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PENTACYCLIC TRITERPENES AND NAPHTHOQUINONES FROM *EUCLEA DIVINORUM*

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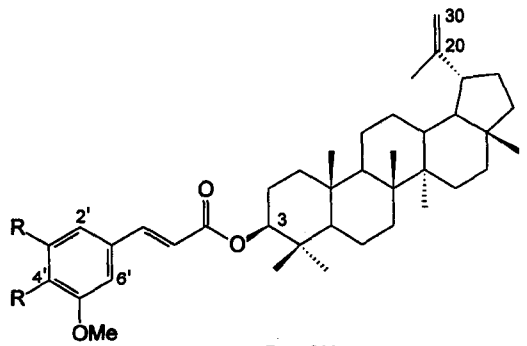
Key Word Index—*Euclea divinorum*; Ebenaceae; pentacyclic triterpenes; 3β-(5-hydroxyferuloyl)lup-20(30)ene; naphthoquinones; 7-methyljuglone, cytotoxicity.

Abstract—Phytochemical studies on *Euclea divinorum* have resulted in the isolation of lupeol, lupene, betulin, 7-methyljuglone, isodiospyrin, shinalone, catechin and 3β-(5-hydroxyferuloyl)lup-20(30)-ene. The structures were assigned on the basis spectral and chemical studies and the compounds were evaluated for their cytotoxic activity. © 1997 Elsevier Science Ltd

INTRODUCTION

The genus *Euclea* of the family Ebenaceae is well presented in Eastern and Southern Africa. *Euclea divinorum* is an evergreen shrub. The bark of the roots is used in traditional medicine for the treatment of diarrhoea, convulsions, cancer, skin diseases and gonorrhoea [1, 2]. Previous chemical studies of *E. divinorum* and other *Euclea* species have yielded some naphthoquinones, triterpenes and flavonoids [3–5]. In this paper, we report the isolation of a new compound, 3β-(5-hydroxyferuloyl)lup-20(30)-ene (**1**) and seven known compounds; isodiospyrin, shinalene, catechin, lupeol, lupene, and betulin [3–5] from the chloroform extract of *E. divinorum*. The reported medicinal importance of this plant prompted us to further investigate the cytotoxic activities of the isolates.



1 R = OH
2 R = OAc

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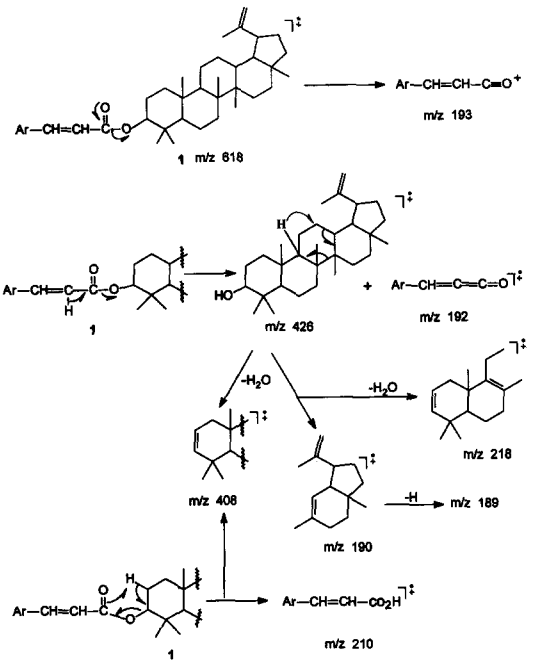
RESULTS AND DISCUSSION

