Four isoflavanones from the stem bark of *Platycelphium voënse*

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**ABSTRACT**

From the stem bark of *Platycelphium voënse* (Leguminosae) four new isoflavanones were isolated and characterized as (5)-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′,4′-dimethylidihydropyranono-[5′,6′:3′,4′]-isoflavanone (platyisoflavonone D). In addition, the known isoflavanones, sophoraisoflavanone A and gasyasperin F, the isoflavone, formomonenit; 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

*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 quinolinizidine alkaloids through GC–MS analysis of the leaves and twigs of plants from the two genera. Quinolinizidine 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 CH₂Cl₂–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 C₂₂H₂₄O₆. The presence of an isoflavonane skeleton was deduced from UV (λmax 288 nm), 1H (δ 4.48, dd, J = 11.1, 11.2 Hz, H-2α; δ 4.66, dd, J = 11.1, 5.6 Hz, H-2β; δ 4.38, dd, J = 11.2, 5.6 Hz, H-3α) and 13C (δ 7.16 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 methoxy (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).

Table 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
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 δH 4.38 (H-3) was enhanced upon irradiation of the methoxyl group at δH 3.71; similarly, NOE interaction was exhibited between a signal at δH 6.67 (H-5′) and the methoxyl group at δH 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 (δH 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 CH3–OCH3 protons of the 3-methylbut-2-enyl moiety with C-2′ and C-4′, implying that the 3-methylbut-2-enyl group is

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**Table 1**

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* Spectra recorded in CD3Cl3.
* Spectra recorded in CD3OD.
* Multiplicity was clear after iteration according to Laatikainen et al. (1996a, 1996b).
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’-methylbutyl-2’-enyl)-isoflavone, hereby named platysiisoflavone A. The CD spectrum of compound 1 showed a negative Cotton effect at 344 nm (n→π* transition) which was consistent with 3S absolute conﬁguration for this isoflavone (Slade et al., 2005).

Compound 2, obtained as a white amorphous solid, was also identiﬁed as an isoflavone derivative from the UV (λmax 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 C21H20O5. The 1H and 13C NMR spectra further revealed the presence of a chelated hydroxy (δ-OH), a methoxyl and a 3-methylbutyl-2-enyl moiety. 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 El-MS, at m/z 218 (2b) (Fig. 1) was in agreement with the placement of the methoxyl, the 3-methylbutyl-2-enyl unit and one hydroxyl groups in the B-ring. The methoxyl group was within the normal range (δc 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 methoxyl protons with C-4’) spectra conﬁrmed the placement of the methoxyl group at C-4’. Therefore this new compound was characterized as 2’,5’-trihydroxy-4’-methoxy-3’-(3’-methylbutyl-2’-enyl)-isoflavone, named platysioslavage A. The nearly zero optical rotation together with insigniﬁcant Cotton effect in the CD spectrum revealed that the compound was isolated as a racemic mixture. Isoflavonanes 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 isoflavonanes 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-methylbutyl-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, C21H22O5), the cyclization has resulted in the formation of a 2-(2-hydroxyisopropyl)-dihydrofuranone moiety in B-ring (Kijoka et al., 1998) as shown from the 1H an ABX system at δ2.97, 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 [δc 9.14 an oxygen methine carbon (C-2’); δc 29.5 (C-3’); δc 26.2 and 25.9 (C-1’ and C-3’)] and δc 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 methylated with the corresponding methoxyl group appearing at δc 3.68 and δc 56.4 in the NMR spectra. In the NOESY spectrum, this methoxyl signal showed NOE interaction with the aromatic proton resonating at δh 6.32 (H-5’), allowing its placement at C-4’, and hence the dihydrofuran ring should be between C-2’ and C-3’. Furthermore, the ‘normal’ methoxyl resonance at δc 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’-5’-dihydroxysopropyl)-dihydrofuran-4’-5’,3’-3’-C5-2’-enyl-isoflavone with a trivial name platysioslavone C. The conﬁguration at C-3’ and C-2’ has not been established.

