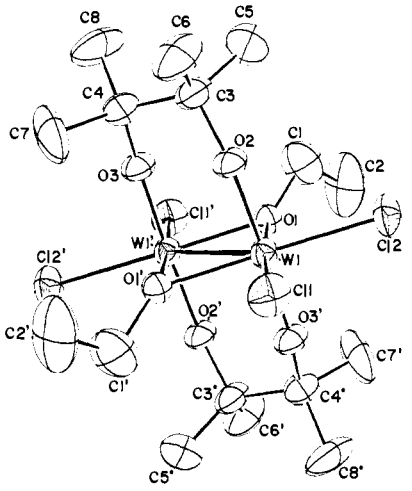


Figure 1. Molecule of $W_2Cl_4(\mu\text{-}OEt)_2[Me_2C(O)C(O)Me_2]$. Atoms are represented by thermal ellipsoids at the 50% probability level. The following mean dimensions were found: $W\text{-}O_B$ = 2.05 [2] Å; $W\text{-}O_T$ = 1.82 [1] Å; W-Cl = 2.364 [7] Å. C-C(ring) = 1.58 (1) Å.

(1) Å. The conformation of the WWOCCO rings is, however, unusual and indicative of strain. The W-O-C angles are 155.9 (6) and 160.4 (6)°, and the torsional angles about the C(3)-C(4) bond are only 33°. From this it would seem likely that **3** would be thermodynamically unstable toward reaction with an alcohol (ROH) to generate pinacol and $W_2Cl_4(\mu\text{-}OEt)_2(OR)_4$.

Additional experiments have shown that a reaction of this type is general for complexes of type **1** and for other ketones.[8] For example, $W_2Cl_4(\mu\text{-}OEt)_2(OEt)_2(EtOH)_2$ reacts with methyl ethyl ketone to produce red crystalline $W_2Cl_4(\mu\text{-}OEt)_2[MeEtC(O)C(O)MeEt]_2$, and the *n*-propoxide complex $W_2Cl_4(\mu\text{-}O\text{-}n\text{-}Pr)_2(O\text{-}n\text{-}Pr)_2(n\text{-}PrOH)_2$ in acetone reacts in an analogous fashion to give $W_2Cl_4(\mu\text{-}O\text{-}n\text{-}Pr)_2[Me_2C(O)C(O)Me_2]_2$.

The stoichiometry and detailed mechanism of these reactions are still obscure. The first step in the reaction between $W_2Cl_4(\mu\text{-}OEt)_2(OEt)_2(EtOH)_2$ and acetone-d_6 in chloroform solutions (^1H NMR spectroscopy) is the displacement, within minutes, of terminal ethanol ligands by acetone, a process greatly enhanced by acid, whose role seems to be protonating and labilizing the terminal ethanol ligands. Identical results were obtained with other acids. Without acid the coupled product $W_2Cl_4(\mu\text{-}OEt)_2[Me_2C(O)C(O)Me_2]_2$, **3**, is formed in lower yield along with an additional compound.[10]

A detailed understanding of the reactions leading to $W_2Cl_4(\mu\text{-}OR'')_2[RR'C(O)C(O)RR']_2$ products will require much further work. At present, however, the following comments can be made. The fact that the yields do not exceed 50% implies that the axial ligands initially present, 2ROH and $2RO^-$, are liberated as such and that per mole of product formed a total of four electrons must be transferred to the ketone molecules. This would require that only half of the starting tungsten atoms go to form a product like **3** while the other half simply supply electrons and end up in some other oxidized form, as is also implied by the formation of substantial quantities of a blue solid.[8] Thus, we suggest the following equation:

$$2W_2Cl_4(\mu\text{-}OR'')_2(OR'')_2(R''OH)_2 + 4RR'CO \rightarrow W_2Cl_4(\mu\text{-}OR'')_2[RR'C(O)C(O)RR']_2 + 4R''O^- + 4R''OH + 2\text{“}W^{V}\text{”}$$

The reaction almost certainly begins with ketone molecules displacing R″OH and $R''O^-$ ligands from a syn pair of axial positions. A two-electron transfer, converting $W^{IV}{=}W^{IV}$ to $W^V\text{-}W^V$ could then occur to give the intermediate $W_2Cl_4(\mu\text{-}OR'')_2(OR'')_2[RR'C(O)C(O)RR']$. This intermediate might then be reduced by other tungsten(IV) atoms and a similar coupling of two RR′CO molecules repeated, but other pathways are possible. Since it has been shown that $W_2Cl_4(OR)_6$ species will not react with pinacol and since the structure of **3** demonstrates that the coordinated $[Me_2C(O)C(O)Me_2]^{2-}$ ligand is in a strained condition, addition of a free $[RR'C(O)C(O)RR']^{2-}$ to a W_2 species is an unlikely event.

