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Selective Toxicity of N-Sulfenylated Derivatives of Insecticidal Methylcarbamate Esters

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An investigation of the toxicological properties of a series of N-arylsulfenyl and N-alkylsulfenyl derivatives of several commercial methylcarbamate insecticides has been carried out. Compared to the parent insecticides, the derivatives display significantly reduced toxicity to the white mouse and S-aryl derivatives are less toxic to the mammal than the alkylsulfenamides. All of the derivatives were more effective as mosquito larvicides than the parent methylcarbamates and many compounds were highly toxic to houseflies. Toxicity to houseflies was enhanced by the synergist piperonyl butoxide. The selective toxicity of the N-sulfenylated carbamates is not directly related to anticholinesterase activities.

The development of effective new insecticides with favorable toxicity to mammals is of continuing significance in insect control. One promising approach to this problem is the design of compounds based on expected differences in the metabolic pathways which may take place in insects and mammals. Previous investigation in our laboratory has shown that selectivity of the desired order between insects and mammals can be achieved upon substitution of the proton on the N-methyl moiety of insecticidal methylcarbamate esters with a dialkoxyphosphinothioyl group, as typified by the structure below (Fahmy et al., 1970).

$$R = (CH_3O)_2P(=S)-$$
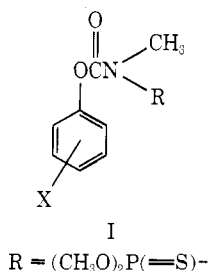
The success of this approach has prompted us to examine other carbamate esters similarly derivatized by substituents capable of modification by oxidative or hydrolyt-

ic processes in the animal. This report is concerned with the selective toxicity of a series of N-arylsulfenyl and N-alkylsulfenyl derivatives of commercial aryl methylcarbamate insecticides, i.e., where R is S-aryl or S-alkyl in structure I. Compounds of this type have recently received some attention in the patent literature (Brown and Kohn, 1972; Farbenfabriken-Bayer A.G., 1972) and one such compound (I, R = S-phenyl and X = 3-s-butyl) has demonstrated outstanding effectiveness against mosquito larvae and adults in the field (Schaeffer and Wilder, 1970).

MATERIALS AND METHODS

The methylcarbamate insecticides used in this study were obtained from their respective manufacturers as previously reported (Fahmy et al., 1970) or were synthesized in this laboratory. The thiols or disulfides were obtained commercially or were synthesized by common procedures. 2,4-Dimethylbenzenethiol (Gilman and Broadbent, 1947), 3,4-methylenedioxybenzenethiol (Dallacker and Zegers, 1965), and bis-(4-cyanophenyl)disulfide (Bauer and Cymerman, 1949) were prepared according to literature methods.

The sulfenyl chlorides were prepared immediately before use by reaction of the appropriate disulfide or thiol with an equimolar amount of sulfuryl chloride in an inert solvent (carbon tetrachloride or benzene), usually at reduced temperatures (Kühle, 1970; Lecher and Holschneider, 1924), although gentle heating was sometimes required to effect the reaction. In some cases, the sulfenyl halides were not distilled before use, owing to thermal instability (Mayer and Frey, 1964).

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Table I. Physical and Biological Properties of N-Arylsulfenyl and N-Alkylsulfenyl Derivatives of Insecticidal Methylcarbamates

