Monitoring of BHT-Quinone and BHT-CHO in the Gas of Capsules of *Asclepias physocarpa*

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Three volatile components, namely benzoic acid ethyl ester (**1**), 2,6-di-*tert*-butyl-*p*-benzoquinone (BHT-quinone) (**2**), and 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde (BHT-CHO) (**3**), were detected from the gas in the capsules of *Asclepias physocarpa* by means of GC/MS analysis. BHT-quinone and BHT-CHO as organic pollutants are the degradation products of the antioxidant 2,6-di-*tert*-butyl-4-methylphenol (BHT). Ground water, lake water and/or rain water are a source of BHT metabolites in the plant *Asclepias physocarpa*.

Key words: *Asclepias physocarpa*, 2,6-Di-*tert*-butyl-*p*-benzoquinone (BHT-Quinone), 3,5-Di-*tert*-butyl-4-hydroxybenzaldehyde (BHT-CHO)

Introduction

Asclepias physocarpa (E. Meyer) Schltr., native to southern Africa, naturalized in Hawaii, Jamaica and most parts of Australia, now introduced into Yunnan, China as an ornamental, belongs to the milkweed family (Asclepiadaceae). It can grow either as annual or short-liked perennial, and prefers a sun position with good drainage. *A. physocarpa* is also called swan plant because the seed of this fantastic plant looks just like a swan. Like all milkweeds, it excretes a poisonous milk sap, which tastes bad and is not the sort of food animals and insects want to eat.

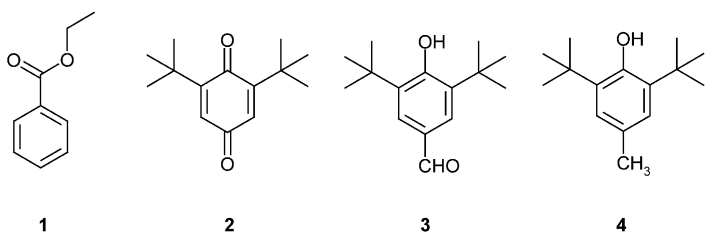
Up to date, there are no reports on the capsules of this plant. We focused on the chemical constituents of the gas in the capsules and attempted to reveal the function of this interesting plant tissue by means of GC/MS analysis. As a result, three volatile components, namely benzoic acid ethyl ester (**1**), 2,6-di-*tert*-butyl-*p*-benzoquinone (BHT-quinone) (**2**), and 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde (BHT-CHO) (**3**), were identified.

Results and Discussion

Mainly three volatile components, namely benzoic acid ethyl ester (**1**), BHT-quinone (**2**), and BHT-CHO (**3**) (Fig. 1), were detected and identified from the gas in the capsules of *Asclepias physocarpa* by means of GC/MS analysis. These three compounds existed either in young or mature capsules of *A. physocarpa*. In the leaves of *A. physocarpa*, the antioxidant 2,6-di-*tert*-butyl-4-methylphenol (BHT) (**4**) was found together with compound **1**. Furthermore, BHT-quinone and BHT-CHO are the metabolites of the antioxidant BHT in terms of the literature (Oikawa *et al.*, 1998; Fries and Püttmann, 2004).

Benzoic acid ethyl ester (**1**) has a common fruity odor, and is found in various fruits, *e.g.* apple, banana. Initially, we paid more attention to the other two compounds and tried to explain the function of these two compounds in capsules of *Asclepias physocarpa*. However, we eventually realized that these two compounds may be the metabolites of the antioxidant BHT after we got enough information about these two compounds from the literature. Further investigation on the volatile components in leaves of *A. physocarpa* was conducted to confirm our hypothesis. Expectably, the parent compound BHT was detected.

BHT has been used as an antioxidant since the 1950s (IARC, 1986) to preserve and stabilize the freshness, nutritive value, flavor and color of food



1 2 3 4

Fig. 1. Structures of benzoic acid ethyl ester (**1**), BHT-quinone (**2**), BHT-CHO (**3**) and BHT (**4**).

and animal feed products (JECFA, 1996). BHT can also improve the stability of pharmaceuticals, fat-soluble vitamins and cosmetics. Additionally, the durability of rubber and plastics is increased by the use of BHT (Sherwin-Williams, 1992). In the European Union, 18,000 t of BHT were produced in 1989 and in the USA, about 12,200 t of BHT were produced in 1998 (BUA, 1991; ChemExpo, 1999). The use of BHT as a food additive does not appear to pose a cancer risk to humans (JECFA, 1996). In contrast to the parent compound, however, BHT metabolites may pose a human health risk. Oikawa *et al*. (1998) suggested for instance that BHT metabolites generate peroxides in mice and rats and may induce cellular DNA damage or have the capacity as a cancer initiator.

Possible sources of BHT-quinone and BHT-CHO in capsules and BHT in leaves of *Asclepias physocarpa* are organic pollutants from river water and ground water around. Nowadays, BHT and its metabolites dissolved in water have entered the plant and food chain. Vallat *et al*. (2005) detected BHT-CHO in volatile emissions from apple trees. Huang *et al*. (2004) detected BHT and BHT-CHO in drinking water in Changsha, China, by GC/MS analysis. Wu *et al*. (2003) conducted an analysis of odors in human armpit by GC/MS with headspace solid phase micro-extraction, and detected BHT-CHO in samples. Our results highlight the need to study and monitor BHT and particularly its metabolites in the aquatic environment in the future.

Experimental

General

Sample analysis was conducted in a GC/MS instrument (HP6890GC/5973MS, Agilent Technologies, Palo Alto, USA). The sample was injected into a capillary column (30 m × 0.25 mm × 0.25 μm film thickness). After injection, the initial temperature (46 °C) was increased at 30 °C/min up to 80 °C, and thereafter at 3 °C/min up to 240 °C. Helium was used as carrier gas, and the flow was 1 ml/min.

Individual compounds were identified by comparison with standards of a mass spectrum database (WILEY7N.L and NIST98.L) and spectral data in the literature.

Plant material

The fresh *Asclepias physocarpa* was collected at Jiangchuan County of Yunnan province, China, in October 2005. The botanical identification was made by Prof. Dr. Hua Peng, Kunming Institute of Botany, the Chinese Academy of Sciences. A voucher specimen (Peng and Liu 2005001) was deposited in the Herbarium of Kunming Institute of Botany, the Chinese Academy of Sciences (KUN).

Analysis of volatiles in capsules and leaves of Asclepias physocarpa

To trap the gas in capsules, an injector was used with 2 g charcoal in the top. It was carefully sticked in the capsule to draw out the gas inside, *ca*. 20 ml, and then lain for air circulation maintained for 10 h, while the volatiles of the capsule were continuously absorbed on the charcoal trap. Following desorption from the charcoal with methylene chloride (2 × 0.5 ml) the samples were directly analyzed by GC/MS. Three components, namely benzoic acid ethyl ester (**1**), BHT-quinone (**2**), and BHT-CHO (**3**), were identified.

The same analytical methods were used for the young and mature capsules. These three compounds mentioned above were also detected, and there was no distinct difference in content.

Four leaves of *Asclepias physocarpa* chosen randomly were extracted with methylene chloride (2 × 2 ml) for 1 h, and the combined extracts were directly analyzed by GC/MS. Fifteen peaks were detected and identified, among them, benzoic acid ethyl ester (**1**) and BHT (**4**).

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