Synthesis of Methylene-C^{14}—Dioxyphenyl Compounds: Radioactive Safrole, Dihydrosafrole, Myristicin, Piperonyl Butoxide, and Diastereoisomers of Sulfoxide

SHOZO KUWATSUKA[1] and JOHN E. CASIDA

Division of Entomology and Acarology, University of California, Berkeley, Calif.

Methylene-C^{14} iodide, prepared by reduction of iodoform-C^{14} was made to react with the appropriate catechol to yield the following methylene-C^{14}–dioxyphenyl compounds with a specific activity of 1.0 millicurie per millimole: safrole, dihydrosafrole, myristicin, sulfoxide synergist {1,2-methylenedioxy-4-[2-(octylsulfinyl)propyl]benzene}, and piperonyl butoxide {α-[2-(2-butoxyethoxy)ethoxy]-4,5-methylenedioxy-2-propyltoluene}. Yields from iodoform-C^{14} were 31 to 55% on a 0.5- to 0.8-mmole scale except with piperonyl butoxide, where the yield appeared to be related to the specific activity of the methylene-C^{14} iodide used. The octylthio, octylsulfinyl, and octylsulfonyl analogs of sulfoxide synergist, substituted on the 1-, 2-, or 3-position of the propyl group, were prepared in nonradioactive form for comparative purposes. Sulfoxide synergist and the 1-octylsulfinyl analog were resolved by chromatography into the enantiomorphs of the diastereoisomers about the sulfoxide grouping and the asymmetric carbon of the propyl grouping. The potency as synergists for the insecticidal activity of carbaryl (1-naphthyl methylcarbamate) and pyrethrum with houseflies (*Musca domestica* L.) was compared for all the nonradioactive methylenedioxyphenyl compounds prepared.

THE METHYLENEDIOXYPHENYL GROUP is present in many natural and synthetic compounds of importance such as perfumes, flavorings, and insecticide synergists. Certain methylenedioxyphenyl compounds, such as safrole and dihydrosafrole, have carcinogenic activity when fed to rats at high dosages for protracted periods (*12*, *13*). Although the methylenedioxy group has been postulated as the synergistic part of the molecule (*4*, *9*, *10*, *14-16*, *24*), the reactions it undergoes in biological systems are largely unknown. Piperonyl butoxide with radioactive carbon in the ether side chain, α-[2-(2-butoxyethoxy)-ethoxy] - 4,5 - methylenedioxy - 2 - propyltoluene-α-C^{14}, has been prepared and its fate in Madeira roaches, *Leucophaea maderae* (F.), investigated without identification of metabolites formed (*20*). Future studies on the metabolic fate and mechanism of synergistic and/or carcinogenic action of this type of chemical would be greatly facilitated by the availability of methylene-C^{14}–dioxyphenyl compounds.

In this study, five radiolabeled methylenedioxyphenyl compounds, all active as insecticide synergists, were prepared: safrole (4-allyl-1,2-methylene-C^{14}-dioxybenzene); dihydrosafrole (4-propyl-1,2-methylene-C^{14}-dioxybenzene); myristicin (5-allyl-1-methoxy-2,3-methylene-C^{14}-dioxybenzene); piperonyl butoxide {α-[2-(2-butoxyethoxy)ethoxy]-4,5-(methylene - C^{14} - dioxy) - 2 - propyltoluene}; and sulfoxide synergist {1,2-(methylene-C^{14} - dioxy) - 4 - [2 - (octylsulfinyl)propyl]benzene}. The synthetic route selected for maximum potential radiochemical yields involved the conversion of iodoform-C^{14} to methylene-C^{14} iodide and subsequent reaction with the appropriate catechol to form the methylene-C^{14}–dioxyphenyl compound. The catechols were prepared by scission of the methylenedioxy group of the methylenedioxyphenyl compounds or by direct synthetic approaches. Two major components of sulfoxide synergist were resolved by paper and thin-layer chromatography (*2*, *3*), but their chemical nature was not determined. The identification of these components was considered to be a necessary preliminary step to the synthesis of radioactive sulfoxide synergist.

Experimental

Materials and Methods. Iodoform-C^{14} was obtained from the Volk Radiochemical Co., Burbank, Calif. The following methylenedioxyphenyl compounds were used as comparison materials for the identification of the radioactive products from synthesis or as starting materials for preparation of the catechols: safrole, dihydrosafrole, and isosafrole (Aldrich Chemical Co., Milwaukee, Wis.); piperonyl butoxide of technical and purified grades (U. S. Industrial Chemicals, New York, N. Y.); sulfoxide synergist of technical and purified (*8*) grades (S. B. Penick and Co., New York, N. Y.) through the courtesy of R. W. Price; and myristicin as separated from parsnips (*11*) through the courtesy of E. P. Lichtenstein, University of Wisconsin, Madison, Wis.

For column chromatography, silicic acid (analytical reagent for chromatography, 100-mesh, Mallinckrodt Chemical Works, activated by heating at 110° C. for 16 hours) columns were prepared by slurrying the silicic acid in the appropriate solvent and pouring into the column. The column size was 2.2 × 25 cm. and it was filled with 40 grams of silicic acid unless specifically noted otherwise. Elution was accomplished with the solvents indicated in Table I using nitrogen pressure to adjust the elution rate to 1 to 5 ml. per minute. An automatic fraction collector was used to collect 10-ml. fractions. The position of elution for the various compounds was ascertained by determining the light absorption at 285 mμ (in the same solvent in which they were eluted) and the radioactivity for aliquots from each fraction. Fractions were also analyzed by silica gel thin-layer chromatography (TLC) to ascertain their homogeneity prior to combining them for the resolved components.

