Antimicrobial Glycosides and Derivatives from Roots of *Picralima nitida*

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Abstract

Phytochemical screening was performed on the roots of *Picralima nitida*, resulting in the isolation of three new coumestan glycosides, 3-hydroxy-9-methoxy-2-[2'(*E*)-3'-methyl-4'-*O*-*β*-D-galactopyranosylbutenyl]-8-isoprenylcoumestan (**1**), 3-hydroxy-9-methoxy-2-[2'(*E*)-3'-methyl-4'-*O*-*β*-D-glucopyranosylbutenyl]-8-[2"(*E*)-3"-methyl-4"-oxobutenyl]coumestan (**2**), and 3-hydroxy-9-methoxy-4-[2'(*E*)-3'-methyl-4'-*O*-*β*-D-glucopyranosylbutenyl]-8-[2"(*E*)-3"-methyl-4"-oxobutenyl]coumestan (**3**). Acid hydrolysis of **1**, **2** and **3** afforded three new coumestan, 3-hydroxy-9-methoxy-2-[2'(*E*)-4'-hydroxy-3'-methylbutenyl]-8-isoprenylcoumestan (**4**), 3- hydroxy-9-methoxy-2-[2'(*E*)-4'-hydroxy-3'-methylbutenyl]-8-[2"(*E*)-3"-methyl-4"-oxobutenyl]coumestan (**5**), and 3-hydroxy-9-methoxy-4-[2'(*E*)-4'-hydroxy-3'-methylbutenyl]-8-[2"(*E*)-3"-methyl-4"-oxobutenyl]coumestan (**6**), respectively. Structures of these compounds were elucidated on the basis of spectroscopic data and chemical transformations. Compounds **1** - **6** showed antimicrobial activities against *Escherichia coli*, *Staphylococcus aureus*, and *Proteus vulgaris*.

1. Introduction

Picralima nitida Stapf (Apocynaceae) is an entirely glabrous shrub of 3-10 m high. Its fruits are ovoid and yellowish at maturity (Adjanohoun et al. 1996). This plant is widely distributed throughout Africa forest regions. Throughout its distribution area the seeds, bark and roots of *P. nitida* have a reputation as a febrifuge and remedy for malaria (Kouitcheu et al. 2008). They are also extensively used for pain relief and to treat chest and stomach problems, pneumonia and intestinal worms. Usually, the seeds or bark are crushed or chewed and eaten for this purpose, or a decoction from the roots, seeds or bark is drunk (Adjanohoun et al. 1996; Ezeamuzieet al. 1994). As part of our continuing study on searching for the bioactive flavonoids constituents from West Africa medicinal plants (Kazie et al. 2009), phytochemical screening was performed on the roots of *P. nitida*, resulting in the isolation of three new coumestan glycosides **1**, **2** and **3**. Acid hydrolysis of these products yielded three new coumestan derivatives **4**, **5** and **6**, respectively. These six compounds showed antimicrobial activities against

E. coli, *S. aureus*, and *P. vulgaris*. We herein describe the isolation, structural elucidation and biological activities of these compounds.

2. Experimental

2.1 General Procedures

Melting points were determined on X-4 digital micro-melting point apparatus and were uncorrected. Optical rotations were measured with a Perkin-Elmer 341 digital polarimeter. The NMR spectra were recorded with a Bruker AMX-500 (500 MHz for ^{1}H-NMR and 125 MHz for ^{13}C-NMR). Samples were run in DMSO-d_6 or CDCl$_3$. Chemical shifts were given in (ppm) with tetramethylsilane as an internal standard (0.00 ppm). The HRFABMS spectrum was obtained with a Kratos MS 25 instrument with a DS-55 data system, and collision gas Xe (ion gun conditions 6KV and 10 mA). The EI mass spectra (at 70 eV) were recorded on a JEOLMSRoute mass spectrometer. IR spectra were run from KBr pellets on a Perkin-Elmer 577 spectrometer. HPLC was performed by using a system comprised of a CCPM pump, a CCP PX-8010 controller, an RI-8010 detector and a Shodex OR-2 detector, and a Rheodyne injection port with a 20 μl sample loop. Sephadex LH-20, Si gel GF$_{254}$ (Merck) and Si gel 60 (70-2 mesh ASTM) (Merck) were used for CC. Silica gel 60 (0.25 mm, Merck) was used for TLC.

