Phytochemistry 98 (2014) 236–242

Contents lists available at ScienceDirect

Phytochemistry

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

Acylated oleanane-type saponins from *Ganophyllum giganteum*

Elier Galarraga Montes[a,b], Anne-Claire Mitaine-Offer[a], Juan Manuel Amaro-Luis[b], Thomas Paululat[c], Clément Delaude[d], Laurent Pouységu[e], Stéphane Quideau[e], Luis B. Rojas[b], Stéphanie Delemasure[f], Patrick Dutartre[f], Marie-Aleth Lacaille-Dubois[a,*]

[a] *Laboratoire de Pharmacognosie, EA 4267 (FDE/UFC), Faculté de Pharmacie, Université de Bourgogne, 7, Bd. Jeanne d'Arc, BP 87900, 21079 Dijon Cedex, France*
[b] *Laboratorio de Productos Naturales. Departamento de Química, Facultad de Ciencias, Universidad de Los Andes, Mérida 5101, Venezuela*
[c] *Universität Siegen, OC-II, Naturwissenschaftlich-Technische Fakultät, Adolf-Reichwein-Str. 2, D-57076 Siegen, Germany*
[d] *Centre de Recherche Phytochimique, Université de Liège, Institut de Chimie-B6, Sart Tilman, B-4000 Liège I, Belgium*
[e] *Institut des Sciences Moléculaires, CNRS–UMR 5255 et Institut Européen de Chimie et Biologie, Université de Bordeaux, 2 rue Robert Escarpit, 33607 Pessac Cedex, France*
[f] *Cohiro, UFR Médecine, 7, Bd. Jeanne d'Arc, BP 87900, 21079 Dijon Cedex, France*

ARTICLE INFO

Article history:
Received 4 March 2013
Received in revised form 25 October 2013
Available online 30 December 2013

Keywords:
Ganophyllum giganteum
Sapindaceae
Saponins
Zanhic acid
Medicagenic acid

ABSTRACT

Five oleanane-type saponins, 3-*O*-β-D-glucuronopyranosylzanhic acid 28-*O*-β-D-xylopyranosyl-(1→3)-[α-L-rhamnopyranosyl-(1→2)]-(4-*O*-acetyl)-β-D-fucopyranosyl ester (**1**), 3-*O*-β-D-glucopyranosylzanhic acid 28-*O*-β-D-xylopyranosyl-(1→3)-[α-L-rhamnopyranosyl-(1→2)]-(4-*O*-acetyl)-β-D-fucopyranosyl ester (**2**), zanhic acid 28-*O*-β-D-xylopyranosyl-(1→3)-[α-L-rhamnopyranosyl-(1→2)]-(4-*O*-acetyl)-β-D-fucopyranosyl ester (**3**), zanhic acid 28-*O*-α-L-rhamnopyranosyl-(1→2)-4-*O*-[(3′-hydroxy-2′-methyl-butyroyloxy)-3-hydroxy-2-methyl-butyroyloxy]-β-D-fucopyranosyl ester (**4**), medicagenic acid 28-*O*-α-L-rhamnopyranosyl-(1→2)-4-*O*-[(3′-hydroxy-2′-methyl-butyroyloxy)-3-hydroxy-2-methyl-butyroyloxy]-β-D-fucopyranosyl ester (**5**), were isolated from the root barks of *Ganophyllum giganteum*. Compounds **4** and **5** possessed an unusual substitution of the C-4 position of the β-D-fucopyranosyl moiety by a C10 ester group formed by two symmetrical C5 nilic acid. From a chemotaxonomic point of view, their structures are in accordance with the previous saponins isolated from the Doratoxyleae tribe of the Sapindaceae family. Their cytotoxicity and anti-inflammatory activity were also evaluated.

