moderate purity (70–>86%). It is not known to what extent the reported potency values for these compounds are due to the assigned structures as opposed to impurities. However, it is clear that each of these compounds is of low toxicity relative to B, 8-Cl-B, and 9-Cl-B.

In general, the potency of compounds formed on introducing one chlorine substituent into B decreases in the order: 9-chloro > 8-chloro > none > 3-*exo*-chloro or 5-*exo*-chloro or 10-chloro. It appears that PB-sensitive mechanisms detoxify B more readily than its 8-chloro- and 9-chloro derivatives.

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α- and β-Alkyl Substituted Cinnamates as Pyrethrum Synergists

R. Vaidyanathaswamy, B. S. Parmar, B. S. Attri, R. P. Singh, and S. K. Mukerjee*

Series of α- and β-alkyl substituted cinnamates have been investigated as pyrethrum synergists against the red flour beetle, *Tribolium castaneum* Herbst. All the methylenedioxy cinnamates showed synergism, the factors of synergism ranging between 1.71–6.43 and 1.72–4.07 for the α- and β-substituted esters, respectively. The 1:5 insecticide:synergist level invariably showed better activity over 1:1 level. The increased length of the alkyl substitutent at these positions did not correspondingly increase the synergistic activity of these esters, maximum activity being attained with a C-2 substituent. On the contrary, lipophilicity of such molecules increased directly with the increased number of carbon atoms of the alkyl substituent, indicating thereby that a C-2 substituent provides enough lipophilicity for such molecules to exhibit maximum synergism. The esters without the methylenedioxy group, mostly, showed antagonism.

Importance of methylenedioxyphenyl compounds as synergists for oxidatively metabolized insecticides is well known. Moore and Hewlett (1958) investigated the effect of different side chains on the synergistic properties of such compounds. Schroeder et al. (1948) and Carson and Eddy (1949) reported that the methylenedioxy cinnamates also possessed synergistic properties toward pyrethrum. However, information on the effect of alkyl substitution on this property of cinnamates is, in general, lacking and the same is reported here.

MATERIALS AND METHODS

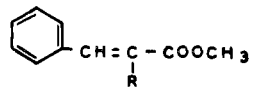
n-Alkanoic acids from C_3 to C_{10}, methyl iodide, *n*-alkyl bromides from C_2 to C_4, and piperonal were procured and used without further purification. Benzaldehyde was washed with sodium bicarbonate solution followed by water, dried, and distilled. Anhydrides of *n*-alkanoic acids from C_3 to C_8 were prepared from the corresponding acid chlorides and their potassium or sodium salts by the general procedure detailed by Vogel (1948). 3,4-Methylenedioxyphenyl alkyl ketones were made by Jones oxidation (Bowden et al., 1946) of the corresponding carbinols obtained by condensing piperonal with the corresponding *n*-alkylmagnesium bromides. Structures of all new compounds agreed with their spectral and analytical data.

Infrared spectra were recorded on a Perkin-Elmer Model 437 spectrometer either as neat film or solution in CCl_4. Nuclear magnetic resonance spectra were recorded on a Varian A-60 spectrometer using Me_4Si as internal standard.

Synthesis of Test Chemicals. *α-Alkylcinnamic Esters.* The α-alkylcinnamic acids were prepared by the

Division of Agricultural Chemicals, Indian Agricultural Research Institute, New Delhi-110012, India.

Table I. Some Pertinent Information about the Prepared Alkyl Cinnamic Acids/Esters



Sample no.	Substitution in benzene ring	R	Corresponding acid Mp, °C	Corresponding acid Yield, %	Corresponding ester Mp/bp, °C	Corresponding ester Yield, %	Literature reference
I		CH_3	80–81	43	40–41	80	Bogert and Davidson (1932)
II		C_2H_5	104–106	34	125 (0.5 mm)	83	Bogert and Davidson (1932)
III		n-C_3H_7	93	30	128 (0.5 mm)	90	Bogert and Davidson (1932)
IV		n-C_4H_9	80–82	25	135 (0.5 mm)	90	Bogert and Davidson (1932)
V		n-C_5H_{11}	84–86	14	140 (0.5 mm)	95	Bogert and Davidson (1932)
VI		n-C_8H_{17}	83–84	15	160 (0.1 mm)	90	New[a]
VII	3,4-methylenedioxy	CH_3	198–199	29	76–78	83	Gensler and Berman (1958)
VIII	-do-	C_2H_5	130–132	25	56–58	80	Gensler and Berman (1958)
IX	-do-	n-C_3H_7	127–128	11	58–60	94	New[b]
X	-do-	n-C_4H_9	120–122	15	180 (0.5 mm)	95	New[c]
XI	-do-	n-C_5H_{11}	102–105	13	185 (0.4 mm)	95	New[d]
XII	-do-	n-C_8H_{17}	103–104	17	220 (0.1 mm)	95	New[e]

