SYNTHESIS OF SOME ANILIDES OF 2-ALKYL-4-PYRIDINECARBOXYLIC ACIDS AND THEIR PHOTOSYNTHESIS-INHIBITING ACTIVITY

Miroslav MILETIN[a1], Jiri HARTL[a2] and Milos MACHACEK[b]

[a] *Department of Medicinal Chemistry and Drug Control, Faculty of Pharmacy, Charles University, 500 05 Hradec Kralove, Czech Republic; e-mail: [1] miletin@faf.cuni.cz, [2] hartl@faf.cuni.cz*

[b] *Department of Inorganic and Organic Chemistry, Faculty of Pharmacy, Charles University, 500 05 Hradec Kralove, Czech Republic; e-mail: machacek@faf.cuni.cz*

Received August 15, 1996
Accepted December 27, 1996

Homolytic alkylation of 4-pyridinecarbonitrile with radicals generated by silver nitrate catalyzed oxidative decarboxylation of alkanoic acids and subsequent alkaline hydrolysis afforded 2-alkyl-4-pyridinecarboxylic acids **2a–2e**. These were converted into the halogeno-hydroxy substituted anilides **3a–3r** *via* acyl chlorides by reaction with the respective aminophenols at 10 °C. Of the compounds tested, 5-chloro-2-hydroxyphenylamide of 2-butyl-4-pyridinecarboxylic acid **3h** showed the highest effect upon oxygen evolution rate in spinach chloroplasts system. The structure–photosynthesis-inhibiting activity relationships are briefly discussed.

Key words: Homolytic alkylation; Pyridinecarboxylic acids; Photosynthesis inhibition.

Studies of relationships between chemical structure and biological activity have shown that many herbicides acting as photosynthesis inhibitors possess in their molecules an >N–C(=X)– group (X = O or N, not S) and a hydrophobic residue in close vicinity to it[1,2]. Shipman[3,4] concluded that the hydrophilic part of a herbicide binds electrostatically to the terminus of an α-helix at a highly charged amino acid, whereby the hydrophobic part of the inhibitor extends into the hydrophobic part of the membrane. Recently, pronounced photosynthesis-inhibiting activity has been found for alkoxy substituted phenylcarbamates[5,6], as well as for the local anaesthetic of anilide type – trimecaine[7–9], *i.e.*, for compounds with a –CONH– group in their molecules.

The present paper deals with the study of (di)halogeno-hydroxy substituted anilides of 2-alkyl-4-pyridinecarboxylic acids **3a–3r** as photosynthesis inhibitors. Starting 2-alkyl-4-pyridinecarbonitriles **1a–1e** were synthesized by homolytic alkylation of 4-pyridinecarbonitrile with radicals generated by oxidative decarboxylation of alkanoic acids, catalyzed with silver nitrate, according to Wang *et al.*[10,11]. Alkaline hydrolysis of 2-alkyl-4-pyridinecarbonitriles **1a–1e** afforded 2-alkyl-4-pyridinecarboxylic acids **2a–2e** in 81–86% yield. On treatment with thionyl chloride at reflux, the acids gave acyl chlorides which were in turn converted into the title anilides **3a–3r** (Scheme 1, Table I) by reaction with 2-amino-5-methylphenol, 2-amino-4-chlorophenol, 4-amino-2-chlorophenol, 4-amino-

