Communications to the Editor

Lifetime and Reactivity of an Ultimate Tamoxifen Carcinogen: The Tamoxifen Carbocation

Cristina Sanchez,[†] Shinya Shibutani,[‡] Lakkaraju Dasaradhi,[‡] Judy L. Bolton,[§] Peter W. Fan,[§] and Robert A. McClelland*[,†]

Department of Chemistry, University of Toronto Toronto, Ontario M5S 3H6, Canada
Department of Pharmacological Sciences State University of New York at Stony Brook, Stony Brook New York 11794-8651
Department of Medicinal Chemistry and Pharmacognosy University of Illinois at Chicago, Chicago Illinois 60612-7231

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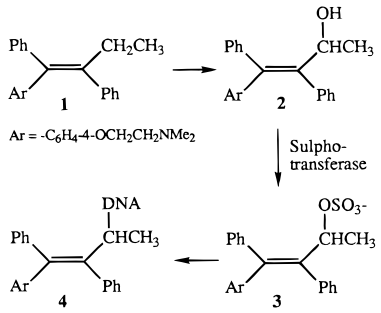
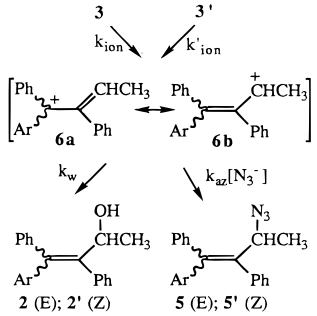
The antiestrogen tamoxifen **1** is widely used in the treatment of breast cancer,[1] and has received considerable recent publicity as a prophylactic agent for women considered at risk of this disease. Concerns have been expressed since the compound is a hepatocarcinogen in rats,[2] and there is an increase in the incidence of endometrial cancer in women treated with the drug.[3] Like most carcinogens, tamoxifen gives rise to DNA adducts, both in animal models[4] and in human patients.[5] Miscoding[6] and mutagenic[7] events have been associated with these adducts. Metabolic activation is required before adduct formation, and while there are several metabolic pathways, much recent research has focused in Scheme 1 as the principle source of DNA binding. The key intermediate is α-hydroxytamoxifen **2**, a known metabolite[8] that produces a pattern of DNA adducts similar to those of the parent.[8c] A further activation event, sulfation, is also indicated by such observations as DNA adducts being increased by sulfotransferase[9] or inorganic sulfate,[10] but reduced by sulfotransferase inhibitors.[10,11]

Scheme 1



Scheme 2



DNA binding occurs principally with the NH_2 group of guanine.[8e,8f,12] This is typical of delocalized carbocations[13] and is consistent with a model[8a] where sulfation provides a leaving group for an S_N1 solvolysis. In fact the cation so formed appears to be a relatively stabilized allylic system. In this paper we report the direct observation of this cation in aqueous solution, using the technique of laser flash photolysis (LFP). The cation is indeed relatively long-lived, at least on the scale of other S_N1 intermediates.

The sulfate **3** and its (*Z*)-isomer **3′** solvolyze in water/acetonitrile mixtures to the same 2:1 ratio of **2** and its (*Z*)-isomer **2′** (Scheme 2). Rate constants[14] depend on solvent polarity; plots of log k_{solv} versus the solvent polarity parameter Y[15] have slopes of 0.7. A short extrapolation gives half-lives in water alone of 6 (**3**) and 10 (**3′**) seconds at 20 °C.

While the solvent dependence and geometric isomerization are clearly indicative of S_N1 solvolysis, as noted previously,[8e] convincing evidence comes from experiments in the presence of sodium azide (50−200 mM). The products **5** and **5′** (1:1 ratio

[†] University of Toronto.
[‡] SUNY Stony Brook.
[§] University of Illinois at Chicago.

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(14) Sulfates were prepared as described in ref 12. Rate constants for ground state solvolysis were obtained from 30 to 80% (by volume) acetonitrile by following the disappearance of the sulphate by HPLC. LFP experiments were performed by injecting the substrates (∼10 mM in dry DMSO) directly into the LFP cuvette to give a solution ∼50 μM and irradiating the solution within 15 s. In 40% acetonitrile, half-lives of the ground-state solvolysis are ∼100 s.
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Table 1. Cation λ_{max} and Lifetime[a] in Water

cation	λ_{max}	lifetime
4-$MeOC_6H_4CH_2^+$	320[b]	5 ns[c,d]
4-$MeOC_6H_4PhCH^+$ [e]	455	500 ns
4-$MeOC_6H_4Ph_2C^+$ [f]	470	700 μs
$ArPhC^+CPh{=}CHCH_3$ (**6**)	460	125 μs[g]
		22 μs[h]
Ph_2CH^+	440[e]	0.8 ns[i]
Benzo[*a*]PyrDiolC^+ [i]		50 ns[j]
2-$FlNH^+$	440	30 μs[k]

[a] The lifetime is the reciprocal of the first-order rate constant for the decay of the cation in the solvent. Lifetimes in acetonitrile/water mixtures are very similar to ones in pure water (refs 16c,h). [b] Reference 16a. [c] Reference 16b, in 1:1 water/2,2,2-trifluoroethanol. [d] Azide-clock method. [e] Reference 16c. [f] Reference 16d. [g] Neutral amine in Ar. [h] Ammonium form in Ar. [i] Reference 16e. [j] Cations obtained on ring opening of benzo[*a*]pyrene diol epoxides, refs 16f,g. [k] The 2-fluorenylnitrenium ion, ref 16h.

from both precursors) are now also formed but with no change in the rate constant for disappearance of the sulfate. Thus, the rate-determining ionization occurs before the product-determining step, the capture of the carbocation. The selectivity k_{az}/k_w is $(4.6 \pm 0.5) \times 10^4$ M^{-1} measured with **3** and $(4.3 \pm 1.1) \times 10^4$ M^{-1} with **3′** (in 40% acetonitrile, 20 °C, pH 9.3).

