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Eucleanal A and B: Two new naphthalene derivatives from *Euclea divinorum*

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Abstract

Two new naphthalene derivatives named eucleanal A and B (**1** and **2**) were isolated from *Euclea divinorum*. The structures of the new compounds were elucidated by detailed spectroscopic analysis such as ¹H, ¹³C NMR, COSY, HMQC, HMBC and HREIMS mass spectrometry.

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Keywords: *Euclea divinorum* Hiern; Ebenaceae; NMR; Structure elucidation

Euclea divinorum Hiern bark is used as a condiment in the preparation of a fatty meat and milk soup in East Africa [1]. The fruits of *E. divinorum* Hiern are used as a purple dye for basket fibres by the Kwanyama Ovambos of northern Namibia and also boiled to produce purple ink. They are applied in the fermentation process to make beer. The roots and twigs of *E. divinorum* Hiern are popular as toothbrushes and roots are chewed as a disinfectant and to colour the lips and mouth red. In traditional medicine root extracts and dried powdered roots of *E. divinorum* Hiern are applied for the treatment of gastro-intestinal disturbances, cancer, ulcers, wounds, arthritis, miscarriage, jaundice, snakebites and gonorrhoea [1]. In south-western Ethiopia *E. divinorum* Hiern is used to purify drinking water by adding branches to the gourds or pots and leaving them to soak in the water for several hours. In the course of phytochemical studies of medicinal plants from Africa and Pakistan [2–10] with the aim of isolation and structure elucidation of novel bioactive compounds, we have investigated *E. divinorum* and report on the structure elucidation of two new isolated naphthalene derivatives, named eucleanal A and B (**1** and **2**).

1. Experimental

The UV (MeOH) and IR (KBr) spectra were measured on Hitachi U-3200 and shimadzu-8900 spectrophotometers respectively. The electron mass spectrum [EI-MS] was recorded on a Finnegan MAT-112 spectrometer coupled with a

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PDP 11/34 computer system. ^1H and ^{13}C NMR, COSY, HMQC, and HMBC spectra were recorded in CDCl_3 at 500 and 125 MHz respectively. Chemical shifts δ are in ppm and coupling constants in Hz with tetramethylsilane (TMS) as an internal standard. Column chromatography (CC) was performed on Merck, silica gel 60 (70–230 mesh sizes and 230–400 mesh size was used for flash chromatography (FC). TLC was performed on precoated Merck silica gel 60 F_{254} plates and detection was achieved at 254 nm and by spraying with ceric sulfate in 10% H_2SO_4 solution followed by heating.

The plants of *E. divinorum* (Ebenaceae) were collected from the Masaai land in the Kajiado district of the Rift valley province in December 2004 and authenticated by Mr. Simon Mathenge (Plant taxonomist), Department of Botany, University of Nairobi. A voucher specimen (MM/12/04) is deposited in Nairobi University herbarium, Chiromo Campus.

1.1. Extraction and isolation

The air-dried leaves of *E. divinorum* (1.25 kg) were ground and extracted sequentially to afford crude extracts of petroleum ether (23 g), dichloromethane (28 g), ethyl acetate (33 g) and methanol (52 g). Ethyl acetate crude extract (33 g) was subjected to VLC using petroleum ether, petroleum ether: CH_2Cl_2 , CH_2Cl_2 :MeOH in gradient elution and afforded 60 fractions (FVC₁₋₆₀). The fractions FVC₁₅₋₂₂ (2.1 g) were combined and subjected to gradient column chromatography using petroleum ether and acetone to afford red crystalline solid of eucleanal A (**1**, 3.5 mg). Repeated column chromatography of FVC₂₈₋₄₀ (5.0 g) by gradient elution using pet ether, pet ether: CH_2Cl_2 and then acetone yielded a mixture which on repeated crystallization using pet ether: acetone (8:2) mixture gave red crystals of eucleanal B (**2**, 2.5 mg).

Eucleanal A (1): M. p. 207–210 °C; Red solid. UV (CHCl_3): λ_{max} (lg ϵ) 234 (2.78), 345 (3.55) nm; IR (CH_2Cl_2): 3390, 1630, 1580, 1420 cm^{-1} ; ^1H NMR (500 MHz, CDCl_3): δ 10.06 (CHO), 9.29 (OH), 7.93 (d, 1H, $J = 1.5$ Hz, H-2), 7.48 (dd, 1H, $J = 1.5, 8.0$ Hz, H-5), 7.43 (dd, 1H, $J = 1.5, 8.0$ Hz, H-7), 7.23 (d, 1H, $J = 1.5$ Hz, H-4), 7.08 (t, 1H, $J = 8.0$ Hz, H-6), 4.14 (s, 3H, OMe); ^{13}C NMR (125 MHz, CDCl_3): δ 191.7 (CHO), 157.3 (C-3), 154.8 (C-8), 135.8 (C-1), 134.4 (C-10), 130.4 (C-6), 129.0 (C-5), 120.8 (C-4), 117.6 (C-2), 116.1 (C-9), 114.5 (C-7), 56.7 (OMe); MS (EI, 230 °C): m/z (%) 202.0640 (24) $[\text{M}]^+$; HREIMS: m/z 202.0630 (Calcd. for $\text{C}_{12}\text{H}_{10}\text{O}_3$ 202.0630).

