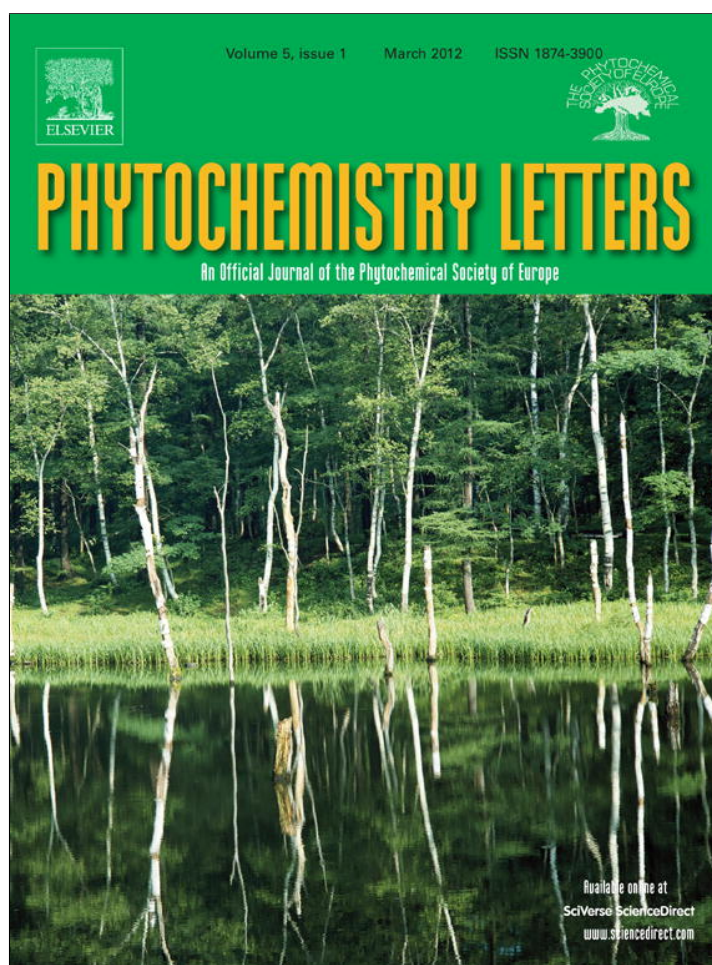


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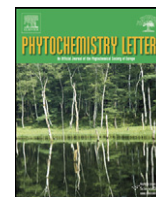
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Phytochemistry Letters

journal homepage: www.elsevier.com/locate/phytolFour isoflavanones from the stem bark of *Platyclaphium voëense*Ivan Gumula^{a,b}, Matthias Heydenreich^c, Solomon Derese^a, Isaiah O. Ndiege^b, Abiy Yenesew^{a,*}^a Department of Chemistry, University of Nairobi, P.O. Box 30197-00100, Nairobi, Kenya^b Department of Chemistry, Kyambogo University, P.O. Box 1, Kyambogo, Kampala, Uganda^c Institut für Chemie, Universität Potsdam, P.O. Box 60 15 53, D-14415, Potsdam, Germany

ARTICLE INFO

Article history:

Received 29 October 2011

Received in revised form 26 November 2011

Accepted 28 November 2011

Available online 9 December 2011

Keywords:

Platyclaphium voëense

Stem bark

Leguminosae

Isoflavanones

Platyisoflavanone

Mycobacterium tuberculosis

ABSTRACT

From the stem bark of *Platyclaphium voëense* (Leguminosae) four new isoflavanones were isolated and characterized as (S)-5,7-dihydroxy-2',4'-dimethoxy-3'-(3"-methylbut-2"-enyl)-isoflavanone (trivial name platyisoflavanone A), (±)-5,7,2'-trihydroxy-4'-methoxy-3'-(3"-methylbut-2"-enyl)-isoflavanone (platyisoflavanone B), 5,7-dihydroxy-4'-methoxy-2''-(2'''-hydroxyisopropyl)-dihydrofurano-[4'',5'':3',2']-isoflavanone (platyisoflavanone C) and 5,7,2',3''-tetrahydroxy-2'',2''-dimethyldihydropyrano-[5'',6'':3',4']-isoflavanone (platyisoflavanone D). In addition, the known isoflavanones, sophoraisoflavanone A and glyasperin F; the isoflavone, formononetin; two flavones, kumatakenin and isokaempferide; as well as two triterpenes, betulin and β-amyrin were identified. The structures were elucidated on the basis of spectroscopic evidence. Platyisoflavanone A showed antibacterial activity against *Mycobacterium tuberculosis* in the microplate alamar blue assay (MABA) with MIC = 23.7 μM, but also showed cytotoxicity (IC₅₀ = 21.1 μM) in the vero cell test.

