Isolation of (−)-Loliolide (I)—The clarified aqueous citric acid solution was cooled and made basic with ammonia (pH 8.5–9.0), and this solution was extracted with chloroform (five 50-ml portions). The chloroform extract was washed and dried, and the solvent was removed; a straw-colored amorphous solid (0.51 g) was obtained. It was again dissolved in chloroform (10 ml) and chromatographed over alumina (30 g). The elution was carried out with benzene. Fractions (50 ml) were collected and monitored by analytical TLC. *n*-Butyl alcohol–acetic acid–water (4:1:2) was used as the solvent system, and iodine vapor was used for staining.

Fractions 9–11 were combined and evaporated. The residue crystallized from acetone–petroleum ether (1:2) as colorless fine needles (143 mg), mp 149–150° [lit. (3, 4) mp 149°, 149–151°]; R_f 0.82; $[\alpha]_D^{25}$ −116.3° (c 0.98, $CHCl_3$); UV: λ_{max} (ethanol) 216 nm (log ϵ, 4.17); IR: ν_{max} (mineral oil) 3425, 1720, and 1622 cm^{-1}; mass spectrometry: *m/e* 196 (M^+, 36%), 181 (7), 179 (5), 178 (50, m* 162, $178^2/196 = 161.6$), 163 (15, m* 149.5, $163^2/178 = 149.2$), 153 (16), 140 (50), 135 (15), 112 (18), and 111 (100).

Anal.—Calc. for $C_{11}H_{16}O_3$: C, 67.34; H, 8.16. Found: C, 67.32; H, 8.16.

The acetyl derivative, prepared with pyridine and acetic anhydride (in equal proportions) at ordinary temperature for 40 hr, was purified by column chromatography over alumina[4]. Benzene–ether (1:1) was used as the eluent. The middle eluates furnished loliolide acetate, which crystallized from hexane–methylene chloride as colorless prisms, mp 86–87° [lit. (3, 4) mp 86–87°, 86.5°]; $[\alpha]_D^{25}$ −74.7° (c 0.77, $CHCl_3$); UV: λ_{max} (ethanol) 214 nm (log ϵ, 4.28); mass spectrometry: *m/e* 238 (M^+, 21%), 223 (4), 195 (12), 178 (54), 163 (11), 135 (8), and 111 (100).

Autoxidation of Violaxanthin (II)—Violaxanthin (52 mg), obtained from *C. decussata* (10), was dissolved in benzene (50 ml) containing traces of methanol and was kept at room temperature for about 3 weeks. The yellow color of the solution faded gradually, and the solution was practically colorless at the end of the reaction. After evaporation of the solvent, the residue was partitioned between aqueous methanol and petroleum ether (bp 60–80°). The residue from the methanol layer was dissolved in benzene and chromatographed over a silicic acid (100-mesh) column, using benzene–ethyl acetate (2:1) as the eluent.

Fractions (50 ml) were collected, and fractions 10–15 showed the presence of loliolide and an aldehyde (2,4-dinitrophenylhydrazine reagent positive) component. The latter had a slightly lower R_f, 0.62, in acetone–petroleum ether (1:3). These compounds were separated by preparative TLC (plate thickness, 2 mm) with the same solvent system. The component having the lower R_f value was identified as violoxin.

Violoxin (III)—This compound was obtained as an oil (11 mg); UV: λ_{max} (ethanol) 230 (0.56), 262 sh (0.38), and 300 (0.08) nm; IR: ν_{max} (liquid) 3450, 1658, 1628, and 972 cm^{-1}; mass spectrometry: *m/e* 408 (51%), 393 (26), 390 (5), and 375 (12).

[4] Brockman alumina, grade IV.

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ACKNOWLEDGMENTS AND ADDRESSES

Received September 10, 1975, from the *Pharmaceutical Chemistry Research Laboratory, Department of Pharmaceutics, Banaras Hindu University, Varanasi-5, India.*

Accepted for publication November 14, 1975.

The authors are grateful to Dr. F. W. Wehrli, Varian AG, Zug, Switzerland, Dr. Nitya Nand, Central Drug Research Institute, Lucknow, India, and Dr. B. C. Das, CNRS, Gif-Sur-Yvette, France, for the NMR and mass spectral data. Financial assistance from the Council of Scientific and Industrial Research, New Delhi, India, is also gratefully acknowledged.

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Antimicrobial Activity of Newly Synthesized Isothiocyanate Derivatives against Pathogenic Plant Microorganisms

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Abstract □ Fifteen reaction products of isothiocyanates with cysteine, seven reaction products of isothiocyanates with 2,3-dimercapto-1-propanol, and four reaction products of isothiocyanates with sulfanilamide were synthesized. Their antimicrobial activity against pathogenic plant microorganisms was investigated.

