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Anticholinesterase Activity and Knock-Down of Substituted Phenyl *N*-Methylcarbamates to Honey Bee[1]

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Although carbamates are considered to be insecticidal by virtue of their ability to inhibit insect cholinesterase (ChE), the toxicity of these compounds to insects cannot be predicted as easily from their potencies as anti-ChEs. Even in the presence of an active synergist, such as piperonyl butoxide, only about 50% of the variation in toxicity of substituted phenyl N-methylcarbamates to houseflies (JONES et al. 1969) and to honey bee (ABD-ELROAF et al. 1977) can be attributed to their anti-ChE potencies.

The present report is concerned with the effect of structure of a series of substituted phenyl N-methylcarbamates on the knock-down and in vivo inhibition to honey bee workers.

MATERIALS AND METHODS

Alkylphenyl N-methycarbamates were synthesized from methylisocyanate and the corresponding phenol as described by KOLBEZEN et al. (1954). Methoxy and chlorophenyl methylcarbamates were obtained through the courtesy of Dr. T. R. Fukuto (Dept. of Entomology, Univ. of Calif., Riverside, Calif., U.S.A.). Carbaryl and carbofuran were purified from commercial forms to their reported melting points.

Bimolecular inhibition reaction constant (K_i) and dissociation constant (K_d) were obtained by the kinetic procedure of MAIN and HASTINGS (1966). The detailed procedure was as previously described (ABD-ELROAF et al. 1977).

Knock-down and in-vivo inhibition: One hundred insects pretreated before by piperonyl butoxide (16 µg/bee) were treated topically with a dose of carbamate which by previous experimentation resulted in 95% mortality in the presence of the synergist. The insects were treated during stupefaction with carbon dioxide. After treatments, insects were confined in a funnel and then the upper side of the funnel was sealed by muslin. At zero time the funnel tube was inserted in a glass container to receive the knocked-down insects. The bee workers were classified either as "knocked-down" or "non-affected"; all the

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insects partially paralysed, but still able to fly or crawl on the sides of the funnel, were classified as non-affected. The number of knocked-down insects at each inspection time were recorded, collected immediately and then decapitated and their heads were stored at -20°C until use. Percent in vivo inhibition in bee heads was determined by the colorimetric method of ELLMAN et al. (1961) in the usual manner as previously described ABDEL-AAL (1977).

RESULTS AND DISCUSSION

Table 1 shows the biological activity of substituted phenyl N-methylcarbamates. As seen in this table, paralytic symptom in the survivors was associated with in vivo honey bee acetyl ChE inhibition. Usually more than 60% of the enzyme was inhibited at the time of knock-down. The amount of in vivo inhibited enzyme was different among the compounds tested and ranged from 56.7% for p-ethylphenyl N-methylcarbamate to 91% for m-isopropyl-phenyl N-methylcarbamate. It is of interest to note that maximum in vivo inhibition of bee head acetyl ChE was associated with the meta substituted derivative as compared with its other isomeric analogues (ortho and para). Also, among the meta alkyl derivatives, in vivo anti ChE activity increases with increase in the size of the alkyl group, i.e., m-CH_3, m-C_2H_5, m-iso-C_3H_7.

TABLE 1

Biological activity of substituted phenyl N-methylcarbamates (x-C_6H_4O C (O) NH CH_3) to honey bee.

