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FLAVONOIDS FROM *EUCLEA DIVINORUM*

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ABSTRACT. Chemical investigation of the aerial parts of *Euclea divinorum* resulted in the isolation of four flavonoids, namely aromadendrin-3-*O*-ß-L-arabinopyranoside (+)catechin, quercetin-3-*O*-α-L-rhamnopyranoside and myricetin-3-*O*-α-L-rhamnopyranoside. Aromadendrin-3-*O*-ß-L-arabinopyranoside is a new natural product.

INTRODUCTION

A few members of the genus *Euclea* (Ebenaceae), have yielded some naphthoquinones, naphthols and triterpenes [1,2,3].

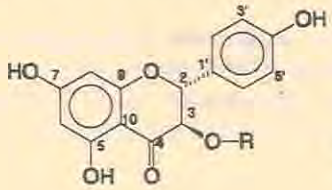
Euclea divinorum Hiern grows widely in Central and Southern Ethiopia and also in many other parts of Africa. Previous chemical investigations of the roots of this plant, collected from Mozambique and S. Africa, revealed the occurrence of two triterpenes, six naphthoquinones and related compounds [2,4]. We report here the isolation and characterization of (2R:3R)-aromadendrin-3-*O*-β-L-arabinopyranoside and three known flavonoids from the aerial parts.

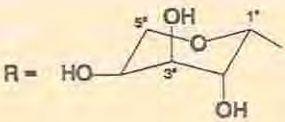
RESULTS AND DISCUSSION

The aerial parts of *Euclea divinorum* were extracted with EtOH in a Soxhlet apparatus. The resulting residue, obtained after removal of the solvent, was partitioned between EtOAc and H_2O. The EtOAc extract was subjected to column chromatography over silica gel 60 to give compounds **1** to **4**.

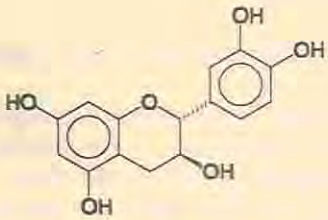
The HRMS (FAB) of compound **1** established the molecular formula $C_{20}H_{21}O_{10}$. The ^1H NMR spectrum showed a typical AB spin system at δ 4.96 and 5.28 exhibiting coupling constants of 11 Hz, which together with UV absorption bands at 290 and 330 (sh) nm suggested a dihydroflavonol skeleton [5]. This was further corroborated by the ^{13}C NMR spectrum which displayed signals at δ 81.5 and 94.8 assignable to C-3 and C-2, respectively [6]. In addition, the ^1H and ^{13}C NMR spectra indicated the presence of a 4'-hydroxylated ring B, a 5,7-dihydroxylated ring A and an *O*-pentoside. The pronounced deshielding of the C-3 resonance requires placement of the sugar unit at this position [6]. The sugar moiety was identified as L(+)arabinose on the basis of the spectral data and by co-PC of the sugar obtained after acid hydrolysis of **1** with authentic arabinose. The pyranose ring form of arabinose was derived from the

resonances of C-2" and C-4" which appeared at δ 71.2 and 69.4, respectively [7]. Furthermore the values of $J_{1''-2''}$ = 3.90 Hz and $J_{2''-3''}$ = 6.6 Hz of the peracetate of **1** and comparison of these with values of model compounds suggested the ß-L-arabinopyranosyl linkage. Comparison of the $[\alpha]_D$ of the aglycone (+27.5 c 0.04 in MeOH) with that reported [8] for (2R:3R)-aromadendrin (+26.5 c 2 in MeOH) showed a very good agreement. The configuration of **1** can also be assigned (2R:3R) as the glycosylation at the 3-OH group will only cause little change on the CD curve. On the basis of CD data analogous dihydroflavonol-3-*O*-glycosides such as the 3-*O*-rhamnosides of aromadendrin and dihydroquercetin have also been assigned the (2R:3R) configuration [9]. Based on the combined evidence presented above **1** was characterized as the new compound (2R:3R)-aromadendrin-3-*O*-ß-L-arabinopyranoside.

