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A new δ-tocotrienolic acid derivative and other constituents from the cones of *Cedrus atlantica* and their *in vitro* antimicrobial activity

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ABSTRACT

The phytochemical and antimicrobial properties of the cones of *Cedrus atlantica* (Endl) Manetti ex Carrière were investigated. Two new compounds (**1,2**) and nineteen known compounds (**3–21**) were isolated. Their structures were established by mass spectrometry (HRESIMS), 1D, 2D NMR and by comparison with literature data. Antimicrobial activity of hydromethanolic extract against a panel of 22 bacteria and yeasts showed an interesting antimicrobial activity. All compound purified from this extract were tested against *S. aureus* by bioautography. MIC values of the most active compounds were determined using a serial dilution technique. The results showed strong antibacterial activity of the abietane diterpenes **10, 11, 14, 15, 16** and **17**. Dehydroabietic acid (**17**) was the most potent against *Enterococcus faecalis* (MIC = 15.1 = μg/mL), a multi-resistant commensal bacterium which can cause the fatal infections in humans.

1. Introduction

Cedrus atlantica (Endl.) Manetti ex Carrière (Pinaceae) is a large and exceptionally long-lived conifer only distributed in mountain range of Morocco and Algeria (Begona et al., 2005; Sugita et al., 2004). Essential oil of *C. atlantica* has been already studied and shown that it possessed anti-inflammatory (Sugita et al., 2004), antifungal (Bouchra et al., 2003), antimicrobial (Hammer et al., 1999; Zrira and Ghanmi, 2016) properties. It's also used in the treatment against hair loss (Ormerod et al., 2000). Phytochemical study of cones of *Cedrus atlantica* has previously exhibited the richness of abietane diterpenoids. Diethylether extracts contained diterpene acids bearing the abietane and pimarane skeletons (Norin et al., 1971), five oxygenated abiet-8(14)-ene derivatives were isolated from the neutral part of the *n*-hexane extract of cones (Barrero et al., 2005) and four abietatrienoid, also isolated from the *n*-hexane extract of the cones, exhibited significant antibacterial activity against Gram (±) bacteria (Dakir et al., 2005). More recently, abietanes diterpenes and lignans were identified in resins of *C. atlantica* (Nam et al., 2011).

Herein, as a result of our investigation on the hydromethanolic extract of the cones of *C. atlantica*, we report the isolation, structure elucidation of a new tocotrienolic acid derivative and a new *O*-acylated flavonol glycoside together with nineteen known compounds. In addition the antimicrobial activity of the hydromethanolic extract was evaluated against 22 micro-organisms and the purified compounds were evaluated against *E. faecalis* ATCC 1034, *S. aureus* CIP 53.154, *S. epidermidis, E. coli* CIP 54.127 and *P. aeruginosa* ATCC 9027.

2. Results and discussion

Compounds **1–21** were isolated from hydromethanolic extract of the cones of *C. atlantica* by successive chromatographic separation, including Vaccum Liquid Chromatography, flash chromatography and semi-prep HPLC. The known compounds **3–21** have been identified as: two tocotrienol derivatives, γ-tocotrienolic acid (= γ-garcinoic acid) (**3**) (Alsabil et al., 2016) δ-(E)- deoxy- amplexichromanol (**4**) (Lavaud et al., 2015), two flavonoids: daglesioside IV (**5**) (Krauze-Baranowska et al., 2013), and (+) taxifolin (**6**) (Agrawal et al., 1981), three neolignans: *ent*- cedrusin (**7**) (Agrawal et al., 1980; Kim et al., 2013), isomassoniaside B (**8**) (He et al., 2011; Kim et al., 2013), and (7*R*, 8*S*) dihydrodehydrodiconiferyl alcohol (**9**) (Miyase et al., 1989), and twelve abietane diterpenoids: pomiferin A (**10**) (Fraga et al., 1994; Yang et al., 2010), 8,11,13-abietatriene-7α,18-diol or 7α-hydroxydehydroabietinol (**11**) (Barrero et al., 1992; Yang et al., 2010), 7-oxodehydroabietinol (**12**) (Tanaka et al., 1997; González et al., 2010), abiesadine F (**13**), abiesadine L (**14**), abiesadine R (**15**), abiesadine Q (**16**) (Yang et al.,

