Phytochemistry Letters 3 (2010) 70–74

Contents lists available at ScienceDirect

Phytochemistry Letters

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

Acylated flavonol pentaglycosides from *Baphia nitida* leaves

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ARTICLE INFO

Article history:
Received 6 November 2009
Received in revised form 9 December 2009
Accepted 17 December 2009
Available online 29 December 2009

Keywords:
Baphia nitida
Fabaceae
Acylated kaempferol pentaglycosides

ABSTRACT

Two new acylated flavonol pentaglycosides were isolated from the butanolic extract of *Baphia nitida* leaves by Sephadex LH-20 and preparative HPLC. Structural elucidation of kaempferol 3-*O*-β-D-xylopyranosyl(1 → 3)-(4-*O*-*E*-*p*-coumaroyl-α-L-rhamnopyranosyl(1 → 2))[β-D-glucopyranosyl(1 → 6)]-β-D-galactopyranoside-7-*O*-α-L-rhamnopyranoside (**1**) and kaempferol 3-*O*-β-D-xylopyranosyl(1 → 3)-(4-*O*-*Z*-*p*-coumaroyl-α-L-rhamnopyranosyl(1 → 2))[β-D-glucopyranosyl(1 → 6)]-β-D-galactopyranoside-7-*O*-α-L-rhamnopyranoside (**2**) was achieved using UV, NMR, and mass spectrometry, indicating the presence of *trans* or *cis* isomers of *p*-coumaric acid moiety in these novel structures. The antioxidant activity of the two compounds was assessed in the peroxynitrite assay.

1. Introduction

Baphia nitida Lodd. (Fabaceae) is a plant widely distributed in the coastal rainy forests of Africa and Madagascar. The species is growing as a small shrubby hard-wooded tree. It is known as camwood or African sandalwood and provides a red santarubin C containing dyewood. Former phytochemical studies on this species have demonstrated the presence of isoflavonoids (sativan, medicarpin, 6,7,3′-trihydroxy-2′,4′-dimethoxyisoflav-3-ene) in the heartwood (Arnone et al., 1981; Omobuwajo et al., 1992) as well as iminosugars (*2R*,*5R*-dihydroxymethyl-*3R*,*4R*-dihydroxy-pyrrolidine (DMDP), 1-*O*-β-D-fructofuranosyl-DMDP, 3-*O*-β-D-glucopyranosyl-DMDP) in the leaves (Kato et al., 2008).

B. nitida leaves are used in traditional medicine of many African countries, particularly for gastro-intestinal complaints (Anderson and Mills, 1876; Bouquet and Debray, 1974; Kone-Bamba et al., 1987; Onwukaeme and Lot, 1991, 1992). The butanolic leaf extract exhibited anti-inflammatory activities on mice and rats, due to the presence of flavonoids (Onwukaeme, 1995). The ethyl acetate leaf extract was investigated in mice and showed neurosedative, anxiolytic, skeletal muscle-relaxant effects and antidiarrhoeal activity (Adeyemi et al., 2006; Adeyemi and Akindele, 2008). Skeletal neuromuscular blocking properties of the aqueous leaf extract were also described (Adeyemi and Ogunmakinde, 1991) as well as negative chronotropic and inotropic effects on isolated cardiac preparations (Adeyemi, 1992).

Recent phytochemical studies in the genus *Baphia* led to the isolation of three new isoflavonoid glycosides from the roots of *B. bancoensis* (Yao-Kouassi et al., 2008). In this paper, we report the structural and chemical elucidation of two acylated flavonol pentaglycosides isolated from the leaves of *B. nitida*.

2. Results and discussion

Compounds **1** and **2** were obtained from the butanolic leaf extract of *B. nitida* after purification on Sephadex LH-20 followed by preparative RP-HPLC.

