

Photosynthetic Electron Transport Inhibition by 2-Substituted 4-Alkyl-6-benzylamino-1,3,5-triazines with Thylakoids from Wild-Type and Atrazine-Resistant *Chenopodium album*

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The effect of 2-benzylamino-1,3,5-triazines on photosynthetic electron transport (PET) was measured with thylakoids isolated from atrazine-resistant, wild-type *Chenopodium album*, and spinach to find novel 1,3,5-triazine herbicides bearing a strong PET inhibition. The PET inhibition assay with *Chenopodium* (wild-type and resistant), yielded a resistance ratio ($R/W = I_{50}(\text{resistant})/I_{50}(\text{wild-type})$) of 324 for atrazine while for benzylamino-1,3,5-triazine derivatives of diamino-1,3,5-triazines a R/W of 11 to 160 was found. The compounds having a benzylamino group at one of the amino groups in the diamino-1,3,5-triazines have a resistant ratio down to one half to 1/30 of the atrazine value. The average resistance ratio of 21 benzylamino derivatives of monoamino-1,3,5-triazines was found to be about 4.0. The inhibition of 21 benzylamino-1,3,5-triazines assayed with atrazine-resistant *Chenopodium* thylakoids, indicated by $pI_{50}(R)$ -values, correlated well with the PET inhibition $pI_{50}(W)$ of wild-type thylakoids from *Chenopodium*.

Introduction

The triazine herbicides like atrazine, simetryne and simazine, were introduced in the early 1950s and contributed strongly to the general practice of chemical weed control (Esser *et al.*, 1975). Nowadays, this type of herbicides including atrazine has been phased out for several reasons, such as appearance of herbicide-resistant weeds and environmental pollution. Kuboyama *et al.* (1998) have recently found novel 2-alkyl-4-benzylamino-6-trifluoromethyl-1,3,5-triazines, e. g. 2-(4-bromobenzylamino)-4-methyl-6-trifluoromethyl-1,3,5-triazine, which exhibit strong photosynthetic electron transport (PET) inhibition with thylakoids from spinach and atrazine-resistant *Chenopodium al-*

bum (Kuboyama *et al.*, 1998, 1999; Ohki *et al.*, 1999).

In this paper, PET inhibitory activities of a number of novel 1,3,5-triazines have been assayed using thylakoids isolated from both atrazine-resistant and wild-type of *Chenopodium album*, and compared with conventional diamino-1,3,5-triazine herbicides, such as atrazine and simazine.

Materials and Methods

Chemicals

All reaction products were purified through column chromatography and/or recrystallization, and their structures were confirmed by IR-, ¹H-NMR- and mass spectroscopy. Melting points (uncorrected) were measured with a Yanagimoto-Seisakusyo melting point apparatus. IR-spectra were recorded on a JASCO FT/IR-420 spectrophotometer and ¹H-NMR spectra were measured in CDCl₃ on a JEOL JNM-GX400 spectrometer at

Abbreviations: PET, photosynthetic electron transport; R, atrazine-resistant *Chenopodium album*; W, wild-type *Chenopodium album*; pI_{50} , the logarithm of the reciprocal I_{50} ; PSII, Photosystem II; R/W, the ratio of the molar concentration producing 50% (I_{50}) inhibition with resistant thylakoids over the wild-type ones.

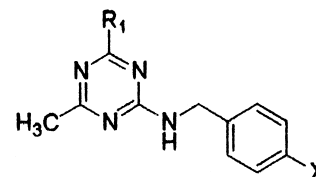
400 MHz using tetramethylsilane (TMS) as an internal standard.

The 1,3,5-triazines tested, excluding atrazine and simazine, were synthesized by a nucleophilic substitution reaction of corresponding chloro-1,3,5-triazines or trichloromethyl-1,3,5-triazines with the appropriate amines according to our previous paper (Kuboyama *et al.*, 1998). The physical and/or spectroscopical data of known benzylamino-1,3,5-triazines, i.e. **8**, **12**, **14**, **26**, **27**, **28** & **29** (Kuboyama *et al.*, 1998) ; **9** (Ursprung, 1966) and **13**, **22**, **23**, **24** & **25** (Inoue *et al.*, 2000) used in this study were reported elsewhere. For spectroscopical data of newly prepared benzylamino-1,3,5-triazines, see Table I. Typical procedures of the nucleophilic substitution reaction are given below.

