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PRENYLATED CHALCONES AND FLAVONES FROM THE LEAVES OF *DORSTENIA KAMERUNIANA*

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Key Word Index—*Dorstenia kameruniana* ; Moraceae ; leaves ; prenylated-flavones ; prenylated chalcones.

Abstract—Two novel flavonoids : 6,7-(2,2-dimethylchromano)-5,4′-dihydroxyflavone and 3,4-,4′,5′-*bis*-(2,2-dimethylchromano)-2′-hydroxychalcone together with the known 6-(3-methylbut-2-enyl)apigenin and two chalcones (*E*)-1-[2,4-dihydroxy-3-[3-methylbut-2-enyl]phenyl]-3-[4-hydroxyphenyl]-prop-2-en-1-one and (*E*)-1-[2,4-dihydroxy-5-[3-methylbut-2-enyl]phenyl]-3-[4-hydroxy-3-[3-methylbut-2-enyl]phenyl]-prop-2-en-1-one were characterised from leaf tissue of *Dorstenia Kameruniana*. The structures were established on the basis of spectroscopic data.

INTRODUCTION

Dorstenia (Moraceae) is a mostly tropical genus of some 170 species [1]. There are twenty three species in Cameroon where a decoction of the leaves is administered for the treatment of cough, headache and stomach pain [2]. The literature on the chemistry of this genus is scanty with only three reports appearing on *D. barnimiana* from Ethiopia [3], *D. contrajerva* from USA [4] and *D. brasiliensis* from Brasil [5] from which various vinyl substituted benzofuran derivatives and monoterpenoid substituted furocoumarins have been reported [3, 4, 5]. Recently two flavones and four phenyl propanoid derivatives were reported from the roots of *D. psilurus* [6]. *Dorstenia kameruniana* Engler is a small herb growing in the forest undergrowth. As part of our continuing systematic studies on Cameroonian *Dorstenia* species we have carried out a phytochemical analysis of the leaves of *D. kameruniana* and identified three chalcones and two flavones containing prenyl substituents.

RESULTS AND DISCUSSION

The non-polar portion obtained by flash chromatogrphy of the residue from the dichloromethane/methanol extract of the leaves of *D. Kameruniana* was found to contain sitosterol and a mixture of hydrocarbons which was not investigated further. Examination of the most polar fraction led to the isolation of copious amounts of sitosterol 3-β-D-glucopyranoside which posed some difficulties to get in pure form. This was circumvented by acetylating the crude fraction and subsequent column chromatography which yielded crystalline tetraacetate that was easily identified spectroscopically. The less polar fractions were passed through Sephadex LH-20 column followed by successive silica gel CC and preparative TLC separations to yield **1–5**.

Compound **1** was isolated as plates. Its molecular formula $C_{20}H_{18}O_5$ was derived from the NMR and EI-MS data. The colour test with magnesium and concentrated hydrochloric acid (pink), together with the NMR and UV spectral data (Experimental), showed that **1** was a 5-hydroxyflavone [7]. Also the chelated hydroxyl proton at δ 13.30 and the carbonyl group at δ 183.1 were consistent with a 5-hydroxyflavone. The ^{1}H NMR showed the presence of a 2,2-dimethylchroman group (see below) and a sharp signal of one proton at δ 6.58 which was appropriate for a proton located at C-3. The ^{1}H NMR of compound **1** further displayed signals for four aromatic protons which form an AA′BB′ system δ 6.97 (2H, *d*, $J = 9.0$ Hz), 7.89 (2H, *d*, $J = 9.0$ Hz), which can only be located on ring B. The signals of benzyl protons at δ 2.71 coupled to a methylene at δ 1.91 (*t*, $J = 6.8$ Hz) and two methyl protons at δ 1.32 (*s*, 6H) could be assigned to a 2,2-dimethylchroman. From the above observations and spectral data, two possibilities were considered, one with a linear chroman ring (**1**) or an alternative structure with an angular 7,8-chroman

