Synthesis and Photosynthesis-Inhibiting Activity of Some Anilides of Substituted Pyrazine-2-carboxylic Acids

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Alkylation of pyrazine-2-carboxylic acid and 6-chloropyrazine-2-carboxylic acid with radicals generated by silver nitrate-catalyzed oxidative decarboxylation of pivalic acid afforded 5-(1,1-dimethylethyl)pyrazine-2-carboxylic acid and 6-chloro-5-(1,1-dimethylethyl)pyrazine-2-carboxylic acid. Condensation of chlorides of the latter three halogenated and/or alkylated pyrazine-2-carboxylic acids with ring-substituted anilines yielded a series of anilides of 6-chloropyrazine-2-carboxylic, 5-(1,1-dimethylethyl)pyrazine-2-carboxylic or 6-chloro-5-(1,1-dimethylethyl)pyrazine-2-carboxylic acids. Some of these anilides showed a moderate inhibitory effect upon the oxygen evolution rate in spinach chloroplasts. The most active inhibitor was 5-chloro-2-hydroxyanilide of 6-chloropyrazine-2-carboxylic acid (IC_{50} = 0.008 mmol dm^{-3}). The introduction of chlorine in the pyrazine moiety led to an increased photosynthesis-inhibiting activity.

Earlier studies [1—4] have described synthesis and tuberculostatic activity of several anilides of unsubstituted pyrazine-2-carboxylic acid. Halogenated anilides of pyrazine-2-carboxylic acid were evaluated for their anthelmintic activity [5]. Many herbicides acting as photosynthesis inhibitors have in their molecules an >N—(C=X)— grouping (X = O or N, not S) and a hydrophobic residue in close vicinity [6, 7]. We have recently reported [8] the synthesis and the photosynthesis-inhibiting effect [9] of a series of some anilides of 2-alkylpyridine-4-carboxylic acids. Using ESR spectroscopy, it was confirmed that the anilides inhibit photosynthetic electron transport in spinach chloroplasts and the site of their inhibitory action is the intermediate Z^+/D^+, *i.e.* tyrosine radicals, which are located in D_1 and D_2 proteins on the donor side of photosystem 2 [10].

In pursuing our research on the synthesis of pyrazine derivatives, we wish to describe here synthesis of some anilides of substituted pyrazine-2-carboxylic acids, *i.e.* the compounds with a similar grouping to that mentioned above. The aim of this work is (i) alkylation and/or halogenation of the pyrazine ring, (ii) synthesis of some anilides, (iii) to study the inhibitory effects of the anilides upon the oxygen evolution rate in spinach chloroplasts, and (iv) to determine the structure—activity relationships.

EXPERIMENTAL

Melting points were determined on a Kofler apparatus. Purity of intermediates and products was checked by TLC on Silufol UV 254 plates (Kavalier, Votice) using toluene—acetone (φ_r = 1 : 1) or light petroleum—ethyl acetate (φ_r = 1 : 1). Samples for elemental analysis were vacuum-dried at about 100 Pa over phosphorus pentoxide at room temperature. Elemental analyses were obtained using an EA 1110 CE instrument (Fisons Instruments S.p.A., Milan). The IR spectra were recorded on a Nicolet Impact 400 spectrometer in KBr pellets. The ^1H NMR spectra were measured in $(CD_3)_2SO$ solutions (unless otherwise stated) with tetramethylsilane as internal standard using a BS 494 (Tesla, Brno) 100 MHz apparatus. Hydrophobicity of compounds was computed using the program ACD/Log *P* version 1.0 (Advanced Chemistry Development Inc., Toronto).

5-(1,1-Dimethylethyl)pyrazine-2-carboxylic Acid (*III*) and 6-Chloro-5-(1,1-dimethylethyl)-pyrazine-2-carboxylic Acid (*IV*)

Method A. Silver nitrate (1.7 g; 0.01 mol) and pivalic acid (10.2 g; 0.1 mol) were added to a stirred solution of 0.1 mol of pyrazine-2-carboxylic acid (*I*,

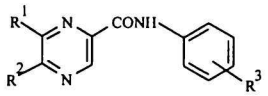
12.4 g) [12] or 6-chloropyrazine-2-carboxylic acid [11] (*II*, 13.9 g) in water (300 cm^3) at 80 °C. Then a solution of ammonium peroxydisulfate (25 g; 0.11 mol) in water (70 cm^3) was added dropwise and the reaction mixture was stirred for 1 h at 80 °C. After cooling, the mixture was made alkaline by sodium hydroxide (5 %) and continuously extracted with chloroform. The extract was washed with water, dried over anhydrous sodium sulfate, filtered and the solvent evaporated at reduced pressure. The residue was recrystallized from aqueous ethanol.

