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Synthesis and Antimicrobial Activity of Salicylanilide Derivatives. II[1)]

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The condensation of 4-halo-*o*-toluidine with salicylic acid or 5-halosalicylic acid was carried out by the use of phosphorus trichloride in xylene to obtain the salicylanilides **1**—**13**. 4-Halo (or nitro)-*o*-toluidine, 4-halo-*o*-nitroaniline or 2,4-dihaloaniline was condensed with 3,5-dihalosalicylic acid to provide the salicylanilides **14**—**30** by the same method. The salicylanilides **2**—**15**, **26** and **27** gave the acetylated compounds **31**—**46** on treatment with acetic anhydride and pyridine. The salicylanilides **26** and **27** gave the methylated compounds **47** and **48** on treatment with dimethyl sulfate.

In the antimicrobial activity tests of the synthesized compounds, 4′,5-dihalo-2′-methylsalicylanilides **1**—**13** and the acetylated compounds **31**—**42** showed strong antimicrobial activity against some *Eumycetes* at the minimum inhibitory concentration (MIC) of 0.8 μg/ml. Compound **3** was shown to have a strong preventive activity against downy mildew of cucumber.

Keywords——salicylanilide; acetylation; antimicrobial activity; antifungal activity; downy mildew; late blight

In the previous work, we synthesized various salicylanilides of diphenyl ethers or diphenyl sulfides in the hope of finding new antimicrobial agents. 2-Acetoxy-5′-bromo-5-chloro-2′-(*p*-chlorophenoxy)salicylanilide showed strong antimicrobial activity against *Staphylococcus aureus*, but a compound effective against *Trichophyton* was not obtained.[2)] It has recently been reported that strong antimicrobial activity appeared when a methyl, nitro or methoxy group was introduced into the *ortho* or *para* position of salicylanilide derivatives.[3)] Therefore, various salicylanilides (A- or B-type) as shown in Chart 1 were synthesized in the present investigation. Some of the synthesized compounds were found to have strong activity and are potentially useful as antimicrobial agents and agricultural chemicals.

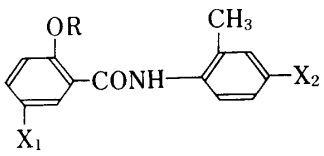
Syntheses

For the synthesis of A-type compounds, 4-halo-*o*-toluidine was condensed with salicylic acid or 5-halosalicylic acid by using phosphorus trichloride in xylene at 140 °C for 4 h to form the salicylanilides **1**—**13**. The products are shown in Chart 2 and Table I.

For the synthesis of B-type compounds, 4-halo (or nitro)-*o*-toluidine, 4-halo-*o*-nitroaniline or 2,4-dihaloaniline was condensed with 3,5-dihalosalicylic acid to form the salicyl anilides **14**—**30** under reaction conditions similar to those described above. The results are shown in Chart 2 and Table I.

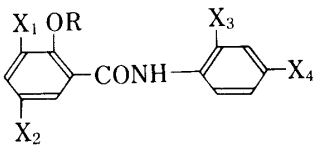
Salicylanilides **2**—**15**, **26** and **27** gave the acetylated compounds **31**—**46** with acetic anhydride and pyridine, and salicylanilides **26** and **27** gave the methylated compounds **47**, **48**

