Phytochemistry Letters 10 (2014) cxli–cxliv

Contents lists available at ScienceDirect

Phytochemistry Letters

journal homepage: www.elsevier.com/locate/phytol

Bioactive acetophenones from *Plectranthus venteri*☆

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ARTICLE INFO

Article history:
Received 17 September 2014
Received in revised form 14 October 2014
Accepted 16 October 2014
Available online 31 October 2014

Keywords:
Plectranthus venteri
Lamiaceae
Acetophenones
Bacterial plasmid transfer inhibition

ABSTRACT

In a project to investigate the chemistry of South African *Plectranthus* species, from the dichloromethane extract of the aerial parts of *Plectranthus venteri*, we isolated two known natural product acetophenones, namely 2-hydroxy-3,4,5,6-tetramethoxy-acetophenone (**1**) and 2-hydroxy-4,5,6-trimethoxy-acetophenone (**2**). Structures were assigned using NMR spectroscopy and HRTOFESIMS. Compound **1** was previously synthesised as a precursor to the polymethoxylated flavone nobiletin. The acetophenones exhibited remarkable inhibitory activities against the transfer of the IncW plasmid R7K in a bacterial plasmid transfer inhibition assay.

1. Introduction

Plectranthus is the largest genus of the Lamiaceae and is represented by approximately 350 species, mostly occurring in Africa, India, Japan, Malaysia and Australia. Fifty-three of these species occur abundantly in South Africa (Van Jaarsveld, 2006). Several species are used as traditional medicine in South Africa for the treatment of various conditions ranging from coughs, wounds, gastrointestinal disorders, skin infections and for pain (Hutchings et al., 1996; Lukhoba et al., 2006). *Plectranthus* is considered paraphyletic as all *Plectranthus* species have a common ancestor but the genus does not include all the descendants of the shared ancestor (Potgieter et al., 2009). *Plectranthus* differs from the other members of the Lamiaceae as the two lipped corolla has exerted stamens attached to its throat, the bracts are smaller than the leaves and the 5-toothed calyx enlarges after fertilisation (Van Jaarsveld, 2006). The infrageneric taxonomy of *Plectranthus* remains challenging due to the lack of well-defined morphological characters. As a result of taxonomic ambiguity, numerous species have been incorrectly placed in closely related genera such as *Coleus*, *Solenostemon* and *Englerastrum* (Lukhoba et al., 2006). Furthermore, species previously assigned to *Plectranthus* now form part of the distant genus *Isodon* (Paton et al., 2004). In a project to study the chemistry of this interesting genus, we conducted a phytochemical investigation of *Plectranthus venteri* van Jaarsv. & L. Hankey, a narrow endemic to the Sekukuniland region of South Africa, which was only discovered in 1997. In order to justify the traditional uses against infectious diseases, we also focused on the antibacterial activities of the compounds isolated from this plant. Purified compounds were evaluated in a bacterial plasmid transfer inhibition assay with plasmids harbouring antibiotic-resistance genes. The rationale for this was that a reduction in plasmid transfer could result not only in a reduction of antibiotic resistance in a bacterial population, but also contribute to a reduction of virulence of a selected bacterial species.

2. Results and discussion

Compound **1** was isolated as an orange oil and HRQTOFESIMS gave an *m/z* at 257.1012 $[M+H]^+$, which indicated that its molecular formula was $C_{12}H_{16}O_6$. The ^1H NMR spectrum (Figure 1, supporting information) was very simple, accounted for all 16 hydrogens and was reminiscent of a methoxylated acetophenone (Parsons et al., 1994) (Fig. 1). An acetyl methyl group (δ_H 2.66), four methoxyl groups (δ_H 3.79, 3.84, 3.94 and 4.07) and a highly deshielded hydrogen-bonded hydroxyl group (δ_H 13.23) accounted for all positions of the aromatic acetophenone core. Given that the hydroxyl group had to be *ortho* to the acetyl group for maximal H-bonding with the carbonyl oxygen of the acetyl group, the structure of the compound could readily be assigned as

☆ This paper forms part of a special issue of Phytochemistry Letters dedicated to the memory of Andrew Marston (1953–2013), outstanding Phytochemist who is much missed by his friends.

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http://dx.doi.org/10.1016/j.phytol.2014.10.021
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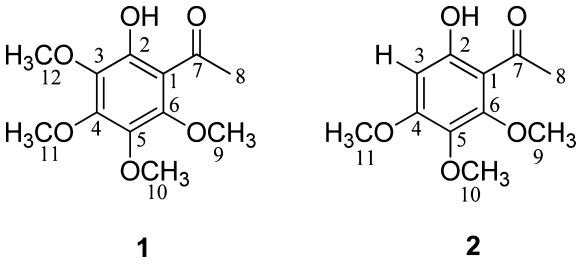

Fig. 1. Structures and numbering for compounds **1** and **2**.

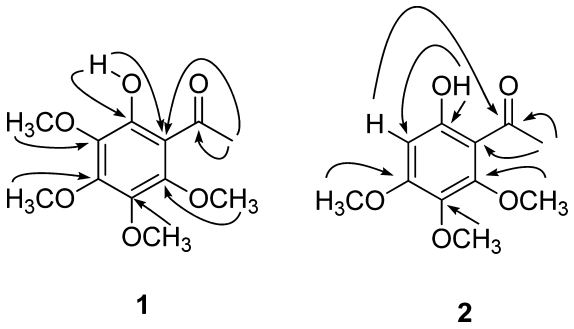

Fig. 2. HMBC correlations for compounds **1** and **2**.

