J. Heterocycl. Chem. 1978, 15, 677.
Giri, S.; Nizamuddin. Synthesis of Some Bis-(Oxadiazolyl)Sulphides Sulphones and 2-Substituted-[1,3,4-]-oxadiazolo-[2,3,4-]quinazol-5-ones as Potential Fungicides. Agric. Biol. Chem. 1978, 42, 41–44.
Hannah, J.; Johnson, C. R.; Wagner, A. F.; Walten, E. Quaternary Heterocyclic β-Lactams. J. Med. Chem. 1982, 25, 457.
Hedrich, L. W.; Neighbores, R. Control of Unwanted Vegetation with N-Carbanylindolines. U.S. Patent 4,428,881, 1943; Chem. Abstr. 1984, 100, 1746622.
Kobayashi, G.; Matsuda, Y.; Tominaga, Y. Antiinflamatory activity of Indole derivatives. Yakugaku Zasshi 1977, 97, 1033.
Mazid, A.; Wahab, A.; Hassan, K. M.; EL-Ezbawy, S. R. Synthesis and Biological Activity of Some New Benzothiazolosulphonamidoazetidinones. Indian J. Chem. 1979, 18B, 467.
Mueller, B.; Nickl, J.; Roch, J.; Narr, B., Haarmann, W.; Wersenberger, J. M. Indolinones and their Pharmaceutical Use. Ger. Offen DE 3,143,327, 1982; Chem. Abstr. 1982, 97, 144768.
Mukherjee, A. K.; Singh, A. K. β-Lactams: Retrospects And Prospects. Tetrahedron Rep. 1978, No. 52.
Osman, A. M.; Hasian, Ph. M. Synthesis of Some New β-lactams. J. Prakt. Chem. 1978, 82, 482.
Rovnyak, G. C.; Narayanan, V. L.; Hougnitz, R. D. 1,3-Thiazolinyl and 1,3-thiazinyl substituted-indolinones. U.S. Patent 4,053,613, 1977; Chem. Abstr. 1978, 88, 22889.
Wolf, M.; Mascitti, A. A. Spiro[indoline-3,2-thiazolidine]-2-ones. U.S. Patent 3,458,525, 1969; Chem. Abstr. 1970, 72, 21715.

Received for review March 3, 1988. Revised manuscript received August 10, 1988. Accepted December 5, 1988.

Metabolism of Dibutyltin Dichloride in Male Rats

Takashi Ishizaka, Takashi Suzuki,* and Yukio Saito

When dibutyltin dichloride was administered intraperitoneally to male rats, butyl(3-hydroxybutyl)tin dichloride, butyl(4-hydroxybutyl)tin dichloride, and butyltin trichloride were detected as acid-stable metabolites. The major acid-stable metabolite, butyl(3-hydroxybutyl)tin dichloride, showed a tendency to accumulate in the kidney. Butyl(4-hydroxybutyl)tin dichloride was detected only in urine extracts. Dibutyltin dichloride, butyl(3-hydroxybutyl)tin dichloride, and butyltin trichloride were found in the brain in spite of differences in their relative polarities.

Butyltin compounds have been widely used as stabilizers for chlorinated polymers, catalysts for a variety of chemical reactions, and biocides for boat paints or fishing nets. Recently, pollution of the environment and foods, caused by these compounds, has become the object of public concern (Mueller, 1984; Maguire and Tkacz, 1985; Takami et al., 1987), and much attention has been focused on the biological effects of butyltin compounds (WHO, 1980; Wada et al., 1982). Informed discussion of these effects, however, requires the clarification of the metabolic fate of these compounds and the elucidation of the biological effects of each individual metabolite.

Some studies have been conducted on the metabolism of these compounds in microsomal monooxygenase systems (Fish et al., 1976; Kimmel et al., 1977). Dibutyltin compounds have been identified as the main metabolic intermediate of tributyltin chloride (Kimmel et al., 1977) and also as a contaminant in reared and natural fish (Sasaki et al., 1988a,b). Information on butyltin compounds metabolism in vivo, accumulations in organs, and their excretion is, however, lacking.

The aim of this study was to clarify the reasons for tributyltin chloride toxicity in animals including man. This paper describes identification of the metabolites of dibutyltin dichloride in vivo and their distribution in organs after intraperitoneal administration to male rat.

MATERIALS AND METHODS

Chromatography. Column chromatography was carried out with silica gel (Kieselgel 60, Art. 7734, E. Merck; Wakogel C-100; Wako Pure Chemical Industries Ltd.) and Florisil (100–200 mesh; Yoneyama Chemical Industries Ltd.). Silica gel (Kieselgel 60) was activated at 130 °C. Silica gel (Wakogel C-100) was made 50% (v/w) with hydrochloric acid (HCl, 36%), equilibrated overnight, and activated for ca. 4 h at 120 °C (Hatorri et al., 1984). Florisil was activated at 130 °C and used without further modification.

High-performance liquid chromatography (HPLC) was performed with use of two systems, a postcolumn Morin reagent modified fluorescence detection system (Yu and Arakawa, 1983; method I) and a fluorescence detection system with Morin in eluent (Langseth, 1984; method II).