2.2 Plant Material

Roots of *P. nitida* were collected in Zo-Etélé, south region of Cameroon in January 2002. The plant was identified at the National Herbarium, Yaounde, where a voucher specimen is deposited (No.2136/SRFK)

2.3 Extraction and Isolation of Compounds

Dried ground roots of *P. nitida* (13 Kg) was immersed in MeOH (35 L) and kept for 72 h at 25 °C. After filtration the solvent was removed by rotary evaporator under reduce pressure. The extract obtained (302 g) was partitioned with 2%H$_2$SO$_4$/EtOAc. The EtOAc phase (102 g) was chromatographed over silica gel (400 g). Elution with Hexane, Hexane- EtOAc (75/25), (50/50), (25/75), EtOAc, EtOAc-MeOH (25/75) and MeOH gave seven fractions I (7.6 g), II (5.5 g), III (11.2 g), IV (10.8 g), V(17.5 g), VI(15.4 g) and VII(29.7 g), respectively. Fraction V was again chromatographed on Sephadex LH20 (100 g) using methanol as eluent. Ninety-five fractions, each of 300mL, were collected and combined on the basis of TLC evidence. Fractions 8-21 (3 g) was purified, using preparative TLC and MeOH/CHCl$_2$/H$_2$O (5/4.75/0.25) as the development solvent, to give compound **1**(56.2 mg), **2**(48.5 mg) and **3**(52.8 mg).

2.4 Physical and Spectroscopic Data of Compounds **1-3**

Compound **1**

M.p. 259-260 °C.-Rf. 33% .- UV/ (MeOH) λ_{max} (log ε_{max}): 244 nm (4.40), 315 nm (4.05), 355 nm (4.46) .- $[\alpha]^{22}_D$ = -44°(c = 0.11, MeOH) .- IR (KBr) v_{max} = 3405-3200, 1724, 1715, 1625, 1600,1504, 1222, 1035, 810 cm^{-1} . - ^{1}H-NMR(Measured in DMSO-d_6) (See Table 2). - ^{13}C-NMR(Measured in DMSO-d_6) (see Table 1). - HRMS (FAB, 6 kV): m/z = 596.2120 [M]$^+$ (calcd. for C$_{32}$H$_{36}$O$_{11}$: 596.2121).

Compound **2**

M.p. 264-265 °C.-Rf. 31%. – UV/(MeOH) λ_{max} (log ε_{max})**:** 245 nm (4.42), 314 nm (4.06), 355 nm (4.44). - $[\alpha]^{22}_D$ = -53°(c = 0.11, MeOH) .- IR (KBr) v_{max} = 3405-3200, 1725, 1715, 1625, 1605,1505, 1220, 1034, 810 cm^{-1} . - ^{1}H-NMR(Measured in DMSO-d_6) (See Table 2) . - ^{13}C-NMR(Measured in DMSO-d_6) (see Table 1). - HRMS (FAB, 6 kV): m/z = 610.1912 [M]$^+$ (calcd. for C$_{32}$H$_{34}$O$_{12}$: 610.1914).

Compound **3**

M.p. 261-262 °C.-Rf. 32%. - UV/(MeOH) λ_{max} (log ε_{max})**:** 245 nm (4.41), 315 nm (4.07), 354 nm (4.42) . - $[\alpha]^{22}_D$ = -36°(c = 0.11, MeOH) .- IR (KBr) v_{max} = 3405-3200, 1724, 1712, 1625, 1605,1504, 1220, 1035, 810 cm^{-1} . - ^{1}H-NMR (Measured in DMSO-d_6) (See Table 2) . - ^{13}C-NMR (Measured in DMSO-d_6) (see Table 1). - HRMS (FAB, 6 kV): m/z = 610.1911 [M]$^+$ Calcd for C$_{32}$H$_{34}$O$_{12}$: m/z = 610.1914

2.5 Acid Hydrolysis and Identification of Sugar

Compounds **1**, **2** and **3** (16.5 mg each) were separately refluxed with 15% HCl/MeOH (12 ml) at 80°C for 4 h. After cooling, each reaction mixture was concentrated and the residue partitioned with CHCl$_3$/H$_2$O. The organic layer was concentrated to dryness to yield 9.8 mg of white material (M1) from **1**, 10.1mg of white material(M2) from **2** and 9.9 mg of white material(M3) from **3**. After purification by preparative-TLC, using silica gel and MeOH/CH$_2$Cl$_2$ (0.25:9.75) as development solvent, M1, M2 and M3 yielded compounds **4** (8.1 mg), **5** (7.2 mg) and **6** (7.8 mg), respectively. Each aqueous layer was evaporated and the residue was analysed by HPLC under

the following conditions: column, Aminex HPX-87H (7.8 mm i.d. x 300 mm); solvent, 5 mM H_2SO_4; flow rate, 0.6ml/min; detection, refractive index and optical rotation. The sugars were confirmed as D-galactose, and D-glucose by comparison of their retention times and optical rotations with those of authentic samples: retention times (min), 9.62 (D-galactose, positive optical rotation), 8.99 (D-glucose, positive optical rotation).

