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TWO PRENYLATED ISOFLAVANONES FROM *MILLETTIA PERVILLEANA**

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Key Word Index—*Millettia pervilleana*; Leguminosae; isoflavanones; pervilleanone; 3′-*O*-demethylpervilleanone; cytotoxic activity.

Abstract—From the root bark of *Millettia pervilleana*, which showed high cytotoxic activity, two prenylated isoflavanones were isolated. Their structures were determined by means of chemical and spectroscopic properties to be (3*R*)-2′,7-dihydroxy-3′,4′-dimethoxy-5′-α,α-dimethylallylisoflavanone, named pervilleanone, and its 3′-*O*-demethyl derivative. © 1997 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Millettia pervilleana Viguier (Leguminosae) is a shrub from Madagascar, used as a fish poison and hitherto not studied phytochemically. Some isoflavonoids, which are the predominant class of phytoalexins, have been isolated from other *Millettia* spp., e.g. *M. auriculata* [2], *M. ferruginea* [3], *M. ovalifolia* [4], *M. racemosa* [5], *M. thonningii* [6], *M. pachycarpa* [7], *M. pulchra* [8] and *M. dura* [9]. The cytotoxicity of the chloroform extract of *M. pervilleana* (0.12 μg ml^{-1} on KB cells) led to the present phytochemical study which resulted in the isolation of two uncommon prenylated isoflavanones: pervilleanone (**1**) and its 3′-*O*-demethyl derivative (**2**).

RESULTS AND DISCUSSION

The chloroform extract of *M. pervilleana* was submitted to countercurrent distribution (CCD) with biphase system water–acetone–ethanol–cyclohexane and the five fractions obtained were evaluated for their cytotoxic activity to cell line KB. The most active fractions B and C (IC_{50} = 0.047 and 0.08 μg ml^{-1}, respectively) are still being studied, but from fraction D (IC_{50} = 0.57 μg ml^{-1}) two isoflavanones, pervilleanone (**1**, $C_{22}H_{24}O_6$, [M$^+$] *m/z* 384) and its *O*-demethyl derivative (**2**, $C_{21}H_{22}O_6$) were isolated. Compounds **1** and **2** were converted into the same methyl derivative (**3**, $C_{24}H_{28}O_6$) by methylation with ethereal diazomethane, thereby strongly supporting the close relationship between the two isoflavanones.

The UV spectrum of **1** (see Experimental) and the presence in its ^{1}H and ^{13}C NMR spectra (see Table 1) of the signals of the O–CH_2–CH–CO sequence accounted for its isoflavanone structure, while two methoxyl groups (δ 3.76 and 3.77), two hydroxyl groups (δ 6.21 and 8.05, detectable in $CDCl_3$) and a *C*-linked α,α-dimethylallyl chain were also evident. The aromatic ring with three hydrogen atoms in position 1, 2 and 4 was identified as ring A and the highly deshielded hydrogen at 7.75 (*d*, *J* = 8 Hz) was assigned to position 5, *peri* to the carbonyl group. The COLOC experiments allowed the assignment of the two low-field carbon atoms (δ 166.2 and 165.5) *ortho* to C-8 (δ 103.5), one (C-7) bearing a hydroxyl group, and the other (C-9) showing a three-bond connectivity with H_a-2 (δ 4.40, *dd*). The B ring of pervilleanone (**1**) is 1,2,3,4-tetrasubstituted, bearing one hydroxyl group, two methoxyl groups and a prenyl chain. The presence in the mass spectrum of the complementary peaks at 137 (62%) and 248 (49%) due to a retro Diels–Alder reaction (see the fragmentation lines in the formula) was in agreement with this substitution. The low chemical shift of C-1′ (δ 117.5) accounted for an *ortho* oxygen substitution. Of the two carbon atoms at δ 148.7 and 153.0, which showed a three-bond connectivity with the aromatic hydrogen (δ 6.72, *s*), only the latter corresponds to a methoxyl group. The

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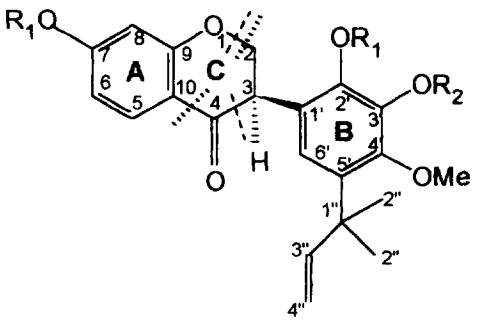

	R_1	R_2
1	H	Me
2	H	H
3	Me	Me

---- retro Diels-Alder fragmentation lines

····· alternative fragmentation lines

other methoxyl group is represented by the higher field (and therefore *ortho,ortho* dioxygen substituted) carbon atom (δ 142.5) at C-3′.