Method I. A Shimadzu LC-6A for mobile phase and a Hitachi 635 for Morin reagent were used as the pumps for the solvents. A stainless-steel column (25 cm × 4.6 mm (i.d.), packed with Unisil Q CN (5 μm, cyanopropyl-bonded phase; Gasukuro Kogyo), coupled with the precolumn (5 cm × 4.6 mm (i.d.)) with the same packing, were used. The mobile phases of *n*-hexane–ethyl acetate (EtOAc)–acetic acid (80:20:5) for quantification and 95:5:5 for identification were used at a flow rate of 1.2 mL/min. Detections were carried out by postcolumn Morin reagent modification (0.005% Morin in ethanol at a flow rate of 0.5 mL/min), followed by monitoring the fluorescence (excitation 420 nm, emission 500 nm; Shimadzu RF-535).

Method II. A Shimadzu LC-6A was used as the solvent pump. The analytical column and the precolumn were the same as for method I. The mobile phase of toluene–acetic acid–methanol (95:3:2) containing 0.0015% Morin was used at a flow rate of 1.0 mL/min. The detector and operating conditions were the same as for method I.

Gas Chromatography/Mass Spectrometry (GC/MS). GC/MS spectra were obtained by a JEOL JMS-DX 300 in the electron-impact (EI) mode. Operating conditions were as follows: GC column, CBP 10 (12 m × 0.53 mm (i.d.), fused silica capillary column, OV-1701 equivalent; Shimadzu); helium gas flow, 15 mL/min; injection temperature, 240 °C; column temperature, 90 °C (0 min) to 230 °C (2 min) at 16 °C/min; separator temperature,

Division of Food, National Institute of Hygienic Sciences, 18-1, Kamiyoga 1-chome, Setagaya-ku, Tokyo 158, Japan.

0021-8561/89/1437-1096$01.50/0

240 °C; ion source temperature, 210 °C.

Nuclear Magnetic Resonance (^{1}H NMR) Spectroscopy. NMR spectra were recorded on a JEOL JNX-FX 200S NMR spectrometer (200 MHz) in $CDCl_3$ with tetramethylsilane as an internal standard (δ value): s, singlet; d, doublet; t, triplet; m, multiplet; br, broad.

Infrared (IR) Spectroscopy. IR spectra were measured with a Jasco A-102 spectrometer.

Authentic Standards. *Dibutyltin Dichloride (I).* Dibutyl tin dichloride of 97% purity was purchased from Wako Pure Chemical Industries Ltd.

Dibutyl(3-hydroxybutyl)tin Chloride (II), Dibutyl(3-oxobutyl)tin Chloride (III), and *Dibutyl(4-hydroxybutyl)tin Chloride (IV).* The materials were synthesized according to the method described by Fish et al. (1976) and then purified without distillation using the solvent system of *n*-hexane–ethyl ether (Et_2O) (8:2) on silica gel (Kieselgel 60).

Butyl(3-hydroxybutyl)tin Dichloride (V). Bromine in chloroform ($CHCl_3$, 10 mg/mL, 18 mL, 2.3 mmol) was added to dibutyl(3-hydroxybutyl)tin chloride (371 mg, 1.1 mmol; II) in $CHCl_3$ (30 mL) dropwise with continuous stirring over 1 h at 0 °C. The solution was stirred continuously overnight at room temperature, and the solvent was evaporated in vacuo. The residue was dissolved in Et_2O (50 mL), and the solution was washed with 4 N HCl (50 mL) containing sodium chloride (NaCl; 5 g). The solution was dried over anhydrous sodium sulfate (Na_2SO_4) followed by filtration and evaporation of the solvent. The residue was purified on silica gel (Kieselgel 60) with the solvent system of *n*-hexane–Et_2O (6:4) to give a colorless solid, V: 102 mg, 29%; IR (film) 3450 cm^{-1} (OH); NMR δ 0.95 (t, 3 H, J = 7.2 Hz, CH_3CH_2), 1.31 (d, 3 H, J = 6.1 Hz, CH_3CHOH), 1.3–2.2 (m, 10 H, CH_2 group), 2.40 (br s, 1 H, OH), 3.90 (m, 1 H, CHOH). Anal. Calcd for $C_8H_{18}Cl_2OSn$: C, 30.04; H, 5.67. Found: C, 30.02; H, 5.70.

Butyl(3-oxobutyl)tin Dichloride (VI). Dibutyl(3-oxobutyl)tin chloride (144 mg; III) was reacted and processed in the same way as described for the synthesis of V to give a colorless solid, IV: 63 mg, 47%; IR (film) 1670 cm^{-1} (C=O); NMR δ 0.96 (t, 3 H, J = 7.2 Hz, CH_3CH_2), 1.3–2.2 (m, 10 H, CH_2 group), 2.35 (s, 3 H, CH_3C=O), 3.11 (t, 2 H, J = 6.9 Hz, CH_2C=O). Anal. Calcd for $C_8H_{16}Cl_2OSn$: C, 30.23; H, 5.08. Found: C, 30.21; H, 5.08.

Butyl(4-hydroxybutyl)tin Dichloride (VII). Dibutyl(4-hydroxybutyl)tin chloride (378 mg; IV) was reacted and processed in the same way as described for the synthesis of V to give a colorless oil, VII: 177 mg, 50%; IR (film) 3400 cm^{-1} (OH); NMR δ 0.95 (t, 3 H, J = 7.2 Hz, CH_3), 1.2–2.4 (m, 12 H, CH_2 group), 2.40 (br s, 1 H, OH), 3.90 (t, 2 H, J = 5.0 Hz, CH_2OH). Anal. Calcd for $C_8H_{18}Cl_2OSn$: C, 30.04; H, 5.67. Found: C, 29.43; H, 5.61.