Compd	R	Mp (bp/mm), °C	n^{25}D	Analysis Calcd	Found	Housefly LD_{50}, μg/g Alone	1:5 PB	Culex fatigans LC_{50}, ppm	Mouse (oral), mg/kg
				2,2-Dimethyl-2,3-dihydrobenzofuranyl-7-OC(=O)N(CH₃)SR					
1	Carbofuran	150–152				6.7	2.5	0.052	2
2	Phenyl	66–67		C, 65.65	C, 66.0	9.3	5.0	0.0045	25–50
				H, 5.78	H, 5.67				
3	4-Tolyl	78		C, 66.47	C, 66.71	9.0	3.3	0.0045	100–125
				H, 6.12	H, 5.72				
4	3-Tolyl	(185/0.005)	1.5758	C, 66.47	C, 66.48	6.5	3.0	0.004	25–50
				H, 6.12	H, 5.84				
5	2-Tolyl	64–65		C, 66.47	C, 66.29	3.7	1.8	0.004	100–125
				H, 6.12	H, 6.21				
6	2,4-Xylyl	(182/0.005)	1.5165	C, 67.23	C, 66.73	9.0	4.0	0.003	50–100
				H, 6.44	H, 6.89				
7	4-*tert*-Butylphenyl	122–123		C, 68.57	C, 68.38	2.7	2.3	0.0025	75
				H, 7.01	H, 7.12				
8	2-Me-4-*tert*-butylphenyl	109		C, 69.17	C, 69.60	7.5	3.3	0.002	75–125
				H, 7.27	H, 6.57				
9	2-Isopropylphenyl	70–71		C, 67.92	C, 68.03	8.3	4.0	0.003	50–75
				H, 6.74	H, 6.85				
10	4-Cl-phenyl	73		C, 59.44	C, 59.38	12.5	4.6	0.002	50
				H, 4.95	H, 4.92				
11	4-Br-phenyl	60–60.5		C, 52.94	C, 53.09	9.0	4.6	0.004	50–75
				H, 4.41	H, 4.44				
12	4-Br-2-Me-phenyl	81		C, 54.03	C, 54.31	11.3	3.3	0.0025	100–150
				H, 4.72	H, 4.52				
13	Penta-Cl-phenyl	153–154		C, 43.11	C, 42.74	26.5	6.0	0.0085	50
				H, 2.79	H, 3.07				
14	4-MeO-phenyl	79–80		C, 63.51	C, 63.77	7.8	3.0	0.006	25–50
				H, 5.85	H, 6.09				
15	3,4-OCH₂O-phenyl	80		C, 61.13	C, 61.10	5.0	3.3	0.0065	10–25
				H, 5.09	H, 5.14				
16	4-CN-phenyl	126		C, 64.41	C, 64.98	22.5	3.9	0.022	25–50
				H, 5.08	H, 5.20				
17	2-Naphthyl	78–79		C, 69.66	C, 69.68	8.0	4.3	0.0032	40
				H, 5.54	H, 5.49				
18	2-Benzothiazolyl	97		C, 59.07	C, 58.82	8.5	4.4	0.0095	50
				H, 4.66	H, 4.76				
19	CH₃	70–71		C, 58.43	C, 58.66	4.9	1.65	0.026	20
				H, 6.37	H, 6.52				
20	C₂H₅	(122/0.005)	1.5353	C, 59.79	C, 60.44	12.8	6.0	0.024	10–15
				H, 6.76	H, 6.96				
				2-Isopropoxyphenyl-OC(=O)N(CH₃)SR					
21	Propoxur	85–87				24.0	5.6	0.33	24
22	Phenyl	(164/0.005)	1.5698	C, 64.35	C, 64.45	36.0	8.0	0.039	300
				H, 5.99	H, 6.05				
23	4-Tolyl	(169/0.005)	1.5668	C, 65.26	C, 65.34	36.0	3.9	0.028	350–400
				H, 6.34	H, 6.17				
24	3-Tolyl	(178/0.005)	1.5670	C, 65.26	C, 65.53	23.5	6.7	0.020	300–400
				H, 6.34	H, 6.37				
25	2-Tolyl	71.5–72.5		C, 65.26	C, 65.65	24.0	3.9	0.024	400
				H, 6.34	H, 6.45				
26	2,4-Xylyl	39–40		C, 66.09	C, 66.47	27.5	7.8	0.014	400
				H, 6.67	H, 6.68				
27	2-Isopropylphenyl	(175/0.005)	1.5600	C, 66.85	C, 67.28	28.5	8.5	0.013	500
				H, 6.96	H, 6.91				
28	4-*tert*-Butylphenyl	(183/0.005)	1.5562	C, 67.56	C, 68.07	9.0	4.5	0.013	750–1000
				H, 7.23	H, 7.12				
29	2-Me-4-*tert*-butylphenyl	89–90		C, 68.22	C, 68.09	24.5	6.5	0.014	>850
				H, 7.49	H, 7.31				
30	4-Br-phenyl	64–65		C, 51.52	C, 52.40	26.5	3.7	0.010	300–400
				H, 4.55	H, 4.58				
31	4-Br-2-Me-phenyl	50–51		C, 52.68	C, 53.26	37.0	5.6	0.020	400
				H, 4.88	H, 4.95				
32	CH₃	53		C, 56.47	C, 56.95	65.0	4.5	0.105	300–400
				H, 6.67	H, 6.92				

Table I (Continued)