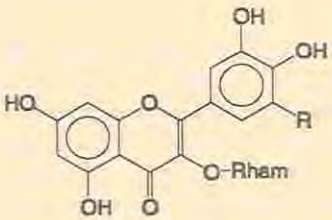




1



2



3 R = H
4 R = OH

The HRMS of compound **2** showed a M⁺ at *m/z* 290 analyzing for $C_{15}H_{14}O_6$ and its ^1H and ^{13}C NMR spectra suggested a flavan-3-ol skeleton. Accordingly the MS spectrum displayed the two RDA fragment ions at *m/z* 137 and 152 indicating the presence of five hydroxyl groups [5], whose positions were determined to be at C-3, 5, 7, 3' and 4' from ^1H and ^{13}C NMR spectra. The ^1H NMR spectrum revealed a doublet at δ 4.52 which is assignable to H-2. $J_{2,3}$ had a value of 8 Hz in accord with a *trans* relationship between H-2 and H-3 [10]. The $[\alpha]_D$ was +9 (in MeOH, c 0.005) indicating a (2R:3S) configuration [10]. Compound **2** was thus identified to be catechin.

The ^{13}C NMR spectrum of compound **3** indicated the presence of 21 carbons and its molecular weight was found to be 448 as determined by FABMS. These findings together with the IR and ^1H NMR spectra suggested a molecular formula of $C_{21}H_{20}O_{11}$ corresponding to a flavonol-3-*O*-glycoside skeleton. Furthermore, the UV spectrum of **3** and its changes after addition of shift reagents [5] revealed the presence of free hydroxyl groups at C-5, 7, 3' and 4' on a 3-*O*-substituted flavonol framework. Accordingly ^1H and ^{13}C NMR spectra of **3** established the aglycone and the sugar

moieties to be 3,5,7,3',4'-pentahydroxyflavone or quercetin and L(+)rhamnose, respectively. Further support was provided by co-chromatography of the hydrolysis products of **3** with authentic quercetin and L(+)rhamnose. The resonance of the anomeric proton of the rhamnose unit appeared at δ 5.52 as a doublet (J = 1.1 Hz) indicating the *eq-eq* relationship to the vicinal H-2". This suggested the α-rhamnopyranosyl linkage [11], which is also consistent with resonances of C-3" and C-5" [12]. On the basis of the above **3** was characterized as the known compound quercetin-3-*O*-α-L-rhamnopyranoside, also known as quercitrin.

Table 1. ^{13}C NMR data of compounds **1**, **2**, **3** and **4**.

C	1	2	3	4
2	94.8	79.8	158.0	157.8
3	81.5	66.1	135.4	134.7
4	194.1	28.5	178.6	178.1
5	163.3	154.6	162.4	161.6
6	96.0	94.0	98.6	99.1
7	166.5	154.3	164.5	164.5
8	94.8	93.4	94.1	94.0
9	162.1	154.9	157.4	156.8
10	100.0	98.3	115.8	104.5
1'	126.5	129.5	122.3	120.2
2'	128.5	112.9	116.5	108.5
3'	114.8	143.1	145.2	146.1
4'	157.0	143.1	148.5	136.8
5'	114.8	113.8	116.0	146.1
6'	128.5	117.8	122.3	108.5
1"	100.6	-	102.3	102.2
2"	71.2	-	70.5	70.8
3"	74.3	-	71.0	70.8
4"	69.4	-	71.7	71.8
5"	62.3	-	70.5	70.4
6"	-	-	17.3	17.8

The FABMS of compound **4** indicated a MH^+ at *m/z* 465 corresponding to a molecular formula of $C_{21}H_{20}O_{12}$. A detailed examination of the spectral data and comparison with that of **3** established compound **4** to be a flavonol-3-*O*-α-L-rhamnopyranoside. The UV spectrum confirmed the presence of C-5,7-hydroxylation in ring A and three adjacent free hydroxyl groups in ring B [5]. A C-3',4',5'-hydroxylation pattern was evident from the ^{1}H and ^{13}C NMR spectra, in which the singlet at δ 7.1 integrating for two protons was due to H-2' and 6' and the two sets of superimposable carbon resonances appearing at δ 108.5 and 146.1 were assignable to C-2',6' and C-3',5', respectively. The above spectral findings together with chemical degradation evidence established **4** to be the known compound myricetin-3-*O*-α-L-rhamnopyranoside, also known as myricitrin.

EXPERIMENTAL

General. ^1H and ^{13}C NMR spectra were measured on Varian XL-300 and Jeol FX90Q (22.5 MHz) NMR spectrometers, respectively. IR and UV spectra were recorded as KBr discs and methanolic solutions, respectively.