Butyltin Trichloride (VIII). Butyltin trichloride of 95% purity was purchased from Aldrich Chemical Co.

Handling and Care of Animals. Rats were individually housed in metabolic cages at 23 ± 2 °C at 50% relative humidity and allowed to acclimate to a 12 h day/night cycle for 7 days prior dosing. Feed and water were provided ad libitum.

Treatment. Dibutyltin dichloride (I) was administered intraperitoneally to 40 male, Wistar rats (8 weeks, 180–210 g) at a dose of 4 mg/kg in soybean oil (0.5 μL/g of body weight of rat). Rats were decapitated 6, 24, 48, 72, 96, 120, 144, and 168 h after treatment without anesthesia. Liver, kidney, brain, spleen, and blood were removed and prepared for HPLC analysis. Urine was separated from feces in the cages and collected 24, 48, 72, 96, 120, 144, and 168 h after administration of dibutyltin dichloride (I).

Sample Preparation. *Organs.* Excised kidney, brain, and spleen from each rat were homogenized in 0.9% saline solution (10 mL) and then transferred to 50-mL centrifuge tubes with ground-glass stopper. HCl (8 mL) was added to the homogenate, and the mixture was vigorously shaken and allowed to stand for 5 min. Et_2O (20 mL) and NaCl (2 g) were added, and the mixtures were shaken for 5 min on a KM Shaker (Iwaki Co., Ltd.), followed by centrifugation for 5 min at 3000 rpm. This extraction procedure was repeated twice. [liver (4 g) was also extracted in the same way.] The supernatant extract was evaporated in vacuo, and the residue was dissolved in *n*-hexane (5 mL). The solution was transferred to a 1-cm-i.d. chromatographic column containing 5 g of HCl-treated silica gel, prepared with *n*-hexane, with 1 cm of anhydrous Na_2SO_4 on the top. The column was washed first with *n*-hexane (50 mL), and then elution was performed with a mixture of *n*-hexane–EtOAc (2:1, 50 mL). The eluate was evaporated in vacuo, and the residue was dissolved in *n*-hexane (5 mL) in preparation for HPLC analysis.

Blood. HCl (20 mL) was added to blood (about 5 mL) from each rat and the mixture diluted with distilled water to 50 mL, extracted with Et_2O (40 mL) and NaCl (5 g) by mechanical shaking for 5 min, and then centrifuged for 5 min at 3000 rpm. This extraction procedure was repeated twice. The combined supernatants containing Et_2O extracts were washed with 4 N HCl (50 mL) containing NaCl (5 g) and evaporated in vacuo. The residue was dissolved in methanol (0.5 mL), and then a mixture of *n*-hexane–EtOAc (2:1, 4.5 mL) was added. This mixture was transferred to a 1-cm-i.d. chromatographic column containing HCl-treated silica gel (5 g) with 1 cm of anhydrous Na_2SO_4 on the top, prepared with a mixture of *n*-hexane–EtOAc (2:1). The column was then eluted with a solvent system, *n*-hexane–EtOAc (2:1, 50 mL), and the collected eluate was concentrated in vacuo to 5 mL for HPLC analysis.

Urine. Urine (about 4–7 mL) from each rat was diluted with water to 30 mL, and HCl (50 mL) was carefully added. The mixture was mixed thoroughly and allowed to stand for 5 min. The mixture was shaken with Et_2O (40 mL) and NaCl (5 g), followed by centrifugation for 5 min at 3000 rpm at 0 °C. This extraction procedure was repeated twice. The combined Et_2O extracts were evaporated in vacuo and treated in the same way as blood samples described above.

Recoveries (mean ± SD, n = 3) of I and V–VIII added to organs (liver, kidney, brain, spleen) at a level of 2 μg/g of samples were 105.5 ± 7.7 to 94.0 ± 5.7, 97.0 ± 2.3 to 92.3 ± 2.1, 92.4 ± 3.4 to 90.4 ± 2.8, 96.0 ± 4.2 to 89.8 ± 4.0, and 74.1 ± 4.3 to 70.1 ± 1.4%, respectively. Recoveries (mean ± SD, n = 3) of I and V–VIII to urine and blood at a level of 2 μg/mL were 79.5 ± 5.5 and 88.1 ± 4.0, 98.3 ± 9.6 and 92.5 ± 4.9, 90.6 ± 2.9 and 94.7 ± 2.9, 91.3 ± 10.0 and 94.9 ± 3.1, and 102.1 ± 7.4 and 72.4 ± 10.3%, respectively.

Pentylation of Metabolites. HPLC sample solution was evaporated in vacuo, and the residue was dissolved in a mixture of *n*-hexane (5 mL) and Et_2O (3 mL) in a 25-mL glass tube with a ground-glass stopper. *n*-Pentylmagnesium bromide (ca. 2 M, 2 mL) was added carefully to the solution described above, mixed thoroughly, and then allowed to stand for 10 min. The mixture was cooled in an ice bath, water (5 mL) and HCl (0.5 mL) were carefully added, and the mixture was vigorously shaken. The separated organic layer was removed, and the remaining aqueous layer was extracted twice with Et_2O (1 mL). The combined organic layers were dried over calcium chloride, neutralized by addition of a piece of sodium hydroxide pellet to prevent the destruction of newly formed tetraalkyltin derivatives (Sasaki et al., 1988a), evaporated in vacuo below 40 °C, and dissolved in *n*-hexane (1 mL). The solution was transferred to a 1-cm-i.d. chromatographic column containing Florisil (5 g) prepared with *n*-hexane. The column was first eluted with *n*-hexane (50 mL) followed by a mixture of *n*-hexane–EtOAc (2:1; 50 mL) to prevent the elution of impurities. Eluates were combined and evaporated in vacuo, and the residue, dissolved in *n*-hexane, was used for GC/MS analysis. Standard compounds were also treated in the same manner.

