Propynyl Naphthyl Ethers as Selective Carbamate Synergists

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Thirty derivatives of propynyl naphthyl ether were synthesized and evaluated as synergists for carbaryl (1-naphthyl *N*-methylcarbamate) against the housefly (*Musca domestica* L.). The most active compound was 1-naphthyl 3-butynyl ether with a synergistic ratio of 176.5. These compounds did not show synergistic activity with carbaryl against the white mouse and provide an interesting approach to selective insect control. It is suggested that the propynyloxyaryl synergists complex in vivo with an essential metal cofactor of the mixed function oxidases, retarding the oxidative degradation of carbaryl and other foreign compounds.

The discovery by Moorefield (1958) that piperonyl butoxide was a very effective synergist for carbaryl and 3-*tert*-butylphenyl *N*-methylcarbamate led to the synthesis and evaluation of numerous methylenedioxyphenyl compounds (1,3-benzodioxoles) as carbamate synergists (Metcalf *et al.*, 1966; Moorefield and Weiden, 1964; Wilkinson *et al.*, 1966). The interesting discovery by Kooy (1966) of the synergistic activity of various propynyl aryl ethers afforded new opportunities for investigations of the mode of action of carbamate synergists. When used at 5 to 1 with carbaryl the following synergistic ratios (SR, ratio between LD_{50} of insecticide alone to LD_{50} of insecticide applied with synergist) were determined for the S_{NAIDM} housefly: 2-(2-nitro-4-chlorophenyl)-propynyl ether, 219; 2-(2,4,5-trichlorophenyl)-propynyl ether, 182; 2-(2,4-dichlorophenyl)-propynyl ether, 142; and 2-(3,4-methylenedioxyphenyl)-propynyl ether, 116. As part of an investigation of the methylenedioxynaphthalenes as carbamate synergists, it was of interest to investigate the synergistic effects of propynyloxy groups attached to the naphthalene nucleus; the effects of varying the relative position of the triple bond in relation to the aromatic nucleus; the effects of nuclear substitution on the activity of propynyl naphthyl ethers; and the metabolic pathways of these compounds in insects and mammals. Since this research was completed, Fellig and Rachlin (1968) have described a large number of propargyl aryl ethers, including two of the naphthyl derivatives investigated here.

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EXPERIMENTAL

Synthesis. The ethers of Table I to III were synthesized from the corresponding naphthols and appropriate alkyl or alkynyl halide in the presence of base. Physical constants and elemental analyses have been summarized in the tables.

Propargyl bromide was commercially available. 1-Bromo-3-butyn (b.p. 103–6° C., n_D^{25} 1.4681) was synthesized from 3-butyn-1-ol in 20% yield by the method described by Eglington and Whiting (1950) using PBr_3 and the corresponding alcohol. 1-Bromo-4-pentyn was synthesized similarly in 13% yield (b.p. 60° C./15 mm. Hg, n_D^{25} 1.4622).

1-Naphthyl 2-butynyl ether (XII) was obtained from 1-naphthyl 2-propynyl ether (I) as follows. The terminal acetylenic hydrogen of I was made to react with sodamide in liquid ammonia to form the sodium salt which subsequently reacted with methyl iodide to form the 2-butynyl derivative. After two vacuum distillations, 36% of light yellow oil (b.p. 85° C./0.05 mm. Hg) was recovered. The NMR spectrum showed proton resonance corresponding to the aromatic, methylene, and terminal hydrogens at τ 2.0 to 4.0, 6.05, and 9.1 with integrated ratios of 7:2:3, respectively.

N-(2-Propynyl)-1-naphthylamine (VII) was prepared by melting 1-naphthylamine and dropwise addition of 0.25 molar equivalent of propargyl bromide. The resulting pasty material was extracted with hexane in a Soxhlet extractor for 24 hours. The extract was washed with water and dilute ammonia, evaporated, and distilled under reduced pressure. The product was obtained in 23% yield (b.p. 84° C./0.11 mm. Hg, n_D^{25} 1.6449).

1-(Thiocyanomethyl)-naphthalene (IX) was obtained in 65% yield by dropwise addition of 1-chloromethylnaphthalene to potassium thiocyanate in *N*,*N*-dimethyl-

formamide. The product was recrystallized from ether-benzene (m.p. 83–86° C.).

The dipropynyloxynaphthalenes (XV to XXII) were prepared from the corresponding naphthalenediols, 2 mole equivalents of sodium metal, and 2.2 mole equivalents of propargyl bromide. 1,2-Dihydroxynaphthalene (m.p. 60–62° C.,) and 1,4-dihydroxynaphthalene (m.p. 173–76° C.) were obtained according to Fieser *et al.* (1939) by reduction of the corresponding naphthoquinones.

