Phytochemistry 152 (2018) 174–181

Contents lists available at ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

Cytotoxic and other withanolides from aeroponically grown *Physalis philadelphica*

Ya-Ming Xu [a], E.M. Kithsiri Wijeratne [a], Alan D. Brooks [b, c], Poonam Tewary [b, c], Li-Jiang Xuan [d], Wen-Qiong Wang [d], Thomas J. Sayers [b, c], A.A. Leslie Gunatilaka [a, *]

[a] Natural Products Center, School of Natural Resources and the Environment, College of Agriculture and Life Sciences, The University of Arizona, 250 E. Valencia Road, Tucson, AZ 85706, United States
[b] Basic Research Program, Leidos Biomedical Research, Inc., Frederick National Laboratory for Cancer Research, National Cancer Institute-Frederick, Maryland 21702, United States
[c] Cancer and Inflammation Program, National Cancer Institute-Frederick, Maryland 21702, United States
[d] State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 501 Haike Road, Zhangjiang Hi-Tech Park, Shanghai 201203, PR China

ARTICLE INFO

Article history:
Received 8 October 2017
Received in revised form
16 April 2018
Accepted 30 April 2018

Keywords:
Physalis philadelphica
Solanaceae
Aeroponic cultivation
Chemical investigation
Withanolides
Cytotoxic activity

ABSTRACT

Eleven withanolides including six previously undescribed compounds, 16β-hydroxyixocarpanolide, 24,25-dihydroexodeconolide C, 16,17-dehydro-24-*epi*-dioscorolide A, 17-*epi*-philadelphicalactone A, 16-deoxyphiladelphicalactone C, and 4-deoxyixocarpalactone A were isolated from aeroponically grown *Physalis philadelphica*. Structures of these withanolides were elucidated by the analysis of their spectroscopic (HRMS, 1D and 2D NMR, ECD) data and comparison with published data for related withanolides. Cytotoxic activity of all isolated compounds was evaluated against a panel of five human tumor cell lines (LNCaP, ACHN, UO-31, M14 and SK-MEL-28), and normal (HFF) cells. Of these, 17-*epi*-philadelphicalactone A, withaphysacarpin, philadelphicalactone C, and ixocarpalactone A exhibited cytotoxicity against ACHN, UO-31, M14 and SK-MEL-28, but showed no toxicity to HFF cells.

1. Introduction

The genus *Physalis* (Solanaceae) contains over 75 species some of which are used as foods and medicines (Whitson and Manos, 2005; Kindscher et al., 2012). Of these, the most widely distributed and cultivated species is *Physalis philadelphica* Lam. (Solanaceae) also known as tomatillo (Wang et al., 2012). Tomatillo is one of the basic ingredients of fresh and cooked Mexican and Central American green sauces. In South American traditional medicine, the fruits of *P. philadelphica* are used to relieve fever, cough, and amygdalitis, the leaves as a remedy for gastrointestinal disorders, and the calices for the treatment of diabetes (Maldonado et al., 2011). Plants of the genus *Physalis* have also received considerable attention because of their constituent withanolides, a class of polyoxygenated steroids based on C_{28} ergostane skeleton (Glotter, 1991). Withanolides, which also occur in other genera of Solanaceae including *Acnistus*, *Datura*, *Dunalis*, *Jaborosa*, and *Withania* are classified into two main classes, one containing the parent skeleton of withaferin A (group I) and the other with a modified skeleton (group II) (Yang et al., 2016). Many withanolides belonging to group I contain a 2,3-enone moiety in ring A and a δ-lactone moiety in their side chains. Based on their oxygenation patterns and the orientation of the side chain, group I withanolides belong to four basic classes to include withaferin A, withanolide A, withanolide D, withanolide E and their structural analogues. Among these, only those of withanolide E class contain an α oriented side chain and a β-hydroxy group at C-17 and are referred to as 17β-hydroxywithanolides.