Pinacols have been synthesized by the reductive coupling of ketones by using active metals such as sodium and aluminum[11] and by electrochemical[12] and photochemical[13] means. The present results provide the first examples of multiply bonded dinuclear complexes promoting carbon-carbon bond formation by the reductive coupling of ketones. The scope of these reactions is under further investigations.

Acknowledgment. Support of this work by the National Science Foundation through Grant CHE82-06117 to R.A.W. and Grant No. CHE 77-00448 to F.A.C. is gratefully appreciated.

Supplementary Material Available: Tables of positional parameters and isotropic-equivalent temperature factors and listings of $10F_o$, $10F_c$, and $10\sigma(F_o)$ for the crystal structure of $W_2Cl_4(\mu\text{-}OEt)_2(C_6H_{12}O_2)_2$ (10 pages). Ordering information is given on any current masthead page.

(9) Data collected on an Enraf-Nonius CAD-4 diffractometer showed that **3** forms monoclinic crystals in space group $P2_1/n$ with unit cell parameters a = 8.681 (2) Å, b = 15.743 (3) Å, c = 9.235 (2) Å, β = 90.98 (2)°, Z = 2. With 1753 reflections with $F^2 \geq 3\sigma(F^2)$ the structure was refined to R_1 = 0.0375 and R_w = 0.0496. A table of atomic positional parameters is available as supplementary material.

(10) This compound may be $W_2Cl_4(\mu\text{-}OEt)_2(OEt)_2[Me_2C(O)C(O)Me_2]$, probably an intermediate preceding the formation of $W_2Cl_4(\mu\text{-}OEt)_2[Me_2C(O)C(O)Me_2]_2$.

(11) Schreibmann, A. A. P. *Tetrahedron Lett.* **1970**, *49*, 4271.
(12) Baizer, M. M.; Petrovich, J. P. *Prog. Phys. Org. Chem.* **1970**, *7*, 189.
(13) Kan, R. O. "Organic Photochemistry"; McGraw-Hill, New York, 1966.

Squalene Synthetase. Inhibition by an Ammonium Analogue of a Carbocationic Intermediate in the Conversion of Presqualene Pyrophosphate to Squalene

Ronda M. Sandifer, Michael D. Thompson, Roger G. Gaughan, and C. Dale Poulter*

Department of Chemistry, University of Utah Salt Lake City, Utah 84112

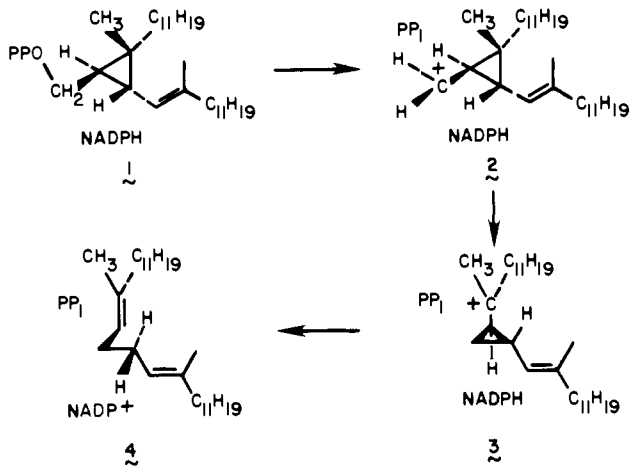
Received June 24, 1982

Squalene synthetase (farnesyl diphosphate:farnesyl diphosphate farnesyltransferase, E.C. (2.5.1.21)), the first pathway-specific enzyme in sterol metabolism, catalyzes the 1′-1 condensation[1] between two molecules of farnesyl PP[2] to yield squalene.[3,4] This reaction is the composite of two distinct transformations—the insertion of C(1) of one molecule of farnesyl PP into the C-(2)-C(3) double bond of the second molecule to generate presqualene PP (**1**), followed by conversion of cyclopropylcarbinyl PP **1** into squalene (**4**). Recent experiments suggest that both steps are catalyzed by a single protein (M_r 55 000) that possesses a catalytic site for each reaction.[5,6] It was recognized by several groups that the reductive rearrangement of **1** to **4** can be rationalized by the bond reorganizations typically observed for cyclopropylcarbinyl cations,[7-11] and we proposed the three-step

