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Synthesis of 6α-Hydroxypaclitaxel, the Major Human Metabolite of Paclitaxel

Haiqing Yuan and David G. I. Kingston*

Department of Chemistry, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061-0212

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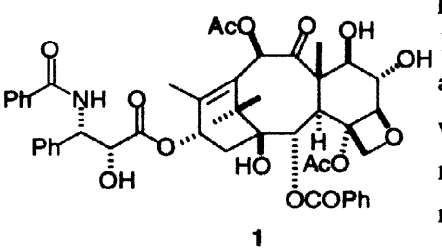
Abstract: 6α-Hydroxypaclitaxel, the major human metabolite of paclitaxel, was synthesized via epimerization of 6α-hydroxy-7-*epi*paclitaxel, which was prepared from paclitaxel in four steps in high yield. Various epimerization conditions were investigated, and the optimum conditions using DBU in xylenes afforded 80-88% isolated yield based on unrecovered starting material, along with 4-deacetyl analogs as minor products. The stereochemistry of paclitaxel 6,7-epoxide was revised in the course of this work.

Paclitaxel, as one of the most important anti-cancer drugs currently on the market for ovarian and breast cancer, has generated wide interest in various areas,[1] and extensive chemical and SAR studies have been carried out.[2] Extensive pharmacological investigations concerning the metabolism of paclitaxel have also been performed, especially after it entered Phase II clinical trials in the late 1980's.[3-5] Both *in vitro* and *in vivo* studies on its metabolism in animals and humans have been reported recently.[4,6-10] 6α-Hydroxy-paclitaxel (**1**) was reported to be the principal metabolite of paclitaxel in human hepatic microsomes, human liver slices, and patient biliary excretions, while in rats it is apparently not a biotransformation product.[7-9] Due to the small quantity of purified materials (usually sub-nanomolar) that could be isolated through biological pathways, a synthetic method for the preparation of 6α-hydroxypaclitaxel from the parent compound paclitaxel in relatively large amounts was desirable for studies of biological activity as well as for providing an HPLC standard for monitoring drug disposition in patients who take paclitaxel.



1

The other major metabolites of paclitaxel are hydroxylated on its aromatic rings, and their syntheses could thus be easily effected using known chemistry such as 2-debenzoylation followed by 2-aroylation[10,11] or through the synthesis and attachment of appropriately modified side chains.[12] In contrast, the preparation of 6α-hydroxypaclitaxel requires modification of the taxane ring system itself, and proved to be a difficult one.

Our initial approach was to open the epoxide ring of 2'-TBDMS-6β,7β-epoxypaclitaxel (**2**), which was prepared from paclitaxel as previously described.[13] It was envisioned that this transformation would occur under acidic conditions, and this strategy was expected to be both straightforward and stereoselective. In the event, however, the oxirane ring proved to be much more stable under acidic conditions than the oxetane ring, and various attempts to carry out a selective oxirane ring-opening only yielded products with opened oxetane rings. This result thus confirms the great susceptibility of the oxetane ring of paclitaxel to electrophilic reagents, as observed previously for paclitaxel itself.[14]

In the course of our investigations on the ring opening of **2** we became aware that its stereochemistry might have been assigned incorrectly. We thus carried out further studies of this question, and found that a NOESY experiment, far from indicating the β-configuration of the epoxide as previously proposed,[13] in fact gave

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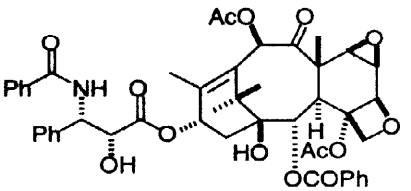
unambiguous support for the alternative α-configuration, and showed that paclitaxel 6,7-epoxide has the structure and stereochemistry **3**. The most convincing correlation observed was between H-7β at 3.0 ppm and CH_3–19 at 1.86 ppm. No correlations were observed between H-6 or H-7 and H-3, and the NOE correlations previously reported[13] must thus have been due to some spectroscopic artefact. The stereochemistry of the 6,7-epoxide in a related compound has been confirmed independently by X-ray crystallography.[14]



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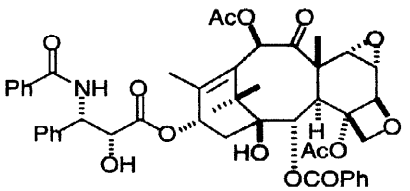
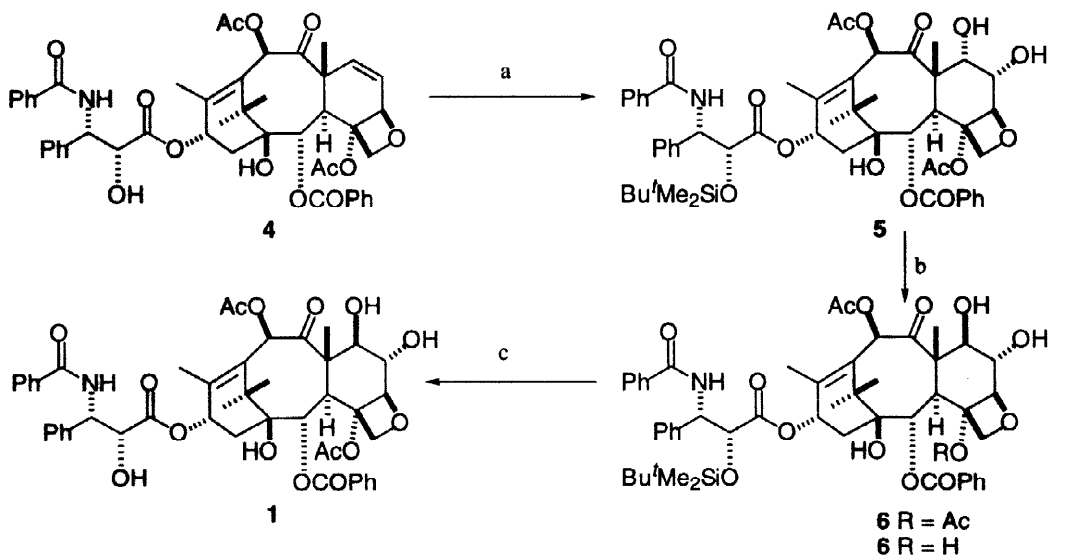