Plant material. Aerial parts of *Euclea divinorum* were collected from Gelgel-Ghibe, alt. 1800 m, in September 1991. A voucher specimen, Ensermu 2257, has been deposited in the National Herbarium, Addis Ababa University.

Extraction and isolation of compounds. Powdered aerial parts (400 g) were extracted with EtOH in a Soxhlet apparatus for 12 hrs. The solvent was removed *in vacuo* and the residue was partitioned between EtOAc and H_2O. The EtOAc extract upon concentration yielded 30 g of a dark oily residue which was subjected to column chromatography over silica gel 60 (150 g, Merck). Elution was carried out using $CHCl_3$-MeOH mixtures of increasing polarities. A total of 55 fractions, each 150 ml, were collected (frs. 1-13 ($CHCl_3$), 14-24 (5% MeOH), 25-44 (15%) and 45-55 (25%)). Fractions 2-3 and 5-8 yielded lupeol (200 mg) and ß-sitosterol (500 mg), respectively. Fractions 30-31 were rechromatographed over silica gel eluting with petrol-EtOAc, 1:2 and then passed through Sephadex LH-20 ($CHCl_3$-MeOH, 1:1) to give compounds **1** (400 mg) and **2** (300 mg). Analogous treatment of fractions 32-34 and 47-53 afforded compounds **3** (200 mg) and **4** (1.2 g), respectively.

Acid Hydrolysis of compounds 1, 3 and 4. A solution of the sample and 2 N HCl in MeOH was refluxed on a water bath for 1 hr, which was then cooled and evaporated to dryness. The resulting residue was partitioned in EtOAc-H_2O system to give the aglycone and sugar moieties. The aglycones were also characterized after hydrolysis by comparison of their physical and spectral data with those reported in the literature, whereas the sugars were identified by co-PC with authentic specimens.

(2R:3R)-Aromadendrin-3-O-ß-L-arabinopyranoside (**1**). Oil; $[\alpha]_D$ -17 (MeOH, c 0.001); Found $[M+H]^+$ 421.1147 $C_{20}H_{21}O_{10}$, requires 421.1140; UV λ_{max} nm: 294, 330 (sh); IR ν_{max} cm^{-1}: 3500, 1650, 1470, 1370, 1260, 1170, 1080; ^1H NMR ($CDCl_3$-MeOH-d_4) δ: 3.3 -3.8 (5H, *m*, arabinopyranosyl), 3.89 (1H, *d*, *J*=3.7 Hz, H-1"), 4.96 (1H, *d*, *J*=11 Hz, H-3), 5.28 (1H, *d*, *J*=11 Hz, H-2), 5.97 (1H, *d*, *J*= 2.1 Hz, H-6), 6.01 (1H, *d*, *J*=2.1 Hz, H-8), 6.91 (2H, *d*, *J*=8.7 Hz, H-3',5'), 7.45 (2H, *d*, *J*=8.7 Hz, H-2',6'), 11.69 (1H, *s*, 5-OH). ^{13}C NMR (Me_2CO-d_6) see Table 1; FABMS *m/z* (rel. int.): 421 $[M+H]^+$ (43), 288 (37).

Acetate derivative of **1**. Treatment of **1** with Ac_2O/Py at RT followed by the usual workup yielded hexaacetate derivative. Oil; ^1H NMR ($CDCl_3$) δ: 1.98, 2.06, 2.09, 2.30, 2.31, 2.40 (18H, 6 X *s*, 6 X OAc), 3.47 (1H, *dd*, *J*=12.0, 3.5 Hz, H-5"$_{eq}$), 3.83 (1H, *d*, *J*=3.9 Hz, H-1"), 4.23 (1H, *dd*, *J*=11.9, 6.8 Hz, H-5"$_{ax}$), 4.50 (1H, *d*, *J*=11 Hz, H-3), 4.92 (1H, *dd*, *J*= 6.7, 3.3 Hz, H-3"), 4.95 (1H, *dd*, *J*=6.6, 3.7 Hz, H-2"), 5.15 (1H, *m*, H-4"), 5.45 (1H, *d*, *J*= 11 Hz, H-2), 6.58 (1H, *d*, *J*=2.2 Hz, H-8), 6.75 (1H, *d*, *J*=2.2 Hz, H-6), 7.15 (2H, *d*, *J*=8.5 Hz, H-3',5'), 7.47 (2H, *d*, *J*=8.5 Hz, H-2',6').

