Allylic and Propargylic Phenyl Selenide Oxygenation by Cyclohexanone Oxygenase: [2,3]-Sigmatropic Rearrangement of the Enzyme-generated Selenoxide

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Enzymic oxidation of propargylic and allylic selenides has been carried out and the resulting selenoxides found to readily undergo 2,3-sigmatropic rearrangement; the propargylic product undergoes fragmentation while the allylic product yields racemic alcohols.

The bacterial cyclohexanone oxygenase is a highly versatile flavin-linked oxygenation catalyst. In addition to catalysing Baeyer–Villiger oxidations of cyclic ketones, acyclic ketones, and aldehydes, it also converts aryl and allyl boronic acids into phenols and alcohols. The carbon centres in both the enzymic Baeyer–Villiger and the boronic acid conversions have been observed to migrate with retention of configuration. This flavoenzyme will also transfer oxygen to nucleophilic substrates including conversion of thiane into thiane sulphoxide and of thiane sulphoxide to thiane sulphone. Also, trimethylphosphite is converted into trimethylphosphate.[1]

In a test for potential [2,3]-sigmatropic rearrangements that

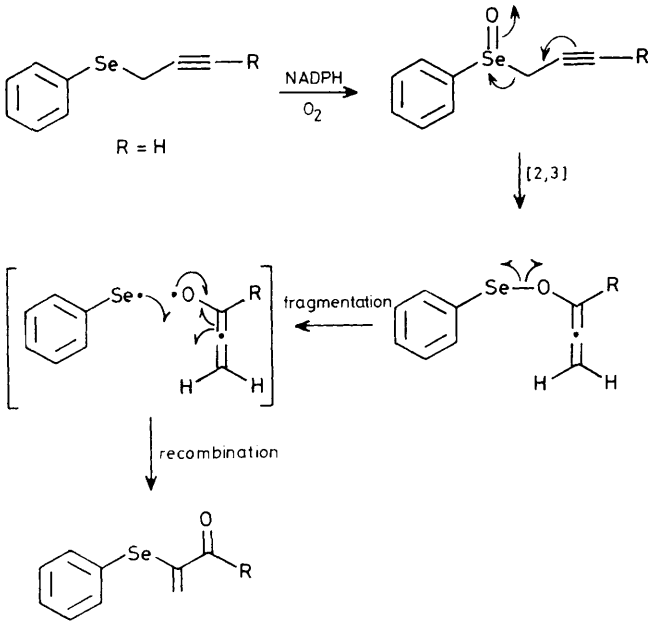

Figure 1

might also subsequently inactivate the enzyme, allyl phenyl sulphides were observed to undergo smooth conversion into the sulphoxides but without loss of enzyme activity. Since the allylic sulphoxides rather than the electrophilic allylsulphenate esters are the major components in the equilibrium under physiological conditions in aqueous solution, we turned to the corresponding selenides.[1c,2] Almost no enzymic studies on selenides have been reported but chemical oxidation studies by Reich's group reveal the allylic selenoxides rearrange much faster than the corresponding allyl sulphoxides, and further, the resulting electrophilic allylselenate esters may be the more favoured 2,3-sigmatropic rearrangement partner by factors of as much as 10^6.[3]

We have prepared and tested phenyl propargyl selenide as a cyclohexanone oxygenase substrate and observed a $k_{cat.}$ value of 585 min^{-1} (85% the value for 4-methylcyclohexanone) and K_m of 128 μM.† No detectable enzyme inactivation ensued in several thousand turnovers. However, product analysis revealed an interesting and unusual chloroform extractable product. ^1H N.m.r. spectroscopic analysis showed signals at δ 9.65 (s, 1H), 7.1—7.7 (m, 5H), 6.66 (d, 1H), and 6.06 (d, 1H). This material was identified as α-phenylselenoacrolein by comparison with an authentic sample.[4] This is also the product that accumulates on nonenzymic ozone treatment of phenyl propargyl selenide in dry dichloromethane as reported by Reich and co-workers.[3] The α-phenylselenoacrolein must have derived from E·FAD-4a-OOH mediated generation of the selenoxide followed by sigmatropic rearrangement to the allenic selenic ester (Figure 1). At this point, fragmentation, possibly to a radical pair, and recombination would yield the accumulating product. No diphenyl diselenide was detected. It is clear that the initial selenoxide formation is enzymic. The rate of the [2,3]-rearrangement is not known accurately but is likely to exceed the turnover rate of 9 s^{-1}, so the allenic selenic ester should begin to accumulate in the enzyme active site. Whether the proposed fragmentation–recombination to the observed α-phenylselenoacrolein is occurring in the enzyme active site is not clear. When the corresponding 1-bromopropargyl phenyl selenide was tested as substrate, oxygen consumption occurred without loss of catalytic activity. If an acyl bromide product were generated by an analogous sigmatropic rearrangement and fragmentation–recombination epiphenomena after oxygen transfer, no essential enzymic groups were covalently captured.

