Phytochemistry 93 (2013) 222–229

Contents lists available at SciVerse ScienceDirect

Phytochemistry

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

Component analysis of propolis collected on Jeju Island, Korea

Kohsuke Shimomura [a], Yasumasa Sugiyama [b], Jun Nakamura [c], Mok-Ryeon Ahn [d], Shigenori Kumazawa [a,*]

[a] Department of Food and Nutritional Sciences, University of Shizuoka, 52-1 Yada, Suruga-ku, Shizuoka 422-8526, Japan
[b] Bioorganic Chemistry Research Group, Faculty of Fisheries, Kagoshima University, 4-50-20 Shimoarata, Kagoshima 890-0056, Japan
[c] Honeybee Science Research Center, Tamagawa University, 6-1-1 Tamagawa Gakuen, Machida, Tokyo 194-8610, Japan
[d] Department of Food Science and Nutrition, Dong-A University, 840 Hadan 2-dong, Saka-gu, Busan 604-714, Republic of Korea

ARTICLE INFO

Article history:
Received 8 August 2011
Received in revised form 4 February 2012
Available online 5 April 2012

Keywords:
Angelica keiskei
Propolis
Jeju Island
Korea
Chalcone
Umbelliferae

ABSTRACT

A study of propolis from Jeju Island, located off the southern tip of Korea, led to the isolation and identification of eight chalcones: (±)-(*E*)-4′-methoxy-4,2′-dihydroxy-3′-(2″,3″-dihydroxy-3″-methylbutyl)-chalcone, (*E,E,E*)-4,2′,4′-trihydroxy-3′-(7″-hydroxy-3″,7″-dimethyloct-2″,5″-dienyl)-chalcone, (±)-(*E,E*)-4,2′,4′-trihydroxy-3′-(5″-hydroxy-3″,7″-dimethyloct-2″,6″-dienyl)-chalcone, (±)-(*E*)-4′-methoxy-4,3″,4″-trihydroxy-2″,2″-dimethyldihydropyrano-(2′,3′)-chalcone, (±)-(*E*)-4′-methoxy-4,3″-dihydroxy-2″-(1‴-hydroxyisopropyl)-dihydrofurano-(2′,3′)-chalcone, (−)-(*E*)-4,4′-dihydroxy-2″-(1‴-hydroxy-1‴,5‴-dimethylhex-4‴-enyl)-dihydrofurano-(2′,3′)-chalcone, (+)-(*E*)-4,2′-dihydroxy-2″-methyl-2″-(3‴,4‴-dihydroxy-4‴-methylpentanyl)-2*H*-pyrano-(3′,4′)-chalcone and (−)-(*E*)-4,2′-dihydroxy-2″-methyl-2″-(3‴,4‴-dihydroxy-4‴-methylpentanyl)-2*H*-pyrano-(3′,4′)-chalcone. Nineteen other known compounds were also isolated. Their structures were determined by spectroscopic analyses and comparison with literature data. The propolis from Jeju Island contained compounds not present in propolis from other regions.

1. Introduction

Propolis is a natural substance that honeybees, *Apis mellifera*, collect from buds and exudates of certain trees and plants. Propolis has various biological activities such as antibacterial, anti-inflammatory, antioxidant and anticancer properties, and has long been used as a folk medicine in many regions of the world (Bankova et al., 2000, 2001; Marcucci, 1995). In recent decades, propolis has attracted much attention, and is extensively used in foods, beverages, supplements and cosmetics intended to prevent diseases such as inflammation, heart disease and cancer, and as a cosmetic (Lotfy, 2006; Salantino et al., 2011; Sforcin and Bankova, 2011).

The chemical components of propolis depend on the vegetation at the region of collection, since honeybees preferentially target plants grown near beehives as sources of propolis. For example, propolis collected in temperate regions contains many kinds of flavonoids and phenolic acid esters, particularly pinocembrin, pinobanksin, galangin, chrysin and caffeic acid phenetyl ester, as the major source of the propolis is bud exudates of the *Populus* species (Bankova et al., 2000; Kumazawa et al., 2004b). On the other hand, green propolis from Minas Gerais State, Brazil, contains many kinds of terpenoids and prenylated derivatives of *p*-coumaric acids, such as artepillin C and (*E*)-3-prenyl 4-(dihydrocinnamoyloxy)-cinnamic acid, as the source of the propolis in young leaves of *Baccharis dracunculifolia* (Kumazawa et al., 2003). Furthermore, differences in plant origin also cause the variations in propolis properties such as biological activity, texture, flavor and color.

Salantino et al. (2011) have stated that although the focus of propolis research has centered mainly on Brazilian green propolis and temperate poplar propolis, propolis collected on many other regions are also promising. Previously, it was found that propolis from Okinawa, which is the southern-most prefecture in Japan, has many prenylflavonoids with strong antioxidant activity not present in propolis from other regions (Kumazawa et al., 2004a, 2007). Moreover, the components of Korean propolis were also studied from various geographical locations and it was found that the components of propolis from Jeju Island, located off the southern coast of Korea, differed from propolis from other regions (Ahn et al., 2004; Kumazawa et al., 2006). In these previous studies, three compounds were isolated and identified from the propolis collected on Jeju Island; however, the other components in it have remained unknown.

