Table 1. PTS Fluorescence Quenching Results at 25 °C

Quencher	"k_Q" × 10^{-9} M^{-1} s^{-1} [a,b]		
	Water	In the presence of CTAC micelle[c]	In the presence of SDS micelle[c]
1	70	$\lesssim 1.0$[d]	$\lesssim 1.0$[d]
2	2.6	66	2.4
3	5.0	5.0	5.0
4		320	$\lesssim 1.0$[d]

[a] *Apparent* k_Q's from the steady-state Stern-Volmer slopes using measured τ_f's as follows: water, 13 ns; CTAC micelle, 8 ns; SDS micelle, 16 ns. All experiments were carried out on nondegassed samples. [b] Estimated error limits ±10%. [c] [CTAC] = 1.0×10^{-2} M which is well above the critical micelle concentration of 1.0×10^{-3} M. [SDS] = 1.5×10^{-2} M which is well above the critical micelle concentration of 8.0×10^{-3} M. P. Mukerjee and K. Mysels, "Critical Micelle Concentration of Surfactant Systems", National Bureau Standards Reference Data Series, National Bureau of Standards, Washington, D.C., 1971. [d] No quenching was observed but upper limit was estimated from the sensitivity limit of the steady-state experiment.

$< [Q] < 1.0 \times 10^{-2}$ M using independently measured τ_f's.

The fluorescence quenching order is **1** ≫ **3** > **2** in pure water, **2** ≫ **3** > **1** in the presence of CTAC micelles and **3** > **2** > **1** in the presence of SDS micelles. The results in the absence of micelles show the expected influence of electrostatic interactions between the excited fluorophor and the charged quenchers. In the presence of the CTAC micelles, where PTS is bound to the micelle surface[11,12] the reactivity order is determined by the *relative binding efficiencies of the nitroxyl radicals to the micelle.* In the environment of the cationic micelle, the reaction between the similarly charged reactants (PTS and **2**) is strongly enhanced and that between the two oppositely charged reactants (PTS and **1**) is strongly retarded relative to the results in pure water.

In the presence of the SDS micelle, PTS appears to be dissociated from the micelle surface. Under this condition, **2** and **3** quench the excited PTS as efficiently as they do in pure water. The striking result is the inhibition of the reaction between excited PTS and **1** which presumably results because of strong binding of the latter to the anionic micelle.

The results obtained with the surfactant nitroxyl radical **4** support the interpretations given above. This quencher is incorporated into both the CTAC and SDS micelles[13] and is a model for the micelle-bound quencher. In the presence of the CTAC micelle, where the fluorophor also is associated with the micelle, very efficient fluorescence quenching is observed. However, in the presence of the SDS micelle, where the fluorophor is dissociated, very inefficient fluorescence quenching is noted.[14]

At this time, we stress only the qualitative interpretation of these results. Because both static-like and dynamic quenching mechanisms[15] can operate, the absolute magnitudes of the k_Q's in Table I should be cautiously interpreted. Further work is in progress to sort out these pathways and to explore the usefulness of this quencher system for studying interactions between fluorophors solubilized in the hydrophobic core of micelles and reactants in the aqueous medium.