Compd	R	Mp (bp/mm), °C	n^{25}D	Calcd	Found	LD$_{50}$, g/g Alone	1:5 PB	Culex fatigans LC$_{50}$, ppm	Mouse (oral), mg/kg
				3-Isopropylphenyl-OC(=O)N(CH$_3$)SR					
33	MIP	72–73				41.0	11.0	0.038	16
34	Phenyl	49		C, 67.80	C, 67.30	75.0	16.0	0.006	150–200
				H, 6.32	H, 6.02				
35	4-Tolyl	(172/0.005)	1.5672	C, 68.57	C, 69.09	92.5	19.5	0.005	50–100
				H, 6.67	H, 6.76				
36	4-tert-butylphenyl	a	1.5570	C, 70.59	C, 70.57	92.4	18.5	0.005	150
				H, 7.57	H, 7.74				
37	2-Me-4-tert-butylphenyl	a	1.5550	C, 71.16	C, 71.60	59.0	18.0	0.009	100–150
				H, 7.81	H, 8.08				
38	4-Cl-phenyl	(156/0.01)^b	1.5809	C, 60.80	C, 60.40	145	17.5	0.006	125
				H, 5.37	H, 5.37				
39	CH$_3$	a	1.5283	C, 60.25	C, 59.95	240	19.5	0.028	50–100
				H, 7.11	H, 6.90				
				CH$_3$SC(CH$_3$)$_2$CH=NOC(=O)N(CH$_3$)SR					
40	Aldicarb	99–100				5.5	3.4	0.16	0.3–0.5
41	2-Tolyl	a	1.5642	C, 56.37	C, 55.47	12.5	8.8	0.084	3–5
				H, 6.71	H, 6.86				
42	4-tert-Butylphenyl	54.5–55		C, 60.00	C, 59.71	7.5	3.2	0.014	10
				H, 7.64	H, 7.59				

^{a} Purified by column chromatography. ^{b} Purified by falling-film molecular distillation.

The following procedure for the synthesis of 2,3-dihydro-2,2-dimethylbenzofuranyl-7 N-methyl-N-(4-toluenesulfenyl)carbamate is typical for the synthesis of the N-sulfenyl methylcarbamates. Freshly distilled 4-toluenesulfenyl chloride (7.9 g, 0.05 mol) was added dropwise to a stirred solution of 11.0 g (0.05 mol) of 2,3-dihydro-2,2-dimethylbenzofuranyl-7 methylcarbamate (carbofuran or 1) in 100 ml of anhydrous pyridine. After stirring for 4 hr, 500 ml of water was added, and the product was extracted in 500 ml of hexane and washed free of pyridine with chilled dilute hydrochloric acid. After drying over sodium sulfate, concentration of the hexane solution gave a yellow product in 74% yield which was recrystallized from a mixture of ether–hexane, mp 64–65°. Pmr spectrum showed the following absorptions (chloroform-d TMS): δ 6.7–7.4 (m, 7 H, aromatic protons), 3.4 (s, 3 H, NCH$_3$), 3.0 (s, 2 H, -CH$_2$), 2.3 (s, 3 H, -CH$_3$), and 1.5 (s, 6 H, gem-di-CH$_3$). The downfield shift and singlet for NCH$_3$, which for carbofuran occurs as a broad doublet at δ 2.9, is consistent with substitution of the proton of the carbamoyl moiety. Infrared spectrum showed a strong carbonyl absorption at 1715 cm^{-1}. Elemental analysis is given in Table I.

Products were purified by crystallization from the solvents hexane, ether–hexane, and ethanol or aqueous ethanol. Liquid products were purified by distillation and, in some cases, by column chromatography on silica (Mallinckrodt SilicAR CC-7), eluting with ether–hexane (2:1).

Pmr spectra were recorded with a Varian T-60 spectrometer (chloroform-d TMS) and infrared spectra were recorded on a Perkin-Elmer 700A spectrophotometer using Nujol mulls.

Bimolecular rate constants (k_i) for the inhibition of housefly head and bovine erythrocyte acetylcholinesterase (AChE) were determined at 30.0°, pH 7.0, by procedures previously established for phosphate esters (Aldridge, 1950) using acetylthiocholine as the substrate (Ellman et al., 1961). The preparation of housefly head AChE has been described (Fukuto and Metcalf, 1956) and bovine erythrocyte AChE was purchased from Sigma Chemical Co. Mammalian toxicity was determined orally on Swiss white mice as described previously (Hollingworth et al., 1967). Mortalities were recorded after 72 hr. Insecticidal activities were de-termined against the susceptible S$_{NAIDM}$ strain of houseflies, Musca domestica, and 4th-instar mosquito larvae, Culex fatigans, according to usual procedures (Metcalf and March, 1949; Mulla et al., 1966).