*Synthesis of 2-chloro-4-(4-chlorobenzylamino)-6-methyl-1,3,5-triazine (cpd. **10**)*

4-Chlorobenzylamine (4.3 g, 0.03 mol) was reacted with 2,4-dichloro-6-methyl-1,3,5-triazine (4.7 g, 0.03 mol), prepared from cyanuric chloride and CH_3MgBr according to Hirt *et al.* (1950), in the presence of triethylamine (3.0 g, 0.03 mol) in tetrahydrofuran (120 ml). After stirring at room temperature for 22 h, the mixture was concentrated under reduced pressure. The residue was washed with water and then recrystallized from *n*-hexane to give 2-chloro-4-(4-chlorobenzylamino)-6-methyl-1,3,5-triazine as a white solid, mp 127–129 °C, yield 4.2 g (66.7%). IR λ_{MAX} (KBr) cm^{-1} : 1531, 1568 (1,3,5-triazine ring). NMR δ_{H} (CDCl_3) ppm : 2.39 and 2.46 (3H in total, each s, CH_3), 4.63 and 4.66 (2H in total, each d, $J = 6.1$ Hz, NHCH_2), 6.05 and 6.34 (1H in total, each br, NH),

Table I. Physical data of new amino-1,3,5-triazines synthesized for this study.



No.	R ₁	X	mp (°C)	IR λ_{MAX} (KBr) cm^{-1}	NMR δ_{H} (CDCl_3 , TMS) ppm
15	C ₂ H ₅	Cl	120–122	1532	1.26 (3H, t, $J = 7.6$ Hz, CH_2CH_3), 2.38 and 2.44 (3H in total, each s, CH_3), 2.62 and 2.68 (2H in total, each q, $J = 7.6$ Hz, CH_2CH_3), 4.64 (2H, d, $J = 6.1$ Hz, NHCH_2), 5.70 and 5.76 (1H, each br, NH), 7.26 (2H, d, $J = 8.5$ Hz, H-2 and H-6 of C ₆ H ₄), 7.30 (2H, d, $J = 8.5$ Hz, H-3 and H-5 of C ₆ H ₄)
17	C ₃ H _{7-n}	Cl	117–118	1537, 1550	0.96 (3H, t, $J = 7.3$ Hz, $\text{CH}_2\text{CH}_2\text{CH}_3$), 1.77 (2H, m, $\text{CH}_2\text{CH}_2\text{CH}_3$), 2.38 and 2.44 (3H in total, each s, CH_3), 2.56 and 2.62 (2H in total, each t, $J = 7.1$ Hz, $\text{CH}_2\text{CH}_2\text{CH}_3$), 4.64 (2H, d, $J = 6.1$ Hz, NHCH_2), 5.69 and 5.76 (1H in total, each br, NH), 7.25 (2H, d, $J = 8.5$ Hz, H-2 and H-6 of C ₆ H ₄), 7.30 (2H, d, $J = 8.5$ Hz, H-3 and H-5 of C ₆ H ₄)
18	C ₃ H _{7-i}	H	60–63	1518, 1548	1.26 (6H, d, $J = 6.6$ Hz, $\text{CH}(\text{CH}_3)_2$), 2.38 and 2.44 (3H in total, each s, CH_3), 2.84 (1H, m, $\text{CH}(\text{CH}_3)_2$), 4.66 (2H, s, NHCH_2), 5.58 and 5.67 (1H in total, each br, NH), 7.30 (5H, m, C ₆ H ₅)
19	C ₃ H _{7-i}	Cl	104–105	1556	1.24 (6H, d, $J = 7.1$ Hz, $\text{CH}(\text{CH}_3)_2$), 2.38 and 2.44 (3H in total, each s, CH_3), 2.86 (1H, m, $\text{CH}(\text{CH}_3)_2$), 4.63 (2H in total, d, $J = 6.1$ Hz, CH_2), 5.61 and 5.74 (1H in total, each br, NH), 7.26 (2H, d, $J = 8.5$ Hz, H-2 and H-6 of C ₆ H ₄), 7.30 (2H, d, $J = 8.5$ Hz, H-3 and H-5 of C ₆ H ₄)
20	C ₄ H _{9-t}	H	85–86	1522, 1555	1.30 (9H, s, $\text{C}(\text{CH}_3)_3$), 2.39 and 2.45 (3H in total, each s, CH_3), 4.66 (2H in total, d, $J = 5.6$ Hz, NHCH_2), 5.50 and 5.65 (1H in total, each br, NH), 7.31 (5H, m, C ₆ H ₅)
30	CCl ₃	Cl	137–139	1539, 1568	2.53 and 2.59 (3H in total, each s, CH_3), 4.68 and 4.69 (2H in total, each d, $J = 6.1$ Hz and $J = 5.6$ Hz, NHCH_2), 6.00 and 6.05 (1H in total, each br, NH), 7.31 (4H, m, C ₆ H ₄)

