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Isolation of diterpenes and flavonoids from a new type of propolis from Saudi Arabia

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ABSTRACT

The chemical composition and biological activity of a sample of Saudi Arabian propolis were investigated. A new diterpene propsiadin ((ent)-2-oxo-kaur-16-en-6,18-diol) (**3**) along with the flavonoids 3,4-dihydro-2-(3,4-dihydroxyphenyl)-2H-chromene-3,7-diol (**1**), psiadiarabin (**2**) and the diterpene psiadin (**4**) were isolated from a Saudi Arabian propolis. Their structures were determined by HR-ESI-MS, 1D and 2D NMR. Compound **1** was inactive against *Trypanosoma brucei* and *Mycobacterium marinum* while compounds **2**–**4** had MICs of 30.9, 78.1 and 78.1 μM against *T. brucei* respectively and 312.1, 312.1 and 69.1 μM against *M. marinum*. The likely sources of the propolis are *Psiadia arabica* and *Psiadia punctulata* and it represents a new type of propolis not described before.

1. Introduction

Propolis is used in traditional medicine in many countries (Popova et al., 2010). Recently it has become popular as a component of health food and an alternative medicine (da Silva Frozza et al., 1997; Petrova et al., 2010). Honey bees (*Apis mellifera*) collect exudates from various buds and flora in order to seal the walls of the hive and protect it from micro-organisms (Piccinelli et al., 2011). Propolis composition varies according to geographic region (Bertelli et al., 2012; Salatino et al., 2011; Watson et al., 2006). Propolis has antimicrobial, antimycotic, anti-inflammatory, antioxidant, antitumor, anti-neurodegenerative, antituberculosis, antiviral, cytotoxic, anti-inflammatory, and immunomodulatory activities (Falcao et al., 2010; Yildirim et al., 2004). It contains many chemical constituents and to date more than 300 compounds have been identified as constituents including polyphenols, flavanones, flavonols, flavones, dihydroflavonols, chalcones, phenolic acids and their esters, monoterpenes, sesquiterpenes, diterpenes and triterpenes, steroids, aromatic aldehydes, alcohols, naphthalene and stilbene derivatives (Abu-Mellal et al., 2012; da Silva Frozza et al., 1997). This study reports the characterisation of a new type of propolis from Saudi Arabia and its activity on *Trypanosoma brucei brucei* the causative organism of African sleeping sickness. A new diterpene propsiadin ((ent)-2-oxo-kaur-16-en-6,18-diol) (**3**) along with the flavonoids 3,4-dihydro-2-(3,4-dihydroxyphenyl)-2H-chromene-3,7-diol (**1**), psiadiarabin (**2**) and the diterpene psiadin (**4**). The likely sources of the propolis are *Psiadia arabica* and *Psiadia punctulata* and it represents a new type of propolis not described before.

2. Results and discussion

The isolated compounds (Fig. 1) were obtained from an ethanol extract of the Saudi Arabian propolis. Compounds **3** and **4** were obtained by column chromatography followed by medium pressure flash chromatography on silica gel. The elemental compositions for the compounds were obtained from LC–MS analysis using an Orbitrap mass spectrometer. The structures were identified by 1D and 2D NMR analysis as summarised in Tables 1 and 2.

Compound **1** gave a molecular ion $[M+H]^+$ at *m/z* 275.0910 HR-ESIMS suggesting a molecular formula of $C_{15}H_{15}O_5$. The NMR data for **1** were comparable to that of anadanthoside (Piacente et al., 1999) previously isolated from the bark of *Anadenathera macrocarpa*. Compound **1** differed from anadanthoside by the presence of a free hydroxyl group which in the pyranoside anadanthoside is

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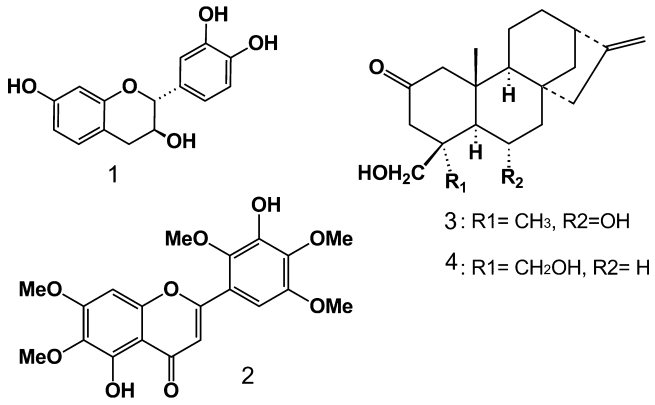

Fig. 1. Structures of compounds isolated from Saudi Arabian propolis.

linked to a xylose unit at C-3 (δ_C = 67.26) with the hydroxyl proton of the C-3 occurring δ_H 4.96 (d, J = 7.08 Hz). The COSY spectrum showed coupling between this proton and the proton at δ_H 3.87 (m) and they were both coupled to the C-4 protons at 2.60 ppm (dd, J = 8.0, 15.9 Hz). In addition, the OH-proton gave a long range (HMBC) correlation to C-2. The rest of NMR data in Table 1 correspond to those reported previously for the compound anadanthoside (Piacente et al., 1999). Thus the structure of compound **1** was elucidated as 3,4-dihydro-2-(3,4-dihydroxyphenyl)-2H-chromene-3,7-diol.

