The tamoxifen cation reacts to give indene products

Cristina Sanchez and Robert A. McClelland

Abstract: The tamoxifen carbocation (Ph(Ar)C=CPh-CH^+-CH_3, Ar = 4-$Me_2NCH_2CH_2OC_6H_4$) is generated from acetate and sulfate precursors by S_N1 ionization in water. The cation exists in (*E*) and (*Z*) forms which equilibrate before reaction. The major products are the α-hydroxytamoxifens Ph(Ar)C=CPh-CHOH-CH_3, both (*E*) 64% and (*Z*) 29%, with the ratio independent of the configuration of the starting ester. Two minor products with a total yield of 7% account for the rest of the products. These have been characterized as indenes derived from intramolecular cyclization, a 4.5% yield of the indene derived from cyclization into the Ar ring with 2.5% due to cyclization into the phenyl ring. Experiments in acid solutions (0.01–0.1 M HCl) starting with pure (*E*)- or (*Z*)-α-hydroxytamoxifen reveal that the two alcohols equilibrate. This occurs by H^+-catalyzed formation of the carbocation followed by water capture. Occurring about 10-fold slower than this isomerization is an irreversible process resulting in the two indenes. This cyclization will result in the destruction of the α-hydroxytamoxifens upon exposure to acidic conditions and also makes the direct observation of the tamoxifen carbocation under super-acid conditions difficult, if not impossible. The indenes do form in low yield whenever the tamoxifen carbocation is generated from an S_N1 precursor. Thus these products could serve as markers for the formation of the tamoxifen carbocation in cellular systems or in in vivo experiments.

Key words: carbocation, indene, allylic cation, isomerization.

Résumé : On a généré le carbocation tamoxifène, [Ph(Ar)C=CPHC$H^+$$CH_3$, Ar = 4-$Me_2NCH_2CH_2OC_6H_4$] à partir de précurseurs acétate et sulfate, par le biais de réactions S_N1, dans l'eau. Le cation existe dans les formes (*E*) et (*Z*) qui se mettent en équilibre avant de réagir. Les produits principaux sont les α-hydroxytamoxifènes Ph(Ar)C=CPH-CHOH-CH_3, *E*, 64 % et *Z*, 29 %, dont le rapport est indépendant de la configuration de l'ester de départ. Deux produits mineurs, qui correspondent à un rendement total de 7 %, forment le reste des produits. Il s'agit d'indènes qui découlent d'une cyclisation intramoléculaire, dont 4,5 % de l'indène dérivé d'une cyclisation dans le noyau aromatique et 2,5 % dû à une cyclisation dans le noyau phényle. Des expériences faites en milieu acide (0,01 à 0,1 M, HCl) faites à partir des (*E*)- ou (*Z*)-α-hydroxytamoxifènes purs révèlent que les deux alcools se mettent en équilibre. Ceci résulte d'une formation acidocatalysée du carbocation, suivie par une fixation d'eau. La vitesse de réaction du processus irréversible conduisant aux deux indènes est dix fois plus faible. Cette cyclisation conduit à la destruction des α-hydroxytamoxifènes lorsqu'ils sont exposés à des conditions acides et elle rend pratiquement impossible toute observation directe du carbocation tamoxifène dans des conditions superacides. Les indènes se forment avec de faibles rendements chaque fois que le carbocation tamoxifène est généré par le biais d'une réaction S_N1. Ces produits pourraient donc servir de marqueurs pour la formation du carbocation tamoxifène dans des systèmes cellulaires ou dans des expériences in vitro.

Mots clés : carbocation, indène, cation allylique, isomérisation.

We have recently reported the direct observation of the tamoxifen carbocation in aqueous solution through the use of the technique of laser flash photolysis (LFP) (1). This cation appears to be responsible for the formation of DNA adducts observed in both animal models (2, 3) and in women treated with the drug tamoxifen (4–7). Such adducts could explain the hepatocarcinogenicity observed in rats (8–10), and the small number of endometrial cancers in women (11–13). The metabolic pathway of Scheme 1 (14–20) accounts for the formation of the cation. The initial metabolic event involves allylic hydroxylation of the parent drug (**Tam**) to form *E*-α-hydroxytamoxifen **E-TOH**, a known metabolite. This is followed by sulfotransferase-mediated conversion to the sulfate **E-TOSO$_3^-$** (21–24). This ester is quite labile in water, undergoing relatively rapid S_N1 ionization, for example, with a half-life around 10 s in water at 20°C (1).

In comparison with other carbocations (25), the tamoxifen intermediate is relatively long-lived. Limiting lifetimes (1/k(decay)) are 160 μs (side-chain amine as free base) and 40 μs (amine protonated).[2] An interesting feature is that the

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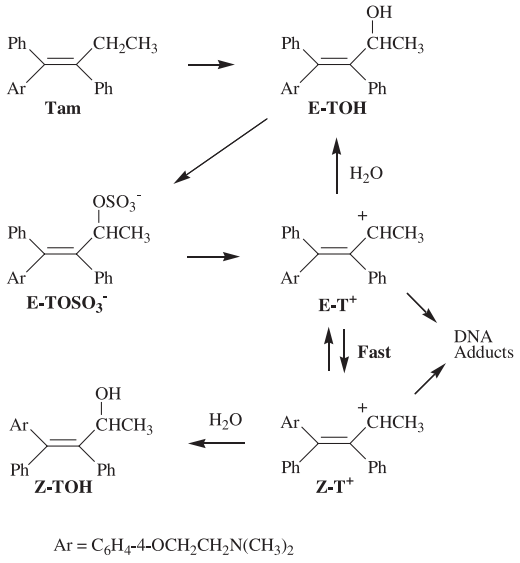
[2]These numbers are based on measurements in wholly aqueous solutions (E. Sauer, C. Sanchez, and R.A. McClelland, unpublished data). Our previously reported lifetimes (1) of 125 μs and 22 μs refer to a 60:40 water:acetonitrile mixture.

