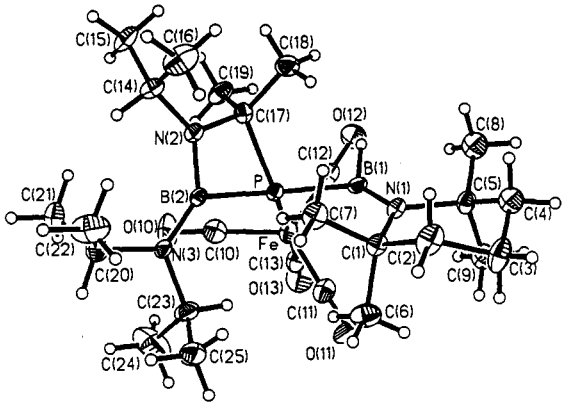

Figure 1. Molecular structure and atom labeling scheme for [tmpB(H)][$Fe(CO)_4$]PB(*i*-Pr_2N)N(*i*-Pr)C(Me_2) (**5**). Selected bond distances (Å): Fe–P = 2.297 (2); P–B(1) = 1.965 (8); P–B(2) = 1.964 (8); P–C(17) = 1.900 (7); B(1)–N(1) = 1.362 (10); B(2)–N(2) = 1.417 (10); B(2)–N(3) = 1.398 (10); N(2)–C(14) = 1.478 (9); N(2)–C(17) = 1.493 (9).

1,2-addition with a C–H bond from an amino isopropyl group on B(2).[9] It is interesting that a related C–H bond addition to a transient phosphinoborane [$Br_2P{=}BMes_2$] occurs in an opposite sense with a mesityl methyl H atom migrating to the P atom. The resulting CH_2 group bonds to the boron atom, forming a five-membered P–B–CH_2–C–C ring.[10]

Several structural features in **5** are of interest. The exo P–B(1) and endo P–B(2) distances are identical, and they are comparable with the P–B(N-*i*-Pr_2)$_2$ distance in **3**, 1.979 (5) Å.[3] The exo B(2)–N(3) distance is identical to the endo B(2)–N(2) distance, and these are similar to the average *i*-Pr_2N–B distance in **3**, 1.426 Å. The B(1)–N(1) distance involving the tmp group is shorter than the B–N(tmp) distance, 1.397 (5) Å, in **3**, and this is consistent with greater B–N π overlap in the tmpB(H)P fragment of **5**. The P–C(17) bond length is comparable with the average endo P–C distance, 1.92 Å, in [Me_2CC(H)(Me)C(Me_2)P(Me)(Ph)$^+$](I^-).[11] The P–Fe bond distance is relatively long compared to the P–Fe distance in $(CO)_4$Fe·PPh_3,[12] 2.244 (1) Å. These distances and carbonyl stretching frequencies suggest that **4** is a slightly better σ donor than PPh_3.

The results of this study suggest that an interesting distinction in the reactivity of transient boraphosphene fragments may be induced by substituent group variations on a progenitor diborylphosphane. Efforts to isolate or trap boraphosphenes and additional studies of factors that influence the formation and reactivity of X_2BP=BY species are in progress.

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Supplementary Material Available: Tables of X-ray data, collection parameters, atom coordinates, bond distances and angles, hydrogen atom coordinates, and thermal parameters for **5** and spectroscopic data (16 pages); listing of observed and calculated structure factors for **5** (15 pages). Ordering information is given on any current masthead page.

(9) The difference in behavior between **1** and **2** is consistent with the expected stability of alkyl carbocations. In an alternative mechanism, steric crowding may reduce the operational acidity of the P–H bond in **3**, and the first step could involve deprotonation of one *i*-Pr on B(2). Subsequent cyclization could occur by carbanion attack on the phosphane center accompanied by hydride transfer from the P atom to the B atom with elimination of LiCl.

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Photosensitized Formation of 7,8-Dihydro-8-oxo-2′-deoxyguanosine (8-Hydroxy-2′-deoxyguanosine) in DNA by Riboflavin: A Non Singlet Oxygen Mediated Reaction

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Increasing attention is devoted to the elucidation of the biological role of 7,8-dihydro-8-oxo-2′-deoxyguanosine[2] (8-oxodG, 8-hydroxy-2′-deoxyguanosine), an important oxidation product of the guanine moiety within DNA.[3] Hydroxyl radicals have been shown to induce the formation of 8-oxodG within both isolated and cellular DNA exposed to ionizing radiation.[4] In addition, several oxidizing agents, including hydrogen peroxide, Fenton type reagents, and radiomimetic agents, induced the formation of 8-oxodG in naked and cellular DNA.[3,5] Another interesting possibility to generate 8-oxodG within DNA is provided by the use of photodynamic agents such as methylene blue,[6] thiazines,[7] and phthalocyanines.[8] The type II mechanism for photooxidation, which involves the formation of singlet oxygen as the reactive intermediate,[9] appears to be the likely process for the photooxidized formation of 8-oxodG.[10]