The chloroform extract of the root bark of *E. divinorum* was purified by chromatography and afforded the new pentacyclic triterpene **1**. Compound **1** gave a positive LB test and a violet colouration with ferric chloride (phenolic OH). The IR spectrum exhibited absorption bands (cm^{-1}) at 3540–3320 (*br*, OH), 1690 (conjugated ester C=O), 1370 (*gem* methyl), 1180 (ester C—O), 970 (*trans* CH=CH) and 885 (C=CH_2). The UV spectrum showed absorption maxima (λ_{max}) at 235 nm (log ε 3.29) and 326 nm (log ε 3.22). The absorption at 326 nm was shifted to 331 nm (log ε 2.60) and 386 nm (log ε 2.16) in alkaline solution (phenolic OH groups). The mass spectrum displayed a $[M]^+$ ion at *m/z* 618 analysing for $C_{40}H_{58}O_5$. The ^1H NMR spectrum showed the presence of six tertiary methyl groups on saturated carbons, one isopropylidene group, C(Me)=CH_2 [δ 1.69, (*s*, vinyl Me), δ 4.58 and 4.70 (*br d*, *J* = 2.4 Hz, two geminal olefinic protons)], a deshielded methine proton on C-3 at δ 4.50–4.60 (*m*) partially obscured by the C-30 protons, *trans* substituted protons on the group Ar—CH=CH—CO_2— (δ 6.29, 1H, *d*, *J* = 15.8 and δ 7.54, 1H, *d*, *J* = 15.8), two aromatic methine protons (δ 6.65, *d*, *J* = 1.8 and δ 6.82, *d*, *J* = 1.8), a methoxyl singlet at δ 3.88 and two phenolic hydroxyl groups at δ 8.21 and 8.65 (D_2O exchangeable). The presence of the hydroxyl groups was further confirmed by the formation of a diacetate (**2**) whose acetate groups showed two 6H singlets at δ 2.32 and 2.35.

The mass spectrum show significant and diagnostic fragmentation ions that are rationalized in Scheme 1. The ions at *m/z* 193 (100%) and 210 (57%) are attributed to the feruloyl fragments (Ar—CH-



Scheme 1. Diagnostic ions and fragments in the mass spectrum of compound **1**

=CH—C≡O$^+$ and Ar—CH=CH—CO_2H, respectively). The ion at *m/z* 426 is assigned to lupeol which on loss of H_2O gave an ion at *m/z* 408. The fission of ring C gave an ion peak at *m/z* 190 arising from the ring D and E fragment and another ion peak at *m/z* 218 due to the fragment from rings A and B after the loss of H_2O. The subsequent hydrogen loss from this fragment gave another ion peak at *m/z* 189. McLafferty rearrangement of **1** produced fragment ions at *m/z* 210 and 408. Thus the foregoing mass spectral evidence confirm the presence and connection at C-3 of the feruloyl and lupene moieties in **1**.

The ^{13}C NMR spectrum showed characteristic carbon signals for the lupene moiety. The only difference was that the C-3 signal was deshielded to δ 80.9, as compared to the free alcohol (δ 78.8), confirming the linkage between the lupene and feruloyl moieties to be at C-3. For the substituted cinnamoyl moiety a detailed ^{13}C and ^{1}H NMR study, and the selective INEPT experiments [6–8] were fully in agreement with the presence of a 5-hydroxyferuloyl structure.

On alkaline hydrolysis, compound **1** afforded lupeol and 5-hydroxyferulic acid, identified by direct comparison with authentic samples. Thus the structure of **1** is concluded to be 3β-(5-hydroxyferuloyl)lupen-20(30)-ene.

The isolates from *E. divinorum* were tested for their cytotoxic activity (ED_{50} < 20 μg ml^{-1}) against a panel of cell lines: BC-1 (human breast cancer), Col-2 (human colon cancer), HT (human fibrosarcoma), KB (human nasopharyngeal carcinoma), KB-V (vinblastine resistant KB evaluated in the presence and absence of vinblastine), Lu-1 (human lung cancer), Me-1 (human melanoma), P-388 (murine lymphocytic leukemia), A431 (human epidermoid carcinoma), LNCaP (human prostate cancer), ZR-75-1 (human breast cancer) and U373 (human glioblastoma). The new compound **1** and 7-methyljuglone (**3**) showed cytotoxic activity, while the rest of the compounds were classed as inactive. Compound **3** was cytotoxic against all cell lines and its most intense responses were observed with KB, P-388, LNCaP, ZR-75-1 and U373 cells at 4.8, 0.1, 0.8, 2.2 and 2.7 μg ml^{-1}, respectively. However, compound **1** was selective, and only showed activity against two cell lines: P-388 and ZR-75-1 at 2.1 and 4.2 μg ml^{-1}, respectively.

EXPERIMENTAL

General. UV: EtOH; IR: KBr; ^{1}H NMR: (400 MHz in $CHCl_3$) and ^{13}C NMR: 100 MHz in $CHCl_3$; EI-MS: 70 eV.