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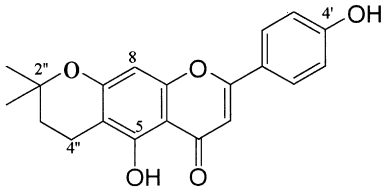

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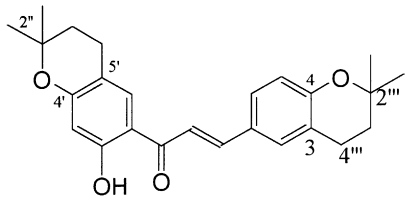

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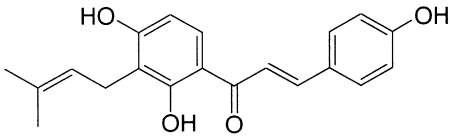

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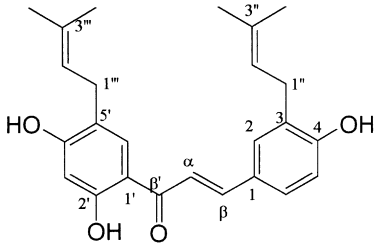

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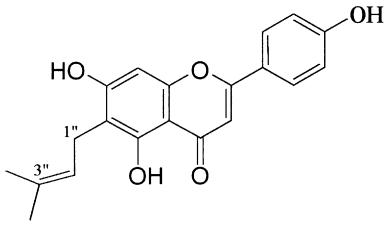

5

ring. The ^{13}C chemical shift at δ 95.5 ppm as a doublet strongly favours the linear 6,7-chroman junction [8]. The proposed structure **1** was further confirmed by ^{13}C NMR data (Table 1) which was fully assigned using DEPT spectra and by comparison of measured values with those reported for apigenin [9] and 6-(3-methylbut-2-enyl) apigenin (**5**) [10].

Compound **2** was isolated as an oil and its molecular formula was determined as $C_{25}H_{28}O_4$ from the NMR and EI-MS. The UV–vis absorptions at 378, 244 and 205 were suggestive of a chalcone skeleton. The bathochromic shift in the UV spectrum of compound **2** induced by aluminum chloride and the IR absorption at 1640 cm^{-1} indicated that **2** was a 2′-hydroxychalcone [7]. The chelated 2′-hydroxyl proton appeared at δ 13.16 and the chemical shift of the carbonyl group at δ 191.8 was consistent with a 2′-hydroxychalcone. Its ^1H NMR showed the presence of two 2,2-dimethylchroman groups (see below) and seven aryl proton resonances. Two of them form an AB system at δ 7.39 and 7.82 (*d*, $J = 15.2$ Hz), the large coupling constant indicating the *trans* geometry; a set of three protons at δ 6.82 (*d*, $J = 8.5$ Hz), 7.37 (*brs*) and 7.47 (*dd*, $J = 8.5$ and 2.2 Hz) which could be located in ring B. The remaining two proton singlets at δ 7.63 and 7.45 ppm must therefore, be located in ring

Table 1. ^{13}C NMR data of compounds **1**, **5** and apigenin (75 MHz, in $CD_3CO\,CD_3$, CD_3OD and DMSO-d_6, respectively)

