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ORIGINAL ARTICLE

Prenylated flavonoids from the stem wood of *Commiphora opobalsamum* (L.) Engl. (Burseraceae)

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Abstract In this study, fractionation of acetone extract of the stem wood of *Commiphora opobalsamum* (L.) Engl. (Burseraceae) has been carried and two new prenylated flavonoids, 6-(3,3-Dimethylallyl)-2,3-dihydrokaempferol-3-β-O-glucoside **5** and 6-(3,3-Dimethylallyl) naringenin-7-O-β-glucoside **6**, together with four known flavonoids 6-(3,3-Dimethylallyl)-2,3-diyhdrokaempferol **1**, aromadendrin **3**, kaempferol **2** and quercetin **4** were isolated. These compounds except quercetin are reported from this plant for the first time. Their structures were elucidated on the basis of spectroscopic analysis and comparison with published data for the known compounds.

1. Introduction

The genus *Commiphora* (Burseraceae), comprising more than 150 plant species, is distributed in the tropical and subtropical regions, especially occurring in north eastern Africa, southern Arabia and India (Langenheim, 2003). The plants of *Commiphora* species are characterized as small trees or shrubs with spinescent branches, pale-gray bark and reddish-brown resinous exudates.

Previous phytochemical investigations of this genus, afforded isolation and identification of more than 300 terpenoid molecules (Dekebo et al., 2002a,b; Meselhy, 2003; Abbas et al., 2007; Fraternale et al., 2011; Shen et al., 2007, 2012a; Xu et al., 2012) as the major constituents in this genus. Flavonoids are found in the flower, stem and bark (Fatope et al., 2003; Abbas et al., 2007), and lignans commonly occurred in the bark or stem (Francis et al., 2004). Steroids and polypodane triterpenoids, characteristically present in the resin of *Commiphora mukul*, might be significant chemotaxonomic markers to identify plants of genus *Commiphora* (Shen et al., 2012b).

Commiphora opobalsamum (L.) Engl., locally known as Gafal (Sudan), is an ancient herb used in Arabian folk medicine for the treatment of various diseases including sore throat, cough, laryngitis, chronic bronchitis and inflammations due to rheumatism and arthritis (Al-Howiriny et al., 2005). It is widely distributed in the western Sudan through Kordofan and Darfur states. The plant produces soft, lightweight and aromatic wood that is used locally to make household utensils (cups and pots), furniture (stools) and tools (hammers).

The resins but not leaves, barks and stems of *C. opobalsamum* are the most commonly investigated target product for potential bioactive compounds and the presence of cycloartane-type

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triterpenoids, an aliphatic alcohol glycoside, and eudesmane-, guaiane-, germacrane-, and cadinane-type sesquiterpenoids in the resinous exudates have been established (Yang and Shi, 2012). The present paper describes isolation and structure elucidation of two new prenylated flavonoids along with four known flavonoids from stem wood acetone extract of locally grown *C. opobalsamum*.

2. Materials and methods

2.1. General

Proton and Carbon-13 nuclear magnetic resonance (NMR) were recorded at 200 and 50 MHz, respectively using a Mercury-200BB apparatus with tetramethylsilane (TMS) as internal reference. A low resolution mass spectrum (LR-MS) was produced on a Finnigan SSQ 700 mass spectrometer. Infrared (IR) spectra were recorded on FTIR-8400 spectrophotometer and UV spectra were measured on a Shimadzu UV-240, following Mabry et al. (1970). Column chromatography was carried out using Sephadex LH-20 and silica gel (230–400 mesh). Analytical TLC and PTLC were performed on precoated Merck F_{254} silica gel plates and visualized first under vis–UV light (254 and 366 nm), then sprayed with vanillin–H_2SO_4 and heated at 105 °C (Harborne, 1998).

