Phytochemistry xxx (2016) 1–11

Contents lists available at ScienceDirect

Phytochemistry

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

A sedge plant as the source of Kangaroo Island propolis rich in prenylated *p*-coumarate ester and stilbenes

Colin C. Duke [a, *], Van H. Tran [a], Rujee K. Duke [b], Abdallah Abu-Mellal [a, c], George T. Plunkett [d], Douglas I. King [a], Kaiser Hamid [a], Karen L. Wilson [e], Russell L. Barrett [f, g, h], Jeremy J. Bruhl [d]

[a] *Faculty of Pharmacy, The University of Sydney, New South Wales, Australia*
[b] *Department of Pharmacology, Faculty of Medicine, The University of Sydney, New South Wales, Australia*
[c] *Faculty of Pharmacy, Al Ain University of Science and Technology, Abu Dhabi, United Arab Emirates*
[d] *Botany, School of Environmental and Rural Science, University of New England, Armidale, New South Wales, Australia*
[e] *National Herbarium of New South Wales, Royal Botanic Gardens and Domain Trust, Sydney, New South Wales, Australia*
[f] *Kings Park & Botanic Garden, West Perth, Western Australia, Australia*
[g] *The University of Western Australia, Nedlands, Western Australia, Australia*
[h] *Australian National Herbarium, Centre for Australian National Biodiversity Research, CSIRO, Canberra, Australian Capital Territory, Australia*

ARTICLE INFO

Article history:
Received 10 February 2016
Received in revised form
10 November 2016
Accepted 16 November 2016
Available online xxx

Keywords:
Lepidosperma
Cyperaceae
Propolis
Honey bee
Prenylated hydroxystilbenes
Anticancer activity

ABSTRACT

Propolis samples from Kangaroo Island, South Australia, were investigated for chemical constituents using high-field nuclear magnetic resonance spectral profiling. A type of propolis was found containing a high proportion of prenylated hydroxystilbenes. Subsequently, the botanical origin of this type of propolis was identified using a beehive propolis depletion method and analysis of flora. Ligurian honey bees, *Apis mellifera ligustica* Spinola, were found to produce propolis from resin exuded by the Australian native sedge plant *Lepidosperma* sp. Montebello (Cyperaceae). The plants, commonly known as sword sedge, were found to have resin that matched with the propolis samples identified as the most abundant propolis type on the island containing *C*- and *O*-prenylated tetrahydroxystilbenes (pTHOS) in addition to a small amount of prenylated *p*-coumarate. The isolation of five pTHOS not previously characterized are reported: (*E*)-4-(3-methyl-2-buten-1-yl)-3,4′,5-trihydroxy-3′-methoxystilbene, (*E*)-2,4-*bis*(3-methyl-2-buten-1-yl)-3,3′,4′,5-tetrahydroxystilbene, (*E*)-2-(3-methyl-2-buten-1-yl)-3-(3-methyl-2-butenyloxy)-3′,4′,5-trihydroxystilbene, (*E*)-2,6-*bis*(3-methyl-2-buten-1-yl)-3,3′,5,5′-tetrahydroxystilbene and (*E*)-2,6-*bis*(3-methyl-2-buten-1-yl)-3,4′,5-trihydroxy-3′-methoxystilbene. A National Cancer Institute 60 human cell line anticancer screen of three of these compounds showed growth inhibitory activity. The large Australasian genus *Lepidosperma* is identified as a valuable resource for the isolation of substances with medicinal potential.

© 2016 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

1. Introduction

Propolis is resinous material produced by honey bees, *Apis mellifera* L., to fill holes and cracks in the hive, reduce the size of the hive entrance, provide a sterile coating on the walls of the hive, or to embalm dead animals too large for removal from the hive. The material is collected as a sticky exudate from different parts of specific plant species (Bankova et al., 2006). Common plant parts from which bees collect are leaf or flower buds, leaf glands, exudates, wounds in the bark or stems of plants and exudates produced in response to microbial infection or insect attack. Bees remove the resinous material with their mandibles and transfer it to their hind leg 'pollen baskets' where they form it into a mass that typically appears as smooth, shiny, semi-transparent droplets. The resinous material is not ingested by bees, and it appears that bees do not alter the material other than mixing with beeswax in the hive (Daugsch et al., 2008; Park et al., 2004; Piccinelli et al., 2011; Simone-Finstrom and Spivak, 2010; Tran et al., 2012). For thousands of years propolis has been used for human medicinal purpose and many medicinal applications for propolis are supported by