Silica gel G (Kensington Scientific Corp., Berkeley, Calif.) was used for thin-layer chromatography with plates of 0.25-mm. thickness for analytical and 1.5-mm. thickness for preparative scale separations. Catechols were detected as blue or brown spots by spraying with 2% ferric chloride in methanol. Methylenedioxyphenyl compounds (and the dibenzyl ether intermediates for the preparation of the piperonyl butoxide catechol) were detected as brown or black spots by the chromotropic acid reagent described

[1] Present address, Department of Agricultural Chemistry, Kyushu University, Fukuoka, Japan.

Table I. Chromatographic Characteristics and Methylenation Yields for Safrole, Dihydrosafrole, Myristicin, Piperonyl Butoxide, and Sulfoxide Synergist

	Safrole	Dihydro-safrole	Myristicin	Piperonyl Butoxide	Sulfoxide A	Sulfoxide B
THIN-LAYER CHROMATOGRAPHY–SILICIC ACID						
	R_f Values for Catechols[a]					
Ether	0.93	0.93	0.90	0.82	0.27	0.27
Hexane-ether (1 to 1)	0.46	0.48	0.48	0.17	0.02	0.02
Benzene-ether (10 to 1)	0.28	0.28	0.28	0.06	0.03	0.03
	R_f Values for Methylenedioxyphenyl Compounds					
Hexane	0.25	0.29	0.08	0.00	0.00	0.00
Ether	0.98	0.98	0.98	0.98	0.53	0.45
Hexane-ether (10 to 1)	0.80	0.89	0.54	0.08	0.02	0.02
Hexane-ether (1 to 1)	0.98	0.98	0.93	0.65	0.10	0.10
Benzene	0.87	0.90	0.60	0.12	0.05	0.05
Benzene-ether (10 to 1)	0.98	0.98	0.89	0.45	0.10	0.10
Column Chromatography–Silicic Acid						
Eluting solvent for methylenedioxyphenyl compounds, ratio	Hexane 20 Ether 1	Hexane 20 Ether 1	Hexane 15 Ether 1	Hexane 3 Ether 2	Ether	Ether
	Methylenation Yields, %					
0.005 mc./mmole						
By weight	46	45	36[b]	23[c]	18	16
By radioactivity	45	45	35[b]	22[c]	17	15
1.0 mc./mmole						
By weight	34	38	58	9.6	23	18
By radioactivity	33	37	58	8.4	21	16

[a] Catechol intermediates which on methylenation yielded appropriate methylenedioxyphenyl compounds.
[b] From reaction of equimolar catechol and methylene iodide. In all other cases, two molar equivalents of catechol reacted with one molar equivalent of methylene iodide.
[c] Methylene-C^{14} iodide of 0.01 mc./mmole used.

by Beroza (*3*); when C^{14}-labeled, the methylenedioxyphenyl compounds were detected by radioautography using x-ray film. R_f values for the pure catechols and methylenedioxyphenyl compounds are given in Table I.

Infrared spectra were determined as 4% solutions in chloroform or carbon tetrachloride using the Beckman IR 4 infrared spectrophotometer with sodium chloride optics. Nuclear magnetic resonance spectra were determined as 1% solutions in deuterated chloroform with a Varian A-60 nuclear magnetic resonance spectrometer using tetramethylsilane as an internal standard. Radioactive measurements were made with the Packard Tri-Carb Model 3003 liquid scintillation spectrometer. Melting points (uncorrected) were determined with the crystals between microscope cover slips on a hot block by observing single crystals with a microscope. Elemental analyses and molecular weight determinations were made by the Microchemical Analytical Laboratory, Department of Chemistry, University of California, Berkeley, Calif.

Female houseflies, *Musca domestica* L. (17 mg., 4 to 5 days after emergence, SCR strain originally obtained from Stauffer Chemical Co., Mountain View, Calif.), were used for assaying the potency of the various nonradioactive methylenedioxyphenyl compounds as synergists for the toxicity of carbaryl (1-naphthyl methylcarbamate) and pyrethrum. The insecticide was applied to the notum in 1.0 µl. of acetone and, 10 to 60 minutes later, the potential synergist was also applied in 1.0 µl. of acetone to the notum. Mortality counts were made 24 hours after treatment.

Preparation and Characterization of Sulfoxide Synergist Components. Technical and purified (*8*) sulfoxide synergist were resolved into almost equal amounts of two components by column or thin-layer chromatography, using silicic acid and ether. Technical sulfoxide synergist in hexane, at −20° C., yielded crystals which also contained equal amounts of the two components. The nature of these components was further investigated.

1,2 - Methylenedioxy - 4 - [2 - (octylthio)propyl]benzene and its sulfoxide and sulfone were prepared according to Synerholm, Hartzell, and Cullman (*22*). The sulfide was obtained in 70% yield and it distilled at 140–44° C. at 0.25 mm. [The reported boiling range is 183–86° C. at 1 mm. (*22*).] It had the appropriate infrared spectrum and, based on TLC, consisted of a single major component. Oxidation with hydrogen peroxide in acetone gave a material identical to the purified sulfoxide synergist (*22*), in respect to infrared spectrum, and almost equal amounts of two components resolved by TLC with ether. Both components had R_f values and infrared spectra that differed from those found for the original sulfide, the more oxidized sulfone, and 1,2-methylenedioxy-4-[1-(octylsulfinyl)propyl]benzene discussed later. The two-component sulfoxide mixture was oxidized with hydrogen peroxide in acetic acid to give the sulfone (80% yield; m.p. 60° C., single component on TLC). [This sulfone (m.p. 58° C.) has previously been prepared by direct oxidation of the sulfide (*22*).]

