Synthesis, Mechanism of Action, and Herbicidal Activity of New Aryl and Alkyl *N*-(4-Pyridyl)carbamates

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A series of aryl and alkyl *N*-(4-pyridyl)carbamates was synthetized and screened for potential inhibitory activity on the germination of wheat seedlings. Activity was compared with that of isopropyl 3-chlorocarbanilate (chlorpropham). The mechanism of hydrolysis of these carbamates was also investigated and was found to follow either an E1cB or $B_{Ac}2$ mechanism depending on the possible formation of an isocyanate intermediate. The reactivities of two series, the *N*-(4-pyridyl)- and *N*-phenylcarbamates, were also compared in order to assess the influence of structure on the biological activity of these new compounds.

Substituted carbamic acid esters, carbamates, R'NHCOOR, are widely used in agriculture as pesticides (Melnikov, 1971). Carbanilic acid aliphatic esters, such as isopropyl carbanilate (propham, IPC) and isopropyl 3-chlorocarbanilate (chlorpropham, CIPC), are selective pre- and postemergence herbicides used for the control of annual grasses and broad-leaf species in a variety of tolerant crops. 3,4-Dichlorocarbanilic acid methyl ester (swep) is also used on rice crops in Japan (Kuwatsuka, 1972). Due to their antimitotic activity (Marth and Schulz, 1952), propham and chlorpropham are widely used as sprout inhibitors during the storage of potato tubers, especially in France (Bailly and Dubois, 1981).

However, the carbamates derived from 2-aminopyridine (Toyo Soda Manufacturing Co.) have rather weak herbicidal activity. We therefore decided to attempt the synthesis of aryl and alkyl *N*-(4-pyridyl)carbamates and screen them for potential herbicidal activity. Compounds with structures similar to that of propham were sought initially. The phytotoxicity of these compounds was also determined in a variety of experimental situations (cultured cells, isolated organites, germinating seeds) and compared with the activity of chlorpropham employed as a reference standard.

The mechanism of hydrolysis of carbamates was also investigated. Two main mechanisms, E1cB and $B_{Ac}2$, differing in the formation or absence of an isocyanate intermediate were observed. Such intermediates can have biological activity due to their strong reactivity toward nucleophilic groups such as NH_2 and OH (Bergon and Calmon, 1983; Bergon et al., 1985). The presence of a 4-pyridyl nucleus suggested a kinetic study of the hydrolysis of these new carbamate derivatives. Finally, the reactivities of two series, the *N*-(4-pyridyl)- and *N*-phenylcarbamates, were compared in order to assess the influence of structure on the biological activity of these new compounds.

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MATERIALS AND METHODS

Chemicals. The alkyl and aryl *N*-(4-pyridyl)carbamates were synthetized by the action of an alkyl or aryl chloroformate on 4-aminopyridine in the presence of triethylamine in chloroform or benzene (Matzner et al., 1964). Phenyl *N*-methyl-*N*-(4-pyridyl)carbamate was obtained by action of phenyl chloroformate on 4-(*N*-methylamino)-pyridine, after N-methylation of the 4-aminopyridine (Matondo, 1987).

The physicochemical characteristics of the resulting compounds are shown in Table I. Their structures were confirmed by IR and NMR spectroscopy.

Biological Test. Wheat seeds (*Triticum aestivum* L. var. Tallent) were rinsed with sodium hypochlorite and placed on filter paper in sterilized Petri dishes (10-cm diameter). Final concentrations were obtained by adding 0.1 M solutions of the carbamates in dimethyl sulfoxide (DMSO) to 10 mL of water. Ten seeds were put in each dish, and each experiment was repeated four times. The untreated seeds were put in Petri dishes with 0.01%, 0.1%, or 1% DMSO in water. The experiments were carried out in the dark at 25 °C and lasted 6 days.

