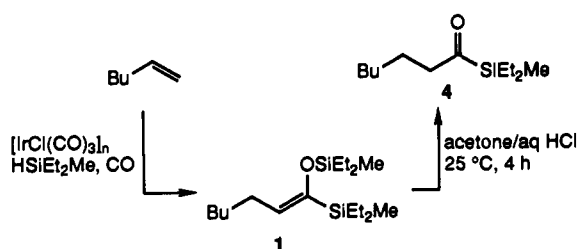


Scheme 1



atm (initial pressure at 25 °C) of carbon monoxide for 48 h in the presence of $[\text{IrCl}(\text{CO})_3]_n$ (2 mol %) as a catalyst. Incorporation of a siloxy(silyl)methylene unit, derived from one molecule of CO and two molecules of HSiEt_2Me , into the terminal carbon atom of the alkene took place to afford a 1-silyl enol silyl ether (**1**, $R' = \text{Bu}$, 74% ($E/Z = 79/21$); **2**, $R' = \text{Cy}$, 66% ($E/Z = 86/14$); **3**, $R' = t\text{-Bu}$, 60% ($E/Z = 69/31$)) as the sole product.⁶ No regioisomer was detected. Dimethylphenylsilane (HSiPhMe_2) and triethylsilane (HSiEt_3) can also be used for the reaction of 1-hexene to give the corresponding products in 65% ($E/Z = 87/13$) and 50% yields ($E/Z = 66/34$), respectively. Several iridium complexes were examined for their catalytic activity. While neither $\text{IrCl}(\text{CO})(\text{PPh}_3)_2$ nor $\text{IrH}(\text{CO})(\text{PPh}_3)_3$ was effective, $\text{Ir}_4(\text{CO})_{12}$ exhibited catalytic activity affording **1** in 65% yield.

The new catalytic reaction can be applied to a wide variety of terminal alkenes (Table I). Note that functional groups, such as acetal,⁷ cyano,⁸ and epoxide,⁹ known to react in transition metal catalyzed reactions with HSiR_3 or with HSiR_3/CO remain intact. While norbornene was reactive, cyclohexene reacted only sluggishly under those conditions. The multifunctionality of the products is synthetically attractive and will no doubt find application in the future. The products obtained can be easily hydrolyzed to acylsilanes.^{5i,10} For example, treatment of **1** with acid (acetone/HCl (0.2 M) = 4/1) at 25 °C for 4 h gave an acylsilane **4** in quantitative yield (Scheme I).

The stoichiometry of the reaction **1** indicates that two hydrogen atoms in reactants are not incorporated in the product. These are incorporated into another molecule of the starting alkene. Thus reaction of vinylcyclohexene gave ethylcyclohexane (64% yield based on HSiEt_2Me) in addition to **2** (66%).¹¹

The mechanism of the catalytic reaction is not known at present. The possibility that an acylsilane intermediate gives the observed product by dehydrogenative silylation was eliminated. Thus reaction of **4** with 1 equiv of HSiEt_2Me in the presence of $[\text{IrCl}(\text{CO})_3]_n$ resulted in 93% recovery of **4**.¹² We are now examining the possible intervention of a siloxycarbyne complex ($\text{Ir}=\text{COSiR}_3$) intermediate.

Acknowledgment. This work is supported by a Grant-in-Aid for Scientific Research on a Priority Area, Activation of Unreactive Small Molecules, Grant No. 04241106, from the Ministry

(6) All new compounds were characterized by NMR, IR, and mass spectral data and by elemental analyses or high-resolution mass spectra. See the supplementary material. The reaction was slow at 80 °C. A decrease in the ratio of 1-hexene/ HSiEt_2Me to 3/1 resulted in a decrease in the yield of **1** to 34%. The reactions run equally well in toluene as a solvent. The use of CH_2Cl_2 or THF in place of benzene gave **1** as a main product along with many unidentified products.

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(11) In this reaction, ethylidenecyclohexane was also obtained in 52% yield (based on vinylcyclohexane) as the result of isomerization of vinylcyclohexane, and 41% of vinylcyclohexane was recovered.