Two other sulfides were also prepared: 1,2 - methylenedioxy - 4 - [1 - (octylthio)propyl]benzene which distilled at 129–134° C. at 0.25 mm. [reported value: 180–85° C. at 2 mm. (*22*)]; 1,2-methylenedioxy - 4 - [3 - (octylthio)-propyl]benzene which distilled at 155–58° C. at 0.25 mm. [reported value: 195–98° C. at 1 mm. (*21*)]. These sulfides, which showed up as single components on TLC, were oxidized to their sulfoxides and sulfones. 1,2-Methylenedioxy - 4 - [3 - (octylsulfinyl)propyl]-benzene, m.p. 61° C., contained only one major component, R_f 0.30, based on TLC with ether; however, 1,2-methylenedioxy - 4 - [1 - (octylsulfinyl)propyl]-benzene was resolved into two components (R_f values of 0.73 and 0.60) on TLC with ether and these components were found to be present in a 1 to 4 ratio, based on column chromatography. The following sulfone products were found to be single components on TLC: 1,2-methylenedioxy - 4 - [3 - (octylsulfonyl)-propyl]benzene (83% yield; m.p. 70–70.5° C.); 1,2-methylenedioxy-4-[1-(octylsulfonyl)propyl]benzene (57% yield from the mixture of the two components of the sulfinyl compound after purification of the oil by column chromatography).

A 200-mg. quantity of technical sulfoxide synergist was partially resolved into two components on a 150-gram silicic acid column (3.4 × 35 cm.) with ether. The curve for resolution of the components from technical sulfoxide synergist (based on ultraviolet absorption) was similar to that for sulfoxide-C^{14} synergist resolution (which gave identical curves in the sulfoxide elution region based on both ultraviolet absorption and radioactivity) (Figure 1). Each column eluate fraction was assayed by TLC. The two major components eluted from the column were identical to the purified sulfoxide synergist components resolved by TLC. The first eluted component, that with the higher R_f value (0.55) on TLC, was designated as sulfoxide A, and the second, with the lower R_f (0.45), as sulfoxide B. Three columns as described were used to purify technical sulfoxide synergist (600 mg.) and the intermediate fractions, containing components not completely resolved, were rechromatographed until pure sulfoxide A (180 mg.) and pure sulfoxide B (240 mg.) were obtained. (As used here, pure designates material with a single component on TLC.)

Sulfoxide A was an oil. Sulfoxide B crystallized as white needles from hexane, and on recrystallization three times from hexane, a material with a melting point of 35–36° C. was obtained. The crystalline sulfoxide B yielded the same R_f value as one component of the sulfoxide synergist and the molecular weight (mol. wt.) and elemental analyses (per cent) were similar to those calculated for sulfoxide synergist: found, mol. wt. 302, C 66.17, H 8.64, S 9.36; calculated, mol. wt. 324, C 66.63, H

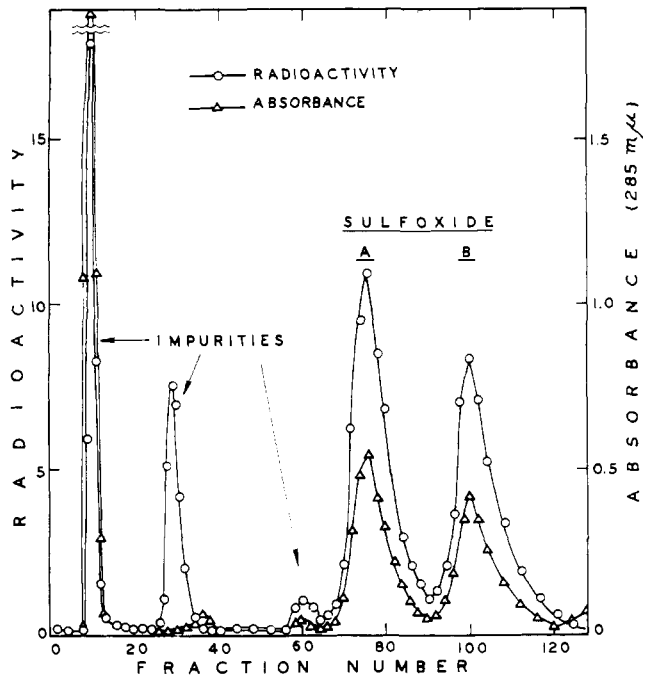

Figure 1. Chromatographic purification of crude 1,2-(methylene - C^{14} - dioxy) - 4 - [2 - (octylsulfinyl)propyl] - benzene and resolution of diastereoisomers (sulfoxide A and B) on a silicic acid column with ether

Each 10-ml. fraction analyzed for total radioactivity (plotted as c.p.m. × 10^{-6}) and absorbance at 285 mμ for a 0.5-ml. aliquot diluted to 3.0 ml. with ether

8.69, S 9.88 (as $C_{18}H_{28}O_3S$). Since some loss occurred on isolation of the pure components, the ratio of sulfoxide A to sulfoxide B in the technical and the purified sulfoxide synergists was determined in separate experiments using TLC. Based on the absorption of the resolved materials at 289 mμ, the ratio of sulfoxide A to sulfoxide B was 51 to 49 in both the technical and purified material.

