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BIOORGANOTIN CHEMISTRY: REACTIONS OF TRIBUTYLTIN DERIVATIVES WITH A CYTOCHROME *P*-450 DEPENDENT MONOOXYGENASE ENZYME SYSTEM *

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Summary

The biological oxidation of several tributyltin derivatives, by a cytochrome *P*-450 dependent monooxygenase enzyme system with reduced nicotinamide-adeninedinucleotidephosphate as the essential cofactor, produced carbon-hydroxylated compounds identified as α-, β-, γ- and δ-hydroxybutyldibutyltin derivatives. The hydroxylation pattern and the lack of oxidative cleavage of tin—carbon bonds strongly suggest a free radical rather than an oxenoid mechanism, while the predominance of β-carbon-hydroxylation further implies some role of the tin—carbon σ electrons in directing the site of hydroxylation.

Introduction

Organotin compounds are known to have interesting biological properties [2a,b], while studies of their metabolic reactions have been somewhat limited [1]. Molecular oxygen plays a major role as an important reactant in biological systems [3], consequently studies of the biological oxidation reactions of organotin compounds are important to the basic understanding of their fate in biological systems.

Previous work in this area with Group IV metals such as organosilicon [4a,b], organotin [5a,b] and organolead compounds [5b] tentatively suggested that oxidative cleavage of the metal—carbon bond and carbon hydroxylation of alkyl side chains were important reactions.

Since these results, particularly with the organotin compounds, were not

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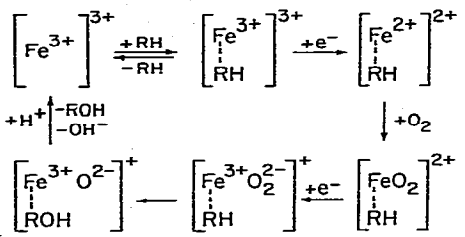
definitive, we established a bioorganotin program to delineate, among other reactions, the role of the tin atom in the biological oxidation reaction.

Of the many oxygenases which convert carbon—hydrogen bonds to carbon—hydroxyl bonds, the cytochrome *P*-450 dependent monooxygenases are the most important and widely studied enzyme systems in the metabolism of organic compounds [3]. These iron—porphyrin complexes are thought to undergo the following reactions for the activation of oxygen in the presence of the essential cofactor reduced nicotinamideadeninedinucleotidephosphate (NADPH) (Scheme 1) [3].

SCHEME 1



In this paper we wish to describe the full account [1] of our study on the reaction of several tributyltin derivatives as model substrates with a cytochrome *P*-450 dependent monooxygenase enzyme system. The identification of carbon-hydroxylated organotin metabolites and the mechanistic implications are presented.

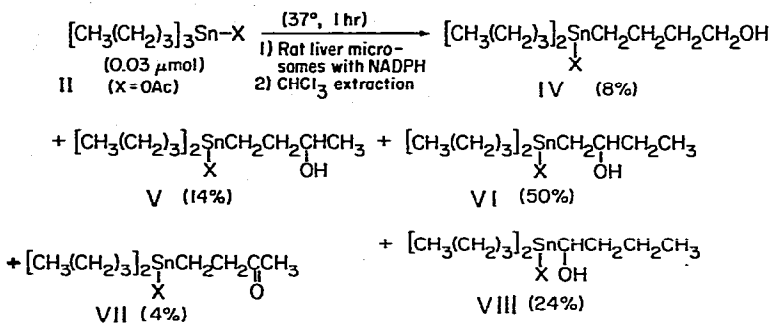
Results

In our biological oxidation reactions we utilized rat liver microsomes as the source of the cytochrome *P*-450 dependent monooxygenase enzyme system. We ascertained by two criteria that a cytochrome *P*-450 monooxygenase enzyme system and not a lipid peroxidase system was responsible for our results. Firstly, this microsomal oxidation was totally inhibited by carbon monoxide and to a large extent by 4(5)-α-naphthylimidazole (0.02 μmol) and secondly it was not affected by ethylenediaminetetraacetic acid (2 μmol) or manganese (2^+) (1 μmol) [6a—c].

The tributyltin derivatives of the general formula $[CH_3(CH_2)_3]_3SnX$ (0.5 μmol), where X is equal to Cl (I), OAc (II) or OSn $[(CH_2)_3CH_3]_3$ (III), were incubated with rat liver microsomes in phosphate buffer (pH 7.4) containing NADPH as the essential cofactor. After 1 h at 37°C, and extraction with chloroform, the product mixture was analyzed by thin-layer chromatography (TLC). Pertinently, we found that all the tributyltin derivatives studied (I—III) gave a similar mixture of identifiable metabolites, however, in low yield (<10%). The low yield is probably a consequence of nonspecific binding of the tributyltin group to other sites on the protein not in proximity to the iron—oxygen complex. We were able, however, to quantify and identify the carbon-hydroxylated tributyltin metabolites by preparing [1-^{14}C]tributyltin acetate and using it as the substrate (0.03 μmol) in all subsequent experiments.

