Comparative evaluation of new synergists containing a butynyl-type synergophore group and piperonyl butoxide derivatives

László Pap,* Géza Árvai, Béla Bertók, Zsuzsanna Kuruczné Ribai and Ildikó Bakonyvári
Agro-Chemie Ltd, Budapest, H-1780, PO Box 49, Budapest, Hungary

Abstract: Cross-substituted derivatives of piperonyl butoxide (PBO) and MB-599 (proposed common name: verbutin) were synthesized and investigated as carbofuran and permethrin synergists against housefly, *Musca domestica* L. The majority of PBO and MB-599 derivatives were significantly more potent synergists for carbofuran than for permethrin. PBO, the most important representative of this series was not the most potent synergist for carbofuran or for permethrin. Cleavage of the methylenedioxy ring of methylenedioxyphenyl (MDP) polyether compounds resulted in complete loss of synergistic activity with both insecticides, but it could be restored or even improved by incorporating an alkynyl ether moiety into the molecule. The improved synergistic activity was found to be closely associated with the 2-butynyloxymethyl side-chain, suggesting that this can be regarded as a characteristic synergophore group. MB-599, one of the most promising compounds bearing this group showed considerably higher activity with carbofuran (synergist ratio, SR = 37.8) than with PBO (SR = 6.4). There was no significant difference between synergistic activities of MB-599 (SR = 4.6) and PBO (SR = 4) for permethrin.

Keywords: butynyl ether synergists; MB-599; piperonyl butoxide; carbofuran; permethrin

1 INTRODUCTION

Oxidative metabolism mediated by cytochrome-P450 monooxygenases is one of most important detoxification routes of insecticides,[1] and can be inhibited by the methylenedioxyphenyl (MDP) synergists, which include piperonyl butoxide (PBO).[2] A theoretical study of the mode of action of PBO at molecular level has been published recently.[3]

Currently, PBO and a few closely related MDP compounds (sesamex, sesamine, sesamoline, sulfoxide, propyl isome) are the most important insecticide synergists. Synergists from other compound groups, such as propynyl esters, propynyl ethers and the substituted imidazoles, with inhibiting monooxygenation activity have failed to achieve commercial success.[4]

We have recently reported a new family of alkynyl synergists having butynyl side-chains as the synergophore group.[5–7] High synergist potency with a number of active ingredients characterizes these compounds. They are supposed to be particularly effective in preventing resistance evolution and controlling resistant populations.[8] The field performance of MB-599 (proposed common name: verbutin), one of the most effective members of this series, is currently under evaluation.

The objective of the present study was to identify and characterize the butynyl side-chain as a new synergophore group. The synergist potency of related MDP and 1,2-dimethoxybenzene homologues and the influence of increasing amounts of co-applied synergists were investigated in housefly using carbofuran and permethrin insecticides.

2 MATERIALS AND METHODS

2.1 Synthesis of synergists tested

Safrol and methyleugenol were hydrogenated over 10% Pd–C[9,10] in methanol to yield the corresponding 4-propyl-1,2-methylenedioxy- and 1,2-dimethoxybenzenes (**1** and **11**), respectively. Benzyl propargyl ethers **10** and **20** were prepared according to a method described in the literature.[11] The structures of the synergists studied are shown in Table 1. Syntheses of other benzyl ether derivatives have been published elsewhere.[12,13]

2.2 Insecticide and synergist standards

Technical grade carbofuran (>95%, Chinoin) and permethrin (>95%, with 40:60 ratio of *cis:trans* isomers, Chinoin) were used as insecticides. Analytical

* Correspondence to: László Pap, Agro-Chemie Ltd, Budapest, H-1780, Po Box 49, Budapest, Hungary
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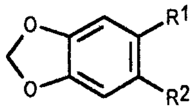
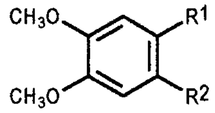
Table 1. Structures of tested synergists