Scheme 1.



intermediate appears to be a rapidly equilibrating mixture of the two isomers **E-T**$^+$ and **Z-T**$^+$. Thus starting from **E-TOSO$_3^-$**, a mixture of the **E-TOH** and **Z-TOH** is observed (1, 18). The same ratio of these two alcohols is obtained with the (*Z*)-sulfate as the starting material (1). Mixtures of (*E*):(*Z*) products are also observed when the cation reacts with azide ion (1) and with the NH_2 group of guanine (18, 19, 26).

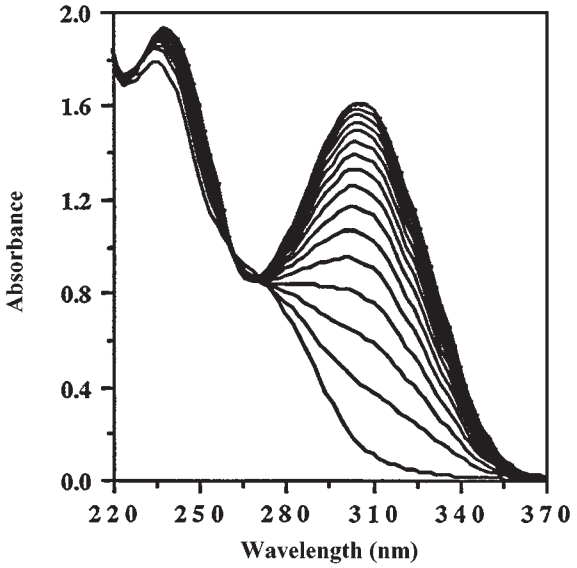
The rapid interconversion can be explained by noting that the cation is better viewed as an α-vinyl substituted diarylmethyl cation such as **E-T**$^{+\prime}$, i.e., where there is little double bond character in the central C—C bond. In fact AM1 calculations show that steric congestion forces the vinyl group to rotate 60–70 degrees out of the plane of the C$^+$-center shown in structure (1). Despite this rotation however we have been unable to detect any products derived from addition of nucleophiles to the C$^+$-center α to the the two aryl groups. In searching for these, we did note two minor peaks in the HPLC chromatogram eluting at considerably longer retention time than the alcohols **E-TOH** and **Z-TOH**. Experiments under acidic conditions where the two alcohols interconvert then revealed that with time an irreversible reaction occurs to produce these same peaks, ultimately as the only peaks in the chromatogram. This provided conditions for isolation of the products responsible. In this paper we provide the details of these experiments, including the characterization of the new products. They turn out to be derived from intramolecular cyclization in the tamoxifen cation. A kinetic analysis shows that the cyclization competes with water addition. Thus, the cyclic products can be taken as an indicator of the formation of the tamoxifen cation.

Results and discussion

Reaction of α-hydroxytamoxifens in acid

The reactions of **E-TOH** and **Z-TOH** in acidic aqueous solutions were first seen in absorption spectra recorded as a function of time in such solutions. As illustrated by the example in Fig. 1, these show the slow growth of a new peak

Fig. 1. Spectra obtained with 2×10^{-4} M (*E*)-α-hydroxytamoxifen (**E-TOH**) in 0.1 M HCl (20°C). Spectra were recorded every 20 min. The absorbance increase at 310 nm fits to a single exponential with a rate constant of 1.1×10^{-4} s^{-1}.