Several methods were used to identify the metabolites, i.e., synthesizing

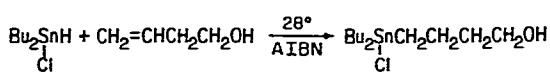
authentic samples for use in conjunction with thin-layer cochromatography techniques; degradation reactions; and chemical ionization mass spectrometry (CIMS). By these methods the following metabolites were characterized and quantified using [1-^{14}C]tributyltin acetate at 0.03 μmol (eq. 1) *:



(1)

The two-dimensional acidic TLC system used in this study completely resolved all the authentic compounds (II, IV—VII, and dibutyltin diacetate, IX). This enabled us to cochromatograph authentically synthesized compounds with the metabolites, thus establishing one criterion for metabolite identification.

Metabolite IV was not formed in sufficient amounts at high tributyltin acetate concentrations (0.5 μmol) for isolation and direct structural identification. However, we were able to synthesize an authentic sample of IV (X = Cl) via a hydrostannation reaction (eq. 2) and then cochromatograph it with metabolite IV (OAc) to provide one criterion for identification. Interestingly, we have never seen the anion on the tin make a difference in R_f values of the metabolites (TLC) and we presume that anion exchange on the tin atom takes place on the TLC plate converting all the anions (e.g., Cl, $OSnBu_3$, etc.) to acetates (OAc), since acetic acid is one of the cosolvents used in the TLC analysis [7] **.

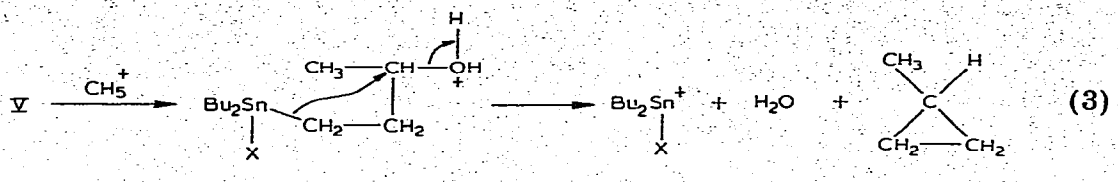


(2)

Metabolite V was also completely resolved (TLC) from the other metabolites and fortuitously at high tributyltin concentration (0.5 μmol) a sufficient amount could be isolated via preparative TLC to obtain a CIMS spectrum [8]. The CIMS of V was conclusive for its composition (*m/e* 307, $[M - OAc]^+$). We have used the CIMS of carbon-hydroxylated organotin compounds as a diagnostic tool for determining the position of the hydroxyl group relative to the tin atom [8]. The γ-hydroxyl organotin compounds such as V undergo a 1,3-deoxystannylation reaction in the gas phase under CIMS conditions and can be readily differentiated from the δ isomers (such as IV) (eq. 3).

* The percentages that we report are normalized values of identified metabolites and account for 10% of the starting [1-^{14}C]tributyltin acetate. The remainder was ~65% starting material, ~6% dibutyltin diacetate, ~5% butyltin triacetate and ~14% unidentified compounds.

** Ref. 7 supports this postulation of anion exchange with the isolation (TLC) of chlorides from exchange of other anions attached to the tin atom in the presence of hydrochloric acid.

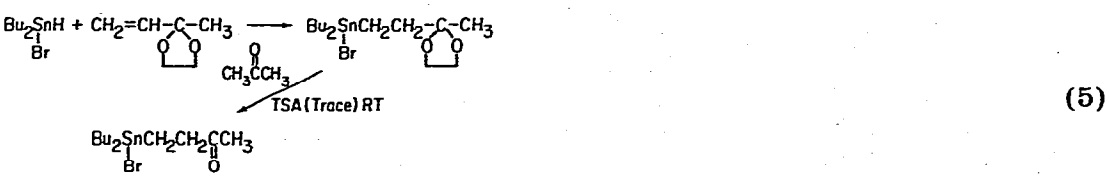


(3)

In this example, the loss of the γ-hydroxybutyl group to the unsubstituted butyl group (γ BuOH/Bu) is ~3.0 times greater in V than it is for the loss of δ-hydroxybutyl to unsubstituted butyl (δ BuOH/Bu) in IV [8] *. We were able to synthesize an authentic sample of V (X = Cl) via the following hydrostannation reaction (eq. 4) and use TLC cochromatography to further define the metabolite structure as V.

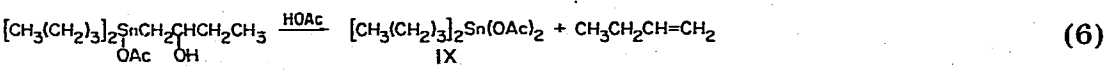
(4)

It was also observed that metabolite V was further oxidized to metabolite VII to the extent of 22% (based on 100% of V from tributyltin acetate). We were able to verify this conversion by using authentic V (X = Cl) in the biological oxidation reaction and observing the formation of VII, which cochromatographed with an authentic sample (X = Br) prepared via the following route (eq. 5).



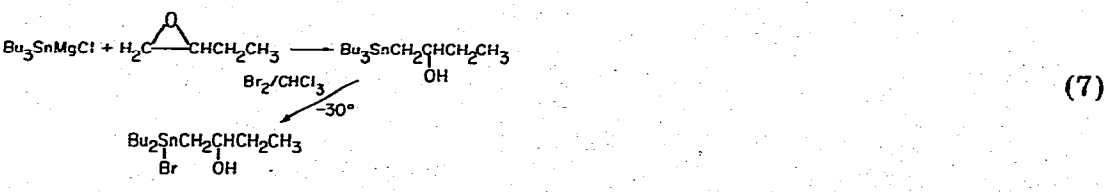
(5)

Metabolite VI, the major product formed in this biological oxidation reaction, was separated from IV, V, and VII by the same TLC system, however, it rapidly undergoes a destannylation reaction under the more acidic conditions in the second direction of TLC development to yield 1-butene and IX (eq. 6).