† Values determined by NADPH and O_2 consumption studies.

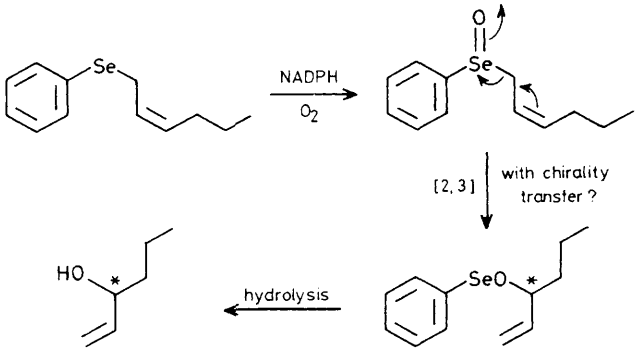

Figure 2

The isomeric *cis*- and *trans*-hex-2-enyl phenyl selenides were prepared‡ and also tested as cyclohexanone oxygenase substrates. NADPH consumption studies indicated that the two selenides did not cause inactivation but rather were slow substrates with a $k_{cat.}$ value of 41 min^{-1} (9% of the $k_{cat.}$ of 4-methylcyclohexanone) and K_m of 9.6 μM for the *cis* isomer and $k_{cat.}$ of 55 min^{-1} (12% 4-methylcyclohexanone) and K_m of 6.8 μM for the *trans* isomer.

Previous studies have shown that cyclohexanone oxygenase oxidizes ethyl *p*-tolyl sulphide to the sulphoxide with 4:1 (*S*):(*R*) enantioselectivity.[5] Given the ease of selenoxide 2,3-rearrangements, we reasoned that enzymic selenide oxidation might provide a means for preparation of chiral allylic alcohols. If the initial enzymic oxidation were chiral, the isomeric *cis*- and *trans*-selenoxides would be expected to have diastereoisomeric transition states which would lead to enantiomeric alcohol products, as exemplified for the *cis* isomer in Figure 2.

cis-Hex-2-enyl phenyl selenide (15 mg, 61 μmol) was incubated with 1.2 mg enzyme in the presence of catalase (90 μg, 17 000 Sigma units) (with 60 μmol NADPH in 50 ml 80 mM glycine–NaOH buffer, pH 9.0) for 19 h. The incubation was brought to saturation by addition of solid NaCl and the product mixture extracted with pentane. Product analysis showed three compounds identifiable (by t.l.c. comparison with authentic samples) as starting hexenyl phenyl selenide, diphenyl diselenide, and hex-1-en-3-ol. The crude mixture was treated with *p*-bromobenzoyl chloride and the allylic ester isolated. C.d. analysis of the *p*-bromobenzoyl ester showed no Cotton effects near the $\lambda_{max.}$ (247 nm), suggesting no chiral induction in the oxidation–rearrangement–hydrolysis process.[6] Analogous treatment of the *trans* isomer yielded a similar result.

To corroborate these findings, the product alcohols were converted into their (−)-methoxyphenyl(trifluoromethyl)-acetyl (MPTA) esters and the esters separated by h.p.l.c. (0.4% diethyl ether–hexane).[7] Comparison with the (−)-

‡ The *cis*- and *trans*-hex-2-enyl phenyl selenides were prepared by treating the corresponding phenyl methanesulphonates with sodium phenyl selenate; *trans* isomer, ^{13}C n.m.r. ($CDCl_3$) δ 133.7, 133.4, 128.8, 126.9, 126.0, 34.3, 30.2, 22.4, and 13.5; *cis* isomer, ^{13}C n.m.r. ($CDCl_3$) δ 133.5, 132.8, 128.8, 127.0, 125.2, 28.9, 24.8, 22.6, and 13.7.

MPTA esters of an authentic sample prepared from racemic alcohol again revealed that no transfer of chirality had occurred. The results suggest the product alcohol is formed from racemic selenoxide. It is not yet clear that the initial oxidation at selenium is not enantioselective since selenoxides readily racemize through their hydrates in aqueous media and this process may outcompete the 2,3-rearrangement rate.[8]

Cyclohexanone oxygenase provides the first example of enzymic oxygenation at selenium to selenoxides, which are then capable of undergoing rapid and quantitative [2,3]-sigmatropic rearrangement.

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