7.24 and 7.25 (2H in total, each d, $J = 8.3$ Hz, H-2 and H-6 of the benzene ring), 7.31 and 7.32 (2H in total, each d, $J = 8.3$ Hz, H-3 and H-5 of the benzene ring). MS: m/z 268 (M^+). Intensities of isotope peaks of the compounds relative to M^+ -peak for Cl_2 are found; Calcd. (found): M^+ , 100% (100%); $M^+ + 2$, 65 (66) and $M^+ + 4$, 11 (8). Isotope abundances were calculated according to $^{35}Cl:^{37}Cl = 100.0 : 32.5$.

Synthesis of 2-(4-chlorobenzylamino)-4-methoxy-6-methyl-1,3,5-triazine (cpd. 11)

To a solution of sodium methoxide (0.04 g, 1.74 mmol) in 30 ml of methanol, 2-chloro-4-(4-chlorobenzylamino)-6-methyl-1,3,5-triazine (0.50 g, 1.86 mmol) in 20 ml of methanol was added at 0 °C. After stirring at room temperature for 20 h, the reaction mixture was concentrated under reduced pressure. The residue was washed with water and then recrystallized from *n*-hexane to give 2-(4-chlorobenzylamino)-4-methoxy-6-methyl-1,3,5-triazine as colorless crystal, mp 144–147 °C, yield 0.33 g (77.5%). IR λ_{MAX} (KBr) cm^{-1} : 1553, 1573 (triazine ring). NMR δ_H ($CDCl_3$) ppm: 2.32 and 2.40 (3H in total, each s, CH_3), 3.90 and 3.93 (3H in total, each s, OCH_3), 4.62 and 4.65 (2H in total, each d, $J = 6.1$ Hz and $J = 5.9$ Hz, $NHCH_2$), 5.93 and 6.22 (1H in total, each br, NH), 7.24 (2H, d, $J = 8.3$ Hz, H-2 and H-6 of the benzene ring), 7.30 (2H, d, $J = 8.3$ Hz, H-3 and H-5 of the benzene ring). MS: m/z 264 (M^+).

Synthesis of 2-(tert-butyl)-4-(4-chlorobenzylamino)-6-methyl-1,3,5-triazine (cpd. 21)

4-Chlorobenzylamine (1.2 g, 8.5 mmol) was reacted with 2-(4-*tert*-butyl)-4-methyl-6-trichloromethyl-1,3,5-triazine (2.0 g, 7.5 mmol) in tetrahydrofuran (10 ml). After stirring at room temperature for 45 h, the mixture was concentrated under reduced pressure. The residue was washed with water and then purified by silica gel column chromatography using ethyl acetate : dichloromethane = 1:10 (v/v) to give the 2-(4-chlorobenzylamino)-4-(*tert*-butyl)-6-methyl-1,3,5-triazine as colorless crystal, mp 79–81 °C, yield 0.8 g (36.7%). IR λ_{MAX} (KBr) cm^{-1} : 1530, 1555 (triazine ring). NMR δ_H ($CDCl_3$) ppm: 1.28 (9H, s, C (CH_3)₃), 2.40 and 2.44 (3H in total, each s, CH_3), 4.62 (2H, d, $J =$

6.1 Hz, $NHCH_2$), 5.50 and 5.63 (1H in total, each br, NH), 7.28 (4H, m, benzene ring). MS: m/z 290 (M^+).

Preparation of spinach thylakoids

Thylakoids were prepared from spinach (*Spinacia oleracea*) leaves according to the method of Böger (1993). After removal of the midribs the leaves were homogenized in a cooking mixer using a medium containing 0.4 M sucrose, 50 mM Tricine (*N*-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]-glycine) (pH 8.0), 10 mM NaCl and 5 mM $MgCl_2$. The homogenate was filtered through eight layers of cheese cloth and centrifuged for 1 min at $4000 \times g$. The pellet was resuspended in the same medium for determination of PET inhibitory activity.