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2010), dehydroabietic acid (**17**) (Cheung et al., 1993), 12-hydroxydehydroabietic acid (**18**) (Kinouchi et al., 2000), 7α,15-dihydroxydehydroabietic acid (**19**) (Prinz et al., 2002), 7-oxodehydroabietic acid (**20**) (Yang et al., 2010), and 8,11,13- abietatriene-7α-ol (**21**) (Conner et al., 1980). Among them, three abietane diterpenes (**10–12**) were previously isolated from the *n*-hexane extracts of the cones of *C. atlantica* (Barrero et al., 2005; Dakir et al., 2005) with dehydroabietic acid (**17**) isolated from the cone and resin of *C. atlantica* (Nam et al., 2011; Norin et al., 1971), *C. deodara* Loud (Ohmoto et al., 1987), and *C. libani* (Avcibasi et al. (1988)). In addition, (+) taxifolin (**6**), cedrusin without stereochemistry **(7)** and 7α-hydroxydehydroabietinol (**11**) were isolated from *C. deodara* (Agrawal et al., 1980; Awad et al., 2015; Ohmoto et al., 1989). All other compounds (**1–5, 8, 9, 13–16, 18–21**) were isolated for the first time in the *Cedrus* genus and *ent*-cedrusin **(7)** was identified in *Abies holophyla* Maxim. (Kim et al., 2013). The two known tocotrienols derivatives (**3**,**4**), previously isolated in *Garcinia amplexicaulis* (Alsabil et al., 2016; Lavaud et al., 2015), were isolated for the first time in Pinaceae family. The other known compounds (**5–21**) were previously isolated from the Pinaceae family, for example, the eleven abietane diterpenes (**10–20**) were isolated from *Abies georgei* Orr (Yang et al., 2010) and the abietane **21** from the trunk of *Abies holophyla* (Kim et al., 2016) and the bark of *Pinus monticola* Dougl. (Conner et al., 1980).

Compound **1** (Fig. 1) was isolated as a white powder with optical rotation $[\alpha]_D^{20}$ −0.8 (MeOH). The compound gave a pseudomolecular ion $[M + Na]^+$ at *m/z* 479.2766 suggesting a molecular formula of $C_{28}H_{40}O_5$ which requires 9° of insaturations. The ^{1}H NMR (Table 1), exhibited signals characteristic of the presence of an aromatic proton at δ_H 6.51 (1H, s, H-7) from a pentasubstituted aromatic ring, a benzylic methyl group at δ_H 2.06 (3H, s, H-27), a methoxyl group at δ_H 3.76 (3H, s, H-26) and four methylene protons of a chroman ring [δ_H 1.71 (1H, dd, *J* = 13.7, 6.8 Hz, H-3a), 1.79 (1H, dd, *J* = 13.7, 6.8 Hz, H-3b), and 2.73 (2H, td, *J* = 6.8, 1.5 Hz, H-4)] (Alsabil et al., 2016; Lavaud et al., 2015). The presence of a tri-isoprenyl side chain was deduced from the observation of three olefinic protons at δ_H 5.27 (1H, t, *J* = 7.6 Hz, H-11), 5.25 (1H, t, *J* = 7.4 Hz, H-15) and 6.74 (1H, t, *J* = 6.6 Hz, H-19) and three singlets of allylic methyl groups at δ_H 1.82 (3H, s, H-22), 1.62 (3H, s, H-23), and 1.60 (3H,s, H-24) (Alsabil et al., 2016; Min-Cheol et al., 2011). In addition, it's ^{1}H and ^{13}C NMR data showed signals of an oxyquaternary methyl group at δ_H 1.27 (3H, s, H-25, δ_C 24.4) and a carbonyl group at δ_C 172.4 as in tocotrienolic acid derivatives (Alsabil et al., 2016) (Table 1). The 12,16,20-trimethyl-11,15,19-tridecatrienoic acid side chain of **1** was established by COSY correlations of H-9 (δ_H 1.56 and 1.62) with H-10 (δ_H 2.14), H-11 (δ_H 5.27) with H-10 and H-24 (δ_H 1.60), H-13 (δ_H 2.01) with H-14 (δ_H 2.10), H-15 (δ_H 5.25) with H-14 and H-23 (δ_H 1.62), H-17 (δ_H 2.10) with H-18 (δ_H 2.29), and H-19 (δ_H 6.74) with H-18 and H-22 (δ_H 1.82) (Fig. 2). In addition, ^3J HMBC correlations between H-11/C-13, H-15/C-17 and from the carbonyl group C-21 to H-22 and H-19 indicated that the carbonyl group was located at the end of the prenyl side chain (Fig. 2). The COSY correlation of H-3 with H-4 (δ_H 2.73), and the HMBC cross peaks between H-3/C-2 (δ_C 75.9), C-4a (δ_C 116.1), C-9 (δ_C 40.3) and oxyquaternary methyl C-25 (δ_C 24.4) confirmed the presence of a chroman ring and the attachment of both 12,16,20-trimethyl-11,15,19-tridecatrienoic acid side chain and oxyquaternary methyl at C-2 of chroman moiety. The location of the substituent on the aromatic ring was deduced from analysis of correlations in the HMBC spectrum. The HMBC correlations between H-4/C-5 (δ_C 144.4) and the methoxyl H-26 (δ_H 3.76) with C-5 indicated that the methoxyl group was located at C-5. Otherwise, the aromatic proton at δ_H 6.51 was assigned as H-7 by its ^3J HMBC correlations with C-5 and C-8a (δ_C 146.2), and ^2J HMBC correlations with C-6 (δ_C 142.8) and C-8 (δ_C 122.4). The correlations of methyl group H-27 (δ_H 2.06) with C-8, C-7 and C-8a established the linkage of the methyl at C-8 and the chemical shift of C-6 the location of an hydroxyl group at C-6. In the NOESY spectrum, correlations between H-10/H-24, H-14/H-23 and H-18/H-22 and between H-11/H-13 and H-15/H-17 indicating that all the isoprenyl units has *trans* configuration (Min-Cheol et al., 2011). According to the literature, the asymmetric C-2 configuration was defined as *R* (Drotleff and Ternes, 2001). Thus the structure of compound **1** was elucidated as 11*E*-15*E*-19*E*-9-(2,8-dimethyl-5-methoxy-chroman-6-ol)-12,16,20-trimethyl-11,15,19-tridecatrienoic acid, also named 5-methoxy-δ-tocotrienolic acid.

