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Preliminary communication

# BIOORGANOTIN CHEMISTRY: BIOLOGICAL OXIDATION OF TRIBUTYLTIN DERIVATIVES

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## Summary

The biological oxidation of several tributyltin derivatives by a rat liver microsomal mixed function oxygenase produces carbon hydroxylated compounds identified as α-, β-, γ- and δ-hydroxybutyldibutyltin derivatives. The mechanistic implications and the possible role of the tin atom in these oxidations are discussed.

The biological oxidation of organometallic compounds is an area which has been neglected, although it is of importance to the basic understanding of the fate of these compounds in biological systems. The relatively few studies in this area concerning organosilicon [1a, b], organotin [2a, b] and organolead compounds [2b] have shown that oxidative dealkylation and possibly hydroxylation of alkyl side chains are important reactions. Since these results were not definitive, we have concentrated our efforts on the organotin compounds and have initiated a program to delineate the role of the tin atom in their biological oxidation.

In this communication, we describe the first reported identification of hydroxylated organotin metabolites formed in a microsomal oxidation (mixed function oxygenase) reaction utilizing several tributyltin derivatives as model substrates, while also using the tin atom as a probe to better define the mechanistic implications involved in these important reactions.

Thus, 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 [2b] (11 mg of protein in phosphate buffer (0.1 *M*, 2.0 ml, pH 7.4) containing reduced nicotinamide-adenine dinucleotide phosphate (NADPH, 2.0 μmol) as the essential cofactor. After 1 h at 37°, and

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extraction with chloroform, the product mixture was analyzed by thin layer chromatography (TLC). It was found that all the tributyltin derivatives studied (I-III) gave a similar mixture of identifiable metabolites*, however, in low yields (< 10%). In view of this fact, we utilized [1-$^{14}$C]-tributyltin acetate (0.003 μmol, specific activity 9.5 mC/mmol) as the substrate in further experiments to insure the quantitation** and identification of the metabolites which were formed in this microsomal oxidation reaction [3].

The following metabolites were identified using authentic compounds*** in conjunction with thin layer cochromatography techniques, degradation studies, and chemical ionization mass spectrometry (CIMS) [4] (eqn. 1).

$$[CH_3(CH_2)_3]_3SnX \xrightarrow[\text{(2) } CHCl_3\text{ extraction}]{(37^\circ,\ 1\text{ h})\ \text{(1) Rat liver microsomes with NADPH}} [CH_3(CH_2)_3]_2\underset{\displaystyle X}{\underset{|}{Sn}}CH_2CH_2CH_2CH_2OH +$$

(0.003 μmol)
X = OAc

(IV) (12%)

$$[CH_3(CH_2)_3]_2\underset{\displaystyle X}{\underset{|}{Sn}}CH_2CH_2CH_2\underset{\displaystyle OH}{\underset{|}{C}}HCH_3 + [CH_3(CH_2)_3]_2\underset{\displaystyle X}{\underset{|}{Sn}}CH_2\underset{\displaystyle OH}{\underset{|}{C}}HCH_2CH_3 +$$

(V) (16%) (VI) (44%)

$$[CH_3(CH_2)_3]_2\underset{\displaystyle X}{\underset{|}{Sn}}\underset{\displaystyle OH}{\underset{|}{C}}HCH_2CH_2CH_3 \qquad (1)$$

(VII) (28%)

The two-dimensional acidic TLC system we utilized completely resolved all of the authentic compounds****. This enabled us to cochromatograph authentic compounds with the metabolites, thus establishing one criterion for metabolite identification. Unfortunately, the extremely low yield of total hydroxylated metabolites and the fact that metabolite IV was not formed in sufficient amount at high tributyltin concentration (0.5 μmol) precluded its isolation for direct structural identification. We were, however, able to cleanly separate IV from the other metabolites with the acidic TLC system and cochromatograph it with an authentic sample. Metabolite V was also completely resolved from the other

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*We presume that anion exchange on the tin atom occurred in the microsomal reaction mixture and that anion exchange also occurred on the TLC plate, since acetic acid was used, producing the acetates.

