Phytochemistry Letters 15 (2016) 220–224

Contents lists available at ScienceDirect

Phytochemistry Letters

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

A new cycloartane-type triterpene and a new eicosanoic acid ester from fruits of *Paullinia pinnata* L.

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ARTICLE INFO

Article history:
Received 28 October 2015
Received in revised form 14 January 2016
Accepted 2 February 2016
Available online xxx

Keywords:
Paullinia pinnata
Sapindaceae
Cyclopinnatol
Paulliniester
Antibacterial activity

ABSTRACT

A phytochemical study of the MeOH-soluble portion from the CH_2Cl_2/MeOH extract of the fruits of *Paullinia pinnata* resulted in the isolation of a new triterpenoid, cyclopinnatol (**1**), and a new eicosanoic acid ester, paulliniester (**2**), together with five known compounds including cycloart-22(*E*)-ene-3β,25-diol (**3**), cycloartenol (**4**), β-sitosterol (**5**), betulonic acid (**6**) and oleanonic acid (**7**). The structures of the new compounds were elucidated by spectroscopic analyses (NMR and MS) and comparisons with published data. Cyclopinnatol (**1**) and the MeOH-soluble portion exhibited significant and weak antibacterial activities against *Staphylococcus aureus,* with MICs of 32 and 50 μg/mL, respectively.

1. Introduction

Paullinia pinnata L. belongs to the family Sapindaceae, and broadly grows in tropical Africa. The leaves and roots of this plant have been used in traditional medicine for the treatment of malaria (Chhabra et al., 1991), erectile dysfunction (Zamble et al., 2006), and bacterial infections (Lunga et al., 2014a; Kofi et al., 2009) in this region. Previous phytochemical studies of this plant revealed the isolation of terpenoids (Annan and Houghton, 2010; Lunga et al., 2014a,b), flavonoids (Abourashed et al., 1999), steroids and cerebrosides (Dongo et al., 2009; Lunga et al., 2014a,b), and fatty acids (Kofi et al., 2009).

In our ongoing search for new bioactive constituents from medicinal plants collected in Cameroon (Awouafack et al., 2015, 2011, 2008), a new triterpenoid, cyclopinnatol (**1**), and a new eicosanoic acid ester, paulliniester (**2**), along with five known compounds (**3**–**7**), were isolated from the fruits of *P. pinnata* L. In this report, we describe the isolation, structure elucidation, and antibacterial activities of these compounds.

2. Results and discussion

The MeOH-soluble portion prepared from the CH_2Cl_2/MeOH crude extract of the fruits of *P. pinnata* was subjected to repeated silica gel open column chromatography, preparative thin-layer chromatography (TLC), and Sephadex LH-20 chromatography to afford a new triterpenoid, cyclopinnatol (**1**), and a new eicosanoic acid ester, paulliniester (**2**) (Fig. 1), together with five known compounds: cycloart-22(*E*)-ene-3β,25-diol (**3**) (Rasool et al., 1991), cycloartenol (**4**) (Nes et al., 1998), β-sitosterol (**5**) (Kamboj and Saluja, 2011), betulonic acid (**6**) (Melnikova et al., 2012), and oleanonic acid (**7**) (Sung et al., 1991). The known compounds were identified by comparisons of their spectroscopic data with those reported in the literature.

Cyclopinnatol (**1**) was obtained as a white powder, and its IR spectrum exhibited characteristic absorption bands at 3373 (OH). The molecular formula $C_{30}H_{50}O_2$ was deduced from the HREIMS, which displayed the molecular ion peak $[M]^+$ at *m/z* 442.3803 (calculated for $C_{30}H_{50}O_2$, 442.3811), in conjunction with NMR data. The ^1H NMR spectrum of **1** showed five singlet [δ_H 1.35 (H-21), 0.98 (H-28), 0.97 (H-18), 0.89 (H-30) and 0.81 (H-29)] and two doublet [0.88 (d, J = 6.5 Hz, H-26) and 0.87 (d, J = 6.5 Hz, H-27)] signals of three protons each, corresponding to seven methyl groups. The ^1H

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http://dx.doi.org/10.1016/j.phytol.2016.02.002
1874-3900/