Thylakoid preparation of atrazine-resistant and wild-type Chenopodium album

For details of the isolation of thylakoids see van Rensen *et al.* (1977). Leaves from atrazine-resistant and wild-type *Chenopodium* were homogenized by a glass pestle using an isolation medium containing 0.4 M sorbitol, 20 mM Tricine-NaOH (pH 7.8), 10 mM NaCl, 5 mM $MgCl_2$, 2 mM sodium ascorbate and 2 mg/ml bovine serum albumin. After squeezing the homogenate through eight layers of cheese cloth the chloroplasts were collected by centrifugation for 30 sec at $500 \times g$ and 8 min $1000 \times g$, washed once in 50 mM sodium phosphate buffer (pH 7.8) to obtain broken chloroplasts, and finally collected by centrifugation during 8 min at $1000 \times g$. The chlorophyll content was measured according to Bruinsma (1963), the chlorophyll concentration adjusted to 2 mg Chl/ml, and the thylakoids stored at -80 °C.

Determination of PET-inhibitory activity with the oxygen electrode

Photosynthetic electron transport activity was measured according to van Rensen *et al.* (1977; 1978). Oxygen evolution was measured at 25 °C at saturating white light with a Gilson oxygraph provided with a Clark oxygen electrode. For measurement of photosystem (PS) II-dependent electron flow the isolated thylakoids were suspended in 2 ml reaction medium containing 0.3 M sorbitol, 50 mM Tricine-NaOH (pH 7.6), 5 mM $MgCl_2$, 5 mM NH_4Cl , 1 mM potassium ferricyanide and thyla-

koids including 50 µg chlorophyll. The inhibition is expressed as pI₅₀ values, the negative logarithms of the molar concentration at which the compound produced a 50% inhibition.

Results and Discussion

Synthesis of benzylamino-1,3,5-triazines

The benzylamino-1,3,5-triazines assayed were readily synthesized by the nucleophilic amination reaction of 2,4-dichloro-6-methyl-1,3,5-triazine (compounds **9** and **10** in Table III, yield 67–93%) or 2,4-dialkyl-6-trichloromethyl-1,3,5-triazines (compounds **12–30**, yield 45–90%). 2-(4-Chlorobenzylamino)-4-methyl-6-methoxy-1,3,5-triazine (**11**) was prepared by methoxylation of 2-chloro-4-(4-chlorobenzylamino)-6-methyl-1,3,5-triazine (**10**) with sodium methoxide. The ¹H-NMR spectra of 2-substituted-4-alkyl-6-benzylamino-1,3,5-triazines showed a set of resonance for the particular protons. For example, compound **10** exhibited two signals at δ 2.39 and 2.46 for the 2-methyl protons, two signals at δ 4.63 and 4.66 for the CH₂–C₆H₄Cl-4 and two signals at δ 6.05 and 6.34 for the NH proton. This finding in ¹H-NMR spectra of monoamino-1,3,5-triazines was already observed

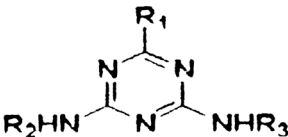
in our previous papers (Kuboyama *et al.*, 1998; 1999; Inoue *et al.*, 2000; Okano *et al.*, 1993). The π-electron on the 1,3,5-triazine ring and the lone electron pair at the (benzyl)amino nitrogen are considered to conjugate each other to form a sort of molecular orbital, rendering the bond between C-6 and nitrogen a kind of partial double bond character. Accordingly, several sets of two resonances in ¹H-NMR spectra can be observed due to a sort of syn-anti isomerism of the C-N bond occurring only in the magnetic field.

PET inhibition of diamino-1,3,5-triazines with Chenopodium thylakoids

In a preliminary study, we examined the PET inhibitory activities of several diamino-1,3,5-triazines with thylakoids from both atrazine-resistant and wild-type *Chenopodium album* as well as from spinach. Results are shown in Table II.

The reference compounds, atrazine and simazine, exhibited strong PET inhibition (pI₅₀ > 6.00) with thylakoids from wild-type of *Chenopodium album* and spinach with a R/W-ratio of more than 300, but only weak inhibition (pI₅₀ about 4) with thylakoids from atrazine-resistant *Chenopodium*.

Table II. PET inhibition by diamino-1,3,5-triazines with spinach and *Chenopodium* thylakoids.



