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Prenylated isoflavonoids from *Millettia pervilleana*☆

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Abstract

From the root bark of *Millettia pervilleana*, which had shown significant cytotoxic activity, a 3-phenylcoumarin, named pervilleanine, two new pterocarpans, pervilline and pervillinine, and one known, emoroidocarpan, were isolated in addition to rotenone and 3α-hydroxyrotenone. The anticancer activity of two previously isolated isoflavanones, pervilleanone and 3′-*O*-demethylpervilleanone is reported.

Keywords: *Millettia pervilleana*; Leguminosae; Coumarins; Pervilleanine; Pterocarpans; Pervilline; Pervillinine; Cytotoxic and anti-cancer activity

1. Introduction

The genus *Millettia* (Leguminosae) includes about 150 subtropical species. Phytochemical research has revealed isoflavonoids as the main constituents of the genus.

Our previous study on the chloroform extract of root bark of *Millettia pervilleana* Viguier of Madagascar (IC_{50} 0.12 μg ml^{-1} on KB cells, Galeffi et al., 1997) resulted in the isolation of two prenylated isoflavanones, pervilleanone (**1**) and 3′-*O*-demethylpervilleanone (**2**) from fraction D endowed with mild cytotoxicity (IC_{50} 0.57 μg ml^{-1}). The anticancer activity of **1** and **2**, performed by NCI on human tumor cell lines of lung, breast and CNS, is now reported (Table 1).

From two more active fractions of the same extract, further isoflavonoids, namely a 3-phenylcoumarin, pervilleanine (**3**), two new pterocarpans, pervilline (**4**) and pervillinine (**5**), and one known pterocarpan, emoroidocarpan (**6**) (Machocho et al., 1995), besides rotenone and 3α-hydroxyrotenone, were isolated.

2. Results and discussion

2.1. Characterization of the constituents

Fraction B (IC_{50} 0.047 μg ml^{-1} on KB cells) obtained from the chloroform extract of *M. pervilleana* (Galeffi et al., 1997) was submitted to counter-current distribution (CCD) and two main substances, **3** and **6**, were thus isolated. The less polar one, pervilleanine (**3**), $C_{22}H_{18}O_6$ (HR–EI–MS, *m*/*z* 378.1124 [M]$^+$, calc. 378.1103), had an UV spectrum (see Section 3.2.1) showing extended conjugation. Its ^{1}H and ^{13}C NMR spectra obtained by HETCOR and DEPT experiments showed the presence of a methylenedioxy group (δ_H 5.97, *s*) and an *ortho*,*ortho* disubstituted methoxy group (δ_H 3.54, δ_C 60.9). The fourth oxygen atom of the molecule belonged to a α,α-dimethylpyrane ring (δ_H 1.43, *s*, 2 Me; 5.69 and 6.89, both *d*, J = 10.2 Hz) originated by cyclisation of a prenyl group, whereas the last two oxygen atoms were those of a coumarin unit (δ_{CO} 163.6). The presence among the ^{13}C NMR signals of **3** of an *ortho*,*ortho*-dioxygen substituted oxygen-bearing carbon, δ_C 132.5, and of two *ortho* hydrogens, δ_H 7.55 and 6.71, *d*, J = 8.2 Hz (the latter shif-

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ted upfield as well the corresponding carbon, δ_C 113.1) accounted for a 2-methoxy-3,4-methylenedioxyphenyl ring linked in position 3 to the coumarin system.

Two of the three remaining aromatic hydrogens, δ_H 6.92, *d*, and 6.86, *d*, in the *peri* position, at C-4 and C-5, were mutually coupled ($J = 1.7$ Hz) whereas the last hydrogen, δ_H 6.84, *s*, was attached to C-8, δ_C 108.2, which is *ortho,ortho*-dioxygen substituted. The structure of pervilleanine, **3**, was thus fully established.

Substance **6**, $C_{21}H_{18}O_5$ (HR–EI–MS, *m/z* 350.1093 $[M]^+$, calc. 350.1154), was emoroidocarpan, a pterocarpan isolated from *Tephrosia emeroides* A. Rich. (Machocho et al., 1995). The complete assignment of its ^{13}C NMR frequencies is reported in Table 2. The absolute *R* configuration of both chiral centres 6a and 11a (which was earlier not reported, as well the specific optical rotation) can be assigned on the basis of *cis*-diaxial relationship of their hydrogens ($J = 6.8$ Hz) and the negative Cotton effect of the CD curve (Baruah et al., 1984).

Fraction C (IC_{50} 0.08 μg ml^{-1} on KB cells) was likewise submitted to CCD and column chromatography and four substances were isolated in order of increasing polarity, two new pterocarpans, pervilline (**4**) and pervillinine (**5**), rotenone and 3α-hydroxyrotenone.

