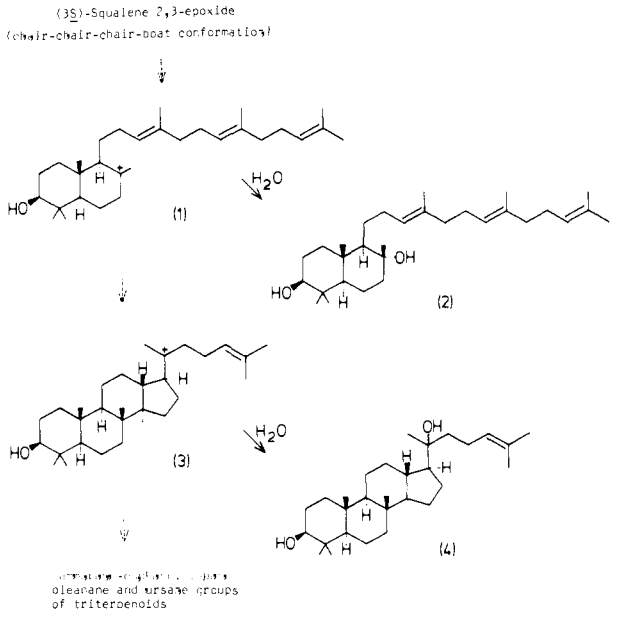
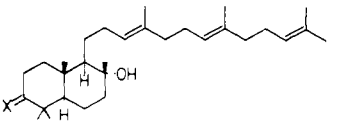
Scheme I



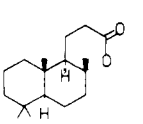
suggests that the cyclization proceeds via a series of discrete, conformationally rigid carbocationic intermediates.[5]

We now report the isolation from gum mastic of a novel bicyclic triterpenoid, **2**,[6] the structure and absolute stereochemistry of which are fully consistent with its formation by interception of the bicyclic carbocation **1** postulated as an intermediate in the cyclization of the chair–chair–boat conformation of (3*S*)-squalene 2,3-epoxide. Significantly, gum mastic also contains a range of *normal* tetracyclic (dammarane, tirucallane) and pentacyclic (lupane, oleanane) triterpenoids. It is interesting to note that the bicyclic diol **2** has the same formal relationship to the C-8 carbocation **1** as the dammarenediols (**4**) have to the C-20 carbocation **3** (Scheme I).

Gum mastic is an abundantly available resin obtained from the Mediterranean shrub *Pistacia lentiscus* L;[7] it has been extensively used as a varnish for paintings.[8] After extensive chromatography of the neutral fraction[9] from gum mastic, we have now isolated, as the third most abundant component (ca. 1.3% of the total resin), compound **2** as a gum. The 400-MHz ^{1}H NMR spectrum of **2** showed the presence of four methyl singlets (δ 0.77, 0.80, 1.00, and 1.14), four vinylic methyl groups (δ 1.60, 1.60, 1.61, and 1.68), a proton geminal to a hydroxy group (δ 3.32, dd, J = 6 and 9 Hz), and three vinylic protons (δ 5.10, 5.12, and 5.17). Treatment with acetic anhydride in pyridine at room temperature readily afforded a monoacetate, $C_{32}H_{54}O_3$ (MS and CI-MS). Comparison of the ^{13}C NMR spectra of the bicyclic diol **2** and its derivatives with spectra of authentic samples of ambrein, sclareol, and *all-trans*-squalene conclusively established the structure, **2**, of the diol. The absolute configuration was established as follows: oxidation of the bicyclic diol **2** (pyridinium chlorochromate–CH_2Cl_2) gave the ketol **5** (99%), which upon Wolff–Kischner reduction afforded the 3-desoxy derivative **6** (63%). Finally, two-phase oxidation



(5) X=O
(6) X=H_2



(7)

of (6) ($KMnO_4$–H_2O/PhH–*n*-$Bu_4N^+Br^-$; room temperature) gave (+)-ambreinolide (**7**), mp 143–144 °C, $[\alpha]_D$ +33° (*c* 0.45 in $CHCl_3$), identical in every respect with an authentic sample.[10]

While other partially cyclized triterpenoids have been described, these normally appear to be products of aberrant modes of cyclization[11] (or laboratory synthesis[12]). One example is the aforementioned ambrein, in which partial cyclization has occurred at both ends of the squalene chain. In contrast, the diol **2** is the first[6] example of a bicyclic triterpenoid that retains all of the regio- and stereochemical features necessary for continued cyclization and occurs in a system that clearly has the enzyme(s) needed for more complete cyclization.

