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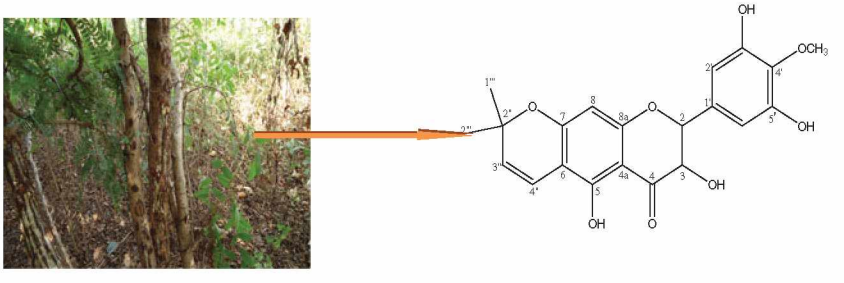
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A novel antimicrobial flavonoid from the stem bark of *Commiphora pedunculata* (Kotschy & Peyr.) Engl.

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A new flavonoid, 2-(3,5-dihydroxy-4-methoxy-phenyl)-3,5-dihydroxy-8,8-dimethyl-2,3-dihydro-8H-pyrano[3,2]chromen-4-one, together with previously reported epicatechin was isolated from the ethyl acetate soluble fraction of the methanol extract of the stem bark of *Commiphora pedunculata*. The structures of these compounds were elucidated based on extensive analysis of their spectral data, including 1 and 2D NMR. The compounds were active against 9 out of 12 tested microorganisms including a resistant strain; vancomycin-resistant *entrococci* (VRE), *Escherichia coli, Staphylococcus aureus* and *Candida albicans*. The zones of inhibition ranged between 22 and 34 mm against the microorganisms. The minimum inhibitory concentration was as low as 6.25 μg/mL against *Shigella dysentriae, Bacillus cereus* and *S. aureus* while the minimum bactericidal concentration was as low as 50 μg/mL against *Pseudomonas aeruginosa,* VRE and *C. albicans*. This is the first report of the isolation of the compound.

Keywords: 2D NMR; *Commiphora pedunculata*; Burseraceae; flavonoids; VRE

1. Introduction

Commiphora pedunculata (Kotschy & Peyr.) Engl. belongs to Burseraceae, a family composed of both trees and shrubs of tropical and sub-tropical geographical distribution (Watson & Dallwitz 1992). Over 200 species of *Commiphora* are recorded to occur in Africa, Arabia, the Indian Ocean Islands and India (Weeks et al. 2005). The Burseraceae family members (also known as the frankinscence or Myrhh family) are characterised by the non-allergic resin produced in virtually all plant tissues and the distinctive smooth, yet flaking aromatic bark (Judd et al. 2008). The resins are of substantial economic, medicinal and cultural value (Langenheim

2003). Traditionally, the stem and root are chewed for treating coughs and the decoction of the leaves and stem bark is used for the treatment of dysentery and diarrhoea (Baba Mai Wada, personal communication). Recently, the antimicrobial activity of the hexane, ethyl acetate and methanol extracts of the stem bark of *C. pedunculata* was reported (Sallau et al. 2014), also two flavonoids with antimicrobial activity have been isolated from the ethyl acetate soluble fraction of the methanol extract of the plant (Tajuddeen et al. 2014). Other species of *Commiphora* are known to produce flavonoids and other phenolic compounds (Hanuš et al. 2005). In this paper, we report the isolation and characterisation of a new flavonoid: 2-(3,5-dihydroxy-4-methoxyphenyl)-3,5-dihydroxy-8,8-dimethyl-2,3-dihydro-8H-pyrano[3,2]chromen-4-one together with a known flavonoid (epicatechin) from the ethyl acetate soluble fraction of the methanol extract of the stem bark.

2. Results and discussion

Compound **1** was isolated as pale yellow crystals. It gave a positive result in the test for flavonoids (Harborne & Mabry 1975). Compound **1** was assigned the molecular formula $C_{21}H_{20}O_8$ based on HR-ESI-MS *m/z* at 401.3787 cald. 400.4896. The existence of a λ_{max} at 265 nm (band II) and a shoulder peak at 320 nm (band I) in the UV spectrum of compound **1** is an indication that it might be a dihydroflavonol (Bohm 1999). The IR spectrum of compound **1** showed a very broad band at 3350 cm^{-1}, characteristic of O—H stretching for phenolic hydroxyl groups, the band at 1680 cm^{-1} is characteristic of carbonyl stretching while the bands observed in the fingerprint region are due to various C—H stretching and bending modes (Wade 2006).