A type



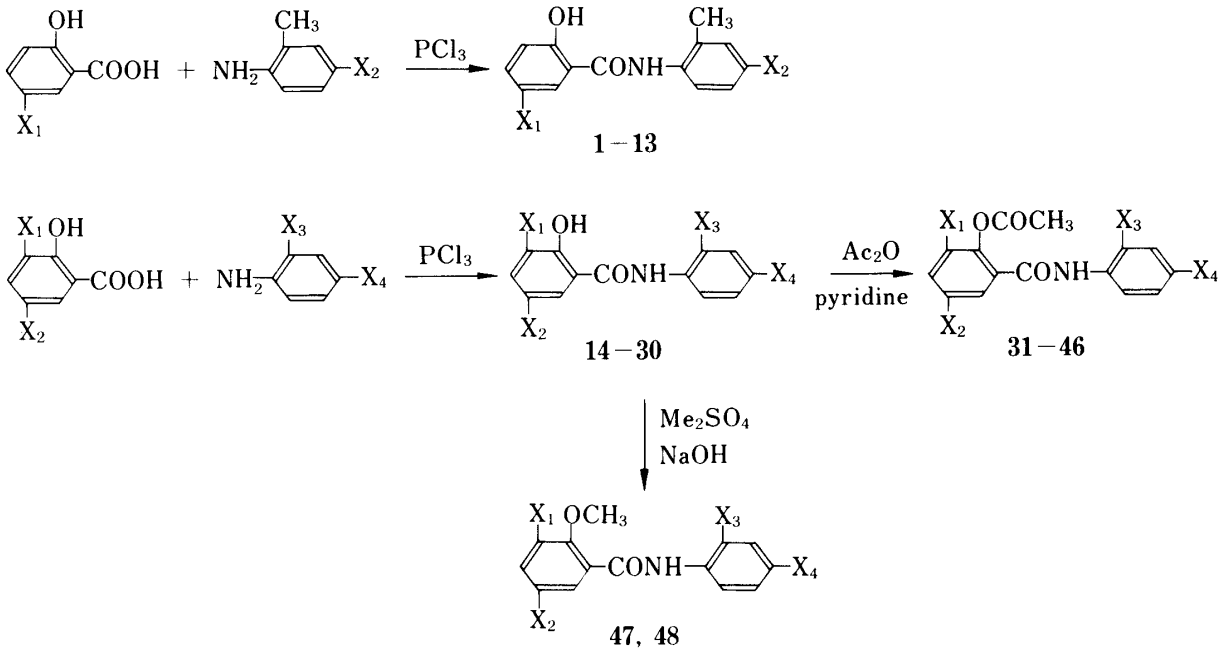
R=H, $COCH_3$
X_1, X_2=F, Cl, Br, I

B type



R=H, CH_3, $COCH_3$
X_1, X_2, X_3, X_4=F, Cl, Br, I, CH_3, NO_2

Chart 1



X_1, X_2, X_3, X_4: see Table II, III, and V

Chart 2

with dimethyl sulfate at 90 °C for 2 h. The results are shown in Chart 2 and Table I.

Antimicrobial Activity

The results of antimicrobial activity testing for the synthesized A-type compounds **1**—**13**, **31**—**42** and some of the B-type compounds **14**—**18**, **43**, **44** are shown in Table II, and those for other B-type compounds **19**—**30** are shown in Table III.

As shown in Table II, a few products showed low activity for *Candida albicans* in Eumycetes, and the minimum inhibitory concentration (MIC) of B-type compounds **15**, **17**, **44** was 25 μg/ml. However, some A- or B-type compounds showed stronger antimicrobial activity than griseofulvin and undecilenic acid against *Trichophyton*, *Microsporum audouini*, or *Epidermo-phyton floccosum*. In compounds **1**—**13**, **31**—**42**, the antimicrobial activity is strongest when the X_1 halogen is a fluorine atom, and the activity seems to be directly proportional to the electronegativity: F > Cl > Br > I. On the other hand, the effect of X_2 is not consistent. The antimicrobial activities of the acetylated compounds **37** and **42** were slightly increased. Antimicrobial activities of these compounds against Gram-positive bacteria were