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Registry No. 2, 89362-84-5; (3*S*)-squalene 2,3-epoxide, 54910-48-4.

Supplementary Material Available: Structures and ^{13}C NMR data of **6**, ambrein, and *all-trans*-squalene and mass measurements on the monoacetate of **2** (2 pages). Ordering information is given on any current masthead page.

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Self-Sensitized Photooxidation of Protoporphyrin IX and Related Free-Base Porphyrins in Natural and Model Membrane Systems. Evidence for Novel Photooxidation Pathways Involving Amino Acids[1]

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Free-base porphyrins and metalloporphyrins have been well established as sensitizers of singlet oxygen in photooxidation processes.[2-4] They are also implicated as photosensitizers in a number of photobiological processes such as the genetic disorder

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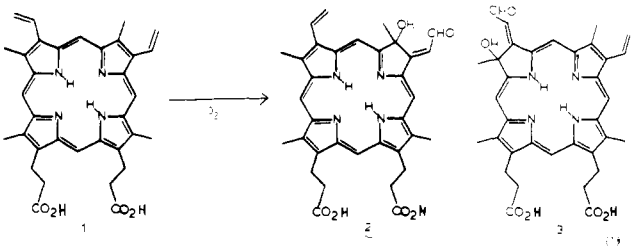
erythropoietic protoporphyria[5,6] and recently developed cancer phototherapy.[7-9] Other studies have shown that various porphyrins such as hematoporphyrin IX, mesoporphyrin IX, and other octaalkylporphyrins can sensitize the photooxidation of amino acids and unsaturated molecules as well as photodynamic membrane damage.[10-15] In previous studies it has been shown that protoporphyrin IX (**1**) sensitizes its own photooxidation to yield products **2–6** as shown in eq 1.[16-19] The relative yields of these products



are strongly medium dependent; in organic solvents it is clear that most of the products arise from singlet oxygen attack on the porphyrins,[17] while in organized media such as micelles or vesicles more complex mechanisms are operative including the intermediacy of superoxide.[19] In the present paper we report results of a study of the photobleaching of **1** in a natural membrane system, erythrocyte ghosts,[20-23] which contains saturated and unsaturated lipids as well as membrane proteins. Interestingly, it is found that the "normal" photooxidation products **2–6** are completely suppressed while photobleaching of **1** involving oxygen is *enhanced* in the "ghosts". The finding that other free-base porphyrins not containing vinyl groups, such as mesoporphyrin IX and hematoporphyrin IX, undergo photobleaching at comparable rates to **1** suggests that the "new" reaction involves photooxidation at the porphyrin macrocycle. The photoreactivity of the free-base porphyrins in ghosts can be mimicked in oil/water microemulsions to which oxidizable amino acids such as methionine, tryptophan, or histidine have been added. The results point to a photooxidation path involving intermediates subsequent to singlet oxygen that may be quite general for the phototherapy process as well as other "photodynamic action" phenomena.

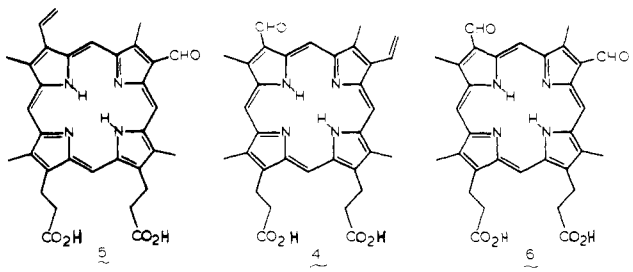


Table I. Comparison of Photobleaching and Product Formation Rates for Protoporphyrin IX in Different Environments

medium[a]	product ratios **1**:(**2** + **3**):(**4** – **6**)	% protoporphyrin reacted
benzene[b]	80:16:4	40
microemulsion[b]	80:17:3	40
erythrocyte ghosts[c]	98:1:2	70
microemulsion + methionine[b,d]	96:–:4	80
microemulsion + tryptophan[b,d]	97:1:2	65
microemulsion + histidine[b,d]	98:1:2	70