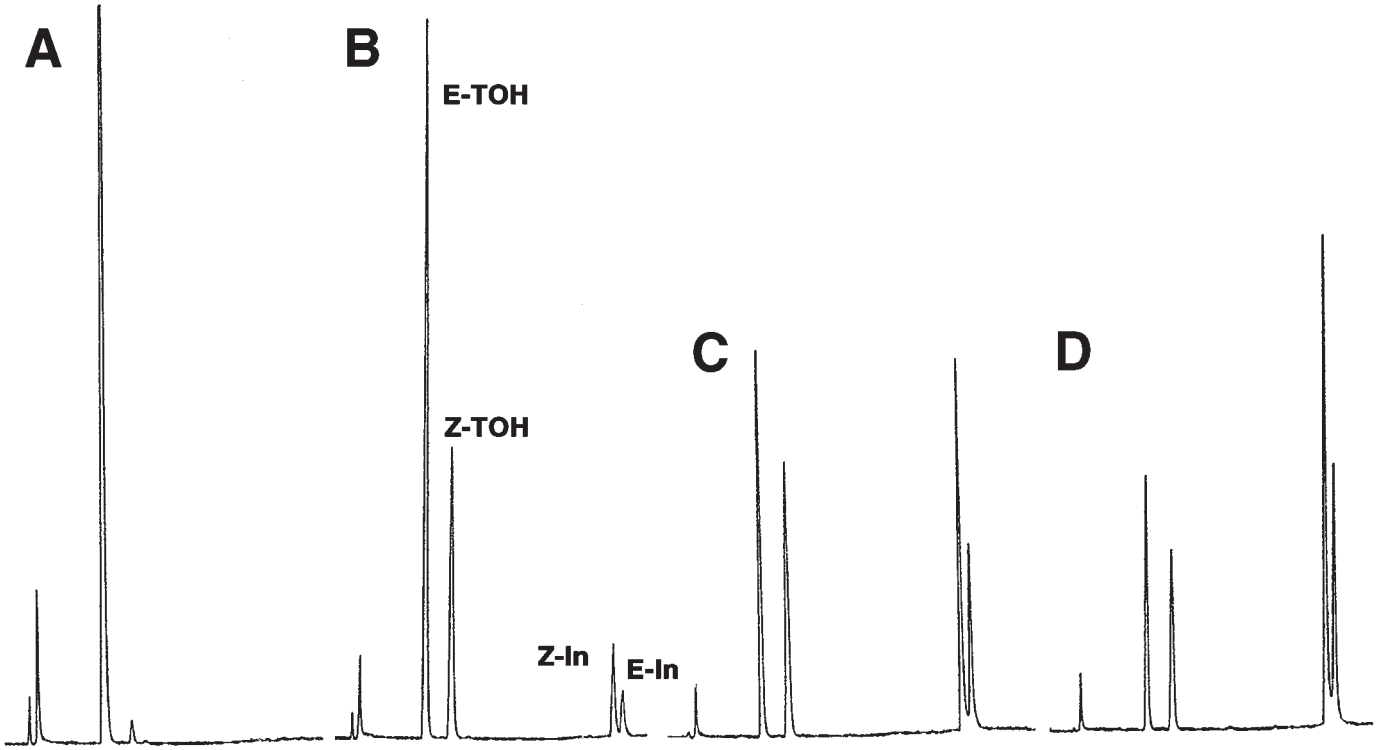


with λ_{max} at 310 nm. The increase at 310 nm follows good single exponential kinetics and there are reasonable isosbestic points at 260 and 270 nm. This suggests a first-order conversion of the starting alcohol to product (or products). However, HPLC chromatograms recorded on the same solution show that the behavior is more complicated. Starting with **E-TOH** as in Fig. 2, this is found to convert to an approximately 1:1 ratio with its (*Z*)-isomer **Z-TOH**, whose HPLC peak appears at a slightly longer retention time (see labels in Fig. 2B). The same interconversion to an ~1:1 **E**:**Z** ratio is obtained with **Z-TOH** as the starting alcohol. At the same time as the isomerization, two new peaks at longer retention time are appearing. This process is considerably slower, and, as will be apparent later, it accounts for the spectral changes in the UV spectra. Ultimately, the two alcohols completely disappear, and the chromatogram contains only the two peaks for the new products.

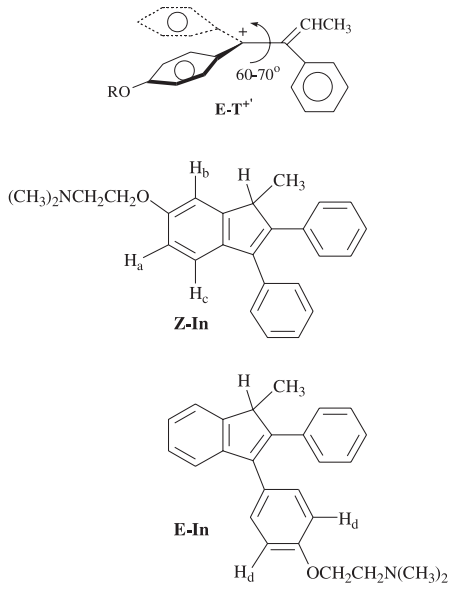
Identification of Indenes

The new products were identified by working on a larger scale in 1 M HCl, and waiting a sufficient time so that there were none of the α-hydroxytamoxifens remaining. An HPLC chromatogram of this mixture was very similar to that obtained at long times on the smaller scales, with the peak at shorter retention time having twice the area of that at the longer retention time. The products were then isolated after neutralization and extraction. Column chromatography unfortunately failed to provide any separation whatsoever, so that identification had to be carried out on the mixture. The mass spectrum showed a molecular ion at *m*/*z* 369 MS, with high resolution MS providing a molecular formula of $C_{26}H_{27}NO$. This indicates that the products are derived from loss of water from the starting material. Considering the chemistry, i.e., that these products arise under acidic conditions where the tamoxifen cations **Z-T**$^+$ and **E-T**$^+$ are being formed, the conclusion is that they are isomeric indenes **Z-In** and **E-In** arising from intramolecular cyclization in the

Fig. 2. HPLC chromatograms (detector at 260 nm) obtained with 2×10^{-4} M (*E*)-α-hydroxytamoxifen (**2**) in 0.1 M HCl (20 ± 1°C). Solutions were injected at 0.8 (**A**), 23.8 (**B**), 95.3 (**C**), and 164.3 (**D**) min after preparation of the solution.



cations. This of course is well established chemistry of phenyl-substituted allylic cations (28–34). The surprising aspect of the present study is that the intramolecular reaction competes with the nucleophilic addition of water. Previous studies of the cyclization have been carried out under strongly acidic conditions where the competing reagents were weakly nucleophilic.