Pervilline (**4**), $C_{21}H_{20}O_5$ (HR–EI–MS, *m/z* 352.1263 $[M]^+$, calc. 352.1311), was a pterocarpan as demonstrated by the sequence O–CH_2–CH(Ar)–CH–O (CH_2,

Table 1
3-Cell line assay for the primary anticancer screening of **1** and **2** at conc. 10^{-4} M

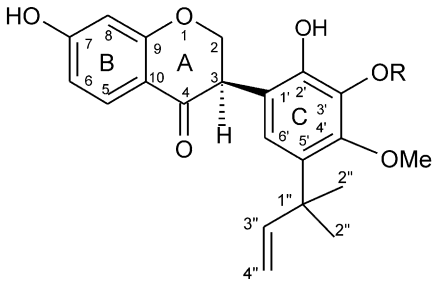
Compound	Growth percentages of the treated cell lines		
	(Lung) NCI-H460	(Breast) MCF7	(CNS) SF-268
Pervilleanone, **1**	−36	−38	−27
3′-*O*-Demethylpervilleanone, **2**	−2	−56	−42

Negative values of the growth percentage correspond to $T_i < T_z$, where T_z is the cell population at time zero and T_i is the cell population in the presence of the drug after 48 h.

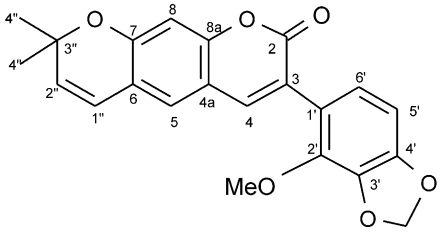
Table 2
^{13}C and ^1H NMR spectral data of pterocarpans **4**, **5** and **6**

Position	**4** ($CDCl_3$)		**5** ($CDCl_3$)		**6** ($CDCl_3$)	
	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
1	126.8	7.33, *s*	126.3	7.33, *s*	126.2	7.20, *s*
1a	111.7		n.o.		111.8	
2	120.7		120.8		120.8	
3	161.2		161.2		161.4	
4	98.1	6.35, *s*	98.2	6.35, *s*	98.2	6.38, *s*
4a	156.0		156.1		156.3	
6	66.5	3.60, *dd* (10.8; 10.6); 4.19, *dd* (10.8; 4.9)	66.6	3.60, *t* (11.0); 4.19, *dd* (11.0; 5.0)	66.5	3.55, *t* (11.4); 4.16, *dd* (11.4; 5.1)
6a	40.1	3.50, *m* (10.6; 6.6;4.9)	40.3	3.50, *m* (11.0; 6.7; 5.0)	40.1	3.40, *m* (11.4; 6.8; 5.1)
6b	121.7		118.1		118.1*	
7	114.7	6.72, *d* (8.0)	110.4	6.79, *s*	104.7	6.67, *s*
8	103.6	6.42, *d* (8.0)	139.9		141.8	
9	148.0		146.9		147.5	
10	130.6		94.7	6.41, *s*	93.7*	6.33, *s*
10a	146.1		152.8		154.1*	
11a	79.8	5.52, *d* (6.6)	78.7	5.52, *d* (6.7)	79.0	5.40, *d* (6.8)
1′	33.8	2.97, *dd* (15.8; 7.6); 3.26, *dd* (15.8; 9.8)	33.9	2.97, *dd* (15.7; 7.7); 3.26, *dd* (15.7; 9.8)	33.8	2.94, *dd* (15.6; 8.0); 3.23, dd (15.6; 9.8)
2′	86.6	5.16, *dd* (9.8; 7.6)	86.7	5.16, *dd* (9.8; 7.7)	86.6	5.12, *dd* (9.8; 8.0)
3′	143.8		143.8		143.7	
4′	17.1	1.76, *s*	17.2	1.76, *s*	17.1	1.70, *s*
5′	112.1	5.05; 4.88, *br s*	112.1	5.05; 4.88, *br s*	112.1	5.01; 4.85, *d* (1.1)
OMe	56.4	3.84, *s*	56.2	3.84, *s*		
OCH_2O					101.2	5.83; 5.89, *d* (2.5)

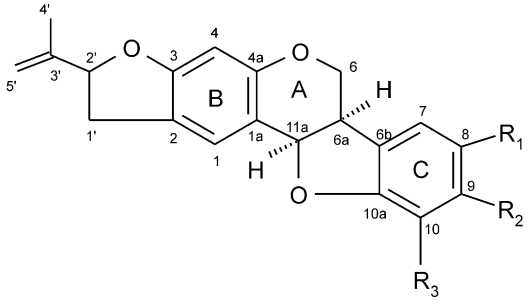
* The assignments of Machocho et al. (1995) for these carbons are not correct.



