ASSESSMENT OF BENZYL AND CINNAMYL SUBSTITUTED PHENOL AND 1,3-BENZODIOXOLE DERIVATIVES AS IGR'S AGAINST MOSQUITOES

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ABSTRACT. The ability of 149 benzyl and cinnamyl substituted phenol and 1,3-benzodioxole derivatives to affect the growth and development of immature mosquitoes was assessed in the laboratory. Several of the more active compounds were also evaluated in small field plots. Nineteen of the materials that were most effective, with LC-90's of 0.1 ppm or less against *Anopheles quadrimaculatus*, were also assessed against *Anopheles albimanus* and *Aedes taeniorhynchus*. In the laboratory and field, the more active compounds in this group of insect growth regulators were similar in effectiveness to the previously reported 2,6-di-tert-butyl-4-(α,α-dimethyl benzyl)phenol (MON-0585).

The ability of benzylphenol compounds to interrupt the development of immature mosquitoes was reported a decade ago by Jakob (1972), who found 2,6-di-tert-butyl-4-(α,α-dimethylbenzyl)phenol (MON-0585) effective against mosquito larvae. Since that time hundreds of candidate insect growth regulators have been assessed for their potential use for mosquito control (Shaefer et al. 1974, Lowe et al. 1975, Mulla and Darwazeh 1975, Dame et al. 1976, McGovern et al. 1980). In 1975 we initiated a study of benzylphenol and benzyl-1,3-benzodioxole derivatives and a number of related cinnamyl compounds to determine the relation between these chemical structures and growth regulator activity. Nineteen of 149 compounds were sufficiently active against *Anopheles quadrimaculatus* Say to warrant further evaluation against other species; the results of tests with these compounds are reported here. The results of the tests conducted with the 130 less active compounds are available upon request.

MATERIALS AND METHODS

The candidate materials were synthesized at the Western Regional Research Center following the general procedures outlined by Jurd et al. (1979). For assessment against mosquito larvae, acetone solutions were prepared at the Insects Affecting Man and Animals Research Laboratory on a weight:volume basis calculated to give the proper concentration following the addition of 1 ml or less to the test container.

Initially, each experimental compound was tested against late third-early fourth-stage larvae of *Anopheles quadrimaculatus*. Most of the exposures were conducted with 25 larvae in 100 ml of well water in a 500-ml glass jar (9 × 8.5 cm diam) (Method A) to which the experimental compound was added in not more than 1 ml of acetone. The larvae were provided with 0.05 g of ground hog supplement and the jars were covered with cloth netting and held at 26–29°C; ca. 0.5 lumen/ft^2 of illumination were provided during nonworking hours. Seven days later the number of dead pupae, exuviae, and adults was observed and recorded. A few (25%) of the materials tested at the beginning of the program were evaluated by exposing 50 larvae to the desired concentration in 1 liter of distilled water in an 18 × 30 cm enamelled pan (Method B; Dame et al. 1976). Dead larvae and pupae were removed daily and live pupae were rinsed and transferred to a 90-ml cup of distilled water. Observations on emergence of adults were made two days after adding the last pupae to the cup. Method A replaced Method B, which gave comparable results, because Method A requires less handling and uses only one-tenth the amount of candiate material.

Untreated controls were run with each replicate and a methoprene standard was assessed at regular intervals. Several concentrations of each compound were tested so that a dose-response relationship could be established, and at least one concentration was replicated with each run within each test series. The data were corrected for control mortality (Abbott's formula) and probit analyses were conducted.

Compounds with LC-90 levels of 0.1 ppm or less were also tested against *Anopheles albimanus* Wied. and *Aedes taeniorhynchus* (Wied.). These tests were conducted with late third-early fourth-stage larvae using the methods (A and B) of exposure and analysis described for *An. quadrimaculatus*.