RESULTS AND DISCUSSION

Identification of Metabolites. Metabolites were identified by comparison of their retention times (RTs) with those of authentic standards by the HPLC–fluorescence detector systems. The identities of metabolites were confirmed by GC/MS after their derivatization to tetraalkyltin compounds.

Figure 1 shows the typical HPLC chromatograms of standard compounds and metabolites extracted from rat organs and urine. These metabolites were designated as M-1, M-2, M-3, and M-4. M-1 was found in every organ, urine, and blood. M-2 was found in every organ and urine. M-3 was found only in urine. M-4 was found in every organ and urine. No other significant metabolite was detected in the extract. The relative retention times

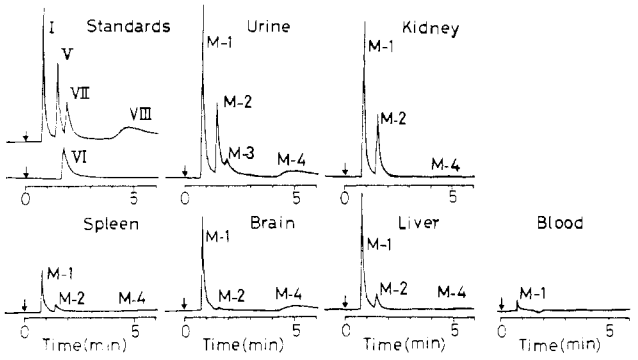


Figure 1. HPLC chromatograms of standard compounds and metabolites extracted from rat organs and urine (method I). Solvent system: *n*-hexane–ethyl acetate–acetic acid (95:5:5).

Table I. Relative Retention Times of Dibutyltin Dichloride Metabolites and Standards

metabolite	standard	RRT[a]	RRT[b]	RRT[c]
M-1	dibutyltin dichloride (I)	1.0	1.0	
M-2	butyl(3-hydroxybutyl)tin dichloride (V)	1.8	1.2	
M-3	butyl(4-hydroxybutyl)tin dichloride (VII)	2.4	1.3	
M-4	butyltin trichloride (VIII)	5.9	6.4	
	butyl(3-oxobutyl)tin dichloride (VI)	2.3	1.2	
PD[d] of M-1	PD of I			1.0
PD of M-2	PD of V			1.3
PD of M-3	PD of VII			1.4
PD of M-4	PD of VIII			1.1
	PD of VI			1.6

[a] RRTs on HPLC (method I), relative to I = 0.8 min. Solvent system: *n*-hexane–ethyl acetate–acetic acid (95:5:5). [b] RRTs on HPLC (method II), relative to I = 4.8 min. [c] RRTs on GC/MS, relative to I = 5.5 min. The column of CBP 10 (fused silica capillary column) was used. [d] PD = pentyl derivative.

(RRTs) of metabolites and standards are shown in Table I. The GC/MS spectra of tetraalkyltin derivatives of metabolites and standard compounds are shown in Figure 2.

(*1*) *M-1.* The RTs of M-1 in HPLC by methods I and II were similar to those of I as shown in Figure 1 and Table I. The mass spectrum of pentylated brain extract at RRT (relative retention time to dibutyldipentyltin) = 1.0 showed fragment peaks at m/z 248, 304, and 318, which are characteristic of pentylated I, although extra peaks were observed in the high-mass range. The characteristic isotope peaks of tin confirm the identity of M-1 with authentic sample I.

(*2*) *M-2.* The RTs of M-2 in HPLC by methods I and II were similar to those of V as shown in Figure 1 and Table I. The mass spectrum of pentylated liver extract at RRT = 1.3 showed almost the same fragment patterns as pentylated V. M-2 was identified as V on the basis of these data.

(*3*) *M-3.* M-3 had the same RTs as those of VII in HPLC by methods I and II. The mass spectrum of pentylated urine extract at RRT = 1.4 exhibited fragment peaks at m/z 320, 334, and their isotope peaks. These peaks were also observed in pentylated authentic VII. The mass spectrum of pentylated M-3 was not superimposable with that of pentylated VII, due to the peaks below m/z 300; however, M-3 was identified as VII when the results of HPLC were taken into consideration.

(*4*) *M-4.* The RTs of M-4 in HPLC were similar to those of authentic VIII, and the mass spectrum of pentylated liver extract at RRT = 1.1 showed characteristic fragment peaks at m/z 248, 262, 318, and 332, which were identical with those of pentylated VIII.

Distribution and Excretion of Dibutyltin Dichloride and Its Metabolites. Figure 3 shows distribution of dibutyltin dichloride (I) and its metabolites in rat organs. These results indicated that I (M-1) was present in liver, kidney, and spleen 6 h after treatment and had been already metabolized to some extent. Brain tissue, on the other hand, showed slower accumulation of I than the other organs. This may be due to slower penetration of the chemical through the blood–brain barrier.