Kinetic Measurements. The time course of the hydrolysis reaction was followed by the change in optical density of substrate or product. This was recorded at a fixed wavelength in a UV spectrophotometer (Cary 210 equipped with thermostated sample holder, ±0.1 °C). The hydrolysis reaction was first order with respect to substrate. The rate constants k_{obsd} were determined graphically from $\log (A_\infty - A_t) = \log (A_\infty - A_0) - k_{obsd}t/2.303$ by plotting $\log (A_t - A_\infty)$ vs time. A_∞ and A_t are the absorbance readings at infinite time and time t, respectively. The biomolecular rate constants k_{OH} were equal to the values calculated from $k_{obsd}/[OH^-]$ for four concentrations of hydroxide ions.

Due to the low solubility of the *N*-(4-pyridyl)carbamates in water, the rate constants of hydrolysis were determined in a mixture of water and dioxane (3/1, v/v).

Nitrogen was bubbled through the distilled water used to make up the sodium hydroxide solutions. The ionic strength of the solutions was kept constant (μ = 1.0) by addition of KCl.

RESULTS AND DISCUSSION

Biological Activity of Isopropyl *N*-(4-Pyridyl)carbamate. Table II and Figure 1 show the effects of this compound on the growth of wheat seedlings after 6 days at 25 °C in the dark. At concentrations of 100 μM and 1 mM, the compound inhibited growth of roots and coleoptile and prevented leaf formation. The meristem re-

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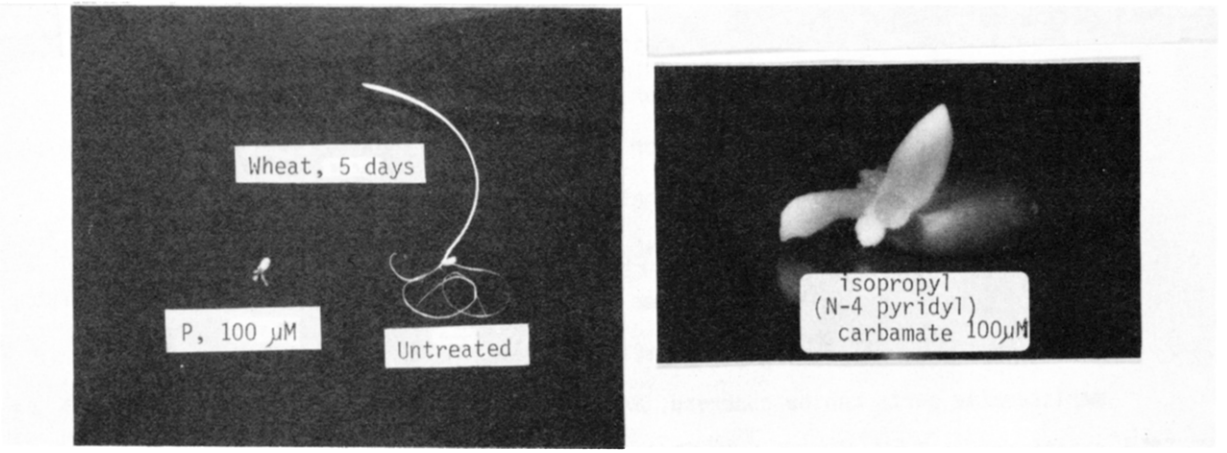

Figure 1. Six-day-old etiolates wheat seedlings treated or untreated with 100 μM isopropyl *N*-(4-pyridyl)carbamate.

Table I. Physicochemical Characteristics of Alkyl and Aryl *N*-(4-Pyridyl)carbamates

		elemental analyses									
		calculated					found				
R	mp, °C	C	H	N	O	Cl	C	H	N	O	Cl
CH_3	162	55.26	5.26	18.42	21.05		54.74	5.39	18.42	21.04	
CH_3CH_2	132	57.83	6.02	16.87	19.28		56.31	5.98	16.30	19.04	
$CH_3OCH_2CH_2$	158	55.10	6.12	14.29	24.49		55.02	6.17	14.16	24.25	
$(CH_3)_2CH$	110	60.00	6.67	15.56	17.78		59.40	6.67	15.26	17.64	
Cl_3CCH_2	257	35.62	2.60	10.39	11.87	38.15	36.61	2.70	10.28	12.26	39.55
C_6H_5	148	67.29	4.67	13.08	14.95		66.63	4.58	12.81	15.08	
m-ClC_6H_4	150	57.95	3.62	11.27	12.88	14.29	57.60	3.02	11.95	12.78	14.19
p-$O_2NC_6H_4$	155	55.60	3.47	16.22	23.97		54.90	3.22	16.66	23.10	