2.2. Plant material and sampling site

The North kordofan region lies between latitude 12° 43′ – 13° 42′ N and longitude 30° 14′ – 31° 55′ E. It is characterized by a dry, hot climate, typically tropical continental with a relatively short rainy season. Plant material was collected from Wad al Baga, a rainy forest about 15 km south of El-Obeid city, capital of the region (Fig. 1) between August and September 2009. Taxonomical identification was determined using the available relevant African Flora (El Amin, 1990; Maydell, 1990) and by means of a comparison with herbarium specimens conserved in the Herbaria of Soba Forests Research Centre, and voucher specimen was deposited in the Herbarium of Botany Department, University of Kordofan (voucher no. B01185).

2.3. Extraction, isolation and characterization

Shade dried stem wood was milled into powder (1300 g) and successively extracted in a Soxhlet with hexane, dichloromethane (DCM), acetone, and methanol (Harborne, 1998). Acetone extract was evaporated in a rotatory evaporator, dried (14 g) and subjected to chromatography on silica gel eluted with hexane–EtOAc and EtOAc–MeOH solvent systems. The 25 column fractions obtained were combined according to their TLC profile into 14 major fractions ($\mathbf{A_1}$ to $\mathbf{A_{14}}$). Further fractionation led to the isolation of two new prenylated flavonoids along with four known flavonoids. Their structures were determined by analysis of their spectroscopic data (UV, IR, MS, ^{1}H- and ^{13}C-NMR) in comparison with those reported in the literature.

2.4. Compound *1* (6-(3,3-dimethylallyl)-2,3-diyhdrokaempferol)

Collected as colorless crystals (20 mg) by repeated crystallization of the residue obtained from fraction $\mathbf{A_3}$; m.p. 200–202 °C. UV/VIS data are shown in Table 1. IR (KBr) cm^{-1}: 3552 (OH), 1616 (C=O), 1521. ^{1}H-NMR (Acetone-d_6), δ: 7.40 (2H, m, H-2′/6′), 6.89 (2H, m, H-3′/5′), 6.01 (1H, s, H-8), 4.64 (1H, d, J = 11.6 Hz, H-3), 5.06 (1H, d, J = 11.6 Hz, H-2), 5.23(1H, m), 3.26 (2H, d, J = 6.8 Hz), 1.75 and 1.64 (s each, all –CH_3). ^{13}C-NMR (Acetone-d_6), δ: 83.7 (C-2), 72.5 (C-3), 161.3 (C-5), 108.7 (C-6), 164.6 (C-7), 94.8 (C-8), 158.2 (C-9), 130.8 (C-2′/6′), 115.2 (C-3′/5′), 158.1 (C-4′), 17.2(CH_3, C-4‴), 25.2 (CH_3, C-5‴), 122.7 (CH, C-2‴), 20.9 (CH_2, C-

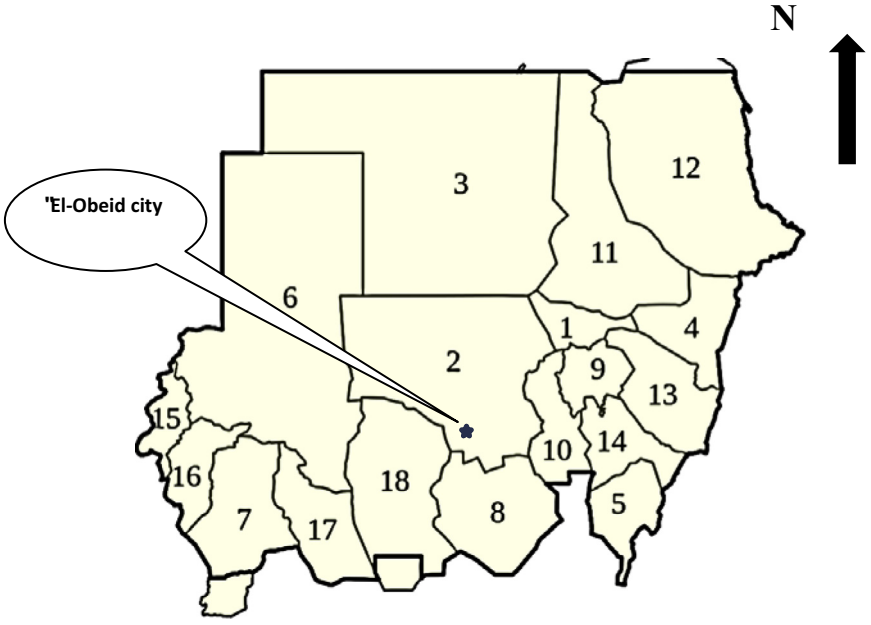


Figure 1 Location map of Northern kordofan, Sudan. **1**: Khartoum state; **2**: North Kordofan state; **3**: Northern state; **6**: Northern Darfur state; **8**: South Kordofan state; **10**: White Nile; **18**: East Kordofan.