The proton NMR spectrum of compound **1** revealed signals for a pair of ortho-coupled unsaturated protons at δ_H 6.61 (1H, d, $J = 10.0$ Hz, H-4″) and 5.61 (1H, d, $J = 10.0$ Hz, H-3″), these signals are typical of the dimethyl substituted pyran ring of 2H-1-benzopyran skeleton (Ellis 1977; Waffo et al. 2000). In addition, two aromatic signals at δ_H 6.55 (2H, s H-2′, 6′) and 5.92 (1H, s, H-8) were revealed by the proton NMR, they were assigned to a tetra-substituted ring B and penta-substituted ring A of a flavonoid. Further observed in the proton NMR of compound **1** are two oxygenated methine proton signals at δ_H 4.54 (1H, d, $J = 11.4$ Hz, H-3) and δ_H 4.90 (1H, d, $J = 11.4$ Hz, H-2), a methoxy proton signal at δ_H 3.83 (3H, s, H—OCH_3) and a methyl signal at δ_H 1.43 (6H, s, H-1‴, 2‴).

The ^{13}C NMR spectrum showed a total of 19 carbon signals (Table S1), 11 of the signals were assigned to a tri-substituted 2,2-dimethylbenzopyran skeleton (δ_C 163.34, 163.60, 159.2, 127.73, 115.97, 104.20, 102.33, 97.13, 79.54, 28.62 and 28.57) (Waffo et al. 2000). It also showed signals for a ketone carbonyl δ_C 198.79 and two oxymethine carbons δ_C 85.04 and 73.71, which together with the aromatic benzene ring of the benzopyran skeleton form a dihydrochromone ring. The remaining aromatic carbon signals at δ_C 151.75, 137.29, 134.02 and 108.27 were assigned to ring B of the resulting dimethylpyran–flavonol. Also revealed by the ^{13}C NMR is a methoxy carbon at δ_C 60.78. All protonated carbons were assigned by analysis of the HSQC and DEPT spectra. The assignment of the ortho-coupled unsaturated protons of the pyran ring was further confirmed by the 2D $^1H-^1H$ COSY of H-3″//H-4″, COSY also confirmed the relationship of H-2//H-3. The methoxy proton was attached to ring B based on the HMBC correlation of H-OCH_3 and C-4′. Other HMBC correlations observed in the HMBC spectrum confirmed the structure of compound **1** and the ^1H and ^{13}C NMR signal assignments, such as the HMBC correlations of H-4″//C-7, 6, 5, 2″, H-3″//C-6, 2″, 1‴, 2‴, H-1‴//C-3″, 2″, H-8//C-8a, 4a, 6, H-2′//C-2, 1′, 3′ 4′ and H-2//C-1′ 4, 3, H-3//C-1′, 4, 2. These and other relevant HMBC correlations are shown in Figure S1. The relative stereochemistry of positions 2 and 3 was established by analysing the coupling constant values of H-2 and H-3 which were both 11.4 Hz, this shows an ax/ax coupling between the two protons. Thus ring B was assumed to be in an equatorial position on a half-chair ring. The structure of compound **1** (Figure 1) was therefore

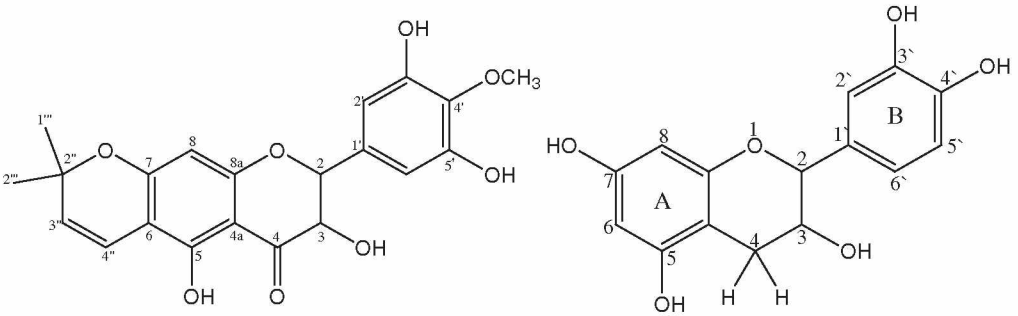


Figure 1. Structures of compounds **1** and **2**.

elucidated as 2-(3,5-dihydroxy-4-methoxy-phenyl)-3,5-dihydroxy-8,8-dimethyl-2,3-dihydro-8H-pyrano[3,2]chromen-4-one.