(6)

Significantly, the authentic VI (X = Br), independently synthesized by the following route (eq. 7), also underwent, as expected [9], a similar destannylation



(7)

reaction in the TLC system employed, further confirming the β-hydroxyl structure of VI. The 1-butene was formed by acidifying the enzyme reaction mix-

* A full account of these gas-phase 1,3-deoxystannylation reactions will be published separately [26].

ture and identified by gas liquid chromatography (GLC). This destannylation reaction, to form 1-butene, is a more accurate method of determining the amount of metabolite VI formed in the biological oxidation reaction than by direct TLC analysis. While GLC identified the 1-butene, we quantified its formation by the reaction of [1-^{14}C]butene with mercuric acetate in methanol/acetic acid (50 : 1) (eq. 8) to provide compound X.

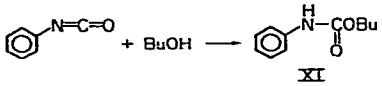
$$CH_3CH_2CH{=}\overset{*}{C}H_2 \xrightarrow[CH_3OH]{Hg(OAc)_2} CH_3CH_2\underset{\displaystyle CH_3O}{\underset{|}{C}}H\overset{*}{C}H_2HgOAc \qquad (8)$$

X

Metabolite VIII was unstable in the buffer system employed in the biological oxidation reaction. On analogy with studies on other α-substituted organotin compounds [10], we presume that the α-hydroxybutyldibutyltin acetate (VIII) reacted via cleavage of the tin—carbon bond (eq. 9).

$$[CH_3(CH_2)_3]_2\underset{\displaystyle OAc}{\underset{|}{Sn}}{-}\underset{\displaystyle OH}{\underset{|}{CH}}(CH_2)_2CH_3 \xrightarrow{pH\,7.4} [CH_3(CH_2)_3]_2\underset{\displaystyle OAc}{\underset{|}{Sn}}OH + CH_3(CH_2)_3OH \qquad (9)$$

Thus it was convenient to isolate the [1-^{14}C]butanol thereby quantifying the formation of metabolite VIII. This was done by reaction of [1-^{14}C]butanol with phenyl isocyanate forming the phenylcarbamate XI (eq. 10) *.



XI (10)

Discussion

Our aim was to not only identify and quantify the organotin metabolites formed in this biological oxidation reaction but also, as stated, to learn more about the role of the tin atom. Characteristically, *P*-450 dependent monooxygenase enzyme reactions encompass a carbon—hydrogen bond selectivity of $3° > 2° > 1°$ for alkanes and an electrophilic aromatic substitution pattern for substituted aromatic hydrocarbons [3].

The hydroxybutyldibutyltin compounds V, VI, VII and VIII formed by 2° carbon hydroxylation accounted for 92% of the identified metabolites and this represents an average 2° to 1° carbon—hydrogen bond reactivity of 5.8. Additionally, a rate study showed that metabolites V and VI are formed approximately nine times faster than metabolite IV after normalizing for the number of hydrogens able to react. A further comparison with a straight-chain hydrocarbon such as butane or pentane shows that these compounds give 2°/1° carbon-hydrogen bond reactivities of > 100 [11a—c]. The smaller 2°/1° ratio we ob-

* It is important to note that within experimental error the dibutyltin diacetate formed can be accounted for by the destannylation reactions of VI (TLC) and VIII (reaction media). The analytical methods for quantitation of 1-butene and especially 1-butanol limit the precision of this relationship.

serve could be rationalized by invoking a steric effect at the enzyme site performing the hydroxylation [11c] which in turn would allow the primary carbon atom to be more reactive than in either butane or pentane. The fact that the 2 position in pentane is more reactive than the corresponding γ-position in tributyltin acetate (II) strongly implies participation of the tin—carbon σ electrons, to some extent, in directing the site of hydroxylation and this is reflected in the predominance of β-carbon-hydroxylation. Thus, at 0.03 μmol tributyltin acetate (II) substrate concentration the normalized ratio of α, β, γ and δ carbon-hydroxylation is 4.5 : 9.4 : 3.4 : 1. If indeed the tin—carbon σ electrons were not participating, in some role, in the stabilization of the intermediate or transition state for this reaction, then the γ-position should be favored for hydroxylation and obviously this is not the case.

By using the tin atom as a mechanistic probe, we wish to evaluate our data with regard to differentiating between a free radical and an oxenoid mechnism for this biological oxidation reaction of compound II.

The reaction mechanism by which cytochrome *P*-450 dependent monooxygenase enzymes convert carbon—hydrogen to carbon—hydroxyl bonds has been postulated to be an oxenoid insertion reaction [3]. For example, the following iron—oxygen species has been postulated [3] (eq. 11).

$$Fe^{2+} + O_2 \rightarrow Fe^{3+}O_2^- \xrightarrow{e^-} [Fe{-}\ddot{O}{-}\ddot{O}{:}]^+ \qquad (11)$$

oxenoid complex

The model systems that have been used to mimic the enzyme reactions include peracids, Fe^{2+}/H_2O_2, Fe^{2+}/2-mercaptobenzoic acid, etc. [3]. All have similar characteristics in that they provide an electrophilic oxygen species (oxenoid). However, electrophilic reagents, such as those mentioned above, cause cleavage of the tin—carbon bond [12], and do not provide carbon-hydroxylation products [27].