Table II. Effects of Isopropyl *N*-(4-Pyridyl)carbamate (10–1000 μM) and of Chlorpropham on the Growth of Wheat Seedlings in the Dark at 25 °C

concn, μM	coleoptile length, cm	leaf 1 length, cm	Σ, root length, cm	fr wt of growing part, g	dry wt of growing part, g
			Isopropyl *N*-(4-Pyridyl)carbamate		
0	3.8 ± 0.2	6.4	47.8 ± 2	0.109	0.018
10	3.1 ● 0.2	0	16.4 ● 2	0.100	0.014
100	0.7 ± 0.2	0	1.9 ± 2	0.030	0.005
1000	0.9 ± 0.2	0	2.3 ± 2	0.026	0.005
			Chlorpropham		
10	3.0 ± 0.2	0	15.1 ± 2	0.100	0.010
100	0.8 ± 0.2	0	2.2 ± 2	0.020	0.006

gion of the root apex and the coleoptile were swollen (Figure 1). These symptoms are typical of inhibition of mitosis. This was confirmed by optical and electron microscopic examination of the root apex (results not shown). At 10 μM, the growth of the seedling was only partially inhibited (root length 34%, coleoptile length 81% of reference). However, no leaves were produced.

In the seedlings treated at concentrations ranging from 10 to 1000 μM, germination itself was not inhibited. The first mitosis took place, but nuclear abnormalities then appeared leading to an arrest of growth.

These results show that, in wheat seedlings, the target most affected by the studied product is mitosis. Additive experiments on *Acer* cell suspension cultures demonstrate the formation of giant cells as for propham itself (Macherel et al., 1986), suggesting a selective inhibition of mitosis (not shown). Furthermore, assays with isolated mitochondria and chloroplasts indicated that high concentrations of the studied compound were needed to obtain significative changes in the activities of these isolated organelles (results not shown). These results demonstrate that the isopropyl *N*-(4-pyridyl)carbamate is a highly selective mitosis inhibitor.

Table III. Effects of the Synthesized Compounds on the Growth of Etiolated Wheat Seedlings (Numbers in the Table Are Percents of the Untreated Seedlings)

R	[C], M	coleoptile length, cm	leaf 1 length, cm	Σ, root length, cm	fr wt of growing part, g	dry wt of growing part, g
CH_3	10^{-4}	100	100	100	100	100
	10^{-3}	45	14	4.2	27	31
CH_2CH_3	10^{-4}	100	100	100	100	100
	10^{-3}	40	15	5	26	29
$CH(CH_3)_2$	10^{-5}	81.6	0	34	92	78
	10^{-4}	18	0	4	27.5	28
	10^{-3}	23.7	0	4.8	24	28
$CH_2CH_2OCH_3$	10^{-4}	100	100	80	85	89
	10^{-3}	100	22	57	46	48
C_6H_5	10^{-4}	100	100	100	55	78
	10^{-3}	75	9	56	29	37
m-ClC_6H_4	10^{-4}	100	100	78	72	71
	10^{-3}	100	50	53	55	56
p-$O_2NC_6H_4$	10^{-4}	100	100	68	78	50
	10^{-3}	71	4	27	38	41

Effects of Other Compounds. The results shown in Table III demonstrate that the isopropyl, methyl, and ethyl derivatives had evident inhibitory activity on wheat seedling growth, as shown by their effects on coleoptile, leaf 1, and root elongation. Of these three, the isopropyl derivative had the strongest inhibitory effect. Substitution by $CH_2CH_2OCH_3$ or *m*-ClC_6H_4 led to almost inactive compounds.