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1. Introduction

Platyclaphium (family Leguminosae, sub-family Papilionoideae, tribe Sophoreae) is a monotypic genus that occurs in the drier parts of Eastern Africa, particularly in Kenya, Ethiopia, Somalia and Tanzania (Gillett et al., 1971). Sophoreae, containing genera of least specialization and diverse morphological features, has been described as “a tribe of convenience” (Gillett et al., 1971; Polhill, 1981). The tribe is considered to be transitional between the subfamilies Papilionoideae and Caesalpinoideae (Bentham, 1841) and DNA sequencing studies have shown that Sophoreae needs taxonomic realignment (Crisp et al., 2000; Doyle et al., 2000; Käss and Wink, 1995; Pennington et al., 2001). From morphological point of view the genus *Platyclaphium* is closely related to the genera *Dicraeopetalum* and *Bolusanthus* all belonging to the Sophora group within the Sophoreae tribe (Polhill, 1994). Whereas, there is some phytochemical information on the genus *Bolusanthus* (Asres et al., 1985; Bojase et al., 2001a,b), the information available on *Platyclaphium voëense* (Asres et al., 1997b; Van Wyk et al., 1993) and *Dicraeopetalum* (Asres et al., 1997a; Van Wyk et al., 1993) is limited to the identification of quinolizidine alkaloids through GC–MS analysis of the leaves and twigs of plants from the two genera. Quinolizidine alkaloids have also been reported from *Bolusanthus* (Asres et al., 1986). With

interest to see if phytochemical information supports the close association among these genera in the Sophora group within the Sophoreae tribe, the stem bark of *P. voëense* was investigated. This paper describes the isolation and characterization of four new prenylated isoflavanones along with seven known compounds (two isoflavanones, an isoflavone, two 3-methoxyflavones and two triterpenes).

2. Results and discussion

Column chromatography of the CH₂Cl₂–MeOH (1:1) extract of the stem bark of *P. voëense*, using n-hexane containing increasing amounts of ethyl acetate as the eluent and subsequent purification of the fractions, resulted in the isolation of eleven compounds including four new isoflavanones, **1–4** (Fig. 1).

Compound **1**, obtained as a white amorphous solid, showed a [M]⁺ at *m/z* 384.1597 in the HREI-mass spectrum suggesting a molecular formula of C₂₂H₂₄O₆. The presence of an isoflavanone skeleton was deduced from UV (λ_{max} 288 nm), ¹H (δ 4.48, *dd*, *J* = –11.1, 11.2 Hz, H-2_{ax}; δ 4.66, *dd*, *J* = –11.1, 5.6 Hz, H-2_{eq}; δ 4.38, *dd*, *J* = 11.2, 5.6 Hz, H-3_{ax}) and ¹³C (δ 71.6 for C-2; 45.9 for C-3 and 198.2 for C-4) NMR spectra. The ¹H NMR spectrum further revealed the presence of two methoxyl (δ 3.71 and 3.80), a chelated hydroxyl (δ 12.18) at C-5 as well as a 3-methylbut-2-enyl moiety (Table 1).

Two *meta*-coupled doublets at δ 5.95 and 5.97 (*J* = 2.0 Hz) were attributable to H-8 and H-6 implying that C-5 and C-7 of A-ring are oxygenated as expected from biogenetic point of view. In the

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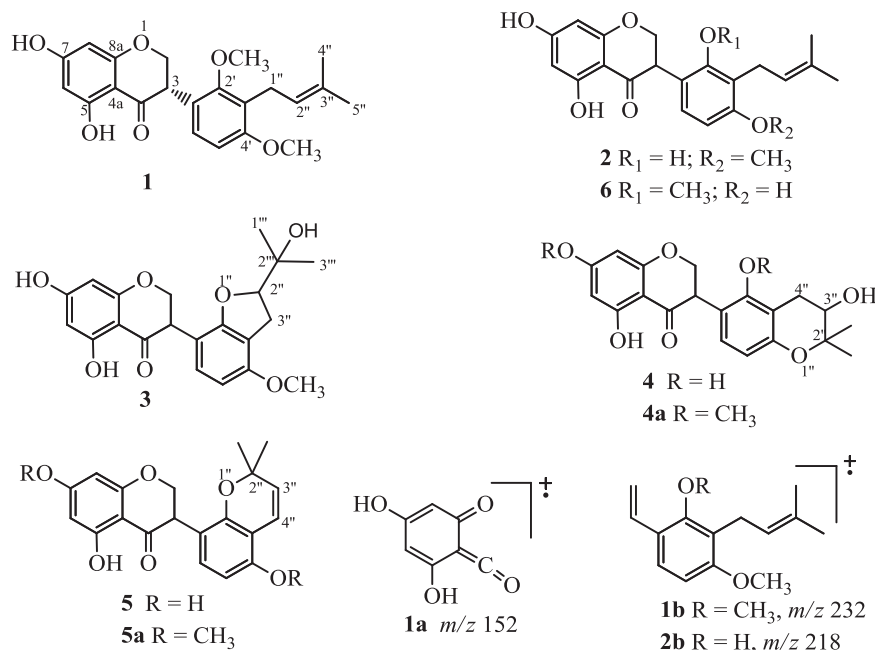


