Phytochemistry Letters 5 (2012) 150–154

Contents lists available at ScienceDirect

Phytochemistry Letters

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

Four isoflavanones from the stem bark of *Platycelphium voënse*

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:
Platycelphium voënse
Stem bark
Leguminosae
Isoflavanones
Platyisoflavanone
Mycobacterium tuberculosis

ABSTRACT

From the stem bark of *Platycelphium voënse* (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 (IC_{50} = 21.1 μM) in the vero cell test.

1. Introduction

Platycelphium (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 *Platycelphium* 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 *Platycelphium voënse* (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. voënse* 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 CH_2Cl_2–MeOH (1:1) extract of the stem bark of *P. voënse*, 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 $C_{22}H_{24}O_6$. 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 ^{13}C (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

* Corresponding author. Tel.: +254 733 832576; fax: +254 204 446138.
E-mail address: ayenesew@uonbi.ac.ke (A. Yenesew).

1874-3900/$ – see front matter © 2011 Phytochemical Society of Europe. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.phytol.2011.11.012

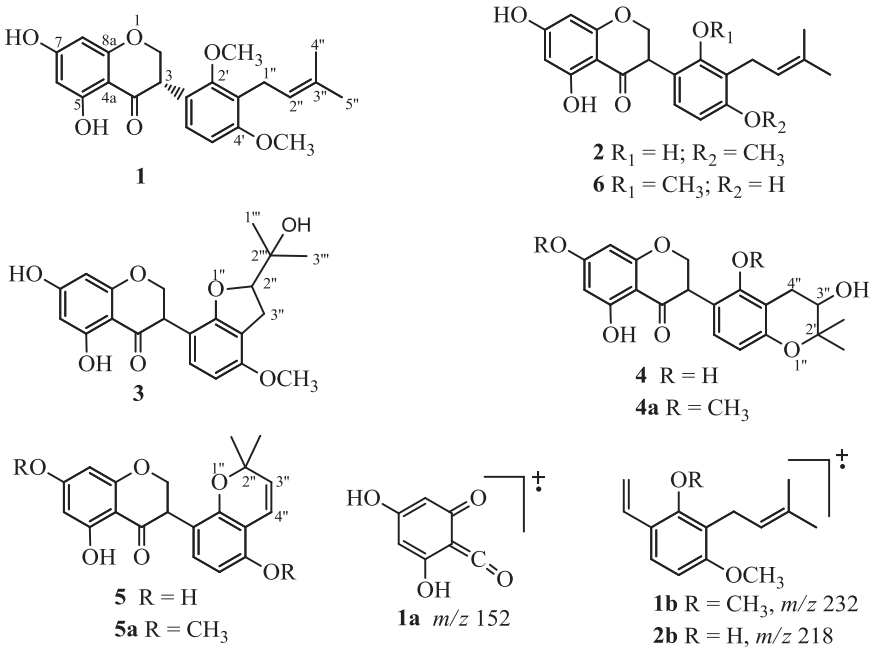

Fig. 1. Structures of compounds **1–6** and RDA fragment ions in the EI-mass spectra of compounds **1** and **2**.

Table 1
^{1}H NMR (600 MHz) spectral data for compounds **1–5**.

Position	δ_H *J* (in Hz)				
	[a]**1**	[a]**2**	[b]**3**	[b]**4**	[b]**5**
2	4.48[c] *dd* (−11.1, 11.2) 4.46[c] *dd* (−11.1, 5.6)	4.62 *dd* (11.6, 5.0) 4.76 *dd* (11.6, 6.4)	4.34 *dd* (11.0, 5.7) 4.42 *dd* (11.2, 11.2)	4.56 *m*/4.57 *m* 4.69 *m*/4.68 *m*	4.41 *dd* (10.9, 5.6) 4.55 *dd* (11.1, 11.1)
3	4.38 *dd* (11.2, 5.6)	4.08 *t* (5.7)	4.06 *dd* (11.4, 5.6)	4.23 *m*/4.22 *m*	4.19 *dd* (11.1, 5.7)
5-OH	12.18 *s*	11.87 *s*	12.15 *s*	12.18 *s*/12.17 *s*	12.40 *s*
6	5.97 *d* (2.3)	5.95 *m*	5.84 *d* (2.2)	5.96 *m*	5.98 *d* (2.2)
7	–	–	–	–	–
8	5.95 *d* (2.3)	5.95 *m*	5.82 *d* (2.2)	5.96 *m*	5.95 *d* (2.2)
5′	6.67 *d* (8.6)	6.48 *d* (8.6)	6.32 *d* (8.4)	6.32 *d* (8.4)	6.40 *d* (8.3)
6′	6.92 *d* (8.5)	7.15 *d* (8.6)	6.83 *d* (8.4)	6.97 *d* (8.4)/6.96 *d* (8.4)	6.86 *d* (8.3)
1″	3.32 *dd* (14.3, 6.6) 3.38 *dd* (14.4, 6.8)	3.39 *m*	–	–	–
2″	5.25 *t* (6.7)	5.17 *t* (7.2)	4.52 *dd* (9.6, 7.1)	–	–
3″	–	–	2.97 *dd* (16.0, 9.6) 3.01 *dd* (16.0, 7.0)	3.78 *m*	5.63 *d* (10.0)
4″	1.77 *s*	1.79 *s*	–	2.62 *dd* (10.2, 10.2)/2.58 *dd* (10.2, 10.2) 2.98 *dd* (8.4, 6.0)/2.96 *dd* (8.4, 6.0)	6.68 *d* (9.9)
5″	1.68 *s*	1.70 *s*	–	–	–
1‴	–	–	1.03 *s*	–	–
2‴	–	–	–	–	–
3‴	–	–	1.04 *s*	–	–
2′-OCH_3	3.71 *s*	–	–	–	–
4′-OCH_3	3.80 *s*	3.78 *s*	3.68 *s*	–	–
2″-CH_3	–	–	–	1.22 *s* 1.33 *s*/1.32 *s*	1.33 *s* 1.35 *s*

