Structure-activity Relationships of Fungicidal *N*-Benzoylanthranilic Esters*

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The antifungal activity of 37 *N*-(methoxy-substituted benzoyl)anthranilic esters was tested on the powdery mildew of barley caused by *Erysiphe graminis* by the pot test. Among the methyl *N*-(methoxy-substituted benzoyl)anthranilates tested, 3,4-dimethoxybenzoyl derivative exhibited the highest activity. The variation in fungicidal activity of *N*-(3,4-dimethoxybenzoyl)anthranilic esters was shown to be related with variation in hydrophobicity and the electronic property of the alcohol moiety of the ester. The branching at the α -position of the alcohol moiety of the ester was detrimental to the activity.

In the accompanying paper,¹⁾ we reported that methyl *N*-(substituted benzoyl)anthranilates exhibit the potent preventive activity against the powdery mildew of various plants. The effect of substitution at the benzoyl moiety was examined for the activity against the cucumber powdery mildew using a number of substituted derivatives. We found that the substituent effect depends upon the substituent position and that the steric dimension of substituents, in particular, those at the 3-position is very important in determining the activity variation. We also found that the methoxy substitution is suitable for the potent activity. In this paper, we have examined the effect of multiple substitution of the methoxy group on the fungicidal activity using various compounds carrying one, two or three methoxy groups on the benzoyl moiety. We have also analyzed the relationship between the structure of *N*-(3,4-dimethoxybenzoyl)anthranilic esters where that of the alcoholic moiety is varied and the activity against the powdery mildew of barley by the Hansch method²⁾ employing the R_M value determined by reversed-phase TLC as the parameter for hydrophobicity of the molecule.

MATERIALS AND METHODS

Synthesis of compounds. The structure of the com-

* Structure-Activity Study of Fungicidal *N*-Benzoylanthranilates. Part II. For Part I, see ref. 1.

pounds was confirmed by IR and NMR spectroscopy in addition to the elementary analyses for C, H and N. Anthranilic esters were prepared by the reaction of isatoic anhydride with each of the various alcohols in the presence of a small quantity of potassium hydroxide according to the method of Heindel *et al.*³⁾ *N*-Methoxybenzoylanthranilic esters were prepared by the reaction of anthranilic esters and methoxybenzoyl chlorides in the presence of a base. Typical procedures are shown below.

1) *Isobutyl N*-(3,4-dimethoxybenzoyl)anthranilate (20). To a mixture of isobutyl anthranilate³⁾ (9.7 g, 0.05 mol), triethylamine (6.1 g, 0.06 mol) and benzene (150 ml), was added 3,4-dimethoxybenzoyl chloride (10.0 g, 0.05 mol) in benzene (50 ml) with stirring at room temperature. After stirring for 3 hr, the reaction mixture was washed successively with water, dilute HCl and aq. Na₂CO₃, and the organic layer was dried over anhydrous Na₂SO₄. After removal of the solvent, the residue was recrystallized from ethanol to give 14.6 g (82%) of colorless needles, mp 87.5~89°C. *Anal.* Found: C, 67.30; H, 6.44; N, 3.97. Calcd. for C₂₀H₂₃NO₅: C, 67.21; H, 6.49; N, 3.92%.

2) 2-(3,4-Dimethoxyphenyl)-4',5'-benzo-1,3-oxazin-6-one. *N*-(3,4-Dimethoxybenzoyl)anthranilic acid (15) (3.0 g, 0.01 mol) was dissolved in acetic anhydride (30 ml) containing sodium acetate (0.1 g) and warmed at 95°C for 3 hr. The reaction mixture was poured into ice and the crystal was collected by filtration. After washing with water, the crude product was recrystallized from ethanol to give 2.1 g (74%) of colorless needles, mp 198~199°C. *Anal.* Found: C, 67.93; H, 4.58; N, 4.93. Calcd. for C₁₆H₁₃NO₄: C, 67.84; H, 4.62; N, 4.94%.

Antifungal activity. Each test compound was formulated as the emulsifiable concentrate and diluted with water according to the method previously re-

ported.¹⁾ The test plants of barley (cultivar; Akashin-riki) at the first leaf stage were sprayed with the test solution 5 hr prior to the inoculation with the conidia of *E. graminis*. The disease assessment was made 8 days after the inoculation. The antifungal activity was expressed as the pI_{50} value (negative logarithm of the molar I_{50} value, the concentration in the sprayed emulsion required for 50% inhibition of the disease development) and is listed in Tables I and II.

