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PHLOROGLUCINOL DERIVATIVES AND FLAVONES FROM *HELICHRYSUM PARONYCHIOIDES*

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ABSTRACT. Investigation of *Helichrysum paronychioides* afforded a total of nine compounds: 4 phloroglucinol derivatives, 2 of which are novel natural products, and 5 flavone derivatives. Structures were established by various spectroscopic techniques (NMR, MS, UV, IR, CD) and by comparison with literature data for the known compounds. The four phloroglucinols, *trans*-(2*R*,3*R*)-5,7-dihydroxy-2,3-dimethyl-4-chromanone (**1**), 2-butanoyl-4-prenyl-1-methoxy phloroglucinol (**2**), 2-(2-methylpropanoyl)-4-prenylphloroglucinol (**3**) and 2-(2-methyl- butanoyl)-4-prenylphloroglucinol (**4**) were screened for antioxidant activity against Cu-induced LDL oxidation. Compound **4** was found to be the most active inhibiting LDL oxidation at all concentrations (0.5-10 μM) while the other three showed moderate to no activity.

KEY WORDS: *Helichrysum paronychioides*, Asteraceae, Phloroglucinol derivatives, CD spectroscopy, Synthesis

INTRODUCTION

The genus *Helichrysum* (Asteraceae, tribe Inuleae), consisting of about 500 species, is widely distributed in Africa, Australia, Europe and Asia [1, 2]. About 264 species are known to occur in southern Africa, eight of which are recorded in Botswana [3, 4]. Studies of several *Helichrysum* species indicate the presence of many metabolites, mainly phloroglucinol derivatives [5-9] and flavonoids [10-13] among other secondary metabolites. Most of the reported phloroglucinols consist mainly of two types of substituents: a prenyl/geranyl group and an acyl group, the most common acyl substituents being butanoyl, isobutanoyl and 2-methylbutanoyl. The prenyl or geranyl groups may undergo cyclization leading to the formation of chromane derivatives [5-9]. The above classes of compounds exhibit antibacterial, antifungal, antiviral and antioxidant activities [11, 13-17]. As part of a program to investigate marketed medicinal plant species of Botswana, we examined *Helichrysum paronychioides* (*phate-ya-ngaka* in Setswana) [18], a mat-forming sub-shrub, locally sold for the treatment of constipation, coughs and as an analgesic. The results of the above investigation are presented in this paper.

RESULTS AND DISCUSSION

Fractionation of the CH_2Cl_2/MeOH extract by flash chromatography followed by various purification processes *viz* Sephadex LH-20 and preparative TLC resulted in the isolation and characterization of two novel phloroglucinol derivatives, (2*R*,3*R*)-5,7-dihydroxy-2,3-dimethyl-4-chromanone (**1**, 110 mg) and 2-butanoyl-4-prenyl-1-methoxyphloroglucinol (**2**, 6 mg) as well as seven known compounds namely, 2-(2-methylpropanoyl)-4-prenylphloroglucinol (**3**, 20 mg), 2-(2-methylbutanoyl)-4-prenylphloroglucinol (**4**, 14 mg) [7], 3-methylquercetin (5.2 mg), 3,3´-dimethylquercetin (3.1 mg), 3,7-dimethylkaempferol (6 mg), penduletin (1.8 mg) and eupalitin (3.2 mg) [19-24] which, however, were isolated from *H. paronychioides* for the first time. They

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were identified by their NMR, MS, UV-Vis and IR spectra data in comparison to the reported data.

Compound **1** was obtained as shiny, green needles from 10% EtOAc/petroleum ether with m.p. of 156-7 ˚C and $[\alpha]_D^{22}$ + 107^o (MeOH, *c* 0.1). The molecular formula of **1** was established as $C_{11}H_{12}O_4$ on the basis of HREIMS which showed a molecular ion at *m/z* 208.07435 (calcd. for $C_{11}H_{12}O_4$ 208.07356). LREIMS showed significant fragments at *m/z* 208 [M^+], 193 [M-15]$^+$, 152 [M-C_4H_8]$^+$ and 124 [M-C_4H_8CO]$^+$. The IR spectrum showed the presence of a hydrogen-bonded –OH (3236 cm^{-1}), a conjugated carbonyl carbon (1638 cm^{-1}) and a C-O stretch (1159 cm^{-1}).