Partition coefficients between 1-octanol and water were determined at room temperature using ^{14}C-radiolabeled compounds. The radioactivity in each phase was determined by means of a Model 3003 Packard Tri-Carb scintillation spectrometer. The synthesis of radiolabeled compounds will be described in a subsequent publication (Black et al., 1973).

RESULTS

Mouse Toxicity. The toxicological data presented in Table I show that, compared to the original methylcarbamates, the sulfenylated derivatives are substantially less toxic to the white mouse. Substitution of the nitrogen proton with an arylsulfenyl moiety reduced oral toxicity to the mouse by 10–50-fold and substitution with alkylsulfenyl was less effective, reducing mouse toxicity by only 5–17-fold. Although the sulfenyl derivatives of aldicarb (41 and 42) displayed the highest toxicity to mice among the various derivatives examined, a direct relationship between the toxicity of the parent methylcarbamate and sulfenylated products was not evident. For example, the derivatives of carbofuran, a highly toxic methylcarbamate, were 12–50-fold less toxic than carbofuran, while derivatives of 3-isopropylphenyl methylcarbamate and propoxur, compounds which are innately less toxic to mice, were made safer by factors of 8–12 and 12–17-fold, respectively. Although a large number of substituted arylsulfenylated derivatives were prepared, particularly with carbofuran and propoxur, no obvious correlation between substituent and mouse toxicity was apparent.

Insecticidal Activity. The derivatized carbamates were variable in their effectiveness against houseflies and mosquito larvae but on the whole were effective insecticides and, in many cases, were more toxic than the parent carbamate, particularly against mosquito larvae. Although no obvious relationship between structure and insecticidal activity was evident, activity appeared to be strongly dependent upon the nature of the ring substituent of the arylsulfenyl moiety. For example, compared to the parent

**Table II. Anticholinesterase Activities of Some N-Arylsulfenyl Derivatives of Aryl Methylcarbamates**

Compd	R	Bovine erythrocyte ChE (k_i M^{-1} min^{-1})	Fly head ChE (k_i M^{-1} min^{-1})
	2,2-Dimethyl-2,3-dihydrobenzofuranyl-7-OC(=O)N(CH₃)R		
1	H (carbofuran)	1.9×10^6	1.3×10^7
2	Benzenethio	8.1×10^5	9.8×10^5
5	o-Toluenethio	1.0×10^5	1.7×10^5
7	p-tert-Butylbenzenethio	7.8×10^4	6.7×10^5
	o-Isopropoxyphenyl-OC(=O)N(CH₃)R		
21	H (propoxur)	4.3×10^4	1.2×10^6
22	Benzenethio	2.9×10^4	9.8×10^6
25	o-Toluenethio	7.0×10^4	1.5×10^5
28	p-tert-Butylbenzenethio	7.4×10^3	5.2×10^4
	m-Isopropylphenyl-OC(=O)N(CH₃)R		
34	H	7.5×10^5	7.7×10^5
35	Benzenethio	1.2×10^5	3.7×10^4
36	p-tert-Butylbenzenethio	1.8×10^4	6.5×10^4

Figure 1. Suggested pathways for the metabolism of an N-aryl-sulfenyl derivative of carbofuran.

carbofuran, the benzenesulfenyl (2) and 4-toluenesulfenyl (3) derivatives were less toxic to the housefly, 3-toluene-sulfenyl (4) derivative was equitoxic, and the 2-toluene-sulfenyl (5) derivative was almost twofold more toxic. Of the various arylsulfenyl ring substituents, the 4-tert-butyl moiety appeared to produce derivatives with highest insecticidal activity. This is especially apparent with the 4-tert-butylbenzenesulfenyl derivatives of carbofuran (7) and propoxur (28). In general, ring substituents which were electron withdrawing gave compounds which were less toxic to houseflies, e.g., compounds 13 and 16. Whether this is attributable to substituent effects on the stability of the nitrogen–sulfur bond is not known.