Compound **2** was isolated as a brown solid. The five aromatic proton signals of compound **2** were assigned to an AB type ring A [δ_H 5.96 (2H, d, $J = 2.1$ Hz, H-6), 5.94 (2H, d, $J = 2.0$ Hz, H-8)] and an ABX type ring B [δ_H 6.9 (2H, d, $J = 1$ Hz, H-2′), 6.7 (2H, d, $J = 8.1$ Hz, H-5′), 6.8 (2H, dd, $J = 8.4$ and 1.6 Hz, H-6′)] (Kazuo et al. 1999). Ring A therefore is a 1,3,4,5-tetra-substituted benzene ring while ring B is a 1,3,4-tri-substituted benzene ring. The signals between 6.7 and 6.9 ppm are characteristic of the ABX spin pattern in the ring B of epicatechin (Antonelli et al. 2007). Also the two methylene protons at δ_H: 2.8 ppm (dd, $J = 16.76$ and 4.5 Hz, ax.) and 2.7 ppm (dd, $J = 16.8$ and 2.6 Hz, eq.) with their small J values are typical of position 4 of epicatechin. The close assemblage of signals about 156 ppm in the ^{13}C spectrum of compound **2** is characteristic of positions 5, 7 and 9 of the ring A of epicatechin. A comparison of the ^{13}C NMR data of compound **2** with data reported in the literature for epicatechin showed very close agreement, for example, 29.3 ppm (C-4), 79.9 ppm (C-2), 67.5 ppm (C-3), 115.9 ppm (C-5′) and 119.4 ppm (C-6′) (Markham & Ternai 1976). Co-TLC of **2** with standard sample of epicatechin showed the two to have the same R_f value. The structure of **2** was therefore elucidated as epicatechin (Figure 1).

The result of the antimicrobial activity of the compounds is shown in Table 1. Twelve microorganisms were screened, nine bacteria (including vancomycin resistant *enterococci* (VRE)) and three fungi. Compound **2** was active against 8 out of the 12 tested microorganisms while compound **1** was active against 6. The highest inhibition zone was observed with **2** against *Shigella dysentriae* (34 mm), followed by *Bacillus cereus* (32 mm) and *Staphylococcus aureus* (27 mm). The highest inhibition zone observed for compound **1** was against *S. aureus* (29 mm), *Escherichia coli* (27 mm) and *Candida albicans* (25 mm). VRE, *S. aureus, E. coli, C. albicans and C. krusei* showed sensitivity to both compounds, with inhibition zones of 24, 29, 27, 25 and 22 mm for compound **1** and 24, 27, 25, 25 and 24 mm for compound **2** against the microorganisms, respectively. The two compounds were inactive against *Streptococcus pyogenes*, *Proteus mirabilis* and *Candida tropicalis*.

The zones of inhibition observed with the compounds were slightly lower than those of the standard drugs used (Ciprofloxacin and Fluconazole). The minimum inhibitory concentration (MIC) value was lowest against *S. dysentriae*, *S. aureus* and *B. cereus* (6.25 µg/mL) with compound **1**. Other MIC values were also considerably low at 12.5, 25.0 and 50 µg/mL for both compounds. The low MIC values suggest that the compound possesses good antimicrobial activity, considering that compounds with MIC values less than 100 µg/mL are regarded as having strong antimicrobial activity (Tang et al. 2003). Although the antimicrobial activity of epictatechin has been previously investigated (Betts et al. 2011), this will be the first report of its

Table 1. Antimicrobial activity of compounds **1** and **2**.