We report that riboflavin, an endogeneous cellular photosensitizer,[11] efficiently photoinduces the formation of 8-oxodG according to a new mechanism which does not involve the participation of any reactive oxygen species. Exposure of calf thymus DNA (260 μg/mL) in phosphate buffer containing riboflavin to visible light was found to generate 8-oxodG,[12] whose formation was quantified by using a modification[13] of the high-performance liquid chromatographic–electrochemical detection assay,[14] subsequent to enzymatic digestion of the irradiated biopolymer. The

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(1) Visiting Scientist at Commissariat à l'Energie Atomique, DRFMC/SESAM/LAN, Centre d'Etudes Nucléaires, Grenoble, France.
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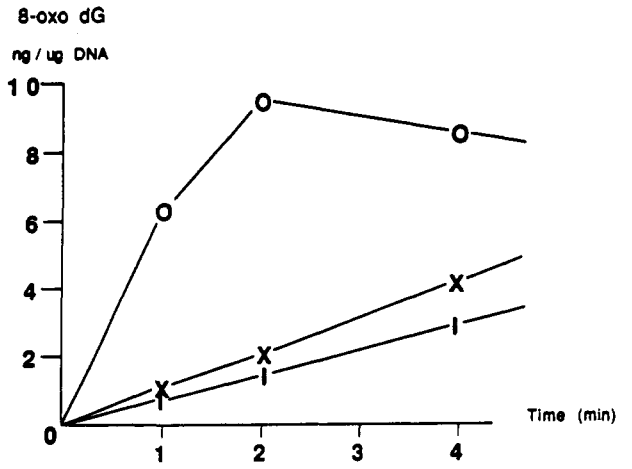

Figure 1. Riboflavin-sensitized formation of 7,8-dihydro-8-oxo-2′-deoxyguanosine within DNA in 10 mM phosphate buffer solutions (pH 7.0) upon exposure to visible light for various periods of time (variations in the measurement of 8-oxodG were found not to exceed 5%): O, under N_2 saturation; ×, under air bubbling; |, in D_2O (air).

yield of 8-oxodG was found to increase linearly with the dose of visible light when the photosensitization experiments were carried out on oxygen-saturated solutions as illustrated in Figure 1. Unexpectedly, irradiation in 80% D_2O aerated solution was found to lead to a slight decrease in the yield of 8-oxodG, suggesting that singlet oxygen is not involved in the riboflavin-mediated induction of this oxidized base damage (vide infra). In contrast it is interesting to note that an about 5-fold enhancement in the formation of 8-oxodG[15] was observed when the photoreactions were performed in oxygen-free aqueous solutions (Figure 1). It should also be added that the presence of either 100 mM mannitol or 10 mM sodium formate, two efficient hydroxyl radical scavengers, in the solution of DNA did not show any significant inhibitory effects on the formation of 8-oxodG (data not shown).

Riboflavin has been shown to be an efficient photosensitizer of the guanine moiety of various nucleic acid components, acting predominantly through type I mechanism as inferred from the results of both final product determination[16] and competitive kinetic analysis[17] experiments. Electron transfer from the guanine moiety to triplet excited riboflavin rather than hydrogen abstraction is likely to be the predominant reaction leading to the formation of a purine radical cation as inferred from photochemically induced dynamic nuclear polarization experiments.[18] As was recently shown on the basis of pulse radiolysis experiments[19] and indirectly confirmed by final product analysis,[5c] the related nucleoside- and nucleotide-derived radical cations predominantly undergo a deprotonation reaction.[20] The resulting neutral radical is identical to those generated by fast dehydration of the hydroxyl radical adduct at the C(4) position of the purine ring of 2′-deoxyguanosine, generating 2,2-diamino-4-[(2-deoxy-β-D-*erythro*-pentofuranosyl)amino]-5(2*H*)-oxazolone as the main final decomposition product in aerated aqueous solutions.[5c] The situation appears quite different for DNA since 8-oxodG becomes a predominant photoproduct, at least in oxygen-free aqueous

