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Short communication

Evaluation of paclitaxel rearrangement involving opening of the oxetane ring and migration of acetyl and benzoyl groups

Sang-Hyun Pyo [a,b], Jin-Suk Cho [a], Ho-Joon Choi [a,*], Byung-Hee Han [b,*]

[a] *Samyang Genex Food & Bio Research Center, 63-2 Hwaam-Dong, Yuseong-Gu, Daejeon 305-717, South Korea*
[b] *Department of Chemistry, Chungnam National University, 220 Gung-Dong, Yuseong-Gu, Daejeon 305-764, South Korea*

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Abstract

The stability of drug is a critical factor in quality control, drug efficacy, safety, storage, and production conditions. The rearrangement of paclitaxel, which involves opening of the oxetane ring and migration of acetyl group occurred on heating a powder of purified paclitaxel. Subsequently, the unusual migration of benzoyl groups progressed rapidly in organic solvents. These rearrangement derivatives were isolated carefully. The structures of the intermediate derivative **A** and the product derivative **B** were confirmed using ^{1}H NMR, high performance liquid chromatography (HPLC), and mass spectrometry. We proposed the rearrangement pathway here for the first time. Neither derivative exhibited bioactivity in SKOV3 (ovarian cancer) or MDA-MB-435 (breast cancer) cell culture assays.

Keywords: Paclitaxel; Oxetane ring opening; Acetyl migration; Benzoyl migration; Rearrangement

1. Introduction

Paclitaxel (Genexol®, Taxol®), a compound originally isolated from the bark of the Pacific yew tree *Taxus brevifolia* in 1971 [1], has been one of the most important anticancer agents in recent decades owing to its unique cytotoxicity mechanism [2,3]. Since the yield of purified paclitaxel from *T. brevifolia* is very low (about 0.04% of the bark dry weight), and bark-stripping leads to the destruction of scarce plant material [4], many attempts have been made to develop new methods for the reliable production of paclitaxel from renewable resources including semi-synthesis, total synthesis, and plant cell culture strategies [5–8]. In the search for other bioactive compounds, many other taxane derivatives have been isolated or synthesized chemically for structure–activity relationship studies [4,9–11]. The degradation of the oxetaine ring of paclitaxel has been studied by treating paclitaxel with acid [12], and Meerwein's reagent [13,14]. The resulting degradation products, such as D-secopaclitaxel, were elucidated using liquid chromatography–mass spectrometry (LC–MS) profiling and NMR analysis. However, in these previous reports, the degradation conditions of paclitaxel were harsh and the chemical reaction methods or the resulting degradation products were not isolated.

The degradation of the final product has a very critical impact on quality control, drug efficacy, safety, storage, and production conditions. Moreover, the identification of degraded compounds and causes have an important role in the drug development process [15,16]. In this report, we describe a new evaluation of paclitaxel rearrangement that yielded two derivatives under heating and in solution (Figs. 1 and 2). Little is known the isolation and characteristics of these compounds, i.e., derivatives **A** and **B** (Fig. 2). The proposed rearrangement mechanism will provide useful knowledge to further the commercial production of paclitaxel.

2. Experimental

2.1. Material and chemicals

Paclitaxel was used as received from Samyang Genex Corporation (Daejeon, Korea). All other reagents and solvents were analytical or HPLC grade.

* Corresponding authors. Tel.: +82 42 821 6547; fax: +82 42 823 1360.
E-mail addresses: shpyo@cnu.ac.kr (S.-H. Pyo), hojoon@genex.co.kr (H.-J. Choi), bhhan@cnu.ac.kr (B.-H. Han).