Carbon	**1**	**5**	Apigenin
2	162.0 (*s*)	163.8 (*s*)	163.8 (*s*)
3	103.7 (*d*)	103.8 (*d*)	102.8 (*d*)
4	183.1 (*s*)	183.9 (*s*)	181.8 (*s*)
5	161.1 (*s*)	162.7 (*s*)	161.1 (*s*)
6	110.4 (*s*)	113.2 (*s*)	98.8 (*d*)
7	165.0 (*s*)	165.8 (*s*)	164.1 (*s*)
8	95.5 (*d*)	94.3 (*d*)	94.0 (*d*)
9	156.3 (*s*)	157.2 (*s*)	157.3 (*s*)
10	105.8 (*s*)	105.2 (*s*)	103.7 (*s*)
1′	123.2 (*s*)	123.4(*s*)	121.3 (*s*)
2′	129.2 (*d*)	129.4 (*d*)	128.4 (*d*)
3′	116.8 (*d*)	117.1 (*d*)	116.0 (*d*)
4′	160.0 (*s*)	160.0 (*s*)	161.5 (*s*)
5′	116.8 (*d*)	117.1 (*d*)	116.0 (*d*)
6′	129.2 (*d*)	129.4 (*d*)	128.4 (*d*)
1″	—	22.4 (*t*)	—
2″	76.8 (*s*)	123.6 (*d*)	—
3″	32.1 (*t*)	132.1 (*s*)	—
4″	16.6 (*t*)	—	—
(*Z*)-Me	—	18.1 (*q*)	—
(*E*)-Me	—	26.1 (*q*)	—
Me_2C	26.8 (*q*) × 2	—	—

Table 2. ^{13}C NMR spectral data of compounds **2**, **3**, and **4** at 75 MHz in CD_3COCD_3 (**2**) and CD_3OD (**3** and **4**)*

Carbon	**2**	**3**	**4**
1	126.7 (*s*)	127.9 (*s*)	127.0 (*s*)
2	130.9 (*d*)	131.8 (*d*)	129.3 (*d*)
3	121.4 (*s*)	116.9 (*d*)	130.2 (*s*)
4	156.8 (*s*)	161.5 (*s*)	159.4 (*s*)
5	117.4 (*d*)	116.9 (*d*)	116.3 (*d*)
6	127.8 (*d*)	131.8 (*d*)	131.3 (*d*)
1′	114.3 (*s*)	116.6 (*s*)	114.3 (*s*)
2′	161.3 (*s*)	163.7 (*s*)	164.7 (*s*)
3′	104.9 (*d*)	114.5 (*s*)	103.4 (*d*)
4′	164.1 (*s*)	165.2 (*s*)	165.8 (*s*)
5′	112.6 (*s*)	108.2 (*d*)	122.0 (*s*)
6′	130.1 (*d*)	130.4 (*d*)	131.9 (*d*)
α	118.1 (*d*)	118.5 (*d*)	117.9 (*d*)
β	144.5 (*d*)	145.3 (*d*)	145.8 (*d*)
β′	191.8 (*s*)	193.7 (*s*)	193.3 (*s*)
1″	—	22.5 (*t*)	29.3 (*t*)†
2″	76.1 (*s*)†	123.6 (*d*)	124.0 (*d*)‡
3″	32.7 (*t*)‡	127.9 (*s*)	133.3 (*s*)§
4″	22.4 (*t*)§	—	—
1‴	—	—	28.8 (*t*)†
2‴	75.5 (*s*)†	—	123.6 (*d*)‡
3‴	32.6 (*t*)‡	—	133.4 (*s*)§
4‴	21.9 (*t*)§	—	—
(*Z*)-Me	—	17.9 (*q*)	18.0 (*q*) × 2
(*E*)-Me	—	26.0 (*q*)	26.0 (*q*) × 2
Me_2C	27.0 (*q*) × 2	—	—
	27.0 (*q*) × 2	—	—

* Signals with the same superscripts in the same column may be interchangeable.

A. The signals that could be assigned to the two 2,2-dimethylchroman groups were as follows: four overlapping benzylic protons signals at δ 2.81 which form a quintet (4H, $J = 6.8$ Hz) coupled to two methylene protons at δ 1.84 (4H, *t*, $J = 6.8$ Hz) and four methyl proton signals at δ1.37 (12H, *s*). The proposed structure of this chalcone as **2** was further confirmed by ^{13}C NMR data (see Table 2) which was fully assigned using DEPT spectra and by comparison of measured values with those reported for **3** and **4** [11, 12, 13].

The ^1H NMR spectrum of **3** was found to be identical to those reported for 4-hydroxyisocordoin isolated from *Cordoa piaca* (*Lonchocarpus* sp., leguminosae) [11] and for isobavachalcone isolated from callus culture of *Glycyrrhiza uralensis* (leguminosae) [12]. Further comparison of physical data reported for these two compounds with those acquired for **3** were also identical.