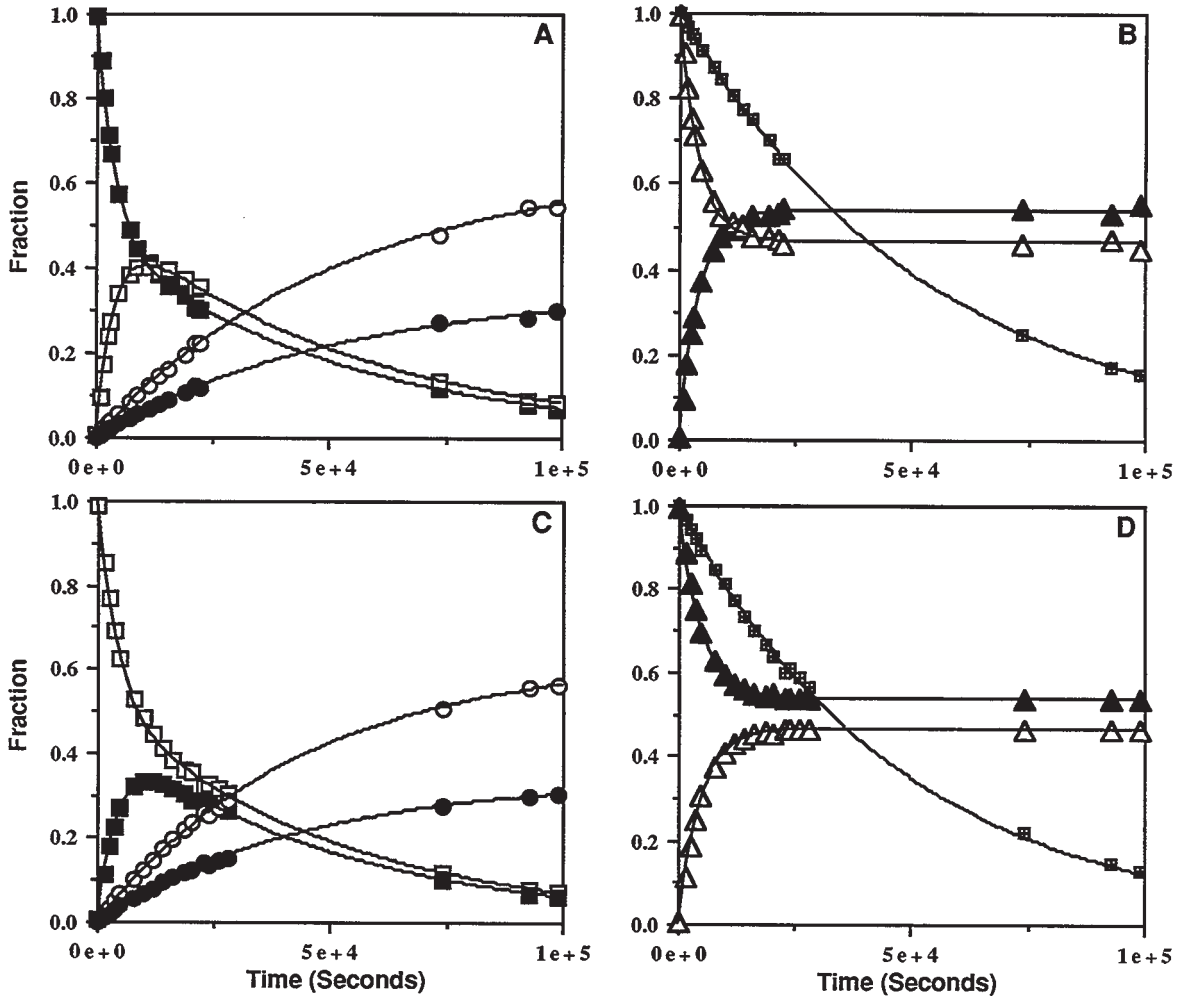
The ^{1}H NMR spectrum of the mixture is consistent with this interpretation. This also shows that the major product at shorter retention time is obtained upon cyclization into the alkoxy-substituted aromatic ring; i.e., the major product is **Z-In**. The indicator here is the aromatic protons next to the alkoxy group, which can be distinguished from the complex multiplet associated with the other aromatic protons. In the indene **Z-In** these protons are nonequivalent. The proton labelled H_a in the representation above appears as a doublet of doublets at 6.87 ppm, with coupling constants of 2.2 and 8.2 Hz representing coupling with H_b and H_c, respectively. The proton H_b is a doublet at 7.18 ppm, with a coupling constant of 2.2 Hz. In **Z-In**, these protons (H_d) are equivalent, and appear as a doublet at 6.95 ppm with a coupling constant of 8.6 ppm. The integration of the doublet of doublets at 6.87 (H_a) vs. the doublet at 6.95 is ~1:1. Since two protons provide the latter doublet, the ratio of **Z**-In**E-In** is ~2:1, so that **Z-In** is the major product eluting at the earlier retention time in the HPLC chromatograms.

Quantitative analysis

The HPLC chromatograms of Fig. 2 show that there are four species present during the reaction that is occurring in acids. The isosbestic point at 260 nm must therefore mean that these four have essentially identical extinction coefficients at this wavelength. This in turn suggests that by operating at 260 nm in the HPLC, the sensitivities of the four peaks are the same, or, in other works, relative peak areas can be converted directly to relative molar amounts. This was verified for **E-TOH** and **Z-TOH** by injecting known amounts of authentic alcohols. It was further observed that the sum of the areas of the four peaks was constant throughout the reaction, within an experimental error of ±3%. This of course means that the products **E-In** and **Z-In** must have the same sensitivity as the alcohols.

Working on the basis of the HPLC sensitivities being the same at 260 nm, Figs. 3A and 3C were constructed. These

Fig. 3. HPLC analysis of solutions of (*Z*)-α-hydroxytamoxifen (**Z-TOH**) (Figs. 3A, 3B) and (*E*)-α-hydroxytamoxifen (**E-TOH**) (Figs. 3C, 3D) in 0.02 M HCl, 0.08 M NaCl (20°C). Legend for Figs. 3A, 3C: (■) — [**Z-TOH**]:[**Total**], (□) — [**E-TOH**]:[**Total**], (○) — [**Z-In**]:[**Total**], (●) — [**E-In**]:[**Total**], where [**Total**] = [**E-TOH**] + [**Z-TOH**] + [**E-In**] + [**Z-In**]. Legend for Figs. 3B, 3D: (△) — [**Z-TOH**]/([**E-TOH**] + [**Z-TOH**]), (▲) — [**E-TOH**]/([**E-TOH**] + [**Z-TOH**]), (⊠) — ([**E-TOH**] + [**Z-TOH**]):[**Total**]. The points are experimental. The lines are based upon fitting the data to a double exponential or single exponential equation (see Table 1).



plot the fractions of each of the four species as a function of time for experiments starting with **Z-TOH** (Fig. 3A) and **E-TOH** (Fig. 3C) in the same acid solution. It can be seen that the two alcohols interconvert, reaching what appears to be equilibrium position with the ratio [**E-TOH**]:[**Z-TOH**] slightly greater than unity regardless of the starting alcohol. Superimposed on this is the slower process in which the alcohols convert to the indenes. The ratio [**Z-In**]:[**E-In**] is constant throughout each of the runs, and, moreover is the same whether **E-TOH** or **Z-TOH** is the starting material.