* Corresponding author.
E-mail address: colin.duke@sydney.edu.au (C.C. Duke).

http://dx.doi.org/10.1016/j.phytochem.2016.11.005
0031-9422/© 2016 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

Please cite this article in press as: Duke, C.C., et al., A sedge plant as the source of Kangaroo Island propolis rich in prenylated *p*-coumarate ester and stilbenes, Phytochemistry (2016), http://dx.doi.org/10.1016/j.phytochem.2016.11.005

scientific evidence. Bees are very selective in the material they collect to produce propolis. In Europe, bees collect from the leaf buds of poplar (*Populus* L.) and beech (*Fagus* L.) (Bankova et al., 2000). These sources are also foraged by bees in other parts of the world where these plants are available. Where the vegetation from which bees normally forage for propolis is not available, bees will forage from other resin producing plants. It has been found that propolis produced by honey bees from plants outside the traditional geographic location of honey bees generally has useful physical and antibiotic properties that effectively serve the purposes of bees. Propolis produced by bees in Europe typically contains polyhydroxyphenolic substances (Bankova et al., 2000) that have potent anti-oxidant and antibiotic properties. These substances include substituted cinnamates and flavonoids that may be methylated or prenylated, imparting them with a relatively non-polar property. The composition of propolis can be very variable as it depends on the plant source. Unique types of propolis have been found in many localities around the world. The vegetation from which they are sourced by honey bees has been identified and well characterized (Popova et al., 2007). For example, Brazilian propolis contains prenylated derivatives of *p*-coumaric acid and acetophenone (Park et al., 2002); Taiwanese propolis, also known as Pacific propolis, contains prenylated flavanones (propolins A-F) as major constituents (Chen et al., 2003); and Cuban propolis contains polyisoprenylated benzophenones as major components (Cuesta Rubio et al., 2002; Trusheva et al., 2004).

From a plant metabolism perspective, the prenylation of aromatic secondary metabolites plays a critical role in the biosynthesis of a wide range of molecules exerting valuable pharmacological effects across phylogenetically different classes of living organisms, from bacteria to mammals and plants. Anticancer potentials of prenylated hydroxystilbenes particularly those from peanut was recently reported. Arachidin-1, a prenylated piceatannol, isolated from germinating peanut seeds, inhibited human leukemia (HL-60) cells growth with an EC_{50} of approximately 4.2 μM, which is 4-fold more potent than resveratrol. That study showed that arachidin-1 induces programmed cell death in human leukemia HL-60 cells via both caspase-dependent and caspase-independent pathways (Huang et al., 2010).

Honey bees were introduced to Australia as part of agricultural practice more than 150 years ago and are now a well-established industry. Vast land areas and a flora largely unique to Australia have provided opportunity for bees to collect resins from non-traditional plant sources to produce propolis with different chemical composition and medicinal properties compared to propolis from other parts of the world. However, despite Australia's unique flora, no propolis of novel chemical constituent composition sourced from an Australian plant, defining both the chemical constituents profile and bee collection behaviour from the plant source, had been reported in detail. A study was undertaken to identify unique propolis types by chemical analysis of propolis samples collected in Australia and to select propolis types to track and characterize the vegetative source of the propolis. After preliminary studies, Kangaroo Island, South Australia, was identified as an area rich in relatively undisturbed endemic flora and as a source of honey bee propolis of unique chemical composition (Abu-Mellal et al., 2012). The variety of honey bee introduced to Kangaroo Island is *Apis mellifera ligustica* Spinola – the Ligurian honey bee, hereafter referred to as 'honey bee' or 'bee'.

Presented here are the isolation and characterization of five new and one known prenylated hydroxystilbenes (pTHOS) from Kangaroo Island propolis samples and the identification of the botanical origin of these propolis samples. Initial screening for inhibitory effect of the isolated prenylated hydroxystilbenes on the growth of cancer cells is also presented.

2. Results

2.1. Identification and characterization of five new prenylated stilbenes

As part of ongoing research into the medicinal properties of propolis, five prenylated stilbenes (Fig. 1), not previously characterized were isolated and characterized by 1D and 2D NMR, low- and high-resolution mass spectrometry. By evaluation of these data structures of **1**, **2**, **3**, **4**, and **5** (Fig. 1) were determined to have molecular formulae $C_{20}H_{22}O_4$, $C_{24}H_{28}O_4$, $C_{24}H_{28}O_4$, $C_{24}H_{28}O_4$ and $C_{25}H_{30}O_4$, respectively.