Method B. 5-(1,1-Dimethylethyl)pyrazine-2-carboxamide [13] (1.8 g; 0.01 mol) or 6-chloro-5-(1,1-dimethylethyl)pyrazine-2-carboxamide [14] (2.1 g; 0.01 mol) was refluxed with 10 % aqueous solution of sodium hydroxide (20 cm^3; 0.05 mol) until the evolution of ammonia ceased. The resulting sodium salt was collected, dissolved in hot water, filtered and the filtrate was acidified with 10 % hydrochloric acid to pH 4—5. The crude product was collected, washed with water, and recrystallized from water. The yields and analytical data are given in Table 1, the IR and ^{1}H NMR spectra are given in Table 2.

Anilides *V—XVI*

A mixture of acid *II*, *III*, *IV* (0.05 mol) and thionyl chloride (5.5 cm^3; 75 mmol) in 20 cm^3 of dry benzene was refluxed for about 1 h. Excess of thionyl chloride was removed by repeated evaporation with dry benzene *in vacuo*. The crude acyl chloride dissolved in 50 cm^3 of dry acetone was added dropwise to a stirred solution of the corresponding aminophenol (50 mmol) in 50 cm^3 of dry pyridine keeping the temperature at 10 °C. After the addition was complete, stirring at 10 °C was continued for another 30 min. The reaction mixture was then poured into 200 cm^3 of cold water and the crude anilide was collected and recrystallized from aqueous ethanol. The yields and analytical data are given in Table 1, the IR and ^{1}H NMR parameters are given in Table 2.

Table 1. Characteristics of Compounds *III—XVI*



V—XVI

Comp.	R^1	R^2	R^3	Formula M_r	w_i(calc.)/% w_i(found)/%				M.p./°C Yield/%
					C	H	N	Cl	
III	–	–	–	$C_9H_{12}N_2O_2$	60.00	6.71	15.55	–	119—120
				180.1	59.98	6.83	15.35	–	80[a], 86[b]
IV	–	–	–	$C_9H_{11}ClN_2O_2$	50.36	5.17	13.05	16.59	176—177
				214.7	50.61	5.12	13.31	16.25	51[a], 81[b]
V	Cl	H	2-OH	$C_{11}H_7ClN_3O_2$	53.14	2.84	16.90	14.26	237—238
				248.6	52.94	2.95	16.62	14.37	63
VI	Cl	H	3-OH	$C_{11}H_7ClN_3O_2$	53.14	2.84	16.90	14.26	212—213
				248.6	53.08	2.92	17.03	14.28	58
VII	Cl	H	4-OH	$C_{11}H_7ClN_3O_2$	53.14	2.84	16.90	14.26	195—196
				248.6	52.98	3.02	17.05	14.48	54
VIII	Cl	H	2-OH, 5-Cl	$C_{11}H_6Cl_2N_3O_2$	46.67	2.14	14.84	25.05	258—260
				283.1	46.73	2.25	14.94	24.88	74
IX	H	$(CH_3)_3C$	2-OH	$C_{15}H_{17}N_3O_2$	66.40	6.32	15.49	–	221—222
				271.3	66.38	6.53	15.61	–	33
X	H	$(CH_3)_3C$	3-OH	$C_{15}H_{17}N_3O_2$	66.40	6.32	15.49	–	173—174
				271.3	66.52	6.39	15.40	–	38
XI	H	$(CH_3)_3C$	4-OH	$C_{15}H_{17}N_3O_2$	66.40	6.32	15.49	–	162—163
				271.3	66.50	6.60	15.20	–	41
XII	H	$(CH_3)_3C$	2-OH, 5-Cl	$C_{15}H_{16}ClN_3O_2$	58.98	5.27	13.74	11.59	166—167
				305.8	59.14	5.44	13.51	11.68	43
XIII	Cl	$(CH_3)_3C$	2-OH	$C_{15}H_{16}ClN_3O_2$	58.92	5.27	13.74	11.59	231—232
				305.8	59.17	5.32	13.66	11.77	45
XIV	Cl	$(CH_3)_3C$	3-OH	$C_{15}H_{16}ClN_3O_2$	58.92	5.27	13.74	11.59	185—187
				305.8	59.09	5.37	13.71	11.77	38
XV	Cl	$(CH_3)_3C$	4-OH	$C_{15}H_{16}ClN_3O_2$	58.92	5.27	13.74	11.59	198—199
				305.8	58.81	5.65	13.70	11.50	32
XVI	Cl	$(CH_3)_3C$	2-OH, 5-Cl	$C_{15}H_{15}Cl_2N_3O_2$	52.96	4.44	12.35	20.84	267—269
				340.2	53.01	4.35	11.95	21.03	27

a) Method *A*; *b*) method *B*.