Interestingly, another electrophilic reagent, dichlorocarbene, has been shown to insert selectively into carbon—hydrogen bonds β to a tin atom [13]. This carbene insertion reaction with trimethylbutyltin was not found to occur with α, γ, or δ carbon—hydrogen bonds and further the reaction was suppressed when phenyl or halogen groups were attached to the metal [13].

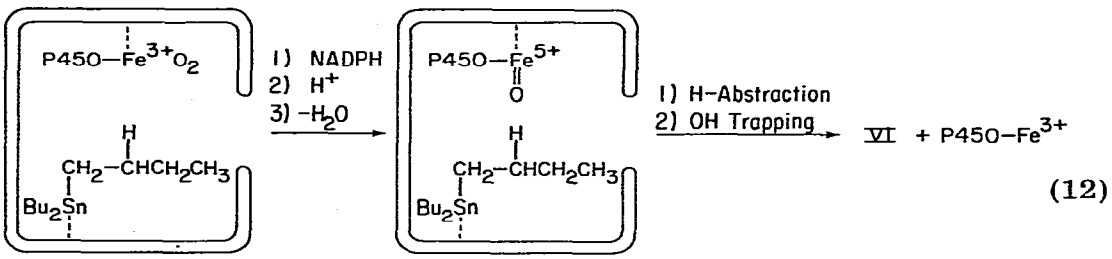
In contrast to this result, all carbon—hydrogen bonds (i.e. α, β, γ and δ to the tin atom) were reactive with the monooxygenase enzyme system we utilized. Additionally, we have observed that diphenylcyclohexyltin acetate readily provides carbon hydroxylation products with our enzyme system [27].

The hydroxylation pattern we observe, and the fact that we see minimal oxidative cleavage of tin—carbon bonds, strongly suggest a free radical rather than an oxenoid mechanism for carbon hydroxylation of tributyltin acetate with the cytochrome *P*-450 dependent monooxygenase enzyme system. Consistent with our free radical mechanism are the recent findings by Kochi [14a—c] and Symons [15a—c] concerning the added stabilization of radicals β to a tin—carbon σ bond and a complementary result pertaining to the ready abstraction of hydrogen on carbons α or β to the tin atom [14a]. Both of these results would help explain the preference for β-carbon hydroxylation and the significant amount of α as well as a lesser extent of γ- and δ-carbon hydroxyla-

tion. Also of significance to our postulated free radical mechanism is the reaction of several organosilicon compounds with sulfuryl chloride. For example, the free radical chlorination of dichloromethylbutylsilane gave an α, β, γ, δ normalized ratio of 1.7 : 3.9 : 5.6 : 1 for the products formed [16].

Thus an important consequence of this work is that the oxenoid mechanism clearly is not always required and that alternative free radical pathways could be invoked to explain results such as we have presented.

A plausible pathway, and one that is still consistent with Scheme I, could involve the abstraction of hydrogen from a carbon atom of the tributyltin derivative by the active iron—oxygen complex to give a radical intermediate of short life-time followed by trapping of the radical with a complexed hydroxyl group [17] (eq. 12).



(12)

Similar model Fe=O complexes have been speculated to be mimics for monooxygenase enzyme systems [18a—c].

Finally, we wish to clarify the previous tentative findings on the biological oxidation reactions of organotin compounds [5a,b] with the fact that carbon-hydroxylation is the primary biological oxidation reaction and that destannylation (i.e. tributyltin to dibutyltin) occurs because the α- and to a lesser extent the β-carbon-hydroxylation products are unstable under the reaction conditions.

We are continuing our studies in this area with other organotin compounds to learn more about the role of the tin atom and the stereochemical consequences of these important reactions.

Experimental

Materials and instrumentation

The nuclear magnetic resonance (NMR) spectra were recorded on a Perkin—Elmer R12B operating at 60 MHz with tetramethylsilane (TMS) as the internal standard. The infrared (IR) spectra were recorded on a Perkin—Elmer 457 IR spectrophotometer. The CIMS were recorded on a Finnigan 1015D instrument (methane at ~1 Torr). The isopropylmagnesium chloride was obtained from Alfa Products while the 3-buten-1-ol, 3-buten-2-ol, 1,2-epoxybutane, and vinyl methylketone were obtained from Aldrich Chemical Co. The dibutyltin dihydride and tributyltin hydride were prepared in the usual manner from the corresponding chloride and lithium aluminum hydride.

Synthesis of dibutylchlorotin and dibutylbromotin hydrides

As described by Sawyer [19], dibutyltin dichloride (0.01 mol) was mixed

with dibutyltin dihydride (0.01 mol) in an ampoule to produce dibutylchlorotin hydride in situ, which was then used in the hydrostannation reactions [20]. A similar procedure for preparing dibutylbromotin hydride, i.e., dibutyltin dibromide and dibutyltin dihydride, was also utilized.