Withanolides have been reported to exhibit a variety of biological activities including cytotoxic, anti-feedant, insecticidal, trypanocidal, leishmanicidal, antimicrobial, anti-inflammatory, phytotoxic, cholinesterase inhibitory and immune-regulatory activities, and effects on neurite outgrowth and synaptic

* Corresponding author. Natural Products Center, The University of Arizona, 250 E. Valencia Road, Tucson, AZ 85706-6800, United States.
E-mail address: leslieg1@email.arizona.edu (A.A.L. Gunatilaka).

https://doi.org/10.1016/j.phytochem.2018.04.018
0031-9422/

reconstruction (Chen et al., 2011). Among these, the most widely investigated are cytotoxic and other activities related to their potential use as anticancer agents. These studies have suggested that unlike the most extensively investigated withanolide, withaferin A, withanolides belonging to withanolide E (**13**) class (17β-hydroxywithanolides) were selectively cytotoxic to only certain cancer cell lines and that these two classes of withanolides may have different molecular targets (Xu et al., 2015, 2017; Tewary et al., 2017). Thus, it was of interest to investigate the cytotoxic activity of withanolides belonging to different structural types.

Previous phytochemical investigations of *P. philadelphica,* also known as *P. ixocarpa* Brot. (Hudson, 1986), have resulted in the isolation of several withanolides, including physalin B (Subramanian and Sethi, 1973), withaphysacarpin (Subramanian and Sethi, 1973; Kennelly et al., 1997; Su et al., 2002), ixocarpalactone A (Kirson et al., 1979; Su et al., 2002; Gu et al., 2003; Maldonado et al., 2011), ixocarpalactone B (Kirson et al., 1979; Su et al., 2002), ixocarpanolide (Abdullaev et al., 1986; Maldonado et al., 2011), 2,3-dihydro-3-methoxywithaphysacarpin and 24,25-dihydrowithanolide D (Kennelly et al., 1997), philadelphicalactone A (Su et al., 2002; Maldonado et al., 2011), philadelphicalactone B, 18-hydroxywithanolide D and withanone (Su et al., 2002), philadelphicalactone C (Maldonado et al., 2011), philadelphicalactone D (Maldonado et al., 2011), 2,3-dihydro-3β-methoxyisocarpalactone A (Gu et al., 2003), 2,3-dihydro-3β-methoxyisocarpalactone B and 4β,7β,20R-trihydroxy-1-oxowitha-2,5-dien-22,26-olide (Gu et al., 2003). In our continuing interest on new and/or biologically active withanolides (Wijeratne et al., 2014; Xu et al., 2015, 2016, and 2017), we have investigated aeroponically grown *P. philadelphica* and herein we report the isolation, identification, and cytotoxic activity of six new and five known withanolides, some of which have not been encountered in wild-crafted plants. The new withanolides were identified as 16β-hydroxyixocarpanolide (**1**), 24,25-dihydroexodeconolide C (**2**), 16,17-dehydro-24-*epi*-dioscorolide A (**3**), 17-*epi*-philadelphicalactone A (**4**), 16-deoxyphiladelphicalactone C (**5**), and 4-deoxyixocarpalactone A (**6**). Comparison of spectroscopic data with those reported led to the identification of the known withanolides as withaphysacarpin (**7**) (Kennelly et al., 1997; Su et al., 2002), philadelphicalactone A (**8**) (Su et al., 2002; Maldonado et al., 2011), philadelphicalactone C (**9**) (Maldonado et al., 2011), and ixocarpalactones A (**10**) and B (**11**) (Kirson et al., 1979; Su et al., 2002).

2. Results and discussion

The aerial parts of aeroponically grown *P. philadelphica* were extracted with methanol and the resulting extract was fractionated by solvent-solvent partitioning with hexanes and 80% aq. methanol followed by 50% aq. methanol and chloroform to obtain hexanes, 50% aq. methanol and chloroform fractions. Of these only the chloroform fraction was found to contain withanolides. Thus, the chloroform fraction was further fractionated by silica gel and reversed phase column chromatography followed by preparative HPLC to afford withanolides **1**–**11** (see Fig. 1) as white amorphous powders.