X	Hansch's	K_i. ($M^{-1}.min.^{-1}$)	K_d(M)	Synergised LD_{95}(ug/bee)	Synergised KT_{50} (min.)	% In-vivo inhibition
H	0.0	$1.44x10^4$	$1.60x10^{-4}$	1.50	47	67.0
o-CH_3	0.68	$2.10x10^5$	$3.00x10^{-4}$	0.60	74	61.5
m-CH_3	0.51	$1.24x10^4$	$1.15x10^{-5}$	0.32	41	81.7
p-CH_3	0.52	$1.76x10^4$	$8.50x10^{-5}$	1.20	46	72.6
o-C_2H_5	1.22	$5.90x10^4$	$2.70x10^{-5}$	2.70	34	89.0
m-C_2H_5	0.97	$3.00x10^5$	$2.00x10^{-6}$	0.26	35	89.6
p-C_2H_5	0.97	$6.18x10^4$	$2.70x10^{-5}$	3.60	52	56.7
o-iso.C_3H_7	-	$5.42x10^5$	$3.60x10^{-6}$	0.035	16	80.5
m-iso.C_3H_7	1.30	$2.39x10^7$	$2.80x10^{-8}$	0.105	26	91.0
o-Cl	0.59	$2.34x10^5$	$6.10x10^{-6}$	1.40	42	67.0
m-Cl	0.76	$4.07x10^4$	$4.10x10^{-5}$	1.50	56	76.2
p-Cl	0.70	$5.71x10^3$	$7.00x10^{-4}$	7.60	72	69.7
o-CH_3O	-0.33	$1.82x10^4$	$4.40x10^{-5}$	7.00	49	69.7
m-CH_3O	0.12	$6.40x10^4$	$2.50x10^{-5}$	0.31	68	89.0
p-CH_3O	-0.04	$5.70x10^3$	$1.00x10^{-4}$	0.55	83	68.7
Carbaryl	-	$4.00x10^6$	$1.80x10^{-6}$	0.32	48	79.0
Carbofuran	-	$8.00x10^6$	$1.50x10^{-6}$	0.035	25	86.5

This indicates that structural complementarity plays an important role in *in vivo* ChE inhibition as well as in *in vitro* in spite of using dosages with the same response (LD_{95}) for all the compounds tested. The widely accepted belief that ChE inhibition is directly related to toxicity does not imply its complete inhibition. Histo-chemical studies of ChE of the central nervous system of houseflies did not show complete inhibition of the enzyme, even after high doses of Diazinon causing over 99% kill (MOLLOY 1961).

The percent of knock-down is a more reliable estimate than observations of the earlier symptoms of poisoning, since the latter are liable to greater personal error. The time percent knock-down curves of the carbamates at the LD_{95} values indicate that all tests followed the general design of the probit assay (FINNEY 1952). Therefore, plotting of percent knocked-down insects against time on a probit-log paper fit a straight line rather well enabling KT_{50} (the time required for 50% knock-down) to be evaluated. As seen in Table 1, there is a lack of any general correlation between the degree of brain ChE inhibition and the occurrence of knock-down symptoms after carbamates poisoning.

Anti-ChE activity (K_i and K_d values) are tabulated together with the appropriate Hansch's π constants (FUJITA et al. 1964) and KT_{50} values in Table 1. This information suggested the feasibility of attempting to correlate chemical structure with KT_{50} values by computing, through multiple regression analysis. The computed equations were:

	n	r	
$\log KT_{50} = 2.27 - 0.12 \log K_i$	14	0.60	..... (1)
or			
$\log KT_{50} = 2.18 + 0.106 \log K_d$	14	0.62	..... (2)

The correlation was increased only to r = 0.61 and 0.65 when term was incorporated in the above equations respectively, showing that rate of penetration to the site of action is essentially equivalent for the compounds studied, none of which were highly ionized. The computed equations in the presence of π term were:

	n	r	
$\log KT_{50} = 2.24 - 0.035\ \pi - 0.11 \log K_i$	14	0.61	..... (3)
$\log KT_{50} = 2.15 - 0.061\ \pi + 0.093 \log K_d$	14	0.65	..... (4)

The similar correlation obtained between $\log KT_{50}$ and $\log K_i$ or $-\log K_d$ is expected since nearly all the variations in K_i are attributable to differences in K_a values (K_a is the affinity constant and equals the reciprocal of K_d) rather than carbamylation rate constants (O'BRIEN et al. 1966; ABDEL-AAL 1977 and ABD-ELROAF et al. (1977)).

Equations (1&2) indicate that anti-ChE activity accounts for only 36% of the variation in the paralytic symptoms (syner-

gised KT_{50} values). The results also indicate that correlation between anti-ChE potency of carbamates and their paralytic symptoms against honey bee is not any better than correlation between any of the various inhibition parameters and synergised toxicity of the same carbamates against the same insect that previously described by ABD-ELROAF et al. (1977).

Nevertheless, one might argue that honey ChE inhibition cannot adequately account for the ultimate cause of death. The possibility of another or complementary mode of action, perhaps involving the acetylcholine receptor as has been suggested (WEIDEN and MOOREFIELD 1965) still cannot be ruled out. The present study does not furnish any clue to this particular point.

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