[a] Spectra recorded in CD_2Cl_2.
[b] Spectra recorded in CD_3OCD_3.
[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 ^{13}C 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 ^{13}C 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 CH_2-1″ 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
^{13}C NMR (150 MHz) spectral data of compounds **1**–**5a**.

Position	δ_C						
	[a]**1**	[a]**2**	[b]**3**	[b]**4**	[a]**4a**	[b]**5**	[a]**5a**
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 CD_2Cl_2.
[b] Spectra recorded in CD_3OCD_3.

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 3*S* 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 ^{13}C (Table 2) NMR spectra. The HREI-mass spectrum of the compound gave $[M]^+$ at *m*/*z* 370.1400 corresponding to molecular formula of $C_{21}H_{22}O_6$. The ^1H and ^{13}C 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 ^{13}C (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 ^{13}C (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 C_5 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, $C_{21}H_{22}O_7$), the cyclization has resulted in the formation of a 2-(2-hydroxyisopropyl)-dihydrofuranyl moiety in B-ring (Kijjoa 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 CH_2-3″; 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 ^{13}C [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, $C_{20}H_{20}O_7$, has a 3-hydroxy-2,2-dimethyldihydropyrano moiety fused to the B-ring, as shown from ^1H (Table 1) and ^{13}C (Table 2) NMR spectra. Two possible structures were considered for this compound – one in which the dihydropyran 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 ^{13}C NMR spectrum

(Table 2) displayed two methoxyl signals at δ_C 56.1 (7-OCH_3) 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′-OCH_3. 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 ^{1}H (Table 1) and ^{13}C (Table 2) NMR signals appeared in duplicates indicating that this compound was isolated as a diasteriomeric mixture.

Compound **5** was identified as the pyranoisoflavanone, glyasperin F, which had already been reported from the roots of *Glyccrhiza 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 ^{1}H 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 ^{13}C 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. voënse*) 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* (Iinuma 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. voënse*.

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-amyrin (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 μM) and weak activity in the low-oxygen-recovery assay (LORA, MIC = 92.2 μM). However, this compound also showed cytotoxicity (IC_{50} = 21.1 μM) 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, IC_{50} = 88.3 μM for **5** and IC_{50} = 50.3 μM for **6**. The rest of the compounds were not tested.

3. Experimental

3.1. General

Analytical TLC: Merck pre-coated silica gel 60 F_{254} 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). ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) were recorded on a Bruker Avance 600 Spectrometer using the residual solvent peaks as reference. For compound **2** ^{13}C 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. voënse* 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. voënse* was pulverized and extracted twice with CH_2Cl_2–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-amyrin (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 (CH_2Cl_2: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, $[\alpha]_D^{24.0} = +14.1$ (CH_2Cl_2, c = 1%, w/v). UV: λ_{max} (CH_2Cl_2) 288 nm. CD (CH_2Cl_2, *c* 0.0047): $[\theta]_{350}$ −100, $[\theta]_{344}$ −120, $[\theta]_{332}$ 0, $[\theta]_{315}$ +490, $[\theta]_{294}$ 0, $[\theta]_{285}$ −340, $[\theta]_{255}$ 0, $[\theta]_{245}$ +50. ^{1}H NMR (Table 1). ^{13}C 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 $C_{22}H_{24}O_6$ (calcd. 384.1567).