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(11) We estimate that PTS has an association constant with the CTAC micelle sufficiently large ($K > 10^5$ M^{-1}) for it to be essentially completely incorporated into the micelle under the reaction conditions. This estimate is based on the report that potassium 2,4-dinitrophenylsulfate binds to cetyltrimethylammonium bromide (CTAB) with $K \sim 1.9 \times 10^5$ M^{-1}. See table 4.1 in J. H. Fendler and E. J. Fendler, "Catalysis in Micellar and Macromolecular Systems", Academic Press, New York, N.Y., 1975.
(12) The absorbance of a 1.0×10^{-5} M PTS solution initially decreases with added CTAC until $\sim 1.0 \times 10^{-3}$ M surfactant. Thereafter, the absorbance increases sharply and levels off $\geq 5 \times 10^{-3}$ M added surfactant. In addition, the λ_m for PTS shifts from 375.5 nm in pure water to 380 nm in the presence of 5×10^{-4} M CTAC and finally to 377 nm $\geq 1 \times 10^{-3}$ M CTAC. These observations indicate some interaction between PTS and CTAC below the cmc point. However, the abrupt spectral changes described near 1×10^{-3} M and the *invariance of the PTS electronic absorption spectrum over a wide* CTAC concentration range above the cmc strongly argue for association of PTS with the CTAC micelle under the conditions of the fluorescence quenching experiments. For similar observations on acridine-type dyes with SDS, see B. H. Robinson, N. C. White, C. Mateo, K. J. Timmins, and A. James in "Chemical and Biological Applications of Relaxation Spectrometry", E. Wyn-Jones, Ed., D. Reidel Publishing Co., Dordrecht, Holland, 1975, p 201.
(13) The incorporation of **4** into the CTAC micelle was studied by ESR spectroscopy.[10] At 25 °C, the association constant is $K = 3.2 \times 10^5$ M^{-1} so that in the present study, where the micelle concentration is $\sim 1.0 \times 10^{-4}$ M, the fraction of **4** incorporated into the micelle is >0.95. Even more efficient incorporation is expected for **4** into the SDS micelle because of electrostatic attraction in addition to the hydrophobic interaction.
(14) Inefficient fluorescence quenching by **4** in the presence of the SDS micelle system could also result from precipitation of aggregates of **4** and SDS. No turbidity was apparent to the eye over the concentrations used in this study. Further, the fluorescence of SDS micelle solubilized pyrene, under experimental conditions similar to the PTS study, is very *efficiently* quenched by **4**, "k_Q" $\approx 8 \times 10^{10}$ M^{-1} s^{-1}, which is consistent with the conclusion presented in the paper.
(15) We use the term "static-like" to describe quenching of micelle-bound excited PTS by quenchers already associated with the same micelle. An example is the quenching of excited PTS by **4** in the presence of CTAC micelles. "Dynamic" quenching results from diffusional encounter of excited fluorophor and quencher where at least one of the two reactants is dissociated from the micelle.

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Stereochemistry of the 1,3-Proton Loss from a Chiral Methyl Group in the Biosynthesis of Cycloartenol as Determined by Tritium Nuclear Magnetic Resonance Spectroscopy

Sir:

The biosynthesis of sterols by photosynthetic organisms[1] proceeds through cyclization of 2,3-oxidosqualene (**1**) to yield cycloartenol (**2**) in contrast to nonphotosynthetic organisms[2] where the cyclization product is lanosterol (**3**) (Scheme I). The last step in the biosynthetic pathway leading to **2** involves a 1,3-proton loss from a methyl group to form the cyclopropane ring. A priori, this process could take in one of two stereochemically defined ways—retention or inversion of configuration around the C-6 methyl group (Scheme II). We now report our results on the stereochemistry of the 1,3-proton loss as determined by ^3H NMR spectroscopy.[3]

The labeled, chiral substrate **12** for cyclization studies was prepared from D-malic acid (**4**) according to Scheme III. ^3H NMR spectra of **10** (Figure 1) confirm the fact that each molecule of **10** labeled with a tritium atom at C-7 was also labeled with one deuterium atom (and one hydrogen atom) at C-7. Approximately thirty percent of all molecules were la-

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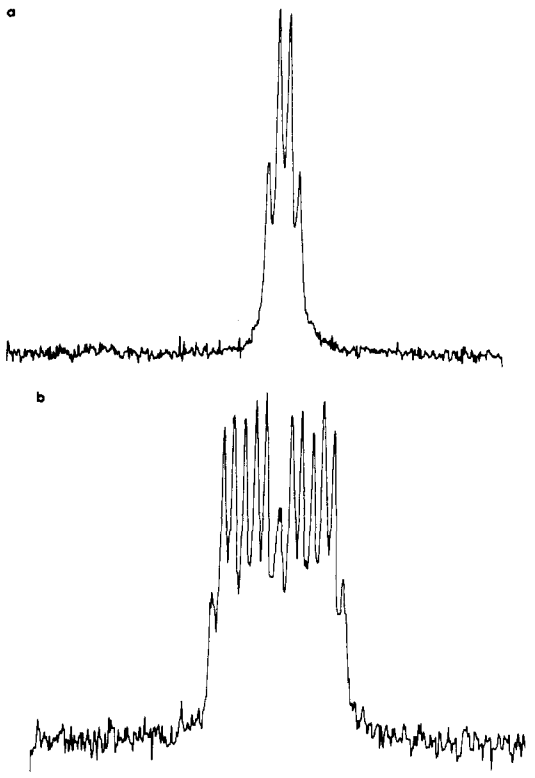


Figure 1. (a) The 106.7-MHz proton decoupled ^3H NMR spectrum of **10** in C_6D_6. Two overlapping 1:1:1 triplets at δ 1.076 and 1.057 are observed (J_{DT} = 2.1 Hz). (b) The 106.7-MHz ^3H NMR spectrum of **10** in C_6D_6. Two overlapping multiplets are observed at δ 1.076 and 1.057 ($J_{HT(gem)}$ = 13.6 Hz; $J_{HT(vic)}$ = 6.5 Hz).