(12) The reaction of **4** with HSiEt_2Me and CO even in the presence of an excess amount of 1-octene under the same reaction conditions also resulted in the recovery of **4** in 85% along with the CO incorporation product derived from 1-octene (**1**, $R' = \text{hex}$, $\text{SiR}_3 = \text{SiEt}_2\text{Me}$, 73% yield).

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Supplementary Material Available: Listings of spectral data and elemental analyses for the products (13 pages). Ordering information is given on any current masthead page.

Nucleotide and Deduced Amino Acid Sequences of the Oxidosqualene Cyclase from *Candida albicans*

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Oxidosqualene lanosterol-cyclase (EC 5.4.99.7) catalyzes a remarkable and crucial cyclization/rearrangement reaction during the course of sterol biosynthesis in fungi and animals (Figure 1).¹ The underlying mechanism of this and related polyene-polycycle conversions has intrigued scientists since the elucidation of the structure of cholesterol.² Decades of synthetic and bioorganic investigations,³ culminating with the most recent work from the Corey laboratory,⁴ have provided a detailed understanding of the substrate structural requirements, specificity, and stereochemistry of cyclization/rearrangement. In stark contrast, little is known about oxidosqualene cyclase enzymes, as they have only recently yielded to complete purification.⁵ We have initiated a program

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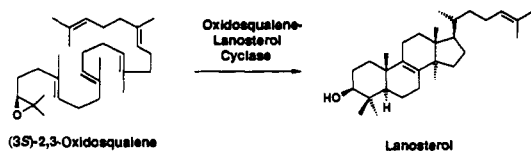


Figure 1.

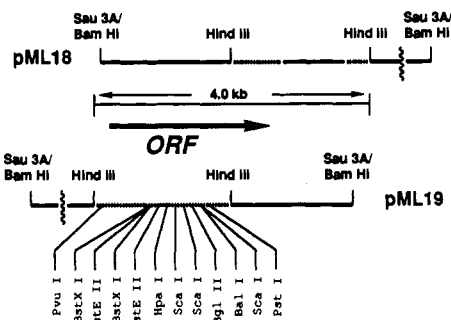


Figure 2. Location of the open reading frame (ORF) encoding the oxidosqualene lanosterol-cyclase within *erg7*-complementing sequences from *C. albicans*. Regions sequenced are indicated by the hashed lines. The sequence-derived restriction map for enzymes *PvuI*, *BstXI*, *BstEII*, *HpaI*, *ScaI*, *BglII*, *BalI*, and *PstI* on the *HindIII* fragment from pML19 differs somewhat from that reported by Kelly et al.⁶

of research directed at the isolation, characterization, and detailed structure/function analysis of oxidosqualene cyclase genes and enzymes. In this communication, we report the first nucleotide and deduced amino acid sequence for an oxidosqualene cyclase enzyme, the oxidosqualene lanosterol-cyclase from the fungus *Candida albicans*.

This work began with the plasmids pML18 and pML19, which were isolated by Kelly et al. and contain overlapping sequences of *C. albicans* genomic DNA that complement cyclase-deficient (*erg7*) mutants of the yeast *Saccharomyces cerevisiae*.⁶ Kelly and co-workers provided genetic and biochemical evidence that these sequences encode an authentic oxidosqualene cyclase enzyme and determined that a 4.0-kb subsequence spanning two adjacent *HindIII* restriction fragments (but not the individual fragments) was sufficient for *erg7* complementation (Figure 2). These *HindIII* fragments, one from pML18 and one from pML19, were subcloned into the vector pHN1+ for sequencing. Both strands of the *HindIII* insert from pML19 were sequenced using the Sanger dideoxy chain extension/termination technology⁷ and a series of overlapping primers. This revealed a potential open reading frame (ORF), which began near the left end of the pML19 *HindIII* fragment and ran toward and through the central, common *HindIII* site. Both strands of the adjoining region of the *HindIII* fragment from pML18 were then sequenced, revealing an in-frame stop codon which terminated the ORF. Sequencing of the opposite end of the *HindIII* fragment from pML18 provided no evidence for the presence of an additional ORF within the 4.0-kb complementing region. The ORF consists of 2187 nucleotides (including the stop codon) and encodes a predicted protein of 728 amino acids with molecular weight 83.7 kDa (Figure 3). A consensus TATA box promoter sequence⁸ and a consensus polyadenylation signal⁹ occur before the initiation codon and after the stop codon, respectively.

