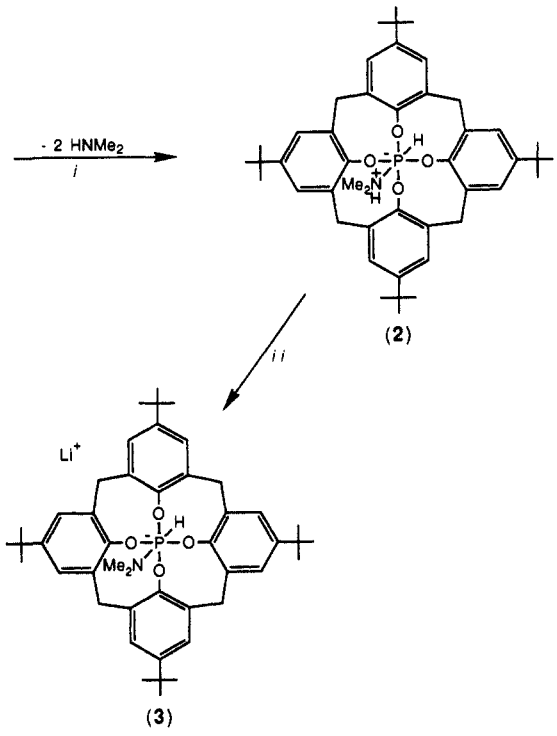
Scheme I[a]



[a] Reagents and solvents: (i) $P(NMe_2)_3$, benzene; (ii) butyllithium, THF.

3 mol of dimethylamine are eliminated and one proton is transferred from nitrogen to phosphorus, as was originally anticipated. However, an additional four-line resonance is seen in the ^1H NMR spectrum of **2** at δ 3.44, as well as a broad peak at δ 7.8. The former integrates for six protons while the latter for one. The multiplicity of the δ 3.44 signal is puzzling since, if it is due to a dimethylamino group bonded to phosphorus, a doublet would be expected. In fact, ^{31}P decoupling and homonuclear ^1H decoupling show that one doublet of this resonance is due to phosphorus coupling while the other is due to coupling from the proton at δ 7.8.

Deprotonation of **2** with butyllithium was attempted, a method that successfully cleaves the P-H bond in cyclenPH to yield a phosphoranide ion.[6] In the present case, the P-H bond is not cleaved: the product **3** has a ^{31}P resonance at δ -113 with an even larger P-H coupling of 836 Hz. The ^1H NMR spectrum of **3** still shows singlets for the *tert*-butyl (δ 1.19) and aromatic (δ 6.97) protons but no longer has the broad peak at δ 7.8; the "dimethylamino" resonance, now at δ 4.61, is a doublet. (In addition, the small phosphorus coupling in the upfield methylene resonance is no longer observed.)

Taken together, the above data indicate that the initial product **2** has a phosphorus connected not only to the four oxygens, but also to hydrogen and dimethylamine, to yield a zwitterionic species. In the initial reaction, the calix[4]arene transfers one proton to the phosphorus and three to the dimethylamino groups; however, only two dimethylamines are given off. Deprotonation of **2** cleaves the N-H rather than the P-H bond to give **3**. These reactions are summarized in Scheme I.

Further support for these formulations comes from a molecular weight determination (vapor pressure osmometry) of **2**, which yielded a value of 701 (calculated 722) indicating that the compound is monomeric.[7] In fact, the ^{31}P NMR chemical shifts of these species are more in the hexacoordinate than pentacoordinate region.[8] Low-temperature ^1H and ^{31}P NMR spectra of **2** to -70 °C are identical with the ambient-temperature spectra, except for some broadening in the ^1H peaks; this suggests that the observed spectra are in fact due to all four oxygens being bound to phosphorus rather than a fast exchange process equilibrating the rings (in which, for example, only three P-O bonds are present at any one time). All attempts at growing X-ray quality crystals of **2** have, so far, been unsuccessful.

As mentioned above, the methylene resonances suggest a cone conformation for **2**, however, at this time, we do not know the orientations of the hydrogen and amine with respect to the cone, although steric factors would argue that the amine is outside and the hydrogen inside.