2.6 Physical and Spectroscopic Data of Compounds **4-6**

Compound **4**

White powder.-M.p. 208-209°C.-*Rf.* 52%. - UV/(MeOH) λ_{max} (log ε_{max})**:** 243 nm (4.40), 315 nm (4.09), 354 nm (4.45).- IR (KBr) ν_{max} = 3400-3200, 1725, 1715, 1624, 1500, 1222, 1034, 810 cm^{-1} . - ^{1}H-NMR(Measured in CDCl$_3$)(See Table 2) . - ^{13}C-NMR(Measured in CDCl$_3$)(see Table 1). - HRMS: *m/z* = 434.1591 [M]$^+$ (calcd. for $C_{26}H_{26}O_6$: 434.1590).

Compound **5**

White powder.-M.p. 204-205°C.-*Rf.* 51%. - UV/(MeOH) λ_{max} (log ε_{max}): 244 nm (4.40), 315 nm (4.09), 354 nm (4.45).- IR (KBr) ν_{max} = 3405-3250, 1725, 1712, 1625, 1450, 1220, 1035, 810 cm^{-1} . - ^{1}H-NMR (Measured in CDCl$_3$)(See Table 2) . - ^{13}C-NMR (Measured in CDCl$_3$)(see Table 1). - HRMS: *m/z* = 448.1381 [M]$^+$ (calcd. for $C_{26}H_{24}O_7$: 448.1382).

Compound **6**

White powder.-M.p. 208-209°C.-*Rf.* 53%.- UV/(MeOH) λ_{max} (log ε_{max})**:** 243 nm (4.40), 315 nm (4.09), 354 nm (4.45)**.** - IR (KBr) ν_{max} = 3405-3200, 1722, 1710, 1625, 1500, 1220, 1034, 810 cm^{-1} . - ^{1}H-NMR (Measured in CDCl$_3$) (See Table 2) . - ^{13}C-NMR (Measured in CDCl$_3$)(see Table 1). - HRMS: *m/z* = 448.1380 [M]$^+$ (calcd. for $C_{26}H_{24}O_7$: 448.1382).

2.7 Antibacterial Assay

Antibacterial activity was determined by the paper disk method. A paper disk (Φ 6 mm, from Whatman number one filter paper), with the sample was incubated on an agar plate containing *E. coli*, *S. aureus*, or *P. vulgaris* at 25 °C. Paper discs containing Amikacin (30 μg), Vancomycin (30μg) and Penicilin (10μg), respectively, were used as positive control. The result recorded for each bioassay was the average of three tests.

3. Results and Discussion

3.1 Structural Elucidation of Compounds **1** *and* **4**

Compound **1** was obtained as white powder. Its molecular formula was derived as $C_{32}H_{36}O_{11}$ by the high resolution–FABMS (HRFABMS) spectrum, showing an [M]$^+$ ion at *m/z* = 596.2120, and broad band-decoupled ^{13}C-NMR spectrum (32 carbon signals). The IR spectrum disclosed absorption bands due to δ-lactone carbonyl (1724 cm^{-1}), aromatic ring (1625, 1600, 1504 cm^{-1}) and aromatic C-O (1222 cm^{-1}). UV spectrum showed absorption maxima at 244, 315, and 355 nm. The IR and UV bands indicated the coumestan nature of **1** (Kouam et al. 2007; Mabry and Markham, 1975). The ^{1}H- and ^{13}C-NMR spectroscopic data of **1**(Table 1 and 2) revealed the presence of a sugar residue in addition to a coumestan aglycone moiety. Upon acid hydrolysis (note 2), **1** afforded D-galactose and compound **4**.

Compound **4** had the molecular formula $C_{26}H_{26}O_6$ on the basis of the HREIMS, exhibiting an [M]$^+$ peak at *m/z* = 434.1591, and ^{13}C-NMR spectrum (Table 1) (26 carbon signals). A distortionless enhancement by polarization transfer (DEPT) NMR experiment permitted differentiation of the 26 resonances into four methyl, three methylene, six methine, and thirteen quaternary carbons. Its UV and IR spectra were similar to those of other coumestans. The ^{1}H-NMR spectrum(Table 2) displayed two para-coupled aromatic protons at δ_H 7.76 (d, 1H, *J* = 1.2Hz, H-1) and 6.96 (d, 1H, *J* = 1.2Hz, H-4). It also showed a peak for another para-coupled protons at δ_H 7.02 (d, 1H, *J*=1.7Hz, H-7), 6.97 (d, 1H, *J*=1.7Hz, H-10). In the ^{1}H-NMR spectrum, further signals were observed that showed the presence of one methoxy group [3.99 (3H,s)], two hydroxyl units [9.55 (1H,s) and 5.34 (1H, s)]**,** a prenyl unit [3.21(2H, d, *J* = 6.9 Hz, H-1"), 5.02(1H, t, *J* = 6.9 Hz, H-2"), 1.81 (3H, s, H-4"), and 1.79 (3H, s, H-5")], and a 4'-hydroxy-3'-methyl-butenyl residue [3.13 (2H, d, *J* = 8.0 Hz, H- 1'), 5.09 (1H, t, *J* = 8.0 Hz, H-2'), 4.55 (2H, s, H- 4'), and 1.78 (3H, s, H-5')]. In the NOESY spectrum, cross peak was observed from H$_2$-4' to H-2', confirming that the relative configuration was *E*. In the heteronuclear multiple bond correlation (HMBC) spectrum, the cross peaks from the proton at δ_H 9.55 to C-3, from the proton at δ_H 5.34 to C-4', and from the proton at δ_H3.99 to C-9, confirmed that two hydroxyl moieties were attached to C-3 and C-4', one methoxy to C-9. The position of the prenyl group was confirmed by HMBC experiment, which showed correlation between a vinylic proton H-2" and C-8, furthermore, the methylene protons H$_2$-1" of the prenyl substituent showed correlations with C-8, C-7, and C-9. Moreover, in HMBC spectrum, the cross peaks from the proton at δ_H