Vogel's (1962) procedure for the synthesis of 1-hexyn was utilized in the synthesis of 1-propargylnaphthalene (VIII) from 1-chloromethylnaphthalene and sodium acetylide. The product, a viscous oil, n_D^{25} 1.6569, was distilled through a falling film molecular still (b.p. 120° C./0.03 mm. Hg).

Tritium-labeled 1-naphthyl 2-propynyl ether was synthesized from ring-labeled 1-naphthol and 3-bromopropyne in the presence of potassium carbonate. The tritiating technique of Yavorsky and Gorin (1962) was used to label 1-naphthol by direct contact with tritiating reagent $H_2TPO_4 \cdot BF_3$. Five hundred milligrams of 1-naphthol were tritiated and used without further purification in the next step. The final product was purified on a 2 × 30 cm. column packed with 60- to 100-mesh silicic acid (Florisil). The sample was eluted from the column with the following sequence of solvents: hexane-ether (3 to 1) 200 ml., hexane-ether (1 to 3) 200 ml., ether 200 ml., and methanol 200 ml. 1-Naphthyl 2-propynyl ether was eluted in the hexane-ether (1 to 3) fraction and the impurities were eluted in the ether and methanol fractions. The compound had the same R_f value as an authentic sample of 1-naphthyl-2-propynyl ether by TLC [R_f = 0.78 with ether-hexane (3 to 1) on silicic acid plates]. The total yield was 150 mg. (23.4%) of light yellow oil of 99.3% purity with specific activity of 0.32 mc. per millimole.

A chromogenic spray reagent was developed for the detection of propynyl naphthyl ether and some of its metabolites by modification of Feigl's (1960) test for terminal acetylenic compounds. This consisted of two solutions: (1) cupric chloride (1.5 grams) and ammonium chloride (3 grams) in 20 ml. of concentrated ammonium hydroxide and water, and (2) hydroxylamine hydrochloride (5 grams) in 50 ml. of water. One part of solution 1 was mixed with 2 parts of solution 2 just before spraying directly on the chromatogram and terminal acetylene groups were detected as bright yellow spots. The limit of detection varied with the compound but was less than 3 μg.

Bioassay. Three-day-old, susceptible female houseflies (*Musca domestica* L., S_{NAIDM} strain) were treated topically with synergist-carbaryl combinations at 5 to 1 ratio (w./v.) in acetone. One-microliter droplets were applied to the thoraces of the flies, which were kept under carbon dioxide anesthesia during the treatment. The treated insects were provided with sugar solution and held in a 20° C. constant temperature room for 24 hours. Carbaryl was chosen because of its low insecticidal activity to the housefly when evaluated alone (extrapolated LD_{50} = 900 μg. per gram). Thus, determination of the synergistic ratio (SR) gives a useful method for comparing a large series of compounds with a broad range of synergistic activity. Each synergist-carbaryl dosage was replicated on three different days; the average per cent mortalities were plotted on log-probit paper and the dosage-mortality regression lines fitted by eye. None of the synergists were toxic at the concentrations employed. The toxicity studies to male Swiss white mice were carried out by oral administration of the compound in olive oil. The treated animals were kept at 80° F. with food and water and observed for toxic effects over a 24-hour period.

DISCUSSION OF RESULTS

Structure-Activity Relationships. Because of the pronounced synergistic activity of 1-propynyloxynaphthalene (SR 58), this compound was considered the reference compound for the comparison with other synergists.

Effects of Changes in Propynyloxy Moiety. Changes were made in both the triple bond and ethereal oxygen (Table I). Compounds I, II, and III show the results of stepwise saturation of the triple bond, which had a profound effect on synergistic activity. Changing the triple bond to a double bond (II) resulted in a decrease to 0.25 of the original activity (SR 58 to 15). Further saturation to *n*-propyloxy (III) caused an additional decrease to SR 7.8. The isopropyloxy analog (IV) was substantially less active (SR 4.0).

Further effects of side chain variation were evaluated by keeping the propynyl moiety constant and replacing the ether oxygen or omitting it entirely. Here again a considerable variation in biological activity results from relatively small structural changes. Table I shows that replacement of oxygen by sulfur (VI) decreased the SR to 0.4 of its original value. This decrease is much greater than that reported by Wilkinson *et al.* (1966) for the substitution of a single sulfur atom into the 1,3-benzodioxole moiety. Substitution of a nitrogen atom (VII) for the oxygen compound (I) reduced activity to about 0.1 of its original value. Compound VIII, where the ethereal oxygen is completely missing (SR 11.9), suggests that the presence of the oxygen atom, although important for high activity, is not essential.

Three additional compounds (IX, X, and XI) were synthesized to establish further the requirements for synergistic activity and to compare the propargyloxy-type synergists with some of the better known compounds such as the organothiocyanates and the methylenedioxynaphthalenes. 1-Naphthyl thiocyanomethane (IX), which synergizes carbaryl 15-fold, is structurally similar to 1-propynyloxynaphthalene in molecular size and in the position of the acetylenic triple bond relative to the cyano triple bond.