Acknowledgment. Acknowledgment is made to the donors of the Petroleum Research Fund, administered by the American Chemical Society (M.L.), the Robert A. Welch Foundation (M.L. and C.D.G.), and National Institutes of Health Grant GM-23534 (C.D.G.) for generous financial support. We thank Dr. Mark O'Neil-Johnson of Bruker Instruments for obtaining the $^1H\{^{31}P\}$ NMR spectra.

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(7) Synthesis of **2**: In an inert atmosphere, a stirred slurry of **1** (130 mg, 0.20 mmol) in benzene (10 mL) was treated dropwise with $P(NMe_2)_3$ (65 μL, 0.36 mmol). After stirring for 24 h, the resulting precipitate was filtered, washed with benzene, and pumped dry, yielding **2** as a white, air-stable solid (115 mg, 80%): mp (nitrogen-filled tube) 385-387 °C; satisfactory elemental analyses (CHN) for **2** were obtained; $^{13}C\{^1H\}$ NMR (THF-d_8), δ 31.8 [C-$(CH_3)_3$, s], 34.6 [$C(CH_3)_3$, s], 36.3 (CH_2, s), 44.6 [$N(CH_3)_2$, d, $^3J_{PC}$ = 4 Hz], 124.7 (CH, s), 138.6 [$C(CH_2)$, d, $^3J_{PC}$ = 9 Hz], 145.7 [$CC(CH_3)_3$, d, $^5J_{PC}$ = 5 Hz], 149.0 (CO, d, $^2J_{PC}$ = 7 Hz).

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Reaction of the Quinone Methide from Reductive Glycosidic Cleavage of Daunomycin with Molecular Oxygen. Evidence for Semiquinone Methide Formation[1]

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Anthracycline antitumor drugs, especially adriamycin and daunomycin (**1**),[2] are proposed to be bioreductively activated to semiquinone, hydroquinone, and quinone methide states through sequential one-electron reductions.[3,4] All of these states are of biological interest with regard to mechanisms of cytotoxicity. Semiquinone and hydroquinone states react rapidly with molecular oxygen to produce reactive oxygen species that inflict oxidative stress.[5] In the absence of molecular oxygen, the hydroquinone state of daunomycin (**1**) undergoes elimination of daunosamine to give the quinone methide **2**.[4,6-8] Quinone methides are of

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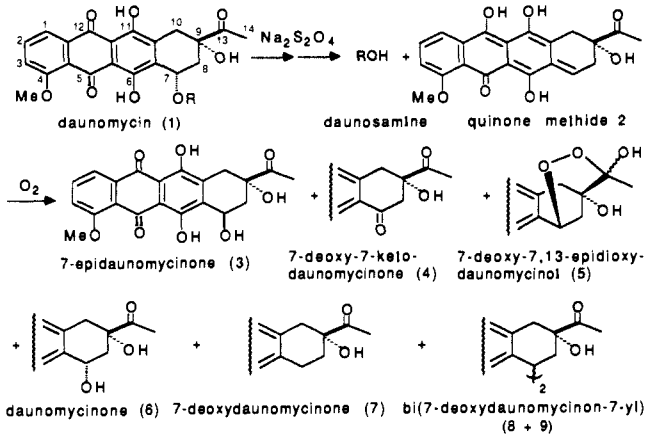
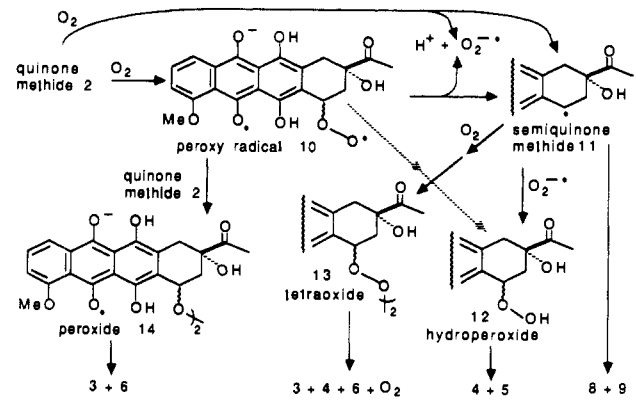
Scheme I



interest because of potential electrophilic reactivity at the 7-position with nucleophilic sites in biological molecules with resulting covalent linkage of the aglycon.[9] Another transient species that has been proposed as a reactive intermediate in coupling the aglycon to biological molecules is the semiquinone methide **11**.[10-12] We now report the structures of products from reaction of **2** with molecular oxygen and argue that some of these structures implicate the intermediacy of semiquinone methide **11**.