Fig. 1. Structures of compounds 1–6 and RDA fragment ions in the EI-mass spectra of compounds 1 and 2.

Table 1

¹H NMR (600 MHz) spectral data for compounds 1–5.

Position	$\delta_{\text{H}}J$ (in Hz)				
	a 1	a 2	b 3	b 4	b 5
2	4.48 ^c <i>dd</i> (–11.1, 11.2) 4.46 ^c <i>dd</i> (–11.1, 5.6)	4.62 <i>dd</i> (11.6, 5.0) 4.76 <i>dd</i> (11.6, 6.4)	4.34 <i>dd</i> (11.0, 5.7) 4.42 <i>dd</i> (11.2, 11.2)	4.56 <i>m</i> /4.57 <i>m</i> 4.69 <i>m</i> /4.68 <i>m</i>	4.41 <i>dd</i> (10.9, 5.6) 4.55 <i>dd</i> (11.1, 11.1)
3	4.38 <i>dd</i> (11.2, 5.6)	4.08 <i>t</i> (5.7)	4.06 <i>dd</i> (11.4, 5.6)	4.23 <i>m</i> /4.22 <i>m</i>	4.19 <i>dd</i> (11.1, 5.7)
5-OH	12.18 <i>s</i>	11.87 <i>s</i>	12.15 <i>s</i>	12.18 <i>s</i> /12.17 <i>s</i>	12.40 <i>s</i>
6	5.97 <i>d</i> (2.3)	5.95 <i>m</i>	5.84 <i>d</i> (2.2)	5.96 <i>m</i>	5.98 <i>d</i> (2.2)
7	–	–	–	–	–
8	5.95 <i>d</i> (2.3)	5.95 <i>m</i>	5.82 <i>d</i> (2.2)	5.96 <i>m</i>	5.95 <i>d</i> (2.2)
5'	6.67 <i>d</i> (8.6)	6.48 <i>d</i> (8.6)	6.32 <i>d</i> (8.4)	6.32 <i>d</i> (8.4)	6.40 <i>d</i> (8.3)
6'	6.92 <i>d</i> (8.5)	7.15 <i>d</i> (8.6)	6.83 <i>d</i> (8.4)	6.97 <i>d</i> (8.4)/6.96 <i>d</i> (8.4)	6.86 <i>d</i> (8.3)
1''	3.32 <i>dd</i> (14.3, 6.6) 3.38 <i>dd</i> (14.4, 6.8)	3.39 <i>m</i>	–	–	–
2''	5.25 <i>t</i> (6.7)	5.17 <i>t</i> (7.2)	4.52 <i>dd</i> (9.6, 7.1) 2.97 <i>dd</i> (16.0, 9.6)	–	–
3''	–	–	3.01 <i>dd</i> (16.0, 7.0)	3.78 <i>m</i>	5.63 <i>d</i> (10.0)
4''	1.77 <i>s</i>	1.79 <i>s</i>	–	2.62 <i>dd</i> (10.2, 10.2)/2.58 <i>dd</i> (10.2, 10.2) 2.98 <i>dd</i> (8.4, 6.0)/2.96 <i>dd</i> (8.4, 6.0)	6.68 <i>d</i> (9.9)
5''	1.68 <i>s</i>	1.70 <i>s</i>	–	–	–
1'''	–	–	1.03 <i>s</i>	–	–
2'''	–	–	–	–	–
3'''	–	–	1.04 <i>s</i>	–	–
2'-OCH ₃	3.71 <i>s</i>	–	–	–	–
4'-OCH ₃	3.80 <i>s</i>	3.78 <i>s</i>	3.68 <i>s</i>	–	–
2''-CH ₃	–	–	–	1.22 <i>s</i> 1.33 <i>s</i> /1.32 <i>s</i>	1.33 <i>s</i> 1.35 <i>s</i>

^a Spectra recorded in CD₂Cl₂.

^b Spectra recorded in CD₃OCD₃.

^c Multiplicity was clear after iteration according to Laatikainen et al. (1996a, 1996b).