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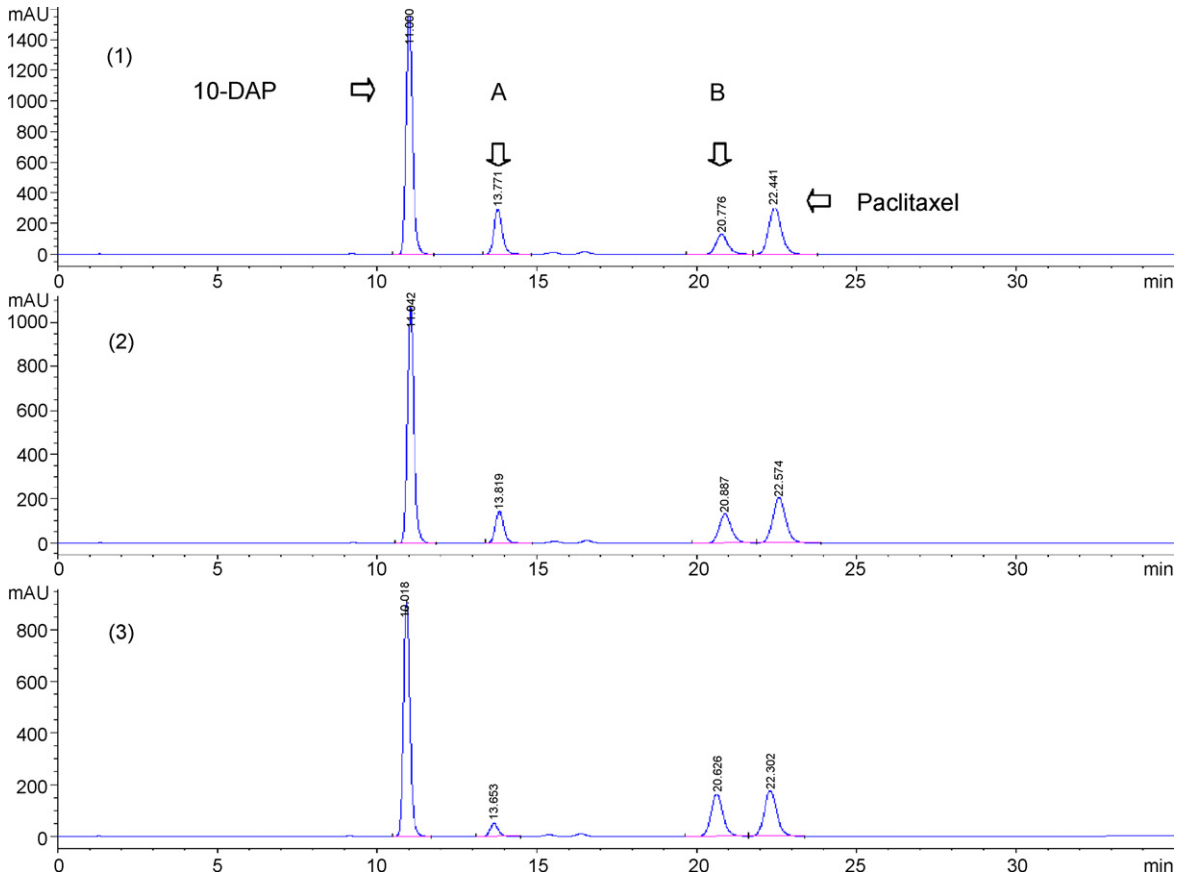

Fig. 1. Chromatograms for a mixture of **A**, **B**, and paclitaxel analyzed by HPLC. (1) Original chromatogram. (2) After 2.5 h at room temperature. (3) After 5.0 h at room temperature. 10-Deacetylpaclitaxel (10-DAP) and paclitaxel were used as internal references. (A) Derivative **A** and (B) derivative **B**.

2.2. Rearrangement of paclitaxel and separation of the resulting product

To prepare **A** and **B**, purified paclitaxel was dissolved in dichloromethane and evaporated under reduced pressure. To accelerate the rearrangement, the recovered paclitaxel was dried and stored in a vacuum oven at 100 °C for 3 days. The amount of **A** increased gradually during storage. A small amount (content 0.4%) of **A** was fractionated using semi-preparative HPLC on a Waters Delta Prep 3000 system fitted with a Curosil PFP column (20 mm × 250 mm, d_p = 5 μm; Phenomenex, Torrance, CA, USA). It was eluted with a gradient of water:acetonitrile from 65:35 to 35:65 over 30 min at a flow rate of 18.0 ml min^{-1} with monitoring at 227 nm. To isolate **A**, the fractionated solution was immediately extracted three times with dichloromethane, and then dried. After the fractionated solution was held for at least 12 h at room temperature, **B** converted from **A** was separated from the solution using dichloromethane extraction, followed by drying.

2.3. Chemical rearrangement of paclitaxel

The oxetane ring-opened derivatives **A** and **C** were prepared using a modification of the method of Chen et al. [17]. Paclitaxel (100 mg, 0.117 mmol) was dissolved in dry dichloromethane (5 ml), cooled to 0 °C, and treated with $SnCl_4$ (130 μl in 0.5 ml dichloromethane, 0.001 mmol) for 15 min (conversion < 40%). The mixture was applied to a silica gel column directly and eluted with 60% ethyl acetate in hexane, affording **A** and **C**.

2.4. High performance liquid chromatography (HPLC) analysis

The fractionated solution was analyzed using a HP1090 HPLC system (Hewlett-Packard, Palo Alto, CA, USA) fitted with an ODS-18 column (4.6 mm × 250 mm, d_p = 5 μm; Shiseido, Tokyo, Japan). HPLC was performed isocratically using solution **A** (water:acetonitrile = 2:3) for 20 min at a flow rate of 1.2 ml min^{-1} followed by a gradient from solution **A** to acetonitrile from 100:0 to 10:90 over 60 min. The elution was monitored at 227 nm.

2.5. Spectroscopy analysis

^{1}H NMR spectra were obtained on a JNM-AL400 (JEOL, Tokyo, Japan) spectrometer operating at 400 MHz. The chemical shifts are reported in ppm relative to that of tetramethylsilane (TMS, $\delta = 0$) as an internal standard, and the coupling constants are given in Hertz.

Liquid chromatography/mass (LC/ESI-MS) spectrometry was performed using an ABI MARINER (Applied Biosystems, Foster City, CA, USA) coupled to a LC-10AD