Anaerobic reduction of daunomycin in pH 8.2 water at 0 °C with 1 equiv of sodium dithionite yielded the transient quinone methide **2**, as indicated by absorption at 375 and 600 nm.[4] When the quinone methide maximized after ~1 min, pure oxygen was rapidly forced into the reaction mixture, whereupon the color instantly changed back to the orange of the anthracycline quinone chromophore. Reverse-phase HPLC analysis of the reaction mixture showed the presence of seven species with absorption in the range 480–495 nm. These materials, in order of their elution from the C-18 column (supplementary material), were indentified as 7-epidaunomycinone (**3**, 5%), 7-deoxy-7-ketodaunomycinone (**4**, 4%), 7-deoxy-7,13-epidioxydaunomycinol (**5**, 20%), daunomycinone (**6**, 12%), daunomycin (**1**, 5%), 7-deoxydaunomycinone (**7**, 29%), and two diastereomers of bi(7-deoxydaunomycinon-7-yl) (**8** + **9**, 21%). Products were separated by a combination of silica gel and C-18 reverse-phase suction chromatography.[13] Compounds **3**, **4**, **6**, and **7** were identified by spectral and chromatographic comparison with authentic samples. Epidioxydaunomycinone (**5**) was identified from UV–vis, IR, ^{1}H NMR, and MS analyses. The important features include the daunomycin UV–vis chromophore, the absence of the IR carbonyl stretching band for the ketone group at the 13-position, an upfield chemical shift for the methyl at the 14-position (δ 1.44) and a chemical shift (δ 5.82) for the proton at the 7-position downfield relative to the chemical shift for the corresponding protons in epidaunomycinone and daunomycinone in the NMR spectrum, and a negative ion FAB molecular ion peak at m/z 413 (M − 1). The other ^{1}H NMR resonances appeared as well-defined patterns consistent with the assigned structure (supplementary material). Precedence for formation of the hemiacetal occurs with the hemiacetal structure assigned earlier to the adduct from **2** reacting with benzaldehyde.[14]

Scheme II. Proposed Mechanism for Product Formation



The aglycon dimers **8** and **9** were similarly characterized. The relative retention times on the C-18 HPLC column were the initial indication of aglycon dimer structure. Both dimers also showed a characteristic daunomycin quinone chromophore in the UV–vis spectrum and FAB mass spectral molecular ions, a negative ion peak at m/z 762 with a strong peak at m/z 381 for cleavage of the 7–7′ bond and a positive ion peak at m/z 763. The ^{1}H NMR spectrum of dimer **8** showed well-defined patterns for the protons at all positions, indicating a symmetrical structure; whereas, the spectrum of dimer **9** showed unresolved multiplet patterns for protons at positions 7 and 8, indicating an unsymmetrical structure (supplementary material).

The effect of some variations in reaction conditions was examined. The product ratio was a function of the partial pressure of the molecular oxygen added to the reaction mixture. When no oxygen was added, 7-deoxydaunomycinone (**7**) was the only aglycon product. With pure oxygen, the ratio of (**8** + **9**)/(**3** + **4** + **5** + **6**) was 0.10; with air, 0.33; and with 5% oxygen in nitrogen, 0.62.[15] The product ratio was not affected by addition of the metal ion chelating agent desferrioxamine. Addition of the reactive radical-scavenging agent α-tocopherol to a similar reduction in methanol-*d* solvent relative to a control had little effect on the product ratio, and the same products were formed when the solvent was methanol.