Introduction

In the order of the Sapindales, the Sapindaceae, known as the saopnut family, is an important family represented by some 150 genera with almost 2000 species (Delaude, 1993). According to the APG III classification, the Sapindaceae is divided into four subfamilies Dodonaeoidae, Hippocastanoideae, Sapindoideae, and Xanthoceroideae for certain authors only (Buerki et al., 2010). Plants of this family are various, from trees to herbaceous plants, and many species possess an economical importance: in food industry with the lychee or rambutan, in phytotherapy with the genus *Paullinia* (Guarana), for their wood (*Acer*), as ornamentals (*Koelroeteria*), and as detergents (*Sapindus*). The latter genera are rich in triterpene saponins, and their fruits were traditionally used in tropical countries to clean fabrics and to kill fish (Delaude, 1993). In the field of our researches on natural saponins, we have investigated the structure and biological activity of some saponins isolated from the roots of a Sapindaceae species named *Ganophyllum giganteum* (A. Chev.) Hauman. This tree, found in the Democratic Republic of Congo, belongs to the Dodonaeoidae subfamily and the Doratoxyleae tribe, and is used in wooden factory (Delaude, 1993). *Ganophyllum giganteum* is also described in Gabonese traditional medicine and previous phytochemical works showed a good cytotoxic activity of an hydromethanolic extract of the leaves against human monocytes (Lamidi et al., 2005). Moreover, investigations of the crude saponin mixture resulted in the characterization of two aglycons: medicagenic acid and zanhic acid, which appeared as chemotaxonomic markers of the Doratoxyleae tribe (Delaude, 1993; Dimbi et al., 1984). In this paper, we described the isolation by successive chromatographic steps of five new triterpene saponins (**1**–**5**) from the aqueous methanolic extract of the root barks (Fig. 1). Their structural determination was performed by a detailed analysis of their spectral data including 600 MHz 2D-NMR (COSY, TOCSY, ROESY, HSQC, HMBC), and mass spectrometry. Cytotoxicity and anti-inflammatory activity of the compounds **2**–**5** were also evaluated.

Results and discussion

After extraction by MeOH 70% of the root bark of *G. giganteum*, the resulting extract was subjected to multiple chromatographic

---

* Corresponding author. Tel.: +33 3 80393229; fax: +33 3 80393300.
E-mail address: m-a.lacaille-dubois@u-bourgogne.fr (M.-A. Lacaille-Dubois).

0031-9422/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.phytochem.2013.11.003

steps over silica gel to yield compounds **1**–**5**. The elucidation of their structures was performed mainly by 600 MHz 2D NMR analysis (^{1}H–^{1}H COSY, TOCSY, ROESY, HSQC, HMBC), in combination with mass spectrometry (HRESIMS and FAB- and ESIMS). All saponins were isolated as white amorphous powders.

The HRESIMS of compound **1** established its molecular formula as $C_{55}H_{84}O_{26}$ and **1** showed in ESIMS (negative-ion mode) a quasi-molecular ion peak $[M-H]^-$ at *m*/*z* 1159 indicating a molecular weight of 1160.

The ^{1}H NMR spectrum of the aglycon part of **1** showed signals of six angular methyl groups as singlets (Table 1), one olefinic proton at δ_H 5.25 (*t*-like), three oxygens bearing methine protons at δ_H 4.00 (*d*, *J* = 3.6 Hz), 4.10 (*m*), and δ_H 4.30 (*m*). The HMBC correlation between one angular methyl group at δ_H 1.15 (*s*, H_3-24) and δ_C 84.1 (C-3) confirmed the location of the first secondary alcoholic function at C-3. The COSY correlation between δ_H 4.00 (*d*, *J* = 3.6 Hz, H-3) and δ_H 4.10 (*m*) allowed the location of the second secondary alcoholic function at C-2. The C-16 position of the third one was deduced by the HMBC correlation between δ_H 4.30 (*m*) and δ_C 40.5 (C-18) and the COSY correlation between δ_H 4.30 (*m*) and δ_H 1.60 (H-15). A cross-peak in the ROESY spectrum between H-3 at δ_H 4.00 and the α-axial H-5 at δ_H 1.39 suggested a α-axial orientation of the H-3. The α-equatorial orientation of the H-2 was determined by the multiplicity and the coupling constant of H-3 as a doublet (*J* = 3.6 Hz) and from the connectivity observed in the ROESY spectrum between H-3 at δ_H 4.00 and H-2 at δ_H 4.10 ppm. For C-16, the ROESY correlations between δ_H 0.69 (s, H_3-26) and δ_H 1.60 ($H_{\beta ax}$-15), and between δ_H 1.60 ($H_{\beta ax}$-15) and δ_H 4.30 (*m*, H-16), suggested a β-equatorial orientation of the H-16 and thus a α-axial orientation of the OH group. The structure of the aglycon of **1** was thus recognized as the triterpene zanhic acid, and was in full agreement with literature data (Lavaud et al., 1998; Tava et al., 2005).