A



B

Structure number	*Aromatic ring*	*R[1]*	*R[2]*	*Reference[a]*
1	A	n-C_3H_7	H	9
2	A	H	$CH_2O(CH_2)_2O(CH_2)_2O(CH_2)_3CH_3$	22
3	A	H	$CH(CH_3)O(CH_2)_2O(CH_2)_2O(CH_2)_3CH_3$	23, 24
4	A	n-C_3H_7	$CH_2O(CH_2)_2O(CH_2)_2O(CH_2)_3CH_3$	
5	A	n-C_3H_7	$CH(CH_3)O(CH_2)_2O(CH_2)_2O(CH_2)_3CH_3$	
6	A	H	$CH_2OCH_2C{\equiv}CCH_3$	
7	A	H	$CH(CH_3)OCH_2C{\equiv}CCH_3$	
8	A	n-C_3H_7	$CH_2OCH_2C{\equiv}CCH_3$	
9	A	n-C_3H_7	$CH(CH_3)OCH_2C{\equiv}CCH_3$	
10	A	H	$CH_2OCH_2C{\equiv}CH$	25, 26, 27
11	B	n-C_3H_7	H	10
12	B	H	$CH_2O(CH_2)_2O(CH_2)_2O(CH_2)_3CH_3$	
13	B	H	$CH(CH_3)O(CH_2)_2O(CH_2)_2O(CH_2)_3CH_3$	
14	B	n-C_3H_7	$CH_2O(CH_2)_2O(CH_2)_2O(CH_2)_3CH_3$	
15	B	n-C_3H_7	$CH(CH_3)O(CH_2)_2O(CH_2)_2O(CH_2)_3CH_3$	
16	B	H	$CH_2OCH_2C{\equiv}CCH_3$	
17	B	H	$CH(CH_3)OCH_2C{\equiv}CCH_3$	
18	B	n-C_3H_7	$CH_2OCH_2C{\equiv}CCH_3$	
19	B	n-C_3H_7	$CH(CH_3)OCH_2C{\equiv}CCH_3$	
20	B	H	$CH_2OCH_2C{\equiv}CH$	28, 11

[a] Unless otherwise noted, the synergists are here reported for the first time.

grade (>98%) PBO was obtained from Riedel–de Haen, Seelze, Germany.

2.3 Bioassay

The synergistic potency of the putative synergists was studied on housefly (*Musca domestica* L, WHO/NTT) by a standard topical method described previously.[14] Dose-mortality tests were carried out for synergized and unsynergized insecticides and assessed by probit analysis.[15] Carbon dioxide anaesthetized adult female flies (3- to 4-day-old, 17–21 mg body-weight) were treated topically by applying insecticide or insecticide plus synergist in cellosolve (2-ethoxyethanol; 0.2 µl) to the dorsal surface of the thorax with a micro-applicator (Microlab P, Hamilton, Reno NV). Control flies were treated with the solvent only. The treated flies were kept in normal laboratory conditions (temperature 22 (±2) °C, relative humidity 60 (±10)%, 12:12 h light:dark). Mortality was assessed after 24 h. Two replicates of 10 flies were tested for each dose level of insecticide or insecticide + synergist. Each test was repeated three times. Synergists were co-administered with insecticides. Synergist activity was screened by application of a constant amount of synergist, 1000 ng per fly. To investigate the effect of synergist dose on insecticidal activity, various quantities of synergist, 250, 500, 1000, 2000 and 5000 ng per fly, were administered. The tested synergists caused no mortality alone even at the highest dose applied, so the synergist ratio (SR) was calculated as the ratio of the LD_{50} of the insecticide alone to that of the insecticide + synergist mixture.

3 RESULTS

3.1 Synergist potency

Tables 2 and 3 show the synergist potencies of MDP compounds with carbofuran and permethrin in comparison with those of the related 1,2-dimethoxybenzene (veratryl) analogues.

3.1.1 MDP compounds

SR values indicate that PBO (**4**) cannot be considered an optimum structure either with carbofuran or with permethrin. The structure of the molecule can be fine-tuned even among the polyether series by removing the 6-propyl group and/or substitution in the benzylic position. Exchanging the polyether chain for an alkynyl ether does not cause a profound improvement in activity with permethrin (Table 3). The best polyethers (**3**, **5**) and alkynyl ethers (**9**) are within a narrow range of SR = 5–6 which does not differ significantly from that of PBO (**4**, SR = 4).

MDP alkynyl ethers (**6**–**10**) of any substitution pattern are superior to the corresponding polyethers (**1**–**5**) when tested with carbofuran (Table 2). Synergist potency is significantly affected by structural variation and the tendency is similar within the corresponding series (**2**–**5** *vs* **6**–**9**). The most active compound **6** (piperonyl 2-butynyl ether) shows an SR