The ^{1}H NMR spectrum of **1** showed a proton signal at δ 12.26 attributed to a chelated hydroxyl group, two *meta* coupled aromatic proton signals resonating at δ 5.94 (1H, d, *J* = 2.0 Hz), and 5.92 (1H, d, *J* = 2.0 Hz), two sp^3 proton signals at δ 4.26 (1H, dq, *J* = 11.0 6.4 Hz) and 2.65 (1H, dq, *J* = 11.0, 7.1 Hz) and, two methyl resonances at δ 1.49 (3H, d, *J* = 6.4 Hz) and 1.19 (3H, d, *J* = 7.1 Hz). The ^{13}C NMR data (see Table 1) indicated the presence of eleven carbons, two of which were methyls and four methine carbons according to the DEPT experiment. On the basis of these spectra data, COSY, HMBC and NOESY experiments, compound **1** was deduced to have the structure shown in Figure 1.

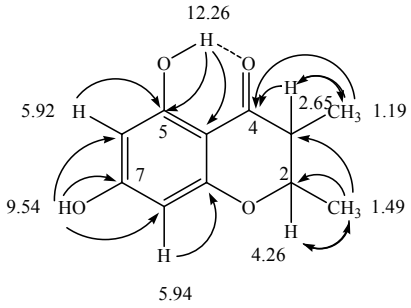


Figure 1. ^{1}H NMR, HMBC (↷) and NOESY (↶) of 5,7-dihydroxy-2,3-dimethyl-4-chromanone (**1**).

Table 1. ^{1}H and ^{13}C NMR spectra data for **1** (CD_3COCD_3, 600 MHz).

Position	^{1}H, δ, (*J* in Hz)	^{13}C NMR
2	4.26, dq, (11.0,6.4)	79.2
3	2.65, dq, (11.0,7.1)	45.8
4	-	199.0
4a	-	101.9
5	-	166.6
5-OH	12.26	-
6	5.91, d, (2.0)	96.2
7	-	164.8
7-OH	-	-
8	5.94, d, (2.0)	94.9
8a	-	163.5
9	1.49, d, (6.4)	19.3
10	1.19, d, (7.1)	9.7

The relative stereochemistry of the proton signals at C-2 and C-3 was deduced to be *trans* from the coupling constant (J = 11.0 Hz) and from nuclear Overhauser enhancements (NOE) observed during irradiation of the proton signals at δ 4.26 and 2.65 which showed enhancement of only the methyl signals at δ 1.49 (0.91%) and 1.19 (1.42%), respectively.

Although compound **1** is herewith reported for the first time as a natural product, it is a principle building block of *Calophyllum* metabolites [25-30] and thus a precursor towards the total synthesis of an anti-HIV-1 compound, (±)-calanolide A [31]. The absolute configuration of **1**, (2*R*,3*R*)-5,7-dihydroxy-2,3-dimethyl-4-chromanone, was inferred from the already established configuration of papuanic acid isolated from *Calophyllum papuanum* [28, 32]. For further proof of the structure, total synthesis of compound **1** was achieved by Friedel-Crafts acylation of phloroglucinol and tigloyl chloride with subsequent Michael Addition type reaction giving a 1:1 mixture of *trans* and *cis* products, as also reported in the synthesis of both papuanic acid [28] and (±)-calanolide A [31].