	Antimicrobial activity of compound **1** (50 μg/mL)			
Test organism	**2**	**1**	CFX (5 μg/mL)	FCZ (5 μg/mL)
S. dysenteriae	34 (6.25/12.5)	–	35 (0.312/1.25)	NT
VRE	24 (12.5/50)	24 (25/50)	32 (0.312/2.50)	NT
S. aureus	27 (6.25/25)	29 (12.5/25)	37 (0.156/1.25)	NT
S. pyogenes	–	–	40 (0.156/0.625)	NT
S. pneumoniae	–	23(25/100)	35 (0.312/2.50)	NT
E. coli	25 (12.5/25)	27 (12.5/50)	32 (0.156/0.625)	NT
B. cereus	32 (6.25/25)	–	42 (0.312/2.50)	NT
P. mirabilis	–	–	35 ()	NT
P. aeruginosa	22 (12.5/50)	–	–	NT
C. albicans	25 (12.5/50)	25 (25/50)	NT	35 (0.312/1.25)
C. tropicalis	–	–	NT	32 (0.312/1.25)
C. krusei	24 (12.5/25)	22 (25/100)	NT	35 (0.625/2.50)

Notes: Key: The values in parentheses indicate minimum inhibitory concentration/minimum bactericidal and fungicidal concentrations for bacteria and fungi, respectively. (MIC/MBC and MIC/MFC, μg/mL), the values outside parentheses are inhibition zones (mm), CFX, Sparfloxacin; FCZ, Fluconazole; VRE, vancomycin-resistant *entrococci*; –, no activity detected; NT, not tested.

efficacy against VRE, also this is the first report of the antimicrobial activity of the isolated new flavonoid. The antimicrobial activity observed with the isolated compounds might be due to the hydroxyl groups present on their structures.

3. Experimental

3.1. *General procedures*

NMR spectra were recorded on a Bruker AVANCE spectrometer (400 MHz) for ^{1}H and (100 MHz) ^{13}C NMR, internal standard was residual solvent signal with methanol as solvent. The IR spectrum was measured on a Shimadzu FT-IR8 400S Fourier Transform Infrared spectrophotometer (Shimadzu Corporation, Kyoto, Japan). The UV spectrum was recorded on a Hitachi U-3200 spectrophotometer (Hitachi High Technologies America, Inc., Schaumburg, IL, USA). Melting points were determined on a Yanaco MP-400 micro melting point apparatus (Yanaco New Science Inc., Kyoto, Japan). Optical rotations were measured on an ADP 440 + Bellingham and Stanley polarimeter (Pt. Global Scientific International, Jakarta, Indonesia). HR-ESI-MS was carried out on a Bruker APEX II mass spectrometer. For thin layer chromatography analysis, silica gel 60 F_{254} (Merck) was used, column chromatography was performed using Merck silica gel (60–120) mesh, while gel filtration chromatography was performed using Sephadex LH-20 (Sigma, Spruce street, St. Louis, MO, USA). Spots on TLC plates were visualised by spraying with 10% H_2SO_4 followed by heating at 100°C for 5 min.

3.2. *Plant sample*

The plant material was collected from the bushes around Basawa in Zaria, Kaduna State, Nigeria in the month of October 2011. It was authenticated by Mallam Galla at the herbarium unit of the Biological Sciences Department, Ahmadu Bello University Zaria-Nigeria, where a sample of it was deposited and assigned the specimen voucher number 219. The stem bark was separated from the stem, air-dried for 21 days and crushed to a coarse powder.

3.3. *Extraction and isolation*

The dried powder (500 g) was extracted with methanol (2.5 L) in a Soxhlet apparatus for 48 h at 65°C. After evaporation of the solvent, the concentrated methanol extract was separated into water-soluble and water-insoluble portions. The water-soluble portion (1 L) was further partitioned with *n*-hexane (2.5 L) and ethyl acetate (5 L) to give 2.0 g of the *n*-hexane fraction and 14.7 g of the ethyl acetate fraction after removal of the solvents. A portion of the ethyl acetate fraction (7.5 g) was chromatographed on silica gel column eluting with hexane 100%, hexane–ethyl acetate mixtures (80:20, 60:40 and 30:70), ethyl acetate 100% and methanol 100% as solvent systems to give 25 fractions. The 25 fractions were pooled together based on similarity in their TLC profile to give four sub-fractions. Repeated Sephadex LH-20 gel filtration chromatography (eluting with MeOH) of sub-fraction 2 led to the isolation of compounds **1** and **2** (6.8 and 7.4 mg, respectively).