Compounds					<i>Chenopodium album</i>			Spinach
No.	R ₁	R ₂	X	mp [°C]	Wild-type pI ₅₀ (W)	Resistant-type pI ₅₀ (R)	R / W	pI ₅₀ (Sp)
Atrazine	Cl	C ₂ H ₅	C ₃ H _{7-i}	175–177	6.72	4.21	324	6.73
Simazine	Cl	C ₂ H ₅	C ₂ H ₅	225–227	6.60	4.10	316	6.28
1* ¹⁾	CF ₃	C ₂ H ₅	C ₃ H _{7-i}	107–108	6.74	4.18	363	5.99
2* ²⁾	CF ₃	C ₂ H ₅	CH ₂ C ₆ H ₅	109–111	6.94	4.72	166	7.14
3* ²⁾	CF ₃	C ₃ H _{7-i}	CH ₂ C ₆ H ₅	oil	6.80	4.59	162	6.51
4* ³⁾	Cl	C ₂ H ₅	CH ₂ C ₆ H ₅	185–189	6.94	5.70	17.3	6.62
5* ³⁾	Cl	C ₃ H _{7-i}	CH ₂ C ₆ H ₅	121–123	6.36	5.26	12.6	6.35
6* ⁴⁾	CH ₃	C ₂ H ₅	CH ₂ C ₆ H ₅	152–154	5.33	4.06	18.6	4.57
7* ⁴⁾	CH ₃	C ₃ H _{7-i}	CH ₂ C ₆ H ₅	98–100	5.05	4.00	11.2	4.71

*¹⁾ Compound **1** was prepared from 2,4-bis(trichloromethyl)-6-trifluoromethyl-1,3,5-triazine according to Tsunoda *et al.* (1977).
*²⁾ Compounds **2** and **3** were synthesized via amination of 2-benzylamino-4-trichloromethyl-6-trifluoromethyl-1,3,5-triazine.
*³⁾ Omokawa and Konnai (1990) have reported 7.09 and 6.66 as pI₅₀ (Sp)-values for compounds **4** and **5**, respectively.
*⁴⁾ Compounds **6** and **7** were prepared from 2-benzylamino-4-trichloromethyl-6-trifluoromethyl-1,3,5-triazine.

In compound **1**, the chlorine atom of atrazine is replaced by a CF₃ group. It also exhibited strong PET inhibition (pI_{50} (W) = 6.74) with wild-type thylakoids, but only weak inhibition with the resistant ones (pI_{50} (R) = 4.18) with the R/W still around 300. Compounds **2** and **3**, in which a benzylamino group was introduced for one amino group of compound **1**, showed a R/W ratio decreased to ca. 160, but kept a strong PET inhibitory activity (pI_{50} (W) > 6.80) with the wild-type thylakoids. Replacement of one N-alkyl of atrazine or simazine by a benzylamino group decreased the R/W-ratio remarkably to 12–17, keeping, however, a strong PET inhibition with the wild-type thylakoids (see compounds **4** and **5** in Table II). Compounds **6** and **7** with the electron-donating CH₃-group as the R₁ substituent also exhibited a lower R/W-ratio (11–18) but PET inhibi-

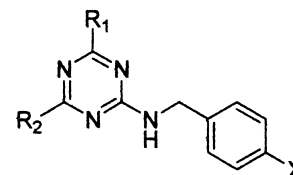
tion by these compounds was too weak to be discussed further. By these findings it can be concluded, that the 1,3,5-triazines with a benzylamino group bring about strong PET inhibition with the atrazine-resistant thylakoids of *Chenopodium*, resulting in a decreased R/W-ratio.

PET inhibition of benzylamino-1,3,5-triazines with Chenopodium thylakoids

Since introduction of a benzylamino group to the 1,3,5-triazine ring appeared to be useful for molecular design of strong PET inhibitors in atrazine-resistant *Chenopodium*, a number of benzylamino-1,3,5-triazines were synthesized and evaluated for PET inhibitory activities (Table III).

The resistance ratios of atrazine and simazine, conventional diamino-1,3,5-triazine herbicides,

Table III. PET inhibition by benzylamino-1,3,5-triazines with *Chenopodium* and spinach thylakoids.