The monosaccharides obtained by acid hydrolysis of **1** were identified by comparison on TLC with authentic samples as glucuronic acid (GlcA), fucose (Fuc), xylose (Xyl) and rhamnose (Rha). The absolute configurations of the sugars were determined to be D for GlcA, Fuc and Xyl, and L for Rha, by GC analysis by a method previously described (Hara et al., 1987). The relatively large $^3J_{H\text{-}1,H\text{-}2}$ values of the GlcA, Fuc, and Xyl (7.3–8.1 Hz) in their pyranose form indicated a β anomeric orientation for GlcA, Fuc, and Xyl (Mimaki et al., 2004). The large $^1J_{H\text{-}1,C\text{-}1}$ value of the Rha (166 Hz) confirmed that the anomeric proton was equatorial (α-pyranoid anomeric form).

The ^{1}H NMR spectrum of **1** displayed in the sugar region signals of four anomeric protons at δ_H 5.37 (*d*, *J* = 8.1 Hz), 5.11 (*br s*), 4.31 (*d*, *J* = 7.3 Hz), and 4.26 (*d*, *J* = 7.7 Hz), which gave correlations, in the HSQC spectrum, with four anomeric carbon signals at δ_C 93.1, 100.5, 104.1 and 101.5, respectively. Complete assignments of the resonances of each sugar were achieved by extensive 2D NMR analyses (COSY, TOCY, HSQC, HMBC) (Table 2). Units of one β-D-fucopyranosyl, one α-L-rhamnopyranosyl, one β-D-xylopyranosyl, and one β-D-glucuronopyranosyl were thus identified. A cross-peak observed in the ROESY spectrum at δ_H 4.26 (*d*, *J* = 7.7 Hz, GlcA-1)/δ_H 4.00 (*d*, *J* = 3.6, H-3), and a HMBC correlation at δ_H 4.26 (*d*, *J* = 7.7 Hz, GlcA-1)/δ_C 84.1 (C-3), revealed a substitution at C-3 of the aglycon by a glucuronopyranosyl moiety. Moreover, the C-28 position at δ_C 174.9 was esterified by an oligosaccharidic chain which was analysed mainly on the basis of ROESY and HMBC spectra: The ROESY cross-peaks at δ_H 5.11 (*br s*, Rha-1)/δ_H 3.68 (*dd*, *J* = 8.9, 8.1 Hz, Fuc-2), and δ_H 4.31 (*d*, *J* = 7.3 Hz, Xyl-1)/δ_H 3.99 (*dd*, *J* = 8.9, 3.3 Hz, Fuc-3) revealed the (1 → 2) linkage

Table 1
^{13}C, ^{1}H NMR spectroscopic data of the aglycon moieties of **1**–**5** in DMSO-d_6 (δ_H ppm, *J* in Hz).[a]

