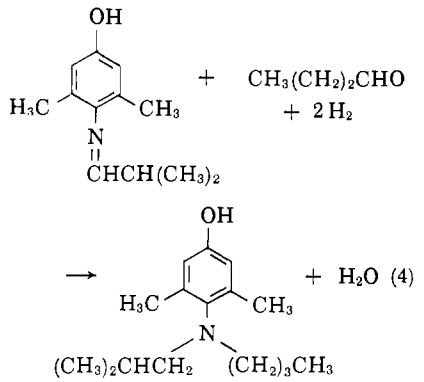
genated at a pressure of 25 to 50 p.s.i.g. at a temperature of 25° to 50° C. (Equation 4). When the theoretical amount of hydrogen was absorbed, the catalyst was removed by filtration, and the solu-



tion was poured over crushed ice. The tan, crude 4-(*n*-butylisobutylamino)-3,5-xylenol was recrystallized twice from pentane to give white needles (melting point, 89–90° C.).

Analysis. Theory for $C_{16}H_{27}NO$: C, 77.05; H, 10.91; N, 5.62. Found: C, 76.60; H, 10.57; H, 5.73.

***N*-Methylcarbamates.** The specific phenol used for preparing each carbamate was dissolved in dry hexane, ether, or methylene chloride to give a solution approaching saturation at room temperature and treated with a 10 to 100% molar excess of methyl isocyanate and a trace of triethylamine catalyst. An exothermic reaction usually took place, and if amounts of phenol in excess of 50 grams were used, an ice bath was necessary to control the temperature. The crude product was purified by recrystallization from an appropriate solvent such as hexane, carbon tetrachloride, or chloroform.

The structures were established by elemental analysis, as summarized in Tables I, II, and III, and by an examination of their infrared spectra. All of the carbamates had characteristic absorption bands at 3300 to 3500 cm.$^{-1}$ (N—H stretching frequency) and 1675 to 1750 cm.$^{-1}$ (carbonyl group) and other distinctive bands for various individual compounds.

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CARBAMATE INSECTICIDES

Hydride-Transferring Ability of Methylenedioxybenzenes as a Basis of Synergistic Activity

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An explanation of the inhibition of the metabolism of carbamates and other pesticides by methylenedioxybenzenes is proposed. Oxidative metabolism of the methylenedioxybenzene with transfer of hydride from the methylenedioxy group is postulated. If the resulting electrophilic benzodioxolium ion were to react with a nucleophilic group in a component of the pesticide-metabolizing enzyme, irreversible inhibition could result. Catechol derivatives as possible products of this process may also compete with the pesticide at some limiting stage of metabolism.

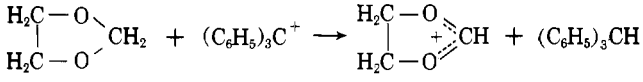
CERTAIN substituted methylenedioxybenzenes are synergists for a number of classes of pesticides of different structural types (*5, 14, 18, 19, 32, 38*), among which are the carbamates (*34, 37*). These same compounds are antagonists (*39, 44*) for a few pesticides and are synergists for some and antagonists for other structurally similar phosphorothioates (*44, 48*). Of the action of these methylenedioxybenzenes, Moorefield (*37*) has said, "This group of compounds must possess a polyvalent and nonspecific potential for blocking enzyme catalysis, or act independently at a less critical site in the insect, or influence the efficiency of an essential property common to the reactivity of all successful insecticides, such as aiding transport." When the antagonistic effect of these same compounds is also considered, the first alternative offered by Moorefield seems most attractive. Inhibition of metabolism as a satisfactory explanation for the synergistic and antagonistic action of the methylenedioxybenzenes has received a measure of experimental support (*4–6, 8, 9, 13, 16, 20, 28, 29, 34, 37, 40, 50, 51*). In higher animals the liver microsomes

have been designated as the locale in which a great variety of reactions chemically alter "foreign" organic compounds (*1*, *10–12*, *21*, *49*). The limited evidence available up to this time points to a microsomal site of metabolism of pesticides in insects, though perhaps not exclusively so (*1*, *11*).

