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Chromenes of polyketide origin from *Peperomia villipetiola*

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Abstract

An extract of leaves and stems of *Peperomia villipetiola* has been found to contain myristicin (3-methoxy-4,5-methylenedioxy-allylbenzene) and seven chromenes, whose structures are methyl 5-hydroxy-7-methyl-2,2-dimethyl-2*H*-1-chromene-6-carboxylate (**1**), methyl 5-methoxy-7-methyl-2,2-dimethyl-2*H*-1-chromene-8-carboxylate (**2**), methyl 7-hydroxy-5-methyl-2,2-dimethyl-2*H*-1-chromene-6-carboxylate (**3**), methyl 7-methoxy-5-methyl-2,2-dimethyl-2*H*-1-chromene-6-carboxylate (**4**), 5-methanol-7-hydroxy-2,2-dimethyl-2*H*-1-chromene-6-carboxylic acid (**5**), 5-methanol-7-methoxy-2,2-dimethyl-2*H*-1-chromene-6-carboxylic acid (**6**), and methyl 5-acetoxymethanol-7-hydroxy-2,2-dimethyl-2*H*-1-chromene-6-carboxylate (**7**). A biosynthetic rationale for **1**–**7** suggests that orsellinic acid may be a common intermediate. The anti-fungal activities of the chromenes were measured bioautographically against *Cladosporium cladosporioides* and *Cladosporium sphaerospermum*: compounds **6** and **7** were found to be the most active.

Keywords: *Peperomia villipetiola*; Piperaceae; Structural elucidation; Anti-fungal activity; Chromenes; Orsellinic acid

1. Introduction

The chemistry of the Piperaceae family is based on the occurrence of phenylpropanoids (Orjala et al., 1993), lignan/neolignans (Monache and Compagnone, 1996; Parmar et al., 1997; Benevides et al., 1999), pyrones (Singh, 1992), aliphatic and aromatic amides (Alécio et al., 1998; Silva et al., 2002), alkaloids (Dodson et al., 2000), polyketides (Cheng et al., 2003) and chromenes (Moreira et al., 1998; Baldoqui et al., 1999; Lago et al., 2004). Most of the studies reported to date have concerned species of the genus *Piper*. Only a few species of *Peperomia* have been subjected to systematic chemical investigation even though many members of this genus are to be found worldwide, particularly in collections of ornamentals.

Of the secondary metabolites that have been isolated from *Peperomia* species, the most noteworthy are the seco-compounds, e.g., the secolignans from *Peperomia glabella* (Monache and Compagnone, 1996) and *Peperomia dindigulensis* (Govindachari et al., 1998), and the cyclobutane compound from *Peperomia pellucida* which seems to be produced by dimerisation of styryl phenol (a seco-phenylpropanoid) (Bayma et al., 2000). Together with these, a number of acylphloroglucinol or phenolic compounds with long aliphatic chains have been isolated from *Peperomia clusiifolia* (Seeram et al., 1998), *Peperomia vulcanica* (Mbah et al., 2002), *Peperomia obtusifolia* (Tanaka et al., 1998), *Peperomia galiodes* (Mahiou et al., 1996) and *Peperomia proctorii* (Seeram et al., 2000).

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In the present work the isolation, structural elucidation and determination of the anti-fungal activities of seven new chromenes from *Peperomia villipetiola* are reported. The bioautographic assay used has been successfully employed in our laboratories over a number of years for screening anti-fungal compounds in Piperaceae species (Alécio et al., 1998; Baldoqui et al., 1999; Navickiene et al., 2000; Silva et al., 2002; Lago et al., 2004).

2. Results and discussion

The CH_2Cl_2:MeOH (2:1) extract of the aerial parts of *P. villipetiola* was fractionated by CC and TLC to yield myristicin (3-methoxy-4,5-methylenedioxy-allylbenzene) and compounds **1**–**7**. The basic skeleton of **1**–**7** was determined to be of the chromene type by virtue of a common set of ^{1}H NMR spectra (Table 1) that displayed an AB system at δ 5.52–5.69 (*d*, 10Hz) and 6.13–6.69 (*d*, 10Hz), and intense singlets at δ 1.39–1.46 (6H) characteristic of the 2,2-dimethyl-2*H*-1-chromene moiety. This structure was further confirmed by inspection of the UV, IR and ^{13}C NMR spectra (see Section 3 and Table 2). MS data, together with elemental analysis and NMR spectra, were employed to determine the molecular formula of each compound. Additionally, a fragment $[M-15]^+$, associated with the loss of a methyl radical and observed in all MS, provided complementary evidence of the identity of the chromene moiety (Diaz et al., 1987). A common feature in the NMR spectra of all compounds was a unique singlet for the aromatic hydrogen (δ 6.19–6.46, *s*, 1H) in the ^{1}H NMR spectra with a corresponding aromatic methine carbon (δ 98.1–111.9) in the ^{13}C NMR spectra. The three remaining substituents on the aromatic ring were determined to be hydroxyl for **1**, **3**, **5** and **7**, methoxyl for **2**, **4** and **6**, methyl for **1**–**4**, carboxyl for **5**–**6**, methyl carboxylate for **1**–**4** and **7**, methanol for **5** and **6**, and acetoxy-methanol for **7**. Assignments of the substituents in each case were achieved by interpretation of spectrometric data including IR, ^{1}H NMR and, especially, HMBC.