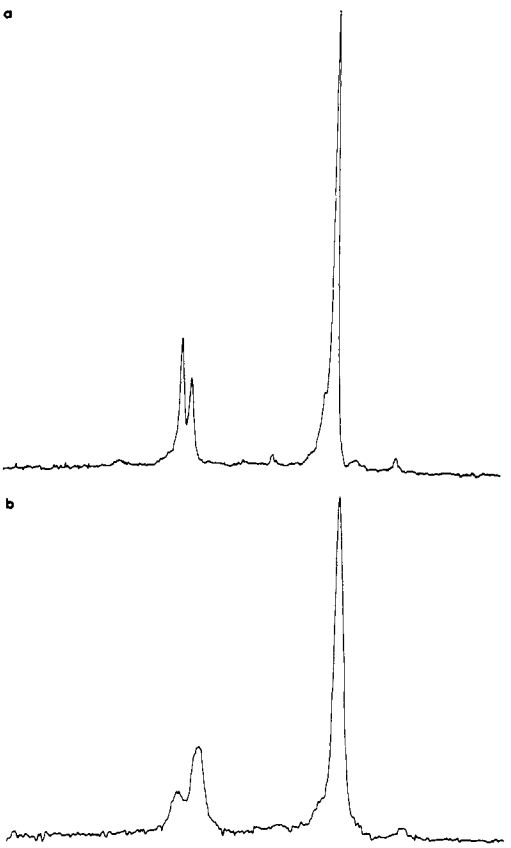
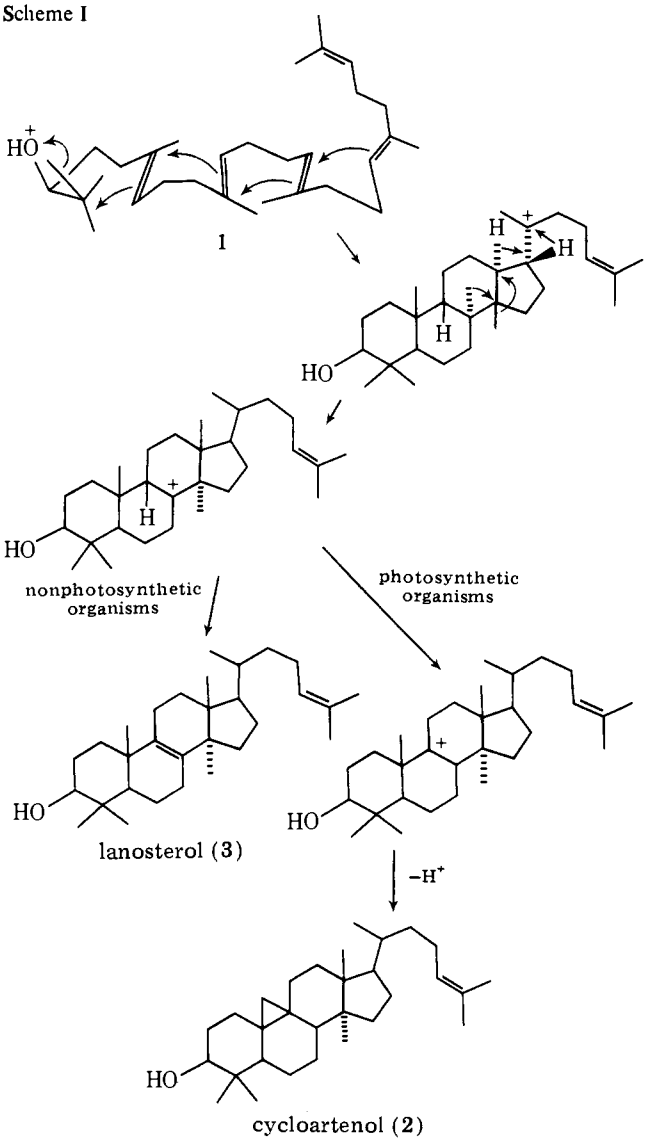
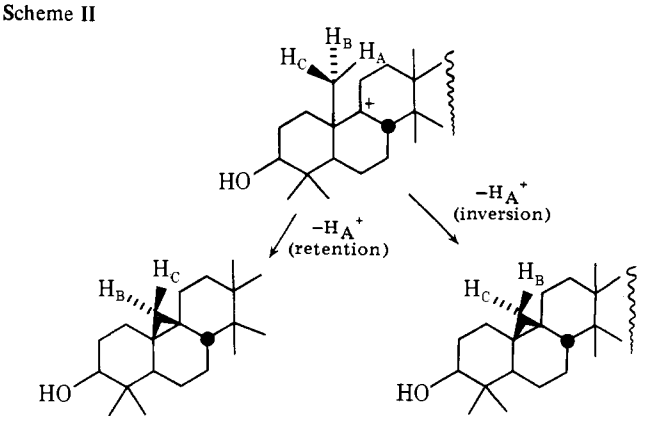