The chemical shifts of the two methoxyl groups (δ 60.2 and 60.4) are in agreement with their *ortho,ortho* disubstituted positions and, therefore, with position 5′ for the side-chain. The hydrogen bearing carbon atom is therefore at position 6′ and is not *ortho* oxygen substituted, in agreement with its chemical shift (δ 123.7). By comparing the same carbon of fraserinone A in the 4′-hydroxy-2′-methoxy-5′-α,α-dimethylallylbenzenic B ring (δ 130.1) [10], it can be seen that C-6′ in **1** is *para* shielded by the additional 3′-methoxyl group. Comparison with fraserinone A also allows one to decide whether there is a hydroxyl at the 2′-position and methoxyl at 4′, or vice versa (the second methoxyl group being unambiguously assigned to the intermediate 3′ position). If the substitution were 2′-methoxy-4′-hydroxy, as in fraserinone A, the chemical shifts of the corresponding carbon atoms would be practically identical (δ 157.5 and 156.8, respectively), whereas in pervilleanone C(OH) is at δ 148.7 and C(OCH_3) at δ 153.0 and, therefore, the substitution in ring B of **1** must be 2′-hydroxy-3′,4′-dimethoxy-5′-α,α-dimethylallyl. Nuclear Overhauser effect (NOE) experiments, showing interactions between the methyl groups of the prenyl chain and H-6′ and the methoxy group at δ 3.76, confirmed the structure (**1**) for pervilleanone. In agreement with this structure, of the four methoxyl groups in the permethylated compound (**3**), only one, that at C-7, is at lower chemical shift (δ 55.6).

In **2**, the only methoxyl group, (δ 60.3) cannot be located at the 2′ position because the corresponding aromatic carbon (δ 147.6) would have an almost identical value to the other mono *ortho*-oxygen substituted carbon (δ 143.1) in fraserinone A. A methoxyl group at the 2′-position also appears unlikely when the ^{13}C NMR spectrum of **2** is compared with that for pervilleanone. Thus, in comparison with the B ring of **1**, almost all signals of **2** undergo a shielding effect; in particular, the shielding of C-3′ (from δ 142.5 to δ 139.0) suggests that it bears a hydroxy rather than a methoxyl group as in **1**. Isoflavanone (**2**) is therefore identified as 3′-*O*-demethylpervilleanone. In the mass spectra of **1** and **2** the peaks at *m/z* 274 (83) and 260 (100), respectively [and the corresponding ones at *m/z* 259 (100) and 245 (100) due to the loss of a methyl group], are the result of the loss of ring A ($M^+ - 110$; see the fragmentation lines in the formula).

In order to establish the absolute configuration at C-3, the CD curves of **1** and **3** were recorded. The positive Cotton effect at 325 nm due to the $n \rightarrow \pi^*$ transition for the carbonyl group and the equatorial position of ring B (in agreement with the *trans*-diaxial relationship between H_b-2 and H-3, $J = 11$ Hz) accounted for the (3*R*) absolute configuration on the basis of the octant rule modified for the cyclic arylketones [11].

The substitution pattern in these two isoflavonones (**1** and **2**) is very uncommon—whereas ring A is monosubstituted, ring B is tetrasubstituted. A related isoflavone, DO-10 described by Yahara *et al.* [12], differs from 3′-*O*-demethylpervilleanone (**3**) only in the absence of the α,α-dimethylallyl chain at position 5′.

Studies are underway to correlate the cytotoxic activity of *M. pervilleana* with the corresponding components, particularly for the high-activity fractions.

EXPERIMENTAL

General. A Craig Post apparatus (200 stages, 10 : 10 ml, upper and lower phase) was used for the separation by CCD. TLC: silica gel, cyclohexane–EtOAc (1 : 1). ^{13}C and ^1H NMR: 125.77 and 500.13 MHz, respectively, Bruker AM 500. Chemical shifts are given in δ (ppm) from internal TMS. EI-MS: 70 eV, HP 5989A. Specific optical rotation: 20 . CD: Jasco 710. The biossay tests were determined on the human oral epidermoid carcinoma cell line, KB.