TABLE I. Yields and Physical Properties of **1**—**48**

Compd. No.	Yield (%)	Appearance	mp (°C)	Formula	Analysis (%) Calcd (Found)		
					C	H	N
1	60	Colorless needles	208—209	$C_{14}H_{12}ClNO_2$	64.25	4.62	5.35
					(64.12	4.51	5.21)
2	73	Yellow needles	210—211	$C_{14}H_{11}ClFNO_2$	60.12	3.96	5.00
					(59.90	3.95	4.95)
3	71	Colorless needles	225—226	$C_{14}H_{11}BrFNO_2$	51.88	3.42	4.32
					(51.74	3.54	4.37)
4	30	Colorless needles	246—248	$C_{14}H_{11}FINO_2$	45.31	2.99	3.77
					(45.29	2.89	3.77)
5	65	Colorless needles	251—252	$C_{14}H_{11}Cl_2NO_2$	56.78	3.74	4.72
					(56.72	3.73	4.71)
6	62	Colorless needles	209—210	$C_{14}H_{11}BrClNO_2$	49.37	3.25	4.11
					(49.21	3.21	4.10)
7	25	Colorless needles	226—227	$C_{14}H_{11}ClINO_2$	43.38	2.86	3.61
					(43.21	2.56	3.51)
8	54	Colorless needles	238—239	$C_{14}H_{11}BrClNO_2$	49.37	3.25	4.11
					(49.35	3.34	4.08)
9[3b]	56	Colorless needles	217—219	$C_{14}H_{11}Br_2NO_2$	43.67	2.88	3.64
					(43.65	2.85	3.65)
10	53	Colorless needles	210—212	$C_{14}H_{11}BrINO_2$	38.92	2.56	3.24
					(38.90	2.50	3.27)
11	74	Colorless needles	209—213	$C_{14}H_{11}ClINO_2$	43.38	2.86	3.61
					(43.32	2.83	3.51)
12	75	White powder	223—225	$C_{14}H_{11}BrINO_2$	38.92	2.57	3.24
					(38.60	2.56	3.23)
13	73	Colorless needles	229—230	$C_{14}H_{11}I_2NO_2$	35.10	2.31	2.92
					(35.11	2.52	2.91)
14	55	Colorless needles	135—136	$C_{14}H_{10}Cl_3NO_2$	50.86	3.05	4.24
					(50.85	3.02	4.21)
15	86	Colorless needles	160—162	$C_{14}H_{10}BrCl_2NO_2$	44.84	2.69	3.73
					(44.82	2.67	3.76)
16	76	Colorless prisms	174—176	$C_{14}H_{10}Cl_2INO_2$	39.84	2.39	3.31
					(39.80	2.39	3.01)
17	60	White powder	193—195	$C_{14}H_{10}Br_3NO_2$	36.24	2.17	3.01
					(36.23	2.16	3.01)
18	91	Yellow needles	165—167	$C_{14}H_{10}I_3NO_2$	27.80	1.67	2.32
					(27.78	1.56	2.30)
19	52	Colorless needles	225—227	$C_{14}H_{11}ClN_2O_4$	54.83	3.61	9.13
					(54.72	3.80	8.98)
20	68	Yellow needles	234—237	$C_{14}H_{11}BrN_2O_4$	47.89	3.16	7.98
					(47.65	3.08	7.87)
21	51	Yellow needles	241—242	$C_{14}H_{10}Cl_2N_2O_4$	49.29	2.95	8.21
					(49.50	2.90	8.19)
22	50	Yellow needles	240—242	$C_{14}H_{10}Br_2N_2O_4$	39.10	2.34	6.51
					(39.32	2.14	6.48)
23	70	Yellow needles	190—191	$C_{13}H_9ClN_2O_4$	53.35	3.10	9.57
					(53.30	3.32	9.25)
24	50	Yellow powder	213—214	$C_{13}H_7Cl_3N_2O_4$	43.18	1.95	7.75
					(43.15	1.80	7.54)
25	89	Yellow needles	227—229	$C_{13}H_7Br_2ClN_2O_4$	34.66	1.57	6.22
					(34.68	1.48	6.10)
26	80	Colorless needles	157—159	$C_{13}H_7Cl_2F_2NO_2$	49.09	2.22	4.40
					(49.35	2.28	4.40)

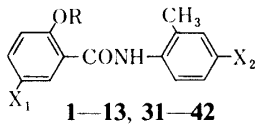
TABLE I. continued.