5.09(H-2') to C-2, from the proton at δ_H3.13 (H-1') to C-2, C-1, and C-3 confirmed that the 4'-hdroxy-3'-methyl-butenyl residue was located at C-2. The complete structural elucidation of **4** was derived from the chemical shifts and coupling constant of the ^{1}H-NMR spectrum and from detailed spectral analysis of HMQC, HMBC experiments (Table 3). Consequently, the structure of **4** was determined as 3-hydroxy-9-methoxy- 2-[2'(*E*)-4'-hydroxy-3'-methylbutenyl]-8-isoprenylcoumestan (**4**) (see Fig. 1).

The molecular formula of **1** indicated that it consisted of one mole each of **4** and D-galactose. For clarification of the location of the sugar moiety in compound **1**, the ^{13}C-NMR and HMBC spectra were inspected. In the ^{13}C-NMR spectrum, the shift values for the carbons of the aglycone residue were in agreement with the corresponding data of compound **4**, except for the C- 4', which was significantly downfield. Furthermore, in the HMBC spectrum of **1**, the anomeric proton signal at δ = 4.81 (d, *J* = 7.8 Hz, H - 1''') exhibited three-bond-coupled carbon signal at δ = 80.4 (C-4'). These results indicated that the C-4' hydroxyl group of 3-hydroxy-9-methoxy- 2-[2'(*E*)-4'-hydroxy-3'-methylbutenyl]-8-isoprenyl coumestan was bound to a sugar through glycosidic linkage. The *J* value (7.8 Hz) of the anomeric proton indicated that galactose moiety was linked *via* a *β* – linkage. Consequently, the structure of **1** was determined as 3-hydroxy-9-methoxy-2-[2'(*E*)-3'-methyl- 4'-*O*-*β*-D-galactopyranosylbutenyl]-8-isoprenylcoumestan (**1**) (see Fig 1).

3.2 Structural Elucidation of Compounds **2** and **5**

Compound **2**, obtained as white powder, had a molecular formula of $C_{32}H_{34}O_{12}$ on the basis of its HRFABMS (*m/z* = 610.1912 [M] [+]) and ^{13}C-NMR data (Table 2) (32 signals). The IR and UV bands indicated the coumestan nature of **2**. Furthermore, the ^{1}H- and ^{13}C-NMR spectroscopic data of **2** (Table 1 and 2) revealed the presence of a sugar residue in addition to a coumestan aglycone moiety. Upon acid hydrolysis (note 2), **2** afforded D-glucose and compound **5**.

Compound **5** had the molecular formula $C_{26}H_{24}O_7$ on the basis of the HREIMS, exhibiting an [M][+] peak at *m/z* = 448.1381, and ^{13}C-NMR spectrum (Table 1) (26 carbon signals). Its UV and IR spectra were almost similar to those of compound **4**. The NMR spectra of **5** (Table 1 and 2) resembled those of **4**, except for the presence of an 4''-oxo-isopentenyl group signals [3.18 (2H, d, *J* = 7.0 Hz, H$_2$- 1''), 5.01 (1H, t, *J* = 7.0Hz, H- 2''), 9.03 (1H, s, H- 4''), and 1.78 (3H, s, H$_3$-5'')] instead of isopentenyl signals in **4** (Table 1 and 2)**.** In the NOESY spectrum, cross peak was observed from H-4'' to H-2''. Based on the chemical shifts and coupling constant of the ^{1}H-NMR spectrum and detailed spectral analysis of HMQC and HMBC experiments (Table 3) the complete structural elucidation of **5** was derived as 3-hydroxy-9-methoxy-2-[2'(*E*)-4'-hydroxy-3'-methylbutenyl]-8-[2''(*E*) -3''-methyl-4''-oxobutenyl]coumestan(**5**) (see Fig. 1).