Infrared spectra of sulfoxide A, sulfoxide B, and the mixture of the two components in purified sulfoxide synergist were completely the same except for a weak absorption band at 993 cm.$^{-1}$ The 993-cm.$^{-1}$ absorption band, absent in sulfoxide A but a distinct peak in sulfoxide B, appeared in the same position when the spectra were prepared from chloroform or carbon tetrachloride solutions. Technical and purified sulfoxide synergist yielded the 993-cm.$^{-1}$ peak at an absorption intensity intermediate between that of sulfoxide A and B. A nuclear magnetic resonance spectrum of crystalline sulfoxide B showed the following peaks: (δ, p.p.m.); 6.7 (3*H*, narrow multiplet, aromatic protons), 5.92 (2*H*, singlet, CH_2 of methylenedioxy group), 2.4 to 3.4 (5*H*, multiplet, protons adjacent to aromatic ring and sulfoxide group), 1.2 to 1.9 (12*H*, multiplet, protons of nonterminal methylene groupings within *n*-octyl group), 0.6 to 1.2 (6*H*, multiplet, methyl protons). Sulfoxide A differed from sulfoxide B in the following manner: (δ, p.p.m.); 6.7 (3*H*, singlet), 2.4 to 3.4 (5*H*, small changes in position of peaks comprising multiplet), 0.6 to 1.2 (6*H*, sulfoxide B showed two distinct peaks at 0.89 and 1.18, while sulfoxide A in addition to these peaks had a third distinct peak at 1.07). Sulfoxide A and sulfoxide B formed the same sulfone (individual and mixed melting point of 60° C.) on oxidation.

A 200-mg. quantity of 1,2-methylenedioxy - 4 - [1 - (octylsulfinyl)propyl]benzene was resolved into two components by column chromatography on silicic acid with ether. The pure components (30 and 130 mg., in the order of elution) were obtained by rechromatography on a column. In a separate experiment, it was determined by column chromatography that the components, designated as component A′ and component B′, were present in a 1 to 4 ratio in the original oxidation mixture. The only difference in the infrared spectra of the two components appeared at 1025 cm.$^{-1}$ (as compared to 993 cm.$^{-1}$ for sulfoxide B). The absorption at 1025 cm.$^{-1}$ was stronger for the second-eluted component, B′, than for the first-eluted component, A′. (This 1025-cm.$^{-1}$ band was stronger for both component A′ and component B′ than the 993-cm.$^{-1}$ band was for sulfoxide B.) On oxidation, both sulfoxide A′ and sulfoxide B′ yielded 1,2-methylenedioxy-4-[1-(octylsulfonyl)propyl]benzene, an oil, which was a single material based on TLC.

These experiments established that the two components of the sulfoxide synergist are enantiomorphs of diastereoisomers about the sulfoxide grouping (*7*) and the asymmetric carbon of the propyl grouping. The infrared and nuclear magnetic resonance spectra could not be interpreted in relation to the structure of the sulfoxide synergist diastereoisomers because comparison spectra for similar compounds were not available.

Isomerization of the sulfoxide synergist diastereoisomers was demonstrated in acid but not in neutral or alkaline solutions. Sulfoxide A-C^{14} or sulfoxide B-C^{14} (20 μg.) was dissolved in 100 μl. of methanol and 4 μl. of acetone. Ten microliters of water or 10% hydrochloric acid or 10% sodium hydroxide were then added and the reaction mixtures were heated for 3 hours at 60° C. in sealed ampoules. The products were recovered by addition of water and extraction into ether. The labeled products were chromatographed on TLC either alone or after fortification with nonlabeled purified sulfoxide synergist containing the mixture of two components.

Three labeled materials were evident. Sulfoxide A-C^{14} (R_f 0.55) and sulfoxide B-C^{14} (R_f 0.45) cochromatographed with the known compounds while a new and unidentified material appeared at R_f 0.95. After reaction under neutral or alkaline conditions, 90 to 97% of the radioactivity from either sulfoxide A-C^{14} or sulfoxide B-C^{14} was recovered as the original diastereoisomer. From 0.4 to 1.4% of sulfoxide B was recovered from the sulfoxide A reactions, and from 0.4 to 1.1% of sulfoxide A was recovered from the sulfoxide B reactions, and no radioactivity was detected, in any case, in the R_f 0.95 region. After reaction under the acidic condition, the products from sulfoxide A were found to consist of 62% sulfoxide A, 22% sulfoxide B, and 6% of the R_f 0.95 component. Sulfoxide B yielded 30% sulfoxide A, 56% sulfoxide B, and 10% of the R_f 0.95 component under the acidic reaction condition. Isomerization of these sulfoxide diastereoisomers occurred readily in acid solution but very slowly, or not at all, in neutral or alkaline solutions.

Preparation and Characterization of Catechol Intermediates. 4-Allylcatechol [m.p. 46–47° C.; reported m.p. 48° C. (*19*) and 46–47° C. (*18*)] was prepared from 4-allylcatechol diacetate [distilled at 168–69° C. at 15 mm.; reported b.p. 148° C. at 2 mm. (*18*)] resulting from refluxing safrole with acetic anhydride and phosphoric acid (*17*, *18*). 4-Propylcatechol [m.p. 59–60° C.; reported m.p. 60° C. (*5*)] was prepared by catalytic hydrogenation with palladium-on-charcoal of eugenol to yield dihydroeugenol [distilled at 129° C. at 15 mm.; reported b.p. 128–30° C. at 13 mm. (*6*)]

which was demethylated by refluxing with hydrobromic, hydroiodic, and acetic acids to yield 4-propylcatechol which was distilled at 148° to 155° C. at 15 mm. 5-Allyl-3-methoxycatechol [distilled at 102–104° C. at 0.2 mm.; reported b.p. 112.5–115° C. at 0.22 to 0.3 mm. (*23*)] was prepared from 3-methoxycatechol by allylation and allylic rearrangement.