Table 1. ^{13}C and ^{1}H spectral data of compounds **1**–**3**

	1 (MeOH-d_4)			**2** ($CDCl_3$ + MeOH-d_4)		**3** ($CDCl_3$)	
Position	C (δ)	H (δ)	COLOC	C (δ)	H (δ)	C (δ)	H (δ)
2	71.6	H_a: 4.40, *dd*, J = 5 and 11 Hz H_b: 4.62, *dd*, J = 11 and 11 Hz	H_a-2, H_b-2	71.1	H_a: 4.39, *dd*, J = 5 and 11 Hz H_b: 4.55, *dd*, J = 9 and 11 Hz	71.4	H_a: 4.42, *dd*, J = 5 and 11 Hz H_b: 4.56, *dd*, J = 11 and 11 Hz
3	49.4	4.15, *dd*, J = 5 and 11 Hz	H-3	48.2	3.92, *dd*, J = 5 and 9 Hz	49.1	4.08, *dd*, J = 5 and 11 Hz
4	194.1		H_a-2, H-3	191.5		191.4	
5	130.3	7.75, *d*, J = 8 Hz	H-5	130.1	7.62, *d*, J = 8 Hz	129.3	7.87, *d*, J = 8 Hz
6	111.6	6.50, *dd*, J = 2 and 8 Hz	H-6	111.5	6.34, *dd*, J = 2 and 8 Hz	109.8	6.57, *dd*, J = 2 and 8 Hz
7	166.2		H-5	165.5		163.8	
8	103.5	6.33, *d*, J = 2 Hz	H-8	103.3	6.20, *d*, J = 2 Hz	100.7	6.39, *d*, J = 2 Hz
9	165.5		H_a-2	164.5		165.7	
10	115.5			114.1		115.4	
1′	117.5		H-3	117.2		122.5	
2′	148.7		H-6′	143.1		150.7	
3′	142.5		MeO-3′	139.0		146.9	
4′	153.0		H-6′, MeO-4′	147.6		153.0	
5′	133.2		3H-2″	133.8		136.8	
6′	123.7	6.72, *s*	H-6′	118.0	6.47, *s*	122.7	6.70, *s*
1″	41.2		3H-2″, H-4″	40.9		40.5	
2″(2)	28.5	1.32, *s*	3H-2″	28.5	1.20, *s*	27.8	1.34, *s*
3″	149.9	6.05, *dd*, J = 10 and 18 Hz	3H-2″, H-3″	149.7	5.91, *dd*, J = 10 and 18 Hz	148.4	6.06, *dd*, J = 10 and 18 Hz
4″	110.1	H_a: 4.90, *dd*, J = 1 and 10 Hz H_b: 4.91, *dd*, J = 1 and 18 Hz	H-4″	109.9	H_a: 4.74, *dd*, J = 1 and 10 Hz H_b: 4.75, *dd*, J = 1 and 18 Hz	109.8	H_a: 4.91, *dd*, J = 1 and 10 Hz H_b: 4.90, *dd*, J = 1 and 18 Hz
MeO-2′						60.1*	3.81, *s**
MeO-3′	60.4*	3.77, *s*				60.5*	3.75, *s**
MeO-4′	60.2*	3.76, *s*		60.3	3.60, *s*	60.3*	3.79, *s**
MeO-7						55.6	3.79, *s**
HO		6.21 and 8.05, *s* (in $CDCl_3$)					

*These signals are interchangeable within the same column.

Plant material, extraction and separation. The root bark of *M. pervilleana* (250 g) was collected in the south-west region of Madagascar, Soalary (Toliara), and a voucher specimen deposited at the Institut Malgache de Recherches Appliquées of Antananarivo.

The alcoholic extract (16 g) was partitioned between water and chloroform. The residue of the organic phase evapn (4.5 g), wherein the biological activity was concd (0.12 μg ml^{-1} on KB cells), was submitted to CCD with solvent system H_2O–Me_2CO–EtOH–cyclohexane (2 : 2 : 3 : 5) and five fractions (A–E) collected.

The IC_{50} of the obtained fractions (μg ml^{-1}): A, >1; B, 0.047; C, 0.08; D, 0.57; E, >1.

Fraction D was submitted to CCD with solvent system H_2O–Me_2CO–EtOH–EtOAc–cyclohexane (7 : 5 : 3 : 1.2 : 10). Two fractions with $K_r = 0.7$ (**1**, 250 mg) and 0.14 (**2**, 240 mg), chromatographically pure, were obtained.

Pervilleanone (**1**). Plates from *n*-pentane, mp 90–92°. $[\alpha]_D = -26.3°$ ($CHCl_3$; *c* 0.5). CD (MeOH; *c* 2.5×10^{-2}): $[\theta]_{326} = +329$. UV λ_{max}^{MeOH} nm (log ε): 309 (3.0), 276 (3.21), 231 (3.33), 208 (3.78). EI-MS, *m/z* (rel. int.): 384 (M^+, 39, $C_{22}H_{24}O_6$), 335 (13), 274 (83), 259 (100), 248 (49), 137 (62).

3′-O-*Demethylpervilleanone* (**2**). Powder from *n*-pentane, mp 195–198°. $[\alpha]_D = -20.4°$ ($CHCl_3$; *c* 0.5). UV λ_{max}^{MeOH} nm (log ε): 310 (2.94), 275 (3.16), 231 (3.31), 209 (3.73). EI-MS, *m/z* (rel. int.): 370 (M^+, 11, $C_{21}H_{22}O_6$), 337 (7), 312 (23), 260 (100), 245 (100), 233 (14), 137 (88).

Dimethyl pervilleanone (**3**). Powder from *n*-pentane, mp 96–98°. $[\alpha]_D = -3.5°$ ($CHCl_3$; *c* 0.3). CD (MeOH; *c* 1.2×10^{-2}): $[\theta]_{325} = +860$. UV λ_{max}^{MeOH} nm (log ε): 311 (2.93), 272 (3.24), 231 (3.36), 210 (3.79). EI-MS, *m/z* (rel. int.): 412 (M^+, 28, $C_{24}H_{28}O_6$), 262 (100), 247 (35), 216 (22), 151 (11).

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