The molecular formula of **2** indicated that it consisted of one mole each of **5** and D-glucose. Comparison of ^{13}C-NMR data of **2** with those of 3-hydroxy-9-methoxy-2-[2'(*E*)-4'-hydroxy-3'-methylbutenyl] -8-[2''(*E*)-3''-methyl-4''-oxobutenyl]coumestan (Table 1) combined with the HMBC spectrum showing three-bond-coupled signal between the anomeric proton signal at δ = 4.34 (d, *J* = 7.7Hz) and carbon signal at δ = 80.5 (C-4') showed that the C-4' hydroxyl group of the aglycone moiety is connected to the glucose molecule through a glycosidic bond. The configuration of the D-glucopyranosyl unit was regarded to be *β* by the *J* value of of its anomeric proton signal. The structure of **2** was finally concluded as 3- hydroxy-9-methoxy-2-[2'(*E*)-3'-methyl- 4'-*O*-*β*-D-glucopyranosylbutenyl]-8-[2''(*E*)-3''-methyl-4''-oxo-butenyl]coumestan (**2**) (see Fig 1).

3.3 Structural Elucidation of Compounds **3** and **6**

Compound **3** was obtained as white powder. Its molecular formula was derived as $C_{32}H_{34}O_{12}$ by the HRFABMS spectrum, showing an [M][+] ion at *m/z* = 610.1912, and broad band-decoupled ^{13}C-NMR spectrum (Table 1) (32 carbon signals). Its IR, UV and NMR data indicated that it was a coumestan glycoside. Acid hydrolysis (note 2) yielded D-glucose along with compound **6**.

Compound **6** had the molecular formula $C_{26}H_{24}O_7$ on the basis of the HREIMS, exhibiting an [M][+] peak at *m/z* = 448.1380, and ^{13}C-NMR spectrum(Table 1) (26 carbon signals). Its UV and IR spectra were almost similar to those of compound 5**.** The ^{1}H-NMR spectrum displayed two ortho-coupled aromatic protons at δ7.79 (1H, d, *J* = 8.2Hz, H-1) and 6.96 (1H,d, *J* = 8.2Hz, H-2). In para position were another two protons at δ7.01(1H, d, *J* = 1.6Hz, H-7) and 6.97(1H, d, *J* = 1.6Hz, H-10). In the ^{1}H-NMR spectrum of **6**, further signals were observed that showed the presence of two hydroxy, one methoxy, one 4'-hydroxy-3'-methyl-butenyl and one 3''-methyl-4''-oxo-butenyl moieties(Table 2). In the NOESY spectrum, cross peak was observed from H-4' to H-2', from H-4'' to H-2'' and from H-1 to H-2. Based on the chemical shifts and coupling constant of the ^{1}H-NMR spectrum and detailed spectral analysis of HMQC, HMBC experiments (Table 3) the complete structural elucidation of **6** was derived as 3-hydroxy-9-methoxy-4-[2'(*E*)-4'-hydroxy-3'-methylbutenyl]-

8-[2"(*E*)-3"-methyl-4"-oxo-butenyl]coumestan (**6**)(see Fig. 1).

The molecular formula of **3** indicated that it consisted of one mole each of **6** and D-glucose. The *β* configuration of the D-glucopyranosyl moiety was deduced from the coupling value of the anomeric proton signal at 4.35 ppm (J = 7.9 Hz). The position of the glycosyl moiety in **3** was decided by the comparison of ^{13}C-NMR data of **3** with those of **6** (Table 1), and the HMBC spectrum in which three-bond-coupled signal between the anomeric proton signal and carbon signal at δ = 80.1 (C-4') was observed. In conclusion, the structure of **3** was determined to be 3- hydroxy-9-methoxy-4-[2'(*E*)-3'-methyl-4'-*O-β*-D-gluopyranosylbutenyl]-8-[2"(*E*)-3"-methyl-4"-oxo-butenyl] -coumestan (**3**) (see Fig 1).

3.4 Antibacterial Assay

Compounds **1 - 6** showed antimicrobial activities against *E. coli*, *S. aureus*, and *P. vulgaris* at the concentration of 13 μg/dis (Table 4).

4. Conclusion

Picralima nitida is known as a rich source of alkaloids**.** Some of them have been reported to show biological activities (Corbett, et al. 1996; Fakeye et al. 2000; Ramirez et al. 2003; Ezeamuzie et al. 1994). However, this paper preliminarily studies the neutral constituents *of P. nitida* roots. The separated products are flavonoids. The use of the roots of *P. nitida* as anti-infective agent may be explained by the presence of antimicrobial coumestan derivatives.

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Notes

Note 1. In memoriam

Note 2. The reason of this acidic treatment was to isolate and identify separately the nature of sugar and aglycone moieties.

