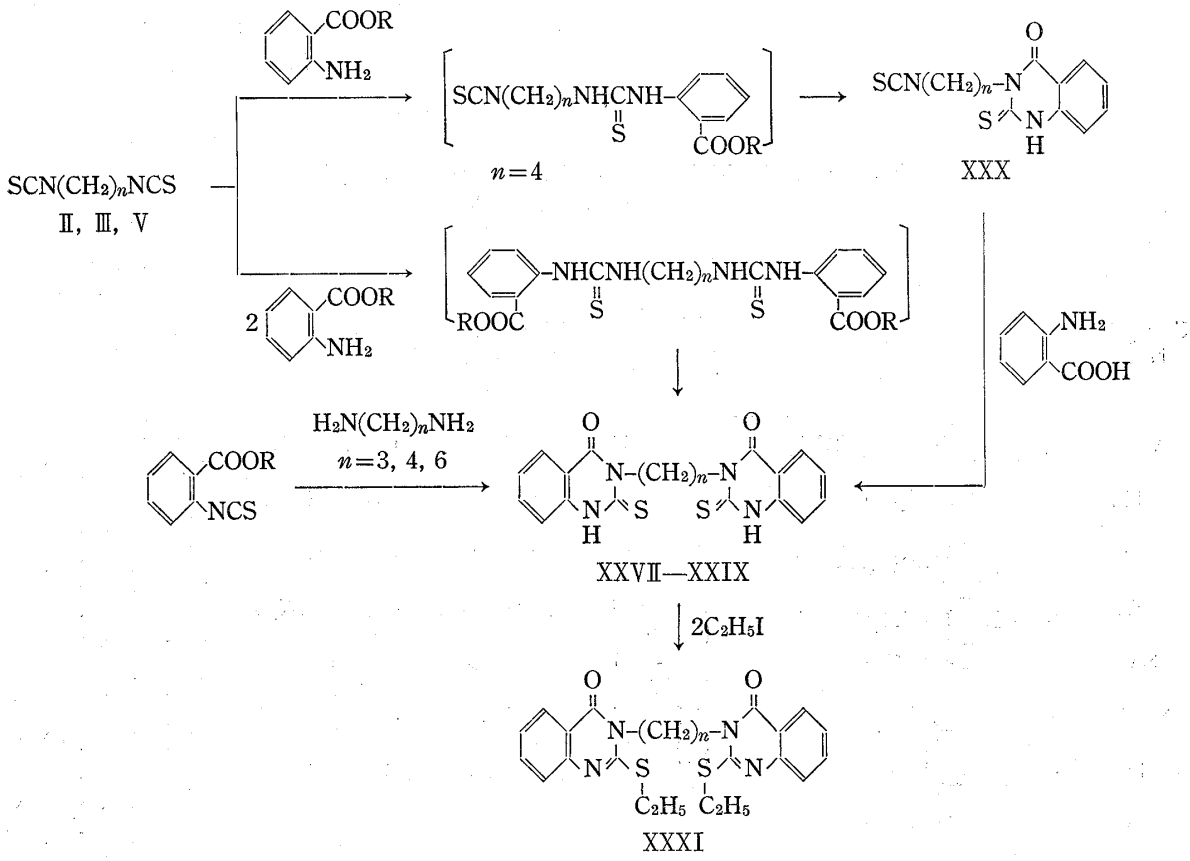
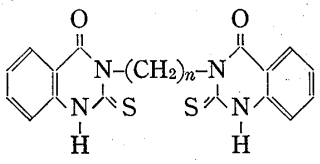


Chart 3

TABLE III. 3,3′-Alkylene-bis[2-thio-2,4-(1H,3H)quinazolinediones]



Compd. No.	*n*	mp (°C) ( ); Cryst. solvent	Appearance (colorless)	Formula	Analysis (%) Calcd. (Found) C	H	N
XXVII[9]	3	300 (DMF)	prisms	$C_{19}H_{16}O_2N_4S_2$	57.62 (57.90)	4.04 (4.10)	14.15 (14.41)
XXVIII	4	300 (DMF)	prisms	$C_{20}H_{18}O_2N_4S_2$	58.58 (58.65)	4.42 (4.48)	13.67 (13.70)
XXIX[9]	6	300 (DMF)	prisms	$C_{22}H_{22}O_2N_4S_2$	60.32 (60.30)	5.02 (5.00)	12.79 (12.60)

9) B. Cherbuliez, B. Willhalm, O. Espejo, S. Jaccard, and J. Rabiwiz, *Helv. Chim. Acta*, **50**, 1440 (1967).