(+)Catechin (**2**). White needles from H_2O, Mp 175-177° (lit. [13] 177°); $[\alpha]_D$ +9 (MeOH, c 0.005), (lit. [13] +10.5 (Me_2CO, c 0.4)); Found $[M]^+$ 290.0778 $C_{15}H_{14}O_6$, requires

290.0794; IR ν_{max} cm^{-1}: 3400, 1620, 1530, 1480, 1380, 1290, 1150, 1050; ^1H NMR (Me_2CO-d_6) δ: 2.53 (1H, *dd*, *J*=16.2, 8.2 Hz, H-4_{ax}), 2.92 (1H, *dd*, *J*=16.2, 5.4 Hz, H-4_{eq}), 4.00 (1H, *m*, H-3), 4.52 (1H, *d*, *J*=8.0 Hz, H-2), 5.88 (1H, *d*, *J*=2.0 Hz, H-6), 6.05 (1H, *d*, *J*=2.0 Hz, H-8), 6.76 (1H, *dd*, *J*=8.5, 2.0 Hz, H-6'), 6.81 (1H, *d*, *J*=8.5 Hz, H-5'), 6.90 (1H, *d*, *J*=2.0 Hz, H-2'); ^{13}C NMR (Me_2CO-d_6) see Table 1, in good agreement with lit. [6]; EIMS *m/z* (rel. int): 290 $[M]^+$ (50), 152 (30), 137 (37), 123 (100), 110 (50).

Quercetin-3-O-α-L-rhamnopyranoside or quercitrin (**3**). Light yellow amorphous solid; mp 185-188° (lit. [14] 176-179°); UV λ_{max} nm: 256, 300, 348; +$AlCl_3$: 264, 320, 430; +$AlCl_3$-HCl: 268, 300, 400; +NaOAc: 262, 300, 350; IR ν_{max} cm^{-1}: 3300, 1660, 1620, 1510, 1460, 1370, 1210, 1180, 1080, 1000; ^1H NMR (Me_2CO-d_6) δ: 0.92 (3H, *d*, *J*=5.8 Hz, 6"-Me), 3.39 (1H, *m*, H-4"), 3.62 (1H, *m*, H-5"), 3.71 (1H, *m*, H-3"), 4.21 (1H, *m*, H-2"), 5.52 (1H, *d*, *J*=1.1 Hz, H-1"), 6.27 (1H, *d*, *J*=2.1 Hz, H-6), 6.47 (1H, *d*, *J*=2.1 Hz, H-8), 7.00 (1H, *d*, *J*=8.4 Hz, H-5'), 7.42 (1H, *dd*, *J*=8.4, 2.1 Hz, H-6'), 7.51 (1H, *d*, *J*=2.1 Hz, H-2'), 12.65 (1H, *s*, 5-OH); ^{13}C NMR (DMSO-d_6): see Table 1, in good agreement with lit. [6]; FABMS *m/z* (rel. int.): 449 $[M+H]^+$ (43), 303 (67).

Myricetin-3-O-α-L-rhamnopyranoside or myricitrin (**4**). Pale yellow solid, mp 196-200° (lit. [15] 196-197°); UV λ_{max} nm : 258, 300, 354; +$AlCl_3$: 268, 312, 420; +$AlCl_3$-HCl: 270, 308, 402; +NaOMe: 260, 294, 400 (dec); +NaOAc: 264, 300, 354; +NaOAc-H_3BO_3: 258, 298, 374, IR ν_{max} cm^{-1}: 3300, 1660, 1620, 1510, 1360, 1300, 1200, 1180, 1040, 1000; ^1H NMR (Me_2CO-d_6) δ: 0.95 (3H, *d*, *J*=5.6 Hz, 6"-Me), 3.35 (1H, *m*, H-4"), 3.52 (1H, *m*, H-5"), 3.75 (1H, *m*, H-3"), 4.20 (1H, *m*, H-2"), 5.50 (1H, *d*, *J*=1.2 Hz, H-1"), 6.25 (1H, *d*, *J*=2.0 Hz, H-6), 6.45 (1H, *d*, *J*=2.0 Hz, H-8), 7.10 (2H, *s*, H-2',6'), 12.60 (1H, *s*, 5-OH); ^{13}C NMR (DMSO-d_6) see Table 1, in good agreement with lit. [6]; FABMS *m/z* (rel. int.): 465 $[M+H]^+$ (47), 319 (71).

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