Treatment[a]	n[b]	LD_{50}	FL 95%		Slope	±SE	χ^2	df	SR[c]
		ng per fly							
Carbofuran alone	480	169.9	143.0	200.4	1.81	0.55	2.713	4	—
1	380	27.0	22.6	32.1	2.12	0.47	6.783	5	6.3
2	420	15.8	12.6	19.3	1.73	0.58	3.856	5	10.7
3	360	16.6	10.6	24.8	2.66	0.37	12.809	4	10.2
4 (PBO)	300	26.4	22.3	30.8	2.57	0.39	1.080	3	6.4
5	300	31.1	19.5	47.1	2.61	0.38	7.168	3	5.5
6	300	4.3	3.7	4.9	2.84	0.27	0.581	3	40.0
7	220	5.2	4.6	5.9	4.46	0.53	2.913	2	32.7
8	300	7.7	6.5	8.9	3.27	0.39	4.738	3	22.1
9	280	8.4	4.2	12.9	3.21	0.31	9.045	3	20.2
10	240	8.6	7.4	9.8	3.60	0.42	0.823	2	19.8
11	300	259.6	215.2	317.0	2.00	0.50	7.370	4	0.7
12	300	261.4	219.9	313.7	2.24	0.23	4.060	4	0.7
13	380	237.3	204.1	274.4	2.34	0.21	2.154	3	0.7
14	300	204.9	175.0	240.0	2.58	0.25	4.802	4	0.8
15	300	170.7	115.1	255.5	2.70	0.25	6.516	3	1.0
16	220	6.4	2.4	12.6	3.67	0.41	5.549	2	26.6
17 (MB-599)	260	4.5	4.0	5.2	3.97	0.43	3.216	3	37.8
18	360	14.6	12.3	17.1	2.46	0.23	3.270	4	11.6
19	280	56.1	47.9	65.3	2.73	0.28	2.522	3	3.0
20	360	19.8	16.5	23.5	2.13	0.19	5.097	4	8.6

[a] Treatments were performed as described in Section 2.3.
[b] Number of flies on which each probit analysis was based.
[c] SR, synergist ratio: LD_{50} of unsynergized divided by LD_{50} of synergized treatment.

Table 2. Synergist potency of tested compounds with carbofuran on housefly

value twice as high as that of the known propargyl homologue **10**.

3.1.2 1,2-Dimethoxybenzene compounds

Opening the methylenedioxy ring results in complete loss of activity in the polyether series (either with carbofuran or with permethrin) as expected from the structure-activity relationship studies by Wilkinson *et al.*[16] Potency is, however, retained by incorporating the alkynyl ether moiety, which confirms that the

Treatment[a]	n[b]	LD_{50}	FL 95%		Slope	±SE	χ^2	df	SR[c]
		ng per fly							
Permethrin alone	300	198.9	169.3	232.5	2.56	0.39	5.604	3	—
1	340	111.7	80.2	162.8	2.86	0.39	9.561	4	1.8
2	360	49.9	43.2	57.7	2.85	0.35	6.729	4	4.0
3	360	34.4	29.4	40.2	2.50	0.40	2.79	4	5.8
4 (PBO)	300	50.2	32.0	79.0	2.65	0.38	7.819	3	4.0
5	360	37.2	26.1	53.1	2.62	0.38	2.774	4	5.4
6	300	51.8	45.3	59.1	3.39	0.30	2.907	3	3.8
7	240	62.5	27.3	126.7	3.08	0.33	5.211	2	3.2
8	300	48.6	41.8	56.6	2.68	0.37	3.755	3	4.1
9	300	33.4	28.8	38.6	2.98	0.34	0.352	3	6.0
10	360	68.5	59.6	78.7	3.04	0.33	2.014	4	2.9
11	300	221.9	19.8	256.3	2.91	0.34	3.811	3	0.9
12	300	225.6	195.9	259.4	3.06	0.33	3.341	3	0.9
13	300	223.8	193.4	258.6	2.90	0.35	1.206	3	0.9
14	300	203.8	178.8	232.0	3.51	0.29	2.381	3	1.0
15	300	189.9	165.7	217.0	2.28	0.30	0.806	3	1.1
16	340	72.8	63.1	84.1	2.91	0.34	2.014	4	2.7
17 (MB-599)	360	44.5	38.3	51.4	2.79	0.36	3.791	4	4.6
18	280	58.9	51.8	66.7	3.81	0.26	5.568	3	3.4
19	340	81.3	70.3	94.2	2.85	0.35	6.111	4	2.5
20	300	94.3	82.9	107.1	3.61	0.28	1.337	3	2.1

[a] Treatments were performed as described in Section 2.3.
[b] Number of flies on which each probit analysis was based.
[c] SR, synergist ratio: LD_{50} of unsynergized divided by the LD_{50} of synergized treatment.

Table 3. Synergistic potency of tested compounds with permethrin on housefly

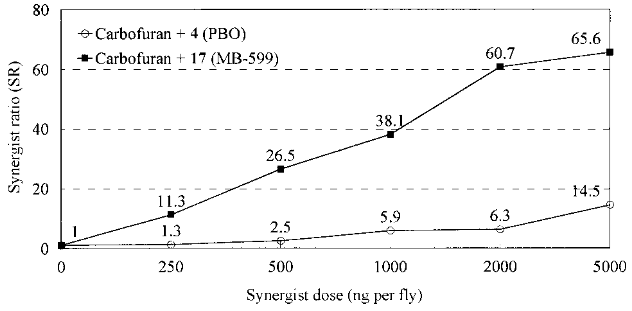


Figure 1. Relationship of synergist ratio (SR) to doses of **4** (PBO) and **17** (MB-599) co-administered with carbofuran in housefly.