Compound **1** may also be envisaged as consisting a chromophore similar to that of 2,3-hydroxyflavanones. It has been reported that flavanones and 3-hydroxyflavanones, which exist in the thermodynamically favored conformation with the 2 or 2,3-substituents equatorial, exhibit four Cotton effects in the order (+), (-), (+) and (+) from 400 to 200 nm and thus have been assigned the 2*R*,3*R* configuration [33]. Comparison of the CD spectra of these compounds to that of **1**, indicates the similarity in the curves hence providing a further confirmation to the assigned absolute configuration of chromanone (**1**) as (2*R*,3*R*)-5,7-dihydroxy-2,3-dimethyl-4-chromanone, Figure 2.

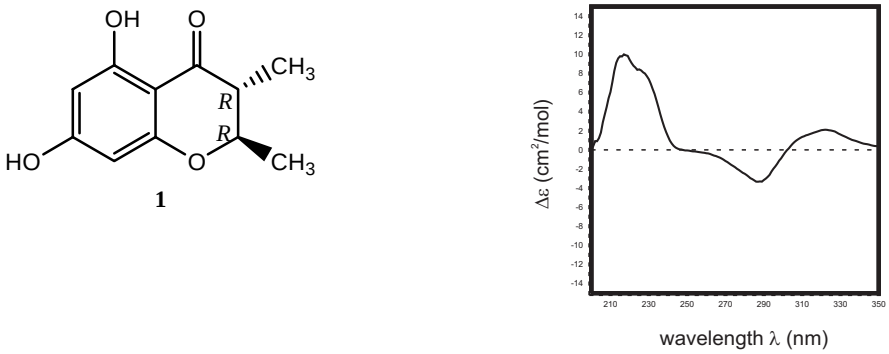


Figure 2. The absolute configuration and CD spectrum of (2*R*,3*R*)-5,7-dihydroxy-2,3-dimethyl-4-chromanone (**1**).

The molecular formula of compound **2** was deduced to be $C_{16}H_{22}O_4$ on the basis of LREIMS, which showed characteristic ion peaks at *m/z* 278 [M^+], 263 [M-15]$^+$, 235 [M-C_3H_7]$^+$ and 179 [235-C_4H_8]$^+$, and ^{13}C NMR data. The ^{1}H NMR spectrum consisted of two proton signals at δ 14.37 and 9.37 representing hydroxy groups; an aromatic proton signal at δ 6.13 (1H, s) and a three-proton signal at δ 3.87 (3H, s) due to a methoxy group. Additional features of the spectrum were proton signals resonating at δ 5.23 (1H, t, J = 7.2 Hz), 3.26 (2H, d, J = 7.2 Hz), 1.75 (3H, s), 1.63 (3H, s) and, 2.96 (2H, t, J = 7.4 Hz), 1.65 (2H, m, J = 7.4 Hz) and 0.96 (3H, t, J = 7.4 Hz) attributed to the presence of a prenyl and a butanoyl group, respectively.

The COSY and DEPT spectra supported the presence of two substituents: in COSY, the proton signals at δ 1.75 and 1.63 both showed correlation to the proton signals at δ 3.26 and 5.23 (prenyl group) whilst for the butanoyl group, the methyl-proton signal at δ 0.96 correlated to the

signals at 1.65 and 2.96. DEPT consisted of eight protonated carbons: 3 methyls, 3 methylenes, a methine and a methxoxy carbon. ^{13}C NMR indicated the presence of three oxygenated carbons at 166.1, 163.1 and 162.6 consistent with a phloroglucinol unit. The position of the methoxy group was identified by HMBC correlations in which the proton signal at δ 3.87 showed a ^{3}J correlation to 162.6 whereas the proton signal of the free hydroxyl group showed a ^{2}J and ^{3}J correlations to 163.1 and 109.1, respectively, Figure 3.

