Table IV. Changes in Phytic Acid Contents (% mfb)[a] during Processing of White Bengal Gram

sample description	moisture, %	phytic acid, %	diff in phytic acid	% loss of phytic acid on processing
whole white grams	12.25	0.80		
soaked white grams in water	29.60	0.70	0.10	12.50
soaked, boiled	35.12	0.60	0.20	25.00
soaked, boiled, fried	22.05	0.50	0.30	37.50
soaked in $NaHCO_3$	28.50	0.40	0.06	7.50
soaked in $NaHCO_3$, boiled	36.00	0.70	0.10	12.50

[a] mfb = moisture free-basis.

Table V. Changes in Phytic Acid Content (% mfb)[a] of Immature Brown Bengal Grams during Preparation of Various Products

sample description	moisture, %	phytic acid, %	diff in phytic acid	% loss on processing
immature grams	24.14	0.15		
immature boiled grams	32.82	0.06	0.09	60.00
fried	21.78	0.07	0.08	53.33
roasted on sand/salt bath	19.14	0.08	0.07	46.66
roasted in pods in sand/salt bath	20.32	0.09	0.06	40.00

[a] mfb = moisture-free basis.

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Registry No. $NaHCO_3$, 144-55-8; phytic acid, 83-86-3.

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Quantitative Structure–Activity Relationships of Photosystem II Inhibitory Anilides and Triazines. Topological Aspects of Their Binding to the Active Site

Ryo Shimizu, Hajime Iwamura,* and Toshio Fujita

We analyzed the quantitative structure–activity relationship for inhibition by meta- and para-substituted anilides and 2-chloro-4,6-diamino-*s*-triazines with sterically different substituents of photosystem II. The results showed that the acyl moiety of anilide compounds and the smaller of the two amine substituents of triazines have features in common in their interaction with the receptor. The steric demands for the meta substituent of anilides and another amine substituent of the triazines were also common. On the basis of these findings and the knowledge that their binding to chloroplasts is competitive, we drew a receptor map that makes visible the structural correspondence between the two series of compounds when bound at the common active site.

Functional binding to spinach chloroplasts of structurally congeneric inhibitors of photosystem (PS) II, anilides, ureas, and carbamates, and chemically different triazines is competitive at a common site (Mitsutake et al., 1986). Studies of the quantitative structure–activity relationship (QSAR) have given a single common equation for the anilide type of compounds, indicating that their modes of action are identical or almost so. In this study, we extended the QSAR study to explore the steric mode of interaction of anilides and structurally different triazines in detail with compounds having various steric dimensions. The analyses were done for the PS II inhibitory activity examined by virtue of DCIP (2,6-dichlorophenolindophenol) reduction, and the results revealed the structural correspondence of the two structures when bound at the common active site. Figure 1 shows the generic formulas of the compounds studied.

Department of Agricultural Chemistry, Faculty of Agriculture, Kyoto University, Kyoto 606, Japan.

MATERIALS AND METHODS

Chemicals. The syntheses or sources of **A1–A5, A8, A9, A12, A14, A16–A20, A22–A26, A33, A34, A36–A38, T1–T6, T10–T15, T17–T23, T25, T26, T28, T29, T37,** and **T38** were reported before (Mitsutake et al., 1986). The rest of the anilides were prepared for this study, mostly by the addition of an acid chloride in dry benzene to a DMF solution of an appropriately substituted aniline and

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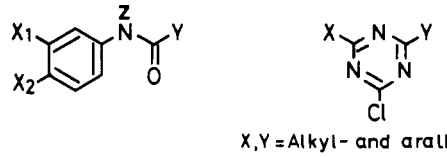

Figure 1. Generic structures of the anilide and triazine compounds studied.

triethylamine at room temperature. *m*- or *p*-Alkoxyanilides with aniline moiety that are not commercially available were prepared by the substitution reaction of an appropriate halide by an *m*- or *p*-hydroxyanilide in the presence of potassium *tert*-butoxide in 1-propanol at about 100 °C. **A73–A76** were synthesized by the methylation reaction of **A4**, **A47**, **A62**, and **A29**, respectively, with methyl iodide in the presence of NaH in dry DMF. **A77** was prepared by the reaction of *m*-(3-phenyl-*n*-propyl)-phenol with cyclopropanecarboxylic acid chloride in dry pyridine. Triazines were synthesized by the reaction of N^2-substituted 2-amino-4,6-dichloro-*s*-triazine with an appropriate amine in the presence of $NaHCO_3$ in water at about 50 °C (Thurston et al., 1951). **T48** and **T49** were prepared by the reaction of 3-phenyl-1-propylamine with 2,6-dichloro-4-ethoxy-*s*-triazine (Dudley et al., 1951) and 2,6-dichloro-4-*n*-propyl-*s*-triazine (Hirt et al., 1950), respectively. The structures of the compounds were identified by ^1H NMR (JEOL Model JNM-PMX60). The analytical results for C, H, and N were within ±0.3% of the theoretical values.

Substituent Parameters. To express the steric features of the molecule, we defined the steric parameters as shown in Figure 2. *D* is the length of a substituent in the extended conformation. It is measured along the *D*-axis that starts at the connecting end and goes forward along the zigzag aliphatic chain. By this definition, the angle between the *D*-axis and the connecting bond becomes 35.4°, and the axis was drawn according to this criterion for substituents other than aliphatic ones. The W_r is the width of the right-hand side of substituents measured from the *D*-axis when viewed from the connecting end, and the W_l is that of the left-hand side. Similarly, T_r and T_l are the thicknesses of the right- and the left-hand sides, respectively. For expressing positional steric effects, we defined W_r3 for acyl substituents of anilides and $W_r\beta$ for amine substituents of triazines. W_r3 is the width W_r at the 3-position (Figure 2B), and $W_r\beta$ is that at the β-position from the connecting triazine carbon (Figure 2C). The steric parameters were calculated on the basis of the CPK model with a computer program made by Verloop et al. (1976) and modified by us for the estimation of the *D* and related dimensional parameters (Asao and Iwamura, 1985).

The logarithm of the partition coefficient between 1-octanol/water, log *P*, has been previously reported for compounds **A1–A5**, **A8**, **A9**, **A12**, **A14**, **A16–A20**, **A22–A26**, **A33**, **A34**, **A36–A38**, **T1–T6**, **T10–T15**, **T17–T23**, **T25**, **T26**, **T28**, **T28**, **T29**, **T37**, and **T38** (Mitsutake et al., 1986).