TABLE I
Characteristic data of 2-alkylpyridine-4-carboxanilides **3a–3r**

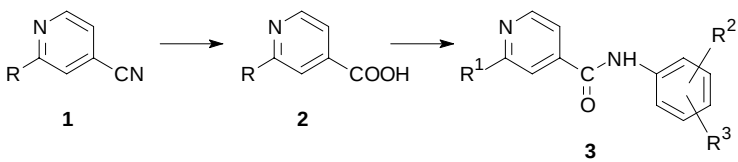
Compound	Formula M.w.	M.p.,°C Yield, %	Calculated/Found				$\tilde{\nu}$(C–H)	$\tilde{\nu}$(C=O)
			% C	% H	% N	% Cl[a]		
3a	$C_{15}H_{15}ClN_2O_2$	191–193	61.94	5.20	9.67	12.19	2 980, 2 940	1 640
	290.8	35	62.08	5.27	9.45	11.99	2 880	
3b	$C_{15}H_{14}Cl_2N_2O_2$	181–183	55.40	4.34	8.61	21.80	2 990	1 660
	325.2	58	55.50	4.39	8.57	21.60		
3c	$C_{16}H_{18}N_2O_2$	141–144	71.09	6.71	10.36	–	2 990	1 640
	270.3	36	71.15	6.79	10.19	–		
3d	$C_{15}H_{15}ClN_2O_2$	169–171	61.94	5.20	9.67	12.19	2 990, 2 970	1 665
	290.8	39	62.07	5.25	9.62	12.05		
3e	$C_{15}H_{14}Cl_2N_2O_2$	182–183	55.40	4.34	8.61	21.80	2 995	1 640
	325.2	60	55.14	4.32	8.57	21.65		
3f	$C_{15}H_{14}Br_2N_2O_2$	129–133	43.51	3.41	6.77	38.59	2 980	1 650
	414.1	58	43.65	3.48	6.81	38.32		
3g	$C_{15}H_{14}I_2N_2O_2$	180–182.5	35.46	2.78	5.51	49.95	2 975	1 640
	508.1	58	35.37	2.71	5.42	50.15		
3h	$C_{16}H_{17}ClN_2O_2$	129–130.5	63.06	5.62	9.19	11.63	2 990	1 680
	304.8	45	63.15	5.71	9.08	11.52		
3i	$C_{16}H_{17}ClN_2O_2$	158–160.5	63.06	5.62	9.19	11.63	2 980, 2 940	1 650
	304.8	42	63.12	5.73	9.06	11.51		
3j	$C_{16}H_{16}Cl_2N_2O_2$	185–187	56.65	4.75	8.26	20.90	2 980	1 640
	339.2	58	56.77	4.81	8.14	20.63		
3k	$C_{16}H_{16}Br_2N_2O_2$	134–137	44.89	3.77	6.54	37.33	2 960, 2 930	1 660
	428.1	56	44.78	3.69	6.48	37.45		
3l	$C_{16}H_{16}I_2N_2O_2$	185–186.5	36.81	3.09	5.37	48.61	2 960	1 640
	522.1	62	36.72	2.94	5.22	48.82		
3m	$C_{16}H_{17}ClN_2O_2$	156–158	63.06	5.62	9.19	11.63	2 980	1 640
	304.8	40	63.12	5.67	9.25	11.47		
3n	$C_{16}H_{16}Cl_2N_2O_2$	182–184.5	56.65	4.75	8.26	20.90	2 970	1 645
	339.2	57	56.61	4.85	8.19	20.71		
3o	$C_{16}H_{17}BrN_2O_2$	150–152	55.03	4.91	8.02	22.88	2 970	1 640
	340.2	42	55.15	4.97	7.89	22.70		

TABLE I
(*Continued*)

Compound	Formula M.w.	M.p.,°C Yield, %	Calculated/Found %C	%H	%N	%Cl[a]	$\tilde{\nu}$(C–H)	$\tilde{\nu}$(C=O)
3p	$C_{16}H_{16}Br_2N_2O_2$	133–136	44.89	3.77	6.54	37.33	2 980	1 640
	428.1	63	44.82	3.85	6.51	37.19		
3q	$C_{17}H_{19}ClN_2O_2$	135–137	64.05	6.01	8.79	11.12	2 985, 2 970	1 645
	318.8	38	63.97	5.98	8.77	11.00		
3r	$C_{17}H_{18}Cl_2N_2O_2$	184–186	57.80	5.14	7.93	20.07	2 990, 2 940	1 660
	353.2	61	58.00	5.26	8.19	19.89		

[a] % Br for **3f**, **3k**, **3o**, **3p**; % I for **3g**, **3l.**

2,6-dichlorophenol, 4-amino-2-bromophenol, 4-amino-2,6-dibromophenol, and 4-amino-2,6-diiodophenol at 10 °C. Keeping the low temperature was essential in order to avoid the partial esterification of acyl chlorides with aminophenols. Structure of the products was proven by IR and ^{1}H NMR spectra. In the ^{1}H NMR spectra (Table II) the protons of alkyl groups as well as the protons of aromatic rings were observed at the expected values with expected splitting patterns and intensities.



