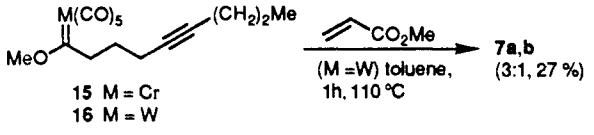
lybdenum carbene complexes are unstable and difficult to handle.[2a,d] We have found that molybdenum carbene complexes **2**, **4**, and **6** are relatively stable. No significant decomposition of these complexes was observed after storage at −10 °C for 1 week.

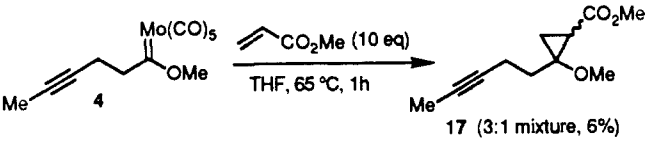
Mild thermolysis (65 °C, THF, 1 h) of complex **2** in the presence of methyl acrylate (10 equiv) led directly to a mixture of vinylcyclopropanes **7a** and **7b** in 71% yield. The presumed pathway for this transformation is outlined in Scheme I. Initial dissociation of CO leads to coordinatively unsaturated complex **9**. Intramolecular cyclization of **9** to **10** and subsequent ring opening generates vinylcarbene complex **11**.[9] Cyclopropanation of methyl acrylate by complex **11** gives vinylcyclopropanes **7a** and **7b**. The stereoselectivity observed in this process is similar to that observed in previous cyclopropanation studies.[10]

Several other electron-poor olefins have been found to readily participate in this transformation. Thermolysis of complex **2** with acrylonitrile, dimethyl vinylphosphonate, and methyl methacrylate led to **12**, **13**, and **14** as mixtures of diastereomers (Figure 2). The major diastereomer in each of these transformations is that in which the cyclopentene ring is anti to the electron-withdrawing group.[10] Cyclization with methyl methacrylate led to the desired cyclopropanes **14a** and **14b** in low isolated yield. Vinylcyclopropanes **14a** and **14b** appear to be less stable than the other vinylcyclopropanes described herein because of the presence of two quaternary centers on the cyclopropane ring.

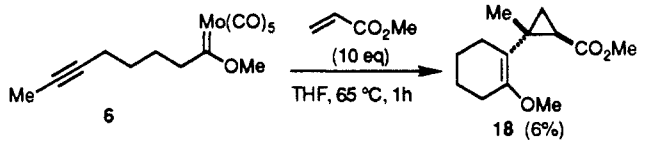
The reactivity of molybdenum carbene complex **2** was compared to that of the analogous chromium- and tungsten-based systems. Carbenes **15** and **16** were prepared by pathways analogous to those presented in Figure 1.[11] Thermolysis of chromium carbene complex **15** in the presence of methyl acrylate (65 °C, 1 h, benzene) led to a complex mixture of products, none of which corresponded to the desired vinylcyclopropane system.[12] Thermolytic chemistry of chromium alkynylcarbene complexes related to **15** has been described.[13] The dominant pathway with these systems appears to be intramolecular cyclization of the carbene complex with the alkyne accompanied by incorporation of carbon monoxide to give a vinylketene complex. In most cases the vinylketene complex undergoes subsequent transformations. In the cyclization of molybdenum carbene complexes **2**, **4**, and **6**, no products resulting from carbon monoxide incorporation were detected. Thermolysis of the more stable tungsten carbene complex **16** in the presence of methyl acrylate (110 °C, 1 h, toluene) led to the desired vinylcyclopropanes **7a** and **7b**. However, the isolated yields of **7a** and **7b** were considerably lower than in the reactions with the analogous molybdenum system.



Cyclization reactions with complexes **4** and **6** were investigated in order to explore the scope of this process. Thermolysis of molybdenum carbene complex **4**, which has a shorter, two-methylene tether between the carbene and the alkyne, did not lead to the desired 2-(1-methyl-2-carbomethoxycyclopropyl)-1-methoxycyclobutene or any identifiable products derived therefrom. Alkynylcyclopropane **17**, resulting from direct cyclopropanation of the carbene complex without initial addition to the alkyne, was the only identifiable product, in 6% isolated yield.



Complex **6**, with the longer, four-methylene tether, when treated with methyl acrylate in THF at 65 °C for 1 h, led to the desired cyclohexenylcyclopropane **18** in 6% yield. This was the only identifiable product that could be isolated from this reaction. Cyclopropanation to give 1-methoxy-1-(5-heptynyl)-2-carbomethoxycyclopropane was not observed. From these studies it appears that the success of the intramolecular cyclization to form the vinylcarbene complex is very dependent on the length of the tether.