Table 1. The ^{13}C-NMR (δ_c in ppm, 125MHz) Data of Compounds **1 - 6**

Position	Compounds					
	1	**2**	**3**	**4**	**5**	**6**
	δ_c(DEPT)	δ_c(DEPT)	δ_c(DEPT)	δ_c(DEPT)	δ_c (DEPT)	δ_c (DEPT)
1	121.9 (CH)	122.2 (CH)	122.2 (CH)	121.7 (CH)	122.2 (CH)	122.2 (CH)
2	111.4(C)	110.2(C)	114.1(CH)	111.2 (C)	110.3(C)	114.2(CH)
3	153.9 (C)	154.2 (C)	154.0 (C)	153.8 (C)	154.1 (C)	153.8 (C)
4	113.9(CH)	113.2(CH)	109.1(C)	113.5(CH)	113.6(CH)	109.2(C)
4a	157.5 (C)	157.4 (C)	157.4 (C)	157.4 (C)	157.4 (C)	157.3 (C)
6	163.2 (C)	163.5 (C)	163.4 (C)	163.2 (C)	163.5 (C)	163.4 (C)
6a	113.5 (C)	113.6 (C)	113.6 (C)	113.5 (C)	113.5 (C)	113.5 (C)
6b	118.4(C)	118.5(C)	118.5(C)	118.2(C)	118.4(C)	118.5(C)
7	123.7(CH)	123.4(CH)	123.5(CH)	123.5(CH)	123.4(CH)	123.4(CH)
8	122.0(C)	121.7(C)	121.7(C)	121.9(C)	121.6(C)	121.8(C)
9	152.6(C)	152.8(C)	152.8(C)	152.5(C)	152.8(C)	152.8(C)
10	99.1(CH)	98.9(CH)	98.8(CH)	99.2(CH)	98.8(CH)	98.6(CH)
10a	157.9(C)	158.1(C)	158.0(C)	157.8(C)	158.0(C)	157.8(C)
11a	160.0 (C)	159.8 (C)	159.8 (C)	159.9 (C)	159.8 (C)	159.7 (C)
11b	103.9 (C)	104.1 (C)	104.0 (C)	103.9 (C)	104.2 (C)	103.9 (C)
1'	30.5 (CH$_2$)	30.5 (CH$_2$)	30.3 (CH$_2$)	30.4(CH$_2$)	30.4 (CH$_2$)	30.1(CH$_2$)
2'	122.9(CH)	123.3 (CH)	123.1 (CH)	122.8(CH)	123.3 (CH)	123.1 (CH)
3'	131.0(C)	130.9(C)	130.9(C)	130.9(C)	130.8(C)	130.8(C)
4'	80.4(CH$_2$)	80.5(CH$_2$)	80.1(CH$_2$)	80.(CH$_2$)	78.2(CH$_2$)	77.9(CH$_2$)
5'	25.8(CH$_3$)	25.6(CH$_3$)	25.4(CH$_3$)	25.7(CH3)	25.5(CH$_3$)	25.3(CH$_3$)
9-OCH$_3$	56.5 (CH$_3$)	56.6(CH$_3$)	56.6(CH$_3$)	56.7(CH$_3$)	56.5(CH$_3$)	56.4(CH$_3$)
1''	29.8(CH$_2$)	30.1(CH$_2$)	30.1(CH$_2$)	30.1(CH$_2$)	30.0(CH$_2$)	30.1(CH$_2$)
2''	122.7(CH)	123.0(CH)	123.0(CH)	123.0(CH)	123.0(CH)	123.0(CH)
3''	130.1(C)	130.4(C)	130.5(C)	130.4(C)	130.4(C)	130.4(C)
4''	18.2(CH$_3$)	204.8(CH)	204.8(CH)	18.1(CH$_3$)	204.7(CH)	204.7(CH)
5''	25.3(CH$_3$)	25.4(CH$_3$)	25.3(CH$_3$)	25.4(CH$_3$)	25.4(CH$_3$)	25.3(CH$_3$)
1'''	100.1(CH)	101.8(CH)	100.7(CH)			
2'''	73.6(CH)	74.5(CH)	74.5(CH)			
3'''	76.9(CH)	77.9(CH)	78.0(CH)			
4'''	70.2(CH)	71.3(CH)	71.2(CH)			
5'''	76.7(CH)	77.6(CH)	77.5(CH)			
6'''	61.3(CH$_2$)	63.5(CH$_2$)	63.4(CH$_2$)			