Compound **1** was isolated as a pale yellow amorphous powder. A molecular formula of $C_{53}H_{64}O_{30}$ was obtained by HRESIMS. The LC-ESI–MS2 in positive mode gave the following protonated fragments at *m*/*z* 1049, 903, 757, 433, 287. The protonated aglycone at *m*/*z* 287 was attributed to the kaempferol moiety. The first fragment (132) suggested a loss of a pentose. The next fragments were interpreted by the successive loss of two residues of 146 indicating the possible loss of deoxyhexoses and/or coumaroyl units, followed by the elimination of two hexose sugars of 324 and finally another residue at 146. This interpretation is in accordance with Schmid and Harborne (1973). The IR spectrum indicated typical absorption bands of OH groups (3350 cm^{-1}), α – β unsaturated ketone (1693, 1650 cm^{-1}), aromatic ketone (1494 cm^{-1}) and *O*-glycosidic linkage (1189–1012 cm^{-1}). The UV spectral data recorded in methanol were similar to the characteristic maxima at 269 and 317 nm of kaempferol 3-*O*-glycoside acylated by a hydroxycinnamic acid. Diagnostic shift reagents suggested the presence of 3,7-disubstituted glycoside with free 5,4′ positions (Mabry et al., 1970).

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doi:10.1016/j.phytol.2009.12.002

Table 1
^{1}H and ^{13}C NMR data of compounds **1** and **2** (CD_3OD, 500 MHz)[a].

Position	**1**		**2**	
	δ_H	δ_C	δ_H	δ_C
2		159.4		159.3
3		135.1		134.9
4		179.6		179.6
5		163.0		163.0
6	6.48 (d, 2.0)	100.7	6.48 (d, 2.0)	100.7
7		163.6		163.6
8	6.75 (d, 2.0)	95.8	6.75 (d, 2.0)	95.8
9		158.0		158.0
10		107.6		107.7
1′		122.7		122.7
2′	8.14 (d, 9.0)	132.5	8.12 (d, 9.0)	132.4
3′	6.90 (d, 9.0)	116.4	6.91 (d, 9.0)	116.4
4′		161.2		161.7
5′	6.90 (d, 9.0)	116.4	6.91 (d, 9.0)	116.4
6′	8.14 (d, 9.0)	132.5	8.12 (d, 9.0)	132.4
3-*O*-β-Gal				
1	5.52 (d, 7.7)	101.7	5.55 (d, 7.7)	101.2
2	3.99 (dd, 9.4, 7.7)	77.6	3.97 (dd, 9.4, 7.7)	77.8
3	3.75 (dd, 9.5, 3.5)	75.6	3.74 (dd, 9.5, 3.5)	75.5
4	3.88 (dd, 3.5, 1.0)	70.5	3.87 (dd, 3.5, 1.0)	70.6
5	3.49 m	75.8	3.50 m	75.9
6	3.68 (dd, 11.7, 4.1)	68.9	3.69 (dd, 11.7, 4.1)	68.8
	3.86 (dd, 11.7, 7.8)		3.84 (dd, 11.7, 7.8)	
2^{Gal}-*O*-α-Rha(I)				
1	5.28 (d, 1.2)	102.1	5.26 (d, 1.2)	102.3
2	4.22 (dd, 3.2, 1.2)	72.1	4.19 (dd, 3.0, 1.2)	72.2
3	4.34 (dd, 9.6, 3.0)	78.6	4.33 (dd, 9.6, 3.0)	78.5
4	5.16 (dd, 9.8, 9.8)	74.4	5.11 (dd, 9.8, 9.8)	74.4
5	4.43 (dd, 9.7, 6.2)	67.9	4.33 (dd, 9.7, 6.2)	68.0
6	0.88 (d, 6.2)	17.4	0.88 (d, 6.2)	17.5
3^{Rhal}-*O*-β-Xyl				
1	4.41 (d, 7.4)	106.3	4.33 (d, 7.4)	106.1
2	3.19 (dd, 8.7, 7.4)	74.7	3.17 (dd, 8.7, 7.4)	74.7
3	3.25 (dd, 8.7, 8.7)	77.4	3.26 (dd, 8.7, 8.7)	77.4
4	3.47 (ddd,11.4,8.7, 5.2)	71.3	3.47 (ddd, 11.4, 8.7, 5.2)	71.4
5	3.90 (dd, 11.4, 5.2)	66.9	3.87 (dd, 11.4, 5.2)	66.9
	3.24 (t, 11.4)		3.21 (t, 11.4)	
4^{Rhal}-*p*-Coumaroyl				
1		127.2		127.6
2,6	7.28 (d, 8.5)	131.2	7.57 (d, 8.5)	133.8
3,5	6.72 (d, 8.5)	116.8	6.68 (d, 8.5)	115.8
4		161.7		161.7
α	7.42 (d, 16.0)	146.6	6.78 (d, 12.8)	145.4
β	6.21 (d, 16.0)	115.5	5.70 (d, 12.8)	116.8
γ		168.8		167.8
6^{Gal}-*O*-β-Glc				
1	4.11 (d, 7.7)	104.3	4.14 (d, 7.7)	104.4
2	3.02 (dd, 9.0, 7.7)	75.0	3.02 (dd, 9.0, 7.7)	75.0
3	3.13 (t, 9.0)	77.8	3.11 (t, 9.0)	77.9
4	3.18 (t, 9.4)	71.4	3.19 (t, 9.4)	71.5
5	2.99 m	77.3	2.99 m	77.7
6	3.75 (dd, 11.8, 2.0)	62.6	3.76 (dd, 11.8, 2.0)	62.6
	3.57 (dd, 11.8, 5.6)		3.57 (dd, 11.8, 5.6)	
7-*O*-α-Rha(II)				
1	5.56 (d, 1.1)	100.0	5.57 (d, 1.1)	100.1
2	4.03 (dd, 3.0 1.5)	71.7	4.02 (dd, 3.0, 1.5)	71.6
3	3.83 (dd, 9.0, 3.0)	72.5	3.82 (dd, 9.0, 3.0)	72.5
4	3.47 (dd, 9.6, 9.6)	73.7	3.48 (dd, 9.6, 9.6)	73.7
5	3.61 (dd, 9.5, 6.2)	71.1	3.63 (dd, 9.5, 6.2)	71.2
6	1.22 (d, 6.2)	18.1	1.27 (d, 6.2)	18.2