Compd. No.	Yield (%)	Appearance	mp (°C)	Formula	Analysis (%) Calcd (Found)		
					C	H	N
27	80	Colorless needles	141—143	$C_{13}H_7Br_2F_2NO_2$	38.36 (38.35	1.73 1.69	3.44 3.21)
28[3c]	60	Yellow needles	199—200	$C_{13}H_7Br_4NO_2$	29.53 (29.51	1.33 1.32	2.65 2.63)
29	84	Yellow needles	179—181	$C_{13}H_7F_2I_2NO_2$	31.17 (31.41	1.41 1.34	2.80 2.66)
30[3c]	65	Yellow needles	135—136	$C_{14}H_{10}Cl_4NO_4$	44.48 (44.34	2.01 2.18	3.99 4.15)
31	81	Colorless needles	171—172	$C_{16}H_{13}FClNO_3$	59.73 (59.72	4.07 4.06	4.35 4.25)
32	79	White powder	179—180	$C_{16}H_{13}BrFNO_3$	52.48 (52.43	3.57 3.56	3.82 3.81)
33	40	Yellow needles	172—175	$C_{16}H_{13}FINO_3$	46.51 (46.50	3.17 3.10	3.38 3.32)
34	36	Colorless needles	190—192	$C_{16}H_{13}Cl_2NO_3$	56.82 (56.81	3.87 3.79	4.14 4.02)
35	52	Colorless needles	179—182	$C_{16}H_{13}BrClNO_3$	50.22 (49.86	3.42 3.25	3.66 3.59)
36	15	Yellow needles	182—185	$C_{16}H_{13}ClINO_3$	44.73 (44.71	3.05 3.03	3.26 3.25)
37	49	Colorless needles	180—182	$C_{16}H_{13}BrClNO_3$	50.22 (50.21	3.42 3.41	3.66 3.56)
38	47	Colorless needles	198—200	$C_{16}H_{13}Br_2NO_3$	45.00 (44.98	3.07 3.06	3.28 3.26)
39	74	Colorless needles	209—211	$C_{16}H_{13}BrINO_3$	40.54 (40.52	2.76 2.76	2.95 2.94)
40	55	Colorless needles	178—180	$C_{16}H_{13}ClINO_3$	44.73 (44.76	3.05 2.95	3.26 3.18)
41	58	White powder	207—209	$C_{16}H_{13}BrINO_3$	40.54 (40.51	2.76 2.75	2.95 2.95)
42	46	Colorless needles	210—211	$C_{16}H_{13}I_2NO_3$	36.88 (36.87	2.51 2.53	2.69 2.67)
43	86	Colorless needles	195—196	$C_{16}H_{12}Cl_3NO_3$	51.57 (51.56	3.25 3.23	3.76 3.76)
44	58	Colorless needles	127—130	$C_{16}H_{12}BrCl_2NO_3$	46.08 (46.07	2.90 2.88	3.36 3.34)
45	70	Colorless needles	166—168	$C_{15}H_9Cl_2F_2NO_3$	50.03 (50.10	2.52 2.56	3.89 3.93)
46	70	Colorless needles	162—163	$C_{15}H_9Br_2F_2NO_3$	40.12 (40.36	2.02 2.15	3.12 3.40)
47	36	Colorless needles	154—156	$C_{14}H_9Cl_2F_2NO_2$	50.63 (50.58	2.73 2.66	4.22 4.25)
48	59	Colorless needles	160—162	$C_{14}H_9Br_2F_2NO_2$	39.94 (40.02	2.15 2.13	3.33 3.18)

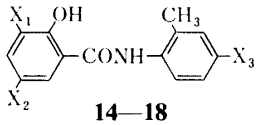
greater than those against Gram-negative bacteria; compounds **1**—**18**, **31**—**42** showed strong antimicrobial activities, the MIC against *Staphylococcus aureus* or *Bacillus subtilis* being 0.4—1.6 μg/ml.