Keyphrases □ Isothiocyanate derivatives—synthesized and screened for antimicrobial activity □ 1-Propanols, 2,3-bis(alkylthiocarbamoylthio)—synthesized and screened for antimicrobial activity □ Cysteines, *N*-(*N*-substituted thiocarbamoyl)—synthesized and screened for antimicrobial activity □ Thioureas, *N*-substituted *N'*-*p*-sulfonamidophenyl—synthesized and screened for antimicrobial activity □ Antimicrobial activity—isothiocyanate derivatives screened □ Structure–activity relationships—isothiocyanate derivatives synthesized, screened for antimicrobial activity

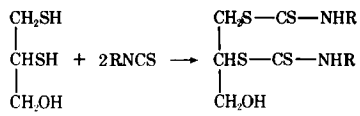
It was reported previously (1) that some reaction products of isothiocyanates with cysteine and 2,3-dimercapto-1-propanol (dimercaprol) exhibited antimicrobial activity against some bacteria and fungi. This paper reports the antimicrobial activity against pathogenic plant microorganisms of these compounds and

Table I—Characteristics of *N*-(*N*-Substituted Thiocarbamoyl)cysteines

Compound[b]	R	Melting Point[c]	Formula	Analysis, %[d] Calc.	Found
VI	$CH_3CH_2(CH_3)CH$	206° dec.	$C_6H_{16}N_2O_2S_2$	C 40.67	40.57
				H 6.83	6.81
				N 11.86	11.77
				S 27.10	27.36
VII	$(CH_3)_3C$	243° dec.	$C_6H_{16}N_2O_2S_2$	C 40.67	40.39
				H 6.83	6.91
				N 11.86	12.05
				S 27.10	27.00
VIII	$CH_3(CH_2)_4$	193° dec.	$C_9H_{18}N_2O_2S_2$	C 43.19	43.19
				H 7.25	7.33
				N 11.20	11.10
				S 25.07	25.55
IX	$(CH_3)_2CH(CH_2)_2$	210° dec.	$C_9H_{18}N_2O_2S_2$	C 43.19	42.81
				H 7.25	7.27
				N 11.20	11.30
				S 25.07	25.40
X	$CH_3CH_2(CH_3)CH(CH_2)_4$	226° dec.	$C_{12}H_{24}N_2O_2S_2$	C 49.30	49.00
				H 8.28	8.51
				N 9.58	10.05
				S 21.89	21.30
XI	$(CH_3)_2CHCH_2C(CH_3)_2CH_2$	235° dec.	$C_{12}H_{24}N_2O_2S_2$	C 49.30	49.51
				H 8.28	8.20
				N 9.58	9.50
				S 21.89	21.50
XII	$CH_2{=}CHCH_2$[e]	185° dec.	$C_7H_{12}N_2O_2S_2$	—	—

[a] Obtained commercially or synthesized from corresponding primary amines by the method of Mumm and Richter (3). [b] Characteristics of I (R = CH_3CH_2), II [R = $CH_3(CH_2)_2$], III [R = $(CH_3)_2CH$], IV [R = $CH_3(CH_2)_3$], V [R = $(CH_3)_2CHCH_2$], XIII (R = C_6H_5), XIV (R = $C_6H_5CH_2$), and XV [R = $C_6H_5(CH_2)_2$] were detailed previously (1). [c] Uncorrected. [d] Carbon and hydrogen were analyzed by the reduction method (5), nitrogen was analyzed by use of a Coleman type-29 nitrogen analyzer, and sulfur was analyzed by the silver absorption method (6). [e] This compound was originally synthesized by Todrick and Walker (4).

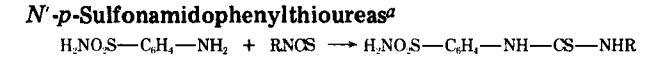
Table II—Characteristics of 2,3-Bis(alkylthiocarbamoylthio)-1-propanols[a]



Compound	R	Melting Point	Formula	Analysis, % Calc.	Found
XVI	CH_3	—	—	—	—
XVII	CH_3CH_2	132–133°	$C_9H_{18}N_2OS_4$	C 36.21	36.09
				H 6.08	6.16
				N 9.39	9.09
				S 42.96	43.38
XVIII	$CH_3(CH_2)_2$	—	—	C 40.45	40.55
				H 6.79	6.77
				N 8.58	8.67
				S 39.27	38.96
XIX	$(CH_3)_2CH$	111–112°	$C_{11}H_{22}N_2OS_4$	C 47.08	46.92
				H 7.91	7.95
				N 7.33	7.42
				S 33.51	33.06
XX	$(CH_3)_2CHCH_2$	—	—		
XXI	$(CH_3)_2CHCH_2$	131–132°	$C_{15}H_{30}N_2OS_4$		
XXII	$CH_2{=}CHCH_2$	—	—		

[a] Characteristics of XVI, XVIII, XX, and XXII were detailed previously (1). Other notations are the same as in Table I.