No.	R ₁	R ₂	X	mp [°C]	<i>Chenopodium album</i>			Spinach
					Wild-type pI_{50} (W)	Resistant-type pI_{50} (R)	R / W	pI_{50} (Sp)
8	H	CH ₃	H	74–75	< 4.00	< 4.00	–	< 4.00
9	Cl	CH ₃	H	98–100	5.24	4.38	7.3	5.33
10	Cl	CH ₃	Cl	127–129	6.65	5.80	7.1	6.97
11	OCH ₃	CH ₃	Cl	149–151	6.28	5.21	11.7	6.09
12	CH ₃	CH ₃	H	113–114	5.32	5.34	1.0	4.19
13	CH ₃	CH ₃	Cl	133–134	5.47	4.75	5.2	5.78
14	C ₂ H ₅	CH ₃	H	71–73	5.25	5.13	1.3	5.16
15	C ₂ H ₅	CH ₃	Cl	120–122	6.64	6.44	1.3	6.45
16	C ₃ H _{7-n}	CH ₃	H	47–49	5.38	5.07	2.0	4.81
17	C ₃ H _{7-n}	CH ₃	Cl	117–118	7.22	6.52	5.0	7.05
18	C ₃ H _{7-i}	CH ₃	H	60–63	6.05	5.60	2.9	5.65
19	C ₃ H _{7-i}	CH ₃	Cl	104–105	7.30	6.92	2.4	7.31
20	C ₄ H _{9-t}	CH ₃	H	85–86	6.55	5.85	5.2	5.99
21	C ₄ H _{9-t}	CH ₃	Cl	79–81	7.13	6.57	3.6	6.86
22	CH ₂ F	CH ₃	H	83–84	4.43	< 4.00	–	4.98
23	CH ₂ F	CH ₃	Cl	110–112	5.64	4.80	7.0	6.15
24	CHF ₂	CH ₃	H	62–63	5.21	4.82	2.5	5.63
25	CHF ₂	CH ₃	Cl	90–91	6.18	5.82	2.3	6.62
26	CF ₃	CH ₃	H	57–59	7.03	6.61	2.6	6.85
27	CF ₃	CH ₃	Cl	74–76	7.19	6.93	1.8	6.98
28	CF ₃	CH ₃	Br	99–100	7.34	7.43	0.8	6.94
29	CF ₃	C ₂ H ₅	H	44–46	5.86	5.40	2.9	5.64
30	CCl ₃	CH ₃	Cl	137–139	7.08	6.55	3.4	7.26
Atrazine	–	–	–	175–177	6.72	4.21	324	6.73

The pI_{50} (Sp) – values of compounds **8**, **12**, **14**, **16** and **22–29**, and pI_{50} (R) and pI_{50} (W) – values of compounds **22–28** were reported briefly in refs. Kuboyama *et al.* (1998; 1999), Inoue *et al.* (2000) and Kohno *et al.* (2000).

were 324 and 316, respectively (Table II). The mean resistance ratio of the 21 benzylamino-1,3,5-triazines was calculated to be about 4.0, indicative of almost no cross-resistance between atrazine and the benzylamino-1,3,5-triazines assayed (Table III). The R/W-ratios show, however, a range of deviation (0.8–11.7) for individual benzylamino-1,3,5-triazines. The ratios were 1.0–11.7 for the benzylamino-1,3,5-triazines bearing electron-donating groups at R₁, i.e. compounds (**11–21**), and were found 0.8–7.3 for the benzylamino-1,3,5-triazines with electron-withdrawing groups at R₁, like compounds (**9**, **10**, **22–30**). The benzylamino-1,3,5-triazines having a CF₃-group at R₁, namely compounds **26**, **27** and **28**, were very active with both their pI₅₀(R) and pI₅₀(W)-values over 6.60. This finding may corroborate our previous observation (Kuboyama *et al.*, 1998) that 2-benzylamino-4-methyl-6-trifluoromethyl-1,3,5-triazines are not only active against atrazine-resistant weeds, but also herbicidal for normal weeds. Introduction of halogen (Cl or Br) at 4-position of the benzene ring resulted in a 2–3 fold increase of PET inhibition, a finding also confirming our results from pot tests (Kuboyama *et al.*, 1998).