[a] Analysis: found: C, 78.4; H, 9.2; $C_{18}H_{26}O_2$ requires C, 78.8; H, 9.5%. [b] Analysis: found: C, 68.9; H, 6.6; $C_{15}H_{18}O_4$ requires C, 68.7; H, 6.9%. [c] Analysis: found: C, 69.2; H, 7.4; $C_{16}H_{20}O_4$ requires C, 69.6; H, 7.2%. [d] Analysis: found: C, 69.8; H, 7.0; $C_{16}H_{20}O_4$ requires C, 69.6; H, 7.2%. [e] Analysis: found: C, 71.4; H, 8.4; $C_{19}H_{26}O_4$ requires C, 71.7; H, 8.2%.

Perkin reaction of benzaldehyde and piperonal and the anhydrides of aliphatic acids. These were converted into the corresponding methyl esters by treatment with methanolic diazomethane. The mp/bp of acids/esters are reported in Table I. The melting points of known compounds agreed with those recorded in literature, and all new compounds gave satisfactory elemental analysis.

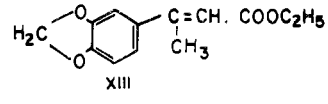
β-Alkylcinnamic Esters. These were obtained from the corresponding phenyl alkyl ketones by the Reformatsky reaction with ethyl bromoacetate, followed by dehydration of the intermediate β-hydroxy esters. The general procedure for this reaction is given below.

A solution of the phenyl alkyl ketone (0.04 mol) in dry benzene containing a few drops of ethyl bromoacetate was stirred with active zinc dust (0.04 mol) and a few crystals of iodine. On heating to about 50 °C, the reaction started; thereafter, ethyl bromoacetate (0.04 mol) in dry ether was slowly added to the gently refluxing mixture. After heating for 2 h, the mixture was cooled, acidified with dilute H_2SO_4, and the organic layer separated. The solvent was removed from the washed organic layer, and the residual crude hydroxy ester containing some unreacted ketone was dehydrated directly by dissolving it in acetic acid (20 mL) and perchloric acid (1 mL) and keeping the solution for 24 h. The acetic acid was removed under vacuum, and the residual mixture of unsaturated esters as well as unreacted ketone was separated by column chromatography on silica gel. Brief details and properties of the individual esters are given below.

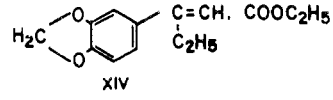
(i) Ethyl β-Methyl-3,4-methylenedioxycinnamate (XIII). XIII was prepared from 3,4-methylenedioxyphenyl methyl ketone obtained by the method of Manchot and Hass (1913), (6.6 g, mp 85–88 °C), ethyl bromoacetate (13.4 g), and zinc dust (5 g). The crude dehydration product (7 g) yielded the above ester (6 g) as the major product: mp 51–53 °C (found: C, 66.5; H, 6.3; $C_{13}H_{14}O_4$ requires, C, 66.7; H, 6.0%); IR, ν_{max} (Nujol) 1700 cm^{-1} (>C=O).

(ii) Ethyl β-Ethyl-3,4-methylenedioxycinnamate (XIV). The crude hydroxy ester (8 g) obtained by reaction of 3,4-methylenedioxyphenyl ethyl ketone prepared by the method of Foulds and Robinson (1914) (5.4 g, mp 38–39 °C), ethyl bromoacetate (10 g), and zinc dust (3.9 g) gave on dehydration a mixture of two products (TLC, benzene–CCl_4, 50:50, v/v) as an oil (7 g). These were separated by column chromatography over silica gel using benzene–carbon tetrachloride (30:70) as the eluent. The cinnamic ester was eluted first as an oil (2 g), bp (bath) 165 °C (0.5 mm); found: C, 67.9; H, 6.8, $C_{14}H_{16}O_4$ requires C, 67.7; H, 6.5%; IR, ν_{max} (Nujol) 1700 cm^{-1} (>C=O). In NMR, it showed a peak at δ 5.80 (S, 1H) besides other peaks, clearly confirming its structure as XIV.