Compound **2** (Fig. 1) was obtained as yellow powder with a molecular formula of $C_{40}H_{34}O_{14}$ deduced from its molecular ion $[M + Na]^+$ at *m/z* 761.1851 in HR-ESI–MS. The ^{1}H NMR spectrum showed characteristics signals of isorhamnetin moiety (Markham and Geiger, 1994; Rosch et al., 2004; Zou et al., 2007). Signals at δ_H 6.15 (1H, d, *J* = 1.9 Hz) and 6.31 (1H, d, *J* = 1.9 Hz) were assigned to H-6 and H-8

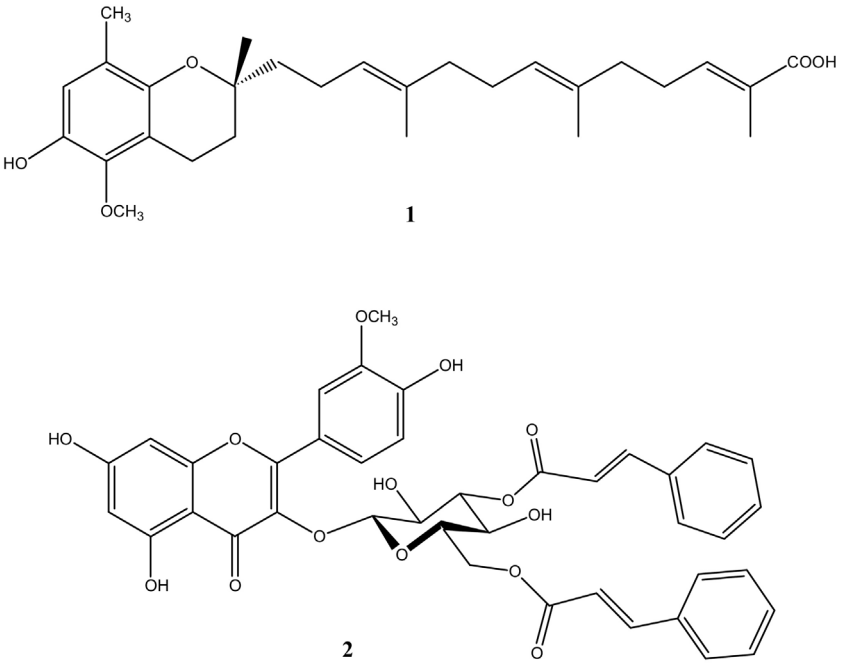

Fig. 1. Structures of compounds **1** and **2**.