Table 1 UV spectral data of isolated flavonoids (λ_{max} nm).

Solvent/reagent	1	2	3	4	5	6
MeOH	295, 328sh	270, 322sh, 365	290, 328sh	257, 269sh, 302sh, 370	285, 328sh	285, 320sh
NaOMe	245, 329	280, 320, 410	245, 325	247sh, 345, 410	250sh, 288, 345	Not done
NaOAc	300sh, 329	275, 305, 385	250sh, 285sh, 325	255sh, 275, 329, 385	288, 315sh, 345	
NaOAc + H3BO3	295, 332sh	267, 295sh, 320sh, 375	292, 330sh	259, 303sh, 385	285, 345sh	
AlCl3	317, 370	269, 305sh, 350sh, 426	315, 375	272 (IIb)	312, 405	
ALCl3 + HCl	317, 370	269, 272, 305sh, 348sh, 425	312, 375	265 (IIb), 305sh, 365 (Ib), 425 (Ia)	315, 403	

1‴), 130.7 (C-3‴). LR-MS (EI, 70 eV): *m/z* (%) = 356.14 (68), $[M]^{\cdot+}$.

2.5. Compound **2** and **3**

A precipitate filtered out from fractions (**A_4** and **A_5**) was separated on a Sephadex LH-20 eluted with DCM/MeOH (9:1) and the obtained fractions were further purified by preparative TLC using DCM/EtOAc (7:3) as developing solvent to yield compound **2** and **3**.

2.5.1. Compound **2** (kaempferol)

Obtained as yellow powder (22 mg); m.p. 271–273 °C. UV/VIS data are shown in Table 1. IR (KBr) cm^{-1}: 3294 (OH), 1658 (C═O), 1616 (C═C), 1508. ^{1}H-NMR (Acetone-d_6), *δ*: 6.27 (1H, d, *J* = 1.8 Hz, H-6), 6.54 (1H, d, 1.8 Hz, H-8), 8.16 (2H, d, *J* = 8.8 Hz, H-2′/6′), 7.01 (2H, d, *J* = 8.8 Hz, H-3′/5′), 12.19 (s, 5-OH). ^{13}C-NMR (Acetone-d_6), *δ*: 176.6 (C-4), 164.9 (C-7), 162.3 (C-5), 160.1 (C-4′), 157.8 (C-9), 141.1 (C-2), 136.6 (C-3), 130.5 (C-2′/6′), 123.3 (C-1′), 116.3 (C-3′/5′), 104.2 (C-10), 99.1 (C-6), 94.5 (C-8). LR-MS (EI, 70 eV): *m/z* (%) = 286.11 (100), $[M]^{\cdot+}$.

2.5.2. Compound **3** (aromadendrin)

Obtained as white powder (48 mg); m.p. 218–220 °C. UV/VIS data are shown in Table 1. IR (KBr) cm^{-1}: 3442 (OH), 1637 (C═O), 1521. ^{1}H-NMR (Acetone-d_6), *δ*: 7.42 (2H, m, H-2′/6′), 6.90 (2H, m, H-3′/5′), 5.94 (2H, d, *J* = 2.2 Hz, H-6), 5.99 (2H, d, *J* = 2.2 Hz, H-8); 5.08 (1H, d, *J* = 11.8 Hz, H-2), 4.66 (1H, d, *J* = 11.8 Hz, H-3). The ^{13}C-NMR (Acetone-d_6), *δ*: 84.3 (C-2), 73.1 (C-3), 198.2 (C-4), 164.9 (C-5), 97.04 (C-6), 167.8 (C-7), 95.9 (C-8), 130.3 (C-2′/6′), 158.8 (C-9), 115.9 (C-3′/5′), 101.9 (C-10), 158.8 (C-4′), 129.1 (C-**1′**). LR-MS (EI, 70 eV): *m/z* (%) = 288.12 (63), $[M]^{\cdot+}$.