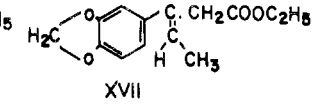
The isomeric ester XVII was eluted next from the column as an oil (4.7 g), bp (bath) 160 °C (0.5 mm); IR, ν_{max} (Nujol) 1725 cm^{-1}. Its NMR spectrum showed a two-proton peak at δ 3.37, characteristic of a methylene group between an ester group and a double bond which agrees with the structure XVII (Benkeser et al., 1963).



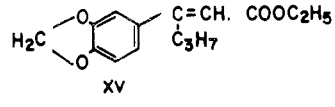
XIII



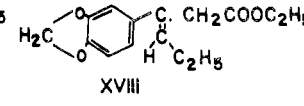
XIV



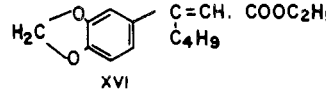
XVII



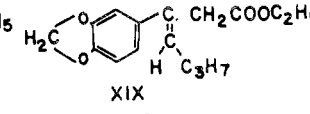
XV



XVIII



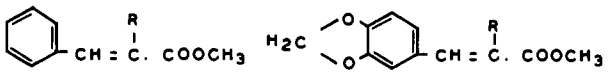
XVI



XIX

(iii) Ethyl β-*n*-Propyl-3,4-methylenedioxycinnamate (XV). The general procedure was followed to obtain the hydroxy ester (8 g) from ethyl bromoacetate (10.02 g, 0.06 mol), zinc dust (3.90 g, 0.06 mol), and 3,4-methylenedi-

Table II. Comparative Performance of α-Substituted Cinnamates and the Corresponding Methylenedioxy Cinnamates as Pyrethrum Synergists against *Tribolium castaneum* Herbst



A B

R	Factor of synergism, compd A Pyr:Syn (1:1)	Factor of synergism, compd A Pyr:Syn (1:5)	Factor of synergism, compd B Pyr:Syn (1:1)	Factor of synergism, compd B Pyr:Syn (1:5)
CH_3	0.75	0.86	1.71	3.00
C_2H_5	0.94		3.21	6.43
n-C_3H_7	0.77	0.57	2.75	4.40
n-C_4H_9	1.10	0.70	2.20	4.12
n-C_5H_{11}	0.83	0.69	2.25	4.00
n-C_8H_{17}	0.69	0.42	2.23	3.62

oxyphenyl n-propyl ketone prepared by the method of Mameli and Alagna (1906) (5.76 g, 0.3 mol, mp 46–47 °C). On dehydration, it gave an oil which separated by column chromatography on silica gel. The cinnamic ester was eluted first as an oil (4.0 g), bp (bath) 168 °C (0.5 mm); found: C, 69.0; H, 6.6; $C_{15}H_{18}O_4$ requires C, 68.7; H, 6.9%; IR ν_{max} (Nujol) 1700 cm^{-1} (>C=O). In NMR it showed a peak at δ 5.81 (S, 1 H), confirming its structure as XV.

The isomeric ester was eluted next from the column as an oil (2.1 g); bp (bath) 165 °C (0.5 mm); IR, ν_{max} (Nujol) 1725 cm^{-1}. Its NMR spectrum showed a two-proton peak at δ 3.37 as expected in structure XVIII.

(v) Ethyl β-n-Butyl-3,4-methylenedioxycinnamate (XVI). From zinc dust (3.25 g, 0.05 mol), ethyl bromoacetate (8.35 g, 0.05 mol), and 3,4-methylenedioxyphenyl n-butyl ketone (5.15 g, 0.025 mol, bp 136–138 °C (3 mm)), an oil (6.0 g) was obtained which on dehydration gave a mixture of two products. The first product (XVI) eluted by column chromatography was on oil (2.8 g); bp (bath) 170 °C (0.5 mm); found: C, 69.2; H, 6.8; $C_{16}H_{20}O_4$ requires C, 69.6; H, 7.2%; IR, ν_{max} (Nujol) 1700 cm^{-1}. In NMR it showed a peak at δ 5.98 (S, 1 H).