The catechol intermediate for sulfoxide synergist was prepared by refluxing 10 grams of sulfoxide synergist (technical purity) with 12 grams of aluminum chloride in 100 ml. of chlorobenzene for 2 hours. After cooling, the reaction mixture was poured over ice and the chlorobenzene removed by steam distillation. The products were then recovered from the residue by extraction into ether, which was washed with water. Acidic products were extracted from the ether into 5% sodium hydroxide and reextracted into ether following acidification. After a final water wash and drying with sodium sulfate, the ether was removed to yield 9.1 grams of brownish oil. Column purification of 3 grams of this oil was accomplished on the silicic acid column using ether for elution to yield impurities in the first 200 ml. of ether and 2.2 grams of oily substance (sulfoxide synergist catechol) in the next 800 ml. of ether.

Attempts to resolve the sulfoxide synergist catechol diastereoisomers by either thin-layer or column chromatography proved unsuccessful. Methylenation of this catechol intermediate for sulfoxide synergist, as described later, yielded the sulfoxide synergist diastereoisomers in almost the same ratio as that resulting from the oxidation of the sulfide resulting from reaction of isosafrole with octylmercaptan, as described earlier.

The catechol intermediate for piperonyl butoxide was not obtained on decomposition of piperonyl butoxide with aluminum chloride or acetic anhydride. Instead, extensive decomposition occurred which probably involved cleavage of the butoxy ether group as well as the methylenedioxyphenyl group. Therefore, the intermediate was prepared by direct synthesis.

The procedure used was based on that reported for preparation of piperonyl butoxide from dihydrosafrole (*20*). 4-Propylcatechol dibenzyl ether was formed by reaction of 30 grams of 4-propylcatechol with 50 grams of benzyl chloride and 50 grams of potassium carbonate in 150 ml. of acetone on refluxing for 24 hours. The dibenzyl ether, which distilled at 175° to 185° C. at 0.2 mm., was recovered in 70% yield. Ten grams of dibenzyl propylcatechol (30 mmoles) were made to react with 1.12 grams of paraformaldehyde (37 mmoles) and 1 gram of fortified hydrochloric acid. The mixture was slowly heated to 80° C. in a period of 2 hours; it was held at 80° C. for an additional 4 hours with stirring. The product was extracted into ether, washed with water, and dried with sodium sulfate, and the solvent removed. The residue was almost completely dissolved in 100 ml. of warm hexane, from which white crystals appeared on cooling. These were repeatedly recrystallized from hexane. The product (m.p. 58° C., 80% yield) was a single material as ascertained by TLC, and was presumably 5-chloromethyl-4-propylcatechol dibenzyl ether, based on the infrared spectrum, molecular weight (mol. wt.) and elemental analyses (per cent): found, mol. wt. 395, C 75.32, H 6.59, Cl 9.28; calculated, mol. wt. 381, C 75.67, H 6.62, Cl 9.31 (as $C_{24}H_{25}O_2Cl$). A 7.6-gram portion of this chloromethyl derivative (20 mmoles) was made to react with sodium butoxyethoxyethoxide [made by adding 0.50 grams (22 mmoles) of sodium to 20 grams of butyl Carbitol] at 120° C. for 2 hours. After cooling, ether was added to the reaction mixture and the organic phase was subsequently washed with water, dilute hydrochloric acid, and water. Evaporation of the ether and residual butyl Carbitol yielded 9.2 grams of brownish oil. A 5-gram portion of the residue was purified on a silicic acid column because the product was not distillable, decomposing at less than 210° C. at 0.4 mm. On elution of the column with benzene-ether (10 to 1), two minor impurities came through initially, followed by a yellow substance, and, subsequently, by the major component (assumed to be the dibenzyl ether of the piperonyl butoxide catechol). The chromatography was repeated three times until the product, recovered in 80% yield, contained a single component based on TLC. This product (oil, 4.0 grams) and 1 gram of 10% palladium-on-charcoal in 100 ml. of diisopropyl ether was hydrogenated at 1.1-atm. pressure for 45 minutes until the theoretical hydrogen was consumed. The reaction mixture was then filtered, the solvent was evaporated, and the product (2.0 grams) was purified in two batches by column chromatography. After two minor components were eluted with hexane-ether (5 to 1), the major component was eluted with hexane-ether (3 to 1). The product was a single component based on TLC and it was obtained in 70% yield based on the dibenzyl ether used. This material, assumed to be the catechol intermediate for piperonyl butoxide, was a yellow oil, not distillable at less than 210° C. at 0.4 mm. It was converted to piperonyl butoxide on reaction with methylene iodide, under the conditions described later, and the piperonyl butoxide so formed was identical with the authentic sample based on TLC and infrared spectra.

