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BIOORGANOTIN CHEMISTRY: SITUS AND STEREOSELECTIVITY IN THE REACTION OF CYCLOHEXYLTRIPHENYLTIN WITH A CYTOCHROME P-450 DEPENDENT MONOOXYGENASE ENZYME SYSTEM

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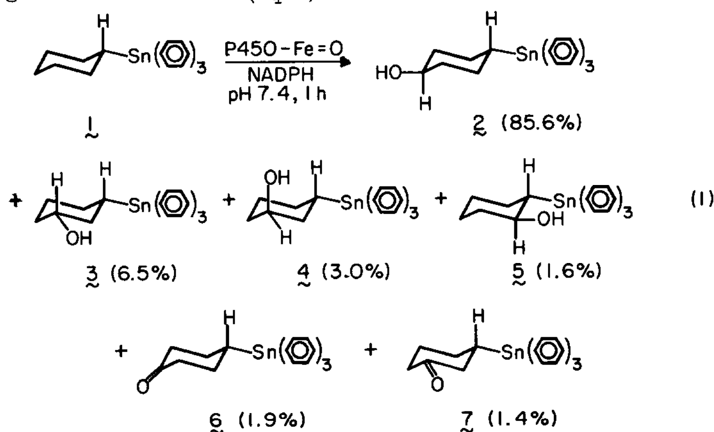
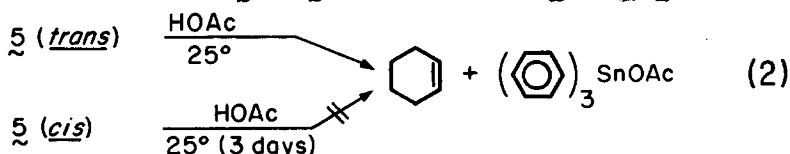
One of the most interesting and important biological reactions is the cytochrome P-450 dependent monooxygenase enzyme reaction with organic and organometallic substrates[1a-d]. The reaction involves a Heme-Iron-Monooxygen complex[2], which converts carbon-hydrogen bonds to carbon-hydroxyl bonds. While several investigations have been concerned with the stereochemistry of this reaction[3], few have concentrated on the cyclohexyl ring system[4a,b]. To our knowledge, no definitive in vitro studies concerning both the situs and stereoselectivity of a cytochrome P-450 dependent monooxygenase reaction with a monosubstituted cyclohexyl derivative have been reported[4a]. Our recent investigation of this reaction with alkylorganotin compounds[1c,d] showed this conversion of C-H to C-OH to be general with this class of compounds and in this communication we wish to provide evidence for both the situs and stereoselectivity using cyclohexyltriphenyltin as a model substrate.

Thus, [1-^{14}C] cyclohexyltriphenyltin, 1[5], (.05 μmole, 1.26 mc/mmole) was incubated with our source of cytochrome P-450 monooxygenase enzymes, rat liver microsomes[1c] (10.6 mg protein), for 1 hr at 37° in phosphate buffer (pH 7.4) containing NADPH (2 μmole) the essential cofactor. After chloroform extraction of the reaction mixture, the metabolites were separated by TLC[6], quantified by liquid scintillation counting, and identified by a combination of TLC cochromatography, preparative TLC in conjunction with 360 ^{1}H FT nmr spectroscopy and degradation reactions (eq 1)[7].



Compounds 2-7 were separated (TLC) and identified by TLC cochromatography with authentically synthesized standards[8] and this constitutes one criterion for metabolite identification[1c]. In addition, we were able to isolate and purify the major metabolite, compound 2, via preparative TLC[9] and obtain a 360 MHz ^1H FT nmr spectrum. The FT nmr spectrum of 2 ($CDCl_3$, TMS) unequivocally assigns its structure as trans-4-hydroxycyclohexyltriphenyltin. The axial methine proton on the carbon (C_4) bearing the hydroxyl group gave the definitive 9 line pattern (δ 3.58 ppm; J_{ax}-J_{ax} = 11.1 Hz; J_{ax}-J_{eq} = 4.0 Hz) consistent with an nmr spectrum of the authentic compound 2[8]. Compounds 3 and 4 were formed in such low concentrations that they could only be identified by two-dimensional TLC which separated them and allowed TLC cochromatography with the known cis and trans-3-hydroxyl compounds; however, we believe this is quite strong evidence for their assigned structures. Compound 5 was also TLC cochromatographed with authentic 5[8], but fortunately, we were able to use its reactivity with glacial acetic acid to assign, unambiguously, the trans stereochemistry. Compound 5 undergoes a facile 1,2-deoxystannylation reaction in the presence of glacial acetic acid to provide [1-^{14}C]cyclohexene and triphenyltin acetate[1c] as was also confirmed with authentic 5 via nmr spectroscopy. Pertinently, the corresponding cis-isomer of 5 (methyl ether) was inert under similar reaction conditions, as was also shown with the corresponding silicon analogs[10]. Thus, if cis 5 was present as a metabolite it would have been detected (eq 2). The ketones 6 and 7 from the alcohols 2 and 3, 4, respectively, were



also identified by two-dimensional TLC cochromatography with authentic standards[8], but again, we are confident of their assigned structures using this technique[1c]. Compound 8, 1-hydroxycyclohexyltriphenyltin, which we would identify if present via an electrophilic cleavage reaction with hydrochloric acid to give [1-^{14}C]cyclohexanol[1c] followed by characterization as its phenylcarbamate, was not detected. In control experiments, we were able to detect the phenylcarbamate of [1-^{14}C]cyclohexanol at levels of 2% but not lower.