3.5. (±)-Platyisoflavanone B (2)

Amorphous white powder, m.p. 133–138 °C. UV: λ_{max} (CH_2Cl_2) 288 nm. $[\alpha]_D^{24.0} = 0$ (CH_2Cl_2, $c = 1\%$, w/v), UV: λ_{max} (CH_2Cl_2) 294 nm: CD (CH_2Cl_2, *c* 0.0046): no Cotton effect. ^{1}H NMR (Table 1). ^{13}C 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 $C_{21}H_{22}O_6$ (calcd. 370.1411).

3.6. Platyisoflavanone C (3)

Amorphous white solid. UV: λ_{max} (CH_2Cl_2) 290 nm. $[\alpha]_D^{24.0} = 0$ (CH_2Cl_2, $c = 1\%$, w/v). ^{1}H NMR (Table 1). ^{13}C 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 $C_{21}H_{22}O_7$ (calcd. mass 386.1366).

3.7. Platyisoflavanone D (4)

Amorphous white solid. UV: λ_{max} (CH_2Cl_2) 289 nm. ^{1}H NMR (Table 1). ^{13}C 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 $C_{20}H_{20}O_7$ (calcd. 372.1209).

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

Amorphous white solid. UV: λ_{max} (CH_2Cl_2) 295 nm. ^1H NMR (600 MHz, CD_2Cl_2): 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-OCH_3), 3.73 (1H, m, H-3″), 3.65 (3H, s, 2′-OCH_3), 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″-CH_3), 1.24 (3H, s, 2″-CH_3). ^{13}C 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 $C_{22}H_{24}O_7$ (calcd. 400.1522).

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

Amorphous white solid. ^1H NMR (600 MHz, CD_2Cl_2): 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-OCH_3), 3.72 (1H, dd, J = 10.8 and 5.4 Hz, H-3″), 3.66/3.65 (3H, s, 2′-OCH_3), 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″-CH_3), 1.24 (3H, s, 2″-CH_3). ^{13}C 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 $C_{22}H_{22}O_6$ (calcd. 382.1416).

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

Five drops of dimethyl sulphate and K_2CO_3 (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 (IC_{50} 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.