B-ring, two *ortho*-coupled aromatic protons at δ 6.67 and 6.92 ($J = 8.6$ Hz) were assigned to H-5' and H-6', respectively, with C-2', C-3' and C-4' being substituted. The ¹³C chemical shift values (Table 2) for the B-ring carbon atoms are consistent with oxygenation at C-2' and C-4', with the 3-methylbut-2-enyl group being at C-3'. The fragment ions at *m/z* 152 (**1a**) and 232 (**1b**) (Fig. 1) in the EI-mass spectrum resulting from a typical *retro*-Diels–Alder (RDA) cleavage confirmed that the A-ring had two hydroxyl groups; and that the two methoxyl together with the 3-methylbut-2-enyl group are located on the B-ring.

In NOE experiments, the peak at δ_{H} 4.38 (H-3) was enhanced upon irradiation of the methoxyl group at δ_{H} 3.71; similarly, NOE interaction was exhibited between a signal at δ_{H} 6.67 (H-5') and the methoxyl group at δ_{H} 3.80 allowing the placement of the two methoxyl groups at C-2' and C-4'. Indeed, the down-field shift of one of the methoxyl group (δ_{C} 62.4) in the ¹³C NMR spectrum, is typical of *di-ortho* substituted methoxyl group (Park et al., 2008), confirming its placement at C-2'. The HMBC experiment revealed a long range correlation of the CH₂-1'' protons of the 3-methylbut-2-enyl moiety with C-2' and C-4', implying that the 3-methylbut-2-enyl group is

Table 2
¹³C NMR (150 MHz) spectral data of compounds **1–5a**.

Position	δ_c							
	a1	a2	b3	b4	a4a	b5	a5a	
2	71.6	70.3	71.5	71.6/71.7	71.5/71.4	71.8	70.8	
3	45.9	46.0	48.7	47.6	46.4/46.0	48.4	47.4	
4	198.2	197.7	198.6	199.1	198.0	199.0	197.8	
4a	103.8	102.6	104.2	103.8	103.8/103.7	104.4	103.8	
5	164.9	165.3	166.4	166.5	164.8	166.3	164.8	
6	96.7	96.8	97.6	97.6/97.7	95.1	97.6	95.0	
7	164.9	165.4	168.0	168.2	168.2	167.6	167.9	
8	95.5	95.4	96.3	96.4/96.3	94.1	96.3	94.1	
8a	163.9	163.7	165.3	165.2	163.6	165.2	163.6	
1'	120.5	115.5	110.6	115.4	119.6	115.5	115.1	
2'	157.9	154.1	160.6	155.3	158.14/158.12	153.1	151.6	
3'	124.2	117.4	115.7	110.7	113.8/113.7	111.0	110.9	
4'	158.9	158.3	157.8	155.0	154.2/154.1	154.3	155.4	
5'	107.2	103.7	104.7	110.6	113.4	109.1	103.3	
6'	127.4	126.2	131.4	129.0/128.9	128.4/128.2	131.9	130.4	
1''	23.7	22.7	–	–	–	–	–	
2''	123.1	122.2	91.4	78.0/77.9	76.9	77.8	76.8	
3''	131.8	134.0	29.5	70.3	69.3/69.2	129.9	129.3	
4''	18.1	17.8	–	28.3	27.3/27.2	118.4	116.8	
5''	25.9	25.7	–	–	–	–	–	
1'''	–	–	26.2	–	–	–	–	
2'''	–	–	72.6	–	–	–	–	
3'''	–	–	25.9	–	–	–	–	
7-OCH ₃	–	–	–	–	56.1	–	56.0	
2'-OCH ₃	62.4	–	–	–	61.2/61.1	–	–	
4'-OCH ₃	56.0	56.0	56.4	–	–	–	55.9	
2''-CH ₃	–	–	–	21.2/21.0	21.8	28.4	27.5	
				26.7/26.6	25.0/24.9	28.9	28.0	

^a Spectra recorded in CD₂Cl₂.^b Spectra recorded in CD₃OCD₃.

placed between the two methoxyl substituents (at C-3') as in structure **1** (Tables 1 and 2). Based on these data the new compound (**1**) was characterized as 5,7-dihydroxy-2',4'-dimethoxy-3'-(3''-methylbut-2''-enyl)-isoflavanone, hereby named platyisoflavanone A. The CD spectrum of compound **1** showed a negative Cotton effect at 344 nm ($n \rightarrow \pi^*$ transition) which was consistent with 3S absolute configuration for this isoflavanone (Slade et al., 2005).

