



Characterisation of triterpenes and new phenolic lipids in Cameroonian propolis



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ARTICLE INFO

Article history:

Received 10 April 2014

Received in revised form 19 June 2014

Available online 4 August 2014

Keywords:

Cameroonian propolis

Triterpenes

Alk(en)ylphenols

Alk(en)ylresorcinols

Phenolic lipids

ABSTRACT

Chemical investigation of a sample of propolis originating from North-Western Cameroon led to the isolation of thirteen alk(en)ylphenols (**1–13**) (inseparable mixture) along with α -amyrin (**14**), β -amyrin (**15**), lupeol (**16**), cycloartenol (**17**), mangiferonic acid (**18**), ambonic acid (**19**), mangiferolic acid (**20**), ambolic acid (**21**), isomangiferolic acid (**22**) and nine alk(en)ylresorcinols (**23–31**) (inseparable mixture). All compounds were identified following analysis of their spectroscopic data and comparison with previously published reports. Compounds (**8**), (**12**), (**13**) and (**30**) are new natural products. GC–MS analysis carried out on the alk(en)ylphenol and alk(en)ylresorcinol mixtures (dimethyl disulphide trimethylsilyl derivatives) revealed the presence of saturated and mono-unsaturated compounds with side chain lengths ranging from C11 to C19 and C15 to C19, respectively. The position of the double bond in mono-unsaturated derivatives was established from the characteristic fragments resulting from the cleavage of the bond between the two methylthio-substituted carbons. The most abundant compound in each mixture was 3-(12'Z-heptadecenyl)-phenol (**10**) and 5-(12'Z-heptadecenyl)-resorcinol (**29**). This study is the first to report the presence of triterpenes (except for lupeol) and phenolic lipids, including eighteen compounds previously unreported in bee glue, in an African sample.

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1. Introduction

Propolis is a natural substance produced by bees upon collection of resins and exudates from plants. Bees use it as antiseptic glue to seal gaps between honeycombs, embalm dead intruders and generally preserve the hive from external contamination (Bankova, 2005a). Propolis has a long history of use in folk medicine and is a popular remedy currently employed to treat a variety of ailments (Castaldo and Capasso, 2002). Numerous scientific studies have been published on the biological properties of propolis and its constituents, including anti-inflammatory (Borrelli et al., 2002) anti-oxidant (Silva et al., 2011), hepatoprotective (Banskota et al., 2001), immunostimulant (Fischer et al., 2007), antitumour (Sawicka et al., 2012), neuroprotective (Nakajima et al., 2009) and antimicrobial activity (Seidel et al., 2008).

The chemical composition of propolis is complex. Different propolis types have been characterised based on the nature of

the plant-derived substances present and the geographical origin of collection. Typically, propolis is broadly characterised into (i) samples from temperate regions mainly originating from poplar tree exudates and rich in phenolics such as flavonoids, aromatic acids and esters (Bankova et al., 2002) and (ii) samples from tropical areas, devoid or containing traces of poplar constituents but rich in other substances including prenylated derivatives of *p*-coumaric acids, diterpenes and lignans (Marcucci and Bankova, 1999), prenylated benzophenones (Cuesta Rubio et al., 2002) and prenylated flavonoids (Raghukumar et al., 2010).

There are numerous reports in the literature on the isolation and structural elucidation of phytochemicals from propolis collected in Europe (Hegazi et al., 2000), South America (Trusheva et al., 2004), Asia and the Pacific region (Chen et al., 2004). Much less is known, however, about the exact chemical constituents of African propolis. In Cameroon, propolis is an important traditional medicine which has antibacterial and antiradical activity (Njintang Yanou et al., 2012; Mbawala et al., 2009, 2010; Talla et al., 2013). We carried out a phytochemical analysis of a propolis sample collected in North-Western Cameroon and report herein the characterisation of nine triterpenes, thirteen alk(en)ylphenols and nine alk(en)ylresorcinols.

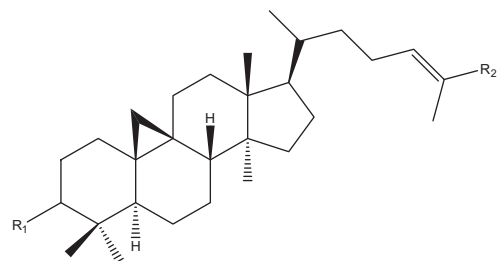
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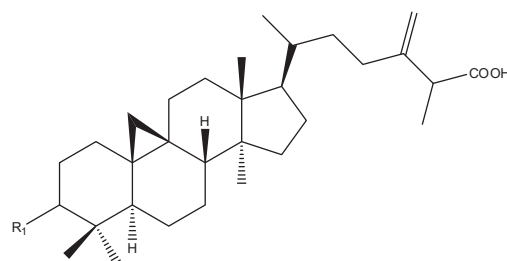
2. Results and discussion

The sample of propolis was extracted with 70% ethanol and the extract obtained was partitioned with *n*-hexane, ethyl acetate and *n*-butanol, respectively. Fractionation of the *n*-hexane and ethyl acetate extracts using repeated chromatographic procedures led to the isolation of a mixture of thirteen alk(en)ylphenols (**1–13**), including the three new structures (**8**), (**12**) and (**13**), along with α -amyrin (**14**) (Hernández Vázquez et al., 2012; Basyuni et al., 2006), β -amyrin (**15**) (Basyuni et al., 2006; Mahato and Kundu, 1994), lupeol (**16**) (Basyuni et al., 2006; Thanakijcharoenpath and Theanphong, 2007), cycloartenol (**17**) (Kamisako et al., 1987; Zhu et al., 2012), mangiferonic acid (**18**) (Escobedo-Martinez et al., 2012), ambonic acid (**19**) (Da Silva et al., 2005), mangiferolic acid (**20**) (Escobedo-Martinez et al., 2012), ambolic acid (**21**) (Escobedo-Martinez et al., 2012), and isomangiferolic acid (**22**) (Escobedo-Martinez et al., 2012). Fractionation of the *n*-butanol extract afforded a mixture of nine alk(en)ylresorcinols (**23–31**), including the new structure (**30**) (Figs. 1 and 2). All known compounds were identified following analysis of their spectroscopic data and comparison with previously published reports.