The log *P* values of most of the other anilide and triazine compounds were calculated by addition of the appropriate π values or multiples of $\pi(CH_3)$, the branch factor F_{cBr}, and the double-bond factor $F_{=}$ to the log *P* values already known or estimated of appropriate compounds. The data necessary for the calculation were taken from the literature (Hansch and Leo, 1979). For compounds **A15**, **A27**, **A31**, **A53**, **A69**, and **A77**, the log *P* values were estimated as follows: log *P* (**A15**; 3,4-$Cl_2C_6H_3NHCOCH_2OPh$) = log *P* (**A14**; 3,4-$Cl_2C_6H_3NHCOCH_2CH_2Ph$) + [log *P* ($PhOCH_2CONH_2$) – log *P* ($PhCH_2CH_2CONH_2$)]; log *P* (**A27**; 3-(isopentyloxy)C_6H_4NHCO-c-Pr) = log *P* (3-$MeOC_6H_4NHCO$-c-Pr) + $4\pi(CH_3)$ + F_{cBr}, where the first term was calculated by log *P* [**A19**; 3-$MeOC_6H_4NHCO$(1-Me-c-Pr)] + [log *P* (3,4-$Cl_2C_6H_3NHCO$-c-Pr) (3.83; Hayashi and Fujita, 1983) – log *P* [**A26**; 3,4-$Cl_2C_6H_3NHCO$(1-Me-c-Pr)]]; log *P* [**A31**; 3-$PhO(CH_2)_3OC_6H_4NHCO$-c-Pr] = log *P* [**A29**; 3-Ph-$(CH_2)_3OC_6H_4NHCO$-c-Pr] + [log *P* (PhO-*n*-Pr) – log *P* (Ph-*n*-Pr); log *P* [**A53**; 3-$MeOCOCH(Me)OC_6H_4NHCO$-*n*-Bu] = log *P* (**A41**; 3-$MeOC_6H_4NHCO$-*n*-Bu) + [log *P* ($PhOCH_2COOMe$) – log *P* (PhOMe)] + $\pi(CH_3)$ + F_{cBr}; log *P* [**A69**; 4-$MeOCOCH(Me)OC_6H_4NHCO$-*n*-Bu] = log *P* (**A55**; 4-$MeOC_6H_4NHCO$-*n*-Bu) + [log *P* ($PhOCH_2COOMe$) – log *P* (PhOMe)] + $\pi(CH_3)$ + F_{cBr}; log *P* [**A77**; 3-$Ph(CH_2)_3OC_6H_4OCO$-c-Pr] = log *P* [**A29**; 3-$Ph(CH_2)_3OC_6H_4NHCO$-c-Pr] + [log *P* (PhOCOMe) – log *P* (PhNHCOMe)]. The hydrophobicity difference between *N*-Me derivatives (**A73–A76**) and the corresponding unsubstituted derivatives was estimated by log *P* (PhNHCOMe) – log *P* [PhN(Me)COMe]. For triazines **T8** (X = $NHCH_2CH_2OMe$, Y = NHEt) and **T9** (X = $NH(CH_2)_2O(CH_2)_2CH_3$, Y = NHEt), the values were calculated by addition of the difference log *P* (MeOMe) – log *P* (propane) to the log *P* values of **T3** (X = NH-*n*-Bu, Y = NHEt) and **T5** (X = NH-*n*-Hx, Y = NHEt), respectively. Similarly, log *P* (**T16**; X = NH-c-pentyl, Y = NHEt) = log *P* (**T14**; X = NH-c-Pr, Y = NHEt) + [log *P* (cyclopentane) – log *P* (cyclopropane)] and log *P* (**T24**; X = $NHCH_2$-biphenylyl, Y = NHEt) = log *P* (**T22**; X = NH-benzyl, Y = NHEt) + π^{arom}(Ph). For compounds **T34**, **T35**, and **T36** where X = (phenylpropyl)amino and Y = NHR, the values were calculated by log *P* (**T28**; X = (phenylpropyl)amino, Y = NHEt) + [log *P* (compound with X = NHR and Y = NHEt) – log *P* (**T1**; X = Y = NHEt)]. Similarly, log *P* (**T39**; X = Y = NH-cyclopentyl) = log *P* (**T16**; X = NH-cyclopentyl, Y = NHEt) + [log *P* (**T16**) – log *P* (**T1**)]. For the compounds with a Y moiety in which the nitrogen atom is methylated, log *P* (**T40**; X = (phenylpropyl)amino, Y = NMe_2) = log *P* (**T28**; X = (phenylpropyl)amino, Y = NHEt) + [log *P* ($PhNMe_2$) – log *P* (PhNHEt)]; log *P* [**T41**; X = (phenylpropyl)amino, Y = N(Me)OMe] = log *P* (**T40**) + [log *P* [PhNHCON(Me)OMe] – log *P* ($PhNHCONMe_2$)] (Kakkis et al., 1984). The values of **T42** [X = (phenylpropyl)amino, Y = N(Me)-*n*-Bu] and **T45** [X = N(Me)-*n*-Bu, Y = NHEt] were calculated by the addition of the

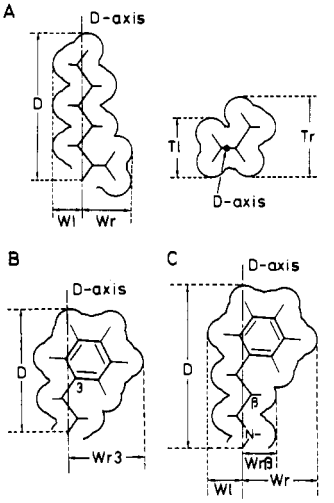

Figure 2. Definition of the steric parameters: A, generic representation; B, acyl moiety of anilides indicating the W_r3 parameter; C, amine substituent of triazines indicating the $W_r\beta$ parameter. The ends of the bars of the molecular model represent hydrogen atoms.

difference log P [HN(Me)-n-Bu] - log P (H_2NEt) to log P (**T28**) and log P (**T1**), respectively, and those of pyrrolidinyl **T43** and piperidinyl **T44** (X = phenylpropyl) were calculated by the addition of the difference log P (pyrrolidine) - log P (H_2NEt) and log P (piperidine) - log P (H_2NEt), respectively, to the value of **T28**. The log P values of methoxy **T48** and n-propyl **T49** (X = phenylpropyl) were estimated by log P (**T28**) + [log P (PhR) - log P (PhNHEt)], where R is OEt and n-propyl, respectively.

Inhibition of Photosystem II Electron Flow. Chloroplasts were isolated from fresh, washed, depetiolated spinach leaves (120 g) that were homogenized in an ice-cold Waring blender with 200 mL of buffer consisting of tricine (50 mM), NaCl (10 mM), and sucrose (0.4 M) at pH 8.0. The homogenate was filtered through four layers of gauze, and the filtrate was centrifuged at 2000g for 10 min. The supernatant was discarded; the pellets were suspended in homogenizing medium and centrifuged again as above. The chloroplasts were suspended in 25 mL of the buffer, added to the same volume of glycerol, mixed well, and stored at -20 °C until use (Asada and Takahashi, 1971). The amount of chloroplasts was measured by the method of MacKinney (1941).

The reaction mixture consisted of 1.0 mL (15 μg) of a chloroplast suspension in a buffer (pH 7.8) of Tris (20 mM), NaCl (10 mM), $MgCl_2 \cdot 6H_2O$ (2 mM), $CH_3NH_2 \cdot HCl$ (10 mM), and sucrose (0.4 M), 1.0 mL of DCIP (2,6-dichlorophenolindophenol) solution (40 μM) in a buffer (pH 7.2) of Tris-HCl (50 mM) and NaCl (10 mM), and 0.5 mL of a solution of a test compound. Test compounds were dissolved in water containing less than 2% (v/v) ethanol or methanol or less than 3% (v/v) Me_2SO, depending on their solubility. The reduction of DCIP was monitored at 600 nm with a Shimadzu UV-300 spectrophotometer modified for illumination with red light through a Toshiba R65 filter. The activity was expressed in terms of the logarithm of the reciprocal of the molar concentration at which 50% inhibition of the photosynthetic DCIP reduction is obtained, pI_{50}; the range of the experimental error was within ±0.08.

RESULTS

Anilide Compounds. Of the 77 compounds listed in Table I, 24 compounds were reported in an earlier paper (Mitsutake et al., 1986). The other 53 compounds were prepared for this study so we could examine the steric mode of interaction with the active site. They possess sterically different X_1, X_2, and Y substituents (Figure 1). Accordingly, we adopted in this study a new set of dimensional parameters, the utility of which has been proved in structure–activity relationship studies of such complex molecules as terpenoid- and non-terpenoid-mimetic compounds of insect juvenile hormones (Nakayama et al., 1984), and bitter amino acids, peptides, and their derivatives (Asao et al., 1987).