In conclusion, we have demonstrated that in situ generated vinylcarbene complexes of molybdenum will react with electron-poor olefins to give vinylcyclopropanes in good yield. Further studies in this area are currently in progress.

Acknowledgment. Support from the Cancer Research Coordinating Committee of the University of California, the donors of the Petroleum Research Fund administered by the American Chemical Society, the American Cancer Society (Junior Faculty Research Award to D.F.H.), and the National Institutes of Health (GM41984-01) is gratefully acknowledged.

Supplementary Material Available: Experimental procedures and spectral data for **2**, **4**, **6**, **7ab**, **12ab**, **13ab**, **14ab**, **15**, **16**, **17ab**, and **18** (9 pages). Ordering information is given on any current masthead page.

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Protein Microencapsulation of Nonaqueous Liquids

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Vesicles have found diverse and important applications, ranging from microencapsulation of dyes, flavors, and fragrances[1] to drug delivery systems,[2] to the study of membrane structure, function, and reactivity.[3] Many such vesicles are made at least in part from proteins,[4,5] but there has been little understanding of the mech-

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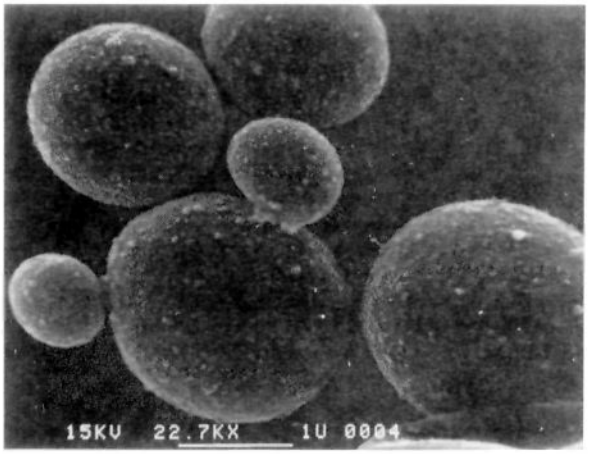

Figure 1. Scanning electron micrograph of a dodecane-filled proteinaceous microcapsule. The microcapsules were prepared for SEM by cross-linking with glutaraldehyde and coating with Au/Pd. Volatile nonaqueous liquids produced deformed microcapsules due to evaporation during sample preparation.

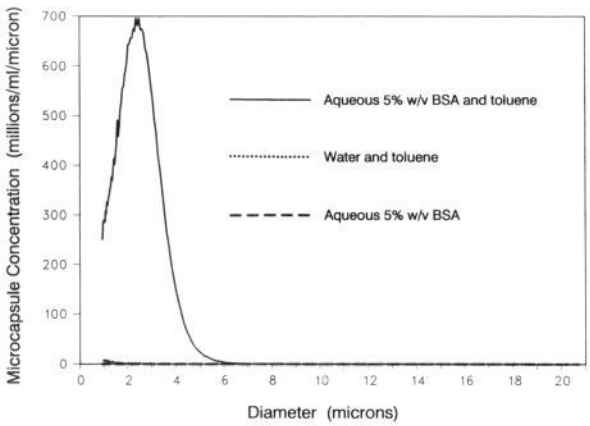

Figure 2. Particle distribution of an aqueous suspension of proteinaceous microcapsules, determined with an Elzone particle counter (Model 180XY). Solutions were irradiated with ultrasound (Heat Systems W375, 20 kHz, 0.5-in. Ti horn) for 3 min at an acoustic power output of $\approx$200 W/cm^2, with an intial cell temperature of 23 °C at neutral pH. The proteinaceous microcapsules can be separated by microfiltration (Anotop filter no. 2134).

anism of their formation. We have developed a method using high-intensity ultrasound to make aqueous suspensions of proteinaceous microcapsules filled with *water-insoluble* liquids and have demonstrated the chemical mechanism of their formation. Scanning electron microscopy, optical microscopy, and particle-counting characterization reveal spherical microcapsules with a narrow size distribution. We find that microcapsule formation is strongly inhibited by free-radical traps, by superoxide dismutase (but not by catalase), by the absence of O_2, and by the lack of free cysteine residues in the protein. We propose that the microcapsules are held together by disulfide bonds between protein cysteine residues and that superoxide, sonochemically produced by acoustic cavitation,[6] is the cross-linking agent.

Proteinaceous microcapsules of bovine serum albumin (BSA) filled with *n*-dodecane, *n*-decane, *n*-hexane, cyclohexane, or toluene have been synthesized with a high-intensity ultrasonic probe.[7] Figure 1, a scanning electron micrograph (SEM), shows their structure, and Figure 2 their size distribution. For preparations carried out under air or O_2, we find concentrations of $\approx 1.5 \times 10^9$ microcapsules/mL with an average diameter of 2.5 μm (Gaussian distribution, $\sigma = \pm 1.0$ μm). This is a rather narrow distribution compared to previously reported microcapsule preparations.

