Photogeneration of an *o*-quinone methide from pyridoxine (vitamin B_6) in aqueous solution

Darryl Brousmiche and Peter Wan*†

Department of Chemistry, Box 3065, University of Victoria, Victoria, British Columbia, Canada V8W 3V6

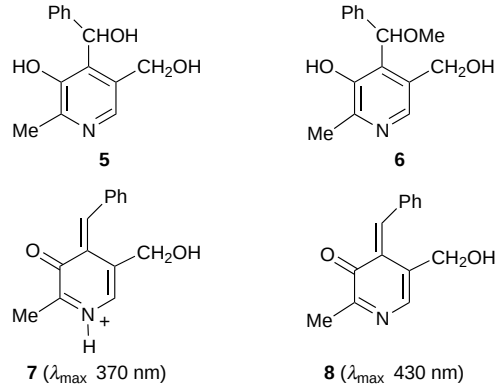
Photolysis (254, 266 or 308 nm) of pyridoxine (vitamin B_6) in aqueous solution gives an *o*-quinone methide efficiently which is trapped by MeOH and ethyl vinyl ether.

Over the past several years there has been a great deal of interest in the biological chemistry of quinone methides‡ (QMs), due mainly to their toxicological properties against both normal and cancerous cells, as well as their proposed intermediacy in the formation of many biologically important polymers (melanin, lignin and insect cuticles).[1,2] These properties have been attributed to the electrophilic nature of QMs which can result in both alkylation of DNA and amino acids, and in self polymerization. QM reactivity in biological systems has been studied extensively with QM precursors such as butylated hydroxytoluene (BHT), eugenol, mitomycin C and the anthracyclines.[1,2] The formation of these QMs has traditionally been *via* thermal reaction, through the use of enzymes *in vivo*, oxidation of phenols, or nucleophilic substitution of silyl or quartenary ammonium groups, amongst others.[1–5] Recently, however, our group has developed a clean and efficient photochemical method for the formation of *o*-, *m*- and *p*-QMs *via* UV (254 nm) photolysis of hydroxybenzyl alcohols.[6] Pyridoxine **1** (vitamin B_6) provides an ideal system for the continued study of photochemically generating QMs as it is a biologically relevant molecule and it contains the required hydroxybenzyl alcohol-type system. Moreover, it allows for a competition between the formation of an *o*-QM *vs*. a *m*-QM due to the presence of a second CH_2OH group *meta* to the aromatic hydroxy group. Formation of a QM from **1** *via* exposure to UV light§ could lead to cell and DNA damage which to the best of our knowledge has not been explored.

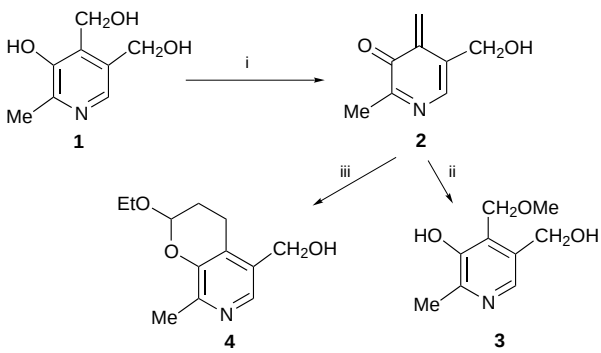
Photolysis of **1** and **5** (10^{-4} M; Rayonet photoreactor; 254 nm; *ca*. 15 °C; argon) in 1 : 1 MeOH–H_2O gave the corresponding methyl ethers **3** and **6** ($\phi \approx 0.2$ for reaction of **5**) cleanly (> 40%) at low conversion (Scheme 1). The location of the methoxy group was unambiguously assigned based on NOE data. Previous work by this group[6] has shown that the quantum yield of methyl ether formation from *m*-QMs is approximately half of that from the corresponding *o*-QM. Thus, if the *m*-QM was formed competitively with the *o*-QM of **1**, it would be easily discernable by formation of the corresponding methyl ether. Interestingly, none of the product studies show any evidence for the formation of the *m*-QM, thereby indicating that *o*-QM **2** is formed selectively.

Photolysis of **1** in 1 : 1 H_2O–MeCN with 0.26 M ethyl vinyl ether (EVE) gave the Diels–Alder adduct **4**¶ cleanly (> 70%) in a regioselective fashion (Scheme 1). Formation of **4** is only possible through the intermediacy of a 1,4-dipolar species and therefore provides conclusive evidence for the efficient photogeneration of *o*-QM **2**.

We have shown[6] that laser flash photolysis (LFP) provides an effective method for the direct detection of appropriately substituted *o*-, *m*- and *p*-QMs. The choice of **5** for these studies



will enable the resulting QM to be more readily detectable due to the expected longer wavelength of absorption and correspondingly larger extinction coefficient. LFP of **5** (λ_{exc} = 266 or 308 nm, YAG or excimer lasers, < 20 mJ per pulse) in 100% H_2O (oxygen purged to remove triplet states and possible radical species) yielded a species at 370 nm, which is observed at pH 7 (τ > 10 ms) and 12 (τ *ca*. 2 ms) (Fig. 1). When LFP experiments were carried out at pH 1, this band can be seen



Scheme 1 *Reagents and conditions*: i, *hν*; ii, MeOH–H_2O (1 : 1); iii, EVE (0.26 M), MeCN–H_2O (1 : 1)