Compound **2** was obtained as a sticky yellow solid $[\alpha]_D$-16° (*c*. 0.1, $CHCl_3$). It yielded a molecular ion $[M+H]^+$ at m/z 405.1170 from its HR-ESIMS suggesting a molecular formula $C_{20}H_{21}O_9$. From its ^{1}H NMR and ^{13}C NMR spectra (Table 1) the presence of five methoxyl groups at C-6, C-7, C-2′, C-3′, C-4′ and C-5′ was clearly observed as well as the presence of a keto group at δ_C 182.5 ppm. Its ^{13}C NMR data exactly matched those reported for psiadiarabin (Al-Yahya et al., 1987; El-Feraly et al., 1990) previously isolated from the aerial parts of *P. arabica*.

Fraction 7 from CC of the ethanol extract of the propolis was further purified by flash chromatography on silica gel to yield compounds **3** and **4** (Fig. 1). Compound **3** was obtained as a yellow solid $[\alpha]_D$ −65° (*c*. 0.1, $CHCl_3$). Its molecular formula $C_{20}H_{30}O_3$ was obtained from HRESI MS for its $[M+H]^+$ ion at 319.2259 ($C_{20}H_{31}O_3$ calc 319.2268). MS^2 – m/z 319.22 $[M+H]^+$, 301 $[M-H_2O]^+$, 282 $[M-2H_2O]$, 265 $[M-(2H_2O, OH)]$, 255 $[M-(CH_2-CH_2)]$, 243 $[M-(CH_2-C=CH_2)]$. Compound **3** showed a set of deshielded protons typical of an exomethylene group at δ_H 4.81 and 4.86 ppm and a methine proton δ_H 4.16 attached to an oxygen bearing carbon at δ_C 68.3 ppm. It also showed two methyl singlets at δ_H 1.26 and 1.35 and a set of methylene protons (on an oxygen bearing carbon at δ_C 69.0) at 3.41 and 4.12 (2H, m). The number of carbons in its ^{13}C spectrum was typical of a diterpene and based on its COSY, HMBC (Fig. 2) and HMQC correlations the compound was identified as a kaurane type diterpene as follows: the methyl group at 1.35 showed long range correlations (HMBC) to the methylene at 52.1 and the oxymethylene carbon at 69.0 thus this methyl group must be at C-4. The correlations from the methylene protons at 4.12 and 3.41 (H-19) to the methyl at δ_C 31.1 ppm (C-18) indicate this oxymethylene group is also at C-4. The position of the second oxygenated carbon was obtained by the correlations from the methine proton at 1.36 (H-5) to an oxygenated carbon at δ_C 68.3 ppm. The COSY spectrum as well as HMBC correlations indicates methylene protons at C-1, C-3 and C-7 therefore this oxymethine carbon must be at C-6. Correlations to and from the rest of the protons and carbons indicated the exomethylene group to be on a kaurane ring moiety and was confirmed by correlations observed for the exomethylene protons as well as correlations to the quaternary double bonded carbon. The NOESY spectrum of the compound gave its relative stereochemistry as follows: The H-20 at 1.26 ppm gave strong correlations to one of the H-19 (CH_2OH) protons and H-6 proton thus they are all axial. Therefore the C-18 methyl and C-6 OH are equatorial. The C-5 proton must be

Table 1
^{1}H and ^{13}C NMR data of compounds **1** and **2** in d_6-DMSO.

Position	**1**			**2**		
	δ_H, mult., J (Hz)	δ_C		δ_H, mult.	δ_C	
1						
2	4.57 d (4.6)	81.9	CH	–	162.9	C
3	3.87 m	67.3	CH	6.81 s	109.2	CH
3-OH	4.96 d (7.1)	–	–	–	–	–
4	2.74 dd (5.1, 15.7) 2.58 dd (8.0, 15.9)	33.2	CH_2	–	182.5	C
5	6.82 d (8.2)	131.1	CH	–	152.4	C
5-OH	–	–	–	12.8 s	–	–
6	6.28 dd (2.4, 8.2)	109.1	CH	–	132.2	C
7	–	158.8	C	–	159.1	C
7-OH	9.16 s	–	–	–	–	–
8	6.18 d (2.4)	103.3	CH	6.90 s	92.1	CH
9	–	155.9	C	–	153.4	C
10	–	112.4	C	–	105.7	C
1′	–	131.7	C	–	119.8	C
2′	6.72 d (2.0)	115.7	CH	–	140.9	C
3′	–	146.5	–	–	145.0	C
3′-OH	8.88 s	–	C	9.45 s	–	–
4′	–	146.3	–	–	142.3	C
4′-OH	8.84 s	–	C	–	–	–
5′	6.67 d (8.0)	116.0	CH	–	149.8	C
6′	6.59 dd (2.0, 8.0)	119.2	CH	6.92 s	102.4	CH
6-OMe				3.74 s	61.0	CH_3
7-OMe				3.93 s	57.0	CH_3
2′-OMe				3.80 s	60.9	CH_3
4′-OMe				3.76 s	60.5	CH_3
5′-OMe				3.87 s	56.6	CH_3