	R	
1	Me	pervilleanone
2	H	3'-*O*-demethylpervilleanone



3 pervilleanine



	R_1	R_2	R_3	
4	H	OMe	OH	pervilline
5	OH	OMe	H	pervillinine
6	O-CH_2-O		H	emoroidocarpan

δ_H 3.60, *dd*, $J = 10.8$; 10.6 Hz, and 4.19, *dd*, $J = 10.8$; 4.9 Hz; CH–Ar, 3.50, *m*, $J = 10.6$; 6.6; 4.9 Hz; CH–O 5.52, *d*, $J = 6.6$ Hz). The substitution of the benzofuran ring C was shown by the COLOC connectivities of C-10a (δ_C 146.1) and C-9 (δ_C 148.0) with the hydrogen at δ_H 6.72 (*d*, $J = 8.0$ Hz) and by the connectivity of C-6b (δ_C 121.7) with the hydrogen at δ_H 6.42 (*d*, $J = 8.0$ Hz). These two *ortho* hydrogens are in position 7 and 8, respectively. The connectivity of C-9 with the methoxy group at δ_H 3.84 and the ^{13}C resonance of the latter (δ_C 56.4) accounted for its mono-*ortho* substituted position 9. The remaining hydroxy group was thus located in position 10 in agreement with the upfield resonance of C-10 (δ_C 130.6) due to the *ortho,ortho*-dioxygen substitution.

Moreover in **4** a prenyl group was linked to ring B to give a methylethenyldihydrofuran-fused ring (H_2-1′, δ_H 2.97, *dd*, and 3.26, *dd*; H-2′, 5.16, *dd*; H-4′, 1.76, *s*; H_2-5′, 4.88 and 5.05, *br s*). The latter is located as in emoroidocarpan in agreement with the presence of an *ortho,ortho*-dioxygen substituted methine (δ_H 6.35, *s*, δ_C 98.1) in position 4.

The absolute configuration of **4** is identical to that of emoroidocarpan on account of the coupling constant $J_{6a/11a}$ (6.6 Hz) and the negative Cotton effect.

Pervillinine (**5**), $C_{21}H_{20}O_5$ (HR–EI–MS, *m/z* 352.1251 $[M]^+$, calc. 352.1311), differed from pervilline on the substitution in ring C (see Table 2). In the former, the two hydrogens at δ_H 6.79, *s* and δ_H 6.41, *s* were located in the *para* positions 7 and 10, respectively, and the methoxy group (δ_H 3.84) in position 9 inasmuch its irradiation by NOE experiments enhanced the signal of the more shielded H-10 (δ_H 6.41). The hydroxy group was thus located in position 8.

2.2. In vitro anticancer activity

Isoflavonoids have shown activity in the search for antiviral and antineoplastic or cancer chemopreventive agents of plant origin. Thus, some isoflavonoids from genus *Millettia* showed inhibitory effects against Epstein–Barr virus (Ito et al., 2000), whereas those of *M. pachycarpa* inhibited the activities of murine retroviral reverse transcriptase and human DNA-polymerases (Ono et al., 1989).

Rotenone and 3α-hydroxyrotenone, which were found in the cytotoxic fraction C of *M. pervilleana*, showed inhibition of TPA-induced ornithine decarboxylase at the level of its mRNA expression and were therefore regarded as promising cancer chemopreventive agents (Gerhauser et al., 1995).

Pervilleanone (**1**) and 3′-*O*-demethylpervilleanone (**2**), isolated from fraction D of *M. pervilleana* endowed with mild cytotoxicity, showed growth inhibition of the

human cancer cell lines of lung, breast and CNS (Table 1) used in the NCI antitumor prescreen.

3. Experimental

3.1. General

A Craig-Post apparatus (200 stages, 10:10 ml, upper and lower phase) was used for the separation by CCD. TLC: silica gel F_{254}, cyclohexane–EtOAc (1:1). CD: Jasco 710. ^1H and ^{13}C NMR: 500.13 and 125.77 MHz, respectively, Bruker AM 500. Chemical shifts are given in δ (ppm) from internal TMS. EI–MS: 70 eV, HP 5989A. HR–EI–MS: VG 7070 EQ-HF.

The bioassay tests on lung, breast and CNS cells were performed by the NCI, Bethesda (USA).

3.2. Separation

Fraction B (1.4 g) was submitted to CCD with solvent system H_2O–Me_2CO-EtOH–cyclohexane (2:6:2:8). Two chromatographically pure fractions, pervilleanine (**3**, 39 mg) and emoroidocarpan (**6**, 68 mg) were thus obtained.