Compound **2**, obtained as a white amorphous solid, was also identified as an isoflavanone derivative from the UV (λ_{\max} 294 nm), ¹H (Table 1) and ¹³C (Table 2) NMR spectra. The HREI-mass spectrum of the compound gave [M]⁺ at *m/z* 370.1400 corresponding to molecular formula of C₂₁H₂₂O₆. The ¹H and ¹³C NMR spectra further revealed the presence of a chelated hydroxyl (5-OH), a methoxyl and a 3-methylbut-2-enyl group. Comparison of the ¹H (Table 1) and ¹³C (Table 2) NMR data of this compound with those of **1** showed identical A-ring, while the B-ring has similar substitution pattern. In fact, the only difference between these two compounds is that **2** has only one methoxyl group. The fragment ion, in EI-MS, at *m/z* 218 (**2b**) (Fig. 1) was in agreement with the placement of the methoxyl, the 3-methylbut-2-enyl unit and one hydroxyl groups in the B-ring. The methoxyl group was within the normal range (δ_c 56.0) suggesting its placement at C-4' rather than at C-2' (Park et al., 2008). NOESY (which showed NOE interaction of the methoxyl protons with H-5') as well as HMBC (correlation of methoxy protons with C-4') spectra confirmed the placement of the methoxyl group at C-4'. Therefore this new compound was characterized as 2',5,7-trihydroxy-4'-methoxy-3'-(3''-methylbut-2''-enyl)-isoflavanone, named platyisoflavanone B. The nearly zero optical rotation together with insignificant Cotton effect in the CD spectrum revealed that the compound was isolated as a racemic mixture. Isoflavanones with free OH at C-4' and/or at C-2', are reported to undergo racemization during extraction and isolation processes (Slade et al., 2005).

Compounds **3–5**, also exhibited ¹H (Table 1) and ¹³C (Table 2) NMR spectral features of isoflavanones with identical A-ring as in **1** and **2**. The B-ring in these compounds also showed similar

substitution pattern (a C₅ substituent at C-3' and oxygenation at C-2' and C-4'), except for the cyclization of the 3-methylbut-2-enyl group at C-3' involving one of the adjacent hydroxyl groups giving rise to three different metabolites (**3–5**).

In the case of compound **3** ([M]⁺ at *m/z* 386.1354, C₂₁H₂₂O₇), the cyclization has resulted in the formation of a 2-(2-hydroxyisopropyl)-dihydrofuranyl moiety in B-ring (Kijjoo et al., 1998) as shown from the ¹H [an ABX system at δ 2.97, *dd*, *J* = -16.0, 9.6 Hz and 3.01, *dd*, *J* = -16.0, 7.0 Hz were attributed to CH₂-3''; δ 4.52, *dd*, *J* = 9.6, 7.1 Hz (H-2'') and a pair of three-proton singlets at 1.03 (H-1'') and 1.04 (H-3'')] and ¹³C [δ_c 91.4 an oxymethine carbon (C-2''); δ_c 29.5 (C-3''); δ_c 26.2 and 25.9 (C-1''' and C-3''') and δ_c 72.6 a quaternary carbon bonded to an oxygen (C-2''')] NMR spectra. Whereas one of the two oxygen groups in B-ring is involved in cyclization, the second oxygen in this ring is methylated with the corresponding methoxyl group appearing at δ_H 3.68 and δ_c 56.4 in the NMR spectra. In the NOESY spectrum, this methoxyl signal showed NOE interaction with the aromatic proton resonating at δ_H 6.32 (H-5'), allowing its placement at C-4', and hence the dihydrofuranyl ring should be between C-2' and C-3'. Furthermore, the 'normal' methoxyl resonance at δ_c 56.4 supports the placement of a methoxyl group at C-4' rather than at C-2' (Park et al., 2008). This compound was therefore characterized as 5,7-dihydroxy-4'-methoxy-2''-(2'''-hydroxyisopropyl)-dihydrofurano-[4'',5'':3',2']-isoflavanone with a trivial name platyisoflavanone C. The configuration at C-3 and C-2'' has not been established.