Methylene-C^{14} Iodide. Iodoform-C^{14} was reduced to methylene-C^{14} iodide by a procedure modified from Adams and Marvel (*1*). Two grams of iodoform-C^{14} (1.0 mc. per mmole) and 1.5 ml. of freshly prepared sodium arsenite aqueous solution (9.5% arsenious oxide and 17.0% sodium hydroxide) were placed in a 10-ml. round-bottomed flask with a stirring bar and a reflux condenser. The reaction temperature was raised to 60–65° C. with stirring, an additional 4.5 ml. of the sodium arsenite solution was added dropwise over a 30-minute period, and the temperature was maintained at 60–65° C. for 4 more hours. After cooling to 40° C., the aqueous layer was pipetted off to leave a yellow oil. This oil was washed three times with 5-ml. portions of water and transferred by a micropipet, weighed, and quickly dissolved in 5 ml. of acetone. On contact with air, the methylene-C^{14} iodide rapidly turned red, even though nonradioactive methylene iodide, prepared in the same manner, remained yellow for several days at room temperature. [This observation is based on three nonradioactive and three radioactive preparations (1 mc./mmole). Methylene-C^{14} iodide (0.005 mc. per mmole) darkened in color at an intermediate rate.] The acetone solution was dried with sodium sulfate, and made up to 10 ml. with acetone washes of the sodium sulfate. The acetone solution of crude methylene-C^{14} iodide was kept at −20° C. and used for methylenation reactions without further purification. Crude methylene-C^{14} iodide was obtained in 97% yield by weight and 95% yield by radioactivity counting.

Methylenation Reaction. General Procedure. Conditions for methylenation (*19*, *23*) were established in three steps. In the first, nonradioactive methylene iodide was used to investigate the optimum reaction conditions and purification procedure. Second, methylene-C^{14} iodide (0.005 or 0.01 mc. per mmole) was used to confirm the identity and purity of the labeled product by cochromatography on TLC and infrared spectroscopy. Yields for each compound based on weight and radioactivity from methylene-C^{14} iodide (0.005 or 0.01 mc. per mmole) are given in Table I. Finally, methylene-C^{14} iodide (1 mc. per mmole) was used with the same conditions found optimal in step two, using methylene iodide of the lower specific activity. The identity and the purity of the final products (1 mc. per mmole) were ascertained by chromatographic characteristics on silicic acid columns and cochromatography on TLC.

Either 0.8 mmole or 0.5 mmole of methylene-C^{14} iodide, twice the millimole amount of the appropriate catechol, and four times the millimole amount of anhydrous potassium carbonate were sealed in a 10-ml. ampoule along with 3 ml. of acetone and a small magnetic stirring bar. Prior to sealing, the air was mostly displaced with nitrogen for the sulfoxide synergist and piperonyl butoxide syntheses. The reaction was allowed to proceed at 70° to 90° C. for 20 to 30 hours, with stirring. Products were recovered on opening the ampoule by addition of 10 ml. of water and extraction into ether (7 ml., three times). The ether was evaporated through a rectification tube at atmospheric or slightly reduced pressure. The crude products were purified by column chromatography, except in the case of piperonyl butoxide, where thin-layer chromatography was also used. The yield of methylene-C^{14}–dioxyphenyl compounds (1.0 mc. per mmole) was 33 to 58%, except with piperonyl butoxide, where the yield was less than 12%. Yields for each compound based on weight and radioactivity from methylene-C^{14} iodide (1.0 mc. per mmole) are given in Table I.

SAFROLE-C^{14}. A 0.8-mmole quantity of methylene-C^{14} iodide was made to react with 1.6 mmoles of allylcatechol at 80° to 85° C. for 24 hours. The crude product was extracted into ether and purified on a 60-gram silicic acid column (2.4 × 35 cm.) from which only a single major component was eluted. Safrole elution was accomplished with hexane-ether (20 to 1), starting after 260 ml. of eluate and being complete after 310 ml.

DIHYDROSAFROLE-C^{14}. A 0.8-mmole quantity of methylene-C^{14} iodide was made to react with 1.6 mmoles of propylcatechol and purified by the same procedure used for safrole. Dihydrosafrole-C^{14} was eluted in the 300- to 340-ml. portion of hexane-ether (20 to 1) eluate.

MYRISTICIN-C^{14}. A 0.8-mmole quantity of methylene-C^{14} iodide was made to react with 1.6 mmoles of 3-methoxy-5-allylcatechol at 70° to 75° C. for 20 hours and purified by the safrole procedure. Myristicin was eluted with hexane-ether (15 to 1) in the 220- to 320 ml. fraction.

SULFOXIDE-C^{14}. A 0.5-mmole quantity of methylene-C^{14} iodide and 1.0 mmole of the catechol intermediate for sulfoxide synergist were made to react at 80° to 85° C. for 20 hours in each of three ampoules. The crude product, in the ether extract from each individual ampoule, was partially resolved on a 60-gram silicic acid column (2.4 × 38 cm.) into components designated as sulfoxide A and B, by developing with 1300 ml. of ether, as shown in Figure 1. The intermediate fractions, which contained a mixture of sulfoxide A and sulfoxide B, were combined from the three columns and rechromatographed for further resolution. Finally, each sulfoxide synergist component from the three original columns and the rechromatography column were combined and the diastereoisomers were isolated in greater than 97% purity (determined by TLC) by one additional pass through the silicic acid column.

PIPERONYL BUTOXIDE-C^{14}. A 0.5-mmole quantity of methylene-C^{14} iodide and 1.0 mmole of the catechol intermediate for piperonyl butoxide were made to react at 65° to 70° C. for 30 hours. The crude product, recovered by ether extraction, was purified on a 40-gram silicic acid column. The column was developed successively with 150 ml. of hexane-ether (3 to 1) and with 400 ml. of hexane-ether (3 to 2), each eluting a major radioactive peak. The peak in the first solvent was equivalent to 10 to 20% of the radioactivity, some of which was methylene-C^{14} iodide and an unknown substance. The second solvent eluted 8.5 to 11.5% of the radioactivity, including mostly piperonyl butoxide-C^{14} mixed with two minor substances. The material eluted by the second solvent was repurified by column chromatography but the product obtained was still impure piperonyl butoxide-C^{14}. Thin-layer chromatography [silica gel G, 1.5-mm. thickness, hexane-ether (1 to 1)] resulted in the final resolution of pure radioactive piperonyl butoxide from the column-purified material. The yields for the high radioactive product (1 mc. per mmole) reported in Table I are based on the combined products from three reactions as described.