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61  AGTGACGCTCTTGGTAGGAAACATGGCCATTATCATCAATCTGAATGTGAAGTGAA
   S D A L G R E T W H Y L S Q S E C E S E
121  CCACAATCAACATTTGCCAATGGCTTTTAGAGTCGCCAGATTTCCCATCCGCCATCG
   P Q S T F V Q W L L E S P D F P S P S
181  TCAGATATTCATCTCAGGCGAGGCAGCAAGAAAGGGAGCTGATTTTTTGAAACTATTG
   S D I H T S G E A A R K G A D F L K L L
241  CAATTGGATAATGGTATCTCCCTGCCAGTACAAAGTCCAATGTTTACAAATGGC
   Q L D N G I F P C Q Y K G P M F M T I G
301  TATGTAACCTGCTAATTATTATAGCAAGACTGAGATACCTGAGCCGATAGGATGGAGATG
   Y V T A N Y Y S K T E I P E P Y R V E M
361  ATACGTTATATGTCAACACTGCACACCCAGTCGATGGTGGTGGGACITCATTCTGTT
   I R Y I V N T A H P V D G G W G L H S V
421  GATAAATCTACTGTTTGGGACAACATGAATTATGATGTCCTCGGTATTAGGAATG
   D K S T C F G T T M N Y V C L R L L G M
481  GAAAAGGATCATCCAGTTTGGTTAAGCGGAGAAAAACATTACATCGTTGGGTGGTGCC
   E K D H P V L V K A R K T L H R L G G A
541  ATTAGAATCCACATTTGGGTAAGGCTGGCTATCTATTTGAAATTTATAGAAATGGGAG
   I K N P H W G K A W L S I L N L Y E W E
601  GGTGTAACCCAGCTCCACCAGAACTTTGGAGATTACCGTACGTTACCAATTTACCA
   G V N P A P P E L W R L P Y W L P I H P
661  CGCAATGGTGGTACATATAGGCTATCTATTTGCCATGGGATATACGCTGCAAAAC
   A K W W V H T R A I I Y L P L G Y T S A N
721  AGAGTTCAATGTGAGCTTGATCCACTTTAAAGAGATCAGAAATGAAATTTACGTTCCA
   R V Q C E L D P L L K E I R N E I Y V P
781  AGTCAATTTACCTATGAGTCAATCAAAATTTGGTAACAGAGAAATGTTTGGTGGTGC
   S Q L P Y E S I K F G N Q R N N V C G V
841  GATTATATACCCACATCAAAAGATCTTGAATTTGCAAAATCTATATTTAGTAAATGG
   D L Y Y P H T K I L D F A N S I L S K W
901  GAAGCTGTAGACCTAAGTGGTTATTGAAATGGGTTAACAGAAAGTTTATGATTTAAT
   E A V R P K W L L N W N K K V Y D L I
961  GTAAGGAGTATCAGAAATCAGAGTACTTGTATTTGCTCCGTGGATTTTCCCTTCAAT
   V K E Y Q N T E Y L L C I A P V S F A F N
1021  ATGGTTGACGCTGTCATTATGAGGCTTCAAGAACTTTAGAAACTCCAAAAC
   M V V T C H Y E G S E S E N F K K L Q N
1081  AGAATGAACGATGTTTGTTCATGGACCTCAGGAAATGACTGTTATGGGACAAATGGT
   R M N D V L F H G P Q G M T V G M G T N G
1141  GTACAAGTTTGGGATGCGCCCTTTATGGTACAGTACTTTTCATGACAGCTTAGTTGAT
   V Q V W D A A F M V Q Y F F M T G L V D
1201  GACCCAAAATATCAGATATGATCAGAAAGAGTTATTTATTTAGTGAGATCTCAATTT
   D P K Y H D M I R K S Y L F L V R S Q F
1261  ACAGAAAATTTGTGTCGATGGAAGTTTACAGATAGACGTAAGGTTGGGCAATTTAGT
   T E N C V D G S F R D R R K G A W P F S
1321  ACTAAGGAACAGGATATCTGTTAGTACTGCTACTCAGAAGCAATGAAGGCTATTATT
   T K E Q G Y T V S D C T A E A M K A I I
1381  ATGGTTAGGAATCAGCAAGTTTGGTGCATCAGGACGCAAAATAGAAATGAAAATTTG
   M V R N H A S F A D I R D E I K D E N L
1441  TTTGATGCTGTTGAAGTTTGTGGAATTTCAAAATGTTGGGAGATGGGAATGGCTCA
   F D A V E V L L Q I Q N V G E W E Y G S
1501  TTCTCGACTTATGAAGTATAAAGCCCTTTGTTGTAGAAAATTTGAATCCTGCCGAG
   F S T Y E G I K A P L L E K L N P A E
1561  GTTTTTAATAATATTGTTGGAGATCCCATACCGTGAATGTACAGACTCTCCGCTTTTA
   V F N N I M V E Y P Y V E C T D S S V L
1621  GGGCTTACTTACTTTGCCAAAATATTACCTTACAGACTACAGCCGAATGATTGCAAAAAC
   G L T Y F A K Y Y P D Y K P E L I Q K T
1681  ATTTCTTCCATCAATATATTCTTGATTCACAAGATAACATTTGATGGCTCTGGTAT
   I S S A I Q Y I L D S Q D N I D G S W Y
1741  GGTGTTGGGAATTTGTTACTTATGCGTCGATGTTGTCATTAGAACCTTTGATACACA
   G C W G I C Y T Y A S M F A L E A L H T
1801  GTTGGCTGATTATGAATCCCTCAGCTGAGAAAAGGATGTTGATTTCTTATTTCA
   V G L D Y E S S S A V K K G C D F L I S
1861  AAACAACCTCCAGCGTGGATGGTCAAGATCAATGAAGGTTGTTGAACACACTTTAT
   K Q L P D G G W S E S M K G C E T H S Y
1921  GTCAATGGTGAACACTCTCTTGTGTTCAATCGGCTTGGGCTTGTAGGTTGATATTG
   V N G E N S L V V Q S A W A L I G L I L
1981  GGAAATTTCTGATGAGGCAATCAAAAGAGGAATTTCAATTTTGTGAAACGACAA
   G N Y P D E E P I K R G I Q F L M K R Q
2041  TTACCGACTGGTGGAGTGAAGTATGAAGTATGAAAGTGTATTAATCAGATTGTCGA
   L P T G E W K Y E D I E G V F N H S C A
2101  ATAGAATATCTTCATATAGGTTTTTTCCAATTAAGCCATTAGGTTTATATAAAAAC
   I E Y P S Y R P L F P I K A L G L Y K N
2161  AAGTATGGTAAAGTGTAGTTTAA
   K Y G D K V L V *