With respect to root inhibition, the estimated values of 50% inhibitory activity could be ranked as follows: (1) $CH(CH_3)_2$, I_{50} = 10 μM; (2) CH_3 or CH_2CH_3, I_{50} = 500 μM; (3) *p*-$O_2NC_6H_4$, I_{50} = 700 μM; (4) *m*-ClC_6H_4, C_6H_5, or $CH_2CH_2OCH_3$, I_{50} = 1000 μM. For inhibition of leaf formation the order was as follows: (1) $CH(CH_3)_2$, I_{50} = 10 μM; (2) *p*-$O_2NC_6H_4$ and *m*-ClC_6H_4, I_{50} close to 500 μM; (3) CH_3, CH_2CH_3, and C_6H_5, I_{50} close to 700 μM. However, for C_6H_5, *m*-ClC_6H_4, and *p*-$O_2NC_6H_4$, no swelling in the meristem regions could be observed. The inhibition of

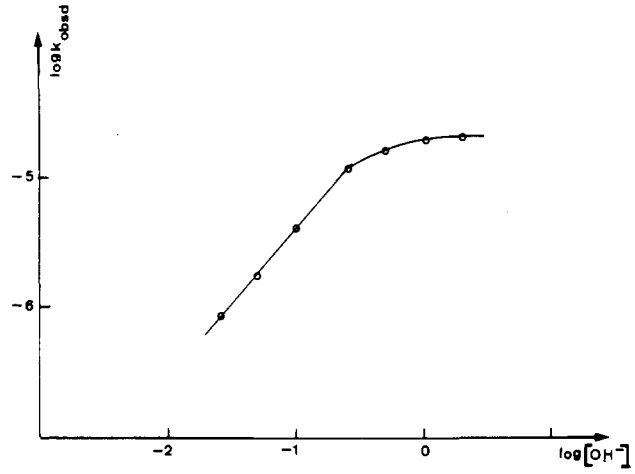

Figure 2. Plot of log k_{obsd} vs log [OH^-] for the hydrolysis of isopropyl N-(4-pyridyl)carbamate at 25 °C.

growth for these substances at a concentration of 1 mM cannot, therefore, be accounted for by specific inhibition of mitosis.

In summary, aryl and alkoxycarbonylation of 4-aminopyridine gives N-(4-pyridyl)carbamates with activities at least equal to that of chlorpropham, when the alkyl group is isopropyl. Replacing the m-chlorophenyl ring with a pyridine nucleus conserves the antimitotic activity. It has been suggested (Tissut et al., 1986) that the para position of the phenyl ring is involved in the binding of chlorpropham to its cellular site of action. It can thus be supposed that the binding of carbamates to their site of action is not unduly hindered by the presence of either a C or N atom at this position.

As for the N-phenylcarbamates, shortening the chain length of the esterifying moiety of the molecule reduced its effectiveness. Moreover, when the esterifying moiety was a phenyl or substituted phenyl nucleus, inhibitory activity was very low. This may be due to an effect of steric hindrance of this side chain, coupled with an effect due to its lipophilic nature. However, it is interesting to note that the highest biological activities were found in derivatives with high pK_a values of the esterifying group.

Alkaline Hydrolysis of a Series of Alkyl and Aryl N-(4-Pyridyl)carbamates and Effects of Sodium Hydroxide Concentration. The rates of hydrolysis were measured spectrophotometrically at 25 °C for concentrations of NaOH ranging from 0.001 to 1.0 M. Figure 2 shows the plot of the logarithm of the observed pseudo-first-order rate constants k_{obsd} against hydroxide ion concentration for isopropyl N-(4-pyridyl)carbamate. The straight line of slope 1.0 was in agreement with the simplified expressions ($k_{obsd} = k_1K_a/a_H$ and $k_{obsd} = k_2[OH^-]$ when $a_H \gg K_a$) of rate laws corresponding to E1cB and $B_{Ac}2$ reaction schemes respectively (cf. Figure 3):

$$k_{obsd} = k_1K_a/(K_a + a_H) \quad (1)$$

$$k_{obsd} = k_2K_w/(K_a + a_H)\gamma_{OH^-} \quad (2)$$

The straight line of unity slope is followed by a plateau at higher values of pH ($a_H \ll K_a$).