The fourth new isoflavanone (compound **4**), [M]⁺ at *m/z* 372.1202, C₂₀H₂₀O₇, has a 3-hydroxy-2,2-dimethyldihydropyrano moiety fused to the B-ring, as shown from ¹H (Table 1) and ¹³C (Table 2) NMR spectra. Two possible structures were considered for this compound – one in which the dihydropyrano ring is between C-2'/C-3' and the other with the dihydropyrano moiety between C-3'/C-4'. In order to decide between the two structures, compound **4** was methylated with dimethyl sulphate in the presence of potassium carbonate and acetone, at room temperature, to give a dimethylated product (**4a**) whose ¹³C NMR spectrum

(Table 2) displayed two methoxyl signals at δ_C 56.1 (7-OCH₃) and at δ_C 61.2; the latter deshielded signal, is typical of a di-ortho substituted methoxyl carbon (Park et al., 2008), and hence assigned to 2'-OCH₃. This implied that C-4', in **4a** and the parent compound **4**, is part of the pyran ring. Therefore, the isolated compound was characterized as 5,7,2',3''-tetrahydroxy-2'',2''-dimethyldihydropyrano-[5'',6'':3',4']-isoflavanone which is also new, with trivial name platyisoflavanone D. Most of the ¹H (Table 1) and ¹³C (Table 2) NMR signals appeared in duplicates indicating that this compound was isolated as a diastereomeric mixture.

Compound **5** was identified as the pyranoisoflavanone, glyasperin F, which had already been reported from the roots of *Glycrrhiza aspera*, by comparison of its spectral features (Tables 1 and 2) with published data (Zeng et al., 1992) and also by conversion to 7,4'-O, O-dimethylglyasperin F (**5a**). The ¹H NMR spectrum (Section 3.9) of compound **5a** revealed a methoxyl signal at 3.72 ppm that exhibited NOE interaction with a one-proton doublet at δ 6.34 ($J = 8.4$ Hz, H-5'), showing that the pyran ring (in both **5** and **5a**) is between C-2'/C-3' rather than between C-3'/C-4'. The presence of two methoxyl signals below δ_C 59 [δ 55.9 (C-2') and 56.0 (C-7)] in the ¹³C NMR of compound **5a** (Table 2) confirmed the placement of the pyran ring between C-2'/C-3'. The sixth isoflavanone was identified as sophoraisoflavanone A (**6**) (Komatsu et al., 1978).

It is worthy to note that the oxygenation pattern in all the six isoflavanones isolated from this plant (*P. voëense*) is identical (at C-5, -7, -2' and -4'), and each with a five-carbon unit at C-3'. Isoflavanones with this substitution pattern have also been isolated from the related genera *Bolusanthus* (Bojase et al., 2001a,b) and *Sophora* (linuma et al., 1993; Komatsu et al., 1978) supporting the placement of the three genera in the same group (Sophora group); the latter two genera also elaborate isoflavanones with different oxygenation and prenylation patterns. It will be interesting to find out if the related genus *Dicraeopetalum* also elaborate isoflavanones, possibly with the same oxygenation pattern as the isoflavanones obtained from *P. voëense*.

Other known compounds identified included the 3-methoxyflavones, kumatakenin (Valesi et al., 1972) and isokaempferide (Yang et al., 1995); the isoflavone, formononetin (Balasubramanian and Nair, 2000); and triterpenes, betulin (Siddiqui et al., 1988) and β -amyirin (Bahato and Kundu, 1994). Neither flavonoids nor terpenoids had, prior to this paper, been reported from this plant.

Compound **1** exhibited moderate in vitro anti-TB activity against *Mycobacterium tuberculosis* in the microplate alamar blue assay (MABA, MIC value of 23.7 μ M) and weak activity in the low-oxygen-recovery assay (LORA, MIC = 92.2 μ M). However, this compound also showed cytotoxicity (IC₅₀ = 21.1 μ M) in the vero cell test. Compounds **5** and **6** were inactive against TB in the two tests but showed moderate cytotoxicity in the vero cell test, IC₅₀ = 88.3 μ M for **5** and IC₅₀ = 50.3 μ M for **6**. The rest of the compounds were not tested.

3. Experimental

3.1. General

Analytical TLC: Merck pre-coated silica gel 60 F₂₅₄ plates. CC and MPLC were carried out on silica gel 60 (70–230 mesh). Gel filtration on Sephadex LH-20. UV spectra were recorded on a Specord S600, Analytik Jena AG, Germany. CD spectra were recorded on JASCO J-710 Spectropolarimeter. EI-MS: direct inlet, 70 eV on Micromass GC-TOFmicro mass spectrometer (Micromass, Wythenshawe, Waters Inc., UK). ¹H NMR (600 MHz) and ¹³C NMR (150 MHz) were recorded on a Bruker Avance 600 Spectrometer using the residual solvent peaks as reference. For compound **2** ¹³C NMR (50 MHz) was recorded on a Varian 200 Spectrometer. HSQC and HMBC spectra were acquired using the standard Bruker

software. The PERCH Program (PERCH solutions Ltd., Kuopio, Finland; Laatikainen et al., 1996a,b) was used for iteration of the ABX spin system of compound **1**.