Table 2 ^{1}H-NMR (Acetone-d_6, 200 MHz) spectral data of compounds **1**, **5** and **6**.

Position	Compounds		
	1	**5**	**6**
2	5.06 (1H, d, *J* = 11.6)	5.10 (1H, m)	5.24 (1H, q)
3	4.64 (1H, d, *J* = 11.6)	5.07 (1H, m)	2.75 (2H, m)
6	–	–	–
8	6.01 (1H, s)	6.27 (1H, s)	6.29 (1H, s)
2′/6′	7.40 (2H, m)	7.42 (2H, m)	7.39 (2H, m)
3′/5′	6.89 (2H, m)	6.90 (2H, m)	6.90 (2H, m)

2.6. Compound **4** (quercetin)

Major fractions **A_6**, **A_7** and **A_8** were repeatedly chromatographed on Sephadex LH-20 eluted with DCM/MeOH (8:2 and 1:1) then purified by preparative TLC to give compound **4**. This compound was obtained as a yellowish-green powder (15 mg); m.p. 310–312 °C. UV/VIS data are shown in Table 1. IR (KBr) cm^{-1}: 3394 (OH), 1654 (C═O), 1514, 1560. ^{1}H-NMR (Acetone-d_6), *δ*: 7.82 (1H, d, *J* = 2.1 Hz, H-2′), 7.70 (1H, dd, *J* = 2.1 and 8.4 Hz, H-6′), 6.99 (1H, d, *J* = 8.4 Hz, H-5′), 6.25 (1H, d, *J* = 2.1 Hz, H-6), 6.52 (1H, d, *J* = 2.1 Hz, H-8), 12.18 (s, 5-OH). LR-MS (EI, 70 eV): *m/z* (%) = 302.12 (100), $[M]^{\cdot+}$.

2.7. Compounds **5** and **6**

Major fractions **A_9**, **A_{10}**, **A_{11}** and **A_{12}** were re-grouped (750 mg) and subjected to a silica gel column eluted with gradient of hexane/EtOAc (7:3, 1:1, 3:7, 1:9 and 100% EtOAc) to afford sixty fractions. Fractions 33–38 and 41–55 were combined to give sub-fractions **f_1** (120 mg) and **f_2** (300 mg), respectively.

2.7.1. Compound **5** (6-(3,3-Dimethylallyl)-2,3-dihydrokaempferol-3-β-O-glycoside)

Further purification of sub-fraction **f_2** on Sephadex LH-20 eluted with DCM/MeOH (19:1 and 9:1) followed by preparative TLC analysis yielding 12 mg of compound **5**. Its UV/VIS data are shown in Table 1. IR (KBr) cm^{-1}: 3419 (OH), 1635 (C═O), 1519, 1579, 1444, 2925, 1070. ^{1}H-NMR (Acetone-d_6), *δ*: 5.23 (1H, m), 3.22 (2H, m), 1.75 (s, CH_3), 1.62 (s, CH_3), 5.07 (1H, m, H-3), 5.10 (1H, m, H-2), 6.27 (1H, s, H-8), 7.42 (2H, m, H-2′/6′), 6.90 (2H, m, H-3′/5′). ^{13}C-NMR (Acetone-d_6), *δ*: 84.4 (C-2), 73.3 (C-3), 160.9 (C-5), 111.3 (C-6), 161.9 (C-7), 95.1 (C-8), 158.8 (C-9), 130.3 (C-2′/6′), 115.9 (C-3′/5′), 158.8 (C-4′), 101.2 (C-1″), 74.5 (C-2″), 77.8 (C-3″), 71.1 (C-4″), 77.9 (C-5″), 62.4 (C-6″) 21.8 (C-1‴), 123.5 (C-2‴), 131.4 (C-3‴), 17.9 (C-4‴), 25.8 (C-5‴). LR-MS (EI, 70 eV): *m/z* (%) = 354.22 (62), $[M\text{-glc}]^{\cdot+}$.