Table III—Characteristics of *N*-Substituted *N'*-*p*-Sulfonamidophenylthioureas[a]



Compound	R	Melting Point	Formula
XXIII[b]	$CH_3(CH_2)_2$	182–183°	$C_{10}H_{15}N_3O_2S_2$
XXIV[b]	$CH_2{=}CHCH_2$	188–189°	$C_{10}H_{13}N_3O_2S_2$
XXV[b]	C_6H_5	190–191°	$C_{13}H_{13}N_3O_2S_2$
XXVI[c]	$C_6H_5CH_2$	176–177°	$C_{14}H_{15}N_3O_2S_2$

[a] Notations are the same as in Table I. [b] Reported by Roth and Degering (2). [c] Reported by Budeanu *et al.* (7).

some additional compounds of the same series.

EXPERIMENTAL

***N*-(*N*-Substituted Thiocarbamoyl)cysteines and 2,3-Bis(alkylthiocarbamoylthio)-1-propanols**—These compounds were prepared by the method described previously (1).

***N*-Substituted *N'*-*p*-Sulfonamidophenylthioureas**—These compounds were obtained by the method of Roth and Degering (2).

Antimicrobial Activity—The antimicrobial activity was determined by obtaining the minimum inhibitory concentration (MIC) macroscopically using the agar dilution method. The tested compound

Table IV—Effects of Isothiocyanate Derivatives on Growth of Pathogenic Plant Microorganisms

Compound	MIC										
	Ascomycetes		Basidiomycetes	Fungi imperfecti					Bacteria		
	Cochliobolus miyabeanus[a]	*Diaporthe citri*[b]	*Corticium rolfsii*[c]	*Gloeosporium laeticolor*[b]	*Pyricularia oryzae*[d]	*Cladosporium carpophilum*[b]	*Alternaria kikuchiana*[b]	*Fusarium oxysporum f. niveum*[e]	*Xanthomonus oryzae*[f]	*Xanthomonus citri*[b]	*Corynebacterium sepedonlicum*[f]
I	10	10	100	10	10	10	100	10	100	100	1000
II	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000
III	1000	100	1000	1000	1000	1000	1000	1000	1000	1000	1000
IV	1000	100	1000	1000	1000	1000	1000	1000	1000	1000	1000
V	100	10	1000	100	10	100	100	10	1000	10	1000
VI	10	10	100	100	10	10	100	100	1000	1000	1000
VII	10	10	1000	100	10	10	1000	100	1000	1000	1000
VIII	10	10	100	10	10	10	100	10	100	100	1000
IX	10	10	100	10	10	10	100	10	100	100	1000
X	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000
XI	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000
XII	10	10	100	10	10	10	10	10	100	10	1000
XIII	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000
XIV	10	10	10	100	10	10	100	10	10	10	100
XV	10	10	100	100	10	10	100	100	10	10	100
XVI	10	10	1000	10	10	10	10	10	100	1000	1000
XVII	100	10	100	100	100	100	1000	1000	100	1000	1000
XVIII	10	10	1000	10	10	10	1000	10	100	1000	1000
XIX	100	10	1000	100	10	10	100	10	1000	1000	1000
XX	10	10	1000	10	10	10	1000	10	100	100	1000
XXI	10	10	1000	1000	10	10	100	10	1000	1000	1000
XXII	100	10	100	100	100	100	100	100	100	100	100
XXIII	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000
XXIV	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000
XXV	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000
XXVI	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000
A[g]	1	1	10	1	1	1	1	1	1	1	10
B[g]	—	—	—	—	—	—	—	—	—	10	—

[a] Supplied by Department of Agriculture, Kyoto University. [b] Supplied by Fruit-tree Institute, Ministry of the Agriculture and Forestry. [c] Isolated from sugar beet. [d] Supported by the Physical and Chemical Research Institute. [e] Isolated from watermelon. [f] Supported by Department of Agriculture, Shizuoka University. [g] Compound A is phenylmercuric acetate, and B is streptomycin sulfate. Both A and B were used as the standard samples.

was added in the agar medium[1] containing 1% soluble starch, 0.2% yeast extract, and 1.5% agar. This medium was solidified in petri dishes (diameter 9 cm), pathogenic microorganisms were seeded on the plates, and the plates were cultured for 3 days at 28°.

DISCUSSION

All experimental compounds were synthesized in this laboratory. Tables I–III show the characteristics of newly synthesized compounds.

Compounds I–XV were used as their hydrochlorides because of their water solubility. Compounds XVI–XXVI were used as dilute ethanol solutions.

The antimicrobial activity of a solution of these compounds is shown as the MIC (micrograms per milliliter) in Table IV.

Compounds V, XII, XIV, and XV exhibited the same potencies as streptomycin sulfate, which was used as the standard sample on *Xanthomonas citri*. Compound XIV also exhibited the same potency as phenylmercuric acetate on *Corticium rolfsii*.

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ACKNOWLEDGMENTS AND ADDRESSES

Received May 12, 1975, from the *Faculty of Pharmaceutical Sciences, Fukuoka University, Nanakuma, Nishi-ku, Fukuoka 814, Japan.*

Accepted for publication January 5, 1976.

The authors thank Mr. K. Narita, Analytical Center, Shizuoka College of Pharmacy, for the elemental analyses.

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