Compounds **19**—**30** (listed in Table III) showed low antimicrobial activities against *Eumycetes*. Although compounds **28** and **30** were reported to be most effective against

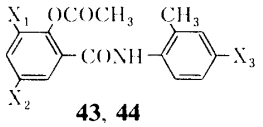
TABLE II. Antimicrobial Activities (MIC: μg/ml)



1—13, 31—42



14—18



43, 44

Compd. No.		Microorganisms[a]										
		1	2	3	4	5	6	7	8	9	10	11
1	$X_1=H$, $X_2=Cl$, R=H	100	1.6	1.6	<0.8	<0.8	>100	100	100	100	1.6	1.6
2	$X_1=F$, $X_2=Cl$, R=H	100	<0.8	<0.8	<0.8	<0.8	100	100	100	100	0.4	0.4
3	$X_1=F$, $X_2=Br$, R=H	100	0.8	<0.8	<0.8	0.8	>100	100	>100	100	0.4	0.4
4	$X_1=F$, $X_2=I$, R=H	100	<0.8	<0.8	<0.8	<0.8	>100	100	100	100	0.4	0.4
5	$X_1=Cl$, $X_2=Cl$, R=H	100	25	25	12.5	0.8	100	100	100	100	0.8	0.8
6	$X_1=Cl$, $X_2=Br$, R=H	100	<0.8	<0.8	<0.8	<0.8	100	100	>100	>100	0.8	0.8
7	$X_1=Cl$, $X_2=I$, R=H	100	0.8	<0.8	<0.8	<0.8	>100	>100	>100	100	0.4	0.4
8	$X_1=Br$, $X_2=Cl$, R=H	100	50	50	<0.8	<0.8	100	100	>100	100	0.8	0.8
9	$X_1=Br$, $X_2=Br$, R=H	100	3.2	0.8	<0.8	<0.8	100	>100	>100	100	0.8	0.8
10	$X_1=Br$, $X_2=I$, R=H	100	1.6	0.8	0.8	<0.8	>100	100	>100	>100	0.4	0.4
11	$X_1=I$, $X_2=Cl$, R=H	100	1.6	1.6	<0.8	<0.8	>100	100	100	>100	0.8	1.6
12	$X_1=I$, $X_2=Br$, R=H	100	12.5	12.5	0.8	0.8	100	100	>100	>100	0.2	0.4
13	$X_1=I$, $X_2=I$, R=H	100	25	6.3	3.2	3.2	>100	>100	>100	>100	0.4	0.4
14	$X_1=Cl$, $X_2=Cl$, $X_3=Cl$	50	3.2	3.2	<0.8	<0.8	100	100	>100	>100	6.3	6.3
15	$X_1=Cl$, $X_2=Cl$, $X_3=Br$	25	3.2	1.6	<0.8	<0.8	100	25	>100	>100	1.6	3.2
16	$X_1=Cl$, $X_2=Cl$, $X_3=I$	50	3.2	3.2	0.8	0.8	100	100	>100	>100	0.8	0.8
17	$X_1=Br$, $X_2=Br$, $X_3=Br$	25	6.3	3.2	1.6	<0.8	100	50	>100	>100	1.6	1.6
18	$X_1=I$, $X_2=I$, $X_3=I$	100	25	12.5	6.3	6.3	>100	>100	>100	>100	1.6	1.6
31	$X_1=F$, $X_2=Cl$, R=Ac	100	<0.8	<0.8	<0.8	<0.8	>100	100	>100	100	0.4	0.4
32	$X_1=F$, $X_2=Br$, R=Ac	100	<0.8	<0.8	<0.8	<0.8	>100	100	>100	100	0.4	0.4
33	$X_1=F$, $X_2=I$, R=Ac	100	<0.8	<0.8	<0.8	0.8	>100	100	>100	100	0.4	0.4
34	$X_1=Cl$, $X_2=Cl$, R=Ac	100	25	25	12.5	0.8	100	100	>100	>100	0.8	0.8
35	$X_1=Cl$, $X_2=Br$, R=Ac	100	<0.8	<0.8	<0.8	<0.8	100	100	>100	>100	0.8	0.8
36	$X_1=Cl$, $X_2=I$, R=Ac	100	0.8	<0.8	<0.8	<0.8	>100	>100	>100	100	0.4	0.4
37	$X_1=Br$, $X_2=Cl$, R=Ac	100	25	25	<0.8	<0.8	100	100	>100	>100	0.8	0.8
38	$X_1=Br$, $X_2=Br$, R=Ac	100	3.2	0.8	<0.8	<0.8	100	100	>100	>100	0.2	0.4
39	$X_1=Br$, $X_2=I$, R=Ac	100	1.6	1.6	0.8	<0.8	>100	>100	>100	>100	0.4	0.4
40	$X_1=I$, $X_2=Cl$, R=Ac	100	3.2	0.8	<0.8	<0.8	100	100	>100	100	0.8	0.8
41	$X_1=I$, $X_2=Br$, R=Ac	100	12.5	12.5	0.8	0.8	100	100	>100	100	0.2	0.4
42	$X_1=I$, $X_2=I$, R=Ac	100	25	6.3	3.2	0.8	100	100	>100	>100	0.4	0.4
43	$X_1=Cl$, $X_2=Cl$, $X_3=Cl$	50	3.2	3.2	1.6	<0.8	100	100	>100	100	1.6	1.6
44	$X_1=Cl$, $X_2=Cl$, $X_3=Br$	25	3.2	3.2	<0.8	<0.8	100	50	>100	100	1.6	1.6
	Griseofulvin	100	3.2	1.6	<0.8	0.8	>100	100				
	Undecylenic acid	100	25	25	25	25	>100	100				