M-2, which is hydroxylated at position 3 of one of the alkyl moieties, was distributed in the kidney at relatively high concentrations compared with the other metabolites, and its concentration increased with time. Kanetoshi (1983) reported the absence of metabolism of tributyltin in isolated viable rat kidney cells. It may be difficult to compare the in vivo result with that of cell culture and to compare the result of dibutyltin dichloride (I) with that of tributyltin chloride. However, it is plausible that M-2 was mainly formed in liver and accumulated in the kidney after transport. Mazaev and Korolev (1969), on the other hand, reported dystrophic changes in the rat kidneys treated with dibutyltin dichloride (I). These changes may be attributable to the accumulation of M-2 in the kidney as M-2 increases with time compared with the decrease of I. Barnes and Magee (1958) reported that dibutyltin compounds cause biliary and hepatic lesions in experimental animals. As relatively high amounts of alkyltin compound are excreted into the bile duct and reabsorbed from small intestine (Iwai et al., 1982), I or its metabolite, especially M-2, might cause these lesions.

M-2 was also found in the brain, although the concentration was low. Iwai et al. (1979, 1980) reported tributyltin chloride was metabolized in the brain to give dibutyltin dichloride (I) and butyltin trichloride (VIII) and the brain may have a butyltin dealkylating system. Also, in the case of dibutyltin dichloride (I), M-2 may be formed in brain by a monooxygenase system and then degraded to M-4. It is difficult to account for the fact that M-4 was already formed 6 h after administration, compared with M-2, which first appeared 2 days after administration and was produced in the same way as M-4 by a monooxygenase system. It is also not clear whether M-4 was transferred to the brain from another part of the body or not.

M-4 was distributed almost at constant levels for 7 days in each organ except spleen in which the proportion of M-4 seemed to be a little higher than in the other organs. As M-4 is very polar and does not seem to have an affinity for organs, it would be excreted easily from each organ into urine as soon as it is formed (Figure 4). This could be the reason why the concentration of M-4 in each organ is low and constant.

Only a small amount of M-1 was detected in blood, and other butyltin metabolites were not detected (Figure 5). The concentration changes of M-1 in liver, kidney (Figure 3), and blood (Figure 5) suggests that the half-lives of I in these organs are about 3–5 days.

M-3, which is hydroxylated at the 4-position, was found in urine, but its concentration was lower than the other metabolites. It is remarkable that although M-1 and M-2 excreted in urine reflected the concentration in the kidney, M-3 did not. This may account for lower metabolic yield of M-3 and rapid excretion due to its high polarity (primary alcohol) compared with the other hydroxylated metabolites. Furthermore, M-4, which had a relatively high concentration among metabolites in urine, was lower in concentration in the kidney.

