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Synthesis, biological evaluation, and modeling of a C,D-*seco*-taxoid

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Abstract—A C,D-*seco*-paclitaxel derivative **12** was prepared and tested for biological activity. The key step in the synthesis was a free radical fragmentation of the bicyclic tertiary alcohol **7** under the conditions of the hypobromite reaction. The compound **12** showed no activity in the tubulin test.

The advent of paclitaxel (taxol) **1**[1] and docetaxel (taxotere) **2**[1,2] is considered as one of the most important recent achievements in the development of anticancer therapeutic agents (Fig. 1). In order to fully understand the compounds' mechanism of action and determine the structural features essential for their biological activity, extensive structure–activity relationship (SAR) studies on taxoids have been performed. These studies suggested that the oxetane ring may be one of the crucial structural units of biologically active taxoids; however, its' exact role is still a matter of debate.[3] The four-membered ring could act as a rigidifying element, which imposes a proper conformational bias to the taxane core, thus holding the functional groups at C-2, C-4, and C-13 at favorable positions for productive interactions with tubulin receptor. Alternatively, electronic effects may be important, with the heteroatom acting as a hydrogen bond acceptor, directly engaged in the interaction with microtubules. For both hypotheses experimental support exists, as well as some contradictory data. Thus, substituting the nitrogen atom for oxygen in the four-membered ring results in reduced activity, although the geometry of the azetidine derivatives does not differ very much, with respect to the parent oxetane.[4] This finding infers the prevalence of the electronic factors. SAR studies on thia-docetaxel and paclitaxel analogues point to a similar conclusion.[5] On the other hand, a recently synthesized taxoid containing cyclopropane ring in place of the oxetane showed the activity comparable to that of docetaxel, pointing to a purely conformational effect of the D-ring.[6] The lack of activity of several D-*seco*-taxoids was explained by inappropriate orientation of the 4-acetyl group.[7,3b] Example is

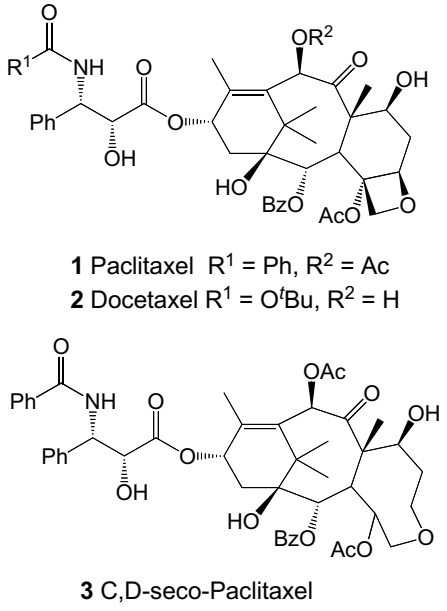

Figure 1.