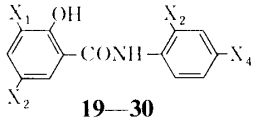
a) 1, *Candida albicans* ATCC 10259; 2, *Trichophyton mentagrophytes* IFO 5812; 3, *Trichophyton rubrum* IFO 9185; 4, *Microsporum audouini*; 5, *Epidermophyton floccosum*; 6, *Aspergillus fumigatus* IFO 8867; 7, *Aspergillus niger* IFO 8541; 8, *Escherichia coli* NIHJ JC-2; 9, *Pseudomonas aeruginosa* NC-5; 10, *Staphylococcus aureus* FDA 209-P; 11, *Bacillus subtilis* PCI-219.

Eumycetes,[3c] the synthesized compounds **1—13, 31—42** showed stronger antimicrobial activities than those of compounds described in the literature[3c] as a result of the introduction of the methyl group into the salicylanilide. However, the antimicrobial activities of **14—25** were decreased by the introduction of the halogen or the nitro group.

Preventive Activity

Some of the A-type compounds were tested for preventive activity against downy mildew of cucumber and late blight of tomato: the results are shown in Tables IV and V.

TABLE III. Antimicrobial Activities (MIC: μg/ml)



19—30

Compd. No.		Microorganisms[a]						
		1	2	3	4	5	6	7
19	$X_1=H$, $X_2=Cl$, $X_3=CH_3$, $X_4=NO_2$	>100	>100	25	50	>100	>100	>100
20	$X_1=H$, $X_2=Br$, $X_3=CH_3$, $X_4=NO_2$	>100	>100	50	>100	>100	>100	>100
21	$X_1=Cl$, $X_2=Cl$, $X_3=CH_3$, $X_4=NO_2$	>100	>100	50	>100	>100	>100	>100
22	$X_1=Br$, $X_2=Br$, $X_3=CH_3$, $X_4=NO_2$	>100	>100	50	>100	>100	>100	>100
23	$X_1=H$, $X_2=H$, $X_3=NO_2$, $X_3=Cl$	>100	10	50	50	25	50	50
24	$X_1=Cl$, $X_2=Cl$, $X_3=NO_2$, $X_4=Cl$	>100	10	50	50	25	50	50
25	$X_1=Br$, $X_2=Br$, $X_3=NO_2$, $X_4=Cl$	>100	>100	50	>100	>100	>100	>100
26	$X_1=Cl$, $X_2=Cl$, $X_3=F$, $X_4=F$	>100	>100	6.3	6.3	10	100	100
27	$X_1=Br$, $X_2=Br$, $X_3=F$, $X_4=F$	>100	>100	6.3	6.3	>100	10	10
28	$X_1=Br$, $X_2=Br$, $X_3=Br$, $X_4=Br$	>100	>100	>10	>10	>100	>100	>100
29	$X_1=I$, $X_2=I$, $X_3=F$, $X_4=F$	>100	>100	6.3	6.3	10	10	10
30	$X_1=Cl$, $X_2=Cl$, $X_3=Cl$, $X_4=Cl$	>100	>100	<10	<10	>100	>100	>100
	Griseofluvin	>100	10	>100	100	10	25	25
	Undecylenic acid	>100	10	>100	100	10	50	50