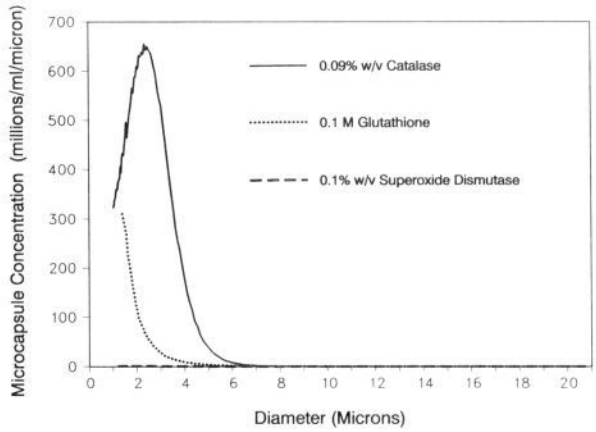

Figure 3. The effect of radical traps on microcapsule formation. Aqueous solutions (5% w/v) of BSA and toluene were irradiated in the presence of catalase, glutathione, or superoxide dismutase. Inhibition of microcapsule formation also occurred with 2,6-di-*tert*-butyl-4-methylphenol.

Fraction V powders of BSA[10] were purchased from Sigma Chemicals. Both BSA and toluene are essential for the formation of microcapsules (Figure 2). The toluene-filled microcapsules show <10% degradation after 1000 h at 2 °C. The size distribution was similar for all nonaqueous liquids examined. Ultrasonic irradiation of human serum albumin (HSA) generates similar microcapsules, as expected, given the high sequence homology.[11,12] Proteinaceous microcapsules can also be formed with hemoglobin (Hb), which has very different physical and chemical properties.[13] Air-filled human serum albumin proteinaceous microspheres have previously been synthesized by sonication and are used as contrast agents in echosonography.[8,9]

To investigate the microcapsule's interior, the water-insoluble 5,10,15,20-tetraphenylporphyrin (H_2TPP) was used as a probe. H_2TPP is soluble in a wide range of medium-polarity liquids, but is completely insoluble in water or aqueous protein solutions. H_2TPP has an intense blue absorption that is blue-shifted in more polar solvents (e.g., 418.0 nm in toluene vs 416.8 nm in dimethylformamide). This absorption band is unaffected by ultrasonic irradiation in the absence of microcapsule formation. The optical spectrum of microcapsules in water, made with H_2TPP dissolved in toluene, shows H_2TPP within the microcapsule. Gas chromatographic analysis confirms the presence of toluene. This band is slightly *red*-shifted (420 nm) and broader than in neat toluene (36 nm vs 12 nm fwhm). Thus, although these protein microcapsules are suspended in water, nonaqueous liquid is present inside.

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How are the microcapsules formed and what holds them together? Clearly, emulsification must occur during the microscopic dispersion of the nonaqueous phase into the aqueous protein solution. Ultrasonic emulsification is a well-known process[14] and does occur in this biphasic system. Emulsification is necessary for microcapsule formation. However, if vortex mixing emulsification is used instead, microcapsules are not formed. Consequently, *emulsification by itself is not sufficient for microcapsule formation.* Denaturation of the protein by thermal or hydrophobic processes might be invoked to hold the microcapsules together after initial emulsification. High concentrations of microcapsules are observed when the mixture is sparged with air or O_2. If the reaction is run under an inert atmosphere (He, Ar, or N_2), however, microcapsules are not formed. Thus, thermal or solvent denaturation (for which O_2, N_2, and Ar should give similar results) cannot explain the microcapsule permanence.

Another, *chemical* process must be involved. There is a wide range of high-energy chemistry associated with ultrasonic irradiation of liquids, arising from acoustic cavitation (the implosive collapse of bubbles).[6] Aqueous sonochemistry produces[15] $OH^{\bullet}$ and $H^{\bullet}$. The radicals so produced by ultrasound[16] form H_2, H_2O_2, and in the presence of O_2, superoxide[17] (HO_2). Hydroxyl, superoxide, and peroxide are all potential protein cross-linking agents.

To identify the specific oxidant involved, the formation of microcapsules was examined in the presence of radical traps. The addition of nonspecific traps, e.g., 2,6-di-*tert*-butyl-4-methylphenol or glutathione, dramatically reduced the number of microcapsules (Figure 3). The effects of catalase (which decomposes hydrogen peroxide to oxygen and water) or of superoxide dismutase (which decomposes superoxide to oxygen and hydrogen peroxide) were tested. Microcapsule formation was inhibited by superoxide dismutase, but *not* by catalase. Therefore, the important oxidant involved in microcapsule formation is superoxide.