(1) See ref 12 for a description of non-head-to-tail attachments of isoprene residues.
(2) Abbreviations used are as follows: farnesyl pyrophosphate, farnesyl PP; presqualene pyrophosphate, presqualene PP; reduced nicotinamide adenine dinucleotide phosphate, NADPH; bicyclo[2.2.1]hept-5-ene-2,3-dicarboxylic acid, BHDA; inorganic pyrophosphate, PP_i; inorganic phosphate, P_i; 2-amino-2-(hydroxymethyl)-1,3-propanediol, Tris; dithiothreotol, DTT.
(3) Poulter, C. D.; Rilling, H. C. "Biosynthesis of Isoprenoid Compounds"; Porter, J. W., Spurgeon, S. L., Eds.; Wiley: New York, 1981; Vol. 1, pp 413-441.
(4) Popjak, G.; Agnew, W. S. *Mol. Cell. Biol.* **1979**, *27*, 97-116.
(5) Agnew, W. S.; Popjak, G. *J. Biol. Chem.* **1978**, *253*, 4566-4573.
(6) Agnew, W. S.; Popjak, G. *J. Biol. Chem.* **1978**, *253*, 4574-4583.
(7) van Tamelen, E. E.; Schwartz, M. A. *J.Am. Chem. Soc.* **1971**, *93*, 1780-1782.
(8) Altman, L. J.; Kowerski, R. C.; Rilling, H. C. *J. Am. Chem. Soc.* **1971**, *93*, 1782-1783.
(9) Rilling, H. C.; Poulter, C. D.; Epstein, W. W.; Larsen, B. R. *J. Am. Chem. Soc.* **1971**, *93*, 1783-1785.

0002-7863/82/1504-7376$01.25/0

Scheme I



sequence shown in Scheme I.[9] Subsequent model studies demonstrated that the rearrangement of **2** to **3** is not kinetically or thermodynamically preferred in solution and that squalene synthetase must exert strict control on the cationic intermediates to achieve the regiospecificity required for biosynthesis of squalene.[10,12-14] We suggested that regiocontrol is in fact the expected consequence when the tight ion pair between **2** and PP_i is generated in an environment that preserves the relative locations of the positive and negative partners shown in Scheme I during the rearrangement of **2** to **3**.[15,16] In this communication we present evidence that squalene synthetase binds an ammonium analogue that mimics the topological and electrostatic properties of the hypothetical tertiary cyclopropylcarbinyl intermediate (**3**) and that the binding is dependent on the presence of PP_i.

The synthesis of ammonium analogue **11** is outlined in Scheme II. Treatment of (±)-ethyl *trans*-2-formyl-1-cyclopropanecarboxylate (Aldrich Chemical Co.) with ((*E*)-6,10-dimethyl-5,9-undecadien-2-ylidene)triphenylphosphorane (**8**) yielded a 1:1 mixture of (*E*)- and (*Z*)-**9**.[17] The desired *E* isomer was separated by column chromatography and converted to the corresponding carbamate by a Curtius rearrangement. Liberation of primary amine **10** followed by successive alkylations with (*E*)-4,8-dimethyl-3,7-nonadienyl iodide (**13**) and methyl iodide gave the desired tertiary amine. Ammonium analogue **11** was prepared by mixing the free amine in an aqueous buffer (pH 6.3) containing Tween-80 (0.6%) by using a vortex mixer or a Vibromill.

Inhibition of squalene synthetase was studied by using a microsomal preparation from bakers' yeast.[5,18] The assay consisted of addition of 0.8–2.5 μg of protein to buffer, pH 7.40, containing 11 mM potassium fluoride, 5.5 mM magnesium chloride, 1 mM NADPH, 50 μM DTT, 0.18% (v/v) Tween-80, and 0.5 μM [1,5,9-$^3H_3$]farnesyl PP (specific activity 100–150 μCi/μmol, New England Nuclear) at 30 °C. The final volume (500 μL) also contained the ammonium analogue and PP_i in the concentrations indicated. The reaction was quenched by addition of 200 μL of 1:1 40% (w/v) aqueous potassium hydroxide and 95% ethanol. Squalene (2 μL) and solid sodium chloride were added, and the

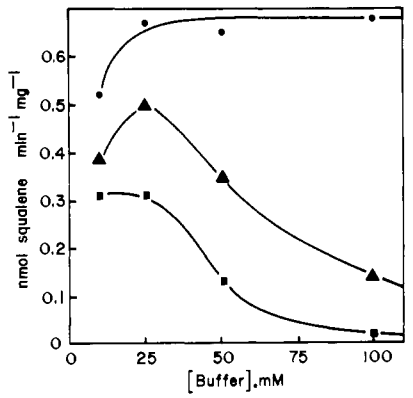


Figure 1. Initial velocities for synthesis of squalene from farnesyl PP (0.5 μM) in the indicated buffers: potassium phosphate, (■), Tris·HCl (▲), potassium BHDA (●).