[a] *J* values are in parentheses and reported in Hz; chemical shifts are given in ppm; assignments were confirmed by ROESY, NOESY, COSY, 1D-TOCSY, HSQC and HMBC experiments.

The structure of the kaempferol moiety for compound **1** was confirmed from NMR spectral data (see Table 1). Two *meta*-coupled proton resonances at δ 6.48 (1H, d, J = 2.0 Hz) δ_C 100.7, and δ 6.75 (1H, d, J = 2.0 Hz), δ_C 95.8, were characteristic for H-6 and H-8 of a flavonoid A-ring, respectively. Similarly the coupled resonances at δ 8.14 (2H, J = 9.0 Hz), δ_C 132.5 and 6.90 (2H, J = 9.0 Hz), δ_C 116.4, were typical of H-2′/6′ and H-3′/5′ of a flavonoid B-ring, respectively. The ^{1}H NMR spectrum exhibited two ethylenic protons at δ 6.21 and 7.42 with a coupling constant of 16 Hz. The stereochemistry of the double bond was deduced from the magnitude of J and attributed to an (*E*)-configuration (*trans*). The four aromatic coupled protons at δ 7.28 and 6.72 (2H

each, J = 8.5 Hz) were attributed to a *p*-coumaroyl unit confirmed by the correlation of the two vinylic protons in HMBC with the carbonyl carbon at δ_C 168.8.