Several compounds were tested outdoors to determine their activity under more natural conditions. Small ponds (ca. 2.7–3.8 m^2), lined with polyethylene under St. Augustine grass recently established from commercial sod, were filled to a depth of ca. 15 cm one day before treatment. The desired amount of each chemi-

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cal was applied manually in 200–300 ml of water and mixed thoroughly in the plot to assure even distribution. During the early portion of the investigation the activity of these materials was determined by bioassay (Method C) of water samples removed from the ponds. In the laboratory the samples were strained through a fine mesh screen to remove most of the detritus, naturally occurring mosquito larvae, and other organisms. Then one liter was placed in an 18 × 30 cm enamelled pan and tests were conducted as described previously with 50 *An. quadrimaculatus* larvae. Subsequently, Method C was replaced by an improved test method (Method D) which provided a direct exposure of larvae in the ponds. With the improved technique, ca. 300–400 fourth-stage larvae of *An. quadrimaculatus* were introduced into each pond soon after treatment and daily for two days to provide information on residual activity of the experimental compound. Twenty-four hr after each introduction of larvae, 50 pupae were randomly collected from each plot; on those occasions when few pupae were available after 24 hr. those collected in 50 dips comprised the sample. Upon return to the laboratory, these samples were rinsed in well water and placed in well water for observation of adult emergence.

On the fifth day the ponds were pumped out and left untreated for two weeks or more before reuse. To monitor the possibility of contamination by a previously used experimental compound, water samples were collected 24 hr after each new flooding and bioassayed in the laboratory. In the event that the bioassay revealed a residual level of activity from a prior treatment, the results of the testing in that plot were discarded and a further two-week period of weathering was programmed before reuse.

RESULTS AND DISCUSSION

The results of 19 of the most active compounds are presented in Table 1. Benzylphenol compounds I, II, and III and benzyl-1,3-benzodioxole materials XIII and XIV provided excellent control of all three test species with LC-90 values of 0.060 ppm or less. The benzylphenols are structurally related to MON-0585 and a few similar benzylphenols previously reported to be effective mosquito growth regulators (Sacher 1971, Hanauye et al. 1975). The level of activity observed in the tests of these compounds was generally slightly greater than that reported for MON-0585 in similar tests (*An. quadrimaculatus* LC-90, 0.099 ppm; Dame et al. 1976).

The benzyl-1,3-benzodioxoles have not previously been reported to possess IGR properties, although some have been shown to be effective sterilants of female house flies (*Musca domestica* Linn., Jurd et al. 1979), the pink bollworm (*Pectinophora gossypiella* (Saunders), Flint et al. 1980) and tsetse flies (*Glossina* spp., P. A. Langley and M. A. Trewern, personal communication). General testing for female sterility was not conducted in our assessment sequence, but cross-mating of treated survivors of unreplicated exposures to compounds II and IV did not reveal any reduction in reproductive capacity. The 2-(phenylmethyl)phenol compound (XX) reported in Table 2 is an effective sterilant of house fly and screwworm fly [*Cochliomyia hominivorax* (Coquerel)] females (Rawlins et al. 1979), although it is only slightly effective as a mosquito IGR.

Methylation of the phenolic hydroxyl group of *ortho*-benzylphenols and *ortho*-cinnamylphenols may result in a very significant increase in the IGR activity of these substances (Table 2). This structure-activity relationship has not previously been noted. Methylation of the phenolic OH group of compound XX results in the formation of the O-methyl derivative, which is one of the most effective mosquito IGR compounds found in the present investigation (Compound I). This increase in IGR activity was found only with methylation. Thus, ethylation or allylation of compound XX to give XXI and XXII, respectively, actually decreased IGR activity.

Similarly, the IGR activity of the 2-(phenylmethyl)phenol (compound XXIII; LC-90, 1.412 ppm) was increased by O-methylation to give the methyl ether (compound VII; LC-90, 0.092 ppm). Methylation of the slightly active ortho-cinnamylphenol (XXIV) yielded the active IGR compound VI. However, with 2-(1-phenylethyl)phenols O-methylation of the phenolic OH group decreased the IGR activity of compounds III (See XXVI) and IV (see XXVIII).

As indicated, a list of less active benzyl and cinnamyl compounds is available upon request. Some generalizations concerning structure-activity relationship are apparent from this list. Thus, in Tables 1 and 2 most of the active benzyl compounds have an alpha-methyl or a 4-methoxy substituent. Replacement of the alpha-methyl by other alkyl groups such as alpha-ethyl, or replacement of the 4-methoxy group by other alkoxy groups such as ethoxy, propoxy, 3,4-methylenedioxy or 3,4-dimethoxy results in a considerable loss of activity. Cinnamyl compounds proved to be generally much less effective than benzyl compounds so that alkyl and alkoxy substituted cinnamyl derivatives were not synthesized and evaluated in this study. As indicated in Table 1 the most active

Table 1. Benzyl and cinnamyl phenol and benzodioxole IGR's most active in laboratory tests.