3

Since 2'-TBDMS-6α-hydroxy-7-*epi*paclitaxel (**5**) can be obtained in high yield in four steps from paclitaxel via the intermediate alkene **4**,[13b] epimerization of the 7α-hydroxyl group in **5** to the normal β-position was also investigated. Thus, treatment of **5** in anhydrous toluene with 1,8-diazabicyclo-[5,4,0]undec-7-ene (DBU) at 80 °C afforded a single product which was isolated and fully characterized[15] as 2'-TBDMS-6α-hydroxypaclitaxel (**6**) in 12% yield, along with 84% unreacted starting material (Scheme).



Scheme (a) OsO_4, NMO, THF/H_2O, 90%; (b) DBU, toluene, 80°C, or entry 2-7 in Table 1; (c) HF/pyridine, THF, 71-82%.

This result parallels a recent study on the epimerization of the 7-hydroxyl group of paclitaxel itself and of other related derivatives.[16] Although the yield obtained was low in absolute terms, the yield based on unrecovered starting material was an acceptable 75%, suggesting that this would be a viable route for the preparation of the desired metabolite. Since the epimerization was essentially an equilibrium reaction, we decided to optimize reaction conditions to achieve the best turnover and yield. The variants investigated included the use of similar hindered bases to DBU such as 1,5-diazabicyclo[4,3,0]non-5-ene (DBN) and 1,4-diazabicyclo[2,2,2]-octane (Dabco™), the use of different temperatures, and the presence of activated 4 Å molecular sieves. In addition to the desired product **6**, small amounts of the 4-deacetyl product **7** were formed under most conditions; the results of these studies are summarized in the Table below.

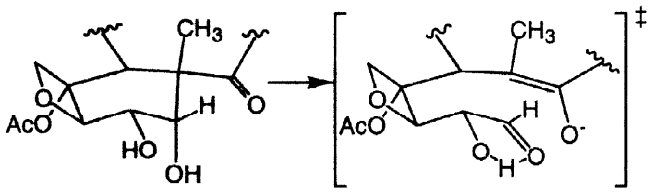
From these studies it is clear that DBU in xylenes at 80 °C (entry 3) gives the best results, with 15%

Table: Isolated Yields of the Epimerization Reaction Under Different Conditions

Entry	Reaction Conditions	5	6	7
1	DBU, toluene, 80 °C, 1.5 h	84%	12%	<2%
2	DBU, toluene, 90 °C, 2.5 h	43%	9-11%	11-12%
3	DBU, xylenes, 80 °C, 1.5 h	83%	15%	<2%
4	DBU, xylenes, mol. sieves, 80 °C, 1.5 h	86%	12%	<2%
5	DBN, xylenes, 80 °C, 1.5 h	92%	7%	<2%
6	Dabco™, xylenes, 80 °C, 1.5 h	99%	0	0
7	DBU, xylenes, 70 °C, 1 h	92%	7%	1%

absolute yield and 88% yield based on unrecovered starting material.[17] Lower temperatures (entry 7) give lower yields for both products **6** and **7**, while higher temperatures and prolonged treatment (entry 2) give larger amounts of 4-deacetyl derivative **7** together with other side products. DBN was worse than DBU in terms of turnover, and Dabco™ gave almost no reaction; this could be attributed to its steric bulk and weaker basicity or both. The use of anhydrous conditions was believed to be critical, but molecular sieves appeared to be unnecessary as long as the solvents and bases were appropriately dried. Attempts were made to trap the 7β-hydroxyl group with chlorotriethylsilane so as to drive the equilibrium to completion, but the use of different bases (DBU or NaH) and different substrates failed to give any of the desired 7β-*O*-(triethylsilyl) derivatives.

Interestingly, the 4-deacetylated product **7** still retained its 7α-hydroxyl group. This is in contrast to the assumption that the strong intramolecular hydrogen bonding observed between the 7α-hydroxyl proton and the acyl oxygen of the C-4 acetate[18] might be the main reason for the facile and favorable epimerization of paclitaxel to 7-*epi*paclitaxel in base. A possible explanation for the preferential formation of **7** is that a hydrogen bond between the 6α-hydroxyl group and the oxygen of the presumed 7-aldehyde intermediate in the transition state of the retro-aldol reaction may lock the conformation in such a way that the following aldol addition regenerates starting material (Figure).



Figure

2'-TBDMS-6α-hydroxypaclitaxel (**6**) was deprotected in the usual way to give the major human metabolite **1** in good yield (Scheme). The method described thus provides a reliable six step synthesis of the major human metabolite of paclitaxel in about 50% overall yield based on unrecovered starting material.

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