Compound **1** was a pentaglycoside as shown by the ^{1}H and ^{13}C NMR spectra with five anomeric proton signals at δ 5.56 (d, J = 1.1 Hz), 5.52 (d, J = 7.7 Hz), 5.28 (d, J = 1.2 Hz), 4.41 (d, J = 7.4 Hz), 4.11 (d, J = 7.7 Hz) and carbons at δ_C 100.0, 101.7, 102.1, 106.3 and 104.3, respectively. Acid hydrolysis afforded the isolation of different sugar units identified by GC-MS analysis of their corresponding trimethylsilylated derivatives. The attribution of the absolute configurations was determined by comparison with authentic samples. The configurations of each anomeric carbons were assigned α or β based on the magnitudes of the corresponding $^3J_{H\text{-}1,H\text{-}2}$ coupling constants. A coupling constant of 1.1 Hz was indicative of a diequatorial configuration between H-1 and H-2 in a sugar unit demonstrating α configuration. This is the case for the anomeric sugar proton at δ 5.56. Assignment of an α-Rha unit at position 7 of the kaempferol was deduced from the following observations: a $^3J_{CH}$ of the anomeric proton at δ 5.56 with C-7 of the kaempferol moiety at δ_C 163.6, downfield shifts observed of H-6 (Δ +0.24 ppm) and H-8 (Δ +0.31 ppm) of the kaempferol (Merfort and Wendisch, 1988), long range connectivities observed from 6-CH_3 to C-4 and C-5 and from H-1 to C-2, C-3 and C-5, the presence of two identical doublets for H-4 and finally the presence of a methyl as a doublet at δ 1.22, δ_C 18.1. A second anomeric sugar proton at δ 5.52 was coupled by $^3J_{CH}$ with C-3 of the kaempferol moiety at δ_C 135.1. This was confirmed by the chemical shift changes for C-2, C-3 and C-4 by comparison with non-substituted kaempferol molecule supporting evidences for 3-glycosylation of compound **1** (Agrawal, 1989). The identification of the sugar unit at C-3 was deduced from COSY, HMQC and HMBC starting from the anomeric proton (Table 1). The primary sugar linked at C-3 was identified as β-galactopyranose. The main HMBC correlations observed were from 6-CH_2OH to C-4 and C-5 and from H-4 to C-2, and finally from H-2 to the anomeric carbon C-1. These hypotheses were supported by the chemical shifts observed for kaempferol 3-*O*-((β-D-glucopyranosyl-(1 → 3)-(4-*O*-(*E*-*p*-coumaroyl))-α-L-rhamnopyranosyl-(1 → 6)-β-D-galactopyranoside))-7-*O*-α-L-rhamnopyranoside (Mariani et al., 2008). The downfield shift resonance of both Gal C-2, δ_C 77.6 and Gal C-6, δ_C 68.9 with respect to their counterparts suggested a glycosylation at these two positions. This was confirmed by the long range correlations of the anomeric proton δ 4.11, δ_C 104.3 to Gal C-6 and assigned as a 6-*O*-linked glucopyranoside. Assignment of an α-Rha unit in position 2 of the Gal was deduced from the following observations: a $^3J_{CH}$ correlation between Gal C-2 and the anomeric proton at δ 5.28, long range connectivities observed from 6-CH_3 to C-4 and C-5 and from H-1 to C-2, C-3 and C-5, the presence of two identical doublets for H-4 and finally the presence of a methyl as a doublet at δ 0.88, δ_C 17.4. The *p*-coumaroyl unit was linked to position 4 of the Rha(I) unit based on the $^3J_{CH}$ correlation of the Rha(I) H-4, δ 5.16 with the ester carbon δ_C 168.8 and $^2J_{CH}$ correlations with Rha(I) C-3 and C-5. The downfield shift of the proton Rha(I) H-4 adjacent to the acylated carbon (Δ +1.7 ppm) compared to kaempferol-3-*O*-{[β-D-xylopyranosyl(1 → 3)-α-L-rhamnopyranosyl(1 → 6)][α-L-rhamnopyranosyl(1 → 2)]}-β-D-galactopyranoside (Semmar et al., 2002) further confirmed the position of the coumaroyl unit. The last sugar unit could be attributed to a pentose because of the presence of five carbons including a CH_2 observed in DEPT. The main correlations observed were from H-5 to C-4 ($^2J_{CH}$) and to C-3 ($^3J_{CH}$) and from H-3 to the anomeric carbon C-1 ($^3J_{CH}$). This sugar unit was attributed to a β-xylopyranoside supported by the ^{13}C NMR chemical shifts (Agrawal, 1992) and acid hydrolysis. The β-xylose unit was connected to the 3-position of Rha(I) based on the ROESY connection between Rha(I) H-3, δ 4.34 and Xyl H-1, δ 4.41 and the long range correlations of the anomeric proton δ 4.41 and carbon Rha(I) C-3, δ_C 78.6 (Semmar et al., 2002). Confirmation of the different ether linkages between sugar units were shown by NOESY correlations. These data demonstrated that a branched tetrasaccharide bearing a *trans p*-coumaroyl unit was linked at C-3 to a kaempferol moiety in addition to a monosaccharide Rha(II) *O*-linked at C-7 (Khan et al., 2009). Thus **1** was identified as kaempferol 3-*O*-β-D-xylopyranosyl(1 → 3)-(4-*O*-*E*-*p*-coumaroyl-α-L-rhamnopyranosyl(1 → 2))[β-D-glucopyranosyl(1 → 6)]-β-D-galactopyranoside-7-*O*-α-L-rhamnopyranoside (see Fig. 1).