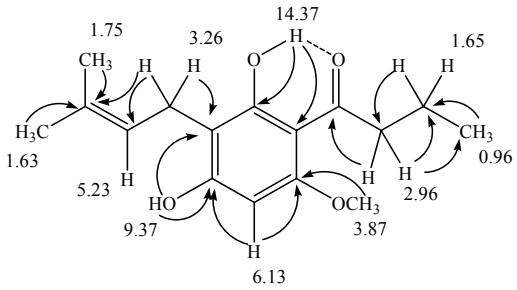
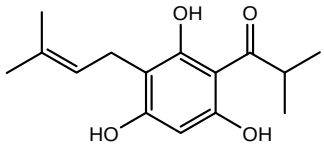


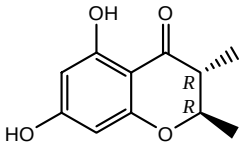
Figure 3. ^{1}H NMR and HMBC ( ) of 2-butanoyl-4-prenyl-1-methoxyphloroglucinol (**2**).

The four phloroglucinol derivatives, (2*R*,3*R*)-5,7-dihydroxy-2,3-dimethyl-4-chromanone (**1**), 2-butanoyl-4-prenyl-1-methoxyphloroglucinol (**2**), 2-(2-methylpropanoyl)-4-prenylphloroglucinol (**3**) and 2-(2-methylbutanoyl)-4-prenylphloroglucinol (**4**) (denoted as HP5, HP57.4, HP10 and HP34.12, respectively, in Figures 4 and 5), were screened for antioxidant activity against Cu-induced Low Density Lipoprotein (LDL). The results obtained showed that compound **4** (HP34.12, Figure 4) was the most active, it inhibited LDL oxidation at all concentrations (from 0.5 to 10 μM) followed by compound **3** (HP10, Figure 5) which was active at doses above 0.5 μM. The activities of these two compounds were comparable to that of quercetin, whose antioxidnat activity has been determined in the same laboratory in previous experiments [34]. Compound **2** (HP57.4) showed moderate activity while **1** (HP5) was inactive at the highest concentration tested (10 μM).

The trend in the antioxidant activities for these compounds may be explained based on their structural features: (1) antioxidant activity is attributed to the ability of the compound to readily donate a phenolic hydrogen to lipid free radicals induced by copper hence the number of free phenolic hydrogen groups is important. In this regard, 2-(2-methylpropanoyl)-4-prenylphloroglucinol (**3**) and 2-(2-methylbutanoyl)-4-prenylphloroglucinol (**4**), possessing three free phenolic hydroxyl groups, were more active than compounds **1** and **2**, with only two phenolic hydroxyl groups. (2) Antioxidant activity also seems to depend on the presence of a prenyl unit on the benzene ring, a characteristic feature which compound **1** lacks. (3) The length of the carbon chain in the acyl group also seems important since 2-(2-methylbutanoyl)-4-prenylphloroglucinol (**4**), with a four carbon chain showed more activity compared to 2-(2-methylpropanoyl)-4-prenylphloroglucinol (**3**) possessing a three carbon chain. Both the prenyl unit and the carbon chain attached to the carbonyl enhance lipophilicity.



HP10



1 (HP5)

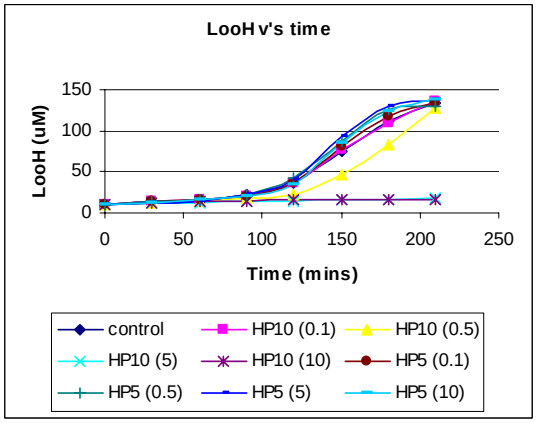
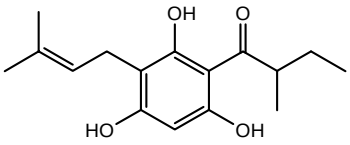
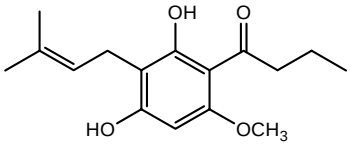


Figure 4. Graph showing the antioxidant activity of 2-(2-methylpropanoyl)-4-prenyl-phloroglucinol (**3**, HP10) and (2*R*,3*R*)-5,7-dihydroxy-2,3-dimethyl-4-chromanone (**1**, HP5).