[a] Samples irradiated at $\lambda > 360$ nm in a merry-go-round appatus in the presence of atmospheric oxygen. [b] Irradiation time 150 min. [c] Irradiation time 78 min. [d] Amino acid concentration 0.013 M.

Protoporphyrin IX is readily incorporated into freshly prepared[21-23] erythrocyte ghosts by gentle stirring. The porphyrin shows characteristic "monomer" absorption and fluorescence spectra and gives no evidence of aggregation. Irradiation of "ghost" solutions of **1** in the presence of molecular oxygen leads to accelerated bleaching of the visible and near-UV absorption of the porphyrin with very little production of the "normal" oxidation products **2–6** (Table I). The accelerated bleaching with no concurrent formation of either hydroxy aldehyde (**2**, **3**)[24] or formyl (**4–6**) products is unprecedented among the wide variety of media studied at date and strongly implies that the change in observed reactivity is due to a mechanism involving direct participation of "ghost" components rather than to a single "solvent" or environmental effect.

Very similar photoreactivity of protoporphyrin IX or its dimethyl ester can be observed when they are solubilized in an oil-in-water microemulsion[25] to which the oxidizable amino acids methionine, histidine, or tryptophan have been added (Table I.).[26] In the absence of the amino acids the porphyrins react about as readily as in benzene to give the usual photoproducts (Table I). With the amino acids present the photodegradation of the porphyrin is enhanced and only small amounts of **2–6** can be detected. The reaction with methionine present has been studied in the greatest detail. For the observation of the "new" photoreaction the presence of molecular oxygen is necessary. Experiments adding independently methionine, methionine sulfoxide, norvaline, and diethyl sulfide to the microemulsion show very clearly that the thioether moiety of methionine is the critical component for the observed photodegradation of the porphyrin. Previous studies have shown that processes with rate-determining interception of singlet oxygen show strong solvent isotope effects upon changing from H_2O to D_2O.[27,28] In accord with previous studies,[18,19] a pronounced "solvent" isotope effect is observed for photooxidation of **1** in the microemulsion alone; in this case the formation of products **2** and **3** and the disappearance of **1** were increased by a factor $\phi_{D_2O}/\phi_{H_2O} = 2.7$, which is reasonable for a microemulsion

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(24) It is found that **2** and **3**, although relatively stable to singlet oxygen in solution, micelles, or microemulsions, are rapidly degraded in microemulsions to which methionine is added. It it thus reasonable that any **2** or **3** formed in the ghosts may be rapidly degraded by rapid subsequent reaction not involving $^1O_2$.
(25) Composition of oil-in-water microemulsion: H_2O = 61.1% w/w; 1-butanol = 19.9% w/w; dodecyltrimethylammonium bromide (or sodium lauryl sulfate) = 10.2% w/w; benzene = 8.9% w/w.
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in which there is reduced contact between **1** and the aqueous phase. In contrast, addition of 0.013 M methionine resulted in a photobleaching with no detectable isotope effect.

Taking into account these observations: a requirement for O_2, non-rate-determining attack of singlet oxygen and (in the case of methionine) the requirement of a thioether group that is a well-known substrate for singlet oxygen;[29] a mechanism involving attack on the porphyrin by an intermediate oxygenated species AO_2 (eq 2–5) can be proposed (where P = **1** or other porphyrin