a) 1, *Escherichia coli* NIHJ JC-2; 2, *Pseudomonas aeruginosa* NC-5; 3, *Staphylococcus aureus* FDA 209-P; 4, *Bacillus subtilis* PCI-219; 5, *Candida albicans* ATCC 10259; 6, *Aspergillus flavus* IFO 8558; 7, *Trichophyton rubrum* IFO 9185.

As shown in Table IV, compound **3** was found to have good preventive activity for downy mildew of cucumber, comparable to that of the commercial product 2,4,5,6-tetrachloroisophthalonitrile (TPN) at the concentration of 25 ppm.

The relationship between the substituent (X_1) and preventive activity indicates that the activity is directly proportional to electronegativity: F>Cl>Br>I. The substituent X_2 affected the preventive activity (for a given halogen of the salicylic acid moiety) in the order Br>Cl>I. The preventive activities of the acetylated compounds were generally decreased. The low activity of acetylated compounds suggests that the free hydroxyl group is necessary for the activity.

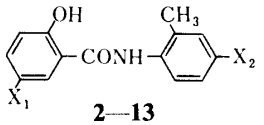
As shown in Table V, compound **20** was found to have a preventive activity comparable to that of TPN against downy mildew of cucumber. The other compounds were not tested at low concentration. The preventive activity decreased with electronegativity in the case of compounds **19** and **20**, in which the nitro group is strongly electron-withdrawing. The existence of the nitro group is very important for activity in organic phosphoric acid ester.[4] In this case, other factors may be important besides the electronegativity of the substituent group.

Salicylanilide derivatives are known to act as uncouplers[5] and an analysis of the structure–activity relationships is in progress. The results of a quantitative approach[6] will be reported in a separate paper.

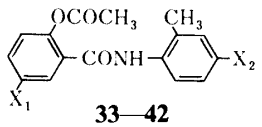
Experimental

Salicylanilide Derivatives (1—30)——A salicylic acid derivative (0.03 mol) and an aniline derivative (0.03 mol) in dry xylene (70 ml, dried over molecular sieve 3A) were heated under reflux. PCl_3 (0.88 g, 0.01 mol) was added to the mixture during 15 min, and the mixture was stirred at 140 °C for 4 h. Evaporation of the solvent left a residue, which was crystallized from Me_2CO. Data for the products: see Table I.

TABLE IV. Preventive Activities



2—13

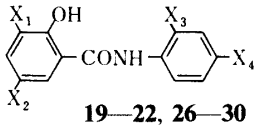


33—42

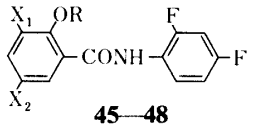
Compd. No.		Downy mildew of cucumber				Late blight of tomato
		500	100	50	25 (ppm)	500 (ppm)
2	$X_1=F$, $X_2=Cl$	0	5	5	10[a]	5[b]
3	$X_1=F$, $X_2=Br$	0	0	5	5	5
4	$X_1=F$, $X_2=I$	5	5	10	30	2
5	$X_1=Cl$, $X_2=Cl$	0	10	90	90	1
6	$X_1=Cl$, $X_2=Br$	0	5	10	20	5
7	$X_1=Cl$, $X_2=I$	5	5	12	65	2
8	$X_1=Br$, $X_2=Cl$	0				4
9	$X_1=Br$, $X_2=Br$	0	10	10	20	1
10	$X_1=Br$, $X_2=I$	5	5	20	50	5
11	$X_1=I$, $X_2=Cl$	0				5
12	$X_1=I$, $X_2=Br$	0				5
13	$X_1=I$, $X_2=I$	30	40	75	80	5
33	$X_1=F$, $X_2=I$	20	30	65	85	5
34	$X_1=Cl$, $X_2=Cl$	60				5
35	$X_1=Cl$, $X_2=Br$	80				5
38	$X_1=Br$, $X_2=Br$	20				5
41	$X_1=I$, $X_2=Br$	20				5
42	$X_1=I$, $X_2=I$	90				5
	TPN[c]	0	0	5	5	0
	Control	90				5