**The percent metabolites that we report are normalized values of identified metabolites and do not take into account unidentified compounds which remained at the origin (TLC). Thus the major metabolites identified amounted to 9%, while starting material recovered amounted to 66%. The remaining 25% consisted of dibutyltin diacetate (4%), butyltin triacetate (5%) and unidentified compounds at the origin (16%).

***All new compounds synthesized gave correct elemental analyses and spectroscopic data for their assigned structures. Compound VII was not independently synthesized but was identified by an electrophilic cleavage reaction with hydrochloric acid, on the reaction mixture, which provided [1-$^{14}$C]-butanol.

****Compounds IV-VI, tributyltin acetate and dibultin diacetate were separated by two-dimensional TLC on silica gel G chromatoplates developed in the first direction with diisopropyl ether/glacial acetic acid (99/1) and in the second direction with hexane/glacial acetic acid (9/1).

metabolites and fortuitously at high tributyltin concentration (0.5 μmol) a sufficient amount could be isolated via preparative TLC to obtain a CIMS spectrum [4]. The CIMS of V was conclusive for its composition (*m/e* 307, $[M-OAc]^+$) while TLC cochromatography with an authentic sample added support to its assigned structure. Metabolite VI was separated from IV and V by the same TLC system, however, the acidic conditions in the second direction of development caused it to destannylate to butene and dibutyltin diacetate (eqn. 2).

$$[CH_3(CH_2)_3]_2\underset{\underset{OAc}{|}}{Sn}CH_2\underset{\underset{OH}{|}}{C}HCH_2CH_3 \xrightarrow{HOAc} [CH_3(CH_2)_3]_2Sn(OAc)_2 + CH_3CH_2CH{=}CH_2 \qquad (2)$$

(VI)

The butene was independently identified via gas liquid chromatography after acidification of the microsomal enzyme reaction mixture. Significantly, authentic VI also underwent, as expected [5], a similar destannylation reaction in the TLC system employed, further confirming the β-hydroxyl structure. It is noteworthy that V, a γ-hydroxybutyldibutyltin, was stable under these TLC conditions. Metabolite VII was unstable in the buffer system employed in the biological oxidation reaction, and it was identified by isolating the [1-$^{14}$C]-butanol, as the phenylcarbamate, formed upon destannylation. Presumably, the α-hydroxybutyldibutyltin reacted via cleavage of the carbon–tin bond [6] (eqn. 3).

$$[CH_3(CH_2)_3]_2\underset{\underset{OAc}{|}}{Sn}\text{—}\underset{\underset{OH}{|}}{C}H(CH_2)_2CH_3 \xrightarrow{pH\ 7.4} [CH_3(CH_2)_3]_2\underset{\underset{OAc}{|}}{Sn}OH + CH_3(CH_2)_3OH \qquad (3)$$

(VII)

It was further observed that metabolite V was oxidized to the extent of 12% (based on 100% of V from tributyltin) forming metabolite VIII which cochromatographed with authentic ketone VIII. The conversion of V to VIII was verified by using authentic V as the substrate in the microsomal oxidation and observing the formation of VIII by TLC cochromatography.

$$[CH_3(CH_2)_3]_2\underset{\underset{OAc}{|}}{Sn}CH_2CH_2\underset{\underset{O}{\|}}{C}CH_3$$

(VIII)

We wish to make several observations pertaining to our results in these biological oxidations. The hydroxybutyldibutyltins formed by secondary (2°) carbon hydroxylation accounted for 88% of the identified metabolites, which represents an average 2° to primary (1°) carbon–hydrogen bond reactivity of 3.7. A similar trend has been noted with butane and pentane which gave a 2°/1° carbon–hydrogen bond reactivity of > 100 [7a-c]. More importantly, at 0.003 μmol tributyltin acetate substrate concentration, the ratio of β to α, γ and δ carbon hydroxylation is 1.6/2.8/3.7 respectively. We believe this hydroxylation pattern is more consistent with a free radical than an ionic process and that the preference for β over α, γ and δ carbon hydroxylation implies some role of the tin atom in directing the site of reaction [8a-c, 9a, b].

We are presently attempting to further elucidate the tin atom's role in these important reactions and are extending our studies to other organotin compounds.

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