The effect of the synergist piperonyl butoxide on house-fly toxicity is of considerable interest. As a rule, deriva-tives with greater intrinsic toxicity to the housefly were synergized to a much lesser extent by piperonyl butoxide than those of poorer toxicity. For example, most of the carbofuran derivatives were synergized about two- to three-fold, but synergism generally was much greater with the less toxic derivatives of propoxur and 3-isopropylphenyl methylcarbamate. The results suggest that activation of the derivatized carbamate, i.e., in vivo generation of the parent carbamate, is not an oxidative process and, also, that oxidative detoxication is the primary cause for the poor housefly toxicities of the less effective compounds, e.g., derivatives of 3-isopropylphenyl methylcarbamate.

Compared to the parent methylcarbamates, the sulfen-ylated derivatives were exceptionally toxic to mosquito larvae, particularly the derivatives of carbofuran and 3-isopropylphenyl methylcarbamate. In a separate test con-ducted for the World Health Organization screen against Anopheles albimanus, LC_{50} values of less than 0.001 ppm were obtained with some of the derivatives (Metcalf, 1972).

In nearly every case, introduction of a hydrophobic sub-stituent to the benzenesulfenyl moiety increased larvicidal activity, suggesting that lipophilicity of the carbamate derivative plays an important role in determining effec-tiveness against mosquito larvae. The high activity of the selective larvicide Abate has been attributed in part to its high lipophilicity, which promotes rapid absorption of Abate by mosquito larvae from water (Leesch and Fukuto, 1972). In this regard, the partition coefficient (1-octanol-water) for 5, the 2-toluenesulfenyl derivative of carbofu-ran, was estimated experimentally to be 2345, a value some 335-fold higher than the corresponding value of 7 for carbofuran. Assuming that the other sulfenylated deriva-tives also have similar partitioning characteristics, such high partition in favor of the nonpolar phase also suggests
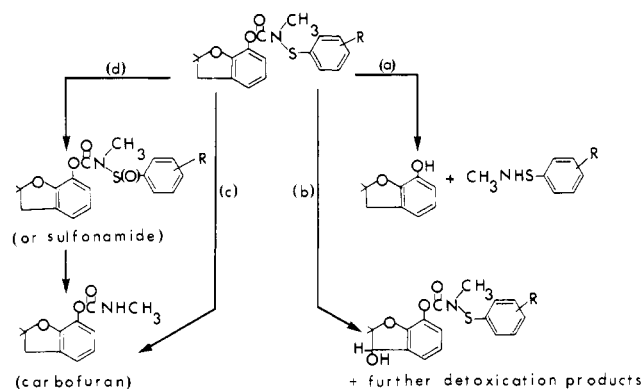
that these compounds are rapidly absorbed by the larvae. This undoubtedly would contribute to their effectiveness as mosquito larvicides.

Anticholinesterase Activity. Anticholinesterase data against bovine erythrocyte and housefly head AChE for a limited number of compounds are presented in Table II. The kinetic data for inhibition showed that the sulfenylat-ed carbamates behave as carbamylating agents, similar to the parent methylcarbamates, and therefore values for the bimolecular inhibition constant (k_i) were determined.

On an overall basis, the sulfenylated derivatives are poorer anticholinesterases than the parent methylcarbam-ates, although there are notable exceptions with the pro-poxur derivatives (22 and 25). In the case of bovine eryth-rocyte AChE, the reduction in activity with derivatization is more or less in agreement with the observed mouse tox-icity. However, there is little, if any, relationship between inhibition of housefly head AChE and toxicity to house-flies, particularly with the carbofuran derivatives. Unex-pectedly, the derivatives of propoxur were relatively strong inhibitors of housefly head AChE. This is contrary to the usual finding that introduction of bulky N substitu-ents diminishes anticholinesterase activity of aryl carba-mate esters (Metcalf and Fukuto, 1965).

DISCUSSION

By analogy with the rationale proposed for the selective toxicity of dialkylphosphinothioyl derivatives of methyl-carbamate esters (Fahmy et al., 1970), it seems likely that sulfenylation of insecticidal methylcarbamates can pro-vide additional opportunities for intoxication and detoxi-cation processes to take place in insects and mammals. The various metabolic pathways which may account for the observed selective toxicity are presented in Figure 1, using as an example a sulfenylated derivative of carbofu-ran.

The generally poor correlation between in vitro anticho-linesterase activity and toxicities to mice and insects suggests that metabolic processes in vivo are responsible for the observed selective toxicities of the derivatized car-bamates, although other factors, e.g., lipophilic properties of the molecule, also must be considered.