Discussion

Comparison of Yields in Radioactive and Nonradioactive Preparations. Safrole, dihydrosafrole, myristicin, and sulfoxide synergist radiosyntheses gave the yields on methylenation anticipated from the preliminary nonradioactive reactions (see Table I). In each case, the pure products, labeled or nonlabeled, were isolated by a single pass through a silicic acid column, except for sulfoxide synergist in which case isomers were also resolved. No isomerization of safrole to isosafrole or of myristicin to isomyristicin occurred during synthesis and purification as ascertained by examination of infrared spectra of the final radioactive products. The synthesis of radioactive piperonyl butoxide gave much lower yields with methylene-C^{14} iodide of 1 mc. per mmole specific activity than with nonradioactive methylene iodide or 0.01 mc. per mmole specific activity. In each of four reactions with nonlabeled or low specific activity methylene iodide, the yields of piperonyl butoxide on methylenation were 17 to 22%. These reactions gave pure piperonyl butoxide after a single pass through the column. Yields of piperonyl butoxide-C^{14} from methylene-C^{14} iodide of 1 mc. per mmole were usually 8 to 12%, never higher, and sometimes practically no piperonyl butoxide-C^{14} was recovered based on eight such radioactive preparations made in the same way, using the identical nonradioactive reagents and the same scale of operation. When methylene-C^{14} iodide of 1 mc. per mmole was used, the column eluate fractions, which with nonlabeled material contained only piperonyl butoxide, consisted of a mixture of materials requiring additional thin-layer chromatography for resolution. The catechol intermediate for piperonyl butoxide appeared to be less stable than the other catechols under the methylenation conditions used. The methylenation reaction mixtures, including those for piperonyl butoxide with nonlabeled or 0.01 mc. per mmole methylene-C^{14} iodide, slowly turned black in the course of the reaction. This reaction to form piperonyl butoxide from 1 mc. per mmole methylene-C^{14} iodide turned black at an early stage of the reaction and produced ether-insoluble polymerized substances in 30 to 50% yield, based on radioactivity. In contrast, methylenation with 0.01 mc. per mmole methylene iodide to give piperonyl butoxide resulted in only 9 to 11% polymerized materials. Thus, the low yield of piperonyl butoxide appears to be associated both with the greater instability of the catechol intermediate for piperonyl butoxide and with radioactive levels of 1 mc. per mmole.

Synergistic Activity of Sulfoxide Synergist Components and Analogs. Carbaryl was highly synergized in its toxicity to houseflies by safrole, dihydrosafrole, and piperonyl butoxide, but less effectively by analogs of sulfoxide synergist [Table II, see also (*15* and *24*)]. The three (octylthio)propyl analogs were more effective than their sulfoxides and sulfones for carbaryl synergism and, in each series, the potency decreased in the order of 1 > 2 > 3 for the position of sulfur attachment to the propyl group. Impurities in technical sulfoxide synergist, such as isosafrole, may have contributed to its apparent synergistic activity with carbaryl. Purified sulfoxide synergist was a less effective

Table II. Synergistic Activity of Nonradioactive Analogs of Sulfoxide Synergist and Other Methylenedioxyphenyl Compounds on Carbaryl and Pyrethrum Toxicity to Houseflies Following Topical Application

Synergist, 10 μg.	LD_{50}, μG./Gram	
	Carbaryl	Pyrethrum
None	>6000	71
Safrole	25	25
Isosafrole	9	23
Dihydrosafrole	25	24
Piperonyl butoxide	15	1.6
Sulfoxide synergist		
Technical	65	4
Purified	129	..
1,2-Methylenedioxybenzene Analogs of Sulfoxide Synergist		
4-[1-(octylthio)propyl]	34	4
4-[2-(octylthio)propyl]	68	6
4-[3-(octylthio)propyl]	90	9
4-[1-(octylsulfinyl)propyl]		
Component A′	157	8
Component B′	78	3
4-[2-(octylsulfinyl)propyl]		
Component A	168	3
Component B	286	2.2
4-[3-(octylsulfinyl)propyl]	>180	4.5
4-[1-(octylsulfonyl)propyl]	78	3
4-[2-(octylsulfonyl)propyl]	180	17
4-[3-(octylsulfonyl)propyl]	180	39

synergist for carbaryl than the technical grade and the components were even less effective. The high synergistic activity of piperonyl butoxide and the sulfoxide synergist analogs but not of safrole or dihydrosafrole for pyrethrum toxicity to houseflies was as anticipated (*10, 14, 16, 21, 22*). Pyrethrum synergism was optimal with the components of the 2-octylsulfinyl analog but was also high with many other analogs. With both the sulfides and the sulfones, pyrethrum synergism decreased in the order of 1 > 2 > 3 for the position of sulfur attachment to the propyl group. Of particular interest was the finding that the more polar component (B or B′) was a more effective pyrethrum synergist than the less polar component (A or A′) of the 2-octylsulfinyl and, especially, the 1-octylsulfinyl compounds. The isomeric configuration about the sulfoxide grouping and the asymmetric carbon of the propyl grouping, therefore, influences the activity for synergism of pyrethrum toxicity.