HP34.12



HP57.4

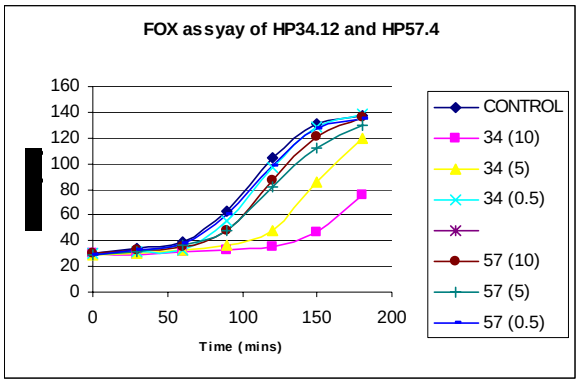


Figure 5. Graph showing the antioxidant activity of 2-(2-methylbutanoyl)-4-prenyl-phloroglucinol (**4**, HP34.12) and 2-butanoyl-4-prenyl-1-methoxyphloroglucinol (**2**, HP57.4).

EXPERIMENATAL

General. Melting points are uncorrected. UV-vis spectra were recorded using a Schimadzu UV-2101 PC UV-vis Scanning Spectrometer. The IR spectrum was measured as a KBr pellet on Perkin Elmer System 2000 FT-IR spectrometer. Optical rotation was determined on a JASCO P-1030 polarimeter using a thermostated cell (20 °C, 10-cm cell). ^{1}H NMR spectra were obtained on a Bruker Avance 300 spectrometer at 300 MHz or on a Bruker Avance 600 spectrometer at 600 MHz. ^{13}C NMR spectra were measured on a Bruker Avance 300 spectrometer at 75 MHz or on a Bruker Avance 600 spectrometer at 150 MHz. MS were measured on a Finnigan Mat SSQ

7000 Single Quadrupole MS using a 70 eV probe. Gel filtration chromatography using Sephadex LH-20 (25-100 μM) was eluted with $CHCl_3$:MeOH (2:1). Column chromatography (CC) was carried out on silica gel 60 (0.040-0.063 mm, Merck). PTLC was carried out on silica gel 60 $PF_{254+366}$ plates (20 ×20 cm, 0.5 mm thick).

Plant material. The plant material was collected from Goodhope, South East of Botswana in February 1999. It is also sold by various vendors at the Gaborone station, Botswana. The plant species was identified by Dr. Gerald Pope and Dr. L. Turton. A voucher specimen is deposited at the Herbarium, Biological Sciences, University of Botswana (Hp 9901) and at the Royal Botanical Gardens in Kew, UK.

Extraction and isolation. The plant material (whole plant) was dried and ground to give powdered plant material (858 g) which was extracted overnight using CH_2Cl_2/MeOH (1:1) followed by MeOH (20 minutes). A portion of the organic extract (52 g) was adsorbed on silica gel (113 g) and placed on a column packed with silica gel (120 g) using petroleum ether. The column was then eluted using petroleum ether with increasing polarity of ethyl acetate. Fifteen fractions (250 mL each) were collected as follows: frs. 1-3 (20% EtOAc/petroleum ether), frs 4-7 (40% EtOAc), frs.8-10 (100% EtOAC), fr. 11 (5% MeOH/EtOAc), fr 12 (10% MeOH/EtOAc), fr. 13 (20% MeOH/EtOAC). TLC of fraction 10-13 showed the presence of similar compounds so the fractions were combined.