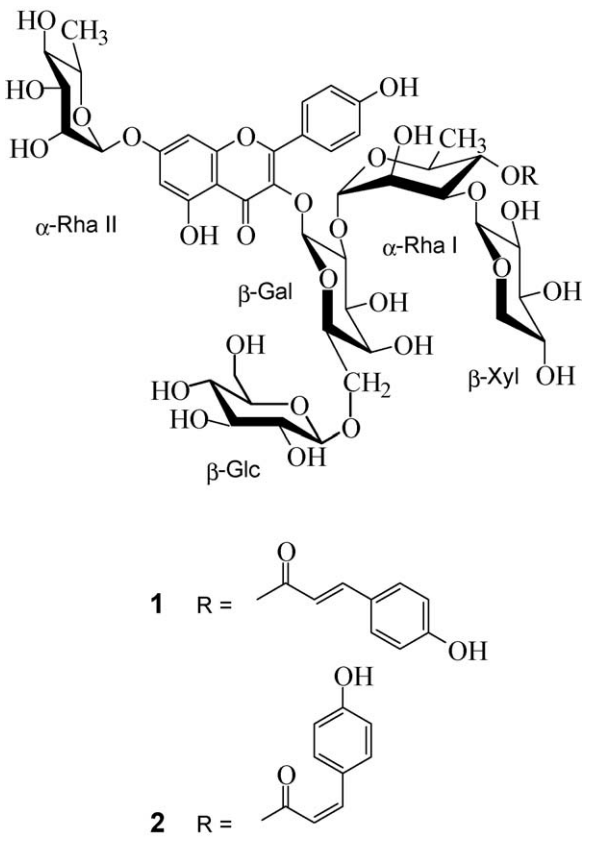

Fig. 1. Structures of compounds **1** and **2**.

Compound **2** was isolated as a pale yellow amorphous powder. The UV spectral data recorded in methanol were also similar to the previous compound **1** with the characteristic maxima at 268 and 317 nm. The mass spectrum by HRESIMS in the positive mode was identical to compound **1**. The similar fragmentations of the two molecules indicated that compound **2** was an isomer of compound **1**. The analysis of ^{1}H and ^{13}C NMR showed the presence of a kaempferol moiety with five anomeric protons signals corresponding to a pentasaccharide. These two compounds differed by the configuration of the *p*-coumaroyl unit with two olefinic protons δ 6.78 and 5.70 (1H each, d, J = 12.8 Hz). The lower coupling constant and chemical shift values indicated a *cis* isomer (Ichiyanagi et al., 2005). Finally, **2** was identified as kaempferol 3-*O*-β-D-xylopyranosyl(1 → 3)-(4-*O*-*Z*-*p*-coumaroyl-α-L-rhamnopyranosyl(1 → 2))[β-D-glucopyranosyl(1 → 6)]-β-D-galactopyranoside-7-*O*-α-L-rhamnopyranoside.

Other rare flavonol tetra- and pentaglycosides in other members of Leguminosae have been characterised by branched tetrasaccharides at C-3 of the aglycone e.g. in *Astragalus caprinus* Maire (Semmar et al., 2002), *Mildbraediodendron excelsum* Harms and the genus *Cordyla* (Veitch et al., 2005, 2008), but acylated forms were only found in *A. caprinus* Maire and the genus *Cordyla*.

Other unusual diacylated kaempferol hexa- and tetraglycosides were isolated in the genus *Planchonia* (Lecythidaceae) (Crublet et al., 2003; McRae et al., 2008).

Compounds **1** and **2** displayed a mild antioxidant activity in the *in vitro* peroxynitrite assay with EC_{50} values of 62 ± 9.3 μM and 19 ± 2.9 μM, respectively. These values were higher than those of the reference compound, gallic acid (4.9 ± 0.4 μM). The isomeric difference of activity might be explained by the higher reactivity of *cis*, compared to *trans*, bonds.