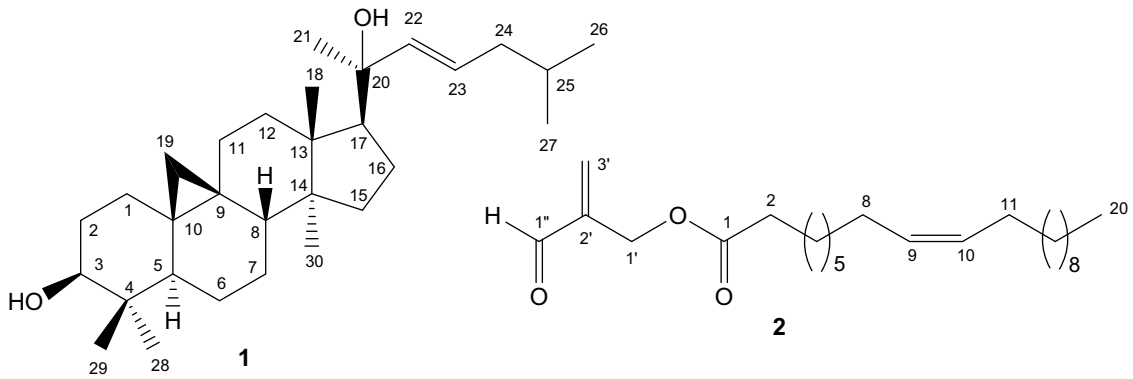

Fig. 1. Chemical structures of new compounds **1** and **2** isolated from *P. pinnata*.

NMR spectrum also exhibited characteristic signals of one methylene at δ_H 0.33 (d, J = 4.0 Hz, H-19$_a$) and 0.55 (d, J = 4.0 Hz, H-19$_b$), an oxygenated methine at δ_H 3.29 (m, H-3), and two olefinic protons at δ_H 5.53 (br.d, J = 15.9 Hz, H-22) and 5.69 (m, H-23) (Rasool et al., 1991; Yang and Shi, 2012; Ayatollahi et al., 1992). The ^{13}C NMR, DEPT, and HMQC data exhibited 30 carbon signals: five aliphatic quaternary carbons (δ_C 48.8, 45.3, 40.4, 26.0, and 19.9), an oxygenated quaternary carbon (δ_C 82.3), four aliphatic methine carbons (δ_C 52.0, 47.0, 47.9, and 36.3), an oxygenated methine carbon (δ_C 78.8), two olefinic methine carbons (δ_C 134.4 and 130.7), ten methylenes, and seven methyl groups. These spectroscopic data of **1** (Table 1) were similar to those of cycloartane-type triterpenoids (Baniadam et al., 2014; Rasool et al., 1991; Ayatollahi et al., 1992). The assignment of all protons and carbons of **1** was performed by analysis of the HMQC, HMBC, and COSY spectra (Fig. 2). In the HMBC experiment, the correlations from the methylene protons at δ_H 0.33 and 0.55 (H$_2$-19) to the quaternary carbons at δ_C 19.9 (C-9) and 26.0 (C-10), and from the methine protons at δ_H 1.31 (C-5) and 1.50 (C-8) to the carbons at δ_C 19.9 (C-9), 26.0 (C-10), and 29.9 (C-19), revealed the presence of a cyclopropane ring. Furthermore, the ^{1}H–^{1}H COSY correlation between the methylene protons at δ_H 1.56 and 1.73 (H$_2$-2) and the oxymethine proton at δ_H 3.29 (H-3), as well as the HMBC correlations from the methyl protons at δ_H 0.98 (H$_3$-28) and 0.81 (H$_3$-29) to the carbons at δ_C 78.8 (C-3) and 40.4 (C-4), confirmed the position of the hydroxy group at C-3 in the A-ring of the triterpenoid. In addition, the HMBC correlations from the methyl protons at δ_H 1.35 (H$_3$-21) to the oxygenated carbon at δ_C 82.3 (C-20) and the olefinic carbon at δ_C 134.4 (C-22), from the olefinic protons at δ_H 5.53 (H-22) and 5.69 (H-23) to the carbon at δ_C 82.3 (C-20), and from the methine proton at δ_H 1.57 (C-17) to the carbon at δ_C 82.3 (C-20) allowed us to locate the hydroxyl group at C-20 (Tsopmo and Kamnaing, 2011; Ayatollahi et al., 1992). The EIMS of **1** had important ion fragments at *m/z* 424 ([M—H_2O]$^+$), 409 ([M—H_2O—CH_3]$^+$), 407 ([M—2H_2O—H]$^+$), 391 ([M—2H_2O—CH_3]$^+$), 315 ($C_{22}H_{35}O^+$, [M—$C_8H_{16}O$]$^+$, cleavage between C-17 and C-20), and 297 ([M—$C_8H_{16}O$—H_2O]$^+$), confirming the presence of two hydroxyl groups on the side chain and the polycyclic moiety. The *E*-configuration of the double bond at C-22 was assigned from the coupling constant value of H-22 (J = 15.9 Hz). Thus, the skeleton of **1** was considered to be 9,19-cycloart-22(*E*)-ene-3,20-diol.