For the mouse, reduced toxicity is realized by metabolic paths (a) and (b). Direct hydrolysis of the derivatized car-bamate at the carbonyl carbon would produce nontoxic phenols and the unstable sulfenamide, route (a). Alterna-tively, oxidative attack at the reactive benzylic carbon in the carbofuran portion of the molecule should yield com-pounds with reduced toxicity which would be available for further metabolism or excretion, route (b). Thus, sulfeny-lation of carbofuran provides the mouse with additional opportunities for detoxication of the derivative.

Route (c), i.e., N–S bond cleavage, could occur prefer-entially in insects and produce the toxic carbamate,

carbofuran, nearer to the site of action. Liberation of carbamate could also occur by hydrolysis of the sulfinamide or the corresponding sulfonamide, produced by oxidation of the sulfenamide sulfur atom, path (d). In either case, predominance of paths (c) and (d) in insects leads to intoxication by carbofuran. The selectivity between mammal and insect is the net result of the relative rates at which these processes occur in each species.

Work on the comparative metabolism of the 2-toluene-sulfenyl derivative of carbofuran (5) in houseflies and mice is in progress.

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Preparation of Tritium-Labeled Disparlure, the Sex Attractant of the Gypsy Moth

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Tritium-labeled disparlure (cis-7,8-epoxy-2-methyloctadecane-7,8-t), the sex attractant of the gypsy moth (Porthetria dispar (L.)), and the olefin precursor ((Z)-2-methyl-7-octadecene-7,8-t) were synthesized. An improved method was devised for preparing the intermediate, 2-methyl-7-octadecyne.

Although many theories relating to the mechanism of olfaction have been advanced, none has been generally accepted, and it is apparent that more scientific data are needed to aid in solving this difficult problem. Toward this end, we synthesized tritium-labeled disparlure (cis-7,8-epoxy-2-methyloctadecane) (Bierl et al., 1970, 1972), the sex attractant pheromone of the gypsy moth (Porthetria dispar (L.)), to gather information about the mechanism of olfaction in the gypsy moth and to determine means of utilizing the pheromone effectively. A pioneer study of the olfactory process of the silkworm moth (Bombyx mori (L.)) along similar lines has been underway for some time (Schneider and Kasang, 1971; Schneider et al., 1968).

The synthesis of disparlure, outlined in Figure 1, is a modification of the method of Eiter (1972) that utilized simpler conditions and produced a higher yield (80%) of the acetylene intermediate, 2-methyl-7-octadecyne (I). Tritiation of I with a modified Lindlar catalyst gave (Z)-2-methyl-7-octadecene-7,8-t (II). Epoxidation of II with m-chloroperbenzoic acid gave disparlure labeled with tritium in the 7 and 8 positions (III). A procedure in which peracetic acid was used for epoxidation is described and suggested for commercial production of disparlure because of the low cost of the peracetic acid.

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EXPERIMENTAL SECTION

Reagent-grade solvents and chemicals were used. For column chromatography, silica gel (J. T. Baker Company, no. 3405) and Adsorbosil-CABN (60/100 mesh, 25% silver nitrate on silica gel, Applied Science Laboratories, State College, Pa.) were used as received. For thin-layer chromatography (tlc), Brinkmann plates (5 × 20 cm, precoated 0.25-mm thick silica gel F254) were used. For AgNO$_3$ tlc, the Brinkmann plates were dipped in a solution of 10% AgNO$_3$ in 75% ethanol in water and dried. Spots were made visible by spraying the plates with a solution of 2% cupric acetate in 8% phosphoric acid and heating them at 110° for ½ hr. For gas chromatographic (glc) analysis, a Hewlett-Packard model 7620A instrument equipped with a flame ionization detector was used with a 50-ft × 0.02-in. i.d. SCOT column containing Carbowax 20M operated at 180° with a helium carrier gas flow rate of 4 ml/min. Infrared spectra were recorded on a Perkin-Elmer model 457A grating spectrophotometer. Nuclear magnetic resonance (nmr) spectra were determined with a Varian T-60 instrument; tetramethylsilane (TMS) was used as the internal standard.

Synthesis of 2-Methyl-7-octadecyne (I). Anhydrous HBr was added to a solution of 5-methyl-1-hexene (Chemical Samples Company, Columbus, Ohio, 26.2 g, 0.267 mol) and recrystallized benzoyl peroxide (1.5 g, 0.0062 mol) in 160 ml of hexane at 10-20° until the olefin content (measured by glc) was negligible. The hexane solution was washed successively with water and aqueous solutions of saturated sodium bisulfite, 7% sodium bicarbonate, and saturated sodium chloride; it was then dried over magne-