Several experiments were performed to identify the specific effect of superoxide. Cysteine is oxidized by superoxide[18] and is present in BSA, HSA, and Hb. In fact, ultrasonic irradiation of proteins has been reported to oxidize cysteine residues.[19] If the microcapsules are held together by protein cross-linking through disulfide linkages from cysteine oxidation, a comparison of Hb and myoglobin (Mb) provides an interesting test. They have similar sequences, except that Mb has no cysteine. Upon ultrasonic irradiation of Mb solutions, there is a substantial decrease in microcapsule yield, compared to Hb. In addition, Hb–toluene or BSA–toluene microcapsules were destroyed by dithioerythritol (a protein disulfide cleavage reagent[20]). Finally, the oxidation of cysteine residues can be inhibited by alkylation with *N*-ethylmaleimide,[21] and microcapsule formation from Hb solutions so treated is greatly reduced. These results confirm the importance of cysteine cross-linking to microcapsule formation.

In summary, ultrasound can produce proteinaceous microcapsules at high concentrations with narrow size distributions. The process involves both emulsification *and* a chemical cross-linking of protein molecules. The cross-linking reaction principally involves disulfide bond formation by sonochemically generated superoxide.

Acknowledgment. We thank R. A. Olson and the Center for Electron Microscopy. This work was supported by the NSF, the NIH, and an NIH Research Career Development Award.

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A Ruthenium-Catalyzed Reconstitutive Condensation of Acetylenes and Allyl Alcohols

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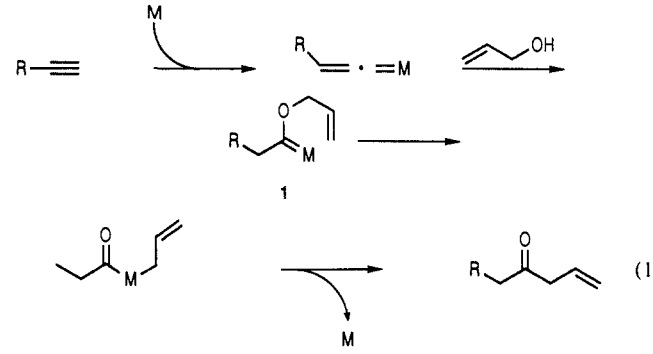
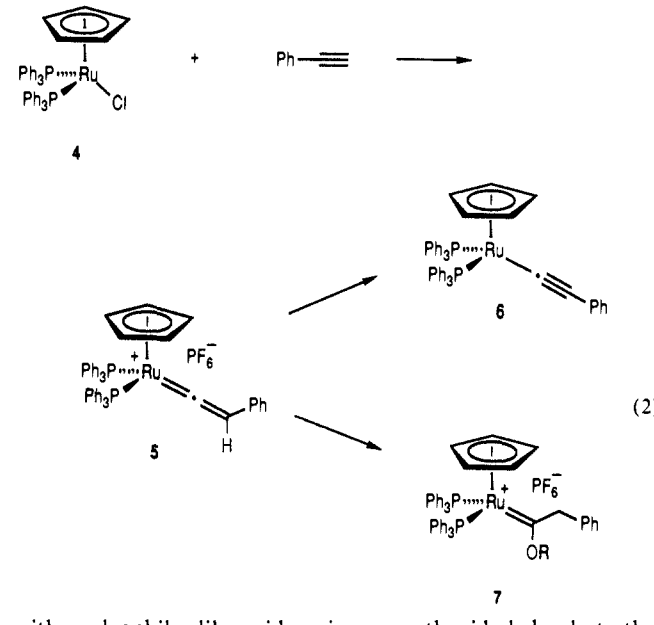
We have initiated a general program to develop carbon–carbon bond forming reactions by condensation, i.e., processes in which the product is the simple sum of the two reactants. In considering terminal acetylenes as one reaction partner, we considered the possibility of developing such condensation reactions invoking vinylidenemetal complexes[1] as reactive intermediates that can readily form under the conditions of a catalytic cycle. Equation 1 outlines one such possibility. The feasibility of such a pathway



is supported by the observation that a stoichiometrically prepared tungsten complex analogous to **1** does generate a β,γ-unsaturated ketone upon thermolysis in which the authors invoked the sequence **1**–**2**–**3**.[2] We report that a ruthenium complex indeed catalyzes the direct condensation of allyl alcohols and terminal acetylenes to generate initially β,γ-unsaturated ketones as outlined in eq 1.

We initiated our study with the known vinylidene complex **5**[3,4] because of its ready availability from the ruthenium complex **4**[3b] and phenylacetylene. Attempts to react the vinylidene complex



with nucleophiles like azide anion or methoxide led only to the

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