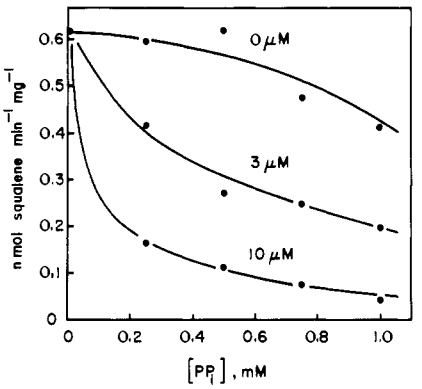


Figure 2. Initial velocities for synthesis of squalene from farnesyl PP (0.5 μM) in 50 mM potassium BHDA buffer containing 0, 3, and 10 μM **11**.

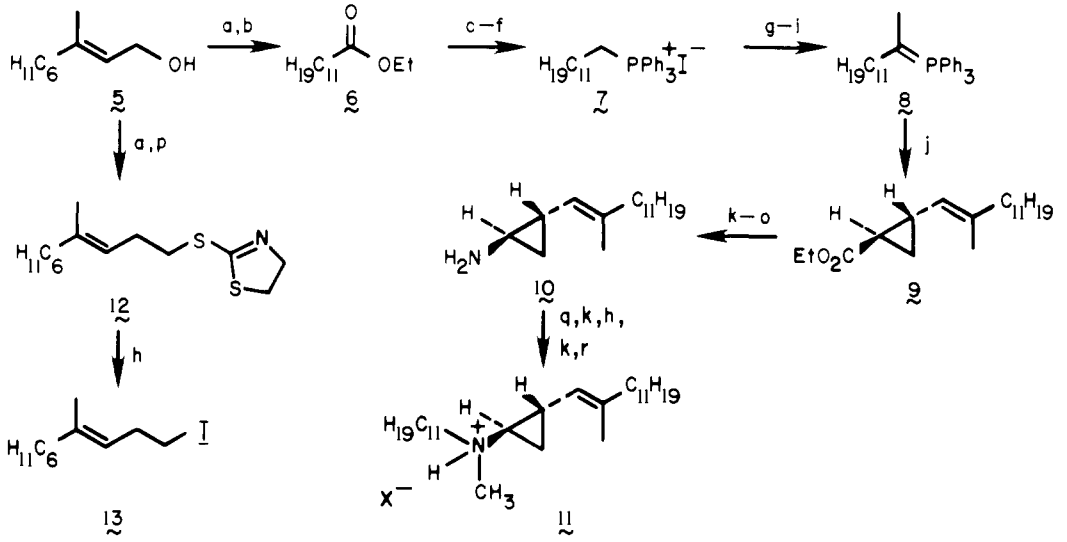
resulting mixture was extracted twice with 1 mL of ligroin (bp 60–90 °C). An 800-μL portion of each extract (80% of total ligroin-soluble radioactivity) was added to a 2-mL column of neutral alumina (activity II), followed by elution with 9 mL of toluene. Omnifluor (1 mL of a 4% (w/v) solution in toluene, New England Nuclear) was added to the eluents, and radioactivity was determined by liquid scintillation spectrometry.[19]

In preliminary experiments using 50 mM phosphate buffer,[5,6,9,11] conversion of farnesyl PP (5 μM) to squalene[20] was inhibited 30% by 10 μM **11**. Addition of PP_i (0.2 μM) further decreased the rate of squalene synthesis to 50%, and at 6 mM PP_i, production of squalene dropped to background levels. Concern that P_i might also inhibit squalene synthetase or mask the effects of PP_i led us to investigate other buffers. As shown in Figure 1, both phosphate and Tris resulted in significant inhibition at concentrations normally used for the assays. In addition, the combination of 100 mM Tris and 1 mM PP_i reduced the production of squalene to background levels. After considerable experimentation we discovered that *endo*-bicyclo[2.2.1]hept-5-ene-2,3-dicarboxylic acid (BHDA)[21] did not inhibit the enzyme at concentrations up to 100 mM.