Eucleanal B (2): M. p. 222 °C; Red solid. UV (CHCl_3): λ_{max} (lg ϵ) 235 (2.70), 343 (3.50) nm; IR (CH_2Cl_2): 3395, 1630, 1583, 1420 cm^{-1} ; ^1H NMR (500 MHz, CDCl_3): δ 10.06, 9.27 (OH), 7.90 (s, 1H, H-2), 7.80 (OH), 7.47 (dd, 1H, $J = 1.5, 8.0$ Hz, H-5), 7.42 (dd, 1H, $J = 1.5, 8.0$ Hz, H-7), 7.04 (t, 1H, $J = 8.0$ Hz, H-6), 4.14 (s, 3H, OMe), 4.00 (s, 3H, OMe); ^{13}C NMR (125 MHz, CDCl_3): δ 191.1 (–CHO), 164.8 (C-4), 152.6 (C-8), 140.5 (C-3), 130.9 (C-2), 127.7 (C-10), 127.5 (C-6), 125.1 (C-1), 118.4 (C-9), 112.8 (C-5), 105.3 (C-7), 56.3 (–OCH₃), 56.2 (–OCH₃); MS (EI, 230 °C): m/z (%) 232.1 (22) $[\text{M}]^+$; HREIMS: m/z 232.0730 (Calcd. for $\text{C}_{13}\text{H}_{12}\text{O}_4$ 232.0736).

2. Results and discussion

A methanolic extract of the air dried leaves of *E. divinorum* was subjected to column chromatography which resulted in the isolation of two new natural compounds viz., eucleanal A and B (**1** and **2**) (Fig. 1).

Eucleanal A (**1**) was obtained as red solid in CH_2Cl_2 /EtOEt, with a melting point of 207–210 °C. The IR spectrum indicated absorption peaks at 1580 and 1420 cm^{-1} suggesting the presence of a benzene ring and absorption bands at 3390 and 1630 cm^{-1} indicated the presence of an hydroxyl and carbonyl group respectively. The UV–visible spectrum exhibited absorption maxima at (λ_{max} , CHCl_3) 234 and 345 nm. The molecular formula of eucleanal A (**1**) was assigned $\text{C}_{12}\text{H}_{10}\text{O}_3$ on the basis of HREIMS with a molecular peak at m/z 202.0640 and ^1H and ^{13}C NMR spectral

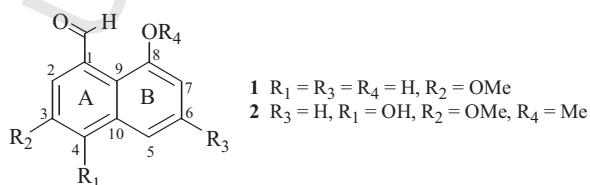


Fig. 1. Structures of compounds **1** and **2**.

76 analyses (see Section 1). The ^1H NMR spectrum (see Section 1) in CDCl_3 revealed the presence of an aldehydic
77 proton at δ 10.06, a hydroxyl proton at δ 9.29 and a 3-proton singlet at δ 4.14 for a methoxy group attached to an
78 aromatic nucleus. In addition, the ^1H NMR spectrum revealed the presence of five aromatic signals at δ 7.93 (d, 1H, J
79 = 1.5 Hz, H-2), 7.48 (dd, 1H, J = 1.5, 8.0 Hz, H-5), 7.43 (dd, 1H, J = 1.5, 8.0 Hz, H-7), 7.23 (d, 1H, J = 1.5 Hz, H-4)
80 and 7.08 (t, 1H, J = 8.0 Hz, H-6). From the ^1H ^1H COSY spectrum, it was evident that two protons (δ 7.93 and 7.23)
81 were coupling to each other *via meta* coupling (J = 1.5 Hz) while the remaining three protons (δ 7.48, 7.43, and 7.08)
82 were correlated to each other *via ortho* (J = 8.0 Hz) as well as *meta* coupling (J = 1.5 Hz). Based on this information,
83 it was evident that compound **1** has a disubstituted A ring and monosubstituted B ring. The ^{13}C NMR and DEPT
84 spectra (see Section 1) displayed twelve signals attributed to one methoxy, one aldehyde, 5 methine and five
85 quaternary carbons. The positions of attachment for the aldehydic, methoxy and hydroxyl carbons in ring A were
86 confirmed from the HMBC correlations: CHO to C-1; H-2 to C-1, C-3, C-4, C-9, and CHO; H-4 to C-2, C-3, C-5, and
87 C-10. Similarly HMBC correlations of H-5 to C-4, C-6, C-7, and C-10; H-6 to C-5, C-7, C-8, and C-10; H-7 to C-5, C-
88 6, C-8, and C-9 confirmed the regiochemistry in ring B. Consequently, the structure was established to be 8-hydroxy-
89 3-methoxy-1-naphthaldehyde (**1**), named eucleanal A, after the producing organism, *E. divinorum*.

90 The molecular formula of eucleanal B (**2**) was assigned $\text{C}_{13}\text{H}_{12}\text{O}_4$ on the basis of HREIMS with a molecular peak at
91 m/z 232.0730. The structure of **2** was determined by comparison of its NMR spectroscopic data with those determined
92 for eucleanal A (**1**). However, two main differences were apparent in the spectrum of compound **2**. A second hydroxy
93 group appeared at δ 7.80 in place of the aromatic proton at δ 7.23 for H-4 found in compound **1**. Secondly, a second
94 methoxy group appeared at δ 4.00 and we suggest this group is at C-8 in compound **2**. The structure of compound **2** was
95 further confirmed by COSY, HMQC, and HMBC spectra. Thus, the structure of eucleanal B (**2**), was established as 4-
96 hydroxy-3,8-dimethoxy-1-naphthaldehyde.

Uncited references

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98Q3 [11–14].

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