The results of kinetic analyses of the data in Figs. 3A and 3C are provided in Table 1. The data for the two alcohols clearly require fitting to an equation that contains two exponential terms, and indeed satisfactory fits are so obtained. The two rate constants determined in such fits differ by a factor of 10–12. The data for the increases in the two indenes, on the other hand, are satisfied by a single exponential. The rate constants so determined are the same as those associated with the slower process of the double exponential fits. Within experimental uncertainty, the same rate constants are obtained whether **Z-TOH** or **E-TOH** is the starting material. Experiments were also performed in solutions with different HCl concentrations with the ionic strength maintained constant at 0.1 M with NaCl. These show that both the fast process and the slow process are first-order in H^+. A final observation is that the slower rate constant obtained from the HPLC data is, within experimental error, the same as the single rate constant obtained from the exponential rise in the UV spectra (obviously for experiments at the same acid concentration). The explanation for this behavior is that the spectra of the two alcohols **E-TOH** and **Z-TOH** are so similar that their interconversion is invisible in the UV. Thus, the spectral change that is observed represents the irreversible process forming the indene products.

Kinetic analysis

Our proposed kinetic model is shown in Scheme 2. This contains the two intermediate carbocations **E-T**$^+$ and **Z-T**$^+$. Each reacts with water to form the corresponding alcohol, and, in competition, cyclizes to an indene. Associated with these reactions are four first-order rate constants k_w(E), k_{cyc}(E), k_w(Z), and k_{cyc}(Z). The carbocations can also form

Table 1. Kinetic analyses of HPLC chromatograms of Fig. 3 obtained with **Z-OH** and **E-TOH** in 0.02 M HCl, 0.08 M NaCl at 20°C.

Constant	Start with **Z-OH**	Start with **E-TOH**
Double exponential decay	k_1 2.57 × 10^4 s^{-1}	2.33 × 10^4 s^{-1}
of starting alcohol[a]	k_2 1.89 × 10^5 s^{-1}	2.01 × 10^5 s^{-1}
Double exponential rise	k_1 2.61 × 10^4 s^{-1}	2.40 × 10^4 s^{-1}
and fall of isomer[b]	k_2 1.87 × 10^5 s^{-1}	2.01 × 10^5 s^{-1}
Single exponential rise of major indene product[c]	1.88 × 10^5 s^{-1}	2.11 × 10^{-5} s^{-1}
Single exponential rise of minor indene product[c]	1.87 × 10^5 s^{-1}	2.07 × 10^{-5} s^{-1}
Ratio of major indene to minor indene[d]	1.86 ± 0.05	1.83 ± 0.04
Single exponential approach to equilibrium — k(isom)[e] [**E-TOH**]/([**Z-TOH**] + [**E-TOH**])	2.37 × 10^{-4} s^{-1}	2.13 × 10^{-4} s^{-1}
[**E-TOH**]([**Z-TOH**]+[**E-TOH**] at equilibrium[f]	0.535 ± 0.02	0.537 ± 0.004
Single exponential decay of ([**E-TOH**] + [**Z-TOH**]):[**total**] – k(cyc)[g]	2.12 × 10^{-5} s^{-1}	1.89 × 10^{-5} s^{-1}

[a]Fit to $A_1 \cdot \mathrm{Exp}(-k_1 t) + A_2 \cdot \mathrm{Exp}(-k_2 t)$ with $A_1 + A_2 = 1$. (Forced to begin at one and end at zero).

[b]Fit to $A_1 \cdot \{\mathrm{Exp}(-k_1 t) - \mathrm{Exp}(-k_2 t)\}$. (Forced to begin and end at zero).

[c]Fit to $(-A_1) \cdot \mathrm{Exp}(-kt) + A_1$. (Forced to begin at zero).

[d]Averaged over all the points.

[e]Fit to eq. [12]. (Forced to begin at zero when **Z-TOH** was starting material and to begin at one when **E-TOH** was starting material).

[f](k_2:(k_1 + k_2)) of eq. [12].

[g]Fit to eq. [13]. (Forced to begin at one and end at zero).

from the appropriate alcohols in H^+-catalyzed processes. The second order rate-constants for these pathways are defined as $k_H(E)$ and $k_H(Z)$; in a solution of a particular acid concentration, the cations will form in pseudo-first-order reactions with rate constants $k_H(E)[H^+]$ and $k_Z(E)[H^+]$.