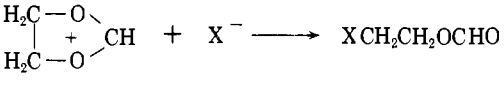
The nature of the substituent groups in methylenedioxybenzenes other than the methylenedioxy moiety may be varied considerably while retaining effectiveness. The role of these variable substituents can only be surmised (*3*, *17*). It is the possible role for the methylenedioxy moiety in both synergism and antagonism, and perhaps in toxicity since the methylenedioxybenzenes have been shown to have some toxicity (*35*, *36*), that is proposed in this paper.

Chemical Activity of Methylenedioxybenzene System

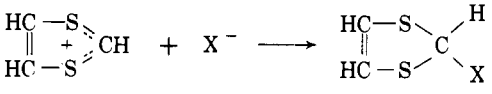
The methylenedioxybenzenes contain a blocked catechol system which is not considered to be particularly sensitive to mild hydrolyzing, reducing, and oxidizing reagents. They are probably metabolized, since when administered to the Madeira roach, piperonyl butoxide was converted about 50% to water-soluble metabolites (*41*). The 1,3-dioxolanes, which are related to the methylenedioxybenzenes, have been shown by Meerwein *et al.* to transfer hydride ion to trityl carbonium ion, forming dioxolenium ion and triphenylmethane (*31*):



Reactions of the dioxolenium ion with nucleophiles (X^-) led to ring opening (*30*):

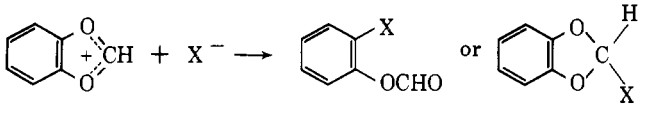


This is in contrast to reactions of the pseudoaromatic 1,3-dithiolium ion

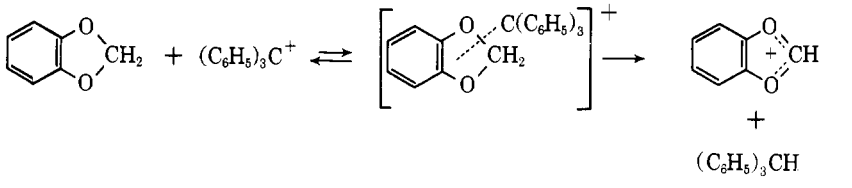


where the electrophilic center is at carbon-2 (*7*, *22*, *23*, *53*) (above).

Benzodithiolium and benzoxathiolium ions also have their electrophilic center at carbon-2 (*42*, *43*, *52*). The benzodioxolium ion (below) if formed might then have its electrophilic center at either a ring carbon or carbon-2.



In an experiment first carried out by the author in the laboratory of R. L. Metcalf in March 1964 methylenedioxybenzene was mixed with trityl fluoborate or perchlorate in acetic acid. An intensely brown-purple solution resulted. This did not deposit any solid triphenylmethane after standing for one hour, even when seeded. When heated for 3 minutes on a steam bath, 80% of the theoretical yield of triphenylmethane was obtained as a crystalline precipitate from the reaction mixture. An uncrystallizable viscous liquid was the other reaction product. This appeared to be a phenolic polymer. It would seem that a charge transfer complex may be formed initially between the trityl ion and the methylenedioxybenzene. The hydride transfer, slow at 25° C., occurred very rapidly at 90° C. Piperonyl butoxide and dihydrosafrole behaved as methylenedioxybenzene, while piperonal showed no significant reaction.



Hydride transfer has been proposed as one mechanism of biological oxidation-reduction (*15*, *24–27*, *45–47*). Ultimately TPN^+ or DPN^+ would act as the hydride acceptor. One or the other of these materials seems necessary for the functioning of several in vitro microsomal enzyme systems.