Fraction 3 (910 mg) was loaded onto a medium pressure liquid chromatography (14 cm x 2.5 cm) and eluted with solvent system of increasing polarity to obtain 115 sub-fractions (SF) (about 10 mL each): SF 1-47 (20% EtOAc), SF 48-66 (50% EtOAc), SF 67-79 (100% EtOAc), SF 80-92 (5% MeOH/EtOAc), SF 93-106 (10% MeOH/EtOAc), SF 107-115 (20% MeOH/EtOAc). TLC analysis of collected fractions led to the combination of various fractions which contained similar compounds. The combined SF 7-13 yielded crystals, which were washed with chloroform, and filtered to give compound **1** (110 mg).

Fractions 4-5 (300 mg) was adsorbed onto silica gel and loaded onto a column which was eluted with petroleum ether with increasing polarity of ethyl acetate. The 20% EtOAc/petroleum ether fraction was subjected to Sephadex LH-20, followed by separation on a column eluted with $CHCl_3$ and about fifteen sub-fractions were collected (about 10 mL each). Sub-fraction 10 gave 2-(2-methylbutanoyl)-4-prenylphloroglucinol (**4**, 14 mg).

Fractions 6-7 (500 mg) were purified on Sephadex LH-20 [$CHCl_3$/MeOH (2:1)] followed by PTLC [$CHCl_3$/MeOH (95:5)] resulting in the isolation of 2-butanoyl-4-prenyl-1-methoxy-phloroglucinol (**2**, 6 mg), 2-(2-methylpropanoyl)-4-prenylphloroglucinol (**3**, 20 mg) and 3,7-dimethylkaempferol (6 mg).

Portions of fraction 8 (1.33 g) were repeatedly subjected to PTLC [$CHCl_3$/MeOH (96:4)] giving 6 bands. Band III (14 mg) was purified further using PTLC [$CHCl_3$/MeOH (48:2)] yielding 3-methylquercetin (5.2 mg). Band IV (20 mg), upon further purification by PTLC [$CHCl_3$/EtOAC (45:5)], gave 3,3´-dimethylquercetin (3.1 mg) and eupalitin (3.2 mg). Band V (10 mg) was also subjected to PTLC [$CHCl_3$/EtOAC (45:5)] and afforded penduletin (1.8 mg).

(2R,3R)-5,7-Dihydroxy-2,3-dimethyl-4-chromanone (**1**). Green crystals, m.p. 156-157 °C, $[\alpha]_D^{22}$ +107° (MeOH, *c* 0.1). UV-vis: λ_{max} (MeOH) nm (log ε): 202 (7.45), 286 (7.16); λ_{max} (MeOH + $AlCl_3$) nm (log ε): 375 (6.32), 309 (7.36), 216 (7.38), 201 (7.49); λ_{max} (MeOH + $AlCl_3$ + HCl) nm (log ε): 374 (6.32), 307 (7.34), 252 (6.27), 215 (7.40), 201 (7.50). CD (MeOH): λ_{max} (Δε) 322 (+2.1), 287 (-3.3), 271 (+10.0). IR ν_{max} (KBr) cm^{-1}: 3236, 2977, 1639, 1596, 1500, 1351, 1294, 1159, 728. EIMS (probe) 70 eV, *m/z* (rel. int.): 208 [M^+] (100) 193 (10), 152 (88), 124 (40). HREIMS *m/z* 208.07435 (calcd. for $C_{11}H_{12}0_4$ 208.07356). ^1H and ^{13}C NMR: see Table 1.

2-Butanoyl-4-prenyl-1-methoxyphloroglucinol (**2**). Yellow gum, UV-vis λ_{max} (MeOH) nm (log ε): 290 (7.20), 214 (7.21). EIMS (probe) 70 eV, m/z (rel. int.): 278 [M^+] (75), 263 [M-15]$^+$ (19), 235 [M-C_3H_7]$^+$ (100), 179 [235-C_4H_8]$^+$ (68). ^{13}C NMR (CD_3COCD_3): 162.6 (C-1), 106.1 (C-2), 166.1 (C-3), 109.1 (C-4), 163.1 (C-5), 91.7 (C-6), 22.4 (C-1′), 124.4 (C-2′), 131.3 (C-3′), 18.2 (C-4′), 26.3 (C-5′), 207.2 (C-1′′), 47.1 (C-2′′), 19.3 (C-2′′), 14.7 (C- 4′′).