Table 2. The ^{1}H-NMR (δ_H in ppm, 500MHz) Data of Compounds **1 - 6**

Position	Compounds					
	1	**2**	**3**	**4**	**5**	**6**
	δ_H (mult, J)	δ_H (mult, J)	δ_H (mult, J)	δ_H (mult, J)	δ_H (mult, J)	δ_H (mult, J)
1	7.77 (1H,d, 1.2)	7.79 (1H,d, 1.5)	7.80 (1H,d, 8.1)	7.76 (1H,d, 1.2)	7.78 (1H,d, 1.4)	7.79 (1H,d, 8.2)
2			6.97 (1H, d, 8.1)			6.96 (1H,d, 8.2)
4	6.95 (1H, d, 1.2)	6.96 (1H, d, 1.5)		6.96 (1H, d, 1.2)	6.95 (1H, d, 1.4)	
7	7.04(1H, d, 1.6)	7.03(1H, d, 1.7)	7.03(1H, d, 1.8)	7.02(1H, d, 1.7)	7.03(1H, d, 1.6)	7.01(1H, d, 1.6)
10	6.98(1H, d, 1.6)	6.97(1H, d, 1.7)	6.98(1H, d, 1.8)	6.97(1H, d, 1.7)	6.98(1H, d, 1.6)	6.97(1H, d, 1.6)
1'	3.15 (2H, d, 8.1)	3.15 (2H, d, 8.2)	3.14 (2H, d, 8.2)	3.13 (2H, d, 8.0)	3.14 (2H, d, 8.0)	3.14 (2H,d, 8.2)
2'	5.10 (1H, t, 8.1)	5.12 (1H, t, 8.2)	5.11 (1H, t, 8.2)	5.09 (1H, t, 8.0)	5.10 (1H, t, 8.0)	5.11(1H,t, 8.2)
4'	5.80 (2H, s)	5.82 (2H, s)	5.81 (2H, s)	4.55 (2H, s)	4.54 (2H, s)	4.51 (2H, s)
5'	1.78 (3H, s)	1.78 (3H, s)	1.78 (3H, s)	1.78 (3H, s)	1.76 (3H, s)	1.77 (3H, s)
1"	3.20(2H, d, 7.0)	3.18(2H, d, 7.1)	3.19(2H, d, 7.0)	3.21(2H,d,6.9)	3.18(2H, d, 7.0)	3.20(2H,d,7.1)
2"	5.01(1H, t, 7.2)	5.02(1H, t, 7.1)	5.01(1H, t, 7.0)	5.02(1H,t,6.9)	5.01(1H, t, 7.0)	5.02(1H, t, 7.1)
4"	1.82 (3H, s)	9.04 (1H, s)	9.01 (1H, s)	1.81 (3H, s)	9.03 (1H, s)	9.02 (1H, s)
5"	1.78(3H, s)	1.78(3H, s)	1.78(3H, s)	1.79(3H, s)	1.78(3H, s)	1.76(3H, s)
1'''	4.81 (1H, d, 7.8)	4.34 (1H, d, 7.7)	4.35 (1H, d, 7.9)			
2'''	3.22(1H, dd, 8.6, 7.9)	3.39 (1H, dd, 8.2, 7.8)	3.37 (1H, dd, 8.4, 7.9)			
3'''	3.41 (1H, m)	3.25 (1H, m)	3.26 (1H, m)			
4'''	3.40 (1H,dd,3.5, 3.6)	3.22 (1H, dd, 7.4, 7.6)	3.22 (1H, dd, 3.4, 3.6)			
5'''	3.32 (1H, m)	3.54 (1H, m)	3.55 (1H, m)			
6'''	3.75(1H,dd,11.4, 5.6) 3.51 (1H, d, 11.4)	4.51 (1H, dd, 10.3, 5.4) 4.54 (1H, dd, 10.3, 5.1)	4.52 (1H, dd, 11.3, 5.4) 4.55 (1H, d, 11.3)			
3-OH	9.57 (1H, s)	9.58 (1H, s)	9.54 (1H, s)	9.55 (1H, s)	9.55(1H, s)	9.53 (1H, s)
9-OCH$_3$	3.98 (3H,s)	4.02(3H,s)	4.01 (3H,s)	3.99 (3H,s)	4.00 (3H,s)	4.01 (3H,s)
4'-OH	5.38 (1H, s)	5.37 (1H, s)	5.38 (1H, s)	5.34 (1H, s)	5.35 (1H, s)	5.37 (1H, s)