2-Methoxymethyloxynaphthalene (X) is a key compound in the comparison of methylenedioxynaphthalene with propynyloxynaphthalene. It has an "open" methylenedioxy grouping, and it also resembles the propynyloxy group. In addition to the aryl-alkyl ether oxygen, an area of high electron density around the second oxygen corresponds to the high electron density of the triple bond, although the 2*p* electrons of oxygen do not form a cylindrical dense cloud similar to that caused by the two π bonds in an acetylenic molecule. As indicated in Table I, 2-methoxymethyloxynaphthalene is inactive as a synergist, suggesting that there is a configurational requirement in addition to the electronic requirement for the activity of

Table I. Effects of Side Chain Variations on the Synergistic Activity of Propynyloxynaphthalene Type Compounds with Carbaryl against S_{NAIDM} Houseflies

	Substituted Naphthalene	B.P., °C./Mm. Hg	Analysis, % Theory	Analysis, % Found	Synergistic Ratio
I	1-$OCH_2C{\equiv}CH$	76/0.12	C 85.67 H 5.54	C 85.92 H 5.09	58
II	1-$OCH_2CH{=}CH_2$	78–80/0.1	C 84.77 H 6.56	C 84.24 H 6.77	15
III	1-$OCH_2CH_2CH_3$	77–78/0.11	C 83.96 H 7.56	C 83.70 H 7.85	7.8
IV	1-$OCH(CH_3)_2$	91/0.4	C 83.86 H 7.56	C 84.05 H 7.79	4.0
V	2-$OCH_2C{\equiv}CH$	58–60 (m.p.)	C 85.67 H 5.54	C 85.80 H 5.63	69.2
VI	2-$SCH_2C{\equiv}CH$	96/0.1	C 78.79 H 5.09	C 79.18 H 5.32	28.1
VII	1-$HN\text{-}CH_2C{\equiv}CH$	94/0.11	C 86.21 H 6.07	C 86.40 H 6.31	7.2
VIII	1-$CH_2C{\equiv}CH$[a]	120/0.03 (FFS)			11.9
IX	1-$CH_2SC{\equiv}N$	83–86(m.p.)	C 72.34 H 4.52	C 72.54 H 4.24	15
X	2-OCH_2OCH_3	192–194(m.p.)	C 76.55 H 6.43	C 77.09 H 6.33	1
XI	2-$OCH_2\underset{\diagdown\,\diagup}{O}CH\text{-}CH_2$[b]				1
XII	1-$OCH_2C{\equiv}CCH_3$	85/0.05	C 85.68 H 6.17	C 85.38 H 6.29	133.5
XIII	1-$OCH_2CH_2C{\equiv}CH$	103/0.05(FFS)	C 85.68 H 6.17	C 86.50[c] H 6.87	176.5
XIV	1-$OCH_2CH_2CH_2C{\equiv}CH$	120/0.01(FFS)	C 85.68 H 6.70	C 85.23 H 6.90	75

[a] Insufficient material for analysis $n_D^{25} = 1.6569$. NMR spectrum consistent with indicated structure.
[b] Compound prepared by J. Hyman.
[c] Insufficient compound for redistillation, analysis on crude product.

methylenedioxynaphthyl synergists, and that there is little if any relationship between this group and the propynyloxynaphthalenes.

Another compound in this series, 3-(2-naphthoxy)-1,2-epoxypropane (XI), is completely inactive as a synergist, stressing the importance of a triple bond system rather than rigidity and electronic density, since the epoxy grouping is rigid and also has high electron density around the oxygen.

Effects of Changes in the Acetylenic Hydrogen and Position of the Triple Bond. Terminal acetylenic compounds are known to react readily with metals such as sodium, copper, and silver to form insoluble salts. It was of interest to determine whether the propynyloxy-containing synergists react similarly with metal ions such as copper and iron, which are essential for the activity of the degrading enzymes. It was shown by Mason *et al.* (1965) that metal-binding reagents will inhibit the final step in the microsomal oxidation of foreign compounds. The reaction of metals with the acetylenic hydrogen would not, of course, be feasible in a nonterminal acetylenic group, although the interaction of transition series metals by ligand bond formation directly with the triple bond is still possible. A compound having a methyl group substituted for the acetylenic hydrogen—i.e., 2-butynyl naphthyl ether (compound XII, Table I)—was prepared. Since this compound showed high synergistic activity (SR 133.5), being almost twice as active as the propynyloxy homolog, it appears that the activity of these compounds is related to their triple bond and not the presence of the acetylenic hydrogen.