The molecular formula of compound **4**, $C_{25}H_{28}O_4$, was deduced from the mass spectrum (HRMS: 392.1988). The IR, UV and ^1H NMR of **4** were consistent with its formulation as a diprenylated chalcone. This compound appears to be identical with a chalcone previously reported from the seeds of *Dalbergia stipulacea* [13] (leguminosae) and named stipulin. The ^{13}C spectra of this compound was initially run in CD_3OD, but was also measured in $CDCl_3$ in order to compare acquired data with those published by Bhatt and Dayal [13] for the same compound. Comparison of the measured chemical shifts in $CDCl_3$ with those reported in reference [13] were identical. But these authors reported a chemical shift value of 25.7 for C-1″ and C-1‴, and values of 29.5 and 28.4 ppm for C-4″ and C4‴ or (E)-Me, respectively. Our analysis of the DEPT spectrum and also predictions based on a literature report [8] led us to conclude that these values have to be revised. The correct assignments are as given in Table 2.

The spectroscopic data generated for **5** (IR, UV, NMR and EIMS) were found to be similar to those reported for 6-prenylapigenin which has been reported recently from the fruits of *Maclura pomifera* (Maraceae) by Delle Monache *et al*. [10]. Although these workers report a slightly lower mp than measured by us for **5**, the spectroscopic data are completely identical.

Compounds **4** and **5** have been tested against growth profiles and viability of HL-60 promyelocytic leukemia cells. 6-(3-Methylbut-2-enyl)apigenin (**4**) was extremely toxic to these cells at the highest concentration tested. It kills approximatley 50% of the cells by day two at only 50 μM. Compound **5** was not as toxic as **4** at low concentration but was extremely toxic above 100 μM.

EXPERIMENTAL

General

Mps were uncorr. UV–vis: MeOH solution. CIMS and EIMS direct inlet. IR: KBr disk. ^1H and ^{13}C NMR ($CDCl_3$, CD_3COCD_3 or CD_3OD) 300 MHz and 75 MHz, respectively, residual solvent peak as int. reference.

Plant material

Leaves of *D. kameruniana* Engler were collected at Kupe Mountain in Cameroon and a voucher specimen (No. 1545) has been deposited at the National Herbarium.

Extraction and isolation

Powdered, air-dried leaves (500 g) were extracted exhaustively with a cold mixture of CH_2Cl_2-MeOH (1:1), MeOH and H_2O successively. Evaporation of the combined organic extracts at reduced pressure gave 55 g of residue which was chromatographed on a silica gel 60 (150 g) column eluted with a hexane-EtOAc mixture to yield several frs of 250 ml. Frs were monitored by TLC and ^1H NMR and similar frs combined. Frs 1–10 (3 g) examined by TLC (hexane-EtOAc 9:1) contained mainly mixtures of hydro-

carbons, and sitosterol. Recryst. of the combined residues gave 80 mg of sitosterol. Frs 11–19 (5 g, hexane-EtOAc 7 : 3) upon examination by TLC did not contain any flavonoids and has not been investigated further. Frs 20–25 (10 g, hexane-EtOAc 3 : 2) were passed through a Sephadex LH-20 column (solvent $CHCl_3$-MeOH 2 : 1) the post chlorophyll fraction was purified successively on CC and PTLC to yield (**1**), 20 mg, (**2**), 15 mg, (**3**), 200 mg and (**4**) 15 mg. Frs 26–35 from the first column were combined and evap. to give 3 g of residue, which was subjected to CC on silica gel 60 (50 g) and eluted with hexane-EtOAc 1 : 1 to yield ca 1 g of **5**. Frs 36–39 yielded a ppt (280 mg) which was insoluble in the usual organic solvents and so was acetylated using boiling 10 ml acetic anhydride for 2 h. The reaction mixture was evaporated in a petri dish to leave a residue which was chromatographed by CC (hexane-EtOAc 3 : 2) to give the white crystalline tetraacetate of sitosterol 3-β-D-glucopyranoside, 150 mg, mp 166–167°.