1, 2	R
a	C_3H_7
b	i-C_3H_7
c	C_4H_9
d	*t*-C_4H_9
e	C_5H_{11}

3	R^1	R^2	R^3
a	C_3H_7	3-Cl	4-OH
b	C_3H_7	3,5-Cl_2	4-OH
c	i-C_3H_7	4-CH_3	2-OH
d	i-C_3H_7	5-Cl	2-OH
e	i-C_3H_7	3,5-Cl_2	4-OH
f	i-C_3H_7	3,5-Br_2	4-OH
g	i-C_3H_7	3,5-I_2	4-OH
h	C_4H_9	5-Cl	2-OH
i	C_4H_9	3-Cl	4-OH

3	R^1	R^2	R^3
j	C_4H_9	3,5-Cl_2	4-OH
k	C_4H_9	3,5-Br_2	4-OH
l	C_4H_9	3,5-I_2	4-OH
m	*t*-C_4H_9	3-Cl	4-OH
n	*t*-C_4H_9	3,5-Cl_2	4-OH
o	*t*-C_4H_9	3-Br	4-OH
p	*t*-C_4H_9	3,5-Br_2	4-OH
q	C_5H_{11}	3-Cl	4-OH
r	C_5H_{11}	3,5-Cl_2	4-OH

SCHEME 1

The photosynthesis-inhibiting activity (Table III) for the chosen anilides was determined by measuring their inhibitory effect upon oxygen evolution rate in spinach chloroplasts system[5]. It was impossible to determine the corresponding IC_{50} values for 3′,5′-dihalogeno-4′-hydroxy derivatives **3b**, **3e**, **3f**, **3g**, **3j**, **3k**, and **3l** due to their too low water solubility. In addition, as seen in comparison of the results obtained with 2-*tert*-butyl and 2-pentyl derivatives **3n**, **3p**, and **3r** with those of 3′-monohalogeno-4′-hydroxy analogues **3m**, **3o**, and **3q**, the introduction of a further halogen into the 5′ position of an aniline moiety was detrimental to the activity in all cases examined. The much lower activity shown by dihalogeno derivatives **3n**, **3p**, and **3r** could be ascribed also to their lower solubility and to their decreased ability to pass through the hydrophilic regions of the thylakoid membranes. In contrast to the 3′-halogeno-4′-hydroxy series, the 5′-chloro-2′-hydroxy derivatives displayed higher photosynthesis-inhibiting activity. Of the anilides tested, the most active compound was the anilide **3h** with an IC_{50} of 31.4 μmol dm^{-3}.

TABLE II
^{1}H NMR chemical shifts of 2-alkylpyridine-4-carboxanilides **3a–3f**, **3h–3r**

Compound	Arom.						Alkyl group(s)		
	H-3	H-5	H-6	H-2′	H-5′	H-6′	CH_2 or CH	other CH_2	CH_3
3a	7.70	7.65	8.66	7.83	6.98	7.51	2.82 t	1.75 m	0.93 t
3b	7.72	7.67	8.68	7.82	–	7.82	2.83 t	1.75 m	0.93 t
3c	7.76	7.67	8.66	–[a]	6.66	7.44	3.13 m	–	1.29 d, 2.25 s
3d	7.75	7.66	8.68	–[b]	–	7.76	3.13 m	–	1.29 d
3e	7.71	7.65	8.69	7.82	–	7.82	3.13 m	–	1.29 d
3f	7.72	7.65	8.69	8.02	–	8.02	3.13 m	–	1.29 d
3h	7.79[c]	7.72	8.69	–[d]	–	7.79[c]	2.87 t	1.72 m, 1.35 m	0.92 t
3i	7.69	7.65	8.66	7.84	6.99	7.51	2.84 t	1.71 m, 1.35 m	0.92 t
3j	7.71	7.66	8.68	7.83	–	7.83	2.84 t	1.71 m, 1.35 m	0.92 t
3k	7.78	7.70	8.71	8.02	–	8.02	2.87 t	1.71 m, 1.35 m	0.92 t
3l	7.71	7.65	8.67	8.22	–	8.22	2.84 t	1.71 m, 1.35 m	0.92 t
3m	7.85[c]	7.65	8.70	7.83[c]	6.99	7.51	–	–	1.37 s
3n	7.82[c]	7.66	8.72	7.82[c]	–	7.82[c]	–	–	1.37 s
3o	7.84[c]	7.65	8.70	7.84[c]	6.98	7.56	–	–	1.37 s
3p	7.85	7.66	8.72	8.01	–	8.01	–	–	1.37 s
3q	7.69	7.64	8.66	7.83	6.98	7.51	2.82 t	1.73 m, 1.33 m[e]	0.88 t
3r	7.70	7.66	8.67	7.82	–	7.82	2.83 t	1.72 m, 1.32 m[e]	0.87 t