Thus, to investigate the potential utility of propolis, the components of propolis collected on Jeju Island, Korea were studied further. Eight new chalcones (**1**, **4**, **5**, **10** and **13**–**16**) and nineteen known compounds were isolated, and their structures were determined by spectroscopic analyses. In this report, the isolation and structural determination of these compounds are described.

* Corresponding author. Tel./fax: +81 054 264 5523.
E-mail address: kumazawa@u-shizuoka-ken.ac.jp (S. Kumazawa).

0031-9422/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.phytochem.2012.02.018

2. Results and discussion

The MeOH soluble fraction of propolis from Jeju Island was subjected to silica gel column chromatography and preparative reversed phase HPLC (RP-HPLC). Eight new chalcones (**1**, **4**, **5**, **10** and **13**–**16**), ten known chalcones (**2**, **3**, **6**–**9**, **11**, **12**, **17** and **18**) and nine known coumarins (**19**–**27**) (Fig. 1) were isolated.

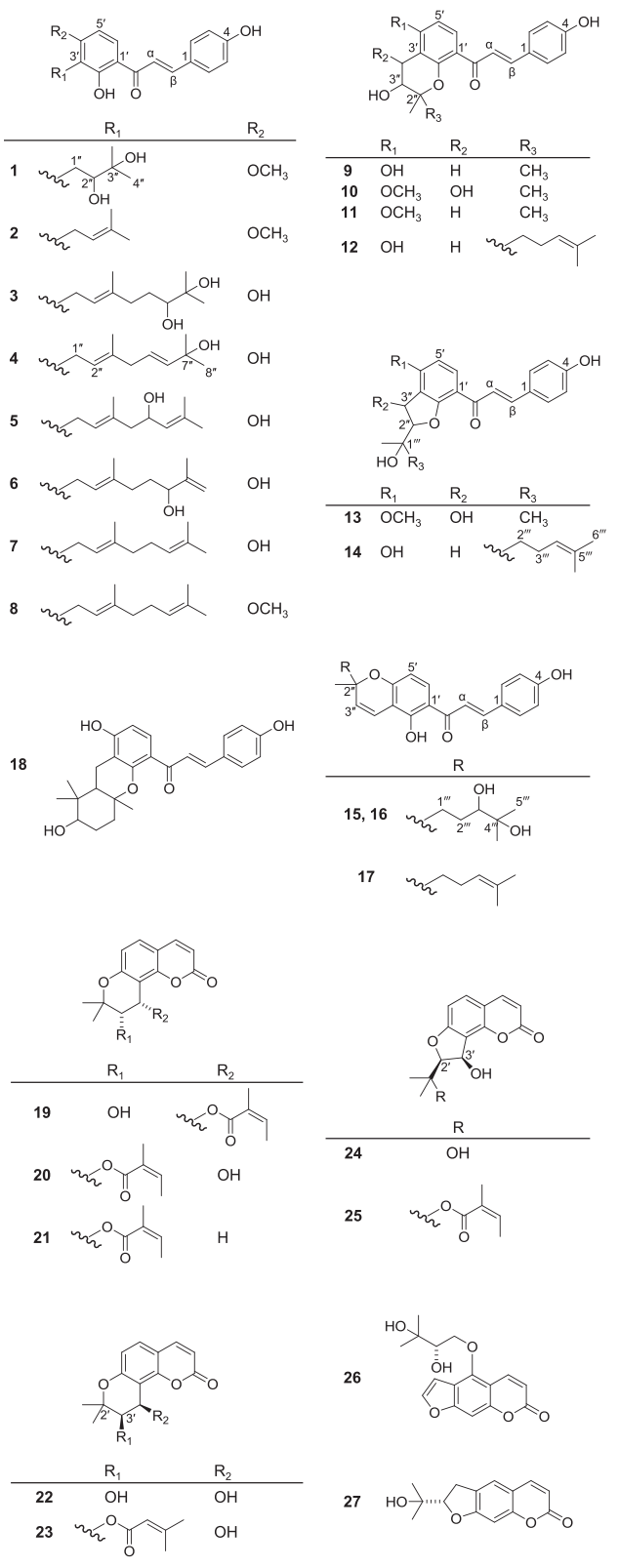

Fig. 1. Structures of compounds **1**–**27**.