The set of compounds **A1–A54** and **A72** having various X_1 and Y substituents was first analyzed on the basis of the bilinear model of Kubinyi (1979).

$$pI_{50} = \underset{(0.15)}{1.02}\log P - \underset{(0.49)}{1.50}\log(\beta 10^{\log P} + 1) - \underset{(0.29)}{0.62}T_1(\mathrm{Y}) - \underset{(0.16)}{0.89}W_r3(\mathrm{Y}) - \underset{(0.34)}{0.77}I_{br}(\mathrm{X_1}) + \underset{(1.43)}{6.48} \quad (1)$$

$n = 55$, $s = 0.38$, $r = 0.93$, $F_{5,49} = 59.4$, $\log \beta = -4.96$, $\log P_o = 5.29$

In this and the following equations, n is the number of compounds analyzed, s is the standard deviation, r is the multiple correlation coefficient, and F is the value of F ratio between variances of calculated and observed activities. The figures in parentheses are the 95% confidence intervals.

The optimum log P (log P_o) was calculated to be 5.29. The T_1(Y) having a negative sign shows that the thickness of the left-hand side of the acyl Y substituent is unfavorable for high activity. During the correlation, the calculated pI_{50} values of the compounds with cyclohexyl, phenetyl, cyclopropyl, or 1-methylcyclopropyl Y tended to deviate from the observed one. Their structural characteristics lie in the thickness or width around the third atom from the connecting end, so we defined the W_r3 parameter as shown in Figure 2. As expected, it was significant in eq 1, the negative coefficient indicating that the compounds with a wider Y are less active. Similarly, the compounds with an X_1 branched at the β-position from the benzene ring had relatively lower activity. We thus introduced the indicator variable term $I_{br}(X_1)$ that takes one for these and zero for the others. None of the D or other dimensional parameters was significant at all for such sterically different meta substituents.

The PS II inhibition of the compounds with a bulky para substituent (X_2) was not very strong and was rather uniform. The analysis in the inclusion of them (compounds **A55–A71**) gave eq 2. The results was essentially the same

$$pI_{50} = \underset{(0.13)}{0.93}\log P - \underset{(0.52)}{1.54}\log(\beta 10^{\log P} + 1) - \underset{(0.28)}{0.62}T_1(\mathrm{Y}) - \underset{(0.16)}{0.89}W_r3(\mathrm{Y}) - \underset{(0.33)}{0.76}I_{br}(\mathrm{X_1}) - \underset{(0.22)}{1.51}I_{OR}(\mathrm{X_2}) + \underset{(1.38)}{6.72} \quad (2)$$

$n = 72$, $s = 0.38$, $r = 0.94$, $F_{6,65} = 84.4$, $\log \beta = -5.25$, $\log P_o = 5.44$

as that of eq 1 except for the significance of the indicator variable term $I_{OR}(X_2)$. It takes one for the compounds having an X_2 larger than ethoxy in terms of the D defined in Figure 2 and zero for others. Again, all of the D and other dimensional parameters were insignificant for the para substituent as for the meta substituents, irrespective of their being sterically so various.

Methylation of the amide nitrogen atom lowered the activity drastically. The pI_{50} values of such compounds, **A73–A76**, calculated by eq 2 gave a rather uniform deviation from the observed activity. We thus incorporated them into the correlation with the use of the indicator variable I_{NMe} that takes one for the N-methylated derivatives and zero for the others. Equation 3 thus formulated provides us with a basis for understanding the mode of interaction of the set of anilide compounds at the active site. The development of eq 3 and the squared correlation matrix of the variables used are shown in Tables III and IV, respectively.

$$pI_{50} = \underset{(0.14)}{0.95}\log P - \underset{(0.49)}{1.39}\log(\beta 10^{\log P} + 1) - \underset{(0.30)}{0.62}T_1(\mathrm{Y}) - \underset{(0.17)}{0.87}W_r3(\mathrm{Y}) - \underset{(0.35)}{0.75}I_{br}(\mathrm{X_1}) - \underset{(0.23)}{1.44}I_{OR}(\mathrm{X_2}) - \underset{(0.42)}{2.59}I_{NMe} + \underset{(1.45)}{6.64} \quad (3)$$

$n = 76$, $s = 0.40$, $r = 0.94$, $F_{7,68} = 75.2$, $\log \beta = -5.08$, $\log P_o = 5.41$

Triazine Compounds. In a preliminary study on triazines (Mitsutake et al., 1986), it was seen that the longer of the two amine substituents (X and Y) behaves differently from the shorter one with respect to the binding to the receptive site. This was not, however, conclusive, as the variation in substituents in the previous set of triazines was insufficient, and the significance of their parameters in the correlation is not fully reliable. To improve the

Table I. Inhibition of Photosystem II Electron Flow and Physicochemical Parameters of Anilide Compounds