A mechanism that rationalizes formation of the various products except **7**, which arises from protonation of the quinone methide **2**,[4,8] is shown in Scheme II. The mechanism is proposed to accommodate formation of all of the products in both water and methanol and the observations that the yield of **3** plus **6** significantly exceeds the yield of **4**, and that desferrioxamine and α-tocopherol do not change the product ratio. The initial step is that of radical addition to form peroxy radical **10**, which can lose superoxide to form semiquinone methide **11**. Semiquinone methide could also arise from **2** by electron transfer to molecular oxygen. The formation of **8** and **9** together with the effect of oxygen partial pressure on the product ratio implicates the intermediacy of **11**. With lower oxygen partial pressure semiquinone methide dimerization competes effectively with combination with molecular oxygen. Biradical **10** is also proposed to add to a second molecular of quinone methide to form peroxide **14**, which is at the proper redox state to fragment to **3** and/or **6**. Combination of two oxygen molecules with two semiquinone methides is shown to form tetraoxide **13**, which can logically fragment to **4** and either **3** or **6** together with singlet or triplet molecular oxygen. A tetraoxide intermediate is precedented in hydrocarbon oxidations.[16] Intramolecular redox of peroxy radical **10** is proposed to give hydroperoxide **12**. If the hydroperoxy group is syn to the acetyl group, **5** is formed, and if the hydroperoxy group is anti to the acetyl group, **4** is formed. The multiple pathways to **4**–**7** ac-

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commodate the observed product ratios; other combinations of the various transients are also conceivable.

Formation of aglycon dimers from reduction of the 11-deoxyanthracyclines (aclacinomycin A,[17] 11-deoxydaunomycin,[18] and menogaril[19]) results from slow, nonradical coupling of quinone methides, one quinone methide serving as a nucleophile and the other as an electrophile. Formation of aglycon dimers from reduction of daunomycin under anaerobic conditions is not observed because of the much shorter lifetime of the quinone methide state due to rapid tautomerization to form 7-deoxydaunomycinone (**7**).[4,20,21]

Earlier, Bachur[10] and Sinha[11] proposed that covalent binding of the aglycon to biological macromolecules occured via a free-radical pathway involving combination of **11** with a radical site on the macromolecule. The semiquinone methide was thought to result from glycosidic cleavage at the semiquinone redox state.[10,11,22,23] Substantial evidence now establishes that semiquinone does not undergo glycosidic cleavage, at least in vitro.[4,17-20] The results described here indicate that the potentially important semiquinone methide is biologically accessible from reaction of the quinone methide, from glycosidic cleavage at the hydroquinone state, with molecular oxygen.

Supplementary Material Available: A reverse-phase HPLC chromatogram showing retention times of the products, ^{1}H NMR data showing chemical shifts and splittings for products **3-6**, **8**, and **9**, and MS data for products **5**, **6**, **8**, and **9** (3 pages). Ordering information is given on any current masthead page.

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First Example of a Peroxo-Bridged Complex Having an Accompanying Metal-Metal Bond

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Transition-metal dioxygen complexes have attracted a great deal of interest owing to their importance in biological systems[1-4] and because of their involvement in the oxidation of organic substrates.[5-8] Although many complexes have been characterized in which the dioxygen molecule bridges the two metals,[9] primarily involving cobalt, there are *no* examples of which we are aware in which the bridging O_2 moiety is accompanied by a metal-metal bond, to give a cyclic dimetallo-peroxide grouping. In this report we outline the preparation, characterization, and some preliminary chemistry of such a species.