2.7.2. Compound **6** (6-(3,3-Dimethylallyl) naringenin-7-O-β-glucoside)

Obtained as a pale pink amorphous solid (10 mg), from sub-fraction **f_1** while eluting the Sephadex LH-20 column with DCM/MeOH (19:1). Its UV/VIS data are shown in Table 1. IR (KBr) cm^{-1}: 3438 (OH), 1635 (C═O), 1519, 1438 ($2CH_3$). ^{1}H-NMR (Acetone-d_6), *δ*: 5.24 (1H, q), 3.22 (2H, m), 1.75 (s, CH_3), 1.62 (s, CH_3), 5.07 (1H, m, H-3), 2.75 (2H, m, H-3), 6.29 (1H, s, H-8), 7.39 (2H, m, H-2′/6′), 6.90

(2H, m, H-3′/5′). ^{13}C-NMR (Acetone-d_6), δ: 80.02 (C-2), 43.6 (C-3), 162.2 (C-5), 110.9 (C-6), 164.3 (C-7), 94.9 (C-8), 161.2 (C-9), 128.8 (C-2′/6′), 116.2 (C-3′/5′), 158.8 (C-4′), 101.3 (C-1″), 74.6 (C-2″), 77.8 (C-3″), 71.1 (C-4″), 77.9 (C-5″), 62.5 (C-6″) 21.8 (C-1‴), 123.6 (C-2‴), 131.4 (C-3‴), 17.9 (C-4‴), 25.8 (C-5‴). LR-MS (EI, 70 eV): m/z (%) = 340.27 (100), [M-glc]$^{\cdot+}$.

3. Results and discussion

The four known flavonoids (**1**–**4**), were identified as: 6-(3,3-Dimethylallyl)-2,3-diyhdrokaempferol (**1**) (Ingham et al., 1986), kaempferol (**2**) (Jiang et al., 2013; Lin et al., 2014), aromadendrin (**3**) (Han et al., 2007) and quercetin (**4**) (Zi et al., 2011; Lin et al., 2014).

3.1. Compound 5

The UV spectrum of this amorphous yellow powder demonstrated a similar profile as compound **1**, and indicated the presence of a flavanonol skeleton with 5,7-dihydroxy groups. The IR spectrum exhibited absorptions at 3419 cm^{-1} (OH), 1635 cm^{-1} (C=O), 1519 and 1579 cm^{-1} for aromatic structure, 1444 cm^{-1} for gem-dimethyl groups and broad bands at 2925 and 1070 cm^{-1} demonstrated the glycosidic nature. The ^{1}H- and ^{13}C-NMR spectra (Tables 2 and 3) showed a typical pattern to compound **1**, except the position of H-3 (5.07 ppm) which indicated the acylation of C_3–OH and this was confirmed further from the absence of substantial chemical shift effects for the aromatic ring protons of compound **5** relative to **1** which according to Moco et al. (2006) suggested attachment of the glucose moiety to the OH group at C-3. The ^{13}C-NMR signals at 101.2 (C-1″), 74.5 (C-2″), 77.8 (C-3″), 71.1 (C-4″), 77.9 (C-5″) and 62.4 (C-6″) along with resonances at 3.5–3.2 ppm and coupling constant 7.2 Hz of H-1‴ proton in ^{1}H-NMR confirmed the presence of the O-sugar moiety in β-configuration. The molecular ion peak at m/z 354.22 corresponding to M$^+$–$C_6H_{11}O_6$ (glucose moiety) and the ^{13}C-NMR, DEPT-135, HMBC and HSQC analysis confirmed the molecular formula $C_{26}H_{30}O_{12}$. Thus, compound **5** was identified as 6-(3,3-Dimethylallyl)-2,3-dihydrokaempferol-3-β-O-glycoside (Fig. 2) and it was the first report of its isolation from natural sources.