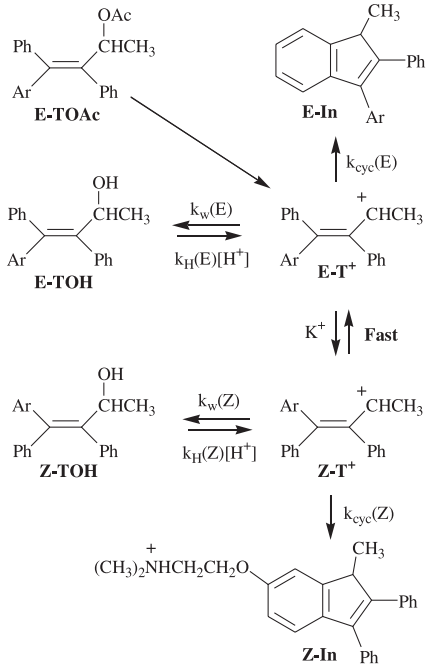
The same carbocations are also the intermediates of the solvolysis of sulfate and acetate precursors. These reactions can be carried out under conditions of kinetic control, i.e., in solutions of very low H^+-concentration such that the alcohol products are stable on the time scale required for complete solvolysis of the ester. An important observation from such experiments is that regardless of whether the ester is (*E*) or (*Z*), **E-TOH** and **Z-TOH** are produced in the same ratio (1), a value of slightly greater than 2:1 in favor of **E-TOH**. The implication is that **E-T**$^+$ and **Z-T**$^+$ achieve equilibrium within their lifetime. These lifetimes (of the equilibrating cations) are known from the LFP experiments, i.e., 40 μs in aq acid. Thus, the (*E*)-(*Z*) interconversion in the cations must be very rapid.[3]

We define the equilibrium constant [**Z-T**$^+$]:[**E-T**$^+$] as K^+. For the solvolysis reactions under conditions of kinetic control, eqs. [1]–[4] can be written for the fraction of each product that is obtained.

[1] $$\mathrm{Fr}(\textbf{E-TOH}) = \left(\frac{k_w(E)}{k_w(E) + k_w(Z)K^+ + k_{cyc}(Z)K^+ + k_{cyc}(E)}\right) = 0.640 \pm 0.005$$

[2] $$\mathrm{Fr}(\textbf{Z-TOH}) = \left(\frac{k_w(Z)K^+}{k_w(E) + k_w(Z)K^+ + k_{cyc}(Z)K^+ + k_{cyc}(E)}\right) = 0.290 \pm 0.003$$

Scheme 2.

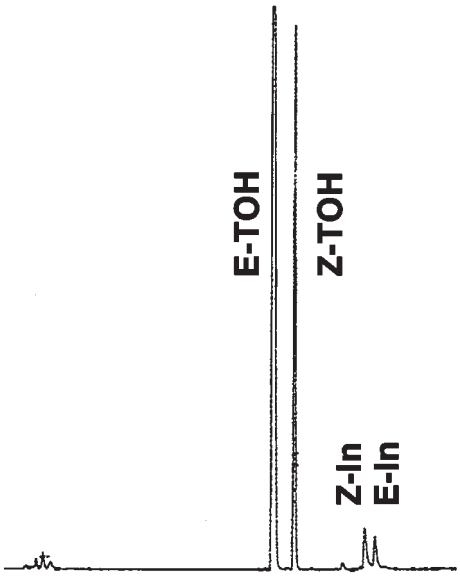


Ar = C_6H_4-4-$OCH_2CH_2N^+H(CH_3)_2$

[3] $$\mathrm{Fr}(\textbf{E-In}) = \left(\frac{k_{cyc}(E)}{k_w(E) + k_w(Z)K^+ + k_{cyc}(Z)K^+ + k_{cyc}(E)}\right) = 0.025 \pm 0.002$$

[3] We have recent evidence that the cations can be trapped before rotation at high concentrations of azide ion, a nucleophile which is a very effective quencher (1).

Fig. 4. HPLC chromatogram obtained after solvolysis of 1×10^{-4} M (*E*)-α-acetoxytamoxifen (**E-TOAc**) in 0.0005 M HCl. Note that the gradient employed to obtain this chromatogram is different from that used in Fig. 2 (see Experimental section). The small peak between the peaks for **Z-TOH** and **Z-In** is due to a small amount of unreacted **E-TOAc**.



[4] $$\mathrm{Fr}(\mathbf{Z\text{-}In}) = \left(\frac{k_{cyc}(Z)K^+}{k_w(E) + k_w(Z)K^+ + k_{cyc}(Z)K^+ + k_{cyc}(E)}\right) = 0.045 \pm 0.002$$

Figure 4 shows a chromatogram obtained in such an experiment, the almost complete solvolysis of (*E*)-α-acetoxytamoxifen (**E-TOAc**) in a very dilute acid solution. The acid was added here to ensure that the products reflect reactions of carbocations where the side-chain amine is protonated. The major products are **E-TOH** and **Z-TOH**, but the peaks for the indenes **Z-In** and **E-In** are present in small amounts. These experiments provide numerical values of the fractions in eqs. [1]–[4]; the ones given above are averages of several experiments. It can be noted that the ratio [**Z-In**]:[**E-In**] is 1.75 ± 0.2, within experimental error the same as the value obtained when these two indenes are formed from alcohol starting materials in more acidic solutions.

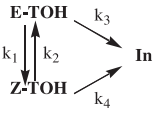
To analyze the experiments with one of the alcohols as starting material, we calculate the steady state concentration of the two carbocations with the assumption that they are in equilibrium. For further simplification we treat the two indene isomers as one, by setting [**In**] = [**E-In**] + [**Z-In**] and Fr(**In**) = (Fr(**E-In**) + Fr(**Z-In**)). This results in the three differential equations of eqs. [5]–[7].