3.2. Plant material

The stem of *P. voëense* was collected from Mwingi District, Eastern Province, Kenya, in January 2009. The plant was identified at the National Museums of Kenya East African Herbarium, Nairobi, where a voucher specimen is deposited (voucher No. Mathenge-2009/568).

3.3. Extraction and isolation

The air-dried stem bark (1.6 kg) of *P. voëense* was pulverized and extracted twice with CH₂Cl₂–MeOH (1:1) at room temperature for 48 h. Evaporation of the solvent afforded brown gummy extract (114 g). A 110 g portion of the extract was subjected to CC on silica gel, using increasing amounts of EtOAc in n-hexane as the eluate, resulting in 10 fractions, each ca. 5.0 L volume-elution.

Fraction 2, eluted with 1% EtOAc in n-hexane, afforded β -amyirin (21 mg). Fraction 3, eluted with 4% EtOAc in n-hexane, contained a mixture of four major compounds which was separated by CC on silica gel (solvent, 0–15% acetone in n-hexane) to give **1** (210 mg), **2** (37 mg), **6** (31 mg) and **5** (36 mg), while fraction 3 eluted with 5% EtOAc was subjected to CC on silica gel (0–30% EtOAc in n-hexane) followed by Sephadex LH-20 (CH₂Cl₂:MeOH, 1:1) yielded betulin (28 mg). Fraction 4 (5% EtOAc in n-hexane) was subjected to Medium Pressure Liquid Chromatography (MPLC, solvent: 0–5% acetone in n-hexane; flow rate: 30 ml/min) to give kumatakenin (29 mg). Fraction 5, obtained after elution with 15–20% EtOAc in n-hexane, was subjected to MPLC (solvent: 0–15% EtOAc in n-hexane; press: 30 ml/min) to give formononetin (10 mg), **3** (40 mg), **4** (15 mg), **5** (25 mg) and isokaempferide (6 mg).

3.4. (S)-Platyisoflavanone A (1)

Amorphous powder, m.p. 160–164 °C, [α]_D^{24.0} = +14.1 (CH₂Cl₂, c = 1%, w/v). UV: λ_{\max} (CH₂Cl₂) 288 nm. CD (CH₂Cl₂, c 0.0047): [θ]₃₅₀ –100, [θ]₃₄₄ –120, [θ]₃₃₂ 0, [θ]₃₁₅ +490, [θ]₂₉₄ 0, [θ]₂₈₅ –340, [θ]₂₅₅ 0, [θ]₂₄₅ +50. ¹H NMR (Table 1). ¹³C NMR (Table 2). EI-MS m/z (rel. int.): 384 (80, [M]⁺), 232 (100), 217 (69), 205 (83), 189 (47), 177 (54), 152 (65), 124 (95), 115 (30), 91 (32), 69 (32), 51 (53), 45 (54). HREI-MS [M]⁺: found m/z 384.1597 for C₂₂H₂₄O₆ (calcd. 384.1567).

3.5. (±)-Platyisoflavanone B (2)

Amorphous white powder, m.p. 133–138 °C. UV: λ_{\max} (CH₂Cl₂) 288 nm. [α]_D^{24.0} = 0 (CH₂Cl₂, c = 1%, w/v), UV: λ_{\max} (CH₂Cl₂) 294 nm; CD (CH₂Cl₂, c 0.0046): no Cotton effect. ¹H NMR (Table 1). ¹³C NMR (Table 2). EI-MS m/z (rel. int.): 370 (92, [M]⁺), 314 (40), 218 (100), 163 (65), 162 (70), 153 (43), 51 (32). HREI-MS [M]⁺: found m/z 370.1400 C₂₁H₂₂O₆ (calcd. 370.1411).

3.6. Platyisoflavanone C (3)

Amorphous white solid. UV: λ_{\max} (CH₂Cl₂) 290 nm. [α]_D^{24.0} = 0 (CH₂Cl₂, c = 1%, w/v). ¹H NMR (Table 1). ¹³C NMR (Table 2). EI-MS m/z (rel. int.): 386 (10, [M]⁺), 328 (100), 234 (8), 201 (14), 180 (30), 176 (60), 161 (25), 83 (26), 59 (28). HREI-MS [M]⁺: found m/z 386.1354 C₂₁H₂₂O₇ (calcd. mass 386.1366).

3.7. Platyisoflavanone D (4)

Amorphous white solid. UV: λ_{\max} (CH₂Cl₂) 289 nm. ¹H NMR (Table 1). ¹³C NMR (Table 2). EI-MS m/z (rel. int.): 372 (60, [M]⁺),

220 (48), 187 (45), 153 (86), 149 (46), 124 (28), 109 (21), 91 (30), 69 (50), 46 (100). HREI-MS [M]⁺: found *m/z* 372.1202 for C₂₀H₂₀O₇ (calcd. 372.1209).