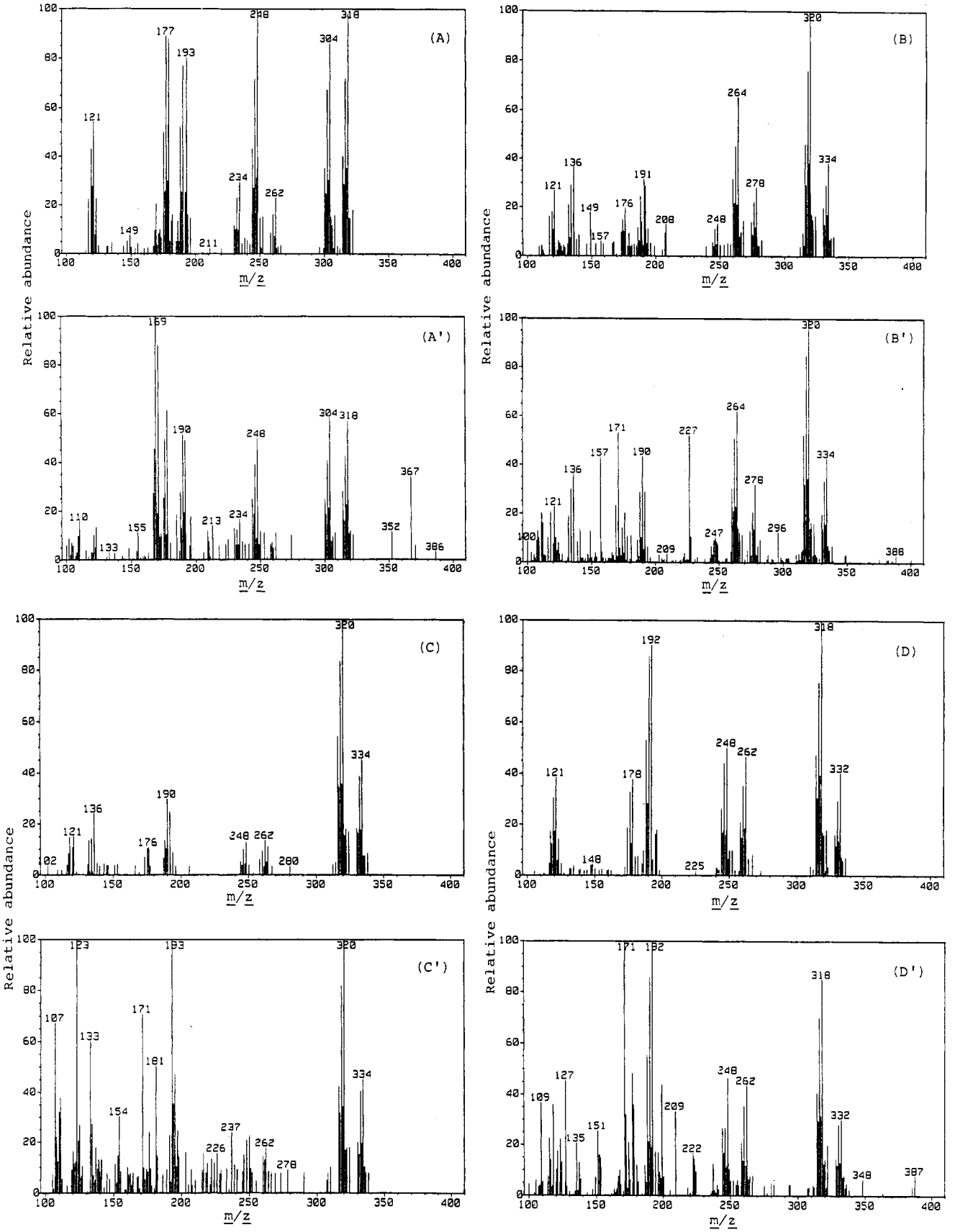


Figure 2. GC/MS spectra of tetraalkyltin derivatives of standard compounds and metabolites: (A) authentic PD (pentyl derivative) of I; (A′) PD of brain extract (RT = 5.5 min); (B) authentic PD of V; (B′) PD of liver extract (RT = 7.1 min); (C) authentic PD of VII; (C′) PD of urine extract; (D) authentic PD of VIII; (D′) PD of liver extract (RT = 6.0 min).

Fish et al. (1976) reported the biological oxidation of tributyltin acetate with the microsomal monooxygenase enzyme system in vitro and identified 1-, 2-, 3-, and 4-hydroxylated tributyltin acetate and dibutyl(3-oxobutyl)tin acetate. They suggested that dibutyl(1-hydroxybutyl)tin acetate and dibutyl(2-hydroxybutyl)tin acetate undergo a destannylation reaction under acidic conditions to form dibutyltin diacetate, 1-butanol, and 1-butene, respectively.

Dibutyltin dichloride (I) may also undergo in vivo metabolism similar to tributyltin acetate by a monooxygenase

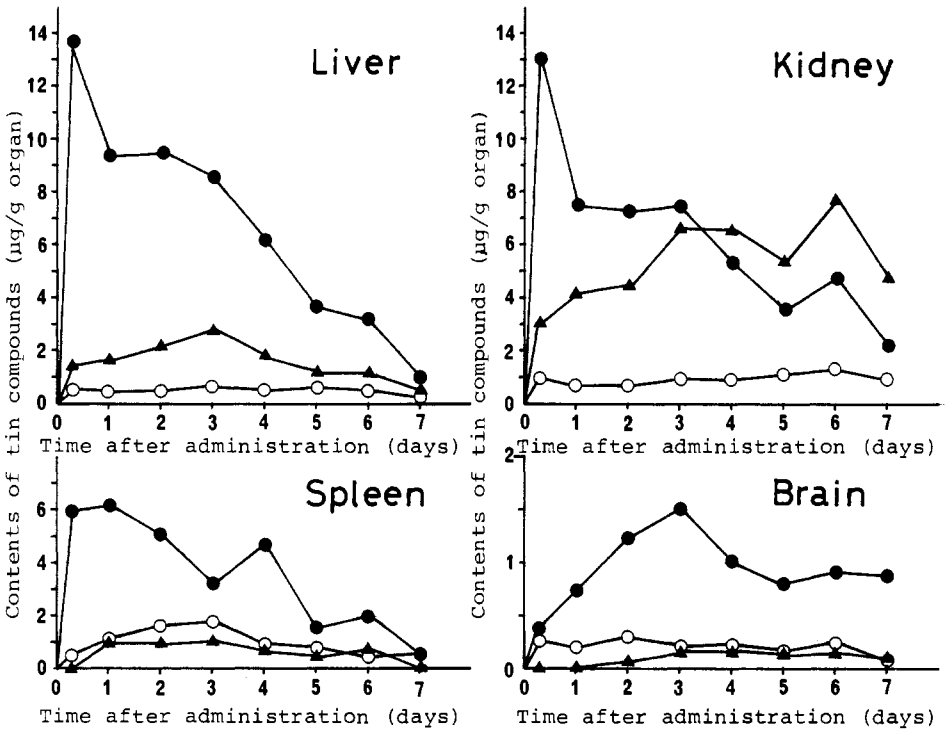


Figure 3. Contents of metabolites in rat organs after administration of dibutyltin dichloride (ip, 4 mg/kg). Results are averages of two to five replications. Key: ●, M-1; ▲, M-2; ○ M-4.