3. Experimental

3.1. General experimental procedures

Optical rotations were measured on a PerkinElmer model 241 MC polarimeter. Melting points were determined on a Buchi melting point apparatus B-545. IR spectra were measured on a Nicolet 5-sxc-FTIR spectrometer. UV spectra were recorded on a CARY 100 Bio UV–vis spectrometer. NMR spectra were recorded on a Bruker AVANCE 500 (500 MHz for ^{1}H and 125 MHz for ^{13}C) and chemical shifts are given in δ (ppm) value relative to TMS as internal standard. HRESIMS spectra were recorded on a micrOTOF ESI-TOF mass spectrometer (Bruker Daltonics, Bremen, Germany) operating in negative or positive modes in separate analyses using a mixture MeOH:H_2O (1:1). LC-ESI–MS2 was performed on a HCT Ultra (Bruker Daltonics) system consisting of a 1200 SL Agilent (Agilent Technologies, Massy, France), an automatic autosampler and a C18 Hypersil (30 × 1.0 mm i.d., 1.9 μm particle size) with a flow rate 0.2 ml/min. Sephadex LH-20 (Pharmacia, Sweden) was used for column chromatography. Reversed-phase HPLC was conducted on a Gilson instrument (Middleton, US) equipped with a 9010 pump, a 115 UV photodiode array detector and a Nucleodur 100-10-C18 (250 × 21 mm id; 10 μm particle size) column for semi-preparative separation, and a Nucleodur 100-10-C18 (150 × 4.6 mm id; 5 μm particle size) (Macherey-Nagel, Dueren, Germany) column for analytical use. Electron impact ionization mass spectra were obtained on a Thermo Fisher Scientific GC-MS Trace DSQ II with a capillary TR-5MS SQC (15 m × 0.25 mm × 0.25 μm) column. All samples were protected from UV-light during handling.

3.2. Plant material

The leaves of *B. nitida* were collected in the classified forest of the Abobo Adjamé University, Ivory Coast in September 2006. The botanical determination was performed by Dr. Laurent Aké Assi at the National Floristic Center, University of Cocody, Abidjan, in the Herbarium of which a voucher specimen (No. 1549) was deposited.

3.3. Extraction and isolation

Air-dried and ground leaves of *B. nitida* (250 g) were extracted by percolation at room temperature with MeOH and concentrated to dryness under vacuum. The residue (36 g) was suspended in H_2O and successively partitioned with cyclohexane (300 ml 3×), CH_2Cl_2 (300 ml 3×), EtOAc (300 ml 3×) and finally with *n*-BuOH (300 ml 3×). Part of the butanolic residue was subjected to column chromatography over Sephadex LH-20 eluted with a H_2O–MeOH mixture of increasing percentage of MeOH. The flavonoid rich fractions were pooled and resubjected to column chromatography over Sephadex LH-20 using 100% MeOH as eluent, yielding 12 subfractions. Dry subfractions 2–4 (45 mg) were redissolved in 1 ml MeOH and filtered through 0.45 μm PTFE filter prior to semi-preparative HPLC. This was performed on a 100-10-C18 Nucleodur Macherey-Nagel (250 × 21 mm id; 10 μm particle size) column, flow rate: 14 ml/min, UV detection 205 nm developed with 0.01% aqueous formic acid (solvent A) and MeOH-acetonitrile (1:1) (solvent B). The following gradient elution was used: t = 0 min 5% B, t = 5 min 5% B, t = 15 min 50% B, t = 20 min 70% B, t = 25 min 80% B, t = 30 min 100% B, t = 35 min 100% B. Under these conditions, two purified compounds were obtained: **1** (15 mg) and **2** (9 mg).

3.4. HPLC analysis of compounds 1 and 2

After separation, the purity of the two compounds was confirmed by analytical HPLC (Macherey-Nagel Nucleodur column 100-10-C18, 150 × 4.6 mm id, 5 μm); solvent A: formic acid 0.01%, solvent B: MeOH using the following gradient of B: 0 min 5%, 10 min 50%, 15 min 70%, 20 min 80%, 25 min 100%, 30 min 100%, 35 min 5% B; flow rate 1.0 ml/min; UV detection between 200 and 600 nm. Compounds **1** and **2** were eluted with retention time of 20.9 and 20.1 min, respectively. Their relative purity was >98%.

