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Stability of Andrographolide in Powdered Andrographis Herb under Accelerated Conditions

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Abstract

The stability of andrographolide in powdered Andrographis Herb – the aerial part of *Andrographis paniculata* (Burm. f.) Nees (Acanthaceae) – was determined using a heat-accelerated experiment to reveal a second-order kinetics of degradation. The fast decomposition was observed regardless of the method of analysis. The rate constant of the decomposition of andrographolide at 25 °C ($k_{25°C}$), predicted from the Arrhenius plot, was 6.58×10^{-6} d^{-1}.

Supporting information available online at http://www.thieme-connect.de/ejournals/toc/plantamedica

Andrographis Herb (APH) or Andrographitis Herba, as officially named in the Thai Herbal Pharmacopoeia (THP), is the dried aerial part of *Andrographis paniculata* (Burm. f.) Nees (Acanthaceae) [1]. The plant is widely known for its wide range of activities (for example, see [2], [3], [4], [5]) and is used in several Asian countries including China, India, and Thailand. In THP [1], APH is categorized as an anti-inflammatory agent for laryngitis, as well as an antidiarrheal and antipyretic agent. The herb is also used for the treatment of liver and cardiovascular diseases in Ayurvedic medicines [6], [7]. The active constituents in *A. paniculata* responsible for the activities are labdane-type diterpene lactones, among which andrographolide (**1**) is the major component (Fig. **1**) [8].

Despite its high potential, *A. paniculata* is one of a few herbal medicines associated with a short shelf life. The shelf life of 12 months was recommended by THP [1], as estimated according to the decrease in the total lactone content by 26% upon 1-year storage of the dried, powdered herb in dry, ambient conditions [9]. The second-order degradation of amorphous andrographolide under a heat-accelerating condition corresponded well with such a recommendation [10]. On the other hand, when in aqueous solution, **1** decomposed through a first-order kinetics

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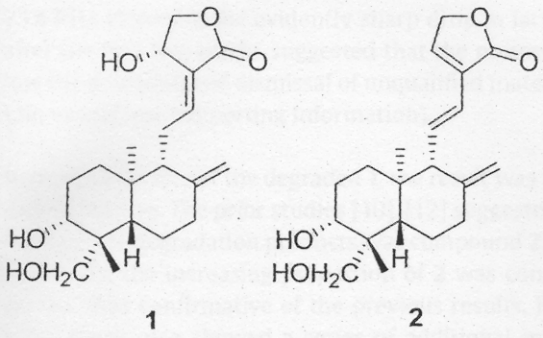

Fig. 1 Chemical structures of andrographolide (**1**) and 14-deoxy-11,12-didehydroandrographolide (**2**).

[11]. The stability of **1** in powdered APH at room temperature was reported to show a rapid decrease (8–25%) after 3 months. However, the total contents of the 3 major diterpene derivatives, as well as that of **1**, otherwise increased after 15 months [12]. The increases in total diterpene contents were partly explained due to the possible transformation of **1** into its related derivatives, namely 14-deoxy-11,12-didehydroandrographolide (**2**) (Fig. **1**) [10], [12].

In order to resolve the arguable conflict due to the inconsistent changes in the contents of each diterpene, particularly that of **1**, the stability determination of **1** in APH under accelerated conditions is reported here. The aim of this study is to acquire the theoretical kinetics that can be a guideline for a more precise real-time stability study and eventually lead to a mandated and effective storage condition for APH.

A simple extraction protocol of 1-h refluxing of the powdered herb in CH_3OH was first devised to couple to the readily validated HPLC procedure [10] without the necessity of pre-chromatographic purification. The linearity of the extraction protocol was met within a range of 0.2–0.7 g of powdered APH (r^2 = 0.9965), and that of the HPLC analysis was examined to be constantly valid in a range of 1–20 µg/mL ($r^2 \geq 0.998$). The average extractable amount of **1** from each sample, with a variable amount of the herb, was 58.01 ± 3.40 mg/g of APH (% R.S.D = 5.86) (see Supporting Information for the quantification procedures).

