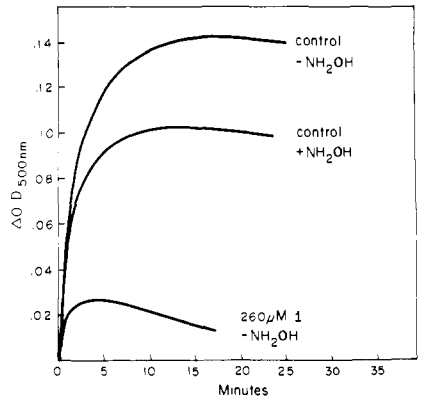

Figure 2. Inactivation of membrane-bound bovine opsin by **1**. Bovine rod outer segments were obtained from frozen retinas (George Hormel Co.) by the published procedure.[8] Control without NH_2OH: To a 500-μL sample containing 100 mM Tris·HCl, pH 7.2, 100 mM $MgCl_2$, 10% sucrose, 4% isopropanol, and bleached rod outer segments incubated in the light for 20 min at room temperature was added 20 μL of egg phosphatidyl choline based liposomes (2 μmol/mL containing 10% 11-*cis*-retinal). Control with NH_2OH: To a 500-mL sample as above containing 40 mM NH_2OH and incubated under the same conditions as above was added 20 μL of the 11-*cis*-retinal bearing liposomes. Inhibitor **1** without NH_2OH: To a 480-μL sample without NH_2OH was added 20 μL of retinoyl fluoride (264 μM) dissolved in isopropanol. The mixture was incubated in the light for 20 min, and then 20 μL of liposomes containing 11-*cis*-retinal was added.

the enzyme utilizes *all-trans*-retinal as a substrate.[5] These experiments taken together are consistent with a specific, active-site mode of inactivation. Inactivation studies with radiolabeled **1** will ascertain the stoichiometry and position of labeling.

Many of the studies of interest using **1** will of necessity take place in the intact rod outer segments. It was therefore of interest to determine whether **1** would inactivate membrane bound opsin. To this end, freshly isolated bovine rod outer segments[6] were bleached and treated with **1**. The results given in Figure 2 show that essentially complete inactivation is achieved in the intact system. Bleached rod outer segments treated with 11-*cis*-retinal show a rapid increase in absorbance as monitored at 500 nm (-NH_2OH). Part of this absorbance increase is caused by nonspecific Schiff's base formation between 11-*cis*-retinal and phosphatidyl ethanolamine.[6] This nonspecific absorbance increase is eliminated by including hydroxylamine in the buffer (+-NH_2OH).[6] Note that treatment of the bleached rod outer segments with **1** in the absence of hydroxylamine leads to about the same absorbance increase as the difference between the two controls. Pretreatment of the rod outer segments with **1** followed by 11-*cis*-retinal and hydroxylamine does not afford a statistically significant absorbance increase at 500 nm. In addition to demonstrating that **1** inactivates opsin in its natural membraneous environment, the data in Figure 2 also suggest that random acylation of amino groups does not occur to a significant extent.

The inactivation of opsin by **1** and isomers of **1** opens up many intriguing questions concerned with the molecular mechanisms of visual transduction. For example, it will be of interest to determine whether the opsins so modified are still photochemically active, whether they will be active in the visual transduction process, and whether this activity is dependent on the stereochemistry of the labeling agent such as 11-*cis*-retinoyl fluoride and 9-*cis*-retinoyl fluoride. On a structural level the active-site sequence of opsin and related molecules can be determined unambiguously.

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Ditungsten(IV) Alkoxides as Reagents for Carbon-Carbon Bond Formation via the Reductive Coupling of Ketones

F. Albert Cotton,*[1a] David DeMarco,[1b] Larry R. Falvello,[1a] and Richard A. Walton*[1b]

Department of Chemistry, Texas A&M University
College Station, Texas 77843
Department of Chemistry, Purdue University
West Lafayette, Indiana 47907

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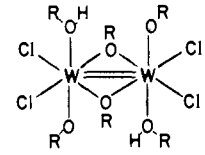
The ditungsten(IV) alkoxides $W_2Cl_4(\mu\text{-}OR)_2(OR)_2(ROH)_2$ (**1**)