The isomeric product was eluted next as an oil; bp (bath) 168 °C (0.5 mm), IR, ν_{max} (Nujol) 1725 cm^{-1}; and NMR peak at δ 3.38 (S, 2 H), confirming its structure as XIX.

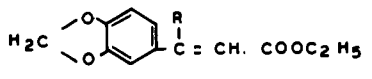
Formulation and Bioassay. The synergistic activity of the test compounds was assessed in pyrethrum-based emulsions at insecticide: synergist ratio of 1:1 and 1:5 (w/w). Benzene (10%) and Tween-80 emulsifier (0.2%) were maintained in the spray emulsions. The tests were conducted in three replications of ten insects each against 2–3-week-old laboratory reared red flour beetle (*Tribolium castaneum* Herbst) as reported earlier by Mukerjee et al. (1973). The data were subjected to probit analysis (Finney, 1971) and factors of synergism (Chadwick, 1963) calculated at LC_{50} level. Piperonyl butoxide was used as a reference synergist.

Lipophilicity of the Cinnamates. Lipophilicity of the test compounds was determined by finding out the R_f values on reverse phase TLC and then calculating the R_M values by using the equation $R_M = \log [(1/R_f) - 1]$ (Biagi et al., 1969). Silica gel plates after activation for 2 h at 110 °C were coated with paraffin (10% paraffin in hexane). The coated plate was spotted with all the test esters at a time and developed in a acetone–water (75:25) mixture. The positions of esters were visualized by spraying with dilute H_2SO_4 and heating, and R_f values were measured.

RESULTS AND DISCUSSION

The results on the synergistic activity of α- and β-substituted esters are reported in Tables II and III.

Table III. Factor of Synergism of β-Substituted Methylenedioxy Cinnamates as Pyrethrum Synergists against *Tribolium castaneum* Herbst



C

Sample no.	R	Factor of synergism, compd C Pyr:Syn (1:1)	Factor of synergism, compd C Pyr:Syn (1:5)
XIII	CH_3	1.72	3.13
XIV	C_2H_5	2.65	3.93
XV	n-C_3H_7	2.35	4.07
XVI	n-C_4H_9	2.79	3.39
	Piperonyl butoxide	2.41	2.29

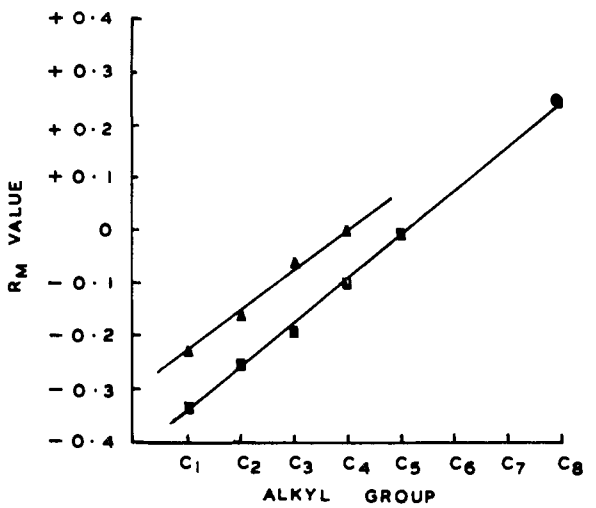


Figure 1. Relationship between the length of alkyl substituent (α and β) of 3,4-methylenedioxy cinnamates and R_M values: (■■■) α-alkyl substituent; (▲▲▲) β-alkyl substituent.

It is seen from Table II that, whereas almost all the α-substituted cinnamates act as antagonists for pyrethrins, the corresponding methylenedioxy cinnamates are synergists, the factors of synergism ranging between 1.71 and 6.43. The 1:5 insecticide:synergist level of methylenedioxy esters was invariable better than the 1:1 level. The increased length of the α-substituent does not lead to increases in synergistic activity, and maximum activity seems to have been attained with a C_2H_5 substituent. All the β-substituted methylenedioxy esters, too, showed synergism, the factors of synergism being between 1.72 and 4.07 (Table III). These esters showed almost the same trend as the α-substituted esters in imparting synergistic properties.

Piperonyl butoxide, which was used as a reference synergist, showed factors of synergism of 2.41 and 2.29 respectively at 1:1 and 1:5 insecticide:synergist levels. In comparison with these values, all the methylenedioxy cinnamates appar to be potential synergists for pyrethrins.