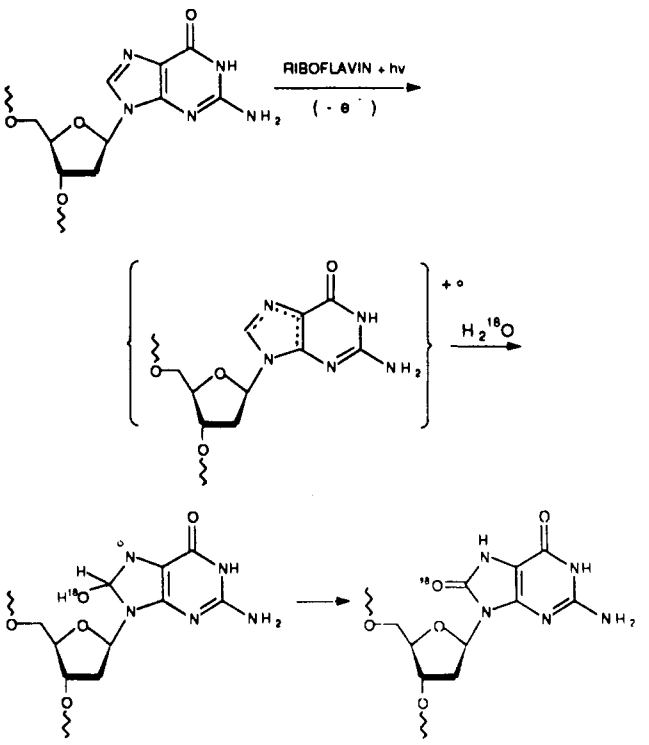

Figure 2. Formation of 7,8-dihydro-8-oxo-2′-deoxyguanosine within DNA upon riboflavin photosensitization.

solution, of the riboflavin-mediated photosensitization reaction. A likely mechanism would be that, in the biopolymer, deprotonation of the guanine radical cation is, at least, partially prevented by base-stacking interactions,[20] allowing a significant hydration reaction to occur. This would lead to the transient formation of 8-hydroxy-7,8-dihydropurin-7-yl radical,[19] the likely precursor of 8-oxodG (Figure 2).

Support for this hypothesis came from the result of an isotopic experiment involving riboflavin-mediated photosensitization of DNA in oxygen-free 98% [^{18}O]H_2O. Electron-impact mass spectrometric analysis of the pentakis(trimethylsilyl) derivative of 8-oxodG obtained by enzymatic hydrolysis of DNA showed characteristic peaks at m/z 645 (M^+) and m/z 630 ($M^+ - CH_3$) together with the base peak at m/z 385 (B + H). The increase by two units in the molecular weight of these peaks with respect to those of unlabeled 8-oxodG (m/z 643, 628, and 383, respectively) is strongly indicative of the incorporation of an ^{18}O atom within the guanine moiety. Similarly, a quantitative incorporation of an oxygen atom from water was observed in the guanine moiety of photosensitized DNA under air, indicating that singlet oxygen is not significantly involved in the riboflavin photoinduction of 8-oxodG.

Further indirect support for the transient formation of the 8-hydroxy-7,8-dihydroguan-7-yl radical is provided by the comparative study of the formation of 8-oxodG within DNA exposed to γ rays in both aerated and oxygen-free aqueous solutions. It is interesting to note that under radiolysis conditions which lead to the transient formation of the above guanine radical through initial OH• addition at C(8), we observed the same oxygen inhibitory effect on the formation of 8-oxodG.[21] However, it should be mentioned that, in the photosensitization experiments, this may be partly explained by the efficient quenching of triplet riboflavin by molecular oxygen as inferred from the results of flash photolysis studies of related lumiflavin mononucleotide.[22]

(15) The percentage of conversion of the guanine moiety into 8-oxodG reaches a maximum of 4% after 2 min of irradiation; then, it is likely that 8-oxodG becomes a competitive substrate with respect to guanine for further photosensitized decomposition.

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(21) Exposure of DNA (260 μg/mL) in phosphate buffer solutions (pH 7.0) to 50 Gy of γ rays was found to generate 8-oxodG in 0.14% and 0.75% yields under oxygen and nitrogen atmospheres, respectively. In contrast, and this illustrates the complexity of the radical reactions of purine components, we observed that the yield of radiation-induced formation of 8-oxodG in 2′-deoxyguanosine is enhanced by a factor of 3–4 when the irradiation is carried out on aerated aqueous solutions.