3.5. Sugar analysis

Acid hydrolysis of **1** and **2** (2 mg of each solution in MeOH) was carried out with 2 ml of 2 M HCl at 85 °C during 2 h. After cooling, the solvent was removed under reduced pressure. The sugar mixture was extracted from the aqueous phase and washed with ethyl acetate to eliminate kaempferol. The absolute configuration of each monosaccharide was determined from GC-MS analysis of their trimethylsilylated derivatives by comparison with authentic samples. Typically, 500 μl of a solution of 1-(trimethylsilyl)imidazole in dry pyridine (1:4 v/v) were added to the standard sugar (2 mg) and heated at 70 °C during 2 h in a glass vial. GC analysis was performed with a capillary TR-5MS SQC (15 m × 0.25 mm × 0.25 μm) column. Operating conditions were as follows: carrier gas, helium with a flow rate of 1 ml/min; column temperature, 1 min in 150 °C, 150–220 °C at 4 °C/min; injector temperature, 250 °C; volume injected, 1 μl of the trimethylsilylated sugar in methylene chloride (0.1%); split ratio, 1:50. The MS operating parameters were as follows: ionization potential, 70 eV; ion source temperature, 230 °C; solvent delay 4.0 min, mass range 100–700. Both **1** and **2** gave D-xylose, D-galactose and D-glucose (t_R = 4.66, 6.21, and 6.74 min, respectively).

3.6. Kaempferol 3-O-β-D-xylopyranosyl(1 → 3)-(4-O-E-p-coumaroyl-α-L-rhamnopyranosyl(1 → 2))[β-D-glucopyranosyl(1 → 6)]-β-D-galactopyranoside-7-O-α-L-rhamnopyranoside (1)

Pale yellow amorphous powder, $[\alpha]_D^{20}$ −97° (c 0.22, MeOH). UV λ_{max} (MeOH) nm: 227 (4.66), 269 (4.49), 317 (3.79), 355 (sh); (MeOH + NaOH): 244, 271, 298, 370; (MeOH + $AlCl_3$): 278, 304, 320, 398; (MeOH + $AlCl_3$ + HCl): 232, 278, 301, 322, 395; (MeOH + NaOAc): 269, 319, 358 (MeOH + NaOAc + H_3BO_3): 270, 319, 352. IR (KBr) ν_{max} cm^{-1}: 3350 (OH), 2930, 1693, 1650 (conjugated ketone C=O), 1633, 1598 (aromatic C=C), 1514, 1494, 1446, 1347, 1258, 1205, 1189, 1012, 890, 832. ^{1}H and ^{13}C NMR: see Table 1. ESIMS *m/z*: 1181 $[M+H]^+$. HRESIMS positive mode *m/z*: 1181.3524 and calcd for $C_{53}H_{64}O_{30}$ + H, 1181.3555 and $[M-H]^-$ HRESIMS negative mode *m/z*: 1179.3410 and calcd for $C_{53}H_{64}O_{30}$ − H, 1179.3399.

3.7. Kaempferol 3-O-β-D-xylopyranosyl(1 → 3)-(4-O-Z-p-coumaroyl-α-L-rhamnopyranosyl(1 → 2))[β-D-glucopyranosyl(1 → 6)]-β-D-galactopyranoside-7-O-α-L-rhamnopyranoside (2)

Pale yellow amorphous powder, $[\alpha]_D^{20}$ −143° (c 0.16, MeOH). UV λ_{max} (MeOH) nm: 268 (4.49), 317 (3.79), 355 (sh); (MeOH + NaOH): 244, 272, 372; (MeOH + $AlCl_3$): 276, 304, 322, 392 (MeOH + $AlCl_3$ + HCl): 277, 302, 325, 396; (MeOH + NaOAc): 268,

318, 354; (MeOH + NaOAc + H_3BO_3): 268, 318, 359. IR (KBr) ν_{max} cm^{-1}: 3350 (OH), 2930, 1693, 1650 (conjugated ketone C=O), 1633, 1598 (aromatic C=C), 1514, 1494, 1446, 1347, 1258, 1205, 1189, 1012, 890, 832. ^1H and ^{13}C NMR: see Table 1. ESIMS *m/z*: 1181 $[M+H]^+$. HRESIMS *m/z*: 1181.3545 and calcd for $C_{53}H_{64}O_{30}$ + H, 1181.3555

3.8. Antioxidant activity

The antioxidant activity of the two isolated compounds was assessed in the peroxynitrite assay according to a previously described method (Choi et al., 2002) using gallic acid as positive standard. All experiments were performed in triplicate.

Acknowledgements

The authors would like to thank retired Dr. Laurent Aké Assi, National Floristic Center for its scientific contribution.

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