The effects of changes in the triple bond with relation to the naphthalene nucleus are also summarized in Table I. Comparison of 1-propynyloxynaphthalene (I) with 2-propynyloxynaphthalene (V) indicates the similarity of their biological activities with synergistic ratios of 58 and 69.2, respectively. The slightly better synergistic activity of the β-substituted naphthalene could be a result of better fit on the carbamate-detoxifying enzyme surface.

Considerable differences in activity were found when

the length of the side chain was varied. 3-Butynyl 1-naphthyl ether (XIII) was the most potent synergist evaluated in the naphthyl ether series (SR 176.5), whereas the activity of the next higher analog—i.e., 4-pentynyl 1-naphthyl ether (XIV)—dropped to SR 75. Synergistic activity in this series is maximum in the compound having a four-carbon side chain, and further increase in the chain length results in decline of activity (Figure 1).

Effects of Substitution with Two Propynyloxy Groups. Eight compounds having two propynyloxy side chains were synthesized and evaluated (compounds XV to XXII, Table II). Since α- and β-propargyloxynaphthalenes have similar SR values of 58 and 69.2, respectively, to find such a broad spectrum of activity among the disubstituted compounds with SR values ranging from less than 8 to as high as 138 was of considerable interest. It appears that introduction of a second propynyloxy side chain either substantially reduces the effectiveness of the compound or increases the activity to twice that of the monosubstituted compound.

The considerable differences in the activity of this series are difficult to explain. It seems that homonuclear substitution is associated with a marked decrease in activity, especially when the two side chains are ortho to each other —i.e., the 1,2-(XV) and 2,3-(XVI) derivatives. With heteronuclear substitution, some of the compounds were better synergists than the monosubstituted analogs. The most active compound evaluated in this series, 1,6-dipropynyloxynaphthalene (XIX), showed an SR value of 138, roughly twice as active as either the α- or the β-propynyloxynaphthalene. Two additional analogs, 1,5- and 1,7-dipropynyloxynaphthalenes (XVIII and XX) with SR values of 94.5 and 72, respectively, were more active than the monosubstituted derivatives. These differences cannot be related to distances between the two side chains, nor do they seem to have any correlation with the ability of certain analogs to resonate and form conjugated systems. Fukuto *et al.* (1964) found extremely wide variations in the toxicity of diethyl mononitronaphthyl phosphates to the housefly, although all had very similar I_{50} values for fly-brain cholinesterase inhibition. Their data showed no direct correlation between toxicity and anticholinesterase activity. The authors explained the differences in toxicity of the nitronaphthyl diethyl phosphates on the basis of in vivo detoxification rates. It may be that the inconsistency in activity of the dipropynyloxy derivatives can also be explained on the basis of differences in in vivo metabolism of the various analogs.

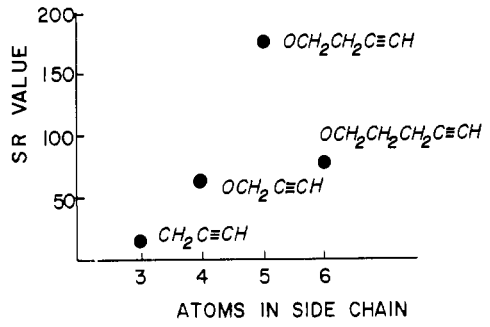


Figure 1. Effects of variations in length of unsaturated side chains on synergistic ratios of naphthyl derivatives with carbaryl

Effect of Nuclear Substitution. Wilkinson *et al.* (1966) demonstrated the importance of nuclear substitution in the case of methylenedioxyphenyl synergists. The unsubstituted 1,2-methylenedioxybenzene is completely inactive as a carbamate synergist, but when substituted in the 5-position with a variety of simple substituents the product is highly effective. The unsubstituted propynyloxybenzene is also inactive but, as in the case of methylenedioxyphenyl derivatives, becomes extremely effective when the benzene nucleus is substituted with chlorine or nitro groups (Kooy, 1966). In the case of naphthalene-based synergists, Metcalf *et al.* (1966) demonstrated that the unsubstituted 1,2- and 2,3-methylenedioxynaphthalenes are among the most active synergists yet evaluated. These authors have shown that the high degree of activity was retained when simple electron-donating groups were incorporated into the naphthalene nucleus.