Figure 2. (a) The 106.7-MHz NOE suppressed, proton-decoupled ^3H NMR spectrum of tritium-labeled biosynthetic cycloartenol (**2**) in C_6D_6. (b) The 106.7-MHz ^3H NMR spectrum of tritium-labeled biosynthetic cycloartenol (**2**) in C_6D_6.

Scheme I



Scheme II

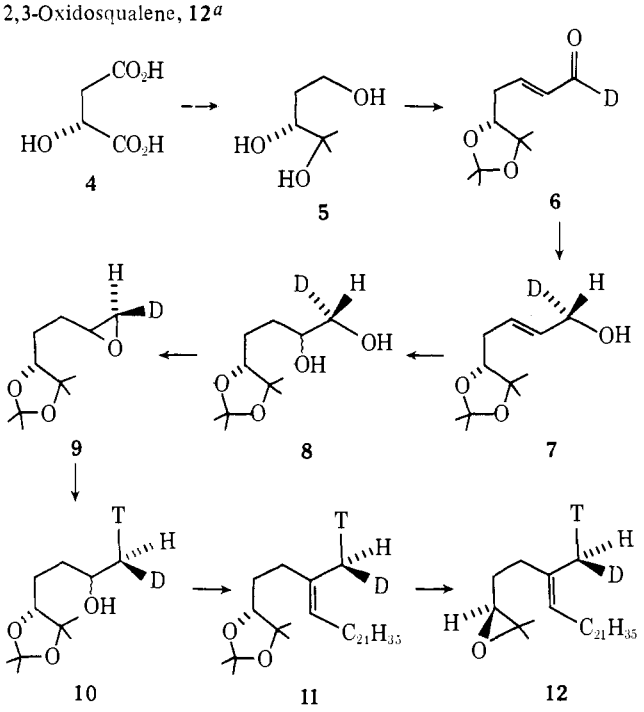


beled with tritium. All subsequent transformations proceeded without exchange of tritium as was demonstrated by the constancy of the specific activity of **10** (11 Ci/mmol), **11** (11.7 Ci/mmol), and **12** (9.4 Ci/mmol).

The conversion of chiral tritium labeled oxidosqualene (**12**) into tritium-labeled cycloartenol (**2**) was accomplished in ~22% yield by incubation with a cell-free microsomal fraction from *Ochromonas malhamensis*.

The biosynthetic cycloartenol showed only one spot on TLC (and by radiochromatogram scanning) and cochromatographed with authentic cycloartenol. The NOE suppressed, proton-decoupled ^3H NMR spectrum (Figure 2a) of tritium-labeled biosynthetic cycloartenol (**2**) shows resonances at δ

Scheme III. Synthetic Pathway for Chiral, Tritium-labeled 2,3-Oxidosqualene, 12[a]



[a] Reagents: 4 → 5, (a)[4] AcCl, (b) EtOH, (c)[5] B_2H_6, (d) CH_3MgBr; 5 → 6, (a) acetone, H^+, (b) $CrO_3 \cdot pyr_2$, CH_2Cl_2, (c)[6] $Ph_3P{=}CHC(=O)D$; 6 → 7,[7] HLADH, NADH, pH 6.8; 7 → 8, (a)[8] t-Bu$(CH_3)_2$SiCl, (b) B_2H_6, (c) H_2O_2, ^-OH, (d)[8] $(n\text{-Bu})_4N^+F^-$, THF; 8 → 9[10,11] (a) TsCl, (b) ^-OH, CH_3OH; 9 → 10,[12,13] $NaBT_4$, Me_2SO; 10 → 11, (a)[14] CBr_4, $(n\text{-Bu})_3P$, pyr, THF, (b) Mg, THF, (c)[15] $C_{21}H_{35}CHO$, (d)[16] $(PhO)_3P^+CH_3I^-$, HMPT; 11 → 12, (a) CH_3OH, H^+, (b) TsCl, (c) ^-OH, CH_3OH.