					activity			physicochemical parameter					
					pI_{50}								
no.	X_1	X_2	Y	Z	obsd	calcd[a]	ΔpI_{50}	log P	$W_r3(Y)$	$T_1(Y)$	$I_{br}(X_1)$	$I_{OR}(X_2)$	I_{NMe}
A1	Cl	Cl	Et	H	6.35	6.32	0.03	3.38	1.31	3.80	0.00	0.00	0.00
A2	Cl	Cl	*i*-Pr	H	6.07	6.63	−0.56	3.72	1.31	3.80	0.00	0.00	0.00
A3	Cl	Cl	1-Me-allyl	H	6.77	6.05	0.72	3.49	2.16	3.20	0.00	0.00	0.00
A4	Cl	Cl	*n*-Bu	H	6.34	6.40	−0.06	4.46	2.25	3.80	0.00	0.00	0.00
A5	Cl	Cl	*i*-Bu	H	4.94	5.36	−0.42	4.11	2.25	5.05	0.00	0.00	0.00
A6	Cl	Cl	1-Me-*n*-Bu	H	7.22	6.63	0.59	4.87	2.25	3.80	0.00	0.00	0.00
A7	Cl	Cl	2-Me-*n*-Bu	H	6.33	5.86	0.47	4.87	2.25	5.05	0.00	0.00	0.00
A8	H	Cl	1,1-Me_2-*n*-Bu	H	5.80	5.90	−0.10	3.83	2.25	3.80	0.00	0.00	0.00
A9	Cl	Me	1-Me-*n*-Bu	H	6.48	6.30	0.18	4.31	2.25	3.80	0.00	0.00	0.00
A10	Cl	Cl	*n*-pentyl	H	6.63	6.68	−0.05	5.00	2.25	3.80	0.00	0.00	0.00
A11	Cl	Cl	*i*-pentyl	H	6.45	5.89	0.56	4.87	3.10	3.80	0.00	0.00	0.00
A12	Cl	Cl	c-Hx	H	5.17	5.04	0.13	4.72	4.00	3.80	0.00	0.00	0.00
A13	Cl	Cl	$(CH_2)_2$-c-Hx	H	4.44	5.21	−0.77	5.80	3.95	3.80	0.00	0.00	0.00
A14	Cl	Cl	$(CH_2)_2$Ph	H	4.77	4.45	0.32	4.85	4.74	3.80	0.00	0.00	0.00
A15	Cl	Cl	CH_2OPh	H	4.42	4.64	−0.22	4.70	4.73	3.40	0.00	0.00	0.00
A16	H	H	1-Me-c-Pr	H	5.04	4.60	0.44	2.01	1.31	4.50	0.00	0.00	0.00
A17	Cl	H	1-Me-c-Pr	H	5.72	5.51	0.21	2.98	1.31	4.50	0.00	0.00	0.00
A18	Br	H	1-Me-c-Pr	H	5.50	5.66	−0.16	3.14	1.31	4.50	0.00	0.00	0.00
A19	OMe	H	1-Me-c-Pr	H	4.57	4.73	−0.16	2.15	1.31	4.50	0.00	0.00	0.00
A20	CF_3	H	1-Me-c-Pr	H	5.32	5.71	−0.39	3.19	1.31	4.50	0.00	0.00	0.00
A21	O-*i*-pentyl	H	1-Me-c-Pr	H	7.05	6.58	0.47	4.18	1.31	4.50	0.00	0.00	0.00
A22	H	F	1-Me-c-Pr	H	5.14	4.77	0.37	2.19	1.31	4.50	0.00	0.00	0.00
A23	H	Cl	1-Me-c-Pr	H	5.80	5.40	0.40	2.86	1.31	4.50	0.00	0.00	0.00
A24	H	OMe	1-Me-c-Pr	H	4.78	4.36	0.42	1.76	1.31	4.50	0.00	0.00	0.00
A25	H	CN	1-Me-c-Pr	H	4.51	4.69	−0.18	2.11	1.31	4.50	0.00	0.00	0.00
A26	Cl	Cl	1-Me-c-Pr	H	6.88	6.11	0.77	3.63	1.31	4.50	0.00	0.00	0.00
A27	O-*i*-pentyl	H	c-Pr	H	6.90	6.92	−0.02	4.38	1.10	4.50	0.00	0.00	0.00
A28	$O(CH_2)_2Ph$	H	c-Pr	H	6.93	7.04	−0.11	4.57	1.10	4.50	0.00	0.00	0.00
A29	$O(CH_2)_3Ph$	H	c-Pr	H	7.09	7.28	−0.19	5.11	1.10	4.50	0.00	0.00	0.00
A30	$O(CH_2)_5Ph$	H	c-Pr	H	6.73	7.15	−0.42	6.19	1.10	4.50	0.00	0.00	0.00
A31	$O(CH_2)_3OPh$	H	c-Pr	H	7.39	7.13	0.26	4.72	1.10	4.50	0.00	0.00	0.00
A32	$O(CH_2)_4OPh$	H	c-Pr	H	6.67	7.30	−0.63	5.26	1.10	4.50	0.00	0.00	0.00
A33	H	H	CH_2Ph	H	4.95	5.06	−0.11	2.72	2.33	3.40	0.00	0.00	0.00
A34	F	H	CH_2Ph	H	5.73	5.46	0.27	3.14	2.33	3.40	0.00	0.00	0.00
A35	O-*i*-pentyl	H	CH_2Ph	H	7.13	6.87	0.26	5.02	2.33	3.40	0.00	0.00	0.00
A36	H	Cl	CH_2Ph	H	5.75	5.89	−0.14	3.61	2.33	3.40	0.00	0.00	0.00
A37	H	Me	CH_2Ph	H	5.13	5.41	−0.28	3.09	2.33	3.40	0.00	0.00	0.00
A38	Cl	Cl	CH_2Ph	H	6.72	6.59	0.13	4.47	2.33	3.40	0.00	0.00	0.00
A39	H	H	*n*-Bu	H	4.12	4.76	−0.64	2.59	2.25	3.80	0.00	0.00	0.00
A40	OH	H	*n*-Bu	H	4.12	4.54	−0.42	2.35	2.25	3.80	0.00	0.00	0.00
A41	OMe	H	*n*-Bu	H	4.87	5.13	−0.26	2.98	2.25	3.80	0.00	0.00	0.00
A42	O-*n*-Pr	H	*n*-Bu	H	6.05	6.10	−0.05	4.06	2.25	3.80	0.00	0.00	0.00
A43	O-*i*-Pr	H	*n*-Bu	H	5.64	5.24	0.40	3.93	2.25	3.80	1.00	0.00	0.00
A44	O-*n*-Bu	H	*n*-Bu	H	6.46	6.49	−0.03	4.60	2.25	3.80	0.00	0.00	0.00
A45	O-*i*-Bu	H	*n*-Bu	H	6.50	6.41	0.09	4.47	2.25	3.80	0.00	0.00	0.00
A46	O-*sec*-Bu	H	*n*-Bu	H	5.76	5.66	0.10	4.47	2.25	3.80	1.00	0.00	0.00
A47	O-*i*-pentyl	H	*n*-Bu	H	7.29	6.68	0.61	5.01	2.25	3.80	0.00	0.00	0.00
A48	O-*n*-pentyl	H	*n*-Bu	H	6.41	6.72	−0.31	5.14	2.25	3.80	0.00	0.00	0.00
A49	O-1-Me-*n*-Bu	H	*n*-Bu	H	5.60	5.93	−0.33	5.01	2.25	3.80	1.00	0.00	0.00
A50	O-*n*-heptyl	H	*n*-Bu	H	6.92	6.57	0.35	6.22	2.25	3.80	0.00	0.00	0.00
A51	O-1-Me-*n*-Hx	H	*n*-Bu	H	6.24	5.87	0.37	6.09	2.25	3.80	1.00	0.00	0.00
A52	OCH_2-c-Hx	H	*n*-Bu	H	6.50	6.50	0.00	6.42	2.25	3.80	0.00	0.00	0.00
A53	OCH(Me)COOMe	H	*n*-Bu	H	3.99	4.11	−0.12	2.69	2.25	3.80	1.00	0.00	0.00
A54	OCH(Me)COOEt	H	*n*-Bu	H	4.19	4.61	−0.42	3.23	2.25	3.80	1.00	0.00	0.00
A55	H	OMe	*n*-Bu	H	4.26	4.76	−0.50	2.59	2.25	3.80	0.00	0.00	0.00
A56	H	OEt	*n*-Bu	H	4.31	3.83	0.48	3.13	2.25	3.80	0.00	1.00	0.00
A57	H	O-*n*-Pr	*n*-Bu	H	4.16	4.32	−0.16	3.67	2.25	3.80	0.00	1.00	0.00
A58	H	O-*i*-Pr	*n*-Bu	H	3.90	4.21	−0.31	3.54	2.25	3.80	0.00	1.00	0.00
A59	H	O-*n*-Bu	*n*-Bu	H	4.49	4.78	−0.29	4.21	2.25	3.80	0.00	1.00	0.00
A60	H	O-*i*-Bu	*n*-Bu	H	4.24	4.68	−0.44	4.08	2.25	3.80	0.00	1.00	0.00
A61	H	O-*sec*-Bu	*n*-Bu	H	4.18	4.68	−0.50	4.08	2.25	3.80	0.00	1.00	0.00
A62	H	O-*i*-pentyl	*n*-Bu	H	5.27	5.07	0.20	4.62	2.25	3.80	0.00	1.00	0.00
A63	H	O-*n*-pentyl	*n*-Bu	H	5.11	5.14	−0.03	4.72	2.25	3.80	0.00	1.00	0.00
A64	H	O-1-Me-*n*-Bu	*n*-Bu	H	4.73	5.07	−0.34	4.62	2.25	3.80	0.00	1.00	0.00
A65	H	$OCH(Et)_2$	*n*-Bu	H	4.57	5.07	−0.50	4.62	2.25	3.80	0.00	1.00	0.00
A66	H	O-*n*-hepty	*n*-Bu	H	5.88	5.25	0.63	5.83	2.25	3.80	0.00	1.00	0.00
A67	H	O-1-Me-*n*-Hx	*n*-Bu	H	5.28	5.28	0.00	5.70	2.25	3.80	0.00	1.00	0.00
A68	H	OCH_2-c-Hx	*n*-Bu	H	5.08	5.20	−0.12	6.03	2.25	3.80	0.00	1.00	0.00
A69	H	OCH(Me)COOMe	*n*-Bu	H	3.46	3.05	0.41	2.30	2.25	3.80	0.00	1.00	0.00
A70	H	OCH(Me)COOEt	*n*-Bu	H	3.63	3.56	0.07	2.84	2.25	3.80	0.00	1.00	0.00
A71	H	OCH(Me)COO-*i*-Pr	*n*-Bu	H	3.89	3.94	−0.05	3.25	2.25	3.80	0.00	1.00	0.00
A72	O-*i*-pentyl	H	1-Me-*n*-Bu	H	6.80	6.74	0.06	5.42	2.25	3.80	0.00	0.00	0.00
A73	Cl	Cl	*n*-Bu	Me	3.88	3.79	0.09	4.42	2.25	3.80	0.00	0.00	1.00
A74	O-*i*-pentyl	H	*n*-Bu	Me	3.65	4.08	−0.43	4.97	2.25	3.80	0.00	0.00	1.00
A75	H	O-*i*-pentyl	*n*-Bu	Me	3.38	2.45	0.93	4.58	2.25	3.80	0.00	1.00	1.00
A76	$O(CH_2)_3Ph$	H	c-Pr	Me	4.09	4.68	−0.59	5.07	1.10	4.50	0.00	0.00	1.00
A77[b]	3-$Ph(CH_2)_3OC_6H_4OCO$-c-Pr				4.28	(7.31)	(−3.03)	5.44	1.10	4.50	0.00	0.00	0.00