These compounds are systematically named as (*E*)-4-(3-methyl-2-buten-1-yl)-3,4′,5-trihydroxy-3′-methoxystilbene (**1**), (*E*)-2,4-bis(3-methyl-2-buten-1-yl)-3,3′,4′,5-tetrahydroxystilbene (**2**), (*E*)-2-(3-methyl-2-buten-1-yl)-3-(3-methyl-2-butenyloxy)-3′,4′,5-trihydroxystilbene (**3**), (*E*)-2,6-bis(3-methyl-2-buten-1-yl)-3,3′,5,5′-tetrahydroxystilbene (**4**) and (*E*)-2,6-*bis*(3-methyl-2-buten-1-yl)-3,4′,5-trihydroxy-3′-methoxystilbene (**5**).

In all five new compounds (**1**–**5**) prenyl groups are clearly recognized from the two vinyl methyl groups appearing as broad singlets in the range 1.6–1.8 ppm and the olefinic hydrogen appearing as a broad triplet in the range 5.0–5.5 ppm (Tables 1 and 2). *O*- and *C*- prenylation are clearly distinguished in the ^{1}H NMR spectra by signals at approximately 4.5 and 3.4 ppm, respectively, for the prenyl CH_2 protons attached to phenolic *O* or aromatic ring *C*. The position of substitution is generally determined from chemical shifts of remaining A-ring aromatic protons and changes in symmetry of the A and B-ring.

^{1}H and ^{13}C NMR spectra for compound **1** revealed a stilbene, evident by the presence of unique doublets at δ_H 6.90 (H-β) and at δ_H 6.77 (H-α); the *E*-configuration is shown by the $J_{\alpha\beta}$ of 16.0 Hz. Similarly, compounds **2** to **5**, were all identified as structures based on an *E*-stilbene configuration.

In compound **1**, the 4 position for the *C*-prenyl group on the A-ring is shown by symmetry resulting in identical chemical shift and lack of coupling of the remaining 2 aromatic hydrogens at 6.47 ppm. The B-ring H-2′, at 7.08 ppm and H-6′ at 6.93 ppm are deshielded by the stilbene double bond and strongly shielded by the oxygen attached at the 3′ position, while H-5′ at 6.76 ppm is mainly affected by strong shielding from the oxygen attached at the 4′ position. The 2 Hz coupling shown by H-2′ indicates a four-bond coupling to H-6′. For H-6′, in addition to the 2 Hz four-bond coupling to H-2′, a three-bond coupling of 8.1 Hz to H-5′ was observed showing a 1′,3′,4′- trisubstituted pattern for the B-ring. Gradient Heteronuclear Multiple Bond Correlation (gHMBC) experiment cross-peaks between the *C*-prenyl CH_2 hydrogens and C-3, C-4 and C-5 indicated the prenyl group was attached to C-4 and the methoxy group was attached to C-3′ (Fig. 2).

Compound **2** was found to have un-symmetrical di-*C*-prenyl substitution on the A-ring from recognition of two sets of ^{1}H and ^{13}C NMR signals for the two prenyl groups and ^{1}H NMR singlet at 6.63 ppm for H-6. The 3,5-dihydroxy substitution on the A-ring was shown by the characteristic deshielded carbon signals at 154.38 and 154.82 ppm for C-3 and C-5, and also was shown by a gHMBC experiment correlation between the *C*-prenyl H-1″, 3.35 ppm, with C-1, C-2 and C-3, and *C*-prenyl H-1‴, 3.40 ppm, with C-4 and C-5 (Fig. 2). A 1′,3′,4′- trisubstituted pattern for the B-ring was determined from the ^{1}H NMR chemical shifts and coupling pattern as for compound **1**.

Compound **3** showed ^{1}H and ^{13}C NMR signals for *O*-prenyl and *C*-prenyl substitution on a piceatannol skeleton. The position of the *C*-prenyl group was established by the chemical shifts and coupling of the two remaining aromatic H on the A-ring at 6.62 ppm, H-6 and 6.32 ppm, H-4, both doublets, J = 2.3 Hz. The position of

Please cite this article in press as: Duke, C.C., et al., A sedge plant as the source of Kangaroo Island propolis rich in prenylated *p*-coumarate ester and stilbenes, Phytochemistry (2016), http://dx.doi.org/10.1016/j.phytochem.2016.11.005

Download English Version:

https://daneshyari.com/en/article/5164022

Download Persian Version:

https://daneshyari.com/article/5164022

Daneshyari.com