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An effective method to produce 7-epitaxol from taxol in HCO_3^-

Yanjie Liu, Xiaoyu Ma, Mengkai Zhou, Xiaoran Hao, Xudong Zhu*

Beijing Key Laboratory of Genetic Engineering Drug and Biotechnology, Institute of Biochemistry and Biotechnology, College of Life Science, Beijing Normal University, Beijing, 100875, P.R. China

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ABSTRACT

It is known that 7-epitaxol has much stronger cytotoxicity than taxol does. However, the content of 7-epitaxol in yew is much less than taxol, which makes it more costly to obtain. We describe here a method to effectively convert taxol to 7-epitaxol. The key condition for reaction needs $NaHCO_3$ in solvent acetonitrile (ACN). The conversion rate can be over 82%.

* Corresponding author. Tel.: +86-010-58804266; e-mail: zhu11187@bnu.edu.cn

[2,3] It was isolated and structurally characterized by Huang *et al.* in 1986 from the bark of yew *Taxus brevifolia*.[1] Also, in the study, they demonstrated that taxol could be transformed to 7-epitaxol via the catalysis of azobis (isobutyronitrile) in toluene at 80°C.[1] It is well established that 7-epitaxol exhibits the same antitumor mechanism, mainly by stabilizing of microtubule polymers,[4] yet shows a higher cytotoxicity than taxol, to a number of tumor types, *e.g.* 12.7 times to murine leukemia L1210 cells and 5.9 times to human epidermoid carcinoma KB cells.[5,6] Still, observations on in vivo biotransformation of taxol revealed the fact that 7-epitaxol is more stable than taxol in patients.[1] When patients were only given with taxol in treatment, 7-epitaxol was formed and detected in both plasma and urine samples,[7,8] suggesting a contribution of 7-epitaxol to treatment. Thus, 7-epitaxol is presumably recognized to own advantages of cure over taxol. Unfortunately, industrial production of 7-epitaxol mainly depend on the raw material of yew tree, and content of 7-epitaxol in the plant is even less than taxol in *Taxus* sp.[1,9] and *Corylus avellana* which are by far the major material supplier for taxol production,[10] resulting in high cost in the production of 7-epitaxol. Here we report a finding that HCO_3^- and some other basic salt could effectively turn taxol into 7-epitaxol in the solvent acetonitrile (ACN) which itself facilitates subsequent purification steps of the product, for instance, via extraction hereby by ethyl acetate or dichloromethane to desalinate.

The reaction was set as the following: 20 μL 0.02 mg/mL of freshly prepared taxol in ACN solution, mixed with an equal volume of $NaHCO_3$ solution at 0.02 mg/mL, was incubated at 60°C for 30 min, then to centrifuge. An aliquot of 20 μL supernatant was taken for HPLC analysis. A new signal peak marking for 7-epitaxol was detected at 16.135 min on HPLC spectrum (Fig 1A).[7,8] The newly formed compound was purified via HPLC (ACN:H_2O, 70:30, 2 mL/min), and subject to HRMS analysis, which gave rise to ions at m/z 854.3382 $[M+H]^+$ and 876.3227 $[M+Na]^+$, consistent with the mass pattern of 7-epitaxol (Figs 1B and 1C).[1,11] ^1H NMR and ^{13}C NMR spectrum in CD_3CN of purified compound were further performed to confirm the structure by comparing with that of taxol. The major difference from purified compound to taxol in NMR spectrum occurred in the protons signals of C-7 (δ_H=3.57 ppm to 4.33 ppm), C-10 (δ_H=6.72 ppm to 6.33 ppm), C-20 (a pair of doublets at δ_H=4.27 ppm and 4.38 ppm to a two-proton singlet peak δ_H=2.00 ppm), and carbon signals of C-7 (δ_C=76.10 ppm to 71.57 ppm) and C-19 (δ_C=15.94 ppm to 9.31 ppm) (Table S1 and Figs S1-S4), similar to the description of Huang *et al.*[1]

To determine the exact role of cation from anion in the reaction, we further tested seven salts, *i.e.* $NaHCO_3$, CH_3COONa, NaCl, Na_2CO_3, Na_2HPO_4, NaOH and NaH_2PO_4, which all had the same cation Na^+, but anion groups are distinct. As shown clearly, $NaHCO_3$ and Na_2HPO_4 displayed high efficiency in the reaction, with a conversion rate of 23.6% and 10.3%, respectively, under such condition, while the other salts hardly drove the conversion (Fig 2A). Extend assays showed that $KHCO_3$ had a similar conversion rate of taxol in reactions with $NaHCO_3$ (Fig S5). These results suggest that the active catalytic ingredient was HCO_3^- rather Na^+, consistent with former conclusion that base promotes the epimerization as well as degradation in aqueous solution though.[12] Considering the conversion rate, $NaHCO_3$ was chosen over Na_2HPO_4 as the reactant in further assay to optimize reaction parameters, including the reaction time,