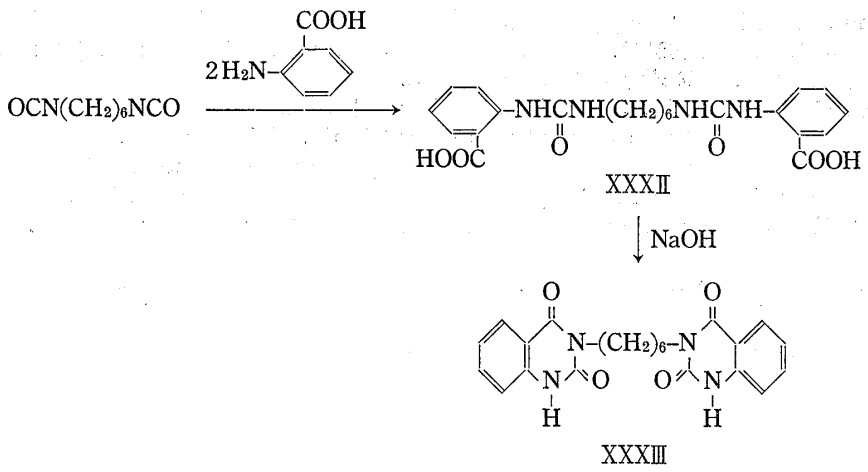


Chart 4

tetramethylene-bis[2,4(1H, 3H)-quinazolinedione] (XXVIII). These reactions and the products are shown in Chart 3 and Table III.

1,1′-Hexamethylene-bis[3,3′-(*o*-carboxyphenyl)urea] (XXXII) was prepared by the reaction of anthranilic acid with hexamethylene-bis(isocyanate) which had a similar structure to that of isothiocyanate, and XXXII was further converted to 3,3′-hexamethylene-bis[2,4-(1H, 3H)-quinazolinedione] (XXXIII) by warming in a diluted aqueous sodium hydroxide solution, as was shown in Chart 4.

Experimental

All melting points are determined in open capillary tubes and are uncorrected. Infrared (IR) spectra are measured with a Shimadzu Model IR-60 spectrophotometer.

Alkylenediisothiocyanates (I to VI)——A solution of alkylene dihalides (1 mole) and KSCN (2.2 mole) in DMF was refluxed for 15 min and the solution was concentrated under reduced pressure. The residue was extracted with $CHCl_3$, and the combined extract was washed with excess water. After being dried over anhyd. Na_2SO_4, $CHCl_3$ was evaporated *in vacuo*, and the residue was distilled under reduced pressure to give the product (see Table I). IR ν_{max}^{liquid} cm^{-1}: 2110, 2200 (–N=C=S).

1-(3-Isothiocyanatopropyl)-3-phenylthiourea (VII)——To a solution of 7.9 g (0.5 mole) of II in 20 ml $CHCl_3$ was added a solution of 4.6 g (0.5 mole) of aniline in 20 ml of $CHCl_3$ at −20°, and the mixture was allowed to stand at room temperature overnight, then concentrated to dryness under reduced pressure. The residue was recrystallized from isoPrOH to colorless prisms, mp 109—110°. Yield, 9.36 g (75%). *Anal.* Calcd. for $C_{11}H_{13}N_3S_2$: C, 52.56; H, 5.21; N, 16.72; S, 25.51. Found: C, 52.77; H, 5.44; N, 16.95; S, 25.35. IR ν_{max}^{liquid} cm^{-1}: 2110, 2200 (–N=C=S), 1560 (C=S).

3,3′-Diphenyl-1,1′-propylene-bis(thiourea) (XIII)——To a solution of 6.3 g (0.02 mole) of VII in EtOH was added a solution of 1.9 g (0.02 mole) of aniline in 10 ml of EtOH at −10°, the reaction mixture was allowed to stand at room temperature overnight and the mixture was evaporated to dryness under reduced pressure. The residue was crystallized from EtOH to colorless prisms, mp 135—137°. Yield, 0.56 g (83%). IR ν_{max}^{liquid} cm^{-1}: 1530 (C=S).

3,3′-Alkylene-bis[2-thio-2,4(1H, 3H)-quinazolinediones] (XXVII to XXIX)——To a solution of 1 mole of alkylenebis(isothiocyanate) (II, III, or V) in 500 ml of EtOH was added a solution of 2 mole of methyl anthranilate in 300 ml of EtOH at −30°, the mixture was allowed to stand at room temperature overnight and the mixture was evaporated to dryness under reduced pressure. The residue was extracted with hot EtOH. The combined ethanolic extracts were evaporated *in vacuo*, and the residue was crystallized from DMF (see Table III).

3,3′-Tetramethylene-bis[2-ethylthio-4(3H)-quinazolinone] (XXXI)——To a solution of 0.8 g (0.0019 mole) of XXX in 20 ml of 10% NaOH was added a solution of 0.6 g (0.0039 mole) of EtI in 30 ml of EtOH. The whole was heated at 70—80° for 10 min. The mixture was filtered, the filtrate was evaporated *in vacuo*, and the residue was washed with excess water and recrystallized from EtOH to colorless prisms, mp 153—154°. *Anal.* Calcd. for $C_{24}H_{26}O_2N_4S_2$: C, 61.77; H, 5.61; N, 12.01. Found: C, 61.88; H, 5.66; N, 12.02. IR ν_{max}^{liquid} cm^{-1}: 1630 (C=O).