[a] 6.77 (H-3′); [b] 6.95 (H-3′), 7.13 (H-4′); [c] overlapping signals; [d] 6.97 (H-3′), 7.14 (H-4′); [e] 4 H.

Turning now to the effect of pyridine nucleus modification, comparison of IC_{50} values of 3′-chloro-4′-hydroxyanilides of 2-alkyl-4-pyridinecarboxylic acids with straight-alkyl chain **3a**, **3i**, and **3q** revealed that increased alkyl chain length caused an enhancement of the activity. This result is expected for good correlation between lipophilicity and photosynthesis-inhibiting activity and has been found previously[5,6,12,13]. The introduction of 2-*tert*-butyl group, as represented by the anilide **3m**, increased activity by factor of 4 relative to **3i**. Neglecting the contribution of branching at the α-carbon, the effect of lipophilicity on photosynthesis inhibition can be illustrated also in 5′-chloro-2′-hydroxy series (compare **3d** with **3h**).

Because the halogeno-hydroxy substituted anilides of 2-alkyl-4-pyridinecarboxylic acids represent a novel structural type of photosynthesis-inhibiting agents, additional work on the structure–activity relationships and the determination of their site of action in photosystem is in progress.

TABLE III
Photosynthesis-inhibiting activity of some 2-alkylpyridine-4-carboxanilides (IC_{50} values from oxygen evolution rate measurement in spinach chloroplasts system)

Compound	**3a**	**3d**	**3h**	**3i**	**3m**	**3n**	**3o**	**3p**	**3q**	**3r**
IC_{50}, μmol dm^{-3}	266.0	78.3	31.4	195.7	50.8	413.5	85.8	207.0	78.1	239.1

EXPERIMENTAL

Melting points were determined on a Boëtius apparatus and are uncorrected. Purity of intermediates and products was checked by TLC on Silufol UV_{254} plates (Kavalier Votice, Czech Republic). Column chromatography was performed on the Silpearl (Kavalier Votice, Czech Republic). Samples for elemental analysis were dried at about 100 Pa over phosphorus pentoxide at room temperature for 6 h. IR spectra ($\tilde{\nu}$, cm^{-1}) were recorded on a Perkin–Elmer model 577 spectrometer in KBr pellets. ^{1}H NMR spectra (δ, ppm; *J*, Hz) were determined for solutions in hexadeuteriodimethyl sulfoxide with tetramethylsilane as the internal standard with a BS 587 (80 MHz) or BS 494 (100 MHz) apparatus (Tesla Brno, Czech Republic).

2-Alkyl-4-pyridinecarbonitriles **1a–1e**

2-Propyl-, 2-isopropyl- and 2-butyl-4-pyridinecarbonitriles (**1a–1c**) were prepared according to Wang *et al.*[10,11]. Crude products were purified by column chromatography using petroleum ether–ethyl acetate (80 : 20) as the eluent. The observed boiling points agreed with those previously described[10,11]. By the same procedure 2-*tert*-butyl-4-pyridinecarbonitrile (**1d**, m.p. 51–53 °C, ref.[14] gives 50–52 °C) and 2-pentyl-4-pyridinecarbonitrile (**1e**, b.p. 134–136 °C/1.06 kPa, ref.[14] gives 116–126 °C/0.93 kPa) were also prepared in 43% and 41% yield, respectively.