6,7-(2,2-Dimethylchromano)5,4′-dihydroxyflavone (**1**). Yellow plates from a mixture of hexane-EtOAc, mp 243–4°, MS *m/z* (rel. Int.): 338 (76, M^+), 323 (15, M^+-CH_3), 295 (36), 283 (72), 282 (34), 156 (100), 155 (58), 128 (28); IR ν_{max}^{KBr} (cm^{-1}): 3480 (—OH), 1660 (C═O), 1620, 1550, 1470, 1380, 1300, 1260, 1200, 1150; UV λ_{max}^{MeOH} (log ε): 215.0 (4.58), 272.0 (4.42), 333.5 (4.50), $\lambda_{max}^{MeOH+AlCl_3}$ (log ε): 220.5 (4.59), 282.5 (4.38), 305.0 (4.38), 356.0 (4.51), $\lambda_{max}^{AlCl_3+HCl}$ (log ε): 216.0 (4.58), 284.2 (4.39), 303.0 (4.39), 351.5 (4.50); ^{1}H NMR (300 MHz, $CD_3CO\,CD_3$) δ: 1.32 (6H, *s*, 2 × Me), 1.91 (2H, *t*, $J = 6.8$ Hz, 2H-3″), 2.71 (2H, *t*, $J = 6.8$ Hz, 2H-4″), 6.40 (*s*, H-8), 6.58 (*s*, H-3), 6.97 (2H, *d*, $J = 9.0$ Hz, 2H-3′,5′), 7.89 (2H, *d*, $J = 9.0$ Hz, 2H-2′,6′), 13.30 (*brs*, 5-OH); ^{13}C NMR: Table 1.

3,4-,4′,5′-*bis*-(2,2-dimethylchromano)-2′-hydroxychalcone (**2**). Yellow oil. MS *m/z* (rel. Int.): 392 (100, M^+), 375 (25), 337 (28), 231 (44), 205 (84), 176 (56), 149 (72), 133 (50); IR ν_{max}^{KBr} (cm^{-1}): 3420 (—OH), 1640 (C═O), 1580, 1500, 1450, 1300, 1260, 1150, 1100; UV λ_{max}^{MeOH} (log ε): 205.0 (4.07), 244.5 (3.77), 378.0 (4.17), $\lambda_{max}^{MeOH+AlCl_3}$ (log ε): 206.2 (4.00), 245.8 (3.69), 341.4 (3.73), 441.6 (4.19), $\lambda_{max}^{MeOH+AlCl_3+HCl}$ (log ε): 213.4 (4.00), 247.6 (3.75), 340.6 (3.75), 397.0 (4.06), 437.0 (4.14); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.37 (12H, *s*, 4 × Me), 1.84 (4H, *t*, $J = 6.8$ Hz, 2H-3″,3‴), 2.81 (4H, *quintet*, $J = 6.8$ Hz, 2H-4″,4‴), 6.82 (*d*, $J = 8.5$ Hz, H-5), 7.37 (*brs*, H-2), 7.39 (*d*, $J = 15.2$ Hz, H-α), 7.45 (*s*, H-3′), 7.47 (*dd*, $J = 8.5$, 2.2 Hz H-6), 7.63 (*brs*, H-6′), 7.82 (*d*, $J = 15.2$ Hz, H-β), 13.16 (*s*, 2′-OH); ^{13}C NMR: Table 2.