3.8. 7,2'-O,O-Dimethylplatyisoflavanone D (4a)

Amorphous white solid. UV: λ_{max} (CH₂Cl₂) 295 nm. ¹H NMR (600 MHz, CD₂Cl₂): δ 12.23 (1H, s, 5-OH), 6.79 (1H, d, *J* = 8.4 Hz, H-6'), 6.51 (1H, d, *J* = 8.4 Hz, H-5'), 5.98 (1H, d, *J* = 1.8 Hz, H-6), 5.95 (1H, m, H-8), 4.38 (1H, m, H-2b), 4.37 (1H, m, H-2a), 4.24 (1H, dd, *J* = 10.8 and 6.0 Hz, H-3), 3.75 (3H, s, 7-OCH₃), 3.73 (1H, m, H-3''), 3.65 (3H, s, 2'-OCH₃), 2.93 (1H, dd, *J* = 11.4 and 11.4 Hz, H-4'a), 2.68 (1H, m, H-4'b), 1.25 (3H, s, 2''-CH₃), 1.24 (3H, s, 2''-CH₃). ¹³C NMR (Table 2). EI-MS *m/z* (rel. int.): 400 (35, [M]⁺), 235 (15), 234 (100), 201 (15), 163 (25), 149 (13), 91 (10). HREI-MS [M]⁺: found *m/z* 400.1510 for C₂₂H₂₄O₇ (calcd. 400.1522).

3.9. 7,4'-O,O-Dimethylglyasperin F (5a)

Amorphous white solid. ¹H NMR (600 MHz, CD₂Cl₂): δ 12.17 (1H, s, 5-OH), 6.79/6.78 (1H, d, *J* = 8.4 Hz, H-6'), 6.55 (1H, d, *J* = 10.2 Hz, H-4''), 6.34 (1H, d, *J* = 8.4 Hz, H-5'), 5.98 (1H, m, H-6), 5.92 (1H, m, H-8), 4.37 (2H, m, H-2), 4.26/4.23 (1H, dd, *J* = 10.7 and 6.3 Hz, H-3), 3.74 (3H, s, 7-OCH₃), 3.72 (1H, dd, *J* = 10.8 and 5.4 Hz, H-3''), 3.66/3.65 (3H, s, 2'-OCH₃), 2.94/2.92 (1H, dd, *J* = 17.4 and 4.8 Hz, H-4'a), 2.69/2.66 (1H, dd, *J* = 17.4 and 5.7 Hz, H-4'b), 1.25 (3H, s, 2''-CH₃), 1.24 (3H, s, 2''-CH₃). ¹³C NMR (Table 2). EI-MS *m/z* (rel. int.): 382 (27, [M]⁺), 368 (35), 367 (100), 201 (68), 186 (24), 45 (25), 43 (37). HREI-MS [M]⁺: found *m/z* 382.1400 for C₂₂H₂₂O₆ (calcd. 382.1416).

3.10. Methylation of platyisoflavanone D (4) and glyasperin F (5)

Five drops of dimethyl sulphate and K₂CO₃ (300 mg) were added to compound **4** (10 mg) in acetone (6 ml) and stirred at room temperature for 24 h. The product was filtered, concentrated and purified by prep. TLC (solvent: 25% EtOAc in n-hexane) to afford compound **4a** (5 mg, 62%). Similarly, compound **5** (10 mg) was methylated using the same conditions and the product purified on prep. TLC (solvent: 40% EtOAc in n-hexane) to give compound **5a** (5.6 mg, 52%).

3.11. Anti-TB assays

The MICs of test samples against *M. tuberculosis* were determined by the microplate alamar blue assay (MABA) as described by Falzari et al. (2005) and by the low-oxygen-recovery assay (LORA) as described by Cho et al. (2007). Rifampicin was used as a standard drug with MIC values of 0.06 and 1.8 μM in the MABA and LORA tests, respectively.

3.12. Cytotoxicity test

The cytotoxicity test was carried on the vero cells as described by Falzari et al. (2005). Rifampicin did not show significant cytotoxicity (IC₅₀ value 183 μM).

Acknowledgements

One of the authors (I.G.) is grateful to the German Academic Exchange Services (DAAD) for a Scholarship that was awarded through the Natural Products Research Network for Eastern and Central Africa (NAPRECA). Mr. S.G. Mathenge is acknowledged for identification of the plant. We thank Mr. Klaus Gast for recording the CD spectra. Dr. Joshua Odingo, of the Infectious Disease

Institute, Seattle, Washington, USA, is acknowledged for facilitating the anti-TB and cytotoxicity tests.

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