1,1′-Hexamethylene-bis(o-carboxyphenylurea) (XXXII)——To a solution of 4.2 g (0.025 mole) of hexamethylenebis(isocyanate) in toluene 6.8 g (0.05 mole) of anthranilic acid in toluene was added dropwise with stirring, while the temperature of the mixture was kept below 0°. Then the reaction mixture was stirred at

40—50° for 30 min, filtered, and the precipitate was recrystallized from iso-PrOH to give colorless prisms, mp 164—166°. Yield, 10.59 g (95%). *Anal.* Calcd. for $C_{22}H_{26}O_6N_4$: C, 47.95; H, 7.60; N, 16.37. Found: C, 48.02; H, 7.59; N, 16.40. IR ν_{max}^{liquid} cm^{-1}: 1660 (C=O).

3,3′-Hexamethylene-bis[2,4(1H, 3H)-quinazolinedione] (XXXIII)——A solution of 3 g (0.01 mole) of XXXII in 8 ml of 10% NaOH was warmed at 50° for 30 min. When cooled, the reaction mixture was acidified with 10% HCl with stirring and the resulting crystals were collected. The crystals were washed with water and recrystallized from DMF–H_2O to colorless prisms, mp>300°. Yield, 3.2 g (78.8%). *Anal.* Calcd. for $C_{22}H_{22}O_4N_4$: C, 65.01; H, 5.45; N, 13.78. Found: C, 64.97; H, 5.44; N, 13.82.

Acknowledgement The authors express their deep gratitude to Professor Haruo Saikachi, Kobe Gakuin University, for his encouragement throughout the course of this work. Thanks are also due to the Analytical Center of Kyoto University for elemental analyses.

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Synthesis of New Antimicrobials. V.[1] Synthesis of Alkylenebis-(thiosemicarbazides) and Their Related Compounds

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(Received June 29, 1974)

Several kinds of alkylenebis(thiosemicarbazide) and alkylenebis(bithiourea) derivatives were synthesized in order to examine their antimicrobial activity. 1,1′-Dibenzylidene-4,4′-alkylene-bis(thiosemicarbazides) were prepared from 4,4′-alkylene-bis(thiosemicarbazides) and arylaldehydes, and 1,1′-diaroyl-4,4′-hexamethylene-bis(thiosemicarbazides) were prepared by the reaction of 4′,4′-hexamethylene-bis(thiosemicarbazide) with aroyl chlorides. 1,1′-Dialkyl- or diaryl-6,6′-alkylene-bis(bithioureas) were synthesized from 4,4′-alkylene-bis(thiosemicarbazides) and alkyl or aryl isothiocyanates. N,N′-Hexamethylene-bis[2-amino-5-(2-methoxyphenyl)thiadiazole] was prepared by the ring closure of 1,1′-bis(2-methoxybenzylidene)-4,4′-hexamethylene-bis(thiosemicarbazide).

The potent antibacterial activity of thiosemicarbazones such as thioacetazone prompted our investigation for producing related compounds which might show improved therapeutic values either by increasing their activity or reducing their toxicity, or both. Alkylenebis-(thiosemicarbazides) and their derivatives, 1,1′-dibenzylidene-4,4′-alkylene-bis(thiosemicarbazides), 1,1′-diaroyl-4,4′-alkylene-bis(thiosemicarbazides), and 1,1′-dialkyl- or 1,1′-diaryl-6,6′-alkylene-bis(bithioureas) were synthesized in our laboratory. N,N′-Hexamethylene-bis[2-amino-5-(2-methoxyphenyl)thiadiazole] was also synthesized.

Synthesis of 4,4′-alkylene-bis(thiosemicarbazides) (I to VIII) was initially carried out in good yield by the reaction between alkylene diisothiocyanate[1] with two equivalents of hydrazine or substituted hydrazines at about −10°. The reaction scheme and the compounds prepared are shown in Chart 1 and Table I, respectively.

1,1′-Dibenzylidene-4,4′-alkylene-bis(thiosemicarbazides) (IX to XXXI) were obtained by the reaction of 4,4′-alkylene-bis(thiosemicarbazides) (I, III, VI) with two moles equivalents of arylaldehyde, 1,1′-diaroyl-4,4′-hexamethylene-bis(thiosemicarbazides) (XXXII to XXXVIII) were prepared from 4,4′-hexamethylene-bis(thiosemicarbazide) (VI) and two moles equivalents of aroyl chlorides, and 1,1′-dialkyl- or 1,1′-diaryl-6,6′-alkylene-bis(thioureas)

1) Part IV: T. Yabuuchi, M. Hisaki, and R. Kimura, *Chem. Pharm. Bull.* (Tokyo), **23**, 663 (1975).
2) Location: *15-Morimoto-cho, Shimogamo, Sakyo-ku, Kyoto.*