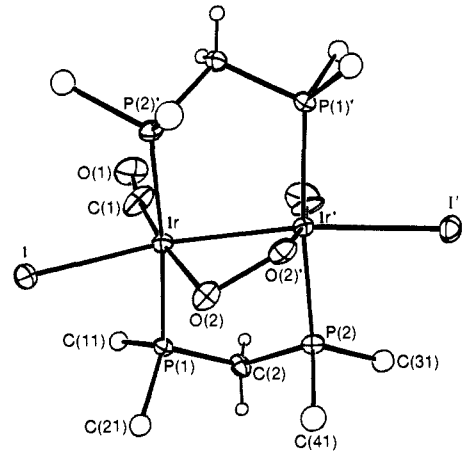

Figure 1. An ORTEP representation of $[Ir_2I_2(CO)_2(\mu\text{-}O_2)(dppm)_2]$ (**1**) in which only the ipso carbons of the phenyl rings are shown. Primed atoms are related by unprimed ones by a crystallographic 2-fold axis bisecting the Ir-Ir' and the O(2)-O(2)' bonds. Thermal ellipsoids are at the 20% level except for methylene hydrogens, which are arbitrarily small. Selected bond distances (Å): Ir-Ir', 2.705 (1); Ir-I, 2.764 (1); Ir-C(1), 1.82 (2); Ir-O(2), 2.04 (1); O(2)-O(2)', 1.58 (2). Interatomic angles (deg): Ir'-Ir-O(2), 71.7 (3); Ir-O(2)-O(2)', 102.0 (4); Ir'-Ir-C(1), 101.3 (7); I-Ir-C(1), 97.8 (7); I-Ir-O(2), 89.4 (3).

The compound in question, $[Ir_2I_2(CO)_2(\mu\text{-}O_2)(dppm)_2]$ (**1**)[10] (dppm = $Ph_2PCH_2PPh_2$), is readily and irreversibly obtained in greater than 70% yield as very insoluble dark purple crystals after exposure of a CH_2Cl_2 solution of $[Ir_2I_2(CO)(\mu\text{-}CO)(dppm)_2]$[11] to air or pure dioxygen for several hours. The $^{31}P\{^1H\}$ NMR spectrum of **1** indicates that all four phosphorus nuclei are chemically equivalent, and the increase in the carbonyl stretching frequencies, from 1948 and 1741 cm^{-1} in the precursor to 2005 and 1979 cm^{-1} in **1**, is consistent with oxidation of the metal centers; no O-O stretch can be unambiguously identified. Confirmation that the complex contains coordinated O_2 comes from the X-ray structure determination (see Figure 1), which shows that the dioxygen ligand bridges the two metals.[12] The short Ir-Ir' separation of 2.705 (1) Å clearly supports the μ-peroxo formulation in which the two Ir(II) centers are joined by an Ir-Ir bond. Although the Ir-O(2) distance (2.04 (1) Å) is normal, the O(2)-O(2)' separation, at 1.58 (2) Å, is extremely long; only $[La_2(N(SiMe_3)_2)_4(O_2)(OPPh_3)_2]$[13] appears to have a longer O-O separation (1.65 (4) Å). By comparison, the O-O distance in Na_2O_2 is 1.49 Å[14] and O-O distances are usually found in the range from 1.40 to 1.50 Å in other μ-peroxo compounds.[9,15] The

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(10) $[Ir_2I_2(CO)_2(\mu\text{-}O_2)(dppm)_2]$: ^1H NMR (CD_2Cl_2) δ 7.1-7.8 (m, 40 H), 5.3 (m, 2 H), 4.0 (m, 2 H); $^{31}P\{^1H\}$ NMR (CD_2Cl_2, vs 85% H_3PO_4) δ -24.4 (s); IR (Nujol) ν(CO) 2005, 1979 cm^{-1}. Anal. Calcd for $Ir_2I_2P_4O_4C_{52}H_{44}$: C, 41.77; H, 2.97. Found: C, 41.44; H, 2.91.
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(12) Crystal data for **1**, $Ir_2I_2P_4O_4C_{52}H_{44}$ (FW = 1495.03): space group $P4_12_12$, a = 14.647 (2) Å, c = 27.973 (4) Å, Z = 4, V = 6001.4 Å^3. Data were collected on an Enraf-Nonius CAD4 diffractometer using Mo Kα radiation. Solved by using direct methods (MULTAN) and refined by using full-matrix least-squares techniques to R = 0.061 and R_w = 0.093 for 3585 unique observations with $F_o^2 \geq 3\sigma(F_o^2)$. The data were corrected for Lorentz and polarization effects and for absorption. The molecule occupies the crystallographic 2-fold axis.
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