PET inhibition of 21 benzylamino-1,3,5-triazines (**9–21**, **23–30**) with atrazine-resistant *Chenopodium* thylakoids, indicated by pI₅₀ (R)-values, correlated well with their PET inhibition (pI₅₀ (W)) in thylakoids of wild-type of *Chenopodium*, as shown by the regression line and equation of Fig. 1A. Furthermore, the PET inhibition (pI₅₀ (W)) of these benzylamino-1,3,5-triazines with wild-type *Chenopodium* thylakoids correlated fairly well with their PET inhibitory activities (pI₅₀ (Sp)) in spinach thylakoids, as shown by the equation of Fig. 1B. Using the pI₅₀ (Sp)-value of newly designed benzylamino-1,3,5-triazines and equations of Fig. 1, A, B, we can now predict the pI₅₀ (W) and pI₅₀ (R)-values of the compounds.

According to the inhibition assay of this study, conventional diamino-type 1,3,5-triazines, such as atrazine and simazine, exhibited weak PET inhibitory activities with thylakoids from atrazine-resistant *Chenopodium*, but benzylamino-1,3,5-triazines revealed a strong PET inhibition with the resistant thylakoids. In atrazine-resistant *Chenopodium* (Bettini *et al.*, 1987), the serine residue no. 264, a constituent amino acid of D1-protein of photosystem II, is mutated to glycine, thus atrazine cannot bind to the D1-protein (Hirschberg *et al.*, 1984). 2-Benzylamino-4-methyl-6-trifluoromethyl-1,3,5-triazines are assumed to bind at the same niche of the D1-protein as atrazine, but in-

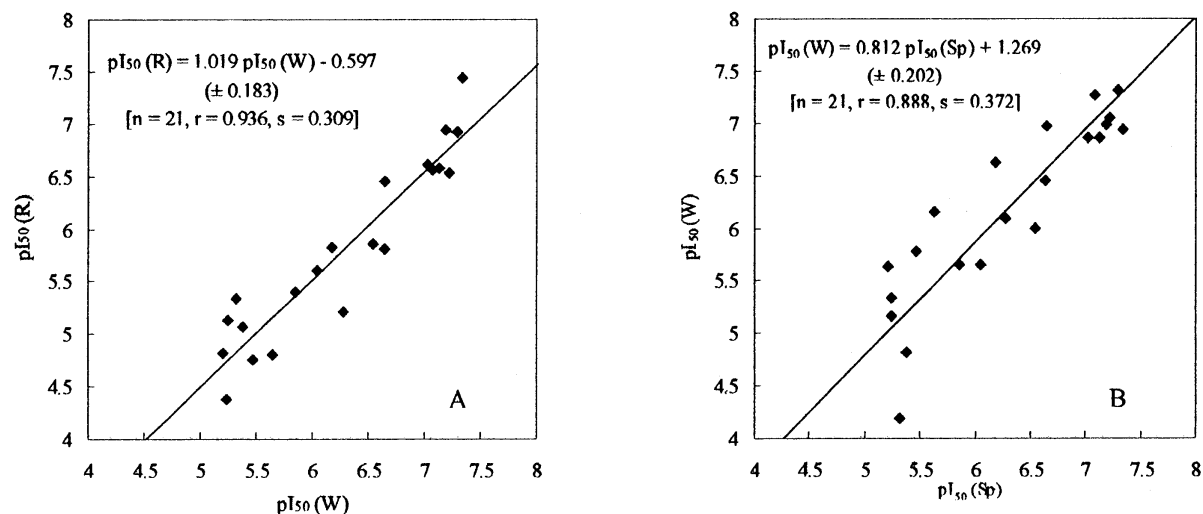


Fig. 1. Correlation of PET inhibitory activities of 21 benzylamino-1,3,5-triazines with *Chenopodium* and spinach thylakoids. (A): pI₅₀ (W) of wild-type vs. pI₅₀(R) of resistant *Chenopodium*, (B): pI₅₀ (Sp) of spinach vs. pI₅₀ (W) wild-type *Chenopodium*.

teract with different amino acid residues, since the mutation of Ser 264 to glycine has no effect on binding of the novel triazines. To study the accurate binding niche of the benzylamino-1,3,5-triazines, replacement assays according to Ohki *et al.* (1999) using both [^{14}C]-atrazine and [^{14}C]-2-benzylamino-4-methyl-6-trifluoromethyl-1,3,5-triazine are now under way with resistant- and wild-type *Chenopodium*.