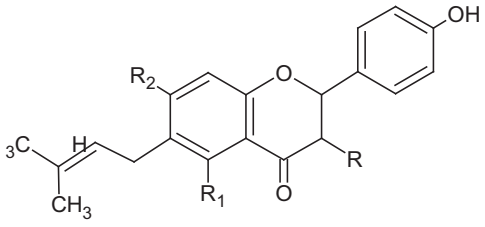
3.2. Compound 6

The UV spectrum (MeOH) of this new prenylated flavanone showed one major peak with a shoulder at 285 and 320 nm corresponding to flavanones (Mabry et al., 1970). The IR spectrum indicated the presence of OH (3438 cm^{-1}), C=O (1635 cm^{-1}), an aromatic structure (1519 cm^{-1}) and dimethyl groups (1438 cm^{-1}). The ^{1}H- and ^{13}C-NMR (Tables 2 and 3) showed spectral data similar to those of compounds **1** and **5**, except the higher shift of C-3 to 43.6 which indicated the absence of OH at that position. This was supported by both the higher shift up field of H-3 (2.75 ppm) relative to that of compound **5** and the presence of cross peak of C-3 with the protons at 2.75 and 3.21 ppm in the HSQC spectra. Low shift of C-7 to 164.3 (~2.4 ppm) relative to that of compound **5** indicated the acylation of the C_7–OH by the glucose moiety which was further confirmed by downfield shift of H-8 to 6.29 ppm relative to 5.9–6.1 ppm, the typical position in 5,7-dihydroxyflavanons (Markham, 1982). The LR-MS analysis showed a base peak at m/z 340.27 (100%) corresponding to M$^+$-glucose moiety and suggested the molecular formula $C_{26}H_{30}O_{11}$ which was confirmed from ^{13}C-, ^{1}H-HSQC and HMBC spectra. Thus, compound **6** was identified as 6-(3,3-Dimethylallyl) naringenin-7-O-β-glucoside (Fig. 2) and to the best of our knowledge it was isolated for the first time from a natural source.

In conclusion, fractionation of the acetone extract of *C. opobalsamum* stem wood afforded six compounds, two new prenylated flavonoids along with known flavonols (kaempferol

Table 3 ^{13}C-NMR (Acetone-d_6, 50 MHz) spectral data of compounds **1**, **5** and **6**.

Position	Compounds		
	1	**5**	**6**
2	83.7	84.4	80.02
3	72.5	73.3	43.6
4	198.0	199.2	198.2
5	161.3	160.9	162.2
6	108.7	111.3	110.9
7	164.6	161.9	164.3
8	94.8	95.1	94.9
9	158.2	158.8	161.2
10	97.1	102.6	104.2
1′	129.6	129.04	129.1
2′/6′	130.8	130.3	128.8
3′/5′	115.2	115.9	116.2
4′	158.1	158.8	158.8
1″	. . ..	101.2	101.3
2″	. . ..	74.5	74.6
3″	. . ..	77.8	77.8
4″	. . ..	71.1	71.1
5″	. . ..	77.9	77.9
6″	. . ..	62.4	62.5
1‴	20.9	21.8	21.8
2‴	122.7	123.5	123.6
3‴	130.7	131.4	131.4
4‴	17.2	17.9	17.9
5‴	25.2	25.8	25.8



Compound	R	R_1	R_2
1	OH	OH	OH
5	Glucose-O	OH	OH
6	H	OH	Glucose-O

Figure 2 Structures of prenylated flavonoids isolated from *Commiphora opobalsamum*.

and quercetin) and flavanonols (6-[3,3-Dimethylallyl]-2,3-diyhdrokaempferol and aromadendrin). To the best of our knowledge all the known isolated flavonoids except quercetin (Abbas et al., 2007) are reported from this plant species for the first time.

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Appendix A. Supplementary data

LR-MS and NMR (^{1}H, HMBC, HSQC and DEPT-135) spectra of compounds **5** and **6**. Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jksus.2014.04.005.

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