Similarities are found between methylenedioxy- and propynyloxynaphthalenes. As evident from 1- and 2-propynyloxynaphthalene (compounds I and V), ring substitution is not essential for high synergistic activity. In contrast to the methylenedioxynaphthalenes, all nuclear substituents other than the propynyloxy moiety itself (compounds XXIII to XXIX, Table III) decreased the synergistic activity of propynyloxynaphthalene. Substitution of either an electron-donating group such as methoxy (XXIII) or weakly electron withdrawing groups such as halogens (XXIV and XXV) brought a drop of activity to approxi-

Table II. Effect of Dipropynyloxy Substitution on Synergistic Activity of Naphthalene Derivatives with Carbaryl against S_{NAIDM} Houseflies

	Substituted Naphthalene	M.P., °C.	Yield, %	Analysis, % (Theory C 81.35, H 5.15)		Synergistic Ratio
XV	1,2-Dipropynyloxy	81–82	30	C 81.17	H 5.41	8
XVI	2,3-Dipropynyloxy	113–15	35	C 81.03	H 4.80	30
XVII	1,4-Dipropynyloxy	90.5–92	23	C 82.07	H 5.60	40
XVIII	1,5-Dipropynyloxy	145–48	36	C 81.73	H 4.95	94.5
XIX	1,6-Dipropynyloxy	134–36	56	C 80.93	H 5.55	138
XX	1,7-Dipropynyloxy	88–89.5	73	C 81.57	H 5.63	72
XXI	2,6-Dipropynyloxy	135–38	39	C 80.78	H 4.92	38
XXII	2,7-Dipropynyloxy	91–95	45	C 81.93	H 5.48	48.6

Table III. Effect of Nuclear Substitution on Synergistic Activity of Propynyloxynaphthalene-Type Synergists

	Compound	M.P., °C.	Analysis, % Theory	Analysis, % Found	Synergistic Ratio
XXIII	3-Methoxy-2-propynyloxynaphthalene	84–86	C 79.26 H 5.66	C 78.91 H 5.88	30
XXIV	6-Bromo-2-propynyloxynaphthalene	89–92	C 59.81 H 3.45	C 59.43 H 3.80	33.3
XXV	4-Chloro-1-propynyloxynaphthalene	128–31	C 72.22 H 4.30	C 72.45 H 4.22	30
XXVI	2,4-Dichloro-1-propynyloxynaphthalene	175 d.	C 62.22 H 3.12	C 62.02 H 3.18	51.2
XXVII	2,4-Dinitro-1-propynyloxynaphthalene	180–85 d.	C 57.37 H 2.94	C 57.72 H 3.05	1
XXVIII	6-Nitro-propynyloxynaphthalene	168–70	C 68.70 H 4.00	C 68.89 H 3.58	1
XXIX	5,6,7,8-Tetrahydro-1-propynyloxy-naphthalene	76–80	C 83.88 H 7.52	C 83.55 H 7.77	25
XXX	4-Benzothienyl propynyl ether	100/0.3 (b.p.)	C 69.12 H 5.79	C 68.83 H 5.59	72

mately 0.5 of the parent compounds. Of special interest is compound XXVI, 2,4-dichloro-1-propynyloxynaphthalene, which had an SR value of 51.2 and a molar activity equivalent to the unsubstituted compound. Similar results were obtained by Fahmy and Gordon (1965), who evaluated a large series of aryloxyalkylamines as carbaryl synergists and found that 2,4-dichloronaphthoxyethyl diethylamine was highly synergistic, being about 100-fold more active than the unsubstituted naphthoxy derivative. The authors could not satisfactorily explain this phenomenon, and only speculated that the high activity of the 2,4-dichloro derivative might be associated with steric fit. Unfortunately, the 2,4-dinitro analog (XXVII) was insoluble in acetone and could not be compared with the dichloro compound. 5,6,7,8-Tetrahydro-1-propynyloxynaphthalene (XXIX), which is an isostere of compound I, has a low SR value of 25.0, indicating that the aromatic nature of the naphthalene nucleus is important for the synergistic activity. Finally, another isostere of compound I, the 4-benzothienyl propynyl ether (XXX), was shown to be slightly better than the parent compound. This observation is consistent with the similar SR values of 2,3-methylenedioxynaphthalene (180) and 5,6-methylenedioxybenzothiophene (173) with carbaryl (Sacher, 1967).

Summary of Structure-Activity Relationships. Maximum synergistic activity is associated with an alkynyloxy side chain attached to the naphthalene nucleus.

The triple bond may be either terminal or nonterminal.

The ethereal oxygen may be replaced by a sulfur atom with but a slight decrease in activity.

Heteronuclear, dipropynyloxynaphthalenes exhibit synergistic activity higher than the monopropynyloxy derivatives, approaching the maximum of twofold increase of activity in the 1,6-analog.

Nuclear substitution is associated with a decrease in synergistic activity in all compounds evaluated in this study of naphthyl ethers.