Synthesis of δ-hydroxybutyldibutyltin chloride (IV, X = Cl)

In a two-necked flask equipped with an inlet for argon and a drying tube was placed 3.04 g (0.01 mol) dibutyltin dichloride and 2.35 g (0.01 mol) dibutyltin dihydride. To the dibutylchlorotin hydride formed in situ was added 2.0 g (0.028 mol) 3-buten-1-ol along with 2 mol % azobisisobutyronitrile (AIBN) as a free radical initiator. The reaction mixture was heated at 35°C for 18 h. The reaction could be conveniently followed to completion by observing the disappearance of the strong Sn—H stretching band at 1850 cm^{-1} in the IR.

Distillation gave four fractions: (1) b.p. 91—92°C/0.15 Torr; (2) 92.5—109°C/0.15 Torr; (3) 110—120°C/0.15 Torr; and (4) 123—126°C/0.15 Torr. The first two fractions (1 and 2) were contaminated with dibutyltin dichloride. Fraction 4 (300 mg) was redistilled, 130°C/0.08 Torr, and gave a NMR spectrum (δ (ppm), $CDCl_3$, TMS) with signals at 3.63 ($C\underline{H}_2OH$, triplet, *J* 4.0 Hz); 2.65 (OH, broad singlet); 1.5 (CH_2 groups, multiplet); 0.83 (CH_3, triplet, *J* 5.0 Hz) in the ratio 2 : 1 : 18 : 6. The CIMS showed *m/e* 307, *M* — Cl, 69% relative abundance (RA), based on ^{120}Sn; *m/e* 269, *M* — BuOH, 18% RA; and *m/e* 285, *M* — Bu, 13% RA. TLC (silica gel plate) using diisopropyl ether/glacial acetic acid (99 : 1) also showed one spot for IV. Anal. Found: C, 42.12; H, 7.92; Cl, 10.29. $C_{12}H_{27}ClOSn$ calcd.: C, 42.35; H, 7.94; Cl, 10.29%.

Synthesis of γ-hydroxybutyldibutyltin chloride (V, X = Cl)

In an ampoule was placed 3.04 g (0.01 mol) dibutyltin dichloride and 2.35 g (0.01 mol) dibutyltin dihydride. To this was added 2.0 g (0.028 mol) 3-buten-2-ol along with 2 mol % AIBN. The ampoule was flushed with argon, cooled and then sealed. The ampoule was heated at 35°C for 36 h. Distillation gave four fractions: (1) b.p. 90—120°C/0.05 Torr; (2) 127°C/0.05 Torr; (3) 128°C/0.05 Torr; and (4) 126.5°C/0.04 Torr. The last fraction (1.0 g) was slightly contaminated (TLC) with dibutyltin dichloride. Thus, it was further purified using a dry column technique. Fraction 4 was placed in a flask and dissolved in 5 ml of ether. The florisil was added and the ether evaporated under a stream of nitrogen. This florisil with V was placed on the top of a florisil column. The column was eluted with diisopropyl ether/glacial acetic acid (99 : 1) and V came rapidly off this column with the first 10 ml of solvent (TLC). The solvent was removed and the residue was distilled in a microdistillation apparatus. The yield of V was ~42%. The NMR spectrum of V (δ(ppm), $CDCl_3$, TMS) had signals at 3.80 (C<u>H</u>OH, multiplet); 3.05 (OH broad singlet); 1.4 (CH_2 groups, multiplet); 0.85 (CH_3, triplet, *J* 6.0 Hz) in the ratio 1 : 1 : 16 : 9. The CIMS showed *m/e* 307, *M* — Cl, 74% RA; *m/e* 269, *M* — BuOH, 21% RA; and *m/e* 285, *M* — Bu, 5% RA. Anal. Found: C, 42.33; H, 7.85; Cl, 10.32. $C_{12}H_{27}ClOSn$ calcd.: C, 42.35; H, 7.94; Cl, 10.29%.

Synthesis of β-hydroxybutyldibutyltin bromide (VI, X = Br)

In a four-necked flask equipped with a nitrogen inlet, two addition funnels

and a drying tube was placed 5.0 ml of a 2.63 *M* solution of isopropylmagnesium chloride in diethyl ether. To this stirring solution was added 3.4 g (0.012 mol) of tributyltin hydride dissolved in 10 ml anhydrous diethyl ether. The solution turned from a gray to white color indicative of the formation of tributyltin magnesium chloride as described by Lahournère and Valade [21]. The reaction mixture was stirred at room temperature for another 2 h after addition of the tributyltin hydride and to this solution was added 1.0 g (0.014 mol) of 1,2-epoxybutane. The reaction mixture was stirred at room temperature for 2 h and then hydrolyzed with saturated ammonium chloride solution. The ether layer was washed thrice with water and then dried over magnesium sulfate. The ether was removed on a rotary evaporator and the product purified via preparative TLC. This preparative TLC procedure utilized six silica gel GF TLC plates (1 mm) with ~137 mg of crude product per TLC plate. The TLC plates were developed with 7 : 3 (v/v) hexane/diisopropyl ether mixture and one edge of the plate was sprayed in the usual manner (see TLC section on metabolites) to visualize the position of the product. The plates were scraped and then the product isolated by extraction of the silica gel with ethyl acetate. After removal of the ethyl acetate, and TLC analysis, 260 mg of ~95% pure β-hydroxybutyltributyltin were obtained. It had a NMR spectrum consistent with data previously reported for this compound [22].