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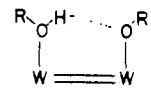

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possess a tungsten–tungsten double bond characterized by the $\sigma^2\pi^2$ ground-state electronic configuration[2-4] and a strong hydrogen bond (**2**) between syn-axial alcohol and alkoxide ligands.[3,4] These molecules undergo facile oxidation to their singly bonded ditungsten(V) congeners, $W_2Cl_4(\mu\text{-}OR)_2(OR)_4$.[3-5] In contrast to most alkoxide complexes of lower-valent molybdenum and tungsten, **1** is stable in air for prolonged periods. In the course of studying the lability of the alcohol ligands of **1** to various nucleophiles we have found that complexes of type **1** bring about the reductive coupling of ketones and lead to metal-containing products in which the resulting organic molecules bridge the ditungsten center.

Solutions of $W_2Cl_4(\mu\text{-}OEt)_2(OEt)_2(EtOH)_2$ in acetone in the presence of a small quantity of acetic acid turned from green to red over a period of 6 h, and a red crystalline product, **3**, separated.[8] Cyclic voltammograms and electronic absorption spectra of dilute CH_2Cl_2 solutions of **3** were characteristic of ditungsten(V) complexes of the type $W_2Cl_4(\mu\text{-}OR)_2(OR)_4$.[3-5,6,7]

An X-ray crystallographic study[9] showed that **3** has the structure shown in Figure 1. The molecule has a center of inversion, and the central $W_2Cl_4(\mu\text{-}OEt)_2O_4$ portion closely resembles $W_2Cl_4(\mu\text{-}OEt)_2(OEt)_4$,[5] with a W–W distance of 2.701

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(7) In a 0.2 M tetra-*n*-butylammonium hexafluorophosphate CH_2Cl_2 solution **3** exhibits two one-electron reductions, one possessing an $E_{1/2}$ value of −0.75 V vs. SCE and the other characterized by $E_{p,c} = -1.30$ V and $E_{p,a} = -1.10$ V with $i_{p,c} \gg i_{p,a}$. In CH_2Cl_2 electronic absorption band maxima λ_{max} are at 503 (sh) and 446 nm.
(8) In a typical reaction for the preparation of these complexes, $W_2Cl_4(\mu\text{-}OEt)_2(OEt)_2(EtOH)_2$ (0.1 g, 0.128 mmol) was treated with acetone (1.0 mL) and acetic acid (0.02 mL, 0.347 mmol) and the resulting mixture set aside under a N_2(g) atmosphere for 6 h. The resulting red crystalline product was filtered off, washed with acetone and vacuum dried, yield ca. 30%. Anal. Calcd for $C_{16}H_{34}Cl_4O_6W_2$: C, 23.08; H, 4.08. Found: C, 22.76; H, 4.04. When the reaction filtrate was set aside, a blue amorphous solid precipitated after several additional hours. The latter material was insoluble in all solvents, and its IR spectrum was indicative of a tungsten blue species.
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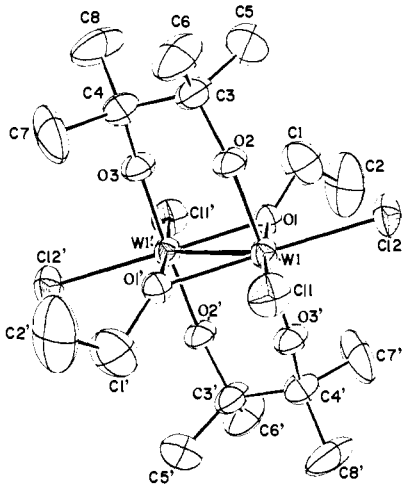

Figure 1. Molecule of $W_2Cl_4(\mu\text{-}OEt)_2[Me_2C(O)C(O)Me_2]$. Atoms are represented by thermal ellipsoids at the 50% probability level. The following mean dimensions were found: $W\text{-}O_B$ = 2.05 [2] Å; $W\text{-}O_T$ = 1.82 [1] Å; W-Cl = 2.364 [7] Å. C-C(ring) = 1.58 (1) Å.

(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.

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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 $W_2Cl_4(\mu\text{-}OEt)_2[Me_2C(O)C(O)Me_2]_2$.

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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

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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.
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