Table 1
^{1}H NMR spectroscopic data (300 MHz, $CDCl_3$) for chromenes **1**–**7** isolated from *Peperomia villipetiola*

Hydrogen	**1**	**2**	**3**	**4**	**5**	**6**	**7**
3	5.52 (1H, *d*, 10)	5.53 (1H, *d*, 10.0)	5.60 (1H, *d*, 10.0)	5.55 (1H, *d*, 10.0)	5.61 (1H, *d*, 10.0)	5.59 (1H, *d*, 10)	5.69 (1H, *d*, 10.0)
4	6.69 (1H, *d*, 10)	6.59 (1H, *d*, 10.0)	6.56 (1H, *d*, 10.0)	6.44 (1H, *d*, 10.0)	6.13 (1H, *d*, 10.0)	6.15 (1H, *d*, 10)	6.53 (1H, *d*, 10.0)
6		6.23 (1H, *s*)					
8	6.19 (1H, *s*)		6.29 (1H, *s*)	6.28 (1H, *s*)	6.33 (1H, *s*)	6.35 (1H, *s*)	6.46 (1H, *s*)
9	2.46 (3H, *s*)	2.29 (3H, *s*)	2.48 (3H, *s*)	2.20 (3H, *s*)	5.23 (2H, *s*)	5.15 (2H, *s*)	5.40 (2H, *s*)
10, 11	1.43 (6H, *s*)	1.39 (6H, *s*)	1.41 (1H, *s*)	1.40 (6H, *s*)	1.45 (6H, *s*)	1.46 (6H, *s*)	1.43 (6H, *s*)
2′	3.92 (3H, *s*)	3.87 (3H, *s*)	3.93 (3H, *s*)	3.89 (3H, *s*)			3.90 (3H, *s*)
1″		3.81 (3H, *s*)		3.77 (3H, *s*)		3.92 (3H, *s*)	
2″							2.07 (3H, *s*)
CH_2OH					7.62 (1H, *s*)		
Ar-OH	11.97 (1H, *s*)		11.39 (1H, *s*)				11.32 (1H, *s*)

Table 2
^{13}C NMR spectroscopic data[a] (75 MHz, $CDCl_3$) for chromenes **1**–**7** isolated from *Peperomia villipetiola*

Carbon	**1**	**2**	**3**	**4**	**5**	**6**	**7**
2	77.3 (C)	76.4 (C)	76.2 (C)	76.0 (C)	77.9 (C)	78.1 (C)	76.6 (C)
3	127.4 (CH)	128.6 (CH)	128.8 (CH)	128.3 (CH)	129.6 (CH)	129.1 (CH)	130.5 (CH)
4	116.4 (CH)	116.5 (CH)	119.1 (CH)	118.8 (CH)	116.4 (CH)	116.4 (CH)	118.1 (CH)
4a	107.3 (C)	108.6 (C)	113.9 (C)	113.1 (C)	108.5 (C)	107.6 (C)	115.4 (C)
5	159.8 (C)	155.7 (C)	137.4 (C)	132.3 (C)	142.8(C)	145.7 (C)	132.2 (C)
6	105.1 (C)	104.8 (CH)	106.4 (C)	117.1 (C)	103.8(C)	106.0 (C)	106.2 (C)
7	142.7 (C)	137.3 (C)	164.0 (C)	157.2 (C)	157.4 (C)	159.6 (C)	163.9 (C)
8	111.9 (CH)	116.0 (C)	102.9 (CH)	98.1 (CH)	103.7 (CH)	100.9 (CH)	105.6 (CH)
8a	157.5 (C)	151.6 (C)	158.9 (C)	155.2 (C)	160.9 (C)	160.0 (C)	158.9 (C)
9	24.4 (CH_3)	20.3 (CH_3)	17.6 (CH_3)	15.4 (CH_3)	69.2 (CH_2)	67.2 (CH_2)	60.2 (CH_2)
10, 11	28.3 (CH_3)	27.57 (CH_3)	28.01 (CH_3)	27.7 (CH_3)	28.26 (CH_3)	28.3 (CH_3)	28.0 (CH_3)
1′	172.4 (C)	168.44 (C)	172.16 (C)	169.0 (C)	172.3 (C)	168.8 (C)	171.0 (C)
2′	51.8 (C)	51.74 (CH_3)	51.9 (CH_3)	52.0 (CH_3)			52.3 (CH_3)
1″		55.6 (CH_3)		55.8 (CH_3)		56.1 (CH_3)	170.6 (C)
2″							20.7 (CH_3)

[a] Multiplicities (CH_n; n = 0–3) given for ^{13}C NMR/Dept 135° spectrum associated with HETCOR data (300 and 75 MHz, $CDCl_3$).