The heat-accelerated degradation experiment for **1** in powdered APH was performed at 3 elevated temperatures; 45, 60, and 70 °C, all under an atmosphere of 75% relative humidity (RH). The storage condition was designed to mimic the average-to-poor storage conditions, i.e., stored in paper sachets that allow free exposure to heat and atmospheric moisture. The degradation kinetics of **1** in powdered APH fitted well with second-order kinetics, in which the linearity was best met when the reciprocals of the remaining percentages of **1** in each sample were plotted against time t (Fig. **2**). The rate constants k of the degradation from each temperature (45, 60, and 70 °C) are 0.2×10^{-3}, 2.6×10^{-3}, and 8.4×10^{-3} d^{-1}, respectively. An Arrhenius plot (Fig. **3**), obtained from the relation of ln k and the reciprocals of the corresponding thermodynamic temperatures T, showed a good linear correlation (r^2 = 0.9942).

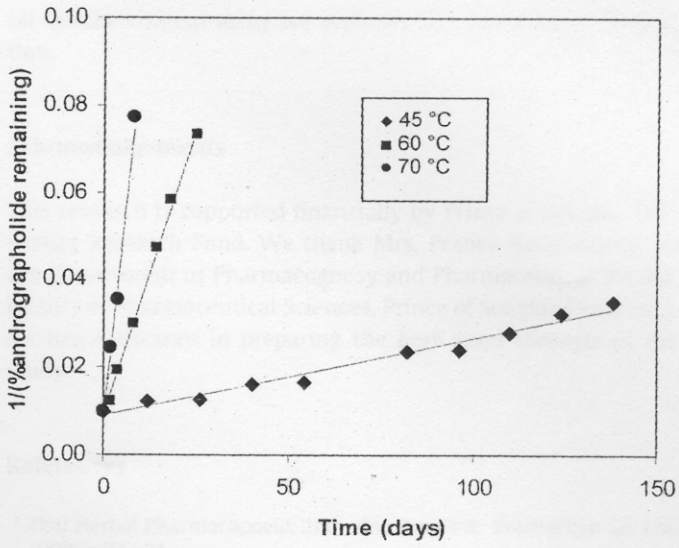

Fig. 2 Second-order plots of degradation of **1** in APH at 45, 60, and 70 °C. The rate equation for each temperature is expressed as $1/c = (0.2 \times 10^{-3})t + 0.0089$, r^2 = 0.9790; $1/c = (2.6 \times 10^{-3})t + 0.0089$, r^2 = 0.9966; and $1/c = (8.4 \times 10^{-3})t + 0.0074$, r^2 = 0.9830, respectively.

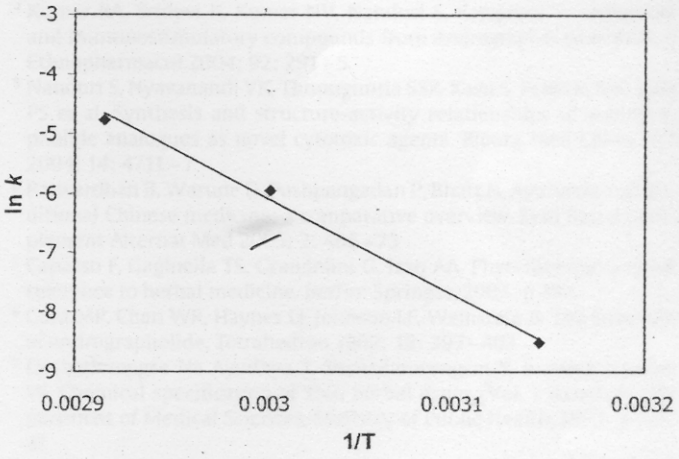

Fig. 3 Arrhenius plot of **1** in APH. The relation is expressed as ln $k = 43.368 - (1.65 \times 10^4)/T$, r^2 = 0.9942.