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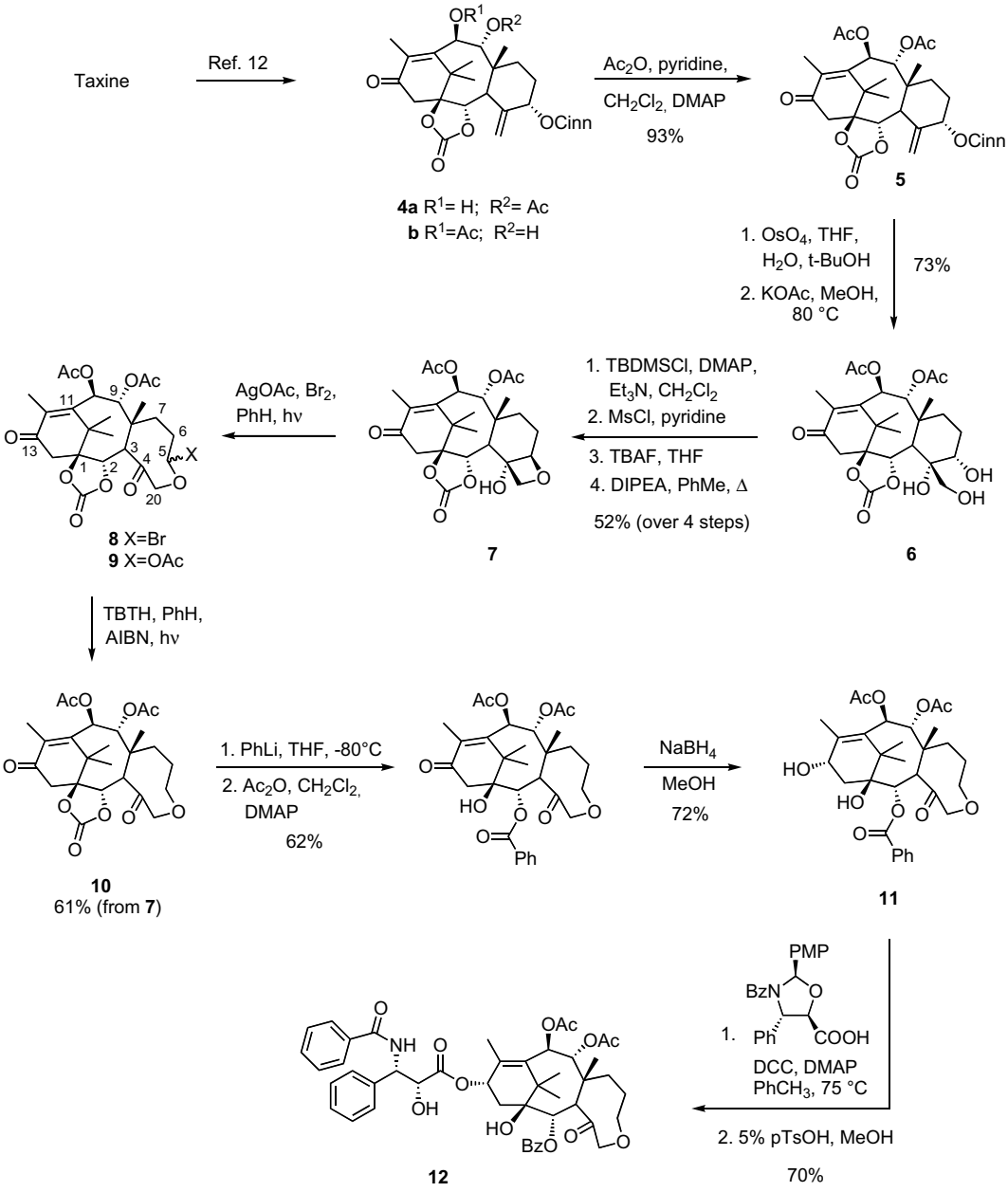
known, however, of a D-*seco*-taxoid, which retains high activity in the tubulin test.[8]

In order to estimate the effect of conformational constraint imposed by the oxetane ring, we set out to synthesize C,D-*seco*-paclitaxel derivatives of type **3** and evaluate their biological activity (Fig. 1). We decided to perform our study on 7-deoxytaxoids, as the hydroxyl group in position C-7 has been shown to be irrelevant for the biological activity of paclitaxel.[9] In addition, some preliminary experiments indicated that the presence of a carbonyl group in position C-9 could complicate some stages of the synthesis. Therefore, we planned to install a 9-α-acetoxy group in place of the carbonyl, as this modification would somewhat simplify the synthetic procedure without affecting substantially the biological activity of the final product.[10]

The synthesis started with the cinnamoyl taxicine derivatives **4a**,**b** whose preparation from taxine B[11]—a pseudoalcaloid readily isolated from the renewable needles of the European yew (*Taxus baccata*)—was described earlier (Scheme 1).[12] Selective cleavage of the cinnamoyl side chain in **5**, in the presence of two acetate units and a cyclic carbonate, was effected using a previously developed procedure:[12] dihydroxylation of **5** with OsO_4 resulted in stereoselective introduction of two requisite hydroxyl groups into the taxane core, while simultaneously activating (by intramolecular hydrogen bonding) the intermediate 2,3-dihydroxy-phenylpropan-