	1		**2**		**3**		**4**		**5**	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	44.0	1.08, 1.95 *br d* (13.8)	43.7	1.94, nd	43.6	1.94, nd	43.6	1.10, 1.94	43.7	1.10, 2.70
2	68.1	4.10 *m*	68.4	4.02 *m*	68.2	4.02 *m*	68.1	4.04 *m*	68.3	4.04 *m*
3	84.1	4.00 *d* (3.6)	84.1	4.00	75.9	3.15	75.9	3.16	76.0	3.16
4	51.1	–	50.7	–	50.8	–	50.7	–	50.7	–
5	51.0	1.39	50.7	nd	50.7	nd	50.7	1.50	50.8	nd
6	19.7	1.32, nd	19.7	1.35, nd	19.7	nd	19.6	1.25, 1.36	19.7	1.34, nd
7	32.3	1.24, 1.34	32.3	1.22, 1.35	32.3	1.22, 1.35	32.3	1.22, 1.36	32.2	1.22, 1.37
8	40.0	–	39.4	–	39.6	–	39.5	–	39.3	–
9	46.4	1.46	46.5	1.47	46.4	1.48	46.3	1.48	47.3	1.43
10	36.0	–	35.6	–	35.6	–	35.7	–	35.7	–
11	23.0	1.85, 1.87	23.0	1.85, 1.87	23.0	1.85, 1.87	23.0	1.84, 1.87	23.0	1.84, 1.91
12	121.4	5.25 *t*-like	123.0	5.24 *t*-like	121.4	5.24 *t*-like	121.5	5.24 *t*-like	121.7	5.20 *t*-like
13	143.3	–	143.3	–	143.3	–	143.2	–	143.1	–
14	41.2	–	41.2	–	41.2	–	41.1	–	41.4	–
15	34.5	1.29, 1.60	34.7	1.27, 1.58	34.6	1.26, 1.60	34.6	1.26, 1.64	27.2	1.04, 1.53
16	72.5	4.30 *m*	72.5	4.31 m	72.3	4.31 m	72.4	4.32 m	22.6	1.54, 1.96
17	48.1	–	48.1	–	48.1	–	48.1	–	46.0	–
18	40.5	2.83 *dd* (12.6, 3.0)	40.4	2.83 *dd* (13.5, 3.0)	40.5	2.83 *dd* (14.2, 3.2)	40.5	2.85	41.1	2.73
19	46.6	0.98, 2.22	46.5	0.98, 2.23	46.6	0.98, 2.23	46.6	0.98, 2.22	45.6	1.07, 1.66
20	30.0	–	30.0	–	30.0	–	30.0	–	30.2	–
21	34.9	1.08, 1.88	34.9	1.09, 1.88	34.9	1.09, 1.86	34.9	1.10, 1.89	33.2	1.17, 1.35
22	30.6	1.69, 1.81	30.5	1.68, 1.80	30.2	1.69, 1.81	30.2	1.69, 1.80	31.4	1.48, 1.65
23	183.0	–	183.5	–	183.0	–	183.5	–	183.0	–
24	14.5	1.15 *s*	13.9	1.17 *s*	13.9	1.18 *s*	13.9	1.18 *s*	13.9	1.19 *s*
25	16.6	1.18 *s*	16.3	1.17 *s*	16.3	1.19 *s*	16.3	1.19 *s*	16.2	1.18 *s*
26	16.7	0.69 *s*	16.6	0.68 *s*	16.7	0.69 *s*	16.7	0.68 *s*	16.7	0.70 *s*
27	26.3	1.30 *s*	26.4	1.29 *s*	26.4	1.30 *s*	26.4	1.30 *s*	25.5	1.08 *s*
28	174.9	–	174.9	–	175.0	–	175.0	–	175.4	–
29	32.7	0.83 *s*	32.7	0.83 *s*	32.7	0.83 *s*	32.7	0.83 *s*	32.7	0.84 *s*
30	24.2	0.90 *s*	24.2	0.90 *s*	24.2	0.90 *s*	24.2	0.91 *s*	23.4	0.88 *s*

[a] Overlapped proton signals are reported without designated multiplicity. Nd: not determined.