[5] $$\frac{d[\mathbf{E\text{-}TOH}]}{dt} = k_2[\mathbf{Z\text{-}TOH}] - (k_1 + k_3)[\mathbf{E\text{-}TOH}]$$

[6] $$\frac{d[\mathbf{Z\text{-}TOH}]}{dt} = k_1[\mathbf{E\text{-}TOH}] - (k_2 + k_4)[\mathbf{Z\text{-}TOH}]$$

[7] $$\frac{d[\mathbf{In}]}{dt} = k_3[\mathbf{E\text{-}TOH}] + k_4[\mathbf{Z\text{-}TOH}]$$

Scheme 3.



where

[8] $$k_1 = \mathrm{Fr}(\mathbf{Z\text{-}ROH})\cdot k_H(E)[H^+]$$

[9] $$k_2 = \mathrm{Fr}(\mathbf{E\text{-}ROH})\cdot k_H(Z)[H^+]$$

[10] $$k_3 = \mathrm{Fr}(\mathbf{In})k_H(E)[H^+]$$

[11] $$k_4 = \mathrm{Fr}(\mathbf{In})k_H(Z)[H^+]$$

These equations are identical to ones that pertain to the kinetic system of Scheme 3, a model where **E-TOH** and **Z-TOH** interconvert with rate constants k_1 and k_2, and react irreversibly to form the indenes with rate constants k_3 and k_4. The HPLC data of Fig. 3 show that the alcohols equilibrate about 10 times faster than they cyclize to form the indenes. Thus, we make the approximation that $k_1,k_2 > k_3,k_4$. This is equivalent to treating the kinetics in two distinct phases — a rapid phase where the alcohols achieve equilibrium and a slow phase where they react irreversibly to form the indenes. This approximation results in eq. [12] for the equilibration and eq. [13] for the cyclization.

[12] $$\left(\frac{[\mathbf{E\text{-}TOH}]}{[\mathbf{E\text{-}TOH}] + [\mathbf{Z\text{-}TOH}]}\right) = Ae^{-k(\mathrm{isom})t} + \left(\frac{k_2}{k_1 + k_2}\right)$$

where k(isom) = ($k_1 + k_2$), and $A = k_1$:($k_1 + k_2$) or $-k_2$:($k_1 + k_2$) for the cases where **E-TOH** and **Z-TOH**, respectively are the starting alcohols.

[13] $$\left(\frac{[\mathbf{E\text{-}TOH}] + [\mathbf{Z\text{-}TOH}]}{[\mathbf{E\text{-}TOH}] + [\mathbf{Z\text{-}TOH}] + [\mathbf{In}]}\right) = e^{-k(\mathrm{cyc})t}$$

where k(cyc) = ($k_2k_3 + k_1k_4$)/($k_1 + k_2$).

As shown in Figs. 3B and 3D, [**E-TOH**]/([**E-TOH**] + [**Z-TOH**]) and ([**E-TOH**] + [**Z-TOH**])/([**E-TOH**] + [**Z-TOH**] + [**In**]) satisfy a single exponential, as required by eq. [12] and eq. [13] respectively. As seen in Table 1, the rate constant k(isom) from the fit to eq. [12] is essentially the same as the larger of the rate constants obtained by fitting the data for the individual alcohols to equations with two exponentials. The rate constant k(cyc) is the same as the smaller of the rate constants and is also identical to the value obtained from the appearance of the indene products.

From experiments at different acid concentrations, both k(isom) and k(cyc) were found to be proportional to [H^+]. Average values of the second-order rate constants obtained by dividing by H^+ are given in eqs. [14] and [18] below. In the case of the isomerization process, the following relations apply:

[14] $$\left(\frac{k(\mathrm{isom})}{[H^+]}\right) = \left(\frac{k_1 + k_2}{[H^+]}\right) = (1.16 \pm 0.06) \times 10^{-2}\ M^{-1}\ s^{-1} = \mathrm{Fr}(\mathbf{Z\text{-}TOH})\cdot k_H(E) + \mathrm{Fr}(\mathbf{E\text{-}TOH})\cdot k_H(Z)$$

The fit to eq. [12] also provides the fraction of **E-TOH** that is present when the **E-TOH**: **Z-TOH** equilibrium is established:

$$[15]\quad \left(\frac{k_2}{k_1 + k_2}\right) = 0.535 = \left(\frac{[\mathbf{E\text{-}TOH}]_{eq}}{[\mathbf{E\text{-}TOH}]_{eq} + [\mathbf{Z\text{-}TOH}]_{eq}}\right) = \left(\frac{[\mathbf{E\text{-}TOH}]_{eq}}{\mathrm{Fr}[\mathbf{Z\text{-}TOH}] \cdot k_H(E) + \mathrm{Fr}[\mathbf{E\text{-}TOH}] \cdot k_H(Z)}\right)$$

With some algebraic manipulation, inputting the values of Fr(**Z-TOH**) and Fr(**E-TOH**) from eqs. [1] and [2] gives the second-order rate constants:

$$[16]\quad k_H(E) = 2.14 \times 10^{-2}\ M^{-1}\ s^{-1}$$

$$[17]\quad k_H(Z) = 8.42 \times 10^{-3}\ M^{-1}\ s^{-1}$$

It is interesting to note that **E-TOH** forms from the equilibrating cations with a slightly greater than 2:1 preference over **Z-TOH**, but it also undergoes H^+-catalyzed ionization over two-fold faster. The consequence is that the ratio of the two alcohols at equilibrium is close to unity.

For the cyclization process,

$$[18]\quad \left(\frac{k(cyc)}{[H^+]}\right) = (1.01 \pm 0.04) \times 10^{-3}\ M^{-1}\ s^{-1} = \mathrm{Fr}(\mathbf{In})\left(k_H(E)\left(\frac{k_2}{k_1 + k_2}\right) + k_H(Z)\left(\frac{k_1}{k_1 + k_2}\right)\right)$$

where the last term is obtained by substituting the various relations for k(cyc) from eq. [13], and k_3 and k_4 from eqs. [10] and [11]. Numerical values for all the quantities except Fr(**In**) are known from the isomerization kinetics (see eqs. [15]–[17]). Substitution gives FR(**In**) = 0.066. The overall consistency in the analysis is seen in the excellent agreement with the value of 0.070 obtained as the sum of Fr(**E-In**) and Fr(**Z-In**) obtained from the product analysis in the acetate solvolysis.