The plot of R_M values vs. the carbon atoms of the alkyl substituent in Figure 1 reveals a linear relationship, implying thereby, that the lipophilicity of these molecules increases directly with increased length of the alkyl substituent. In contrast, maximum synergism is shown by a C_2H_5 substituent in both α- as well as β-substituted esters. This indicates that a C-2 substituent at these positions provides enough lipophilicity for such molecules to exhibit maximum synergism with pyrethrins.

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Mechanisms of Production of Soil-Bound Residues of [^{14}C]Parathion by Microorganisms

Jaacov Katan[1] and E. Paul Lichtenstein*

Unextractable (bound) radiolabeled residues are formed when [^{14}C]parathion is incubated in soil. The role of microorganisms in producing these bound residues was investigated by incubating [*ring*-^{14}C]-parathion in soil-free culture media that had been inoculated with soil microorganisms. The amounts of compounds in culture supernatants, that upon addition to soil became unextractable, increased up to 12 h of microbial culture incubation, when 43% of the added radiocarbon was bound after a 2-h soil incubation period. The increase in soil bound residues was correlated with a decrease in the amount of parathion in the microbial culture and a concomitant increase in the appearance of the major degradation product, aminoparathion. Microbial cells contained only a minor proportion of ^{14}C-bound residues. Addition of natural microbial growth metabolites to the soil did not affect binding of either parathion or aminoparathion. This latter compound was highly bound to both sterile and nonsterile soils. When [^{14}C]parathion, [^{14}C]paraoxon, *p*-[^{14}C]nitrophenol, and their respective ^{14}C-amino compounds were added to soil, the amino compounds were bound to a much greater extent than were the nitro compounds. It appears that the process of parathion degradation has to be separated from that of binding and that the role of the microorganisms in soil binding phenomena consisted in degrading [^{14}C]parathion to compounds which are more tightly bound to soil than the parent insecticide. Once these compounds were formed, their binding to soil was found to be high.

The fate of pesticides in the environment has been the subject of numerous studies. Because of potential hazards to living organisms, some of the more persistent insecticides were replaced by less persistent ones which rapidly "disappear" from environmental components, such as soil. However, the "disappearance" of a pesticide from soil may not only reflect its degradability, but can also reflect our inability to detect its residues by conventional procedures. One reason a chemical is not detected is its potential conversion to compounds which cannot be extracted, thus forming "bound" residues. The formation of these residues in soils can be followed by using ^{14}C-labeled compounds and combusting the previously extracted soil to $^{14}CO_2$. Formation of bound residues was shown with the herbicide, propanil (Bartha, 1971), and with the fungicide, 2,6-dichloro-4-nitroaniline (DCNA) (Van Alfen and Kosuge, 1976). The production of bound residues in soil was recently demonstrated with several insecticides which usually are considered to be nonpersistent (Katan et al., 1976; Lichtenstein et al., 1977). It was shown in this study that the binding of [^{14}C]parathion residues to soil was the result of the activity of soil microorganisms, and it was also suggested that microorganisms degraded [^{14}C]parathion to amino compounds which are rapidly and tightly bound to soil. However, other possibilities, such as binding to microbial cells and binding by means of microbial growth metabolites, should not be excluded.

The study of the mechanisms of binding of pesticides is important since this may assist us in obtaining information pertaining to the problem of the potential release of bound residues. In the present investigations degradation products of [^{14}C]parathion were produced by soil microorganisms in soil-free microbial cultures, followed by a separate study of the binding potential of these compounds to soils. The possible role of microorganisms in binding insecticide residues directly to microbial cells was also studied.

MATERIAL AND METHODS

Chemicals. [*ring*-^{14}C]Parathion (sp act. 2 μCi/mg) was purchased from Amersham-Searle Corporation, Arlington, Ill. Its radio purity was at least 99% after cleanup and isolation by thin-layer chromatography (TLC). In addition, five potential metabolites were prepared from [*ring*-^{14}C]parathion: [^{14}C]paraoxon was prepared as described by Fuhremann and Lichtenstein (1972), [^{14}C]-aminoparathion and [^{14}C]aminoparaoxon were obtained by reducing [^{14}C]parathion or [^{14}C]paraoxon, respectively,

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