2-Alkyl-4-pyridinecarboxylic Acids **2a–2e**

2-Alkyl-4-pyridinecarbonitrile **1a–1e** (60 mmol) in ethanol (10 ml) was mixed with 25% aqueous solution of sodium hydroxide (15 ml, 120 mmol) and refluxed until the evolution of ammonia ceased. The reaction mixture was then diluted with twofold volume of water and acidified with 10% hydrochloric acid to pH 4–5. The crude product was collected, washed with water, and recrystallized from aqueous ethanol. TLC was performed using petroleum ether–ethyl acetate–acetic acid (50 : 45 : 5) as the mobile phase.

2-Propyl-4-pyridinecarboxylic acid (**2a**), m.p. 187–189 °C. Yield 81%. For $C_9H_{11}NO_2$ (165.2) calculated: 65.44% C, 6.71% H, 8.48% N; found: 65.29% C, 6.82% H, 8.27% N. IR spectrum: 2 985, 2 960, 2 895 (C–H); 2 435 (COOH); 1 705 (C=O). ^{1}H NMR spectrum: 0.91 t, 3 H (CH_3); 1.73 m, 2 H (CH_2); 2.83 t, 2 H (CH_2Ar); 7.66 d, 1 H, J = 4.9 (H-5); 7.71 s, 1 H (H-3); 8.69 d, 1 H, J = 4.9 (H-6).

2-Isopropyl-4-pyridinecarboxylic acid (**2b**), m.p. 183–186 °C. Yield 82%. For $C_9H_{11}NO_2$ (165.2) calculated: 65.44% C, 6.71% H, 8.48% N; found: 65.31% C, 6.79% H, 8.26% N. IR spectrum: 2 980, 2 955, 2 890 (C–H); 2 440 (COOH); 1 710 (C=O). ^{1}H NMR spectrum: 1.26 d, 6 H [$(CH_3)_2$]; 3.13 m, 1 H (CH); 7.66 d, 1 H, J = 4.4 (H-5); 7.68 s, 1 H (H-3); 8.67 d, 1 H, J = 4.4 (H-6).

2-Butyl-4-pyridinecarboxylic acid (**2c**), m.p. 179–181 °C (ref.[15] gives m.p. 181 °C). Yield 82%. ^{1}H NMR spectrum: 0.91 t, 3 H (CH_3); 1.31 m and 1.72 m, 2 H (CH_2) each; 2.83 t, 2 H (CH_2Ar); 7.63 d, 1 H, J = 4.9 (H-5); 7.67 s, 1 H (H-3); 8.67 d, 1 H, J = 4.9 (H-6).

2-tert-Butyl-4-pyridinecarboxylic acid (**2d**), m.p. 174–176 °C. Yield 86%. For $C_{10}H_{13}NO_2$ (179.2) calculated: 67.02% C, 7.31% H, 7.82% N; found: 67.15% C, 7.47% H, 7.68% N. IR spectrum: 2 975, 2 890 (C–H); 2 440 (COOH); 1 705 (C=O). ^{1}H NMR spectrum: 1.35 s, 9 H [$(CH_3)_3$]; 7.63 d, 1 H, J = 4.9 (H-5); 7.84 s, 1 H (H-3); 8.71 d, 1 H, J = 4.9 (H-6).

2-Pentyl-4-pyridinecarboxylic acid (**2e**), m.p. 167–169 °C. Yield 86%. For $C_{11}H_{15}NO_2$ (193.2) calculated: 68.37% C, 7.82% H, 7.25% N; found: 68.55% C, 7.91% H, 7.06% N. IR spectrum: 2 980, 2 950, 2 880 (C–H); 2 450 (COOH); 1 750 (C=O). ^{1}H NMR spectrum: 0.93 t, 3 H (CH_3); 1.31 m, 4 H [$(CH_2)_2$]; 1.72 m, 2 H (CH_2); 2.81 t, 2 H (CH_2Ar); 7.63 d, 1 H, J = 4.6 (H-5); 7.66 s, 1 H (H-3); 8.67 d, 1 H, J = 4.6 (H-6).