Table 2. IR and ^{1}H NMR Parameters of Compounds *III*—*XVI*

Comp.	IR	^{1}H NMR, δ										
	$\tilde{\nu}$(ν(C=O))	H-3	H-5	H-6	H-2	H-3	H-4	H-5	H-6	OH	NH	CH_3
	cm^{-1}	pyrazine			benzene							
III	1740	9.05, d J = 1.2 Hz	–	8.82, d J = 1.2 Hz	–	–	–	–	–	–	–	1.32
IV	1680	9.01, s	–	–	–	–	–	–	–	–	–	1.40
V[a]	1660	9.11	9.28	–	–		6.85—7.06, m, 3H		8.28, dd J =7.5 Hz, J = 1.2 Hz	10.38	9.98	–
VI	1660	9.06, d J = 0.5 Hz	9.22, d J = 0.5 Hz	–	7.42, m	–	6.58, m	7.15, m	7.25, m	9.52	10.48	–
VII	1660	9.03	9.21	–	7.64, d J = 8.8 Hz	6.78, d J = 8.8 Hz	–	6.78, d J = 8.8 Hz	7.64, d J = 8.8 Hz	10.40	9.35	–
VIII	1660	9.13	9.29	–	–	6.97, d J = 8.5 Hz	7.08, dd J = 2.5 Hz, J = 8.5 Hz	–	8.33, d J = 2.5 Hz	10.77	9.97	–
IX	1660	9.25, d J = 1.5 Hz	–	8.93, d J = 1.5 Hz	–		6.80—7.10, m, 3H		8.36, d J = 8.0 Hz	10.32	10.21	1.42
X[a]	1670	9.43, d J = 1.5 Hz	–	8.63, d J = 1.5 Hz	7.99, m	–		6.70—7.30, m, 3H		n.o.	9.74	1.46
XI	1670	9.19, d J = 1.2 Hz	–	8.84, d J = 1.2 Hz	7.67, d J = 8.8 Hz	6.79, d J = 8.8 Hz	–	6.79, d J = 8.8 Hz	7.67, d J = 8.8 Hz	10.38	5.9	1.42
XII[a]	1640	9.14	–	8.78	-	7.42, m	6.72, m	–	8.16, m	9.38	9.89	1.39
XIII	1670	9.19	–	–	–		6.85—7.05, m, 3H		8.29, d J = 8.0 Hz	10.36	9.92	1.52
XIV[a]	1690	9.27	–	–	7.89, m	–		6.70—7.30, m, 3H		9.28	9.44	1.56
XV	1660	9.11	–	–	7.61, d J = 8.8 Hz	6.76, d J = 8.8 Hz	–	6.76, d J = 8.8 Hz	7.61, d J = 8.8 Hz	9.38	10.31	1.52
XVI[a]	1660	9.26	–	–	–	7.43, m	7.02—7.12, m	–	7.02—7.12, m	9.39	9.67	1.56

a) In $CDCl_3$; n.o. – signal not observed.

Table 3. Photosynthesis-Inhibiting Activity (IC_{50}[a]) and Lipophilicity (log P[a]) of Compounds *V*—*XVI* in Comparison with Standard (Atrazine)

Compound	IC_{50} / mmol dm^{-3}	log P
V	0.066	1.89 ± 0.42
VI	2.288	1.90 ± 0.42
VII	3.322	1.51 ± 0.41
VIII	0.008	3.27 ± 0.44
IX	0.205	2.46 ± 0.41
X	0.431	2.47 ± 0.41
XI	0.314	2.08 ± 0.41
XII	0.465	3.84 ± 0.43
XIII	0.435	3.58 ± 0.43
XIV	0.262	3.59 ± 0.42
XV	0.043	3.20 ± 0.42
XVI	0.105	4.96 ± 0.45
Atrazine	0.001 [16]	1.03 ± 0.62

a) See Experimental.