Scheme 1.

oate side chain toward alcoholysis in a buffered methanolic solution (73% over two steps). The resulting triol **6** was converted into oxetane **7** by a slightly modified four-step protocol developed by the CNRS group (52% over four steps),[12,13] thus setting the stage for the fragmentation reaction. We planned to effect the crucial skeletal transformation—the scission of the central carbon–carbon bond of the C,D-ring system in **7**—by using radical methodology. To this aim several methods of tertiary alkoxyl radical generation were tried with variable success. Surprisingly, reagents such as HgO/I_2, Ag_2O/Br_2, or DIB/I_2 under photolytic conditions gave mixtures of halide **8** and the corresponding acetate **9** (with the latter predominating), as well as some unidentified products. After considerable experimentation, we found that bromide **8** and acetate **9** could both be obtained chemoselectively with $AgOAc/Br_2$ under carefully controlled conditions. When the reaction was performed with 1.4–1.5 equiv of AgOAc and 2.0–2.5 equiv Br_2 the required bromide **8** could be isolated as the sole product. Using 4 equiv of AgOAc and 2 equiv of Br_2 reverses the chemoselectivity of the reaction and leads to the exclusive formation of acetate **9**. Bromide **8** was obtained as an equimolar mixture of diastereoisomers, which were further reduced (without separation) with Bu_3SnH to furnish the C,D-*seco*-derivative **10** (61% yield, over two steps). Subsequent transformations involved installation of a benzoate ester into the position C-2 (for simplicity and consistency, the taxane numeration, as shown in the structural formula of **8**, is maintained after the fragmentation step) (excess PhLi, 62%, after reacetylation) and reduction of the C-13 carbonyl group ($NaBH_4$, 72%). At this stage we planned the reduction of the C-4 carbonyl group in **11** followed by acetylation. Surprisingly, this ketone proved unreactive toward a variety of reduction agents, such as $NaBH_4$ (20 equiv, rt), $NaBH_4/CeCl_3$, $BH_3{\cdot}Me_2S$ (rt), Li-Selectride ($-70 \rightarrow 0$ °C), Red-Al ($-60 \rightarrow +50$ °C), or Noyori transfer–hydrogenation (performed separately with both (*R*,*R*)- and (*S*,*S*)-enantiomers of TsDPEN ligand). Under reducing conditions with Et_3SiH/BF_3, only products derived from Lewis acid-catalyzed rearrangement were observed. With $LiAlH_4$ a complex mixture of products was obtained. At low temperatures, excess DIBALH induced only the reductive hydrolysis of the acetate groups, while a very complex mixture of products was formed at rt. The attempted Noyori reduction of **10** was very slow and resulted only in reductive deprotection of the cyclic carbonate unit. With other reducing reagents occasional reduction of the C-13 carbonyl group was observed, but the C-4 carbonyl remained intact. Apparently, fragmentation of the central bond in the condensed C,D-system brings about a major conformational change in the taxane core and renders the newly formed carbonyl group (C-4) inaccessible to reducing agents (in paclitaxel it is the carbonyl group at C-9 that is resistant even to $LiAlH_4$).

In order to examine any potential conformational reorganization arising from the ring cleavage, we performed a 10,000 step $MMFF/GBSA/H_2O$[14] conformational search for ketone **11** within 7.0 kcal/mol from the boat–chair (BC) global minimum. The second lowest energy conformer is a crown form at 3.1 kcal/mol, while two twist–chair structures fall higher. Boltzmann populations at 298 K are calculated to be 99.5%, 0.5%, and <0.1%, respectively. The calculated relative stability of conformers is identical to that for cyclooctanone[15] and suggests that attempts to reduce the C-4 carbonyl in **11** operate on the BC conformation shown in as a space-filling model in Figure 2. The C-4 carbonyl (center, yellow arrow) is surrounded and sequestered by the C-2 phenyl group (left), the C-8 CH_2 group (right) and the C-18 Me group (i.e., Me-19, top, magenta arrow). The eight-membered ring oxygen is highlighted by the red arrow. The buried carbonyl carbon is sterically inaccessible on both π-faces to either nucleophile or reducing agent.