Reinvestigation of the conversion of farnesyl PP (0.5 μM) to squalene by the microsomal preparation in BHDA buffer showed *no inhibition* by **11** at concentrations as high as 170 μM. Under similar conditions 1.0 mM PP_i produced a modest decrease in the rate of squalene production (see Figure 2), presumably by product

(10) Coates, R. M.; Robinson, W. H. *J. Am. Chem. Soc.* **1972**, *94*, 5920–5921.
(11) Qureshi, A. A.; Barnes, F. J.; Semmler, E. J.; Porter, J. W. *J. Biol. Chem.* **1973**, *248*, 2755–2767.
(12) Poulter, C. D.; Marsh, L. L.; Hughes, J. M.; Argyle, J. C.; Satterwhite, D. M.; Goodfellow, R. J.; Moesinger, S. G. *J. Am. Chem. Soc.* **1977**, *99*, 3816–3823.
(13) Poulter, C. D.; Hughes, J. M. *J. Am. Chem. Soc.* **1977**, *99*, 3824–3829.
(14) Poulter, C. D.; Hughes, J. M. *J. Am. Chem. Soc.* **1977**, *99*, 3830–3837.
(15) Poulter, C. D.; Muscio, O. J.; Goodfellow, R. J. *Biochemistry* **1974**, *13*, 1530–1538.
(16) Poulter, C. D. *J. Agric. Food Chem.* **1974**, *22*, 167–173.
(17) Satisfactory spectral and analytical data were obtained for all compounds.
(18) Epstein, W. W.; Rilling, H. C. *J. Biol. Chem.* **1970**, *245*, 4597–4605.
(19) Stereospecific removal of the *pro-S* proton at C(1) of one of the farnesyl PP molecules during synthesis of presqualene PP will result in retention of 92% of the radioactivity in presqualene PP and squalene prepared from racemic [1,5,9-$^3H_3$]farnesyl PP.
(20) All rates reported as nanomoles of squalene produced per milligram of protein per minute are initial velocities (10–15% of V_{max}) for runs that were linear with respect to enzyme concentration and time.
(21) BHDA has been used as a nontoxic anionic buffer for fungal and bacterial cultures. Child, J. J.; Knapp, C.; Eveleigh, D. E. *Mycologia* **1973**, *65*, 1078–1086. Mallette, M. F. *J. Bacteriol.* **1967**, *94*, 283–290.

Scheme II



[a] PBr_3. [b] $(EtOOCCH_2)_2CuLi$, -78 °C. [c] $LiAlH_4$. [d] MsCl, pyridine. [e] NaI. [f] PPh_3. [g] BuLi. [h] CH_3I. [i] BuLi. [j] ethyl *trans*-2-formyl-1-cyclopropanecarboxylate. [k] NaOH. [l] $C_2O_2Cl_2$. [m] NaN_3. [n] MeOH, Δ. [o] Me_3SiI. [p] Lithium (2-methylthio)-thiazolide. [q] 1-Iodo-4,8-dimethylnona-3,7-diene. [r] Buffer.

inhibition. The *combination* of ammonium analogue **11** and PP_i, however, resulted in a synergistic inhibition of the enzyme. At 0.25 mM PP_i, a concentration that alone produced a negligible effect, ammonium analogue **11** inhibited the rate of squalene synthesis by 33% at 3 μM and by 73% at 10 μM concentrations.

It is evident that this analogue, which mimics the topological and electrostatic properties of the reactive intermediate **3**, inhibits squalene synthetase.[22] When **3** was first proposed as an intermediate in the reaction,[9] compounds with the 1′-1-2 isoprene linkage[12] were unknown in nature. Recently, however, Epstein and Gaudioso[23] reported the discovery of rothrockene, a monoterpene readily derived from the C_{10} equivalent of **3**, in *Artemisia tridentata rothrockii*. This variety of sage and closely related species are known to produce compounds with irregular isoprenoid carbon skeletons,[24] including rothrockene and chrysanthemol, a C_{10} analogue of presqualene alcohol.[25] Related carbocationic rearrangements were proposed for the biosynthesis of these nonhead-to-tail monoterpenes.[24]