Spectrum of Activity of Propynyloxy-Type Synergists. Table IV shows the effect of 2-propynyl 1-naphthyl ether on the activity of Chlorthion and allethrin. The enzymatic conversion of Chlorthion, *O,O*-dimethyl *O*-3-chloro-4-nitrophenyl phosphorothioate, a P=S compound to the active anticholinesterase chloroxon (P=O analog) is mediated by the mixed function oxidases. The activating enzymes are readily inhibited by synergistic compounds such as piperonyl butoxide, sesamex, MGK 264, SKF-525A, etc. (Metcalf, 1967). In this case, these compounds are acting as "antagonists" by preventing the activation of the phosphorothionates to phosphates. Sun and Johnson (1960) found an SR value of 0.30 when 1% sesamex with Chlorthion was sprayed on houseflies. The data in Table IV show that the same degree of antagonism to Chlorthion was achieved with 5 parts of propynyloxynaphthalene in topical application.

Table IV. Effects of Cotreatment with 2-Propynyloxynaphthalene on Toxicity of Chlorthion and Allethrin to Snaidm Houseflies

	LD_{50} μg./fly	LD_{50} μg./g.	Synergistic Ratio
Chlorthion alone	0.185	9.25	...
Chlorthion + propynyl naphthyl ether[a]	0.48	25.0	0.37[b]
Allethrin alone	0.43	21.5	...
Allethrin + propynyl naphthyl ether[a]	0.144	7.2	3.7

[a] Ratio 1:5 (insecticide:synergist).
[b] Antagonistic ratio 2.7.

The toxicity of allethrin is increased slightly by synergists in comparison to pyrethrins, presumably because of the difference in the side chain of the alcohol moiety (Metcalf, 1967). It has been found in this laboratory that the LD_{50} values for the housefly of allethrin with 5 parts of piperonyl butoxide and 2,3-methylenedioxynaphthalene are 4.5 and 9.5 μg. per gram, respectively. Thus, the 7.2 μg. per gram value shown in Table IV for allethrin with propynyloxynaphthalene indicates once again that propynyloxynaphthalenes have the same spectrum of activity as methylenedioxyphenyl synergists.

Metcalf *et al.* (1967) have shown that piperonyl butoxide, Lilly 18947, and Thanite interfered with the degradation of carbamates in houseflies. A comparative study on the effect of various synergists in retarding the detoxification of a carbamate insecticide is presented in Table V. This effect was studied by pretreating the houseflies with 50 μg. of synergist, applied to the ventral abdomen so as not to influence the application of 2-isoprop-^{14}C-oxyphenyl *N*-methylcarbamate (Baygon), which was applied 1.5 hours later to the dorsal prothorax. The output of $^{14}CO_2$ and other ^{14}C volatiles over the 24-hour period was sharply reduced to 0.30 to 0.55 of the amount produced without synergist. Piperonyl butoxide was most active in this series, while 2-(2-nitro-4-chlorophenyl) propynyl ether was the least effective in retarding the *O*-dealkylation of the side chain of Baygon to $^{14}CO_2$ and other volatiles such as acetone (Shrivastava, 1967). These data suggest that all seven synergists, including the new group of propynyloxyaryl compounds, act on the same detoxification enzyme which is active in *O*-dealkylation.

It appears that the propynyloxy-type synergists are closely related in their action to the methylenedioxyphenyl synergists. The two classes of compounds synergize carbamates and pyrethrins and antagonize certain phosphorothionates. However, the magnitude of the synergistic action of the individual compounds of both types is dependent upon complex factors involving penetration and detoxication of the synergist in the insect and in all probability upon its specific inhibitory effect on the mixed function oxidases responsible for insecticide metabolism (Metcalf, 1967).

Table V. Effects of Synergists on Metabolism of 2-Isoprop-^{14}C-oxyphenyl *N*-Methylcarbamate R_{MIP} Flies Pretreated 1.5 Hours with 50 μg. of Synergist

Pretreatment	Applied Dose, μg./♀	Per Cent of Applied Dose, 24 Hr. Absorbed	$^{14}CO_2$ and other ^{14}C volatiles	Excreted
No synergist[a]	0.5	97.6	31.6	17.4
Piperonyl butoxide	1.0	85.4	6.8	3.1
Lilly 18947[a]	0.5	91.6	16.7	6.0
Thanite[a]	0.5	81.3	14.7	6.4
2,3-Methylene dioxynaphthalene	1.0	94.2	15.0	10.8
2-(2-Nitro-4-chlorophenyl)-propynyl ether	1.0	93.8	18.8	...
2-Propynyl 1-naphthyl ether	1.0	96.8	9.5	7.2

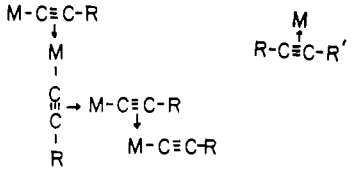
[a] Data from Metcalf *et al.* (1967).

Mode of Action of Naphthyl Propynyl Ether and Related Compounds. There are only few reports in the literature concerning the activity and mode of action of compounds containing a triple bond. Recently, Dillard *et al.* (1967) published a study on the structure-activity relationships of acetylenic carbamates which are active as oncolytic agents. The structural requirements for potent antitumor agents parallel those for synergistic activity. Reduction of the acetylenic group resulted in a large drop in activity. Substitution of the terminal acetylenic hydrogen (with either methyl or bromine) did not appear to alter the antitumor effect.

