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Mechanistic considerations pertaining to the solvolysis of paclitaxel analogs bearing ester groups at the C2′ position

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Abstract—Dilute solutions of paclitaxel-related derivatives having chloroacetyl esters in the C2′ position undergo ready methanolysis according to pseudo first-order kinetics while more concentrated solutions appear to be stabilized, possibly by the formation of hydrophobic aggregates that tend to bury this reaction center. Methanolysis is also attenuated in the presence of weak acid, suggesting that paclitaxel's neighboring benzamide nitrogen may be participating in the reaction by serving as an assisting nucleophile.

Selective manipulation of the hydroxyl groups present in paclitaxel (**PAC**) and in 10-deacetylpaclitaxel (**DAP**) (Fig. 1) to produce stable analogs and water-soluble prodrugs, has received considerable attention over the course of the last twenty years of PAC-related research.[1–11] Deutsch et al.[3] have shown that among all of the hydroxyl groups present in this family of compounds, the C2′ OH is the most reactive toward acylation. When PAC is treated with carbonyldiimidazole, the resulting C2′ acylated intermediate can additionally form an oxazolone derivative, an interesting side-reaction more recently encountered by de Groot et al.[4] as well. Amino acid derivatives connected via ester linkages at the C2′ position are considerably less stable than when connected at C7 such that the C2′ arrangement has been extensively pursued during prodrug strategies. Likewise, Mathew et al.[5] has exploited the instability of C2′ esters to produce C7-amino acid esters of PAC by partial, selective hydrolysis of the 2′,7-bis-substituted PAC analogs at pH 7.4. Harada et al.[6] have speculated that the instability of the C2′ amino acid esters is due to steric repulsion of the bulky groups attached to C2′ and C3′ as well as to an electronic effect from these types of esters′ amino groups. The latter has also been previously implicated by Zhao et al.[7] and more recently by Pendri et al.[8] who suggested more specifically that protonation of the amino group could serve to assist attack of the C2′ acyl functionality by external nucleophiles due to a simple inductive effect. Other investigators have further postulated that C2′ esters of PAC are particularly susceptible to cleavage by various hydrolytic enzymes present in vivo.[9,10]

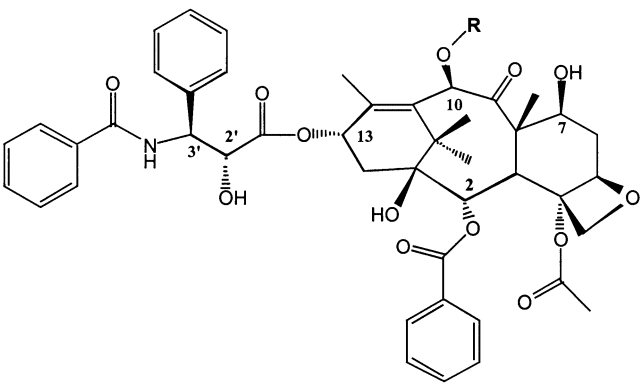

Figure 1. Structures of paclitaxel (PAC), 10-deacetylpaclitaxel (DAP) and selected derivatives. For PAC, R = $COCH_3$; For DAP, R = H; For the monochloroacetyl (CAC) derivatives, a $ClCH_2CO$-adduct replaces one or more of the hydroxy group hydrogens located at positions C2′, C7 and C10.

In an attempt to better understand the interaction of PAC and related compounds with various components within cancer cells,[12,13] we have recently had occasion to deploy monochloroacetyl (**CAC**) protection of both PAC and DAP. During development of an HPLC method to purify 2′,7-bis-monochloroacetyl-10-deacetylpaclitaxel (2′,7-bis-CAC-DAP), we observed that the compound was unstable in methanol solution at a concentration of 1 mg/ml. After confirming this observation and characterizing the breakdown product

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as 7-CAC-DAP we suggested that such treatments might represent a convenient, general method to selectively remove ester functionalities from the 2′-position during synthetic manipulations of the PAC framework.[14] Interestingly, we also found that more concentrated methanolic solutions, namely 20 mg/ml for use in preparative HPLC, did not appear to undergo solvolysis. Likewise, the use of methanol to quench unreacted acylating reagent immediately after chloroacetylation did not appear to disturb the C2′ ester, the latter reflecting a derivatized DAP concentration of 4 mg/ml in total solvent having an acidic pH. Thus, we decided to further investigate the apparent differences in the stability of several PAC and DAP CAC esters relative to concentration and acidity. The compounds we examined are shown in Table 1.