[a] Values were calculated by eq 3. [b] Excluded from regression analysis, but the value calculated with eq 3 shown in parentheses.

Table II. Inhibition of Photosystem II Electron Flow and Physicochemical Parameters of Triazine Compounds

			activity			physicochemical parameter				
			pI_{50}							
no.	X	Y	obsd	calcd[a]	ΔpI_{50}	log P	$W_r\beta$(X)	D(Y)	T_1(Y)	I_{NMe}
T1	NHEt	NHEt	5.84	6.23	−0.39	2.14	2.20	5.20	3.80	0.00
T2	NH-*n*-Pr	NHEt	6.06	6.59	−0.53	2.75	2.20	5.20	3.80	0.00
T3	NH-*n*-Bu	NHEt	6.53	6.88	−0.35	3.25	2.20	5.20	3.80	0.00
T4	NH-*n*-pentyl	NHEt	7.02	7.12	−0.10	3.76	2.20	5.20	3.80	0.00
T5	NH-*n*-Hx	NHEt	7.59	7.27	0.32	4.33	2.20	5.20	3.80	0.00
T6	NH-*n*-octyl	NHEt	6.83	7.18	−0.35	5.27	2.20	5.20	3.80	0.00
T7	NH-*n*-decyl	NHEt	7.17	6.80	0.37	6.46	2.20	5.20	3.80	0.00
T8	$NH(CH_2)_2OMe$	NHEt	5.59	5.53	0.06	0.99	2.20	5.20	3.80	0.00
T9	$NH(CH_2)_3OEt$	NHEt	6.71	6.04	0.67	1.83	2.20	5.20	3.80	0.00
T10	NH-*i*-Pr	NHEt	6.52	6.51	0.01	2.61	2.20	5.20	3.80	0.00
T11	NH-*i*-Bu	NHEt	6.41	6.58	−0.17	3.12	3.11	5.20	3.80	0.00
T12	NH-1-Me-*n*-Hx	NHEt	7.43	7.27	0.16	4.74	2.20	5.20	3.80	0.00
T13	NH-1-Me-*n*-heptyl	NHEt	6.78	7.17	−0.39	5.28	2.20	5.20	3.80	0.00
T14	NH-c-Pr	NHEt	6.17	6.23	−0.06	2.13	2.17	5.20	3.80	0.00
T15	NH-c-Bu	NHEt	7.01	6.52	0.49	2.77	2.57	5.20	3.80	0.00
T16	NH-c-pentyl	NHEt	7.16	6.96	0.20	3.41	2.20	5.20	3.80	0.00
T17	NH-c-Hx	NHEt	6.79	7.06	−0.27	3.63	2.20	5.20	3.80	0.00
T18	$NHCH_2$-c-Pr	NHEt	6.42	6.58	−0.16	2.85	2.51	5.20	3.80	0.00
T19	$NHCH_2$-c-Hx	NHEt	7.28	6.82	0.46	4.17	3.95	5.20	3.80	0.00
T20	$NHCH_2CH(Et)_2$	NHEt	6.40	6.82	−0.42	4.20	3.95	5.20	3.80	0.00
T21	$NHCH(OEt)_2$	NHEt	5.27	5.15	0.12	1.37	4.73	5.20	3.80	0.00
T22	$NHCH_2Ph$	NHEt	6.24	6.20	0.04	3.16	4.75	5.20	3.80	0.00
T23	$NHCH_2$-*p*-tolyl	NHEt	6.71	6.48	0.23	3.72	4.75	5.20	3.80	0.00
T24	$NHCH_2$-*p*-biphenylyl	NHEt	6.58	6.59	−0.01	5.12	4.75	5.20	3.80	0.00
T25	$NHCH_2$-1-naphthyl	NHEt	6.85	6.65	0.20	4.33	4.75	5.20	3.80	0.00
T26	$NH(CH_2)_2Ph$	NHEt	7.08	7.08	0.00	3.70	2.27	5.20	3.80	0.00
T27	$NH(CH_2)_3Ph$	NHMe	7.62	7.34	0.28	3.70	2.20	3.95	3.80	0.00
T28	$NH(CH_2)_3Ph$	NHEt	7.54	7.26	0.28	4.24	2.20	5.20	3.80	0.00
T29	$NH(CH_2)_4Ph$	NHEt	6.88	7.27	−0.39	4.78	2.20	5.20	3.80	0.00
T30	$NH(CH_2)_3Ph$	NH-*n*-Pr	7.61	7.02	0.59	4.78	2.20	6.46	3.80	0.00
T31	$NH(CH_2)_3Ph$	NH-allyl	7.47	7.01	0.46	4.23	2.20	6.41	3.80	0.00
T32	$NH(CH_2)_3Ph$	NH-*n*-Bu	6.19	6.41	−0.22	5.32	2.20	8.98	3.80	0.00
T33	$NH(CH_2)_3Ph$	NH-*i*-Pr	7.49	6.64	0.85	4.65	2.20	5.20	5.05	0.00
T34	$NH(CH_2)_3Ph$	NH-c-Pr	7.85	7.76	0.09	4.23	2.20	4.97	2.90	0.00
T35	$NH(CH_2)_3Ph$	NH-c-pentyl	6.67	6.60	0.07	5.51	2.20	6.14	4.43	0.00
T36	$NH(CH_2)_3Ph$	NH-c-Hx	5.52	6.16	−0.64	5.73	2.20	6.46	5.05	0.00
T37	NH-allyl	NH-allyl	5.55	6.26	−0.71	2.75	2.57	6.41	3.80	0.00
T38	NH-*i*-Pr	NH-*i*-Pr	6.18	6.15	0.04	3.08	2.20	5.20	5.05	0.00
T39	NH-c-pentyl	NH-c-pentyl	6.45	6.77	−0.32	4.68	2.20	6.14	4.43	0.00
T40	$NH(CH_2)_3Ph$	$N(Me)_2$	4.45	4.72	−0.27	4.45	2.20	3.95	3.80	1.00
T41	$NH(CH_2)_3Ph$	N(Me)OMe	4.19	4.25	−0.06	4.76	2.20	6.29	3.80	1.00
T42	$NH(CH_2)_3Ph$	N(Me)-*n*-Bu	4.40	3.75	0.65	5.70	2.20	7.71	3.80	1.00
T43	$NH(CH_2)_3Ph$	pyrrolidinyl	3.93	4.00	−0.07	4.83	2.20	4.98	4.78	1.00
T44	$NH(CH_2)_3Ph$	piperidinyl	3.88	4.38	−0.50	5.21	2.20	5.24	3.80	1.00
T45	N(Me)-*n*-Bu	NHEt	4.52	4.24	0.28	3.60	2.20	5.20	3.80	1.00
T46	N(Me)-*n*-Bu	N(Me)-*n*-Bu	3.88	3.92	−0.04	5.06	2.20	7.71	3.80	1.00
T47	$NHC(Me)_2Ph$	NHEt	6.06	6.57	−0.51	3.98	4.75	5.20	3.80	0.00
T48[b]	$NH(CH_2)_3Ph$	OEt	6.05	(7.25)	(−1.20)	5.01	2.20	5.14	3.80	0.00
T49[b]	$NH(CH_2)_3Ph$	*n*-Pr	4.63	(6.94)	(−2.31)	6.07	2.20	5.20	3.80	0.00