(*E*)-1-[2,4-dihydroxy-3-[3-methylbut-2-enyl]phenyl]-3-[4-hydroxyphenyl]-prop-2-en-1-one (**3**). Yellow solid, mp 171–2° lit. 154–6° [11, 12]. ^{1}H NMR (300 MHz, CD_3OD) δ: 1.65, 1.77 (2 vinyl Me), 5.25 (*brt* $J = 3.3$ Hz, vinyl proton), 6.42 (*d*, $J = 9.4$ Hz, H-5′), 6.83 (2H, *d*, $J = 8.4$ Hz H-3,5), 7.60 (2H, *d*, $J = 8.4$ Hz, H-2,6), 7.61 (*d*, $J = 15.5$ Hz, H-α), 7.78 (*d*, $J = 15.5$ Hz, H-β), 7.82 (*d*, $J = 9.4$ Hz, H-6′). For δ values in CD_3COCD_3, see [12], ^{13}C NMR: Table 2.

(*E*)-1-[2,4-dihydroxy-5-[3-methylbut-2-enyl]phenyl]-3-[4-hydroxy-3-[3-methylbut-2-enyl]phenyl]-prop-2-en-1-one (**4**). ^{1}H NMR (in $CDCl_3$ [13], 300 MHz, CD_3OD) δ: 1.72 (6H, *s*, 2 × CH_3), 1.75 (6H, *s*, 2 × CH_3), 3.24, 3.29 (2H each, *d*, $J = 7.1$ Hz, Ar-CH_2), 5.32 (2H, *brt*, $J = 7.1$ Hz, vinyl proton), 6.29 (1H, *s*, H-3′), 6.79 (*d*, $J = 8.0$ Hz, H-5), 7.34 (*dd*, $J = 8.0$, 2.0 Hz, H-6), 7.37 (*d*, $J = 2.0$ Hz, H-2), 7.42 (*d*, $J = 16.1$ Hz H-α), 7.63 (*d*, $J = 16.1$ Hz, H-β), 7.68 (*s*, H-6′). ^{13}C NMR: Table 1.

6-(3-*Methylbut*-2-*enyl*)*apigenin* (**5**). Yellow solid, mp (EtOAc) 214–6° lit 196–7° [10]. For spectroscopic data see ref 10; ^{13}C NMR: Table 1.

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REFERENCES

1. Mabberley, D. J., *The Plant Book*, Cambridge University Press, 1987, p. 192.
2. Bouquet, A., *Feticheurs et Medecines Traditionnelles de Congo Brazaville*, Memoire ORSTOM, 1969. **36,** 295.
3. Woldu, Y., Abegaz, B. Botta, B., Delle Monache, G. and Delle Monache, F., *Phytochemistry*, 1988, **27,** 1227.
4. Swain, L. A., Quirke, J. M. E., Winkle, S. A. and Downum, K. R., *Phytochemistry*, 1991, **30,** 4196.
5. Kuster, R. M., Bernardo, R. R., Da Silva, A. J. R., Parente, J. P. and Mors, W. B., *Phytochemistry*, 1994, **36,** 221.
6. Ngadjui, B. T., Dongo, E., Happi, E. N., Bezabih, M-T., and Abegaz. B. M., *Phytochemistry*, 1998, in press.
7. Mabry, T., Markham, K.R. and Thomas, M. B. *The Systematic Identification of Flavonoids*, Springer Verlag, New York, 1970, p. 165.
8. Agrawal, P. K., ed. *Carbon*-13 *NMR of Flavonoids*, Elsevier, Amsterdam, 1989, p. 123.
9. Berghöfer, B. and Hölzl, J., *Planta Medica*, 1987, **53,** 216.
10. Delle Monache, G., Scurria, R., Vitali, A., Botta, B., Monacelli, B., Pasqua, G., Palocci, C. and Cernia, E., *Phytochemistry*, 1994, **37,** 893.
11. Delle Monache, G., De Mello, J. F., Delle Monache, F., Marini-Bettolo, G. B., De Lima, G. D. and Coelho, J. S. D. B. *Gazz. Chimica Ital.*, 1974, **104,** 861.
12. Kobayashi, M., Noguchi, H. and Sankawa, U.,*Chem. Pharm. Bull.*, 1985, **33,** 3811.
13. Bhatt, P. and Dayal R., *Phytochemistry*, 1992, **31,** 719.