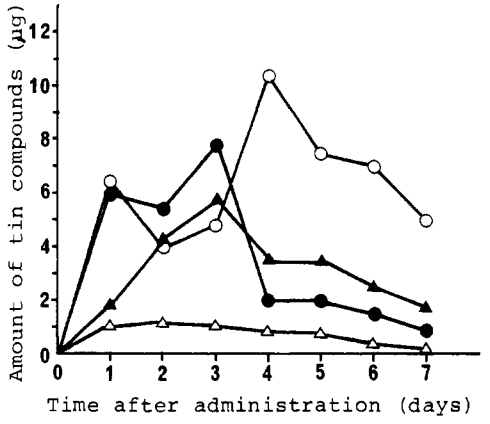


Figure 4. Excretion of metabolites in rat urine after administration of dibutyltin dichloride (ip, 4 mg/kg). Results are averages of six or four replications. Key: ●, M-1; ▲, M-2; △, M-3; ○, M-4.

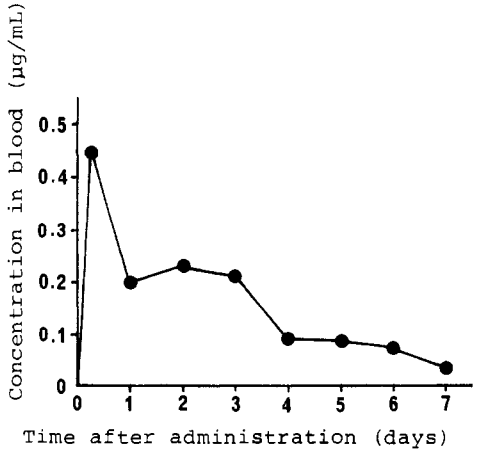


Figure 5. Concentration of M-1 in rat blood after administration of dibutyltin dichloride (ip, 4 mg/kg). Results are averages of two to five replications.

enzyme system. The hydroxylated metabolites at the 1- and 2-positions could not be found, and only the oxidized metabolites at positions 3 and 4 were detected. This may be due to the acid instablity of 1- and 2-oxidized metabolites. Accordingly, most of M-4, which was obtained by metabolism of I, could come from biological or chemical degradation of the oxidized product at positions 1 and 2. Unfortunately this experiment was not designed to assay hydroxylated monoalkyl products, so that the presence of another hydroxylated monoalkyl derivative is possible. Trace amounts of the 3-oxo metabolite might have been formed, although it was not detected in these experiments.

Metabolism of tributyltin chloride is currently under investigation. These results will be reported elsewhere in the near future.

Registry No. I, 683-18-1; II, 53477-39-7; III, 65301-77-1; IV, 53477-40-0; V, 120853-46-5; VII, 120853-47-6; butyltin trichloride, 1118-46-3.

LITERATURE CITED

Barnes, J. M.; Magee, P. N. The Biliary and Hepatic Lesions Produced Experimentally by Dibutyltin salts. *J. Pathol. Bacteriol.* **1958**, *75*, 267–279.

Fish, R. H.; Kimmel, E. C.; Casida, J. E. Bioorganotin Chemistry: Reactions of Tributyltin Derivatives with a Cytochrome P-450 Dependent Monooxygenase Enzyme System. *J. Organomet. Chem.* **1976**, *118*, 41–54.

Hattori, Y.; Kobayashi, A.; Takemoto, S.; Takami, K.; Kuge, Y.; Sugimae, A.; Nakamoto, M. Determination of Trialkyltin, Dialkyltin, and Triphenyltin Compounds in Environmental Water and Sediments. *J. Chromatgr.* **1984**, *315*, 341–349.

Iwai, H.; Manabe, M.; Ono, T.; Wada, O. Distribution, Biotransformation and Biological Half-Life of Tri-, Di-, Monobutyltin in Rats. *J. Toxicol. Sci.* **1979**, *4*, 285.

Iwai, H.; Manabe, M.; Matsui, H.; Ono, T.; Wada, O. Effects of Tributyltin and Its Metabolites on Brain Function. *J. Toxicol. Sci.* **1980**, *5*, 257.

Iwai, H.; Wada, O.; Arakawa, Y.; Ono, T. Intestinal Uptake Site, Enterohepatic Circulation, and Excretion of Tetra- and Trialkyltin Compounds in Mammals. *J. Toxicol. Environ. Health* **1982**, *9*, 41–49.

Kanetoshi, A. The Metabolism of Butyltin Compounds in Isolated Viable Rat Hapatocytes. *Eisei Kagaku* **1983**, *29*, 303–311.

Kimmel, E. C.; Fish, R. H.; Casida, J. E. Bioorganotin Chemistry. Metabolism of Organotin Compounds in Microsomal Mono-