[a] Values were calculated by eq 5. [b] Excluded from regression analysis, but the value calculated with eq 5 shown in parentheses.

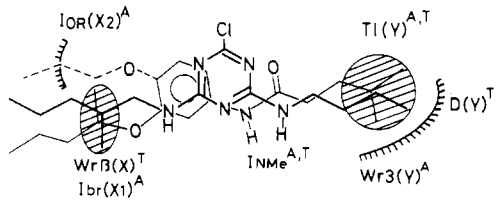

Figure 3. Schematic receptor map for anilide-type compounds and triazines that inhibit PS II. The stippled solid lines show the steric interaction sites or spatial walls, and the striped circle is that located upward or downward. The affixes with superscript A indicate the parameters incorporated into eq 3 for anilide compounds and those with superscript T are the parameters incorporated into eq 5 for triazines.

analysis, we prepared the set of compounds with sterically different amine substituents shown in Table II. The results showed that the steric restriction for the larger of the two amines was very weak. This is exemplified by the high activity of ethylamino compounds **T7** and **T19** with the bulky *n*-decylamine and (cyclohexylmethyl)amine, respectively, as another amine substituent. (Phenylpropyl)amino compounds with smaller methyl-, ethyl-, propyl-, and cyclopropylamines (compounds **T27**, **T28**, **T30**, and **T34**) were similarly highly active, but those with a bit bulkier *n*-butyl- and cyclohexylamines (compounds **T32** and **T36**) were relatively weak in activity. We thus classified the two amine substituents in terms of their length D, defining the one with larger D as X and the other as Y. Analysis of their structure–activity relationship gave eq 4. Coinciding with the observation made above, none

$$pI_{50} = \underset{(0.18)}{0.60}\log P - \underset{(0.53)}{1.08}\log(\beta 10^{\log P} + 1) - \underset{(0.14)}{0.26}W_r\beta(\mathrm{X}) - \underset{(0.18)}{0.30}D(\mathrm{Y}) - \underset{(0.34)}{0.47}T_1(\mathrm{Y}) + \underset{(1.72)}{8.86} \quad (4)$$

$n = 40$, $s = 0.39$, $r = 0.83$, $F_{5,34} = 14.5$, $\log \beta = -4.52$, $\log P_o = 4.63$

of the dimensional parameters except for the position-

Table III. Development of Equation 3

const	$I_{OR}(X_2)$	log P	log ($\beta 10^{\log P}$ + 1)	I_{NMe}	$W_r3(Y)$	$I_{br}(X_1)$	$T_l(Y)$	r	s	$F_{x,y}$[a]
5.75	−1.30							0.49	0.99	$F_{1,74}$ = 22.88
3.48	−1.38	0.58	−0.56					0.68	0.84	$F_{2,72}$ = 15.23
3.05	−1.37	0.73	−1.00	−2.26				0.81	0.67	$F_{1,71}$ = 40.64
3.74	−1.27	0.94	−1.41	−2.51	−0.69			0.91	0.48	$F_{1,70}$ = 72.63
3.78	−1.34	0.94	−1.38	−2.56	−0.68	−0.65		0.93	0.44	$F_{1,69}$ = 11.38
6.64	−1.44	0.95	−1.39	−2.59	−0.87	−0.75	−0.62	0.94	0.40	$F_{1,68}$ = 17.53

[a] F statistic for the significance of the addition of each variable.

Table IV. Squared Correlation Coefficient between the Variables Used in Equation 3

	log P	$W_r3(Y)$	$T_l(Y)$	$I_{br}(X_1)$	$I_{OR}(X_2)$
$W_r3(Y)$	0.07				
$T_l(Y)$	0.02	0.32			
$I_{br}(X_1)$	0.00	0.00	0.01		
$I_{OR}(X_2)$	0.00	0.01	0.04	0.02	
I_{NMe}	0.02	0.00	0.00	0.00	0.00

specific $W_r\beta$ were significant at all for bulkier X, although the two steric parameters $D(Y)$ and $T_l(Y)$ were significant for smaller Y.

We prepared N-methyl derivatives of triazines as we did the anilides. They were of three types: compound **T45** methylated at the X site, compounds **T40**, **T41**, and **T42** methylated at the Y site, and doubly methylated **T46**. Surprisingly, all of them had about the same degree less activity than expected for the corresponding nonmethylated compounds. Moreover, the cyclic pyrrolidinyl (**T43**) and piperidinyl (**T44**) derivatives showed a similar degree of less activity than calculated by eq 4. Thus we

$$pI_{50} = \underset{(0.19)}{0.61} \log P - \underset{(0.48)}{0.96} \log (\beta 10^{\log P} + 1) - \underset{(0.14)}{0.24} W_r\beta(X) - \underset{(0.15)}{0.20} D(Y) - \underset{(0.32)}{0.51} T_l(Y) - \underset{(0.35)}{2.81} I_{NMe} + \underset{(1.66)}{8.44} \quad (5)$$

$$n = 47,\ s = 0.41,\ r = 0.94,\ F_{6,40} = 51.5,\ \log \beta = -4.31,\ \log P_o = 4.55$$

formulated eq 5, adopting the indicator variable I_{NMe} that takes one for these compounds and zero for others. The coefficient of the I_{NMe} term indicates that the activity of the N-methylated compounds is about $^1/_{600}$th that of the nonmethylated counterparts on the average. The optimum log P value was calculated to be 4.6, overlapping with that of eq 3 for anilides, 5.4. The development of eq 5 and the squared correlation matrix of the variables used are shown in Tables V and VI, respectively.

DISCUSSION

In eq 3 for anilides, only I_{br} was significant for the various meta substituents (X_1) with a negative sign. For the larger (X) of the two amine substituents of triazines, the only significant term in eq 5 was $W_r\beta$, which also had a negative sign. This suggests that they correspond with respect to the steric interaction at a common active site. Bulkiness caused by branching at the lower part of the substituents was unfavorable for the activity; the region of the receptor surface where the higher part of both substituents comes was probably flat or deserted. The para substituents (X_2) of anilides gave a different effect; those larger or bulkier than propoxy weakened the activity uniformly, as shown by the indicator variable $I_{OR}(X_2)$ in eq 3. This anomaly is hard to explain, but such substituents would be crowded out of the receptor cavity. We gave the value of one to the I_{OR} of p-ethoxy **A56** in the regression, and the deviation of its observed pI_{50} from the calculated one was positive and rather large. Thus, the ethoxy substituent appears to be borderline.