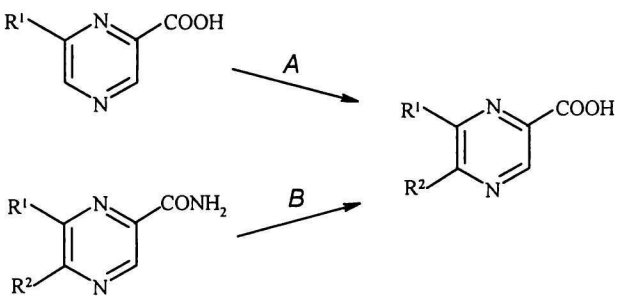
Measurement of Oxygen Evolution Rate in Spinach Chloroplasts

The oxygen evolution rate in spinach chloroplasts was investigated spectrophotometrically (Specord UV VIS, Zeiss, Jena) in the presence of an electron acceptor 2,6-dichlorophenol—indophenol by the method described in Ref. [15]. The compounds were dissolved in dimethyl sulfoxide (DMSO) because of their low water solubility. The used DMSO volume fractions (up to 5 vol. %) did not affect the oxygen evolution. The inhibitory efficiency of the studied compounds has been expressed by IC_{50} values, *i.e.* by molar concentration of the compounds causing 50 % decrease in the oxygen evolution relative to the untreated control. The results are summarized in Table 3.

RESULTS AND DISCUSSION

The starting 5-(1,1-dimethylethyl)pyrazine-2-carboxylic acid (*III*) and 6-chloro-5-(1,1-dimethylethyl)-pyrazine-2-carboxylic acid (*IV*) were prepared by two methods (Scheme 1). Homolytic alkylation (method *A*) of pyrazine-2-carboxylic acid (*I*) [11] or 6-chloropyrazine-2-carboxylic acid (*II*) [12] with radicals generated by silver nitrate-catalyzed oxidative decarboxylation of pivalic acid afforded *III* and *IV*. Alkaline hydrolysis (method *B*) of 5-(1,1-dimethylethyl)pyrazine-2-carboxamide [13] or 6-chloro-5-(1,1-dimethylethyl)-pyrazine-2-carboxamide [14] gave the same products.

On treatment with thionyl chloride at reflux, acids *II*—*IV* gave acyl chlorides which were in turn converted into the title anilides (*V*—*XVI*, Table 1) by reaction with substituted aminophenols at 10 °C. Keeping the low temperature was essential in order to avoid the partial esterification of acyl chlorides with aminophenols. The structure of compounds was confirmed by elemental analysis, IR, and ^1H NMR spectra. The hydrophobicity (log P) of compounds was computed (Table 3).



	R^1	R^2
I	H	H
II	Cl	H
III	H	$C(CH_3)_3$
IV	Cl	$C(CH_3)_3$

Scheme 1

The photosynthesis-inhibiting activity (Table 3) of the anilides was determined by measuring their inhibitory effects upon the oxygen evolution rate in spinach chloroplasts system [15]. The lowest inhibitory activity (IC_{50} = 3.322 mmol dm^{-3}) and the lowest lipophilicity (log P = 1.51 ± 0.44) was shown for compound *VII* (R^1 = Cl, R^2 = H, R^3 = 4-OH). A pronounced increase in inhibitory activity was found with the more lipophilic compound *XI* with R^1 = H, R^2 = $(CH_3)_3C$, R^3 = 4-OH (IC_{50} = 0.314 mmol dm^{-3}, log P = 2.08 ± 0.41). The comparable compound *XV* (R^1 = Cl, R^2 = $(CH_3)_3C$, R^3 = 4-OH) exhibits a high activity and lipophilicity (IC_{50} = 0.043 mmol dm^{-3}, log P = 3.20 ± 0.42). The most active inhibitor was 5-chloro-2-hydroxyanilide of 6-chloropyrazine-2-carboxylic acid (*VIII*, $IC_{50} = 8.0 \times 10^{-3}$ mmol dm^{-3}, log P = 3.27 ± 0.44). Comparable IC_{50} value for a selective herbicide Atrazine [16] is about 1.0×10^{-3} mmol dm^{-3}. In general, it can be concluded that the introduction of chlorine to the pyrazine moiety (*V*, *VIII*, *XV*, *XVI*) leads to an increase in biological activity.

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