The two lines cross at a value of pH equal to the pK_a of the substrate. The pK_a values of the carbamates were determined graphically (Table IV) except for the phenyl, p-nitrophenyl, and m-chlorophenyl esters for which plateaus were not reached due to their lack of stability.

Reaction Mechanism. Two criteria were used to evaluate the reaction mechanism: the parameter β of the Brønsted relationship and the influence of N-methylation.

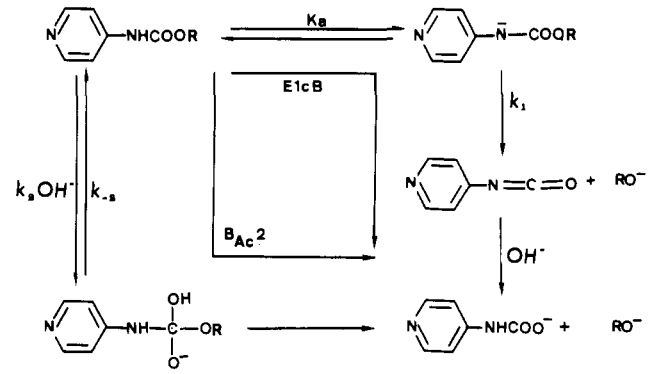

Figure 3. Mechanisms of alkaline hydrolysis of aryl and alkyl N-(4-pyridyl)carbamates.

Table IV. Bimolecular Rate Constants for the Hydrolysis of Aryl and Alkyl N-(4-Pyridyl)carbamates at 25 °C in 3/1 (v/v) Water–Dioxane and pK_a of Carbamate and of Leaving Group ROH

R	pK_a (carbamate)	pK_a(ROH)	k_{OH}, L mol^{-1} s^{-1}	correln coeff (r)
p-$O_2NC_6H_4$		7.15[a]	1.25×10^5	0.988
m-ClC_6H_4		9.13[a]	2.06×10^3	0.999
C_6H_5		10.00[a]	0.91×10^2	0.999
Cl_3CCH_2	12.84	12.24[b]	2.21×10^{-2}	0.999
CH_3	13.80	15.54[b]	3.75×10^{-4}	0.999
CH_3CH_2	13.90	16.00[b]	2.76×10^{-4}	0.998
$CH_3OCH_2CH_2$	13.76	14.82[c]	3.68×10^{-4}	0.998
$(CH_3)_2CH$	14.10	17.1[c]	5.87×10^{-5}	0.999

[a] Barlin and Perrin, 1966. [b] Jencks and Gilchrist, 1968. [c] Ballinger and Long, 1960.

The Brønsted relationship between the logarithm of the bimolecular rate constant k_{OH} and the pK_a of the leaving group can be used to distinguish between E1cB ($\beta < -1.0$) and $B_{Ac}2$ ($\beta > -0.5$) mechanisms (Williams, 1973; Bergon and Calmon, 1976; Sartoré et al., 1977).

In order to plot the Brønsted relationship, log k_{OH} = f(pK_a) (cf. Figure 3), the bimolecular rate constants k_{OH} shown in Table IV were calculated from $k_{OH} = k_{obsd}a_H\gamma_{OH^-}/K_w$ with $K_w = 1.0 \times 10^{-15}$ for a water–dioxane mixture (3/1, v/v) (Harned and Fallon, 1939). The value of the activity coefficient of OH^-, γ_{OH^-}, was determined from pH measurements of solutions of NaOH (0.001–0.01 M) in a mixture of water and dioxane (3/1, v/v), giving a value of 0.31 (Bergon and Calmon, 1983).