$$P \rightarrow {}^1P^* \rightarrow {}^3P^* \quad (2)$$

$$^3P^* + O_2 \rightarrow P + {}^1O_2^* \quad (3)$$

$$^1O_2^* + A \rightarrow AO_2 \quad (4)$$

$$AO_2 + P \rightarrow AO + PO \rightarrow \text{other products} \quad (5)$$

and A is an amino acid or other primary acceptor). In the case of methionine, AO_2 is probably methionine persulfoxide ($R_1R_2S^+OO^-$) and methionine sulfoxide (AO) is detected as a product produced concomitantly with porphyrin photobleaching. Although primary porphyrin degradation products cannot be isolated under the reaction conditions, some clear structural inferences can be obtained. Thus while non-vinyl porphyrins such as mesoporphyrin IX and hematoporphyrin IX are relatively stable when irradiated in benzene or the microemulsion with oxygen, both porphyrins photodegrade rapidly in the microemulsions containing methionine. For all three porphyrins weak absorption near 650 and 360–370 nm grows in during prolonged irradiation. This absorption is characteristic of photolabile biliverdins.[14,30,31]

The rapid loss of absorption in the visible and near-UV is consistent with a reaction in which the porphyrin macrocycle itself is attacked followed by a ring opening to give linear pyrrole derivatives. It has been shown that certain metalloporphyrins are photooxygenated to yield formylbiliverdins as primary products;[31–34] however, in other cases where metal-free porphyrins are degraded in aqueous solution suspected formylbiliverdins cannot be isolated.[31] We suggest that the first product, PO, is probably an epoxide that subsequently converts to a formylbiliverdin hydrolytically. Biliverdins are well-known to degrade rapidly in the presence of singlet oxygen sensitizers.[35,36]

The intermediates, "AO_2", obtained with different amino acids or other membrane components should possess a variety of lifetimes and reactivities. We find, for example, in studies with methionine in the microemulsion, evidence for pronounced differences in reactivity between AO_2 and $^1O_2$. Thus while hematoporphyrin IX, mesoporphyrin IX, and the hydroxy aldehydes **2** and **3** are all relatively stable toward $^1O_2$, they are all rapidly degraded by AO_2 as evidenced by their accelerated bleaching by addition of methionine. In contrast the photobleaching of (tetraphenylporphyrin)zinc, which is rapidly converted to a biliverdin upon irradiation in homogeneous solution[31–34] or the microemulsion without methionine, is strongly quenched by addition of methionine to the microemulsion.

The most striking aspect of these studies to date is thus the finding that several amino acids can evidently generate relatively powerful oxidants subsequent to reaction with $^1O_2^*$. These ground-state reagents may be powerful agents in photodynamic action processes due to their relatively long lifetimes or localization at specific sites in in vivo systems. We are currently extending our studies to include a variety of natural membrane systems and other oxidizable "sensitizer substrates".

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Registry No. 1, 553-12-8; **2**, 70552-66-8; **3**, 89398-63-0; **4**, 89398-64-1; **5**, 10200-02-9; **6**, 60185-98-0; methionine, 63-68-3; histidine, 71-00-1; tryptophan, 73-22-3; oxygen, 7782-44-7.

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Autoxidation of Micelles and Model Membranes. Quantitative Kinetic Measurements Can Be Made by Using Either Water-Soluble or Lipid-Soluble Initiators with Water-Soluble or Lipid-Soluble Chain-Breaking Antioxidants[1]

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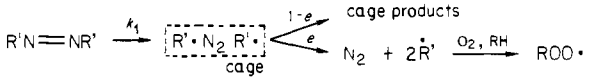
It is well-known that the autoxidation of biological membranes occurs readily and is associated with many important pathological events.[2] In 1980 quantitative kinetic studies of the autoxidation of phospholipid bilayers were first described in two preliminary communications,[3,4] one of which has since been fully amplified.[5] The difficulty that had to be overcome in these studies, as in all quantitative autoxidation kinetics, was that of ensuring that the initiation of the radical chain occurred at a reproducible and known rate. In both cases, this was achieved within the lipid bilayer by the thermal decomposition of a relatively large amount of a lipophilic azo compound.[3,4,6] Rates of initiation, R_i, were determined by the induction-period method[7] using lipophilic phenolic antioxidants (e.g., α-tocopherol, α-T[4]). The efficiencies of chain initiation, e, were also determined by measuring the rate of decomposition of the azo initiator.



$$R_i = 2ek_1[R'NNR']$$

(1) Issued as N.R.C.C. No. 23209.
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