Compound	Chemical name	LC-90 (ppm) *Anopheles quadrimaculatus*	*Anopheles albimanus*	*Aedes taeniorhynchus*
I	2,4-Bis(1,1-Dimethylethyl)-6-[(4-methoxyphenyl)methyl]-1-methoxybenzene	0.032	0.001	0.023
II	2,6-Bis(1,1-Dimethylethyl)-4-[(4-methoxyphenyl) methyl]phenol	0.042	0.005	0.029
III	2,4-Bis(1,1-Dimethylethyl)-6-[1-(4-methoxyphenyl) ethyl]phenol	0.028	0.060	0.137
IV	2,4-Bis(1,1-Dimethylethyl)-6-(1-phenylethyl) phenol	0.052	0.011	0.078
V	2,6-Bis(1,1-Dimethylethyl)-4-(1-phenylethyl) phenol	0.072	0.046	0.154
VI	2,4-Bis(1,1-Dimethylethyl)-6-(3-phenyl-2-propenyl)-1-methoxybenzene	0.089	0.066	0.016
VII	2,4-Bis(1,1-Dimethylethyl)-6-(phenylmethyl)-1-methoxybenzene	0.092	0.025	0.164
VIII	2,6-Bis(1,1-Dimethylethyl)-4-[1-(methoxyphenyl)] ethylidene-2,5-cyclohexadien-1-one	0.095	0.103	0.044
IX[a]	5-Propoxy-6-[(4-methoxyphenyl) methyl]-1,3-benzodioxole	0.043	0.039	0.116
X	5-Butoxy-6-[(4-methoxyphenyl) methyl]-1,3-benzodioxole	0.047	0.102	0.149
XI	5-(3-methyl-butoxy)-6-[(4-methoxyphenyl) methyl]-1,3-benzodioxole	0.035	0.163	0.076
XII	5-(2-methyl-propoxy)-6-[(4-methoxyphenyl) methyl]-1,3-benzodioxole	0.082	0.029	0.047
XIII	5-Propoxy-6-[1-(4-methoxyphenyl)ethyl]-1,3-benzodioxole	0.041	0.052	0.029
XIV	5-Butoxy-6-[1-(4-methoxyphenyl)ethyl]-1,3-benzodioxole	0.025	0.052	0.022
XV	5-Pentoxy-6-[1-(4-methoxyphenyl)ethyl]-1,3-benzodioxole	0.060	0.108	0.064
XVI	5-Butoxy-6-(1-phenylethyl)-1,3-benzodioxole	0.099	0.298	>0.100
XVII[a]	5-(3-phenyl-2-propenyl)-6-(2-propenyloxy)-1,3-benzodioxole	0.092	0.059	0.100
XVIII	5-Propoxy-6-(3-phenyl-2-propenyl)-1,3-benzodioxole	0.057	0.102	>0.100
XIX	5-Butoxy-6-(3-phenyl-2-propenyl)-1,3-benzodioxole	0.042	0.111	>0.100

[a] Method B assessment; other compounds assessed by Method A.

5-alkoxy-1,3-benzodioxole derivatives (compounds IX–XIX) are those in which the alkoxy alkyl group was C_3, C_4, and C_5. Higher alkoxy, as well as methoxy or ethoxy, groups are less active. The phenolic OH group of the 2,6-bis(dimethylethyl) compounds II and V is sterically hindered. Attempts to methylate these compounds were unsuccessful so that it is not known whether methylation of this hydroxyl enhances activity as observed in the case of XX→1.

Under outdoor conditions several materials were similar to MON-0585 (99–100% control at 0.22–0.56 kg/ha; Dame et al. 1976) in activity against *An. quadrimaculatus*. Compounds IX and XVII were completely effective at 0.5 kg/ha immediately after treatment, while compounds II and XIV were highly effective (Table 3). At this application rate none of the compounds tested in the ponds revealed any residual activity; however, of the two materials tested at 1.0 kg/ha, compound IX was highly effective 24 hours posttreatment.