3.4. *Spectral data*

2-(3,5-Dihydroxy-4-methoxy-phenyl)-3,5-dihydroxy-8,8-dimethyl-2,3-dihydro-8H pyrano[3,2] chromen-4-one. Pale yellow crystals, m.p 192–195°C; $[\alpha]_D^{22} = +3.71$ ($c = 0.021$, MeOH) UV (MeOH) λ_{max}: 265, 320 nm (shoulder); IR (KBr): ν_{max}. 3350, 1680 cm^{-1};^{1}H NMR (CD_3OD) δ: 6.61 (1H, d, $J = 10.0$ Hz, H-4″), 6.55 (2H, s, H-2′, 6′), 5.92 (1H, s, H-8), 5.61 (1H, d, $J = 10.0$ Hz, H-3″), 4.90 (1H, d, $J = 11.4$ Hz, H-2), 4.49 (1H, d, $J = 11.4$ Hz, H-3), 3.83 (3H, s, 4′–OCH_3), 1.43 (3H, s, H-1‴), 1.44 (3H, s, H-2‴). ^{13}C NMR (CD_3OD) δ: 198.79 (C-4), 163.64 (C-7), 163.60 (C-8a), 159.21 (C-5), 151.75 (C-3′, 5′), 137.29 (C-4′), 134.01 (C-1′), 127.73 (C-3″), 115.97 (C-4″), 108.27 (C-2′, 6′), 104.20 (C-6), 102.33 (C-4a), 97.13 (C-8), 85.01 (C-2), 79.54 (C-2''), 73.71 (C-3), 60.78 (C–OCH_3), 28.62 (C-1‴), 28.56 (C-2‴); HR-ESI-MS: *m/z* 401. 3787 $[M + H]^+$ (Calcd. for $C_{21}H_{20}O_8$ 400.4896).

3.5. *Antimicrobial assay*

The isolated compound was tested for antimicrobial activity against clinical isolates of VRE, *S. dysentriae*, *S. aureus*, *S. pyogenes*, *S. pneumonia*, *E. coli*, *B. cereus*, *P. mirabilis*, *Pseudomonus aeruginosa*, *C. albicans*, *C. tropicalis* and *Candida krusei*, as described previously (Volleková et al. 2001; Bonev et al. 2008; Londonkar et al. 2013). Ciprofloxacin and fluconazole were used as positive standards. The agar well diffusion and broth dilution methods were used. The standardised inocula of the isolates were uniformly streaked onto freshly prepared Mueller Hinton agar plates. Using a sterile cork borer (6 mm in diameter), appropriately labelled wells were punched into each agar plate. Then 0.2 mL of the appropriate compound concentration was added into each well and then allowed to diffuse into the agar. The plates were incubated at 37°C for 24 h for bacteria, while the incubation period was 48 h at 25°C for fungi. The antimicrobial activities were expressed as diameter of inhibition zones produced by the compounds. The experiment was done in duplicates. The MICs of the compounds were determined using the broth dilution method. Various concentrations of the compound (50–3.125 μg/mL) were prepared by twofold serial dilution in test tubes containing Mueller Hinton broth. The organisms (0.2 mL) were inoculated into each tube containing the compound. The tubes were incubated at 37°C for 24 h for bacteria and 48 h at 25°C for fungi. The lowest concentration in the series showing no visible growth of the test organisms was considered to be the MIC. The contents of the MIC tubes in the serial dilution were sub-cultured onto appropriately labelled Mueller Hinton agar plates and incubated at 37°C for 24 h, then they were observed for colony growth. The lowest concentration of the sub-culture with no growth was considered as the minimum bactericidal concentration (MBC).

4. Conclusions

This study is a continuation of our investigations into the chemistry of *C. pedunculata* from Nigeria. Interestingly it led to the isolation of a new flavonoid with antimicrobial activity, indicating that the ethnomedicinal claims ascribed to the plant have scientific backing.

Supplementary material

Supplementary material relating to this paper is available online, alongside Table S1 and Figures S1–S8.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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