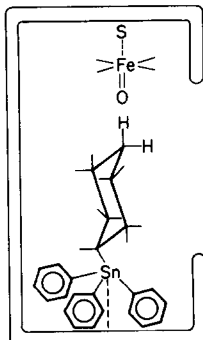
From our results (eq 1) the major site of hydroxylation (including ketones) is C_4 (87.5%) with C_3 (10.9%) and C_2 (1.6%) minor sites and C_1 (~ 0%), with the only methine hydrogen, not hydroxylated to any detectable extent. The situselectivity based on a per hydrogen basis, i.e. $C_4 : C_3 : C_2 : C_1$, is 109 : 7 : 1 : 0. This dramatic situselectivity is complemented by a very high degree of stereoselectivity for predominantly equatorial hydroxylated products with compounds 2, 3 and 5 representing ~ 95% of the metabolites formed.

In contrast to these results with cyclohexyltriphenyltin, the monosubstituted cyclohexyl derivative, methylcyclohexane, which was also studied under in vitro reaction conditions[4a], gave a per hydrogen reactivity for $C_4 : C_3 : C_2 : C_1$ of 5 : 5 : 1 : 11. No stereochemical assignments were made for the 2-, 3- or 4-methylcyclohexanols formed, but the differences in the ratios of hydroxylation at each carbon atom are striking between the two compounds.

While steric effects could be invoked to explain these differences, it should be pointed out that the carbon-tin bond length is 2.18A° as compared with the carbon-carbon bond length of 1.54A° and this factor should reduce the steric bulk of the triphenyltin group.

Consequently, these results point out the highly specific binding of substrate which must exist in proximity to the Fe=O complex[3]. The triphenyltin group could provide a template for the possible positioning of the cyclohexyl group towards the Fe=O monooxygenase as depicted in Figure 1. If one of the phenyl groups is removed and replaced by an acetate group,

Figure 1
One possible conformation of **1**



i.e., cyclohexyldiphenyltin acetate, the pattern of hydroxylation changes dramatically as evidenced by a more equal reactivity of all hydrogens[11]. Thus, the tin atom seems to be able to control the sites of hydroxylation by the way it specifically binds to the protein surface in proximity to the Fe=O complex.

While our previous studies[1c] have convinced us that the monooxygenase carbon-hydroxylation with alkylorganotin compounds is a free radical process, our present results with cyclohexyltriphenyltin reveal that the overall reaction $-\overset{|}{\underset{|}{C}}-H \rightarrow -\overset{|}{\underset{|}{C}}\cdot \rightarrow -\overset{|}{\underset{|}{C}}-OH$ is highly stereospecific with a clear preference for equatorial products predominating, i.e., the ratio of equatorial to axial hydroxyl products (Eq/Ax) based on **2-5** is 59. The important question of whether the P-450 Fe=O complex homolytically removes equatorial hydrogens in a totally stereospecific reaction (as implied in Figure 1) or whether some of the equatorial products come from axial hydrogen removal, recently reported for exo and endo hydrogens[12] in the norbornane system, awaits further clarification.

We are presently continuing our studies of this reaction with cyclohexyldiphenyltin acetate and tricyclohexyltin acetate as substrates with the hope of learning more about the situs and stereochemistry involved.

Acknowledgements

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5. Prepared by reaction of [1-^{14}C]cyclohexylmagnesium bromide with triphenyltin chloride in 33% yield.

6. Separation of 1-4 and 6,7 was achieved on 0.25 mm silica gel G plates using a two-dimensional TLC system consisting of (1st direction) diisopropyl ether/hexane (85:15) and (2nd direction) ethyl acetate/benzene (30:70), while separation of 5 from 1 was best achieved by using diisopropyl ether/hexane (1:1) and ethyl acetate/benzene (5:95). The ^{14}C-labelled metabolites were detected by radioautography, while the unlabelled authentic compounds were detected using 8-hydroxy-5-quinolinesulfonic acid (HQ) and pyrocatecol violet (PCV) as spray reagents (see ref. 1c).

7. The percentages in equation 1 represent normalized values of identified metabolites and account for 10% of the starting [1-^{14}C]cyclohexyltriphenyltin. The remainder was ~ 70% starting material and ~ 20% unidentified materials.

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9. Separation was achieved on 0.25 mm silica gel G plates with hexane/diisopropyl ether (15:85) as solvents. After development, the appropriate band was detected by radioautography then scraped and eluted with chloroform. The corresponding cis-4-hydroxy isomer could not be separated from compound 2 by TLC, but both are readily analyzed by 360 MHz ^{1}H nmr spectroscopy. No cis-4-hydroxycyclohexyltriphenyltin was evident in the nmr spectrum of 2.

10. While the corresponding cis-2-hydroxycyclohexyltriphenyltin compound was not available, the methyl ether was available and does not react with glacial acetic acid even after 3 days. The important point is that the cis-2-alcohol or methyl ether cannot assume the trans diaxial conformation needed for the 1,2 deoxystannylation. This is also consistent with the cis-2-hydroxy and cis-2-methoxycyclohexylsilicon derivatives, which also fail to undergo the 1,2-deoxysilylation reaction under weakly acidic conditions. See W. K. Musker and G. L. Larson J. Amer. Chem. Soc., 91, 514 (1969); M. De Jesus, O. Rosario and G. L. Larson, J. Organometal. Chem., 132, 301 (1977).

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