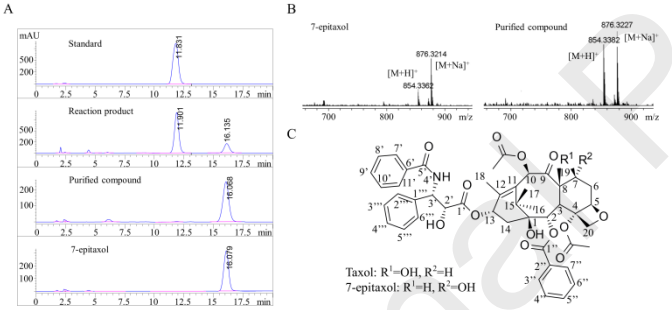

Fig. 1 Characterization of 7-epitaxol from taxol. (A) HPLC analysis of standard Taxol, reaction products, purified 7-epitaxol and 7-epitaxol. The retention time of taxol and 7-epitaxol was at 11.831 min and 16.135 min, respectively. (B) HRMS of standard and purified 7-epitaxol. (C)

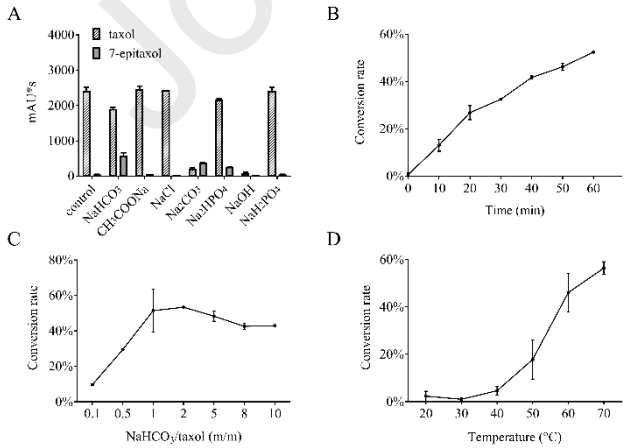

Fig. 2 Effects of reactants (A), reaction time (B), reactant ratio (C) and reaction temperature (D) on the epimerization of taxol to 7-epitaxol. Yield of taxol and 7-epitaxol after reaction was measured by HPLC. Each data was measured in triplicate.

reac and nearly half of taxol was turned to 7-epitaxol in 50 min. The conversion rate naturally depended on initial concentration of $NaHCO_3$ and taxol and their ratio. When the ratio of $NaHCO_3$ to taxol in mass was 2, the highest reaction efficiency (51.4%) was achieved (Fig 2C). The temperature was also a key factor for the reaction. The epimerization required a temperature over 40℃ (Fig 2D). Notably, approximately 82% of transformation rate could be achieved when 10 mg taxol, plus 10 mg $NaHCO_3$ in 1 mL ACN was incubated at 60℃, for 1.5 h, *i.e.* approximately 8.2 mg 7-epitaxol was obtained, and 1.5 mg taxol was recovered, in experiments. Using a taxol sensitive yeast strain *Saccharomyces cerevisiae* AD1-8,[13] our product 7-epitaxol after purified had a stronger cytotoxicity than taxol (Table 1. Strain S288C is the wild type).

Table 1 Cytotoxicity of taxol and our 7-epitaxol against *Saccharomyces cerevisiae* S288C and AD1-8

Compound	S288C IC_{50} (μM)	AD1-8 IC_{50}(μM)
Taxol	>100	>100
7-epitaxol	>100	71.5

There are two possible epimerization routes from taxol to 7-epitaxol (Fig 3). The first one is a retroaldol/aldol reaction under the

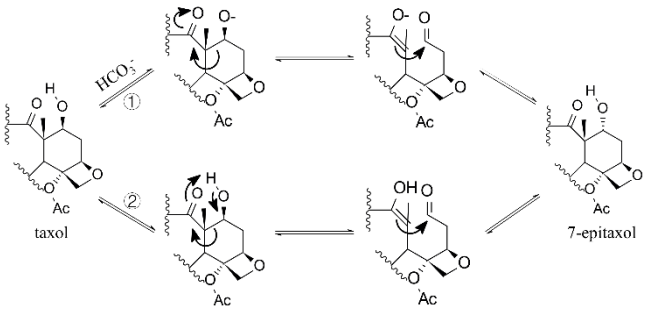

Fig. 3 Deduced reaction routes to epimerization of taxol to 7-epitaxol.

catalysis of HCO_3^- reported by Tian *et al*.[12] (Fig 3). The 7-OH was first deprotonated by Lewis base, HCO_3^-, and turned into $[O]^-$. The deprotonated intermediate made the bond of C-7 and C-8 easier to cleave, resulting in a high possibility to produce epimer, 7-epitaxol. The second route seems plausible to be an intramolecular reaction described by Mclaughlin *et al*.[14] (Fig 3). In this mechanism, the proton of 7-OH was first transferred to [O] at C-9, concomitant the formation of aldehyde group at C-7 and the cleavage of C-7 and C-8. However, as analyzed by Tian *et al*.[12], the secondary mechanism was occurred in a 'closed system', which may depend on a natural or acid environment, rather alkaline environment. Together with that fact that 7-epitaxol was rarely produced when acidic NaH_2PO_4 or neutral NaCl were added in reaction system (Fig 2), the epimerization occurred through more likely via the first mechanism (Fig 3).

In summary, we reported taxol could be converted to the only product 7-epitaxol epimerized in the presence of HCO_3^- and ACN, instead of the aqueous Windows Userphase which may causes simultaneous severe degradation of taxol.[12] Comparatively, our method is straightforward with high efficiency to obtain 7-epitaxol which has a higher bioactivity in respect of stability and a stronger antitumor efficacy.

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Declaration of interests

☐ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☒The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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