Extrapolation of the Arrhenius plot led to the calculated k at 25 °C ($k_{25°C}$) of 6.58×10^{-6} d^{-1}. Using the presumably 100% remaining andrographolide as the initial amount to conjure a kinetic equation at 25 °C, the half life ($t_{1/2}$) and shelf life ($t_{90\%}$) at 25 °C were theoretically estimated to be 4.2 and 0.46 years, respectively. It is noteworthy to mention here that the shelf life of less than 1 year had also been observed when the degradation experiments had been performed with isolated andrographolide using similar accelerated conditions [10].

Due to Thai FDA regulation that requires all APH products in the country to be assayed according to the THP protocol, i.e., the assay of total lactone content [1], [12], we were obliged to determine how the degradation progressed when monitored with the conventional method of analysis. The non-selective nature of the protocol did not facilitate the postulation of a precise kinetics that fitted well with the decline in lactone contents in powdered APH that had been subjected to an accelerated condition (70 °C,

75% RH). However, the evidently sharp drop in lactone content after the first two weeks suggested that the protocol should allow the detection and dismissal of unqualified materials to a certain extent (see Supporting Information).

Regarding the fate of the degraded **1**, the result was unfortunately inconclusive. The prior studies [10], [12] suggested that one of the possible degradation products was compound **2**. Throughout this study, the increasing proportion of **2** was consistently observed, thus confirmative of the previous results. However, the HPLC traces also showed a series of additional minor components, most of which are also gradually increasing, even though unquantifiable. This does not favor the presumption whether a similar pathway predominated throughout the course of degradation of **1**. Furthermore, although unable to concur in such a hypothesis, it is possible to consider the interaction of **1** with other chemical components in the cellular compartments among the possible degradation pathways.

Materials and Methods

Standard andrographolide used throughout this experiment was prepared in-house, isolated from *A. paniculata* according to the isolation protocol previously described [10]. The authenticity and qualitative purity were determined on the basis of spectroscopic identification [8], [13], from which no observable signals of a major impurity were detected.

APH used in the stability study was cultivated in the nursery of the Faculty of Pharmaceutical Sciences, Prince of Songkla University. The authentication as *A. paniculata* was carried out by one of us (TS), and a voucher specimen (SKP 001-01-16) was deposited at the Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkla University. The aerial part of the herb was harvested at the age of approximately 3 months [9]. Once collected, the herb was rinsed, air-dried (24 h), and oven-dried at 40–50 °C (18 h). It was then ground and sifted through a 80-mesh sieve. Once ground, the herb was strictly kept in a dried plastic bag under air-conditioned storage for not longer than 1 week prior to the further experiments.

The elevated-temperature systems were performed using closed desiccators containing saturated NaCl solution to furnish an atmosphere of 75 ± 5% RH. A series of paper sachets (5 × 5 cm) containing 5 g of dried, ground and sieved APH were placed in the aforementioned desiccators, which were separately allowed to stand in ovens with controlled temperatures of 45, 60, and 70 °C (Memmert BP600, B50, and Pr402, respectively) (Memmert; Schwabach, Germany). The sachets were randomly and independently collected in triplicate from each temperature according to the following intervals: d 0, 12, 26, 40, 54, 82, 96, 110, 124, and 138 (45 °C); d 0, 2, 4, 8, 14, 18, and 25 (60 °C); d 0, 2, 4, and 8 (70 °C for the determination of **1**); and d 0, 15, 26, 40, 54, and 68 (70 °C for the determination of total lactone contents).

Supporting information: Extraction and chromatographic conditions, examples of HPLC chromatograms of APH showing the changes in proportion of major constituents, and results from total lactone content assay are available the Supporting Information.

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