Compound VI (X = Br) was prepared in the following manner. In a 10 ml two-necked flask with a sidearm for nitrogen and a dropping funnel was placed 52 mg (95%) of β-hydroxybutyltributyltin dissolved in 3 ml of chloroform. To this cooled solution (~—30°C) was added dropwise over a 1 h period 18 mg of bromine in 3 ml of chloroform and the reaction proceeded to completion as shown by the total disappearance of starting material (TLC). In this procedure only a trace of tributyltin bromide was detected. A NMR spectrum of this chloroform solution confirmed its structure as VI (X = Br) with a signal at (δ(ppm)) 3.9 (quintuplet, *J* 6.0 Hz) for the (HCOH) methine proton and signals at 1.0 (CH_3 groups) and 1.1—2.0 (CH_2 groups) in the correct ratios. Our attempts to purify VI (X = Br) were unsuccessful, due to its instability, and experiments were performed with a chloroform solution of this compound.

Synthesis of γ-ketobutyldibutyltin bromide (VII, X = Br)

In an ampoule was placed 1.0 g (0.0025 mol) dibutyltin dibromide and 0.6 g (0.0025 mol) dibutyltin dihydride. After mixing under argon the ampoule was cooled in a Dry Ice/acetone bath and then 0.6 g (0.0053 mol) of 1-buten-3-one ethylene ketal [23] was added along with 2 mol % AIBN. The ampoule was sealed and placed in an oil bath at 28—30°C for 36 h. The contents of the ampoule were distilled and four fractions were obtained: (1) b.p. 96—100°C/0.03 Torr 0.19 g; (2) 104—116°C/0.03 Torr 0.25 g; (3) 116—120°C/0.03 Torr 0.42 g; and (4) 120—122°C/0.03 Torr 0.25 g. Fractions 1 and 2 were identified by TLC as dibutyltin dibromide. Fractions 3 and 4 were the ethylene ketal of VII, X = Br, which was identified by NMR and CIMS. The NMR spectrum of the ketal of VII, X = Br ($CDCl_3$, TMS) gave signals at (δ(ppm)) 3.95 (OCH_2CH_2O); 1.8 ($Sn—C\underline{H}_2CH_2—C(CH_3)(O(CH_2)_2O)$, triplet, *J* 5.0 Hz); 2.08 ($C\underline{H}_2—C(CH_3)(O(CH_2)_2O)$, triplet, *J* 5.0

Hz); 0.9—1.6 (CH_2 groups multiplet); and 1.3 ppm ($-C(C\underline{H}_3)(O(CH_2)_2O)-$, singlet). The CIMS showed *m/e* 349, *M* — Br, RA 76%; *m/e* 371, *M* — Bu, RA 9%; *m/e* 313, *M* — $[(CH_2)_2-C(CH_3)(O(CH_2)_2O)-]$, RA 15%.

The ketal of VII was deblocked using the following procedure. To 350 mg of the ethylene ketal of VII, as described above, was added 50 ml of acetone and several crystals of *p*-toluenesulfonic acid (TSA). After 3 days stirring at room temperature, TLC [two dimensional: diisopropyl ether/glacial acetic acid (99 : 1) then hexane/glacial acetic acid (9 : 1)] showed the total reaction of the ketal and the appearance of VII, X = Br. The reaction mixture was purified via the dry column technique on florisil using the diisopropyl ether/glacial acetic acid (99 : 1) system. After elution and TLC examination, the fractions containing the ketone VII, X = Br, were combined. A microdistillation gave 50 mg of pure ketone VII. The NMR of VII gave the following signals (δ(ppm), $CDCl_3$, TMS): 2.24 ($CH_3C(O)$, singlet); 2.95 ($CH_2C(O)$, triplet, *J* 6.0 Hz); 1—2 (CH_2 groups, multiplet); and 0.9 ($C\underline{H}_3(CH_2)_3Sn$, triplet, *J* 5.0 Hz). The IR in CCl_4 had a strong C=O stretch at 1690 cm^{-1} and a weak C=O stretch at 1720 cm^{-1}. The CIMS showed *m/e* 305, *M* — Br, 85% RA; *m/e* 337, *M* — Bu, RA 11%; and *m/e* 313, *M* — $[(CH_2)_2C(O)CH_3]$, RA 4%. Anal. Found: C, 37.40; H, 6.50; Br, 20.40. $C_{12}H_{25}BrOSn$ calcd.: C, 37.70; H, 6.54; Br, 20.68%.