In tests made under conditions somewhat similar to those used in this study, myristicin has been found to have a high degree of synergism for carbaryl but not for pyrethrum toxicity (*11*).

Acknowledgment

The authors are indebted to Esaac Esaac of this laboratory for performing the bioassays reported, and W. G. Dauben and G. C. Pimentel, Department of Chemistry, University of California, Berkeley, for assistance in interpretation of spectra.

Literature Cited

(1) Adams, R., Marvel, C. S., *Org. Synth.* **1,** 57 (1921).
(2) Beroza, M., *Anal. Chem.* **28,** 1550 (1956).
(3) Beroza, M., J. AGR. FOOD CHEM. **11,** 51 (1963).
(4) Casida, J. E., *Ann. Rev. Entomol.* **8,** 39 (1963).
(5) Delange, R., *Compt. Rend.* **130,** 659 (1900).
(6) Fitcher, F., Christen, A., *Helv. Chim. Acta* **8,** 332 (1925).
(7) Harrison, P. W. B., Kenyon, J., Phillips, H., *J. Chem. Soc.* **1926,** p. 2079.
(8) Haus, J. B., Manolev, L., Price, R. W., *J. Assoc. Offic. Agr. Chemists* **47,** 264 (1964).
(9) Hennessy, D. J., J. AGR. FOOD CHEM. **13,** 218 (1965).
(10) Hewlett, P. S., *Advan. Pest Control Res.* **3,** 27 (1960).
(11) Lichtenstein, E. P., Casida, J. E., J. AGR. FOOD CHEM. **11,** 410 (1963).
(12) Long, E. L., Jenner, F. M., *Federation Proc.* **22,** No. 684, 275 (1963).
(13) Long, E. L., Nelson, A. A., Fitzhugh, O. G., Hansen, W. H., *Arch. Pathol.* **75,** 595 (1963).
(14) Metcalf, R. L., in "Organic Insecticides," pp. 73–101, Interscience, New York, 1955.
(15) Moorefield, H. H., Weiden, M. H. J., *Contrib. Boyce Thompson Inst.* **22,** 425 (1964).
(16) Negherbon, W. O., in "Handbook of Toxicology, Vol. III. Insecticides," pp. 716–721, W. B. Saunders Co., Philadelphia, Pa., 1959.
(17) Ono, K., Hirayama, S., *J. Chem. Soc. Japan* **58,** 926 (1937).
(18) Ono, K., Oyamada, T., *Ibid.*, **57,** 872 (1936).
(19) Perkin, W. H., Jr., Trikojus, V. M., *J. Chem. Soc.* **1927,** p. 1663.
(20) Schmidt, C. H., Dahm, P. A., *J. Econ. Entomol.* **49,** 729 (1956).
(21) Prill, E. A., Hartzell, A., Arthur, J. M., *Contrib. Boyce Thompson Inst.* **14,** 127 (1946).
(22) Synerholm, M. E., Hartzell, A., Cullman, V., *Ibid.*, **15,** 35 (1947).
(23) Trikojus, V. M., White, D. E., *J. Chem. Soc.* **1949,** p. 436.
(24) Weiden, M. H. J., Moorefield, H. H., J. AGR. FOOD CHEM. **13,** 200 (1965).

Received for review April 5, 1965. Accepted August 27, 1965. Study supported in part by grants from California Division of the American Cancer Society (Grant 307); Cancer Research Coordinating Committee of the University of California, Berkeley; S. C. Johnson and Son, Inc., Racine, Wis.; the U. S. Atomic Energy Commission [Contract AT(11-1)-34, Project Agreement No. 113]; and the U. S. Public Health Service (Grant No. ESGM 00049).

INSECTICIDE RESIDUES IN URINE

Determination of Urinary *p*-Nitrophenol by Thin-Layer Chromatography and Phosphorimetry

H. A. MOYE

Southern Regional Pesticide Residue Research Laboratory, University of Florida, Gainesville, Fla.

J. D. WINEFORDNER

Department of Chemistry, University of Florida, Gainesville, Fla.

Human urine is analyzed for *p*-nitrophenol, a major metabolite of parathion. After acid hydrolysis, the urine is extracted with ether. The ether extract is then cleaned up by thin-layer chromatography. A urine blank is employed to account for the remaining urine background. The final measurement is by the extremely sensitive technique of phosphorimetry. The time required for the entire procedure is only 40 minutes and only 5 ml. of urine is required for the analysis of urine samples containing at least 0.01 μg. of *p*-nitrophenol. The average recovery of *p*-nitrophenol in the concentration range of 0.28 to 142 μg. per 100 ml. of urine is 88%. A relative standard deviation of 2.5% is obtained for a urine specimen containing 7.0 μg. of *p*-nitrophenol per 100 ml. of urine.

ONE of the most commonly used organophosphorus insecticides is parathion. Because of its widespread use and high toxicity, sensitive and accurate analytical techniques are needed in many agricultural and clinical laboratories to protect agricultural workers and consumers. The methods must be not only highly sensitive but also simple enough to permit their use for routine analyses.

The widely used technique of measuring blood cholinesterase activity has numerous faults when applied to methods for monitoring human parathion exposure (*3, 6*). The cholinesterase method, however, does have the advantage of measuring the effect of parathion on enzyme activity. The direct spectrophotometric measurement of *p*-nitrophenol excretion in urine is preferred (*1, 5, 8*) in many instances. These