R_M values. An aliquot (4 μ l) of each compound (1000 ppm in acetone) was applied to a silica-gel plate (Merck 0.25 mm thick) which was previously treated with 5% *n*-paraffin in *n*-hexane and air-dried. Then the compound was chromatographed with aqueous acetone (1:1.3, V/V). After drying, the spot was located under the UV light and the R_M value was estimated according to the equation, $R_M = \log(1 - 1/R_f)$. Each determination was repeated 4 times and the mean R_M values are listed in Table III. The standard deviation involved in the value is less than 0.04.

RESULTS AND DISCUSSION

Effect of the methoxy group on the benzoyl moiety

As shown in Table I, methyl *N*-benzoyl-anthranilate (1) exhibited a potent fungicidal activity against the powdery mildew of barley. The monosubstitution of the methoxy group at 2-, 3- or 4-position of the benzoyl group (compds. 2~4) shows only a slight effect on the activity. While the 3,4-disubstitution (compd. 9) enhances the activity about two-fold, disubstitutions at other positions (compds. 5~8, 10) are unfavorable to the activity. The 3,4-methylenedioxybenzoyl derivative (14) is as active as the 3,4-dimethoxybenzoyl compound (9). The trisubstitution (compds. 11~13) greatly reduced the activity. The activity is highly dependent on the number and the position of the methoxy group.

Structure-activity correlation of the esters of *N*-(3,4-dimethoxybenzoyl)anthranilic acid

Since the 3,4-dimethoxy substitution is most favorable to the activity on the benzoyl moiety, we have synthesized a number of the esters of *N*-(3, 4-dimethoxybenzoyl)anthranilic acid. Their activity varies considerably with variation of the ester group as shown in Table II.

TABLE I. ANTIFUNGAL ACTIVITY OF METHYL *N*-(METHOXY-SUBSTITUTED BENZOYL)ANTHRANILATES AGAINST POWDERY MILDEW OF BARLEY

No.	R	mp (°C) ^a [lit. value] or n_D^{25}	Activity (pI_{50})
1	H	101~102 ^b	4.64
2	2-OMe	1.6109	4.40
3	3-OMe	86~87	4.49
4	4-OMe	113.5~114.5 [113°]	4.74
5	2,3-(OMe) ₂	101~102	2.60
6	2,4-(OMe) ₂	98~99	3.25
7	2,5-(OMe) ₂	1.6025	3.48
8	2,6-(OMe) ₂	167~169	<2.5
9	3,4-(OMe) ₂	109~110 [108°]	4.94
10	3,5-(OMe) ₂	124~126	<2.5
11	2,3,4-(OMe) ₃	88.5~89.5	<2.5
12	2,4,5-(OMe) ₃	164~165	<2.5
13	3,4,5-(OMe) ₃	139~140 [138°]	<2.5
14	3,4-OCH ₂ O-	164~165	5.05

^a Melting points are uncorrected.

^b D. H. Hey and D. G. Turpin, *J. Chem. Soc.*, **1954**, 2471.

^c H. Stephen and G. Wadge, *J. Chem. Soc.*, **1956**, 4420.

Structure-activity relationship has been quantitatively analyzed by the method developed by Hansch and Fujita²⁾ by means of physico-chemical substituent parameters and regression analysis.

Boyce and Milborrow⁴⁾ demonstrated that the R_M value determined from the reversed-phase TLC systems can be used for the hydrophobic parameter of the molecule. As expressed by eq. 1, R_M value is nicely correlated with the π value of the ester substituents estimated by means of the additivity principle⁵⁾ (Table III).

$$R_M = 0.313\pi - 0.465 \quad (1)$$

$$(\pm 0.022) \quad (\pm 0.043)$$

$$n=21, r=0.990, s=0.030, F=896.04$$

In this and the following equations, n is the number of compounds, r is the correlation coefficient, s is the standard deviation, F is the F -value of the correlation and the figures