Synthesis of [1-^{14}C]tetrabutyltin

To 72 mg of [1-^{14}C]butyl bromide (9.5 mCi/mmol; 5 mCi total radioactivity) was added 144 mg of unlabeled butyl bromide. This was done by a vacuum transfer of the [1-^{14}C]butyl bromide to unlabeled butyl bromide. In a four-necked flask equipped with an inlet for argon, reflux condenser and a dropping funnel were placed 90 mg (3.74 g at.) of amalgamated magnesium chips in 20 ml of dry diethyl ether. To this was added all at once 216 mg (1.57 mmol) of butyl bromide (72 mg [1-^{14}C]butyl bromide and 144 mg unlabeled butyl bromide). The reaction mixture was refluxed under argon for 1.5 h to form the [1-^{14}C]butylmagnesium bromide and then allowed to stir at room temperature for 30 min. The Grignard was cooled in an ice bath (0°C) and to this stirring solution was added dropwise over 30 min 171 mg (0.39 mmol) of stannic bromide (weighed out in a glove bag). The reaction mixture was refluxed for 1 h and stirred at room temperature for 1 h. It was then cooled in an ice salt bath and hydrolyzed with a saturated ammonium chloride solution. The condenser was connected to a Dry Ice/acetone trap to collect any [1-^{14}C]butane or [1-^{14}C]butyl bromide that might escape. The ether layer was separated and washed thrice with water and then dried over magnesium sulfate. TLC in hexane showed that [1-^{14}C]tetrabutyltin was the only product formed and it was 99% chemically and radiochemically pure. The ether was removed under vacuum at room temperature and the last traces of ether were removed by flushing the flask with argon. We obtained 61 mg (45%) of [1-^{14}C]tetrabutyltin as calculated from the specific activity (12.7 mCi/mmol) and total activity (2.3 mC).

Synthesis of [1-^{14}C]-tributyltin acetate (II)

In a 10-ml flask was placed 61 mg (0.175 mmol) of [1-^{14}C]tetrabutyltin in

4 ml of carbon tetrachloride. The mixture was cooled to 0°C in an ice salt bath and then 59.4 mg (0.37 mmol) of bromine (excess) was added dropwise over a 2 h period. The solvent was then removed under vacuum to give ~134 mg of product. The product was analyzed by TLC [diisopropyl ether/glacial acetic acid (99 : 1)]. This procedure included development of the TLC plate then scraping, elution of the silica gel with absolute ethanol, and counting an aliquot of the ethanol solution of the organotin acetate. These results showed that we obtained [1-^{14}C]tetrabutyltin (0.0056 mCi, 12.7 mCi/mmol); [1-^{14}C]tributyltin acetate (0.18 mCi, 9.5 mCi/mmol); [1-^{14}C]dibutyltin diacetate (0.88 mCi, 6.3 mCi/mmol); and [1-^{14}C]butyltin triacetate (0.06 mCi, 3.2 mCi/mmol). The mixture of bromination products was then dissolved in 50 ml of hexane and 2-ml aliquots were placed in 10-ml ampoules and stored at —50°C for purification as needed. The [1-^{14}C]tributyltin acetate was obtained chemically and radiochemically pure (99%) by preparative TLC with the diisopropyl ether/glacial acetic acid (99 : 1) system. We assumed that anion exchange took place on the chromatoplate converting the bromide to the acetate [7].

Procedure for rat liver microsomal monooxygenase reactions

The livers of male albino rats (150—160 g) were homogenized with a glass-teflon homogenizer at 20% (w/v) in sodium phosphate buffer (0.1 *M*, pH 7.4) and the homogenate was centrifuged at 15,000 *g* for 15 min at 2°C to sediment the nuclei, mitochondria and debris. The supernatant fraction was centrifuged at 110,000 *g* for 60 min at 2°C to recover the microsomal supernatant (soluble fraction); the sedimented microsomal fraction was reconstituted in phosphate buffer equal to the original homogenate volume.

The standard incubation mixtures in 2 ml sodium phosphate buffer (0.1 *M*, pH 7.4) contained the following components: liver microsomal preparation (5.1 mg protein, 200 mg fresh liver weight equivalent); soluble fraction (5.5 mg protein, 80 mg fresh liver weight equivalent); NADPH (0 or 2 μmol). The substrate [0.5 μmol tributyltin derivative (I, II, III or V) or 0.03 μmol of [1-^{14}C]tributyltin acetate], in 50 μl ethanol, was then added by injecting it below the surface of the aqueous medium with immediate mixing. All incubations were carried out at 37°C with shaking for 60 min in air. Each reaction mixture was then extracted five times with 7 ml volumes of chloroform, a procedure providing almost quantitative recovery of metabolites IV, V, VI and VII.

TLC analysis of the metabolite mixture

The chloroform solution of metabolites was evaporated under nitrogen and spotted at the origin of a chromatoplate (20 × 20 cm, precoated silica gel 60 without fluorescent indicator, 0.25 mm thickness, E. Merck, Darmstadt, Germany) which was then immediately developed twice in the first direction with diisopropyl ether/glacial acetic acid (99 : 1) and twice in the second direction with hexane/glacial acetic acid (9 : 1). When unlabeled substrate was present, or in cochromatography studies, the chromatogram was sprayed with a 0.1% (w/v) solution of 8-hydroxy-5-quinolinesulfonic acid (HQ) in 95% ethanol to detect dialkyltin derivatives which fluoresce under an ultraviolet (UV) lamp. The chromatoplate was then placed under a germicidal UV lamp for 1 h to convert the triorganotin derivatives to dialkyl or monoalkyl derivatives for

visualization as above with the HQ reagent and in addition as blue spots on spraying with a saturated solution of pyrocatechol violet (PCV) in absolute ethanol. [^{14}C]Compounds detected by radioautography were quantified by scraping the appropriate gel regions and liquid scintillation counting. For cochromatography of [^{14}C]metabolites with authentic compounds, 5—10 μg of each unlabeled standard was individually added to the [^{14}C]metabolite mixture prior to spotting. Exact coincidence of spot position and shape, after two-dimensional TLC development, for the unlabeled and labeled compounds was considered to constitute tentative identification of the [^{14}C]metabolites.