2.1. Structure elucidation of the new withanolides *1*–*6*

The ^{1}H and ^{13}C NMR spectroscopic data of withanolides **1**–**3** (Tables 1 and 2) suggested that these are structurally related to each other and contain a carbon skeleton bearing 2,3-enone, 5α-hydroxy, 6α,7α-epoxy moieties in rings A/B and δ lactone moiety in the side chain similar to ixocarpanolide (**12**) (Abdullaev et al., 1986). Withanolide **1** possessed a molecular formula of $C_{28}H_{40}O_7$ as determined by its HRMS and NMR data and indicated nine degrees of unsaturation. The ^{1}H NMR spectrum of **1** (Table 1) displayed signals due to three methyl groups attached to non-protonated carbons [δ_H 1.18 (3H, s, H_3-19)], 1.19 (3H, s, H_3-18),

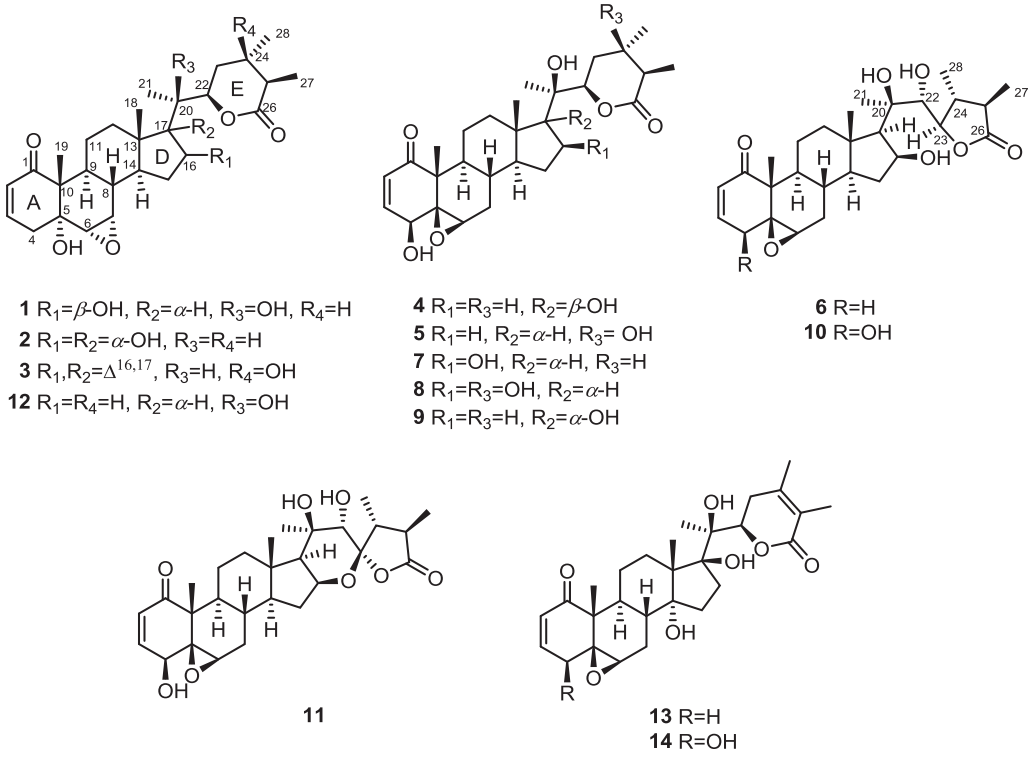

Fig. 1. Structures of withanolides **1**–**11** from aeroponically grown *P. philadelphica*, ixocarpanolide (**12**), withanolide E (**13**), and 4β-hydroxywithanolide E (**14**).