2-butynyloxymethyl side-chain is a characteristic synergophore group. With permethrin, the synergistic effectiveness of analogous MDP and 1,2-dimethoxybenzyl alkynyl compounds (**6–8** *vs* **16–18** and **10** *vs* **20**) does not differ significantly. With carbofuran, the effect of substitution pattern on potency is the greatest. The most active representative among veratryl alkynyl ethers (**17**, MB-599) possesses an SR value more than four times that of the closely related propargyl ether **20**.

3.2 Effect of dose of compounds 4 (PBO) and 17 on synergist ratio

The effect of different quantities of the PBO (**4**) and compound **17** on the activity of carbofuran are presented in Fig 1. Increasing synergist doses generally resulted in higher SRs in both cases, although the increase of insecticide potency of carbofuran was significantly higher at each dose with **17** than with PBO. On increasing the synergist dose from 250 to 5000 ng per fly, the SR was enhanced from 1.3 to 14.5 for PBO and from 11.3 to 65.6 for **17**. The fitted line for **17** is much steeper ($b = 13.8$, $r = 0.991$) than was obtained for PBO ($b = 2.45$, $r = 0.906$). The synergistic activities of both PBO and **17** showed significantly lower dose-dependence with permethrin ($b = 1.25$, $r = 0.988$ and $b = 1.15$, $r = 0.946$, respectively, Fig 2).

4 DISCUSSION

PBO was one of the less effective carbofuran synergists amongst the MDP compounds tested (Table 2).

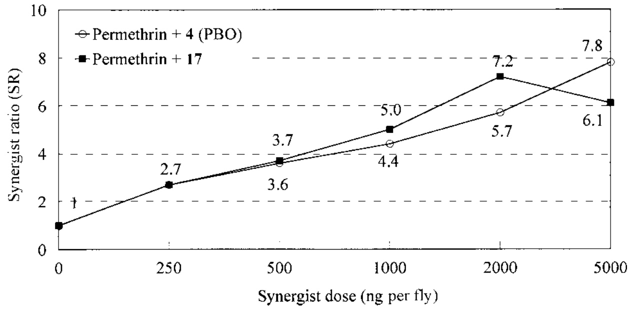


Figure 2. Relationship of synergist ratio (SR) to doses of **4** (PBO) and **17** (MB-599) co-administered with permethrin in housefly.

Within the MDP series, carbamate synergists more potent than PBO have already been reported. When tested on susceptible housefly, isosafrol, safrol and 3,4-methylenedioxyphenyl 2-propynyl ether (RO 7-1366) proved to be better synergists of carbaryl and isolan than did PBO.[17] Similarly, myristicin, isosafrol and 1-nitro-3,4-methylenedioxybenzene were more efficient synergists for carbaryl than PBO in the housefly.[18] A similar superiority of some closely related propynyl aryl ethers over PBO as carbaryl synergists, particularly at lower synergist doses, has been demonstrated on such important crop pests as brown planthopper (*Nilaparvata lugens* Stal), green leafhopper (*Nephotettix cincticeps* Uhler) and tobaco caterpillar (*Spodoptera litura* Fab).[19]

With the exception of compound **5**, all tested MDP derivatives were found to be much more efficient synergists for carbofuran than for permethrin (Tables 2 and 3). In accordance with the results found with the MDP compounds, 1,2-dimethoxybenzene compounds, particularly **16**, **17**, **18** and **20**, showed significantly higher synergistic action with carbofuran than with permethrin. From a theoretical point of view this variance can be attributed either to different potencies of the synergists in inhibiting an identical metabolic process and/or to distinct metabolic routes actually involved in permethrin and carbofuran detoxification.

A striking difference was observed between PBO and **17** in responding to increasing amounts of synergist co-applied with carbofuran, but not with permethrin. Accordingly, it is postulated that PBO and **17** inhibit similar metabolic processes of both carbofuran and permethrin. While carbofuran is metabolized almost exclusively via a single enzyme system, the cytochrome-P450 monooxygenases,[20] permethrin can alternatively be degraded by different classes of detoxification enzymes[21] that cannot be suppressed by either PBO or **17**.

In conclusion, the results presented confirm that the co-applied insecticide and the insecticide–synergist ratio inherently influence the observed synergistic potency. Under our test conditions we found benzyl butynyl ethers to be superior to PBO-type benzyl polyethers. MB-599 (**17**), the most promising candidate of butynyl ethers, potentiates carbofuran at extremely low insecticide–synergist ratios, which is preferable in the ready-mix formulations used in agriculture. The field performance of MB-599 (**17**) with various insecticides is currently being evaluated.

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