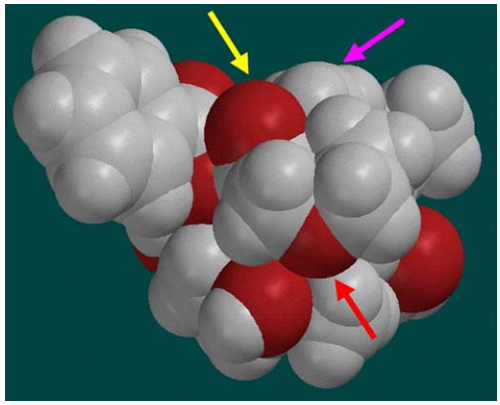


Figure 2.

Thus, introduction of an acetate group into position C-4—a structural feature which is known to be important for the biological activity of taxoids—turned out to be unfeasible for C,D-*seco*-derivative **11**. However, we proceeded further with the appendage of the side chain and the conversion of 4-oxo-derivative **11** into a C,D-*seco*-taxoid **12** suitable for testing of biological activity. This transformation proceeded uneventfully, according to the previously described protocol.[16] It should be noted, however, that prolonged reaction times in the deprotection step lead to the skeletal rearrangement of **12** to the 11(15-1)-abeotaxane.

The result of the tubulin test[17] showed **12** to be devoid of microtubule stabilizing activity. At 11.9 mM concentration, **12** effected only 7% inhibition of the microtubule disassembly process (for comparison, taxol showed 50% inhibition at 1 μM concentration). This finding confirms the importance of both the oxetane ring and the C,D-ring system in taxoids for the biological activity. However, the fact that compound **12** lacks the C-4 acetate group makes this result somewhat inconclusive, as it is difficult to estimate, which of the two structural features of **12** (the conformational change, or the absence of the C-4 acetate) contributes more to the loss of the microtubule stabilizing properties. Another factor concerns the relocation of the oxetane oxygen and the adjacent carbons by the BC conformation placing them deep within the hydrophobic tubulin pocket. The corresponding position of the CH_2–O–CH_2 fragment within

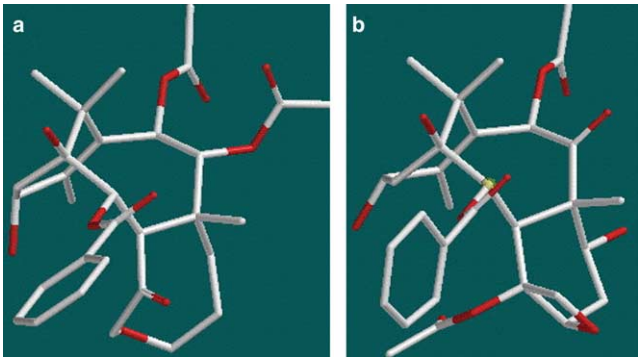


Figure 3.

the concave pocket of the tricyclic core of **11** is illustrated at the bottom of Figure 3a. By contrast the same atoms in the oxetane ring of the tetracyclic baccatin moiety are directed outward from the convex face of the molecule (Fig. 3b). Thus, steric conflicts between **12** and residues in the binding site may also contribute to the lack of activity as speculated for low-activity taxanes that can otherwise achieve the T-taxol binding conformation.[18]

To summarize, the described chemistry allows for the efficient preparation of C,D-*seco*-taxoids. The scission of the C4–C5 bond in the taxane core brings about important modifications of both the chemical reactivity and the biological activity of the starting taxoid.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2005.05.071.

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