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Figure 3. Nucleotide and deduced amino acid sequences for the *C. albicans* oxidosqualene lanosterol-cyclase.

The predicted molecular weight of the *C. albicans* cyclase is somewhat greater than that of the recently cloned squalene hopene-cyclase from the thermophilic bacterium *Bacillus acidocaldarius* (69.5 kDa)¹⁰ and the relative molecular weight reported for purified rat liver oxidosqualene lanosterol-cyclase (75 kDa).^{5d} The *C. albicans* cyclase appears to be substantially larger than purified oxidosqualene cycloartenol- and oxidosqualene amyrin-cyclase enzymes from plants (28-55 kDa)^{5a-c} and the oxidosqualene lanosterol-cyclase from *S. cerevisiae* (26 kDa).^{5e}

The FASTA programs¹¹ were used to search for similarities between the DNA and predicted amino acid sequences of the *C.*

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albicans ORF and sequences in databases. No significant DNA similarities were found with sequences contained in GenBank 71 or EMBL. Searches of protein sequence databases (PIR 31 and SWISS-PROT 21) reported a limited similarity to human cholesterol ester transferase.¹² Significantly greater similarity was obtained by comparing the predicted amino acid sequence of the *C. albicans* oxidosqualene lanosterol-cyclase with the predicted amino acid sequence of the *B. acidocaldarius* squalene hopene-cyclase.¹⁰ Four regions of notable similarity were observed, ranging from 28% identity over 77 residues to 46% identity over 37 residues. Beyond specific sequence identities, both cyclases have regions of primary sequence in which tryptophan and/or tyrosine residues are concentrated. Perhaps the electron-rich aromatic side chains of some of these residues serve to stabilize cationic transition states and/or high-energy intermediates along the cyclization/rearrangement pathway.^{13,14}