Plant material. Roots of *E. divinorum* were collected from the Midlands Province in Zimbabwe and the plant was identified by Mr S. Mavi of the National Herbarium and Botanic Gardens of Harare Zimbabwe. A voucher specimen has been deposited in the National Herbarium and Botanic Gardens in Harare.

Extraction and isolation. The air dried root bark (665 g) was ground and extracted with $CHCl_3$. The $CHCl_3$ extract was evapd under red. pres. to yield a brown residue (12 g). The residue was subjected to CC on silica gel eluted successively with *n*-hexane–EtOAc solvent mixt. of increasing polarity. The eluate from *n*-hexane–EtOAc (9:1) yielded compound **3** (2.2 g). Further eluates gave mixts of compounds which were further purified by repeated silica gel CC. Elution with *n*-hexane–EtOAc (4:1) gave compound **1** (673 mg). The other known compounds: lup-20(30)-ene, lupeol, butelin, catechin, shinalone and isodisopyrin were also isolated in the process with various mixts of the solvent system.

3β-(5-*hydroxyferuloyl*)*Lup*-20(30)-*ene* (**1**). Orange-red powder. UV λ_{max} nm: 235 (log ε 3.29), 326 (log ε 3.22); IR ν_{max} cm^{-1}: 3540–3320, 2935, 2850, 1690, 1370, 1180, 970, 885; ^{1}H NMR: δ 0.76, 0.87, 0.86, 0.89, 0.93 and 1.02 (6 tert-Me), 1.66 (*s*, Me-29), 1.23–2.0 (*m*, CH_2, s and CH's), 2.30 (*m*, H-19), 3.88 (*s*, MeO), 4.50–4.60 (*m*, H-3), 4.58 (*br d*, *J* = 2.4 Hz, Ha-30), 4.70 (*br d*, *J* = 2.4 Hz, Hb-30), 6.29 (*d*, *J* = 15.8 Hz, H_α), 7.54 (*d*, *J* = 15.8 Hz, H_β), 6.79 (*br d*, *J* = 1.8 Hz, H-2′), 6.63 (*br d*, *J* = 1.8 Hz, H-6′); ^{13}C NMR: δ 14.5 (C-27), 16.0 (C-26), 16.2 (C-25), 16.7 (C-24), 18.0 (C-28), 18.2 (C-6), 19.3 (C-29), 20.9 (C-11), 23.8 (C-2), 25.1 (C-12), 27.4 (C-15), 28.0 (C-23), 29.8 (C-21), 34.2 (C-7), 35.6 (C-16), 37.1 (C-10), 38.0 (C-13), 38.4 (C-1), 39.0 (C-4), 40.0 (C-22), 40.8 (C-8), 42.8 (C-14), 43.0 (C-17), 48.0 (C-18), 48.3 (C-19), 50.3 (C-9), 55.4 (C-5), 56.2 (MeO), 80.9 (C-3), 109.3 (C-30), 150.9 (C-20), 102.9 (C-6′), 109.2 (C-2′), 116.8 (C-α), 126.6 (C-1′), 134.5 (C-4′), 143.9 (C-3′), 144.4 (C-β), 146.9 (C-5′), 167.2 (C=O); MS *m/z* (rel. int.): 618 [M]$^+$ (76),

426 (10), 408 (15), 218 (22), 210 (56), 193 (100), 190 (22), 189 (35).

Acetylation of **1**. Compound **1** (10 mg) was treated with 2 ml of Ac_2O–pyridine (1:1) at room temp. for 24 hr. The usual work-up afforded a diacetate **2** (6 mg).

Hydrolysis of **1**. Compound **1** (18 mg) was refluxed with 5% KOH–MeOH soln (10 ml) on a steam bath for 8 hr. The reaction mixt. was diluted with H_2O (25 ml), and extracted with EtOAc to afford lupeol. The aq. layer was neutralized with HCl and evapd. The addition of MeOH and evapn was repeated × 3. The residue was extracted with $CHCl_3$, purified by prep. TLC to give ferulic acid. The products of hydrolysis were identified by direct comparison with authentic samples.

Evaluation of cytotoxic potential. Cytotoxicity of the isolates was evaluated using cell culture systems, according to procedures described in refs [9].

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