Compounds (**1–13**) were obtained as a yellow-coloured oil (R_f 0.49 in hexane–EtOAc, 8:2). The ^1H NMR revealed a 1,3-disubstituted aromatic system with four protons at δ 7.13 (t), 6.74 (d), 6.64 (s) and 6.63 (d); as well as signals typical for a long aliphatic chain including olefinic (δ 5.33, m), benzylic (δ 2.54, t) and allylic (δ 2.04, m) protons. A sharp singlet at δ 4.57, attributable to a phenolic OH group, was also observed. Both ^1H and ^{13}C NMR data showed good correlation with literature reports on 3-alk(en)ylphenols (Saitta et al., 2009; Silva et al., 2008; Franke et al., 2001). In order to determine the composition of the mixture, the compounds were derivatised to their corresponding dimethyl disulphide-trimethylsilyl derivatives and the mixture was separated by GC–MS. The GC-trace (Fig. 3) allowed the unambiguous identification of a total of 13 different compounds with side chains ranging from C11 to C19 and including six (saturated) alkylphenols and seven alkenylphenols showing mono-unsaturation (Table 1). The EI mass spectrum of all compounds showed predominant fragment ions at m/z 179 (benzylic cleavage) and/or 180 (benzylic cleavage with transfer of one proton) (Franke et al., 2001). The position of the double bond in mono-unsaturated dimethyl disulphide derivatives was established from the characteristic fragments resulting from the cleavage of the bond between the two methylthio-substituted carbons (Christie, 1997). Key fragments observed for mono-unsaturated derivatives are reported in Table 2. The fragmentation of compounds (**7**), (**9**) and (**10**) was in agreement with the literature (Saitta et al., 2009). Key fragments for the dimethyl disulphide



$R_1 = \beta\text{-OH}$, $R_2 = \text{CH}_3$ Cycloartenol (**17**)
 $R_1 = =\text{O}$, $R_2 = \text{COOH}$ Mangiferonic acid (**18**)
 $R_1 = \beta\text{-OH}$, $R_2 = \text{COOH}$ Mangiferolic acid (**20**)
 $R_1 = \alpha\text{-OH}$, $R_2 = \text{COOH}$ Isomangiferolic acid (**22**)



$R_1 = =\text{O}$, Ambonic acid (**19**)
 $R_1 = \beta\text{-OH}$, Ambolic acid (**21**)

Fig. 2. Structures of cycloartane triterpenes in Cameroonian propolis.

trimethyl silyl derivative of (**11**) are reported here for the first time. The mass spectrum of (**11**) showed a molecular ion $[\text{M}]^+$ at m/z 496 and two fragments, resulting from α -homolytic cleavage directed by the sulphur atom, at m/z 89 (base peak) and 407, corresponding to the structures $[\text{CH}_3\text{-CH}_2\text{-CHSCH}_3]^+$ and $[(\text{CH}_3)_3\text{SiO-C}_6\text{H}_4\text{-(CH}_2)_{13}\text{-CHSCH}_3]^+$, respectively. A fragment at m/z 359 was attributed to neutral loss of a CH_3SH unit (m/z 48) from the fragment ion at m/z 407. The mass spectrum of compound (**8**) showed a molecular ion $[\text{M}]^+$ at m/z 468 and two diagnostic fragments at m/z 89 (base peak) and 379, corresponding to the structures $[\text{CH}_3\text{-CH}_2\text{-CHSCH}_3]^+$ and $[(\text{CH}_3)_3\text{SiO-C}_6\text{H}_4\text{-(CH}_2)_{11}\text{-CHSCH}_3]^+$, respectively. A fragment at m/z 331 was attributed to neutral loss of a CH_3SH unit from the fragment at m/z 379 (Fig. 4A). The mass spectrum of compound (**12**) showed a molecular ion $[\text{M}]^+$ at m/z 524 and two key fragments at m/z 131 and 393,

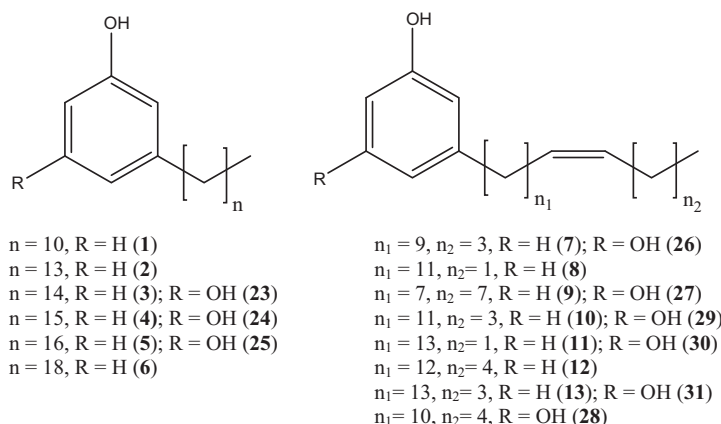


Fig. 1. Structures of alk(en)ylphenols and alk(en)ylresorcinols in Cameroonian propolis.

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