TABLE II. ANTIFUNGAL ACTIVITY OF *N*-(3,4-DIMETHOXYBENZOYL)ANTHRANILATES AGAINST POWDERY MILDEW OF BARLEY

No.	R ^a	mp (°C) ^b of <i>n</i> _D (°C)	Activity (<i>pI</i> ₅₀)	
			Obsd	Calcd ^c
15	H	192~194	<2.0 ^d	—
9	Me	109~110	4.94	4.87
16	Et	110~111	5.44	5.39
17	<i>n</i> -Pr	89~90	5.58	5.51
18	<i>i</i> -Pr	136~137	4.01	4.29
19	<i>n</i> -Bu	91~92	5.30	5.36
20	<i>i</i> -Bu	87.5~89	5.65	5.46
21	<i>s</i> -Bu	67~68	4.29	4.22
22	<i>n</i> -Pent	75.5~76.5	4.63	5.01
23	<i>i</i> -Pent	81~82.5	5.19	5.26
24	<i>s</i> -Pent	1.5783 (24)	4.25	3.93
25	3-Pent	79~81	3.86	3.98
26	Neopent	120~121	5.52	5.32
27	3-Me-2-Bu	1.5820 (21.5)	4.18	4.01
28	<i>n</i> -Hex	115.5~116.5	4.58	4.47
29	3-Hex	1.5771 (21.5)	3.12	3.52
30	4-Me-2-Pent	1.5725 (24)	3.74	3.50
31	<i>c</i> -Pent	100.5~101.5	2.69 ^d	—
32	<i>c</i> -Hex	99~100	2.99 ^d	—
33	2-Propenyl	100~101	5.15	5.22
34	2-Propynyl	148.5~150	4.42	4.37
35	2-Butenyl	125~126.5	5.12	5.27
36	2-Me-2-Propenyl	96.5~97.5	5.29	5.29
37	3-Butenyl	74~75	5.39	5.39

^a *n*, *i*, *s* and *c* denote normal, iso, secondary and cyclo.

^b Melting points are uncorrected.

^c Calculated by eq. 4.

^d Omitted from calculation.

TABLE III. SUBSTITUENT CONSTANTS USED FOR CORRELATIONS

No.	Substituent ^a	π ^b	R _M	X ^c	σ* ^d
9	Me	0.50	-0.31	0	0.00
16	Et	1.00	-0.14	0	-0.10
17	<i>n</i> -Pr	1.50	0.03	0	-0.12
18	<i>i</i> -Pr	1.30	-0.03	1	-0.19
19	<i>n</i> -Bu	2.00	0.17	0	-0.13
20	<i>i</i> -Bu	1.80	0.11	0	-0.13
21	<i>s</i> -Bu	1.80	0.13	1	-0.21
22	<i>n</i> -Pent	2.50	0.31	0	-0.16 ^e
23	<i>i</i> -Pent	2.30	0.23	0	-0.16 ^e
24	<i>s</i> -Pent	2.30	0.26	1	-0.21 ^f
25	3-Pent	2.30	0.25	1	-0.23
26	Neopent	2.10	0.21	0	-0.17
27	3-Me-2-Bu	2.10	0.23	1	-0.21 ^e
28	<i>n</i> -Hex	3.00	0.44	0	-0.17
29	3-Hex	2.80	0.38	1	-0.23 ^g
30	4-Me-2-Pent	2.80	0.38	1	-0.21 ^f
33	2-Propenyl	1.20	-0.10	0	0.15 ^h
34	2-Propynyl	0.98	-0.25	0	0.76 ⁱ
35	2-Butenyl	1.70	0.06	0	0.13
36	2-Me-2-Propenyl	1.50	0.02	0	0.13 ^j
37	3-Butenyl	1.70	0.06	0	0.00 ^k

^a *n*, *i* and *s* denote normal, iso and secondary.

^b Estimated by means of the additivity principle.⁽⁵⁾

^c Indicator variable for α-branching.

^d Unless noted, taken from Ref. 6.

^e A. B. Hoefelmeyer and C. K. Hancock, *J. Am. Chem. Soc.*, **77**, 4746 (1955).

^f Taken from that of *s*-Bu.

^g Taken from that of 3-Pent.

^h J. Sestakova *et al.*, *Coll. Czech. Chem. Comm.*, **31**, 3889 (1966).

ⁱ H. K. Hall Jr., *J. Am. Chem. Soc.*, **79**, 5411 (1957).

^j Taken from that of 2-Butenyl.

^k Taken from that of Me.

in parentheses are the 95% confidence intervals of the corresponding constants.