The activity of anilides soon decreases with the increasing bulkiness of the acyl moiety. This is reflected in eq 3 by $T_l(Y)$ and $W_r3(Y)$ terms, which have negative signs. A similar trend was also observed for the smaller amine substituent (Y) of triazines being reflected by $T_l(Y)$ and $D(Y)$. Both Y substituents were probably cramped, the narrowness of the upward and downward directions being similar, as indicated by the similar coefficient values of the T_l terms in eq 3 and 5. If one supposes common room for both Y moieties, the anilide Y may bump against the wall in the W_r3 direction and triazine Y against the wall in the D direction. This difference in accommodation may be brought about by the differences in the structure of the rest of the molecule.

In triazines, all three kinds of N-methylation (those at X, Y, and both sites) caused a similar degree of decrease in activity, as indicated by the I_{NR} term in eq 5. This may mean that the effect is caused by prevention of the active conformation or the reduction of conformational flexibility for fitting to the receptor rather than by the blocking of presumably site-specific H-bonding interaction with the receptor via the amino hydrogen atom. The effect is probably a conformational one in anilides as well, the coefficient value of I_{NMe} in eq 3 coinciding with that in eq 5 for triazines.

To check the interpretation made above, we prepared a triazine compound **T48** having a non-hydrogen donating OEt instead of $N(Me)_2$. Its activity could not be explained by eq 5 with I_{NMe} = 1, as it was 40 times higher than the calculated activity. This finding suggests that the weakened activity of the corresponding $N(Me)_2$ compound **T40** is not due to its lack of hydrogen-donating ability. On the other side, the potency of compound **T48** was 10–20 times less than the activity calculated by eq 5 with I_{NMe} = 0 on the assumption that the oxygen atom of OEt is equivalent to the nitrogen atom of the corresponding NHEt with respect to inhibition of the PS II electron flow. To examine the effect of the atom at α to the triazine ring, we prepared compound **T49** with n-Pr at the Y position. The activity was about 300 times less than that predicted by eq 5 with

Table V. Development of Equation 5

const	I_{NMe}	log P	log ($\beta 10^{\log P}$ + 1)	$W_r\beta(X)$	$T_l(Y)$	$D(Y)$	r	s	$F_{x,y}$[a]
6.69	−2.51						0.83	0.61	$F_{1,45}$ = 100.00
4.83	−2.72	0.59	−1.08				0.90	0.49	$F_{2,43}$ = 13.30
5.34	−2.82	0.61	−1.19	−0.21			0.91	0.46	$F_{1,42}$ = 6.98
7.32	−2.84	0.62	−1.12	−0.23	−0.50		0.93	0.42	$F_{1,41}$ = 8.86
8.44	−2.81	0.61	−0.96	−0.25	−0.51	−0.20	0.94	0.39	$F_{1,40}$ = 7.18

[a] F statistic for the significance of the addition of each variable.

Table VI. Squared Correlation Coefficient between the Variables Used in Equation 5

	log P	$W_r\beta$(Y)	D(Y)	T_l(Y)
$W_r\beta$(Y)	0.02			
D(Y)	0.12	0.02		
T_l(Y)	0.04	0.02	0.01	
I_{NMe}	0.09	0.04	0.03	0.00

I_{NMe} = 0. Accordingly, some electronic interaction may be at work at this specific position, with the order of N > O >> C for the effects on activity. In line with this, we prepared compound **A77** in the anilide series, in which the amide nitrogen atom of **A27** was replaced with oxygen. Its activity was $^1/_{1000}$th of what was predicted (Table I). This may mean that the oxygen atom in **A77** has a different effect from the oxygen atom in Y moiety of triazine **T48**. On the basis of the results presented above, we drew a receptor map for anilides and triazines (Figure 3) that was an improvement of the previous one (Mitsutake et al., 1986). The stippled lines represent the steric interaction sites or receptor walls located on the plane of the page, and the striped circle expresses that located upward (or downward). The affixes show the corresponding parameters, the superscript A indicating those incorporated in eq 3 for anilides and the superscript T those of triazines in eq 5. The modes of the interaction of urea and carbamate herbicides with the mode of anilides corresponds closely (Mitsutake et al., 1986), so the map represents the overlapping features of triazines with these anilide-type compounds as well.

Our QSAR results and the receptor map should be of help in examination of the roles of amino acid residues that constitute the herbicide-binding niche in PS II, if its three-dimensional features are well-elucidated on the basis of analysis of amino acid sequences of the constituting peptides (Trebst, 1986, and references therein). In the *Rhodopseudomonas viridis* system, the accommodation of the triazine herbicide terbutryn [2-(methylthio)-4-(ethylamino)-6-(*tert*-butylamino)-*s*-triazine] in the niche and the amino acids involved in the binding have been identified by X-ray structure analysis (Michel et al., 1986). Based on the overall analogy of the bacterial and plant system, the region to which the larger amine substituent (X) is directed seems to be hollow, but the alloy into which the smaller one (Y) enters appears to be blind. These observations coincide with the QSAR results that the site of action is rather tolerant to bulkiness of the X moiety while the steric demands for Y substituents are strict. A hydrogen bond may exist between the amino hydrogen of the Y moiety and the oxygen atom of a serine residue located nearby. If the situation is the same in plant systems as well, the effect of N-methylation discussed above may be primarily indirect prevention of bond formation by conformational distortion, rather than by direct blockage of the bonding. The electrostatic interaction that operates at this nitrogen site may be with the arginine side chain located close to the heteroaromatic ring.