A 1 mg/ml solution of 2′,7-bis-CAC-DAP in methanol was allowed to stand at ambient temperature. 20 μl samples were withdrawn at $T=0$, 2, 24 and 48 h. Longer incubations were deployed for compounds showing little breakdown. Aliquots were assayed directly by HPLC after establishing that responses obtained for serial dilutions of a freshly prepared 10 mg/ml stock solution of 2′,7-bis-CAC-DAP were linear throughout the concentration range anticipated for the samples. A decay curve was generated by following the decrease in compound peak area versus time. An apparent half-life ($T_{1/2}$) was then calculated from a semi-log plot using Microsoft Excel. The decay curve for 2′,7-bis-CAC-DAP appears to follow pseudo first-order kinetics typical for solvolysis reactions. The $T_{1/2}$ value for conversion to 7-CAC-DAP is 5.5 h. The product growth curve matches very closely with the compound decay curve. Similar results were obtained for all of the other 2′-chloroacetylated derivatives listed in Table 1 except for 2′-CAC-DAP whose $T_{1/2}$ appears to be about three times longer (18 h). Nevertheless, this derivative is still much more labile than the chloroacetyl esters placed at either the 7- or 10-positions ($T_{1/2}$ values all >>72 h). For example, when 7-CAC-DAP was studied for up to 120 h, no significant breakdown to DAP was detected.

For the more concentrated experiments, a 10 mg/ml solution of 2′,7-bis-CAC-DAP was allowed to stand at ambient temperature. 10 μl aliquots were withdrawn and diluted with 90 μl of acetonitrile such that 20 μl samples could be injected into the HPLC at the same concentrations used in the previous assays. As before, isolation and crystallization of the product followed by complete characterization confirmed its structure as 7-CAC-DAP. It can be noted that the NMR chemical shifts for the C2′ proton among the various family members are diagnostic for loss of the 2′-CAC esters (Table 1). For the more concentrated case, however, the apparent $T_{1/2}$ was found to be 16.2 h suggesting that increased sample concentration can have a protective effect upon solvolysis. Likewise, when the 1 mg/ml studies were repeated in the presence of 0.2% acetic acid, the decay curves again suggested that there is a protective effect on hydrolysis. For the weakly acidic studies, less than 4% breakdown of 2′,7-bis-CAC-DAP was observed over 48 h and the calculated $T_{1/2}$ was determined to be >1,000 h. Thus, the presence of monochloroacetic acid after acetylation fortuitously serves to protect the product when methanol is used to quench this reaction.

When identical functionalities attached to different locations on the same molecular framework display significantly different chemical reactivity, the involvement of neighboring groups as either catalysts or inhibitors of such reactions is likely to be operative.[15] An especially important case for catalysis occurs when the participating groups can adopt orientations analogous to a favorable ring system while the reaction traverses its transition state. In 2′,7-bis-paclitaxel-related esters, the 2′-ester functionality resides in close proximity to the 3′-amide group. The latter can provide nucleophilic catalysis via either its carbonyl oxygen or its nitrogen lone pair.[16] Normally, the electron density of the nitrogen lone pair is partially displaced by its

Table 1. Stability of PAC and DAP CAC analogs in methanol at 1 mg/ml

Compound	RT (min)	NMR C2′H	$T_{1/2}$ (h)	Product
2′,7,10-Tri-CAC-DAP[a]	17.7	5.58	5.6	7,10-Bis-CAC-DAP
2′,7-Bis-CAC-DAP[a]	12.2	5.59	5.5	7-CAC-DAP
2′-CAC-DAP	6.0	5.55	18.1	DAP
2′,7-Bis-CAC-PAC[a]	16.6	5.59	6.4	7-CAC-PAC
2′-CAC-PAC[b]	8.3	5.55	4.3	PAC
7,10-Bis-CAC-DAP	14.2	4.80	>>72[c]	–
7-CAC-DAP	8.2	4.78	>>72[c]	–
7-CAC-PAC	12.7	4.81	>>72[c]	–
DAP	4.2	4.78	>>72[c]	–
PAC	5.5	4.78	>>72[c]	–

Compound abbreviations are provided in Fig. 1. RT = Retention times on an analytical HPLC (Waters) equipped with a reversed-phase column (Supleco Discovery C18) using a gradient elution (40% acetonitrile in water going to 85% over a 45 min time period) at a flow rate of 1 ml/min monitored by UV detection (225 nm). NMR ($CDCl_3$) values for the C2′ proton shifts are in ppm relative to TMS. $T_{1/2}$ = Apparent half-lives (see text). All compounds were independently prepared and characterized by HPLC, NMR and elemental analyses (their syntheses and biological properties will be reported elsewhere).

[a] Previously reported by Rao et al.