Hypotheses of Inhibition of Pesticide Metabolism by Methylenedioxybenzenes

It is proposed then that the methylenedioxybenzene inhibitors reach the site of pesticide metabolism in the microsome where, possibly by reason of the polarizability of their pi electron system which can act as an electron source, complexation occurs with enzyme intermediates which are electron sinks. Such complexation might account at least in part for the inhibition of pesticide metabolism. The need, however, for the methylenedioxy group rather than two methoxy groups or an isopropylidenedioxy group, whose contribution to the electronic effects of the system would be like that of methylenedioxy, suggests some special role for this last group. Steric requirements in addition to the electronic contribution for effective action could offer a reasonable basis for the observed limitation in structure-activity relationship. Consideration, however, of the broad array of pesticide types whose metabolism appears to be affected by the presence of the methylenedioxybenzenes precludes analog competitive inhibition as a reasonable explanation for the observed effect. The inhibition in some fashion of a common mediator for metabolism of all those pesticides whose lethality is influenced by joint action of the methylenedioxybenzenes may be proposed. For this purpose both electronic and steric requirements could be invoked. Such a hypothesis would not necessitate chemical activity on the part of the methylenedioxy system to the extent that a covalent bond in this system would be ruptured.

Finally, the reaction of methylenedioxybenzene with tritylcarbonium ion may be used as a model to suggest another hypothesis to explain the manner in which the metabolism of pesticides is inhibited. The synergist (or antagonist) having reached the location of the pesticide-metabolizing system is itself oxidatively metabolized by transfer of a hydride from its methylenedioxy group to an acceptor in the enzyme system. The resulting electrophilic benzodioxolium ion may then, acting as an acylating agent, attack hydroxyl, amino, sulfhydryl, or other nucleophilic groups in a component of the enzyme chain with consequent irreversible inhibition. This is not to say that by hydrolytic removal of the blocking group the enzyme could not be slowly regenerated. The regeneration would fit the well known fact that while synergists are effective if applied topically prior to application of the pesticide, the effectiveness does not persist too long.

A further effect of the metabolism of the methylenedioxybenzenes at the site of insecticide metabolism could be the result of a nonspecific competition at some limiting step by the catechol derivatives resulting from reaction of the benzodioxolium ion with water. A combination of both of these suggested modes of inhibition is not unreasonable.

The testing of methylene-deuterated methylenedioxybenzene synergists prepared in this laboratory is under way and preliminary data support the view that the synergistic action involves chemical participation of the methylenedioxy group (*33*)

Analogs of the synergistic methylenedioxybenzenes which can be expected by hydride transfer to form cationic electrophiles of reactivity similar (*2*) to the benzodioxolium ions are in the course of preparation for testing.

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The author is indebted to Christopher Wilkinson, graduate student in the Department of Entomology of the University of California, Riverside, for stimulating discussions which led to the hypothesis presented in this paper. The author acknowledges the financial assistance of the New York City Health Research Council under contract HRC U1353 with Fordham University and of a Faculty Fellowship from Fordham University.

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CARBAMATE INSECTICIDES

Effects of Chemical Structure on Intoxication and Detoxication of Phenyl *N*-Methylcarbamates in Insects

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THESE investigations of aryl carbamates as potential insecticides began in 1949 with a study of the insecticidal activities of physostigmine sulfate, the *N*,*N*-dimethylcarbamic acid ester of 3-hydroxy-2-pyridylmethyl dimethylamine dihydrochloride, and other quaternary ammonium derivatives of aryl *N*-methylcarbamates (*34*). The quaternary compounds inhibited fly brain cholinesterase at concentrations of 10^{-8} *M* but were inactive as contact toxicants when applied at dosages of 500 μg. per gram. Therefore it became of interest to determine, from the analogy with the organophosphorus anticholinesterases, whether modification of the structures of these very active carbamate