Although these compounds are not as effective as the currently registered IGR methoprene, they are relatively inexpensive to produce. Some are effective against other organisms, e.g., compounds IV and XXIII protect wood from attack by marine borers (Jurd and Bultman 1977, Bultman and Jurd 1979). Furthermore, a number of representative materials (e.g., compounds I, XX, XIII and XXIII)

from both structural groups have been found to be nonmutagenic by the standard Ames *Salmonella*/microsome procedure (Jurd et al. 1979). Thus, further assessment and development of effective benzyl and cinnamyl substituted phenol and 1,3-benzodioxole derivatives seem warranted.

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Table 2. Effects of 0-alkylation on IGR activity of benzylphenols against *Anopheles quadrimaculatus*.

Compound	Chemical name	LC-90 (ppm)
XX	2,4-Bis(1,1-Dimethylethyl)-6-[(4-methoxyphenyl)methyl]phenol	0.225
I	2,4-Bis(1,1-Dimethylethyl)-6-[(4-methoxyphenyl)methyl]-1-methoxybenzene	0.032
XXI	2,4-Bis(1,1-Dimethylethyl)-6-[(4-methoxyphenyl)methyl]-1-ethoxybenzene	0.285
XXII	2,4-Bis(1,1-Dimethylethyl)-6-[(4-methoxyphenyl)methyl]-1-allyloxybenzene	>1.000
XXIII	2,4-Bis(1,1-Dimethylethyl)-6-(phenylmethyl)phenol	1.412
VII	2,4-Bis(1,1-Dimethylethyl)-6-(phenylmethyl)-1-methoxybenzene	0.092
XXIV	2,4-Bis(1,1-Dimethylethyl)-6-(3-phenyl-2-propenyl)phenol	0.544
VI	2,4-Bis(1,1-Dimethylethyl)-1-methoxy-6-(3-phenyl-2-propenyl)benzene	0.089
XXV	2,4-Bis(1,1-Dimethylethyl)-6-(3-phenyl-2-propenyl)-1-ethoxybenzene	>1.000
III	2,4-Bis(1,1-Dimethylethyl)-6-[1-(4-methoxyphenyl)ethyl]phenol	0.028
XXVI	2,4-Bis(1,1-Dimethylethyl)-6-[1-(4-methoxyphenyl)ethyl]-1-methoxybenzene	0.556
XXVII	2,4-Bis(1,1-Dimethylethyl)-6-[1-(4-methoxyphenyl)ethyl]-1-ethoxybenzene	>1.000
IV	2,4-Bis(1,1-Dimethylethyl)-6-(1-phenylethyl)phenol	0.052
XXVIII	2,4-Bis(1,1-Dimethylethyl)-6-(1-phenylethyl)-1-methoxybenzene	0.678
XXIX	2,4-Bis(1,1-Dimethylethyl)-6-(1-phenylethyl)-1-ethoxybenzene	>1.000

Table 3. Activity of IGR materials applied to outdoor ponds against *Anopheles quadrimaculatus* larvae introduced after treatment (means of 2 replications).

Compound	Test method	Larval introduction (hr)	% Eclosion from samples collected 24 hr after introduction to ponds treated with indicated dosage (kg/ha) 0.0	0.05	0.1	0.5	1.0
I	D	2		31	25	62	
		24		73	70	70	
II	D	2		67[a]	32	11	
		24		91[a]	71	77	
III	D	2		89[a]	44	85[a]	
		24		91[a]	78	89[a]	
IV	D	2		88[a]	75[a]	86	
		24		88[a]	91[a]	74	
IX	C	2		51	3	0	0[a]
		24		94	90	82	6[a]
XI	D	2			68	68[a]	
		24		42[a]	58	91[a]	
XIV	D	2		16[a/b]	26	9	
		24		84[a/b]	72	71	
XVII	C	2		71	34	0	0[a]
		24		86	90	96	60[a]
Methoprene	D	2		0			
		24		0			
Control	C	2	97				
		24	90				
	D[c]	2	91				
		24	92				

[a] 1 replication.
[b] 1 replicate discarded due to prior contamination of plot.
[c] 14 replications.

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