Fraction C (1.4 g) was submitted to CCD with solvent system H_2O–Me_2CO–EtOH–cyclohexane (5:4:4:10) and four fractions were obtained. Each fraction was subsequently purified by column chromatography on silica gel 60 (70–230 mesh), eluent cyclohexane–EtOAc (8:2) and thus pervilline (**4**, 26 mg), pervillinine (**5**, 31 mg), rotenone (89 mg) and 3α-hydroxyrotenone (56 mg) were obtained in order of increasing polarity. The last two substances were identified by comparison of their physico-chemical data.

3.2.1. Pervilleanine (3)

Pale crystals from *n*-hexane, mp 177–179 °C. UV (MeOH): λ_{max} nm (log ε): 324 (4.09), 239 (4.02). ^1H NMR ($CDCl_3$): δ 1.43 (6H, *s*, 2 Me-4″); 3.54 (3H, *s*, OMe); 5.69 (1H, *d*, $J=10.2$ Hz, H-2″); 5.97 (2H, *s*, O–CH_2–O); 6.71 (1H, *d*, $J=8.2$ Hz, H-5′); 6.84 (1H, *s*, H-8); 6.86 (1H, *d*, $J=1.7$ Hz, H-5); 6.89 (1H, *d*, $J=10.2$ Hz, H-1″); 6.92 (1H, *d*, $J=1.7$ Hz, H-4); 7.55 (1H, *d*, $J=8.2$ Hz, H-6′). ^{13}C NMR ($CDCl_3$): δ 28.0 (2 Me, C-4″); 60.9 (OMe); 77.4 (C-3″); 101.2 (O–CH_2–O); 108.1 (C-4a); 108.2 (C-8); 108.8 (C-6); 110.9 (C-1′); 111.4 (C-4); 113.1 (C-5′) 115.4 (C-1″); 123.8 (C-6′); 124.7 (C-5); 125.9 (C-3); 130.3 (C-2″); 132.5 (C-3′); 147.5 (C-7); 147.6 (C-4′); 148.4 (C-2′); 156.2 (C-8a); 163.6 (C-2). EI–MS, *m*/*z* (rel. int.): 378 ($[M]^+$, 44), 363 (100), 320 (17), 187 (13), 159 (15), 145 (10). Molecular formula $C_{22}H_{18}O_6$, HR–EI–MS, *m*/*z*: 378.1124 $[M]^+$, calc. 378.1103.

3.2.2. Pervilline (4)

White powder from *n*-hexane, mp 112–114 °C. $[\alpha]_D^{20}$ −192° ($CHCl_3$; *c* 0.12). CD: $[\theta]_{240}$ −44 600 (MeOH; *c* 0.013). UV (MeOH): λ_{max} nm (log ε): 298 (3.9), 220 (4.1). ^1H and ^{13}C NMR data in Table 2. EI–MS, *m*/*z* (rel. int.): 352 ($[M]^+$, 22), 350 (24), 337 (22), 162 (37), 149 (100). Molecular formula $C_{21}H_{20}O_5$, HR–EI–MS, *m*/*z*: 352.1263 $[M]^+$, calc. 352.1311.

3.2.3. Pervillinine (5)

Yellow powder from *n*-hexane, mp 152–154 °C. $[\alpha]_D^{20}$ −189° ($CHCl_3$; *c* 0.07). CD: $[\theta]_{240}$ −46 800 (MeOH; *c* 0.011). UV (MeOH): λ_{max} nm (log ε): 298 (3.9); 220 (4.1). ^1H and ^{13}CNMR data in Table 2. EI–MS, *m*/*z* (rel. int.): 352 ($[M]^+$, 100), 337 (58), 322 (18). Molecular formula $C_{21}H_{20}O_5$, HR–EI–MS, *m*/*z*: 352.1251 $[M]^+$, calc. 352.1311.

3.2.4. Emoroidocarpan (6)

White crystals from *n*-hexane, mp 189–190 °C. $[\alpha]_D^{20}$ −268° ($CHCl_3$; *c* 0.11). CD: $[\theta]_{240}$ −45 200 (MeOH; *c* 0.012). UV (MeOH): λ_{max} nm (log ε): 300 (2.9); 220 (3.9). ^1H and ^{13}C NMR data in Table 2. EI–MS, *m*/*z* (rel.int.): 350 ($[M]^+$, 100), 335 (87). Molecular formula $C_{21}H_{18}O_5$, HR–EI–MS, *m*/*z*: 350.1093 $[M]^+$, calc. 350.1154.

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