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Registry No. **A1**, 709-98-8; **A2**, 882-14-4; **A3**, 74064-59-8; **A4**, 2150-96-1; **A5**, 7017-11-0; **A6**, 2533-89-3; **A7**, 115892-40-5; **A8**, 7287-36-7; **A9**, 2307-68-8; **A10**, 2150-97-2; **A11**, 25251-70-1; **A12**, 15907-85-4; **A13**, 115892-41-6; **A14**, 86886-77-3; **A15**, 51988-04-6; **A16**, 102587-29-1; **A17**, 3022-71-7; **A18**, 102587-30-4; **A19**, 102587-32-6; **A20**, 102587-33-7; **A21**, 115892-42-7; **A22**, 102587-34-8; **A23**, 102587-35-9; **A24**, 102587-36-0; **A25**, 102587-37-1; **A26**, 2790-16-1; **A27**, 115892-43-8; **A28**, 71218-41-2; **A29**, 71219-00-6; **A30**, 115892-44-9; **A31**, 115892-45-0; **A32**, 115892-46-1; **A33**, 621-06-7; **A34**, 5215-27-0; **A35**, 115892-47-2; **A36**, 2990-06-9; **A37**, 6876-65-9; **A38**, 27816-82-6; **A39**, 10264-18-3; **A40**, 55791-89-4; **A41**, 115892-48-3; **A42**, 115892-49-4; **A43**, 115892-50-7; **A44**, 115892-51-8; **A45**, 115892-52-9; **A46**, 115892-53-0; **A47**, 115892-54-1; **A48**, 115892-55-2; **A49**, 115892-56-3; **A50**, 115892-57-4; **A51**, 115892-58-5; **A52**, 115892-59-6; **A53**, 115892-60-9; **A54**, 115892-61-0; **A55**, 73644-87-8; **A56**, 88552-40-3; **A57**, 115892-62-1; **A58**, 115892-63-2; **A59**, 115892-64-3; **A60**, 115892-65-4; **A61**, 115892-66-5; **A62**, 115892-67-6; **A63**, 115892-68-7; **A64**, 115892-69-8; **A65**, 115892-70-1; **A66**, 115892-71-2; **A67**, 115892-72-3; **A68**, 115892-73-4; **A69**, 115892-74-5; **A70**, 115892-75-6; **A71**, 115892-76-7; **A72**, 53916-15-7; **A73**, 2150-96-1; **A74**, 115960-24-2; **A75**, 115960-25-3; **A76**, 71219-00-6; **A77**, 115892-77-8; **T1**, 122-34-9; **T2**, 90952-64-0; **T3**, 49624-63-7; **T4**, 102587-51-9; **T5**, 102587-52-0; **T6**, 102587-57-5; **T7**, 115892-78-9; **T8**, 1824-11-9; **T9**, 115892-79-0; **T10**, 1912-24-9; **T11**, 74150-96-2; **T12**, 102587-55-3; **T13**, 102587-58-6; **T14**, 22936-85-2; **T15**, 102587-50-8; **T16**, 84712-73-2; **T17**, 84712-77-6; **T18**, 40533-52-6; **T19**, 102587-54-2; **T20**, 102587-53-1; **T21**, 115892-80-3; **T22**, 102587-56-4; **T23**, 115892-81-4; **T24**, 115892-82-5; **T25**, 102587-62-2; **T26**, 102587-59-7; **T27**, 116004-73-0; **T28**, 102587-60-0; **T29**, 102587-61-1; **T30**, 115892-83-6; **T31**, 115892-84-7; **T32**, 115892-85-8; **T33**, 115892-86-9; **T34**, 115892-87-0; **T35**, 115892-88-1; **T36**, 115892-89-2; **T37**, 15468-86-7; **T38**, 139-40-2; **T39**, 85196-54-9; **T40**, 110231-79-3; **T41**, 115892-90-5; **T42**, 110231-78-2; **T43**, 115892-91-6; **T44**, 110231-80-6; **T45**, 115892-92-7; **T46**, 100544-24-9; **T47**, 111535-30-9; **T48**, 115892-93-8; **T49**, 115892-94-9; *m*-(3-phenyl-*n*-propyl)phenol chloride, 115892-95-0; 3-phenyl-1-propylamine, 2038-57-5; 2,6-dichloro-4-ethoxy-*s*-triazine, 18343-30-1; 2,6-dichloro-4-*n*-propyl-*s*-triazine, 30894-73-6.

Supplementary Material Available: Table of analytical data for anilide and triazine compounds in this study (3 pages). Ordering information is given on any current masthead page.

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Production of Valinomycin, an Insecticidal Antibiotic, by *Streptomyces griseus* var. *flexipertum* var. *nov.*†

Rod M. Heisey,*[1] Jamin Huang,[2] Saroj K. Mishra,[3] James E. Keller, James R. Miller, Alan R. Putnam, and Themistocles D. J. D'Silva[2]

A screening program to discover microorganisms that produce novel pesticides has yielded a new streptomycete strain that produces valinomycin, an insecticidal and acaricidal antibiotic. Bioassays of the crude culture broth produced by this strain demonstrated an LC_{50} to mosquito larvae of 10^{-3}–10^{-4} dilution. Bioassays of the purified insecticide yielded LC_{50} values of 2–3 pm for mosquito larvae, 3 ppm for two-spotted spider mites, and 35 ppm for Mexican bean beetle larvae. Taxonomic studies indicated the valinomycin-producing microorganism was an atypical variant of *Streptomyces griseus*, which is hereby named var. *flexipertum* var. *nov.* Morphology and physiology of the new microorganism and production, isolation, and identification of the insecticidal metabolite are described.

Much interest currently exists in discovering metabolites of microorganisms that have potential for use as pesticides and plant growth regulators (American Chemical Society, 1987). We have been testing soil microorganisms for the production of such compounds (Heisey et al., 1985, 1988; Heisey and Putnam, 1986; Mishra et al., 1987a,b, 1988; Huang et al., 1988). One isolate, an atypical strain of *Streptomyces griseus*, produced culture broth that was strongly active against mosquito larvae. Chemical analyses revealed the presence of valinomycin, an insecticidal antibiotic (Figure 1). Valinomycin has previously been reported as a product of *Streptomyces fulvissimus* and a similar strain (Brockman and Schmidt-Kastner, 1955; Brown et al, 1962) and *Streptomyces roseochromogenes* (Patterson and Wright, 1970). It has been considered for insecticidal, nematocidal, and acaricidal use (Patterson and Wright, 1970; Pansa et al., 1973). Valinomycin has not heretofore been reported from *S. griseus* strains. This paper describes a new valinomycin-producing variant of *S. griseus* and the isolation, identification, and characterization of the insecticidal metabolite.

Pesticide Research Center, Michigan State University, East Lansing, Michigan 48824 (R.M.H., S.K.M., J.E.K., J.R.M., A.R.P.), and Union Carbide Agricultural Products Company, Box 12014, T. W. Alexander Drive, Research Triangle Park, North Carolina 27709 (J.H., T.D.J.D.).

[1] Present address: Department of Biological Sciences, Fordham University, Bronx, NY 10458.

[2] Present address: Rhone-Poulenc Ag Co., P.O. Box 12014, T. W. Alexander Dr., Research Triangle Park, NC 27709.

[3] Present address: Krug International, Suite 120, 1290 Hercules Dr., Houston, TX 77058.

† The nucleic acid sequence in this paper has been submitted to In Vitro International under Accession Numbers 10129 and 10130.

MATERIALS AND METHODS

Isolation of Microorganism. The valinomycin-producing strain was isolated from surface soil collected in 1982 in Ingham County, Michigan, near a manure pile in an outdoor cattle feeding area. The soil was mixed with calcium carbonate (1 g:1 g) and incubated 7–10 days at room temperature in a sterile Petri dish containing water-saturated filter paper above the mixture to maintain high humidity (El-Nakeeb and Lechevalier, 1963). Serial dilutions were plated onto arginine–glycerol–nutrient salts agar (El-Nakeeb and Lechevalier, 1963) and incubated at 28 °C. Actinomycete colonies that developed were transferred to other plates and used to inoculate liquid cultures for tests of insecticidal activity. The valinomycin-producing strain was distinguished on the basis of the potent insecticidal activity it produced in shaken broth culture. It is deposited with In Vitro International (611 P Hammondsferry Road, Linthicum, MD; accessions 10129, 10130).

Taxonomy of Microorganism. Growth of *S. griseus* var. *flexipertum* var. *nov.* was tested on glycerol–casitone (GC) agar (glycerol, 70 mL; Bacto casitone, 5 g; Bacto agar, 15 g; distilled water, 1 L; pH 7.0) and yeast extract–malt extract–glucose (YMG) agar (Mishra et al., 1980; Mishra and Gordon, 1986). Species identification was according to Mishra et al. (1980) and Mishra and Gordon (1986).

Spore chain morphology and spore surface texture were examined with scanning electron microscopy (Kutzner, 1982). The occurrence of diaminopimelic acid isomers was determined by paper chromatography according to Becker et al. (1964).

Culturing for Insecticide Production. The producer microorganism was grown in A-9 medium (Bacto peptone, 5 g; glucose, 10 g; Brer Rabbit green label molasses, 20 g) (Warren et al., 1955). Antifoam-A (Sigma Chemical Co.,

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