The structure-activity relationships of *N*-methyl-*N*-propargylbenzylamine and analogs have shown again the same requirements for the inhibition of monoamine oxidase (Swett *et al.*, 1963). It was suggested that the inhibition occurred by reaction of the triple bond with a sulfhydryl group of the enzyme to form an irreversible complex. Therefore, it is possible that the propynyloxynaphthyl synergists interfere with the mixed function oxidases through the formation of an irreversible complex with an essential —SH group (Brady, 1963).

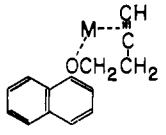
A second, possibly more attractive, suggestion for the mode of action of acetylenic synergists is through complex formation with certain transition metals such as iron, copper, or magnesium. Some of these metals are known to play an essential role in a variety of metabolic pathways, especially as cofactors for some enzymes. Certain metal chelating agents are good inhibitors of the microsomal oxidation of foreign compounds (Mason *et al.*, 1965), suggesting that a metal (probably iron) assumes an essential role in this oxidative process.

Some work has been carried out on acetylene complexes of silver, copper, and other metals. Distribution and solubility studies have been made of the interaction of hex-3-yne, methyl-substituted hex-3-ynes, and hept-2-yne with silver nitrate (Dorsey and Lucas, 1956; Helmkamp *et al.*, 1957). Evidence for the formation of complexes containing one and two molecules of acetylene per silver ion was obtained, and in some cases, solid complexes could be isolated. Black *et al.* (1959) suggested that the complexes of copper and silver of the general formula RC≡CM (R = alkyl, aryl; M = metal) are not simple acetylides, but coordination monomers and polymers in which the triple bond is bound to the metal atom.



The propynyloxyaryl synergists may react in a similar manner to complex the microsomal iron which participates in the electron transfer chain, thus inhibiting the cytochrome P-450 that is responsible for the oxidation of drugs and other foreign compounds. This hypothesis is consistent with the data presented on the structure-activity relationships of these compounds above. It explains the

activity of compounds containing nonterminal acetylenic bonds, the lower activity of the analog containing a double bond, and the high activity of 3-butynyloxynaphthalene. The latter was the most active synergist in this series, while either a decrease or increase in the side chain length was associated with a gradual fall in synergistic activity. The butyn derivative is able to form a strainless ring structure while chelating a metal as follows:



whereas the propynyloxy or pentynyloxy analogs would have either a four- or a six-membered ring, respectively, which are known to be less stable.

Selectivity of Synergism in Fly and Mouse. The comparative effects of carbaryl plus propynyl aryl ether synergist in insect and mammal are of considerable theoretical and practical importance. In contrast to the topical LD_{50} for the housefly of carbaryl plus 1-naphthyl 2-propynyl ether (1 to 5) of 15.5 mg. per kg. as carbaryl, the same combination produced no apparent toxic effects in the male Swiss white mouse when administered orally at 400 mg. per kg. of carbaryl plus 2000 mg. of 1-propynyl naphthyl ether in olive oil. Similarly a mixture of carbaryl and 4-benzothienyl 2-propynyl ether (1 to 1) produced no indication of toxic symptoms in the mouse at 750 mg. per kg. as carbaryl. It appears that the selectivity factor of these propynyloxy synergists for the housefly over mouse is of the order of 50- to 100-fold, of the same order of magnitude as is found in the companion study of 2,3-methylenedioxynaphthalene and carbaryl (5 to 1) which had a topical LD_{50} for the housefly of 5.0 mg. per kg. and an oral LD_{50} to the male mouse of >750 mg. per kg. (as carbaryl) (Sacher *et al.*, 1968). It seemed most probable that this impressive selectivity was related to the comparative metabolism of 1-naphthyl 2-propynyl ether in the fly and mouse and this was investigated in some detail.

Metabolism in Housefly. The penetration of radioactive 2-propynyl 1-naphthyl ether, applied topically at dosages up to 100 μg. per gram, was rapid and complete. Six hours after treatment, 96% of the applied dose had penetrated the insects. The rate of excretion of metabolites in the housefly was slow and did not exceed 5% within the first 24 hours.

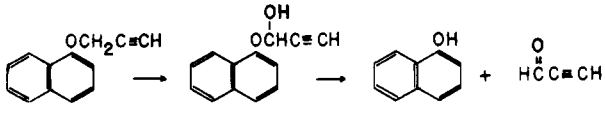
Chromatography of metabolites on a silicic acid column showed that the parent compound is very stable in vivo and is metabolized slowly. Table VI summarizes the results of an experiment in which flies were treated topically with synergist 12 hours prior to extraction of the metabolites. The hexane eluent which represents 75% of the total dose was identified as 2-propynyl 1-naphthyl ether (parent compound) by comparing R_f values, the reaction with chromogenic agent, and the infrared spectrum of the extracted material with an authentic sample. The methanol eluent was partially (30%) hydrolyzed with β-glucosidase, indicating the presence of glucosidic ester of a naphtholic derivative.