References

Asres, K., Mascagni, P., O'Neil, M.J., Phillipson, J.D., 1985. Isoflavonoids from *Bolusanthus speciosus* (Bolus) harms Leguminosae. Z. Naturforsch. 40C, 617–620.
Asres, K., Tei, A., Wink, M., 1997a. Quinolizidine Alkaloids from East-African Legum *Dicraeopetalum* (harms). Biochem. Syst. Ecol. 25, 305–308.
Asres, K., Tei, A., Wink, M., 1997b. Quinolizidine Alkaloids of *Platycelphium voense* (Engl.) Wild (Leguminosae). Z. Naturforsch. Ser. C. 52, 129–131.
Asres, K., Phillipson, J.D., Mascagni, P., 1986. Alkaloids of *Bolusanthus speciosus*. Phytochemistry 25, 1449–1452.
Bahato, S.B., Kundu, A.P., 1994. ^{13}C NMR spectra of pentacyclic triterpenoids: a compilation and some salient features. Phytochemistry 37, 1517–1575.
Balasubramanian, S., Nair, M.G., 2000. One-pot synthesis of isoflavones. Synth. Commun. 30, 469–484.
Bentham, G., 1841. Observations on the distinctive characters of the papilionaceae and caesalpinieae, suborders of Leguminosae. Hooker's J. Bot. 3, 125–133.
Bojase, G., Wanjala, C.C.W., Majinda, R.R.T., 2001a. Flavonoids from the stem bark of *Bolusanthus speciosus*. Phytochemistry 56, 837–841.
Bojase, G., Wanjala, C.C.W., Majinda, R.R.T., 2001b. Two new isoflavanoids from *Bolusanthus speciosus*. Bull. Chem. Soc. Ethiop. 15, 131–136.
Cho, S.H., Warit, S., Wan, B., Hwang, C.H., Pauli, G.F., Franzblau, S.G., 2007. Low-oxygen-recovery assay for high-throughput screening of compounds against nonreplicating *Mycobacterium tuberculosis*. Antimicrob. Agents Chemother. 51, 1380–1385.
Crisp, M.D., Gilmore, S., Van Wyk, B.-E., 2000. Molecular phylogeny of the genistoid tribes of the papilionoid legumes. In: Herendeen, P.S., Bruneau, A. (Eds.), Advances in Legume Systematics (Part 9). Royal Botanic Gardens, Kew, Richmond, pp. 249–276.
Doyle, J.J., Chaphill, J.A., Bailey, D.C., Kajita, T., 2000. Towards a comprehensive phylogeny of legumes: evidence from *rbc*L sequences and non-molecular data. In: Herendeen, P.S., Bruneau, A. (Eds.), Advances in Legume Systematics (Part 9). Royal Botanic Gardens, Kew, Richmond, pp. 1–20.
Falzari, K., Zhu, Z., Pan, D., Liu, H., Hongmanee, P., Franzblau, S.G., 2005. In vitro and *in vivo* activities of macrolide derivatives against *Mycobacterium tuberculosis*. Antimicrob. Agents Chemother. 49, 1447–1454.
Gillett, J.B., Polhill, R.M., Verdcourt, B., 1971. Flora of tropical east africa-subfamily Papilionoideae. In: Brenan, J.P.M. (Ed.), Leguminosae (Part 3). Royal Botanic Gardens, Kew, Richmond, pp. 31–60.
Iinuma, M., Ohyama, M., Tanaka, T., Hegarty, M.P., Hegarty, E., 1993. Isoflavonoids in roots of *Sophora fraseri*. Phytochemistry 34, 1654–1655.
Käss, E., Wink, M., 1995. Molecular phylogeny of the Papilionoideae (Family Legumenosae): *Rbc*L gene sequences versus chemical taxonomy. Bot. Acta 108, 149–162.
Kijjoa, A., Cidade, H.M., Gonzaleze, M.J.T.G., Afonso, C.M., Silver, A.M., Herz, W., 1998. Further Prenylflavonoids from *Artocarpus elasticus*. Phytochemistry 47, 875–878.
Komatsu, M., Yokoe, I., Shirataki, Y., 1978. Studies on the constituents of *Sophora* species XIII Constituents of the aerial parts of *Sophora tomentosa*. Chem. Pharm. Bull. 26, 3863–3870.
Laatikainen, R., Niemitz, M., Weber, U., Sundelin, J., Hassinen, T., Vepsäläinen, J., 1996a. General strategies for total-lineshape-type spectral analysis of NMR spectra using integral-transform iterator. J. Magn. Reson. A120, 1–10.
Laatikainen, R., Niemitz, M., Malaisse, W.J., Biesemans, M., Willem, R., 1996b. A computational strategy for the deconvolution of NMR spectra with multiplet structures and constraints: analysis of overlapping ^{13}C–^2H multiplets of ^{13}C enriched metabolites from cell suspensions incubated in deuterated media. Magn. Reson. Med. 36, 359–365.
Park, Y., Moon, B.-H., Lee, E., Hong, S., Lee, S., Lim, Y., 2008. The ^1H and ^{13}C NMR data of 19 methoxyflavonols derivatives. Bull. Korean Chem. Soc. 29, 81–84.
Pennington, R.T., Lavin, M., Ireland, H., Klitgaard, B., Preston, J., Hu, J.-M., 2001. Phylogenetic relationships of basal papilionoid legumes based upon sequences of the chloroplast *trn*L intron. Syst. Bot. 26, 537–556.
Polhill, R.M., 1994. Classification of the Leguminosae. In: Southon, I.W. (compiler), Phytochemical Dictionary of the Leguminosae. Chapman and Hall, London, pp. 35–57.
Polhill, R.M., 1981. Sophoreae. In: Polhill, R.M., Raven, P.H. (Eds.), Advances in Legume Systematics (Part 1). Royal Botanic Gardens, Kew, Richmond, pp. 213–230.
Siddiqui, S., Hafeez, F., Begum, S., Siddiqui, B., 1988. Oleanderol, a new pentacyclic triterpene from the leaves of *Nerium oleander*. J. Nat. Prod. 51, 229–233.
Slade, D., Ferreira, D., Marais, J.P.J., 2005. Circular dichroism, a powerful tool for the assessment of absolute configuration of flavonoids. Phytochemistry 66, 2177–2215.
Valesi, A.G., Rodriguez, E., Velde, G.V., Mabry, T.J., 1972. Methylated flavonols in *Larrea cuneifolia*. Phytochemistry 11, 2821–2826.
Van Wyk, B.-E., Greinwald, R., Witte, L., 1993. Alkaloids of the genera *Dicraeopetalum* Platycelyphium and *Sakoanala*. Biochem. Syst. Ecol. 21, 711–714.
Yang, S.-L., Roberts, M.F., O'Neill, M.J., Bucar, F., 1995. Flavonoids and chromenes from *Artemisia annua*. Phytochemistry 38, 255–257.
Zeng, L., Fukai, T., Nomura, T., Zhang, R.-Y., Lou, Z.-C., 1992. Five new isoprenoid-substituted flavonoids glyasperins F, G, H, I and J from the roots of *Glycrrhiza aspera*. Heterocycles 34, 1813–1828.