Summary

These experiments show that the equilibrating tamoxifen carbocations undergo intramolecular cyclization in competition with their reaction with aqueous solvent. The cyclizations represent 7% of the fate of the cations under conditions of kinetic control. Thus they occur with a rate constant of $1.8 \times 10^3\ s^{-1}$, i.e., 7% of the rate constant of $2.5 \times 10^4\ s^{-1}$ obtained by LFP for cation decay (where these rate constants refer to acidic conditions where the side chain amino group is protonated).

We also show that acid conditions must be avoided when performing quantitative analysis of α-hydroxytamoxifens. The H^+-catalyzed formation of the tamoxifen carbocations will occur under these conditions, and the cyclizations represent irreversible processes that result in removal of the alcohols. We had also hoped that we would be able to generate the tamoxifen carbocations under weakly nucleophilic superacid conditions. The occurrence of the rapid intramolecular cyclization however has made this impossible to date, even working at lower temperatures.

Small amounts of the indenes also form at neutral pH when the carbocations are generated in S_N1 solvolysis reactions of acetate and sulfate precursors. Observation of these indenes could be used as an indicator of the formation of the tamoxifen carbocations in cellular systems and even in vivo. To date such experiments have relied upon the formation of the DNA adducts to indicate the formation of the cation (26). The indenes with their λ_{max} at 310 nm and their relatively long retention time should be easily detected by reversed-phase HPLC. Their formation would be unambiguous proof of the formation of the tamoxifen carbocation (and the S_N1 precursor to this cation).

Experimental section

(*E*)-α-Hydroxytamoxifen and (*Z*)-α-hydroxytamoxifen ((*E*)- and (*Z*)-1-[4-[2-(dimethylamino)ethoxy]phenyl]-1,2-diphenyl-1-buten-3-ol) were prepared as a mixture and separated by column chromatography following a literature procedure (27). (*E*)-Acetoxytamoxifen was prepared from the (*E*)-alcohol by treatment with acetic anhydride in pyridine, followed by chromatography as described previously (26, 35).

Z-In (6-(2-dimethylaminoethoxy)-1-methyl-2,3-diphenyl-*1H*-indene) and **E-In** (3-[4-(2-dimethylaminoethoxy)phenyl]-1-methyl-2-phenyl-*1H*-indene) were obtained as a mixture by adding 50 mg of (*E*)-α-hydroxytamoxifen to 100 mL of 1 M HCl. After stirring at ambient temperature for 14 h, the pH was adjusted to 10–11 by the addition of sodium hydroxide, and the indenes extracted with dichloromethane. After drying ($MgSO_4$) and filtration, the solvent was removed to yield a viscous oil. This was purified by chromatography using 8:11:1 ether:hexanes:triethylamine. This failed to provide any separation of the two isomers, as was also the case of TLC experiments using various other solvent combinations. MS (EI): *m*/*z* 369 (100%), 324 (5%), 298 (50%), 265 (75%), 233 (45%), 202 (30%). HRMS *m*/*z* 369.2097; $C_{26}H_{27}NO$ requires 369.2093. The ^1H NMR (400 MHz, $CDCl_3$) of the mixture exhibited peaks that could be assigned to the major isomer (**Z-In**) and minor isomer (**E-In**): 1.315 (major) and 1.320 (minor) (3H, d, J = 7.5 Hz) (CH_3), 2.384 (major and minor) (6H, s) ($N(CH_3)_2$), 2.790 (major) and 2.780 (minor) (2H, t, J = 5.6 Hz) (NCH_2), 4.025 (major) and 4.044 (minor) (1H, q, J = 7.6 Hz) (CH), 4.152 (major) and 4.118 (minor) (2H, t, J = 5.6 Hz), 6.87 (major) (1H, dd, J = 2.2 Hz and 8.2 Hz) (5-H of indene ring of **Z-In**), 6.95 (minor) (2H, d, J = 8.2 Hz) (protons *ortho* to $OCH_2CH_2N(CH_3)_2$ group in **E-In**), 7.16–7.14 (multiplet) (all the other phenyl protons, H7 of the indene ring of **Z-In** could be seen as a doublet at 7.18 Hz with J = 2.2 Hz).

HPLC experiments were conducted with a Waters 600E system, a Waters 486 Tunable Absorbance Detector set at 260 nm, and Waters 746 Data Module. The column was a Water Symmetry C_{18} μm column of dimensions 4.6 mm × 150 mm. The experiments with the α-hydroxytamoxifens as starting material were performed with a flow of 1 mL/min using a programmed eluting system of 0–2 min — isocratic 60% buffer:40% acetonitrile; 2–8 min — linear gradient ending at 15% buffer:85% acetonitrile; and 8–18 min — isocratic 15% buffer:85% acetonitrile, where buffer = 0.025 M sodium acetate:0.025 M acetic acid. Retention times were 4.6 min (**E-TOH**), 5.9 min (**Z-TOH**), 14.2 min

(**Z-In**), and 15.4 min (**E-In**). Experiments starting with α-acetoxytamoxifen used a program of 0–1 min — isocratic 70% buffer:30% acetonitrile; 1–11 min — linear gradient ending at 15% buffer:85% acetonitrile; and 8–18 min — isocratic 15% buffer:85% acetonitrile, where buffer = as above. Retention times were 11.3 min (**E-TOH**), 12.1 min (**Z-TOH**), 13.7 min (**E-TOAc**), 14.8 min (**Z-In**), and 15.3 min (**E-In**). Solutions contained 1×10^{-4} M of the substrate with 60 μL being directly injected into the HPLC.

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