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- Bettini P., McNally S., Seignac M., Darmency H., Gasquez J. and Dron M. (1987), Atrazine resistance in *Chenopodium album* – low and high levels of resistance to the herbicide are related to the same chloroplast psbA gene for mutation. *Plant Physiol.* **84**, 1442–1446.
- Böger P. (1993), Inhibition of the photosynthetic electron transport system. In: *Target Assays for Modern Herbicides and Related Phytotoxic Compounds* (P. Böger and G. Sandmann, eds.). Lewis Publ., Boca Raton, FL, pp. 83–91.
- Bruinsma J. (1963), The quantitative analysis of chlorophylls a and b in plant extracts. *Photochem. Photobiol.* **2**, 214–249.
- Esser H. O., Dupuis G., Ebert E., Marco G. and Vogel C. (1975), s-Triazines. In: *Herbicides, Chemistry, Degradation, and Mode of Action* (P. C. Kearney and D. D. Kaufman, eds.). Vol. **1**, Marcel Dekker Inc., New York, pp. 129–208.
- Hirschberg J., Bleeker A., Kyle D. J., McIntosh L. and Arntzen C. J. (1984), The molecular basis of triazine herbicide resistance in higher-plant chloroplasts. *Z. Naturforsch.* **39c**, 412–420.
- Hirt R., Nidecker H. and Berchtold R. (1950), Syntheses with cyanuryl chloride. *Helv. Chim. Acta.* **33**, 1365–1369.
- Inoue H., Ohki S., Kotaka E., Kuboyama N., Ohki A., Koizumi K., Kohno H., Böger P. and Wakabayashi K. (2000), Photosynthetic electron transport inhibitory and herbicidal activities of 2-(fluorinatedmethyl)-4-benzylamino-6-methyl-1,3,5-triazines. *J. Pestic. Sci.* **25**, 101–106.
- Kohno H., Ohki A., Ohki S., Koizumi K., van den Noort M. E., Rodrigues G. C., van Rensen J. J. S. and Wakabayashi K. (2000), Low resistance against novel 2-benzylamino-1,3,5-triazine herbicides in atrazine-resistant *Chenopodium album* plants. *Photosynth. Res.* **65**, 115–120.
- Kuboyama N., Koizumi K., Ohki S. and Wakabayashi K. (1998), Synthesis and herbicidal activity of 2-benzylamino-4-methyl-6-trifluoromethyl-1,3,5-triazine derivatives. *J. Pestic. Sci.* **23**, 268–274.
- Kuboyama N., Koizumi K., Ohki A., Ohki S., Kohno H. and Wakabayashi K. (1999), Photosynthetic electron transport inhibitory activity of 2-arylamino-4-methyl-6-trifluoro-methyl-1,3,5-triazine derivatives. *J. Pestic. Sci.* **24**, 138–142.
- Ohki A., Kuboyama N., Koizumi K., Tanaka A., Sato Y., Kohno H., Böger P. and Wakabayashi K. (1999), Mode of action of novel 2-(benzylamino)-4-methyl-6-(trifluoromethyl)-1,3,5-triazine herbicides: inhibition photosynthetic electron transport and binding studies. *J. Agric. Food. Chem.* **47** (10), 4398–4402.
- Okano N., Murakami M., Miyamoto Y., Koizumi K., Ogawa H. and Wakabayashi K. (1993), Synthesis and structure activity relationship of newer trichloromethyl-1,3,5-triazine nitrification inhibitors. *J. Pestic. Sci.* **18**, 361–368.
- Omokawa H. and Konnai M. (1990), PSII inhibitory activity of 2,4-diamino-6-chloro-s-triazines with a chiral *sec*-butyl and/or α -methylbenzyl group. *Agric. Biol. Chem.* **54**, 2373–2378.
- Tsunoda M., Tobe A., Omata K. and Shirasaka T. (Mitsubishi Chem. Ind., Ltd.) (1977), *Jpn. Kokai Tokkyo Koho JP 52–83582*.
- Ursprung J. J. (Upjohn Company) (1966), U. S. Patent 3270015.
- van Rensen J. J. S., van der Vet W. and van Vliet W. P. A. (1977), Inhibition and uncoupling of electron transport in isolated chloroplasts by the herbicide 4,6-dinitro-o-cresol. *Photochem. Photobiol.* **25**, 579–583.
- van Rensen J. J. S., Wong D. and Govindjee (1978), Characterization of the inhibition of photosynthetic electron transport in pea chloroplasts by the herbicide 4,6-dinitro-o-cresol by comparative studies with 3-(3,4-dichlorophenyl)-1,1-dimethylurea. *Z. Naturforsch.* **33c**, 413–420.