Table VI. Recovery of Radioactive Fractions from S_{NAIDM} Houseflies Topically Treated with 100 μg. per Gram of H^3-Propynyloxynaphthalene

	% of Applied Dose after 12 Hours at 60° F.
Radioactivity remaining on surface	0.4
Column chromatography of internal metabolites	
Hexane eluent (R_f 0.74)[a]	75
Hexane-ether (3:1) eluent	0.02
Hexane-ether (3:1) eluent (R_f 0.55)[a]	0.04
Ether eluent	Trace
Chloroform eluent	Trace
Ethyl acetate eluent	...
Methanol eluent	2.1
Water-extractable metabolites	2.0
Holding-jar wash (excreta)	4.6
Total recovery	87.76

[a] R_f value with ether-hexane (3:1) on TLC.

The fecal extract contained some 1-naphthol (20% of total excreted). A naphtholic derivative may result from an oxidative attack on the α-carbon of the propargyloxy side chain with a subsequent split to yield propargylaldehyde and 1-naphthol:



Five per cent of the parent compound was also present in the feces. Two additional unidentified metabolites with R_f's of 0.00 and 0.17 in ether-hexane (3 to 1) system gave a pink color with diazonium fluoroborate and base. These compounds, which represented the majority of the radioactivity in the excreta, are probably conjugates of the parent compound.

Metabolism in the White Mouse. In contrast to the remarkable stability of 2-propynyl 1-naphthyl ether in houseflies, where 75% of the applied dose (or 87% of the recovered dose) was still intact 12 hours after treatment, the compound is rapidly metabolized in mice to water-soluble products. As much as 72% of the applied dose (10 mg. per kg.) appeared in the urine within 18 hours after dosage. The 30- and 48-hour recoveries were 82 and 90%, respectively.

The partition coefficient of the mixed components in a urine sample between water and chloroform was 31.2, indicating that some of the metabolites were apolar. Analysis of the urine on TLC with butanol-ethanol-water (10:2:3) system revealed four radioactive metabolites with R_f values of 0.33, 0.45, 0.6, and 0.73 in 9:1:3:1.5 ratios, respectively. The first three metabolites gave a positive reaction with diazonium fluoroborate spray and the first reacted also with the cupric-hydroxylamine spray for terminal acetylenic derivatives.

The results of a more elaborate study utilizing ion exchange chromatography are summarized in Figure 2. Two gradients of tris-HCl buffer were used as described by Knaak *et al.* (1965) for the separation of carbaryl metabolites. No trace of 1-naphthyl sulfate could be de-

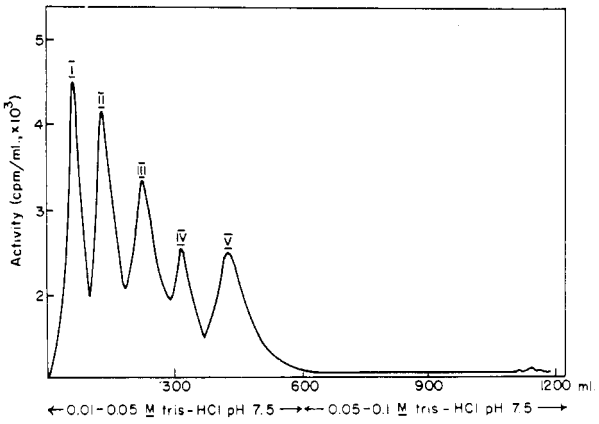


Figure 2. Ion exchange chromatography of urinary metabolites from mice treated with H^3-labeled 1-naphthyl 2-propynyl ether

tected, although appreciable amounts of 1-naphthyl glucuronide were present (peak 5). Knaak *et al.* (1967) found at least four glucuronic acid conjugates of carbaryl and 1-naphthol in guinea pig and rat urine, all of which were eluted in the first 500 ml. of tris buffer. In the present study, as many as five radioactive metabolites were eluted from the column with the first 500 ml. of solvent. One is 1-naphthylglucuronide and the others are probably products of hydroxylation of the aromatic rings and subsequent conjugation.

The striking differences in the metabolism of the synergist between houseflies and mice was demonstrated by the high recoveries of the unchanged parent compound in insects as opposed to its rapid deactivation and excretion by mice. These results are consistent with the finding that propargyloxynaphthalene shows synergistic activity only in insects. This mixture of synergist and carbaryl represents, therefore, an exceptionally interesting example of a highly selective insecticide.

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