0.168, 0.438, and 0.456 in a ratio of 4.64:1:1.46. We assign[18] the resonances as follows: the resonance at δ 0.168 is due to an exo cyclopropyl tritium in molecules which also have an endo deuterium; the resonance at δ 0.438 is due to an endo cyclopropyl tritium in molecules which also have an exo deuterium;[19] and the resonance at δ 0.456 is due to an endo cyclopropyl tritium in molecules which also have an exo hydrogen. These assignments are confirmed by the proton-coupled ^3H NMR spectrum (Figure 2b) in which only the resonance at δ 0.456 has been split (J = 4 Hz).

It is thus immediately apparent that this conversion has proceeded with retention of configuration.[20]

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(17) Based on the 6*E* isomer: G. H. Beastall, H. H. Rees, and T. W. Goodwin, *FEBS Lett.*, **18**, 175 (1971).
(18) The assignment of the low-field (δ 0.45) resonance to the endo cyclopropyl proton (or tritium) is based upon the long-range coupling between $H_{1\alpha}$ and the endo cyclopropyl proton (in unlabeled cycloartenol) which causes a selective broadening of the δ 0.45 resonances. Deuteration of the 1α position removes this broadening. In addition, irradiation at δ 1.39 (in unlabeled cycloartenol) also removes the broadening. The low-field cyclopropyl resonance of 2β-cycloartanol was also selectively broadened. Lanthanide shift experiments utilizing $Pr(fod)_3$ demonstrated that the low-field cyclopropyl resonance in 2β-cycloartanol was due to the endo proton. These assignments (the low-field, broadened cyclopropyl resonances being due to the endo cyclopropyl proton) are further corroborated by T_1 measurements—80-MHz ^1H NMR spectra of unlabeled **2** show T_1 (endo H) = 0.48 s; T_1 (exo H) = 0.68 s. ^3H NMR spectra (106.7 MHz) of tritium-labeled cycloartenol show tritium T_1 (exo T, endo D) = 1.41 s; T_1 (endo T, exo D) = 0.98 s. The additional relaxation of the endo proton (or tritium) we presume to be coming from the 6β proton. The resonance of the 6β proton in 3β-cycloartenol was observed at 270 MHz at δ 0.66 as a quartet of doublets ($J_{6\beta,5\alpha} = J_{6\beta,6\alpha} = J_{6\beta,7\alpha}$ = 12.5 Hz, $J_{6\beta,7\beta}$ = 4.5 Hz) indicating that the 6β proton is in an axial conformation. Models of this conformation place the 6β proton ~0.18 nm from the endo cyclopropyl resonance thus allowing for the 6β proton's candidacy as being capable of selectively relaxing the endo cyclopropyl resonance. The unusual, downfield shift of the resonance of an endo cyclopropyl proton has been observed before by W. G. Dauben and W. T. Wipke, *J. Org. Chem.*, **32**, 2976 (1971).
(19) This resonance is derived from the 20% (*S*)-methyl impurity.
(20) Professor D. Arigoni has independently observed retention of configuration for this transformation using Zea Mays. We thank Professor Arigoni for discussions concerning these results prior to publication.

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Stereospecific Double Alkylation of Diphenylacetylene by η^5-Cyclopentadienyl-(triphenylphosphine)dimethylcobalt(III). Evidence for Noninterconvertible Diastereomeric Complexes in the Cobalt-Catalyzed Isomerization of Alkenes, and Some Comments on Factors Influencing the Rates of Reductive Elimination Reactions

Sir:

Yamazaki and Hagihara reported in 1971 that treatment of η^5-cyclopentadienyl(triphenylphosphine)dimethylcobalt(III) (**1**) with 2.8 equiv of diphenylacetylene (**2**) in refluxing benzene led to metallocycle **3** (Scheme I) and η^4-tetraphenylcyclobutadiene(η^5-cyclopentadienyl)cobalt(I) (**4**) in 49 and 13% yield, respectively.[1] Because this report left the methyl groups in **1** unaccounted for, and **1** "doubly alkylates" CO to give acetone quantitatively,[2] we have reinvestigated this reaction. We find that, when **1** is dissolved in oxygen-free benzene-d_6 and heated at 56 °C with 3.4 equiv of diphenylacetylene, **3** is observed, as reported earlier.[3] However,

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