Compound **1** was isolated as a yellow oil. Its molecular formula was determined to be $C_{21}H_{24}O_6$ by high resolution FABMS (HRFABMS). The ^{1}H NMR spectrum of **1** (Table 1) showed signals assignable to two *ortho*-coupled aromatic protons at δ_H 6.59 (1H, *d*, *J* = 9.1 Hz, H-5′) and 7.97 (1H, *d*, *J* = 9.1 Hz, H-6′) and four protons at δ_H 6.76 (2H, *d*, *J* = 8.7 Hz, H-3 and H-5) and 7.54 (2H, *d*, *J* = 8.7 Hz, H-2 and H-6). These resonances suggested the presence of a 1,2,3,4-tetrasubstituted benzene and a *para*-disubstituted benzene. This was supported by the ^{13}C spectrum of **1** (Table 2) which showed twelve signals assignable to aromatic carbons at δ_C 103.6 (C-5′), 115.8 (C-1′), 116.5 (C-3′), 117.0 (C-3 and C-5), 127.8 (C-1), 131.4 (C-6′), 131.9 (C-2 and C-6), 161.8 (C-4), 164.4 (C-2′) and 165.3 (C-4′). The ^{1}H NMR spectrum also showed signals assignable to an olefin moiety at δ_H 7.57 (1H, *d*, *J* = 15.3 Hz, H-α) and 7.73 (1H, *d*, *J* = 15.3 Hz, H-β). The geometry of this olefin moiety was determined to be an *E* configuration by the vicinal proton coupling constant ($J_{H\text{-}\alpha,\ H\text{-}\beta}$ = 15.3). The ^{13}C NMR spectrum also showed the typical downfield resonance assignable to a ketone group at δ_C 194.3 (C=O). These structural units and the HMBC correlations from the H-α proton to carbons C-1 and C=O, from the H-β proton to carbons C-1, C-2, C-6 and C=O, and from the H-6′ proton to the C=O carbon, established the presence of a chalcone skeleton. The presence of hydroxyl groups at C-4, C-2′, C-2″ (δ_C 79.1) and C-3″ (δ_C 74.2) was confirmed by the carbon chemical shift changes observed following hydrogen–deuterium exchange. The presence of a methoxy group was suggested by a proton signal at δ_H 3.84 (3H, *s*, 4′-OCH$_3$) and a carbon signal at δ_C 56.4 (4′-OCH$_3$). The presence of a 2″,3″-dihydroxy-3″-methylbutyl moiety was suggested by proton resonances at δ_H 1.15 (3H, *s*, H-4″), 1.17 (3H, *s*, 3″-CH$_3$), 2.78 and 2.83 (2H, *dd*, *J* = 13.5, 9.3 and 13.5, 3.6 Hz, H-1″) and 3.54 (1H, *dd*, *J* = 9.3, 3.6 Hz, H-2″), and carbon signals due to two methyl carbons at δ_C 25.2 (C-4″) and 25.7 (3″-CH$_3$), a methylene carbon at δ_C 26.0 (C-1″), an oxygenated methine carbon at δ_C 79.1 (C-2″), and an oxygenated quaternary carbon at δ_C 74.2 (C-3″). The presence of this moiety was established by the COSY correlation between protons H-1″ and H-2″, and the HMBC correlations from the H-4″ proton to carbons C-2″, C-3″ and 3″-CH$_3$. Moreover, the HMBC correlations from the H-1″ proton to carbons C-2′, C-3′ and C-4′, and from the 4′-OCH$_3$ proton to the C-4′ carbon, established the attachment of a 2″,3″-dihydroxy-3″-methylbutyl moiety and a methoxy group to the chalcone skeleton at C-3′ and C-4′, respectively. Based on these spectroscopic analyses, **1** was determined to be (*E*)-4′-methoxy-4,2′-dihydroxy-3′-(2″,3″-dihydroxy-3″-methylbutyl)-chalcone, and was named jejuchalcone A. Since **1** was optically inactive, it was concluded to be a racemic mixture.

Signals assignable to a chalcone skeleton were observed in the 1D and 2D NMR spectra of **4**, **5**, **10** and **13**–**16**, confirming that these compounds are chalcones (Tables 1 and 2). Additionally, the geometry of the olefin moieties in these chalcone skeletons were determined to be in the *E* configuration by the large vicinal proton coupling constants between H-α and H-β (**4** and **15**: *J* = 15.3 Hz, **5** and **16**: *J* = 15.4 Hz, **10**: *J* = 15.8 Hz, **13** and **14**: *J* = 15.6 Hz).

Compound **4** was obtained as a yellow powder. Its molecular formula was determined to be $C_{25}H_{28}O_5$ by HRFABMS. The presence of hydroxyl groups at C-4 (δ_C 161.0), C-2′ (δ_C 165.2), C-4′ (δ_C 162.9) and C-7″ (δ_C 70.1) was confirmed from the carbon chemical shifts upon hydrogen–deuterium exchange. In particular, the presence of a hydrogen bonding hydroxyl group at C-2′ was confirmed from the characteristic downfield signal at δ_H 14.0 (1H, *s*, 2′-OH) in the ^{1}H NMR spectrum. The presence of a 7″-hydroxy-3″,7″-dimethyloct-2″,5″-dienyl moiety was suggested by the 1D NMR spectrum, which showed two overlapping methyl protons at δ_H 1.22 (6H, *s*, H-8″ and 7″-CH$_3$), a methyl proton at δ_H 1.76 (3H, *br s*, 3″-CH$_3$), four olefinic