Table 3. The HMQC and HMBC Data of Compounds **4 - 6**

Proton	Compounds					
	4		**5**		**6**	
	HMQC	HMBC	HMQC	HMBC	HMQC	HMBC
1	C:1	C:2,11b,1',3,4a,11a	C:1	C:2,11b,1',3,4a,11a	C:1	C:2,11b,3,4a,11a
2					C:2	C:1,3, 4,11b
4	C:4	C:3,4a,1',2,11b	C:4	C:3,4a,1',2,11b		
7	C:7	C:6b:8,6a,1",10a,9	C:7	C:6b,8,6a,1",10a,9	C:7	C:6b,8,6a,1",10a,9
10	C:10	C:9,10a,8,6b	C:10	C:9,10a,8,6b	C:10	C:9,10a,8,6b
1'	C:1'	C:2,2',3,1,3'	C:1'	C:2,2',3,1,3'	C:1'	C:4,2',3,4a,3'
2'	C:2'	C:1',3',4',5',2	C:2'	C:1',3',4',5',2	C:2'	C:1',3',4',5',4
4'	C:4'	C:3',5',2'	C:4'	C:3',5',2'	C:4'	C:3',5',2'
5'	C:5'	C:3',4',2'	C:5'	C:3',4',2'	C:5'	C:3',4',2'
1"	C:1"	C:8,2",7,9,3"	C:1"	C:8,2",7,9,3"	C:1"	C:8,2",7,9,3"
2"	C:2"	C:1",3",4",5",8	C:2"	C:1",3",4",5",8	C:2"	C:1",3",4",5",8
4"	C:4"	C:3",5",2"	C:4"	C:3",5",2"	C:4"	C:3",5",2"
5"	C:5"	C:3",4",2"	C:5"	C:3",4",2"	C:5"	C:3",4",2"

Table 4. Results of Antibacterial Activities of Compounds **1-6**

|	Zone of inhibition in mm		
Compounds	*E. coli*	*S. aureus*	*P. vulgaris*
1	11.5	13.8	12.7
2	15.4	14.6	11.5
3	14.5	14.8	12.8
4	10.8	12.7	10.8
5	12.8	13.7	10.8
6	12.7	12.9	10.9
Positive control	Amikacin 16.8	Vancomycin 15.5	Penicilin 13.4
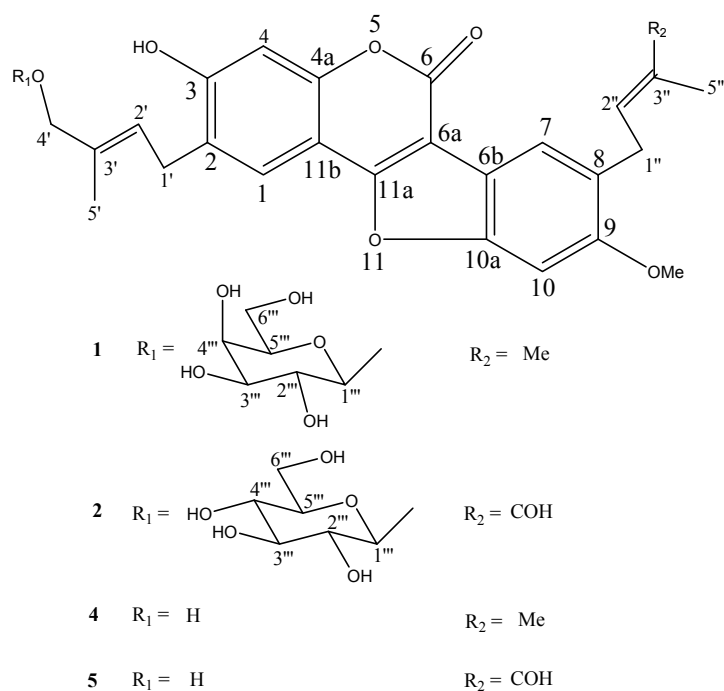
Figure 1. Structures of Compounds **1**, **2**, **4** and **5**
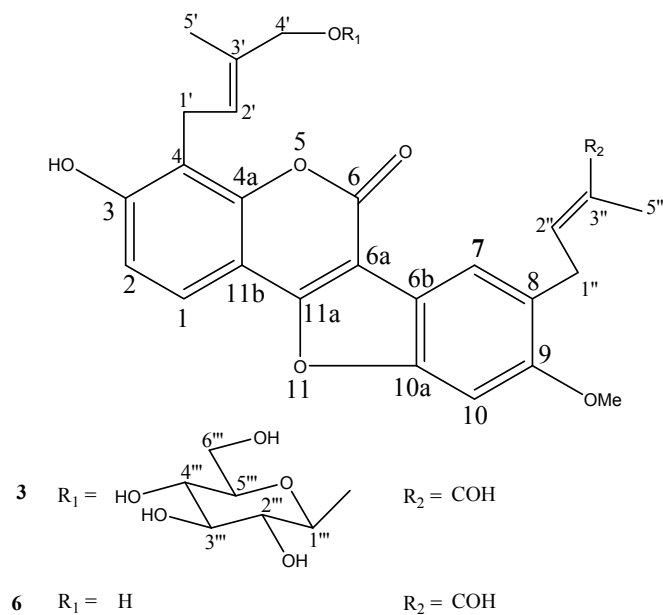
Figure 2. Structures of Compounds **3** and **6**