2-hydroxy-3,4,5,6-tetramethoxy-acetophenone. To confirm this nomenclatural assignment, full 2-dimensional NMR spectral analyses, including HMBC spectroscopy, were carried out to unambiguously assign all ^{1}H and ^{13}C resonances (Figure 2, supporting information). The acetyl methyl hydrogens (H_3-8) showed a ^{2}J correlation to a carbonyl carbon (δ_c 204.5) and to an aromatic quaternary carbon, to which this acetyl group was directly attached (C-1, δ_C 110.4) (Fig. 2). The hydrogen-bonded hydroxyl hydrogen correlated to C-2, C-1 and to an oxygen-bearing aromatic quaternary carbon (C-3), which was also coupled to by the hydrogens of a methoxyl group (H_3-12). The three remaining methoxyl groups each coupled to their respective aromatic oxygen-bearing quaternary carbons (C-4, C-5 and C-6). Full assignment of the precise resonances of each methoxyl was achieved by careful inspection of the NOESY spectrum. Methoxyl H_3-12 showed a correlation to H_3-11, which coupled to H_3-10 which in turn coupled to H_3-9. In combination with the HSQC spectrum, we were therefore able to unambiguously assign all of their respective ^{1}H and ^{13}C resonances (Table 1). Compound **1** was therefore assigned as 2-hydroxy-3,4,5,6-tetramethoxy-acetophenone. This compound had previously been isolated as an alkaline hydrolysis degradation product from two separate studies from a polymethoxylated chromone conyzorigun from *Ageratum conyzoides* (Adesogan and Okunade, 1978) and the flavone 5,6,7,8,3′,4′,5′-heptamethoxyflavone from *Eupatorium coelestinum* (Le-Vam and Pham, 1979). Additionally, it was isolated from the Liverwort *Adelanthus decipens* (Rycroft et al., 1998). This is however, the first report of its full ^{13}C NMR data. This compound was previously synthesised using the microwave-assisted technique in a study on the inhibitory effects on melanogenesis by polymethoxylated acetophenones and polymethoxylated flavones (Tsukayama et al., 2007). The ^{1}H NMR data are in very close agreement with the natural and synthetic compound. **1** was also synthesised as a precursor to the synthesis of the *Citrus* polymethoxylated flavone nobiletin in a study of its use as a stimulator of neuronal cell signalling by positron emission tomography (Asakawa et al., 2011).

The ^{1}H and ^{13}C resonances for compound **2** (Table 1) were highly similar to those of **1** with the exception of the absence of one methoxyl group and the presence of an aromatic hydrogen (δ_H 6.21), again indicating a methoxylated acetophenone. HRQTOFE-SIMS gave an m/z at 227.0920 $[M+H]^+$, which indicated that its molecular formula was $C_{11}H_{14}O_5$. Signals in the ^{1}H NMR spectrum (Table 1 and Figure 3 (supporting information)) included a highly deshielded hydrogen-bonded hydroxyl hydrogen (δ_H 13.49), an aromatic hydrogen (δ_H 6.21), three methoxyl groups (δ_H 3.76, 3.86 and 3.97) and an acetyl methyl group (δ_H 2.63).

Inspection of the HMBC and ^{13}C spectra (Fig. 2 and Figure 4 (supporting information)) allowed unambiguous assignment of all resonances and again confirmed that compound **2**, like compound **1**, was a methoxylated acetophenone. The difference between the two compounds was identified by an HMBC correlation from the phenolic hydroxyl hydrogen to the carbon bearing an aromatic hydrogen (δ_H 6.21), therefore fixing the position of the aromatic hydrogen *ortho* to the hydroxyl group. Compound **2** was therefore assigned as 2-hydroxy-4,5,6-trimethoxy-acetophenone and has been previously isolated from *Erigeron breviscapus* (Chun et al., 2003).

Both compounds were evaluated for their ability to inhibit conjugal transfer of plasmid-mediated antibiotic resistance in *Escherichia coli*. The compounds exhibited significant inhibitory activities against the transfer of the IncW plasmid R7K with an 85% and 87% reduction in the presence of **1** and **2**, at a concentration of 100 mg/L, respectively. This activity was comparable to that of linoleic acid, a known anti-conjugation agent for IncW plasmids (Fernandez-Lopez et al., 2005). At 100 mg/L, linoleic acid gave a 92% reduction in the transfer of the R7K IncW plasmid. The activity of **1** and **2** were found to be specific as they were poorly active (with a 35% or below reduction) in limiting the transfer of the IncN plasmid pKM101, the $IncI_2$ plasmid TP114 and the IncP plasmid pUB307. At a concentration of 10 mg/L, novobiocin significantly

Table 1
^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectral data and HMBC correlations of **1** and **2** recorded in $CDCl_3$.

1					**2**				
Position	^{1}H	^{13}C	^{2}J	^{3}J	Position	^{1}H	^{13}C	^{2}J	^{3}J
1	–	110.4			1	–	108.4		
2	–	154.7			2	–	161.9		
3	–	136.7			3	6.21	96.1	C2, C4	C1, C5
4	–	151.6			4	–	160.1		
5	–	138.0			5		134.7		
6	–	153.8			6	–	155.3		
7	–	204.5			7	–	203.6		
8	2.66	32.6	C7	C1	8	2.63	32.2	C7	C1
9	4.07	61.6		C6	9	3.97	61.1		C5
10	3.79	61.5		C5	10	3.76	61.1		C4
11	3.94	61.3		C4	11	3.86	56.2		C3
12	3.84	61.2		C3	OH	13.49		C2	C1
OH	13.23		C2	C1, C3	–	–			