Table 1
^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data of **1** in $CDCl_3$ [δ (ppm), J (Hz)].

	Cyclopinnatol (1)	
Position	δ_C	δ_H
1	31.9	1.24, m; 1.57, m
2	30.3	1.56, m; 1.73, m
3	78.8	3.29, m
4	40.4	
5	47.0	1.31, dd (2.9, 10.3)
6	21.1	1.59, m; 1.62, m
7	28.1	1.29, m; 1.95, m
8	47.9	1.50, m
9	19.9	
10	26.0	
11	26.4	0.98[a], m; 2.00, m
12	35.5	1.32[a], m; 1.48, m
13	45.3	
14	48.8	
15	32.8	1.34[a], m; 1.61, m
16	24.4	0.96[a], m; 1.35[a], m
17	52.0	1.57, br s
18	18.1	0.97, s
19	29.9	0.33, d (4.0); 0.55, d (4.0)
20	82.3	
21	24.3	1.35, s
22	134.4	5.53, br d (15.9)
23	130.7	5.69, m
24	39.3	1.78, m; 2.23, m
25	36.3	1.28[a], m
26	25.4	0.88, d (6.5)
27	26.0	0.87, d (6.5)
28	18.3	0.98, s
29	14.0	0.81, s
30	19.3	0.89, s

[a] Overlapping resonances within the same column.

The relative configuration of **1** was determined on the basis of the NOESY experiment (Fig. 2). The NOESY correlations between H-3, H-5, and H$_3$-28 suggested the β and α orientations of 3-OH and H-5, respectively. Furthermore, the NOESY correlations of H-17 to H$_3$-21 and H$_3$-30, of H-8 to H$_3$-18 and H-19$_b$, and of H-19$_a$ to H$_3$-29 indicated the *cis* B/C- and *trans* C/D-ring configurations. Due to the scarcity of compound **1**, the absolute configuration of C-20 was not determined. Based on the spectroscopic data and comparisons with those of related published compounds (Baniadam et al., 2014; Tsopmo and Kamnaing, 2011; Ayatollahi et al., 1992), the structure of cyclopinnatol was assigned as cycloart-22(*E*)-ene-3β,20-diol (**1**).

Paulliniester (**2**) was obtained as a white powder, and its HRESIMS had a pseudo-molecular ion peak [M + H]$^+$ at *m/z* 379.3168, corresponding to the molecular formula $C_{24}H_{42}O_3$, in conjunction with the ^{13}C NMR data (Table 2). The IR spectrum of **2** displayed strong absorption bands, due to the α,β-unsaturated carbonyl (1732 cm^{-1}) and the ester carbonyl (1668 cm^{-1}). The ^{1}H NMR spectrum of **2** exhibited signals of one formyl proton in an aldehyde at δ_H 9.60 (s, H-1"), one exomethylene at δ_H 6.20 (br s, H-3'$_a$) and 6.45 (br t, J = 1.7 Hz, H-3'$_b$), and one oxymethylene at δ_H 4.82 (2H, br t, J = 1.7 Hz, H-1'). The set of proton signals of two olefinic protons at δ_H 5.35 (2H, t, J = 4.6 Hz, H-9/H-10), aliphatic protons at δ_H 2.36 (2H, t, J = 7.5 Hz, H-2), 2.01 (2H, m, H-8/H-11),