The fourth new isoflavone (compound 4), [M]+ at m/z 372,1202, C22H22O5, has a 3-hydroxy-2,2-dimethylidihydroprano 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 dihydroprano ring is between C-2’/C-3’ and the other with the dihydroprano 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**

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(Table 2) displayed two methoxyl signals at δC 56.1 (7-OC₃H₃) and at δC 61.2; the latter deshielded signal, is typical of a di-ortho substituted methoxyl carbon (Park et al., 2008), and hence assigned to 2-OC₃H₃. 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′,3′-dimethylidihydropyrano-[5′,6′:3′,4′]-isoflavanone which is also new, with trivial name platyisoflavonolane 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 pyranoisoflavonol, glyasperin F, which had already been reported from the roots of Glycyrrhiza 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′,0-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 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 isoflavonol was identified as sophoraisoflavonol A (6) (Komatsu et al., 1978).

It is worthy to note that the oxygenation pattern in all the six isoflavonols isolated from this plant (P. voëns) is identical (at C-5, -7, -2′ and -4′), and each with a five-carbon unit at C-3′. Isoflavanols with this substitution pattern have also been isolated from the related genera Bolusanthus (Bojase et al., 2001a,b) and Sophora (Inuma 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 isoflavonols with different oxygenation and prenylation patterns. It will be interesting to find out if the related genus Diceraeopetalum also elaborate isoflavonols, possibly with the same oxygenation pattern as the isoflavonols obtained from P. voëns.

Other known compounds identified included the 3-methoxyflavones, kumatakenin (Valeisi et al., 1972) and isokaempferide (Yang et al., 1995); the isoflavone, forsonnotin (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-BTB 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 (IC₅₀ = 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, IC₅₀ = 88.3 μM for 5 and IC₅₀ = 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 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 Spectrocolor 5600, Analytik Jena AG, Germany. CD spectra were recorded on JASCO J-710 Spectropolarimeter. EI-MS: direct inlet, 70 eV on Micromass GC-TOF Micro 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. HMQC 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. voëns 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. voëns was pulverized and extracted twice with CH₂Cl₂-MeOH (1:1) at room temperature for 48 h. Evaporation of the solvent afforded brown gumy 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 (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 formononettin (10 mg). 3 (40 mg), 4 (15 mg), 5 (25 mg) and isokaempferide (6 mg).

3.4. (5)-Platyisoflavonolane A (1)

Amorphous powder, m.p. 160–164 °C, [α]D²⁴ = +14.1 (CH₂Cl₂, c = 1%, w/v). UV: λmax (CH₂Cl₂) 288 nm. CD (CH₂Cl₂, < 0.0047); [θ]₃⁵⁰ = 100, [θ]₃⁴⁴ = 120, [θ]₃₂₀ = 0, [θ]₂₅⁵ = +90, [θ]₂₈⁵ = 340, [θ]₂₉₅ = 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 (55), 115 (30), 91 (32), 69 (32), 51 (53), 45 (54). HREI-MS [M]+: found m/z 384.1597 for C₂₇H₂₃O₂ (calcd. 384.1567).

3.5. (±)-Platyisoflavonolane B (2)

Amorphous white powder, m.p. 133–138 °C. UV: λmax (CH₂Cl₂) 288 nm. [α]D²⁴ = 0 (CH₂Cl₂, c = 1%, w/v), UV: λmax (CH₂Cl₂) 294 nm: CD (CH₂Cl₂, < 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 C₂₉H₂₂O₃ (calcd. 370.1411).

3.6. Platyisoflavonane C (3)

Amorphous white solid. UV: λmax (CH₂Cl₂) 290 nm. [α]D²⁴ = 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 C₂₉H₂₂O₂ (calcd. mass 386.1366).

3.7. Platyisoflavonane D (4)

Amorphous white solid. UV: λmax (CH₂Cl₂) 289 nm. 1H NMR (Table 1). 13C NMR (Table 2), EI-MS m/z (rel. int.): 372 (60, [M]+), 153 (43), 51 (32). HREI-MS [M]+: found m/z 372.1404 C₂₉H₂₂O₃ (calcd. mass 372.1411).
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