Preparation of tigloyl chloride. Tiglic acid (20.03 g) and phosphorus trichloride (9.53 mL) were refluxed together at 70-80 ˚C for 2 hours. After cooling, the upper, slightly yellow, layer was decanted from the lower syrupy layer [35]. The tigloyl chloride (upper layer, 89% yield) was used directly for synthesis without further distillation.

Synthesis of 5,7-dihydroxy-2,3-dimethyl-4-chromanone (**1**). A mixture of anhydrous phloroglucinol (2.5 g), anhydrous powdered aluminium chloride (3.8 g) and carbon disulphide (25 mL) were stirred for 30 minutes after which nitrobenzene (17.5 mL) was added and the mixture stirred for another 30 minutes. The reaction mixture was then placed in an oil bath (60-70 °C) and tigloyl chloride (2.5 mL) was added. After heating for about one hour, the viscous residue was poured onto crushed ice and Rochelle salt (sodium potassium tartarate, 33 g) was added. The solution was then neutralized with 40% sodium hydroxide. Nitrobenzene and carbon disulphide were removed by vigorous steam distillation [35]. After cooling, the solution was acidified with 6 M HCl and extracted with dichloromethane (5 x 20 mL). About 3 mg of sodium hydrogen carbonate (dissolved in a minimum amount of water) was added and the compound was extracted with dichloromethane (5 x 20 mL) and dried with sodium sulfate. The dried organic extract was evaporated to give a mixture of two compounds (1.9 g): the *trans* and *cis* isomers of 5,7-dihydroxy-2,3-dimethyl-4-chromanone in a 1:1 ratio. A portion of the extract (300 mg) was separated by column chromatography using petroleum ether/ethyl acetate (95:5) giving pure *trans*-(2*R*, 3*R*)-5,7-dihydroxy-2,3-dimethyl-4-chromanone (**1**, 88 mg) and *cis*-(2S,3R)-5,7-dihydroxy-2,3-dimethyl-4-chromanone (35 mg).

trans-(2R,3R)-5,7-Dihydroxy-2,3-dimethyl-4-chromanone (**1**)**.** ^{1}H NMR (CD_3COCD_3): δ 12.25 (1H, s), 5.92 (2H, d, *J* = 2.3 Hz, H-6, H-8), 4.26 (1H, dq, *J* = 10.7, 6.3 Hz, H-2), 2.64 (2H, dq, *J* = 10.7, 7.0 Hz, H-3), 1.48 (3H, d, *J* = 6.3 Hz, 2-CH_3), 1.18 (3H, d, *J* = 7.0 Hz, 3-CH_3). ^{13}C NMR (CD_3COCD_3): 79.2 (C-2), 45.7 (C-3), 198.9 (C-4), 101.9 (C-4a), 166.6 (C-5), 96.2 (C-6), 164.7 (C-7), 95.0 (C-8), 163.4 (C-8a), 19.3 (C-9), 9.7 (C-10).

*cis-(2S,3R)-5,7-Dihydroxy-2,3-dimethyl-4-chromanone***.** ^{1}H NMR (CD_3COCD_3): δ 12.19 (1H, s), 5.92 (2H, d, *J* = 2.3 Hz, H-6, H-8), 4.65 (1H, dq, *J* = 6.6, 3.1 Hz, H-2), 2.64 (1H, dq, *J* = 7.2, 3.1 Hz, H-3), 1.38 (3H, d, *J* = 6.6 Hz, 2-CH_3), 1.13 (3H, d, *J* = 7.2 Hz, 3-CH_3). ^{13}C NMR (CD_3COCD_3): 76.5 (C-2), 44.4 (C-3), 200.9 (C-4), 101.2 (C-4a), 166.7 (C-5), 96.3 (C-6), 165.1 (C-7), 95.1 (C-8), 163.3 (C-8a).

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