2-Alkylpyridine-4-carboxanilides **3a–3r**

A mixture of 2-alkyl-4-pyridinecarboxylic acid **2a–2e** (50 mmol) and thionyl chloride (5.5 ml, 75 mmol) in dry benzene (10 ml) 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 ml of dry acetone was added dropwise to a stirred solution of the corresponding aminophenol (50 mmol) in dry pyridine (50 ml) keeping the temperature at 10 °C. After the addition of aminophenol was complete, stirring at 10 °C continued for another 30 min. The reaction mixture was then poured into cold water (200 ml). Crude anilide was collected and recrystallized from aqueous ethanol. TLC was performed in petroleum ether–ethyl acetate (50 : 50) as the mobile phase. The yields, melting points, elemental analyses, and IR spectral data of 2-alkylpyridine-4-carboxanilides **3a–3r** are given in Table I and their ^{1}H NMR chemical shifts in Table II.

Measurement of Oxygen Evolution Rate

The oxygen evolution rate in spinach chloroplasts was investigated spectrophotometrically (Specord UV-VIS, Zeiss Jena, Germany) in the presence of electron acceptor 2,6-dichlorophenolindophenol according to method described in ref.[5]. Due to low water solubility the studied compounds were dissolved in dimethyl sulfoxide (DMSO) so that the applied DMSO concentration (up to 5% v/v) did not affect 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 activity with respect to the untreated control. The results are summarized in Table III.

This study was supported by the Grant No. 204/96 from the Grant Agency of Charles University. The authors thank Dr K. Kralova, Institute of Chemistry, Comenius University, Bratislava, for providing data of the photosynthesis inhibition. They also thank Mrs D. Karlickova (Department of Medicinal Chemistry and Drug Control, Faculty of Pharmacy, Charles University) and Mrs J. Zizkova (Department of Inorganic and Organic Chemistry of the same Faculty) for performing the elemental analyses and recording the IR spectra.

REFERENCES

1. Harth E., Oettmeier W., Trebst A.: FEBS Lett. *43*, 231 (1974).
2. Hauska G. A., Oettmeier W., Reimer S., Trebst A.: Z. Naturforsch., C *30*, 37 (1975).
3. Shipman L. L.: J. Theor. Biol. *90*, 123 (1981).
4. Shipman L. L. in: *Biochemical Responses Induced by Herbicides* (D. E. Moreland, J. B. St. John and F. D. Hess, Eds), p. 23. ACS Symposium Series 181. Washington 1982.
5. Kralova K., Sersen F., Cizmarik J.: Gen. Physiol. Biophys. *11*, 261 (1992).
6. Kralova K., Bujdakova H., Cizmarik J.: Pharmazie *50*, 440 (1995).
7. Sersen F., Kralova K.: Gen. Physiol. Biophys. *13*, 329 (1994).
8. Semin B. K., Chudinovskii M. N., Ivanov I. I.: Biokhimiya *52*, 1279 (1987).
9. Semin B. K., Chudinovskii M. N., Ivanov I. I.: Gen. Physiol. Biophys. *9*, 233 (1989).
10. Wang C. H., Horng J. M., Hwang F. Y. : Bull. Inst. Chem. Acad. Sin. *26*, 67 (1979).
11. Wang C. H., Hwang F. Y., Horng J. M. : Heterocycles *12*, 1191 (1979).
12. Kralova K., Bujdakova H., Loos D., Sidoova E.: Cesk. Farm. *43*, 15 (1994).
13. Kralova K., Bujdakova H., Kuchta T., Loos D.: Pharmazie *49*, 460 (1994).
14. Liebermann D., Rist N., Grumbach F., Cals S., Moyeux M., Rouaix A.: Bull. Soc. Chim. Fr. *52*, 687 (1958).
15. Phelisse J. A., Brossard B., Gruffaz M., Quiby C. (Rhone-Poulenc Chem. Company): Fr. 1.292.234 (1962); Chem. Abstr. *57*, 16572 (1962).