We have used the experimentally determined *R*_M value as the hydrophobic parameter in the following analyses. The *pI*₅₀ values are plotted against the *R*_M values in Fig. 1 showing a roughly parabolic relationship. However, the correlation is very poor with the *R*_M parameter alone as represented by eq. 2.

$$pI_{50} = -7.378R_M^2 + 5.137 \quad (2)$$

$$(\pm 5.091) (\pm 0.384)$$

$$n=21, r=0.571, s=0.597, F=9.20$$

substituent is α-branched is always lower than that of the others. Moreover, the plot for esters of unsaturated alcohols is lower than that of the saturated alcohols. Thus, the variation in the activity is expected to be determined not only by the hydrophobicity but also by the steric effect of α-branching as well as the unsaturation effect of the ester substituents. We have tried to delineate these additional substituent effects with steric and electronic parameters. We have found that the α-branching effect is best represented by the indicator variable, *X*, which takes 1 for the α-branching alkyl esters and 0

FIG. 1. Antifungal Activity and the R_M Value of *N*-(3,4-Dimethoxybenzoyl)anthranilic Esters.

●, α -unbranched alkyl esters; ■, α -unbranched alkenyl and alkynyl esters; ○, α -branched alkyl esters.

for the others. Since unsaturated alkyl groups are supposed to show the higher electron withdrawing effect than the saturated, we have applied the Taft σ^* parameter⁶⁾ to represent the effect of unsaturation.

$$pI_{50} = -5.212R_M^2 - 1.104X + 5.390 \quad (3)$$

$$(\pm 2.424) \quad (\pm 0.275) \quad (\pm 0.189)$$

$$n=21, r=0.927, s=0.276, F=56.96$$

$$pI_{50} =$$

$$-5.624R_M^2 - 1.287X - 0.902\sigma^* + 5.410 \quad (4)$$

$$(\pm 1.840) \quad (\pm 0.230) \quad (\pm 0.492) \quad (\pm 0.143)$$

$$n=21, r=0.963, s=0.207, F=72.45$$

Equation 3 shows that the indicator variable X for the α -branching is highly significant in correlating the activity value of the whole series of compounds. Although the Taft-Hancock corrected steric parameter, E_s^c , has been suggested to emphasize the α -branching effect,⁷⁾ the use of E_s^c instead of X yielded only poorer correlation. Equation 4 clearly indicates that the addition of the σ^* parameter for the electron withdrawing effect of ester substituents is also highly significant. All of the terms in eq. 4 are justified at better than 99.5% by *t*-test. The squared correlation matrix of the variables used in eqs. 3 and 4 is shown in Table IV.

According to eq. 4, the fungicidal activity is really related parabolically to the hydropho-

TABLE IV. THE SQUARED CORRELATION MATRIX OF VARIABLES USED IN EQS. 3 AND 4

	R_M^2	σ^*	X
R_M	1.000	0.043	0.050
σ^*		1.000	0.212
X			1.000

bicity of the ester moiety and reaches a maximum when R_M is 0. The parabolic relationship seems to indicate that the compounds should traverse a number of lipoidal-aqueous interfaces to reach a critical site for the fungicidal activity.⁸⁾

The negative sign of the σ^* term means that the activity decreases with increasing the electron withdrawing property of the ester substituents. *N*-(3,4-Dimethoxybenzoyl)anthranilic esters may be hydrolyzed spontaneously and/or biologically in the plant and fungus bodies. The hydrolyzed compound, *N*-(3,4-dimethoxybenzoyl)anthranilic acid (15), did not exhibit the activity. The electron withdrawing effect may facilitate the attack of the bio-nucleophiles against the ester carbonyl leading to hydrolysis of the esters. Intramolecular cyclization which eliminates the alcohol moiety is also likely to occur similar to the quinazoline formation.⁹⁾ The pI_{50} value of the cyclization product, 2-(3,4-dimethoxyphenyl)-4,5-benzotriazin-6-one, was lower than 2.0. Therefore, the negative sign of the σ^* term suggests that detoxication processes by the hydrolysis and/or the cyclization is important in governing the activity variation.

The fact that the coefficient of the X term is -1.287 means that the activity of the α -branched ester derivatives is about 20 times lower than that of the unbranched compounds, other things being equal. The possibility that this type of the steric effect is operative in the detoxication processes is defined, since, if so, the coefficient should be positive. The α -branching steric effect is expected to retard the hydrolysis. Thus, it is most probable that the effect reflects the unsuitable fit of the branched compounds with the critical site of the fungicidal action. The ester linkage ortho to the peptide bridge of this series of compounds may

play a critical role for the fungicidal activity. Cyclopentyl (31) and cyclohexyl (32) derivatives were not included in the analyses. These cyclic compounds are only weakly active compared with the uncyclic α -branched esters. The rigidity of the ester substituents may decrease the activity.

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