LFP of **3** in 40% acetonitrile[14] gives a transient species with absorbance in the region 400−550 nm, λ_{max} at ∼460 nm and a single-exponential decay. The identical transient is obtained with **3′** as the precursor. No such transient is seen with **2** and **2′** or with aged solutions of the sulfates, indicating that its formation requires the leaving group. Azide ion shows effective quenching, with a ratio k_{az}/k_w at pH 9.3 of $(4.6 \pm 0.2) \times 10^4$ M^{-1}, calculated from the measured absolute rate constants of $(3.62 \pm 0.05) \times 10^8$ M^{-1} s^{-1} and $(7.84 \pm 0.05) \times 10^3$ s^{-1}. This is unequivocal evidence that the transient is the cation **6**, since the ratio is the same as that observed in the ground-state solvolysis. Rate constants do depend on pH, with k_{obs} following the equation $(k'_w[H^+] + k_wK_a)/([H^+] + K_a)$. This behavior is associated with protonation of the tertiary amine group. The constant k_w corresponds to a lifetime of 125 ms and represents the decay at high pH where this amine is in the free base form. The constant k'_w (lifetime = 22 ms) refers to the decay in acid where the amine is protonated, so that the intermediate is a dication. The constant K_a is the acidity constant of the ammonium group in the dication. The pK_a is 6.6; this low value may reflect a substantial localization of the positive charge of the carbocation on the oxygen of the aminoethoxy group. It could also be related to the 40% acetonitrile which is necessary in our experiments.

The identical products and transient being observed with both isomers **3** or **3′** indicate that the isomeric forms of the cation are rapidly equilibrating. On the basis of lifetimes obtained with LFP, 10^6 s^{-1} is a lower limit for this interconversion. Thus, the central bond is largely a single bond, i.e., as if the cation mainly existed as the resonance form **6a**. This is certainly consistent with the λ_{max}, which is very much like that of 1,1-diarylalkyl cations (Table 1). AM1 calculations in fact show that there is considerable steric crowding, such that the $C(Ph){=}CHCH_3$ unit twists considerably out of the plane of the $ArPhC^+$ unit. This would further reduce the contribution of structure **6b**. In this respect it is interesting that reactions with nucleophiles still occur as if **6b** were the intermediate. We, and others, have not detected any products derived from attack at the C^+ center of **6a**.[17]

Previous work by the Stony Brook authors has shown that the sulfate **3** reacts in the presence of 2′-deoxyguanosine (dG) to form four diastereomeric products with the guanine NH_2 group attached at the tamoxifen α-carbon ((*E*)-α(R), (*E*)-α(S), (*Z*)-α(R), and (*Z*)-α(S)).[12] To investigate how dG and water compete for the intermediate cation, LFP experiments were performed with the former present. The nucleoside in fact causes only small increases in rate constant, for example, a 30−40% increase at 20 mM dG. Plots of k_{obs} versus [dG] are linear with slopes $k_{dG} = (2.3 \pm 0.3) \times 10^5$ and $(4.0 \pm 0.6) \times 10^5$ M^{-1} s^{-1} at pH 7.4 and 6.3 respectively, and ratios k_{dG}/k_w of 20 ± 5 (pH 7.4) and 13 ± 5 M^{-1} (pH 6.3), respectively. These are modest selectivities, but they do mean that at 1 mM dG, for example, ∼1−2% of the cation is trapped by dG to form adducts.[18]

In summary, this work reports the direct determination of the aqueous lifetime and dG reactivity of the carbocation obtained by metabolism of tamoxifen. This cation has been implicated as the source of the DNA binding observed with this drug, and the present results add considerable support to this model. As shown in the comparisons in Table 1, the tamoxifen cation is a relatively long-lived electrophile by carbocation standards, approaching the kinetic stability of stabilized triarylmethyl cations. Although there are many factors that determine the carcinogenic potential of a given compound, the present results show that tamoxifen can be metabolized to a cation with a lifetime similar to, or even longer, than those of electrophiles derived from established carcinogens such as 2-aminofluorene and benzo[*a*]pyrene. The cation is also capable of being trapped by the relatively weak nucleophile 2′-deoxyguanosine in an aqueous solution.

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Supporting Information Available: Spectrum of transient tamoxifen carbocation, rate-pH profile for decay, rate constants for reaction in the presence of dG, and k_{obs}-dG plots, and characterization of α-azidotamoxifens (4 pages, print/PDF). See any current masthead page for ordering and Web access instructions.

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(17) With both solvent alone where the alcohols are the products and at an azide concentration where the azides are the predominant products, there are no additional peaks in the HPLC that could be ascribed to such products. Since these still retain a styrene chromophore, they would be detectable, certainly at a 5−10% level.

(18) (a) An interesting comparison is with the 2-fluorenylnitrenium ion, the electrophile derived from the carcinogen 2-aminofluorene. This cation has a lifetime in water of 30 ms, similar to that of the tamoxifen cation, but k_{dG} is 7.6×10^8 M^{-1} s^{-1}.[18b] Thus, $k_{dG}/k_w = 2.5 \times 10^4$ M^{-1}, and 96% of the cation reacts with dG in a 1 mM solution. (b) McClelland, R. A.; Gadosy, T. A.; Ren, D. *Can. J. Chem.*, accepted for publication.