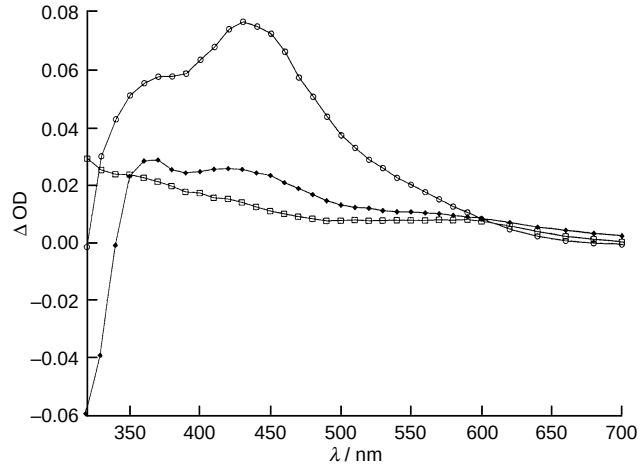


Fig. 1 LFP spectra (λ_{max} 308 nm; 100% H_2O; O_2 purged) of **5** at (□) pH 1, (◆) 7 and (○) 12

below 350 nm (τ < 50 μs). In all three cases, however, there is rapid drop-off signal (bleaching) as the wavelength of observation approaches the region where the ground state material absorbs,‖ thereby masking the true extent of this band. Another species with λ_{max} at 430 nm is apparent in the pH 7 (τ > 10 ms) and 12 (τ *ca*. 2 ms) spectra (Fig. 1). The relative intensities of the bands change with pH (there is no 430 nm band at pH 1), with an 'inflection' point in the pH 4–7 region. We have assigned the 370 nm band to *o*-QM **7** which is protonated at nitrogen. This is clearly the most basic site of the *o*-QM; protonation at oxygen (the other possible basic site) is unlikely at this pH as this would generate a highly reactive diarylmethyl carbocation. The 430 nm band is thus assigned to neutral *o*-QM **8**.

The lifetime of simple QMs is expected to be lower in basic and acidic media than at pH 7, due to attack by either H^+ or OH^- at the appropriate sites of the QM (carbonyl oxygen and exocyclic vinyl carbon, respectively). The transient lifetimes at pH 7 for both **7** and **8** are approximately five times longer than at pH 12, while the lifetime of **7** at pH 1 is approximately two hundred-fold shorter than in neutral solution, consistent with QM reactivity. The heteroatom present in the pyridine ring will also have an influence on the QM lifetime at low pH, where it is fully protonated: it should act a powerful electron-withdrawing group, making the QM more reactive, and this is consistent with the much shorter lifetime observed in pH 1.

It has been shown *via* LFP,[6] in the case of the simple hydroxybenzhydrol systems, that at elevated pH (> 10) QMs are formed more efficiently (higher quantum yields) than at neutral pH, as the phenolate is already present. This appears to be verified in our system as much stronger signals are observed when LFP experiments are carried out at pH 12. Moreover, product studies on the formation of **3** from **1** (1 : 1 MeOH–H_2O) at pH 7 and 12 gave yields of 9 and 15% (performed under low conversion conditions and in which samples received the same UV dose), respectively, consistent with the notion that the QM is more efficiently formed at high pH.

In summary, we have shown that the corresponding *o*-QMs of **1** and **5** can be formed readily in aqueous solution *via* irradiation with UV light. The *o*-QM is formed selectively in all cases, although *m*-QM formation is a possibility. The QM can exist as the free base or in the iminium ion form, which differ in reactivity. The pH of the solvent leads to significant differences in both the lifetime and amount of QM formed, with the QM being longest-lived at pH 7. These results suggest the possibility that **2** can be formed inside biological systems, leading to cell and DNA damage. We believe this to be the first example of the photogeneration of a quinomethane from an important biomolecule (*i.e.* **1**). Since pyridoxal is the active form of pyridoxine, and is known to be extensively hydrated in aqueous solution, we are investigating the possibility of analogous photochemistry for this compound.

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Notes and References

† E-mail: pwan@uvic.ca

‡ IUPAC name: quinomethanes.

§ *o*-QM **2** has been generated thermally from **1** at 130–190 °C and trapped with various nucleophiles (ref. 7).

¶ *Selected data* for **4**: δ_H (300 MHz, $CDCl_3$) 1.07 (t, *J* 7.4, 3 H, CH_3CH_2O), 1.96 (m, 2 H, Ar CH_2CH_2), 2.25 (s, 3 H, $ArCH_3$), 2.70 (t, *J* 7.4, 2 H, $ArCH_2CH_2$), 3.65, 3.78 [two sets of dq (diastereotopic Hs), *J* 7.4, 10.3, 2 H, CH_3CH_2O), 4.50 (s, 2 H, $ArCH_2OH$), 5.33 (t, *J* 3.3, 1 H, CH_3CH_2OCH), 7.78 (s, 1 H, ArH).

‖ According to UV–VIS data, **5** is fully protonated (at the nitrogen) at pH 1 (λ_{max} 290 nm), is in its free base form at pH 7 (λ_{max} 325 nm) and is in its ArO^- form at pH 12 (λ_{max} 310 nm), in accordance with literature data for the parent **1** (ref. 8).

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