Work is currently in progress in our laboratories to further investigate the role of the polymeric structure and conformation of DNA on the chemical reactions of the guanine radical cation, one of the two main intermediates of the direct effects of ionizing radiation.[23]

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Supplementary Material Available: Experimental details of the photosensitization experiments and characterization of 7,8-dihydro-8-oxo-2'-deoxyguanosine (2 pages). Ordering information is given on any current masthead page.

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A Short Synthesis of (+)-Lycoricidine

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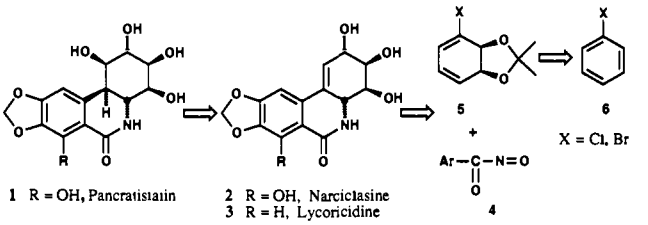
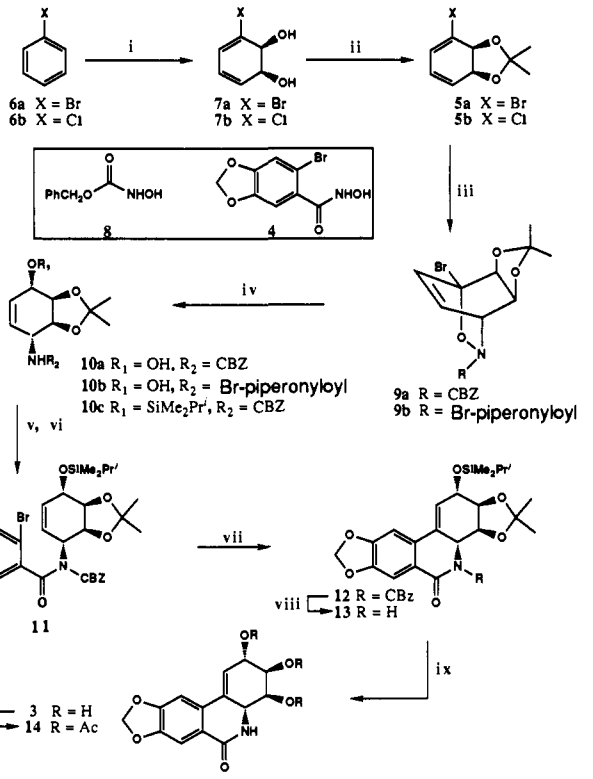
The narcissus alkaloids pancratistatin (**1**), narciclasine (**2**), and lycoricidine (**3**) are members of the Amaryllidaceae family and possess considerable medicinal potential because of their wide range of biological activities. Since their isolation in the late 1960s[1] and subsequent determination of their diverse cytotoxic properties,[2] there has been a focused effort to provide the most promising of these alkaloids, pancratistatin (**1**), to the medical community.[3] Extremely low natural abundance as well as practical complications in separation of the desired compound from other plant constituents diminishes the probability of reasonable supply of this and related compounds by means of isolation.[4] Clearly there is justification for synthetic effort in this area if the following criteria can be met: (a) cost-effective preparation, (b) environmentally benign synthetic protocol that would make the synthesis amenable to a large-scale production, and (c) stereorational and general design for all of the members of this class, especially the compounds named above.

Despite the many valiant synthetic approaches to these alkaloids[5] and several total syntheses,[6] no preparation of fewer than

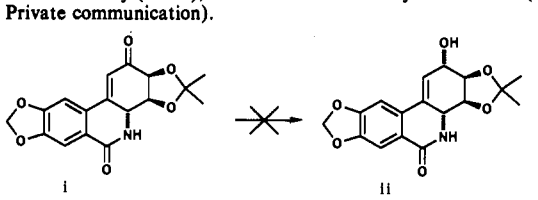
Scheme I. A General Approach to Narcissus Alkaloids



Scheme II[a]



[a] Reagents: (i) *Pseudomonas putida*; (ii) DMP, acetone, *p*-TsOH; (iii) **4** or **8**, Bu_4NIO_4, CH_2Cl_2; (iv) Al(Hg), THF; (v) $ClSiMe_2Pr^i$, Im, CH_2Cl_2; (vi) BuLi, THF, -78 °C; then Br-piperonyloyl chloride; (vii) $Pd(OAc)_2$, Tl(OAc), DIPHOS, anisole; (viii) Pd(C), cyclohexene, EtOH; (ix) CF_3CO_2H, 0 °C, (x) Ac_2O, py.



* To whom correspondence should be addressed. Recipient of the American Cyanamid Faculty Research Award, 1992.

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