[b] Previously reported by Rimoldi et al.[21]

[c] Less than 5% breakdown after 72 h.

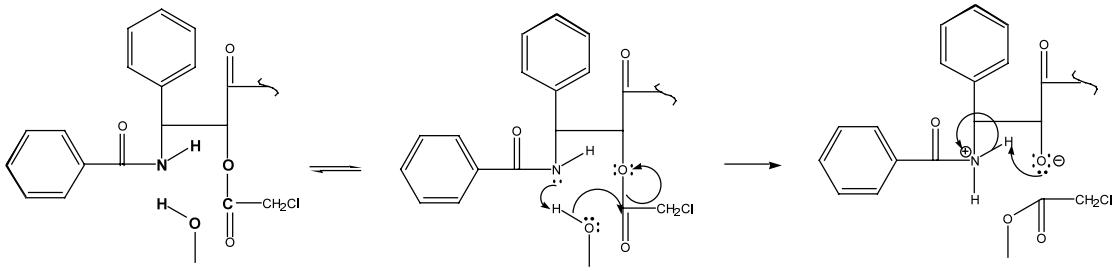

Figure 2. Mechanistic model for the methanolysis of PAC-related 2′-esters wherein the neighboring 3′-amide nitrogen atom catalyzes the reaction by serving as a nucleophilic hydrogen bond/proton acceptor.

resonance relationship with the carbonyl such that the overall system is planar and the oxygen typically serves as the predominant nucleophile. However, in this case the adjacent phenyl ring also donates electrons to the carbonyl such that there is a relative increase in the localization of the lone pair on nitrogen, an increase in the nitrogen′s tetrahedral character, and an increase in its capacity to serve as a nucleophile.[16] That this type of catalysis would be expected to be very sensitive to acidification compared to having the oxygen play such a role, is in line with the experimental observation that the overall solvolysis is dramatically attenuated in weakly acidic solutions. As shown in Fig. 2, the 2′-ester, the 3′-amide nitrogen, and one molecule of methanol can be placed in an arrangement wherein the six atoms that participate in the reaction (bolded) become oriented in a spatial relationship that resembles a six-membered ring. In this model, the amide nitrogen serves as a nucleophilic hydrogen bond acceptor to effect neighboring group catalysis of the methanolysis reaction by enhancing the nucleophilicity of the methanol oxygen. While a concerted mechanism is also possible, the resulting transesterification has been depicted as a two-step process in order to emphasize the catalytic role initially played by the nitrogen lone pair.

The concept of neighboring nucleophile-assisted hydrolysis of 2′-PAC esters was used by Nicolaou et al.[11] in their design of PAC prodrugs. For example, the rate of PAC release was found to increase across the series $HOOCCH_2XCH_2COO$-2′-PAC according to the electron-withdrawing nature of the heteroatom systems placed at X. In this case, however, the proposed mechanism initially involves complete removal of the carboxylic acid proton under basic conditions such that the resulting anion can then effect nucleophilic attack of the ester carbonyl located, by design, to be six atoms away.

The mechanism depicted in Fig. 2 would be expected to be accompanied by pseudo first order kinetics wherein the apparent half-life should be independent of substrate concentration. While this is exactly what appears to be happening in dilute solutions, we also observed that more concentrated solutions have extended half-lives. One possible explanation for this seeming paradox is that the PAC-related materials may be forming aggregates as their concentrations are increased. In this regard, PAC has been observed by others to form concentration-dependent aggregates in non-polar media such as chloroform[17] and to undergo hydrophobic-driven, conformational collapse of the C13 side chain in polar media.[18] While the latter would presumably occur in a concentration independent manner, another way for the lipophilic C13 side chains to avoid protic, polar solvents would be to cluster among two or more PAC molecules, a process that would be promoted by increasing concentration. Clustering of the C13 side chains, in turn, would be expected to limit the access of methanol to the 3′-amide-catalyzed, 2′-ester-reaction area. Additional studies directed toward the further elucidation of the proposed mechanism for solvolysis are underway. The implications of our findings on the pharmacological profiles and structure–activity relationships for the PAC-related family of compounds are also important relative to recent models for PAC′s association with microtubules.[19,20] For example, Snyder et al.[20] has recently reported a binding conformation in which β-tubulin's His-229 imidazole ring appears to become flanked by the phenyl rings from PAC's 3′-benzamido and 2-benzoyl moieties. The chemical model proposed herein suggests that such a stacked arrangement could be further stabilized by hydrogen bonding or even proton transfer between the nucleophilic benzamide nitrogen and an imidazole N-H depending upon the latter's state of protonation.

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