It has been advanced that the *B. acidocaldarius* cyclase associates with membranes by virtue of its richness in arginine residues.¹⁰ The *C. albicans* cyclase is not arginine-rich. A hydrophathy plot indicates that it is a moderately hydrophilic protein with two notable hydrophobic regions (spanning amino acid residues 329-345 and 645-664). These may be involved in anchoring the enzyme to membranes, which would be consistent with the behavior of oxidosqualene cyclase enzymes from plants, mammals, and yeast which reside in the microsomal fractions of cell homogenates and require detergents for their solubilization.⁵

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Total Synthesis of Angular [4]Phenylene and [5]Phenylene

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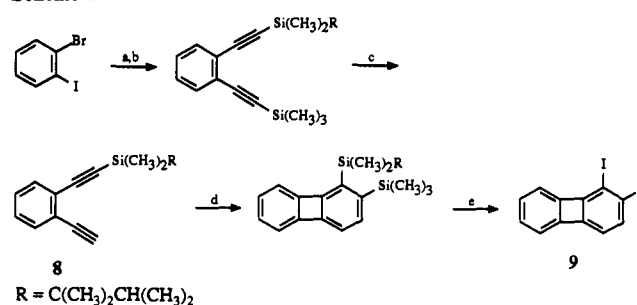
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Biphenylene or [2]phenylene, **1**, is a key polycyclic hydrocarbon with which to probe the effect of fusing a classical antiaromatic nucleus, cyclobutadiene, to the aromatic frame of benzene.¹ Activated molecules of this nature are important fundamentally, because their study sheds light on the limits of chemical bonding to carbon and, in a more practical vein, because they are protagonists in current efforts directed toward the elucidation of the mechanism(s) of carcinogenesis by polycyclic benzenoid hydrocarbons,² the activation of benzene and related petroleum and

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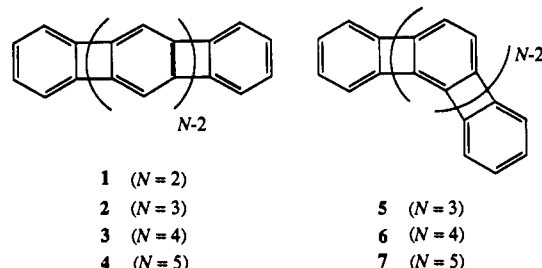
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Scheme 1^a



^a (a) (CH₃)₃SiC≡CH, PdCl₂[P(C₆H₅)₃]₂, CuI, (CH₃CH₂)₃N, 93%; (b) (CH₃)₂CHC(CH₃)₂Si(CH₃)₂C≡CH,⁹ PdCl₂[P(C₆H₅)₃]₂, CuI, azacyclohexane, methylbenzene, Δ, 79%; (c) K₂CO₃, CH₃OH, CH₃C-H₂OH, 100%; (d) (CH₃)₃SiC≡CH, CpCo(CO)₂, 1,3-dimethylbenzene, hν, Δ, 19%; (e) ICl, CH₂Cl₂, 0 °C, 56%.

coal-derived compounds as a source of industrial raw materials,³ and the development of organic electroactive materials, such as potential conductors, ferromagnets, memory storage devices, and so on.⁴ Connected with these topics is the anticipated novel organometallic chemistry of the strained and electronically reactive π-framework. Finally, the direct connections of the component benzene rings might be exploited in the assembly of the shortest "spacer" analogs of the corresponding acenes, a facet that has already been put to use in the synthesis of biphenylene-bridged porphyrins.⁵ The discovery that "CpCo" facilitates the cycloaddition of *o*-diethynylarenes with alkynes to generate this otherwise difficult to assemble structural moiety has led to the construction of a number of biphenylenes, as well as several benzocyclobutadienologs, i.e., the linear [3]-, [4]-, and [5]- and the angular [3]phenylene, 2-5.^{6,7}



In a nutshell, the trends in the physical properties exhibited along the linear series 1-4 appear to strongly indicate complete nonadherence to the Hückel $[4n + 2]$ rule, the electronic spectra reflecting a rapidly diminishing HOMO-LUMO gap, and the ¹H

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