Identification of metabolite V: CIMS

The previous TLC technique was employed, except that the plate was not sprayed and the region of metabolite V was scraped and the silica gel extracted with ethyl acetate. The solvent was evaporated in a capillary for CIMS analysis. The fragmentation pattern for metabolite V was consistent with that for authentic V.

Identification of metabolite VII: biological oxidation of V (X = Cl) to the corresponding ketone VII

Compound V (X = Cl) (0.5 μmol) was incubated with the microsomal enzyme system and the metabolite pattern (TLC) was compared with that obtained from tributyltin chloride under the same conditions. The metabolite mixture derived from each substrate was spotted on a separate TLC plate and, on a third plate, a mixture of the metabolite extracts from tributyltin chloride and γ-hydroxybutyldibutyltin chloride was spotted. Intercomparison of the three TLC plates, after development and detection of the organotin compounds with HQ and PCV, showed that one of the tributyltin chloride metabolites was also derived from γ-hydroxybutyldibutyltin chloride, This common metabolite was identified as compound VII in studies with [1-^{14}C]tributyltin acetate. It should be noted that the conversion of V to VII occurred only to a minor extent and only in the presence of NADPH.

Quantitation of metabolite VI: GLC analysis of 1-butene and reaction of [1-^{14}C]1-butene with mercuric acetate in methanol

The GLC analysis (150 m × 0.2 mm glass capillary column coated with SF 96, flame ionization detector) was performed by acidifying the enzyme reaction mixture and sampling the head space of the flask. The 1-butene formed cochromatographed with an authentic sample (23.0 min) but not with butane (23.4 min) or *cis*- or *trans*-2-butene (24.2 and 25.1 min).

In order to quantitate the formation of metabolite VI, we formed [1-^{14}C]1-butene, via a protodestannylation reaction of VI, and then utilized the reaction of butene with mercuric acetate in methanol (oxymercuration). A special apparatus insured efficient trapping of the small amount of [1-^{14}C]1-butene which was formed. Thus two gas trapping columns were placed in series, with the first column being cooled by an ice bath and the second column by a Dry Ice/acetone bath, and they were connected to a vacuum line to insure a continual air flow through the enzyme reaction flask and the columns. Each column contained 250 mg of mercuric acetate in 50 ml of a methanol/acetic

acid (50 : 1) mixture. The enzyme reaction was placed in a flask with a gas inlet and at zero time 0.03 μmol of [1-^{14}C]tributyltin acetate was introduced via a rubber septum. After incubation for 30 min at 37°C, hydrochioric acid was added to the reaction mixture to bring the pH to ~1. After 10 min the system was flushed with unlabeled 1-butene to react with the excess mercuric acetate.

The oxymercuration product of 1-butene was isolated by rotary evaporation of the methanol/acetic acid solution followed by recrystallization of the product from hexane (two times) until a constant specific activity was obtained. The white crystalline oxymercuration adduct m.p. 39—41°C [24] was conveniently analyzed by TLC [25] for chemical and radiochemical purity using the following solvent systems and an authentic sample: ethyl acetate/methanol/acetic acid (30 : 2 : 1), R_f 0.63; diisopropyl ether/acetic acid (49 : 1), R_f 0.10; and n-propanol/triethylamine/water (2 : 1 : 1), R_f 0.17. *S*-Diphenylcarbazone (0.1% w/v in 95% ethanol) was used to detect the oxymercuration product which gave a deep purple spot on a pink background.

From the radiocarbon content of the oxymercuration product we could calculate the amount of butene and hence the amount of metabolite VI formed in this biological oxidation reaction. Furthermore, it was determined in separate experiments that more than 90% of the [1-^{14}C]1-butene was formed upon acidification and that NADPH was required for formation of the [1-^{14}C]butene.

Identification and quantitation of metabolite VIII: reaction of [1-^{14}C]butanol with phenyl isocyanate

Since metabolite VIII was unstable in the buffer system used in the biological oxidation reaction, it was not possible to identify it directly. We therefore identified and quantified metabolite VIII by the formation of a derivative, since we thought that it would undergo a destannylation reaction to provide [1-^{14}C]-butanol. The incubation mixture was extracted with 2 ml of chloroform to which 500 μl of phenyl isocyanate was then added. After holding this mixture overnight at room temperature and evaporation to dryness under a stream of nitrogen, 1 ml of water and 3 ml of hexane were added. The phenylcarbamate of [1-^{14}C]butanol was readily extracted into hexane, which was then subjected to TLC analysis. The phenylcarbamate of [1-^{14}C]butanol was found to be identical to an authentic sample by one and two dimensional TLC [chloroform in the first dimension (R_f 0.81) and hexane/acetone (4 : 1) in the second dimension (R_f 0.53)]. In order to quantify the formation of [1-^{14}C]butanol the derivative was scraped and counted by liquid scintillation. This in turn enabled us to quantify the amount of metabolite VIII. We also corrected for the evaporation of [1-^{14}C]butanol during incubation by using a known amount of [1-^{14}C]-butanol in a comparable experiment. In this manner we found that ~28% of the [1-^{14}C]butanol was lost, while a similar control with the phenylcarbamate indicated ~50% recovery for this derivative.

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