Duplication of the topological and electrostatic features of **3** is, in itself, insufficient for inhibition of squalene synthetase.[26] The synergism found between **11** and PP_i or, to a lesser extent, P_i suggests that the enzyme binds the carbocation:PP_i ion pair much more tightly than either partner. The strong influence of PP_i on the binding of the ammonium analogue is particularly striking. The pyrophosphate moiety is known to be a major contributor to enzyme-substrate interactions for squalene synthetase[3,4] and other enzymes in the terpene pathway.[27] In the case of farnesyl PP synthetase, Barnard and Popjak[28] found evidence for arginyl residues in the active site that presumably facilitate binding of the negatively charged portions of the substrates. If similar features are present in squalene synthetase, it is not surprising that the enzyme fails to bind the ammonium analogue unless the positively charged region of the active site is shielded by the appropriate anion.

Acknowledgment. We thank the National Institutes of Health for support of this project by postdoctoral fellowship GM 08098 to R.M.S. and Grant GM 21328.

Registry No. 5, 106-24-1; **6**, 19894-83-8; **7**, 32205-44-0; **8**, 83732-41-6; (*E*)-**9**, 83732-42-7; **10**, 83732-43-8; **11**, 83732-44-9; **12**, 83732-45-0; **13**, 22339-13-5; ethyl *trans*-2-formyl-1-cyclopropanecarboxylate, 13949-93-4; lithium (2-methylthio)thiazalide, 57662-52-9; squalene synthetase, 9077-14-9; pyrophosphate, 14000-31-8.

(22) The series of amines examined previously produced only a moderate and nonspecific inhibition of squalene synthetase and other microsomal enzymes: Bertollino, A.; Altman, L. J.; Vasak, J.; Rilling, H. C. *Biochim. Biophys. Acta* **1978**, *530*, 17-23.
(23) Epstein, W. W.; Gaudioso, L. A. *J. Org. Chem.* **1982**, *47*, 175-176.
(24) Epstein, W. W.; Poulter, C. D. *Phytochemistry* **1973**, *12*, 737-747.
(25) Alexander, K.; Epstein, W. W. *J. Org. Chem.* **1975**, *40*, 2576.
(26) Recently Narula and co-workers reported inhibition of (*S*)-adenosyl-L-methionine:sterol C(24)-methyltransferase by an ammonium analogue for the proported C(25) carbocatonic intermediate during side-chain methylation. Their example differs from ours in that no strict anion requirement for inhibition was reported. Narula, A. S.; Rahire, A.; Benveniste, P.; Schuber, F. *J. Am. Chem. Soc.* **1981**, *103*, 2408-2409.
(27) Poulter, C. D.; Rilling, H. C. "Biosynthesis of Isoprenoid Compounds"; Porter, J. W., Spurgeon, S. L., Eds.; Wiley: New York, 1981; Vol. 1, pp 161-224.
(28) Barnard, G. F.; Popjak, G. *Biochim. Biophys. Acta* **1980**, *617*, 169-182.

A Long Si-H Bond or a Short Si-H Nonbond? Neutron Diffraction Study of $(\eta^5\text{-}CH_3C_5H_4)(CO)_2(H)MnSiF(C_6H_5)_2$

Ulrich Schubert,* Klaus Ackermann, and Barbara Wörle

Anorganisch-Chemisches Institut der Technischen Universität München D-8046 Garching, West Germany

Received November 9, 1981
Revised Manuscript Received July 7, 1982

Metal complexes containing both a hydride and a silyl ligand play an important role in hydrosilylation and related reactions. These complexes are formed by oxidative addition of silanes to metal complexes and can be isolated if both the metal complex moiety and the substituents at silicon provide sufficient stabilization. In most cases the reaction is reversible because of facile dissociation of the silane from the complex. A number of hydrido silyl complexes have been investigated by X-ray structure analyses. In some, close contact between the hydride ligand and the silicon atom has been postulated from the evidence of bond lengths and angles involving the silicon and metal atoms,[1-3] or in a few cases,

* To whom correspondence should be addressed at the Institut für Anorganische Chemie der Universität, Am Hubland, D-8700 Würzburg, West Germany.

(1) Auburn, M.; Ciriano, M.; Howard, J. A. K.; Pugh, N. J.; Spencer, J. L.; Stone, F. G. A.; Woodward, P. *J. Chem. Soc., Dalton Trans.* **1980**, 659-666.
(2) Smith, R. A.; Bennett, M. J. *Acta Crystallogr., Sect. B* **1977**, *B33*, 1113-1117.

0002-7863/82/1504-7378$01.25/0