a) Percent of leaf infected.
b) The degree of infection.
c) 2,4,5,6-Tetrachloroisophthalonitrile (TPN).

TABLE V. Preventive Activities



19—22, 26—30



45—48

Compd. No.		Downy mildew of cucumber				Late blight of tomato
		500	100	50	25 (ppm)	500 (ppm)
19	$X_1=H$, $X_2=Cl$, $X_3=CH_3$, $X_4=NO_2$	0	20	30	40[a]	1[b]
20	$X_1=H$, $X_2=Br$, $X_3=CH_3$, $X_4=NO_2$	0	0	20	50	1
21	$X_1=Cl$, $X_2=Cl$, $X_3=CH_3$, $X_4=NO_2$	100				5
22	$X_1=Br$, $X_2=Br$, $X_3=CH_3$, $X_4=NO_2$	80				5
26	$X_1=Cl$, $X_2=Cl$, $X_3=F$, $X_4=F$	0				5
27	$X_1=Br$, $X_2=Br$, $X_3=F$, $X_4=F$	5				5
28	$X_1=Br$, $X_2=Br$, $X_3=Br$, $X_4=Br$	100				5
29	$X_1=I$, $X_2=I$, $X_3=F$, $X_4=F$	5				5
30	$X_1=Cl$, $X_2=Cl$, $X_3=Cl$, $X_4=Cl$	0	10	20	30	5
45	$X_1=Cl$, $X_2=Cl$, $R=Ac$	5				5
46	$X_1=Br$, $X_2=Br$, $R=Ac$	100				5
47	$X_1=Cl$, $X_2=Cl$, $R=CH_3$	60				5
48	$X_1=Br$, $X_2=Br$, $R=CH_3$	100				5

a) Percent of leaf infected.
b) The degree of infection.

Acetylated Derivatives (31—46)——A mixture of a salicylanilide derivative (0.002 mol), Ac_2O (4 ml), and a few drops of pyridine was heated on a water-bath for 1 h with stirring. The reaction mixture was concentrated, and the residue was crystallized from EtOH. Data for the products: see Table I.

Methylated Derivatives (47, 48)——Me_2SO_4 was added to a mixture of a salicylanilide derivative (**26** or **27**, 0.01 mol), 2 N NaOH, and diglyme. The mixture was stirred at 90 °C for 2 h, then water was added. The aqueous solution was neutralized with dil. HCl and the resulting precipitate was collected and crystallized from Me_2CO. Data for the products: see Table I.

Determination of Minimum Inhibitory Concentration (MIC)——The MIC was determined by the agar dilution method according to the Japanese standard procedure.[7] Heart infusion agar was used for antimicrobial tests and Sabouraud's glucose agar for *Eumycetes*. A loopful of precultured microbial cells was inoculated on agar plates containing test compounds. The MIC was determined by visually judging the microbial growth after incubation for 24 h at 37 °C, or for 2 weeks at 27 °C in the case of *Eumycetes*.

Prevention of Downy Mildew of Cucumber——A solution of each chemical at the indicated concentration was sprayed on young cucumber plants with one or two main leaves. After 24 h, downy mildew spores (5×10^5/ml) were sprayed on the plants, which were incubated at 20—25 °C for 4 h, at the humidity of 90—100%. After subsequent incubation at 25—30 °C for a week, the infected area was measured.

Prevention of Late Blight of Tomato——The test method was the same as that used on cucumber. Tomato plants of 10 cm in hight were used.

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References and Notes

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