On figure 4, the points corresponding to the phenyl, p-nitrophenyl, m-chlorophenyl, and 2,2,2-trichloroethyl derivatives lay on a straight line from log k_{OH} = −1.38 pK_a + 15.05 (r = 0.87, s = 0.08) while the methyl, ethyl, methoxyethyl, and isopropyl N-(4-pyridyl)carbamates characterized by a "poor" leaving group (pK_a(ROH) > 13) lay on a straight line from log k_{OH} = −0.37pK_a + 2.15 (r = 0.918, s = 0.19). These lines intersected at a point represented by a pK_a value of 13.00. The abrupt change of slope from −1.38 to −0.37 observed in the Brønsted plots indicated a changeover in the reaction mechanism from E1cB to $B_{Ac}2$. The $B_{Ac}2$ mechanism is represented by the line of highest slope and is thought to be when hydroxyl ion attack on the carbonyl group is the determining factor (Bergon and Calmon, 1983).

Furthermore, the bimolecular rate constant $k_{OH} = 4.79 \times 10^{-3}$ mol^{-1} s^{-1} of phenyl N-methyl-N-(4-pyridyl)carbamate indicated that this derivative is much less reactive than its nonmethylated homologue ($k_{OH} = 9.1 \times 10$ mol^{-1} s^{-1}). Since the N,N-disubstituted compound can only be hydrolyzed via a $B_{Ac}2$ mechanism, we concluded that phenyl N-(4-pyridyl)carbamate, along with the other de-

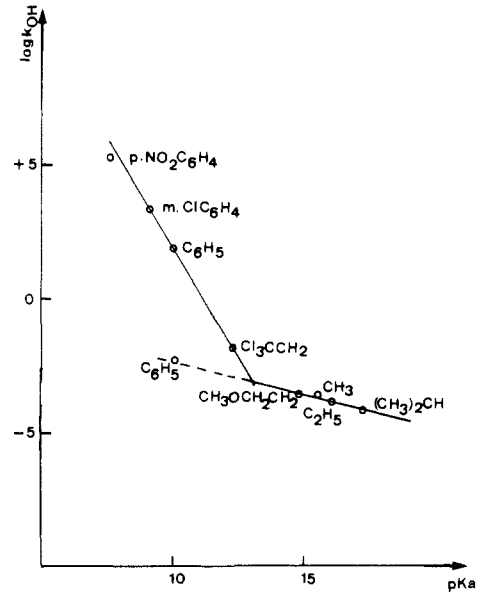

Figure 4. Brønsted plot of log k_{OH} vs pK_a of leaving group for the hydrolysis of alkyl and aryl *N*-(4-pyridyl)carbamates at 25 °C.

rivatives lying on the Brønsted line of slope −1.38, are hydrolyzed via an E1cB mechanism, while the carbamates including phenyl *N*-methyl-*N*-(4-pyridyl)carbamate lying on the line of slope $\beta = -0.37$ are hydrolyzed via $B_{Ac}2$ mechanism.

In conclusion, the results showed that the isopropyl derivative of *N*-(4-pyridyl)carbamate had phytotoxic activity similar to that of chlorpropham. The similar chemical reactivity of the two compounds with respect to hydrolysis would indicate that they have a similar mechanism of action in vivo.

Registry No. Methyl *N*-(4-pyridyl)carbamate, 79546-31-9; ethyl *N*-(4-pyridyl)carbamate, 54287-92-2; isopropyl *N*-(4-pyridyl)carbamate, 117652-47-8; methoxyethyl *N*-(4-pyridyl)-carbamate, 117652-48-9; phenyl *N*-(4-pyridyl)carbamate, 20951-01-3; *m*-chlorophenyl *N*-(4-pyridyl)carbamate, 117652-49-0; *p*-nitrophenyl *N*-(4-pyridyl)carbamate, 117652-50-3; 2,2,2-trichloroethyl *N*-(4-pyridyl)carbamate, 117652-51-4.

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