oxygenase Systems and in Mammals. *J. Agric. Food Chem.* **1977**, *25*, 1–9.
Langseth, W. Determination of Diphenyltin and Dialkyltin Homologues by HPLC with Morin in the Eluent. *Talanta* **1984**, *31*, 975–978.
Maguire, R. J.; Tkacz, R. J. Degradation of the Tri-*n*-butyltin species in Water and Sediment from Toronto Harbor. *J. Agric. Food Chem.* **1985**, *33*, 947–953.
Mazaev, V. T.; Korolev, A. A. Experimental Basis for the Maximum Permissible Concentration of Dichlorodibutyltin in Reservoir Waters. *Prom. Zagryaz. Vodoemov* **1969**, *9*, 15–37.
Mueller, M. D. Tributyltin Detection at Trace Levels in Water and Sediments Using GC with Flame-Photometric Detection and GC–MS. *Fresenius' Z. Anal. Chem.* **1984**, *317*, 32–36.
Sasaki, K.; Ishizaka, T.; Suzuki, T.; Saito, Y. Determination of Tri-*n*-Butyltin and Di-*n*-Butyltin Compounds in Fish by Gas Chromatography with Flame Photometric Detection. *J. Assoc. Off. Anal. Chem.* **1988a**, *71*, 360–363.
Sasaki, K.; Suzuki, T.; Saito, Y. Determination of Tri-*n*-butyltin and Di-*n*-butyltin Compounds in Yellowtails. *Bull. Environ. Contam. Toxicol.* **1988b**, *41*, 888–893.
Takami, K.; Yamamoto, H.; Okumura, T.; Sugimae, A.; Nakamoto, M. Application of "Clean-up" Cartridge for Gas Chromatographic Determination of Di- and Tri-*n*-butyltin in Fish. *Anal. Sci.* **1987**, *3*, 63–67.
Wada, O.; Manabe, S.; Iwai, H.; Arakawa, Y. Recent Progress in the Study of Analytical Methods, Toxicity, Metabolism and Health Effects of Organotin Compounds. *Jpn. J. Ind. Health* **1982**, *24*, 24–54.
WHO. *Environmental Health Criteria 15: Tin and organotin compounds*; World Health Organization: Geneva, 1980.
Yu, T.; Arakawa, Y. High-Performance Liquid Chromatographic Determination of Dialkyltin Homologues Using Fluorescence Detection. *J. Chromatogr.* **1983**, *258*, 189–197.

Received for review July 18, 1988. Accepted January 30, 1989.

Insect Juvenile Hormone Mimics: Synthesis of Some 5-Oxa-3,8,12-trimethyltridecenoates

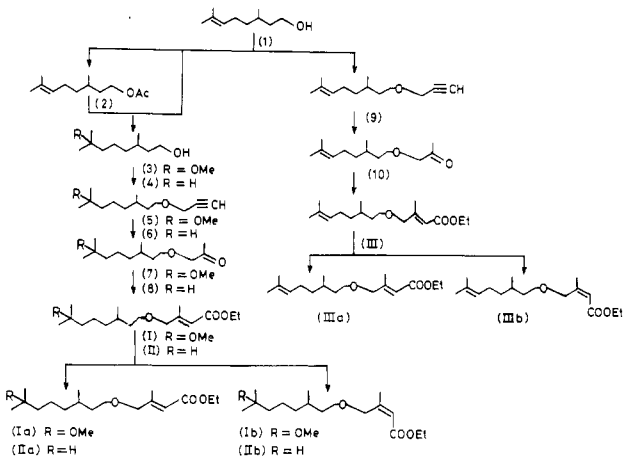
Ramakrishnan R. Iyer and Vasant R. Mamdapur*

Syntheses of some 5-oxahomofarnesanes have been described and shown to be active at micrograms/nymph against *Dysdercus koenigii*, and the activity is comparable with that of methoprene (Altozar IGR; ZR 515). Stress was also laid on the separation of *Z* and *E* isomers, thereby confirming their structural integrity.

Insect growth regulators (IGRs) are potent growth regulators (Williams, 1956) of certain essential processes in the life cycle of insects. Many insects are remarkably sensitive to the external application of suitable IGRs at critical stages in their life cycle. Though the question of structure–activity relationship in the area of juvenile hormone (JH) mimics is very complex because of a large number of variables (Slama et al., 1974), the compounds with optimum activity are as a rule based on the farnesane skeleton. Oxafarnesanes and oxahomofarnesanes have been reported as juvenoids. However, the methods of synthesis are either patented or low yielding. The detailed JH activity studies of 4-oxafarnesanes along with their synthesis have been reported in the literature (Patwardhan et al., 1976). Jarolim and Sorm (1974) have reported the synthesis of oxahomofarnesanes and have mentioned the separation of *Z* and *E* isomers on a silica gel column, but no further evidence has been attributed to confirm their structural identity. We report herein a novel approach to the synthesis of these oxahomofarnesanes (Scheme I) starting with the easily accessible citronellol (1) and their separation. However, the method of separation of *E* and *Z* isomers as reported by the authors failed in our hands. Hence, the separation was carried out on a silica gel column impregnated with silver nitrate (10–15%) to afford the pure *E* and *Z* isomers. The salient features of our approach are (i) preparation of propargyl ether, (ii) catalytic hydration of terminal acetylene to the desired methyl ketone, and (iii) separation of *E* and *Z* isomers on a silica gel column impregnated with silver nitrate.

Bio-Organic Division, Bhabha Atomic Research Centre, Bombay 400 085, India.

Scheme I



EXPERIMENTAL SECTION

IR spectra were recorded on a Perkin-Elmer Infracord spectrophotometer (Model 783). ^1H NMR spectra were obtained in $CDCl_3$ with tetramethylsilane as the internal reference in a Varian A-60A spectrometer. GLC was carried out on a Schimadzu GC-7A chromatograph fitted with hydrogen flame detector and glass columns. HPLC analyses were performed on a Water Associates instrument (Model ALC/GPC 244) equipped with a solvent delivery system (Model 6000 A) and U6K injector and detector (Model 440).

7-Methoxycitronellol (3). To a stirred suspension of mercuric acetate (16.0 g, 0.05 mol) was added citronellyl acetate (**2**; 9.8 g, 0.05 mol) in dry methanol (130 mL). The reaction mixture was stirred for 0.5 h at ambient temperature to complete the meth-

0021-8561/89/1437-1101$01.50/0