



First isolation of acetovanillone and piceol from *Crinum buphanoides* and *Crinum graminicola* (I. Verd.) Amaryllidaceae



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ABSTRACT

Screening of three native South African Amaryllidaceae bulbs, aimed at finding new metabolites for their promising biological activities, led to the initial discovery of two interesting non-alkaloid compounds viz., acetovanillone **1** (also known as apocynin) and 4-hydroxyacetophenone **2** (also named piceol) isolated from *Crinum buphanoides*, while only the former was isolated from *Crinum graminicola*. This is the first time that acetovanillone **1** and piceol **2** were isolated from *C. graminicola* and *C. buphanoides*, respectively. Acetovanillone **1** was recently reported as a metabolite of *Boophane disticha* (L.f.), another South Africa Amaryllidaceae species.

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1. Introduction

Plants belonging to the Amaryllidaceae family are well known for their beautiful flowers and medicinal use in folk medicine. These plants, classified into 60 genera, are distributed through both tropical and subtropical regions of the world and are dominant in Andean South America, the Mediterranean basin, and southern Africa, where one third of the 1000 known species grow. Amaryllidaceae plants have been shown to be a good source of different metabolites (many of them alkaloids) with a large spectrum of biological activity – in addition, many of these compounds have been shown to have practical and potential application in medicine (Nair et al., 2013; Nair and Van Staden, 2014; Cimmino et al., 2017). Amaryllidaceae plants, on the other hand, are also known to be poisonous and these toxic effects need to be investigated as a major requirement in their total evaluation (Nair and Van Staden, 2013). Recently, the native South Africa Amaryllidaceae plant *Nerine sarniensis* was studied and from the acid extract of its bulbs, sarniensine and sarninsinol, both belonging to the mesembrine compound class found in Amaryllidaceae alkaloids were isolated as well as, crinsarnine belonging to the *Crinum* subgroup of the same plant family. Of additional significance was that sarniensine and

crinsarnine both showed strong adulticidal activity against *Aedes aegypti*, the vector of yellow and dengue fevers and the Zika virus (Masi et al., 2016, 2017).

For this present study, two other native South Africa Amaryllidaceae plants, namely *Crinum graminicola* and *Crinum buphanoides* were selected, with the main aim of discovering new bioactive metabolites. We report here on the first identification of acetovanillone **1** (Fig. 1) from both *C. buphanoides* and *C. graminicola*, while piceol **2** (Fig. 1) was isolated only from *C. buphanoides*.

2. Methods

The bulbs of *Crinum buphanoides* and *Crinum graminicola* were purchased from the South African Bulb Company (<http://www.thesabulbcompany.co.za/>) and three live specimens of each are growing in the Botanical Garden at the University of Stellenbosch, Stellenbosch, South Africa. Fresh bulbs of *C. buphanoides* (1.0 Kg) and *C. graminicola* (1.0 Kg) were dried at 40 °C for 48 h and then finely minced. The resulting material (191.7 g) of *C. buphanoides* (CB) was extracted using a protocol previously reported (Masi et al., 2016). The organic extract appeared as a brown oil (266.6 mg) and was further fractionated by column chromatography and eluted with CHCl₃-EtOAc-MeOH (2:2:1) affording 7 fractions (CBF₁₋₇). CBF₃ was further purified on a TLC plate with CHCl₃-i-PrOH (98:2) as eluent to afford apocynin **1**

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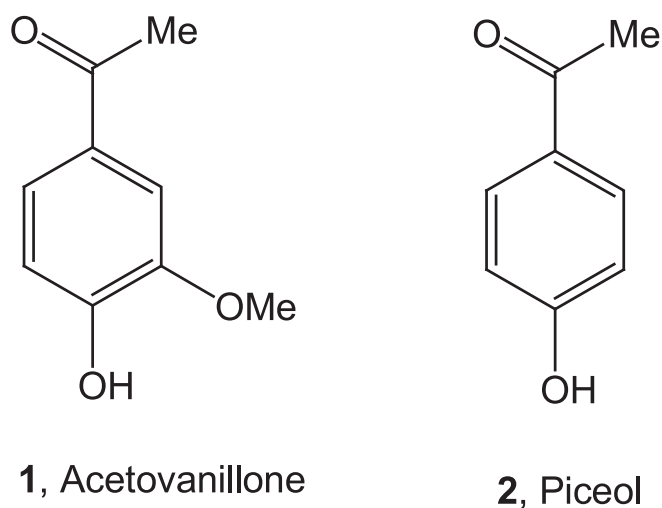


Fig. 1. Structures of acetovanillone **1** and piceol **2**.

(15.0 mg) and piceol **2** (2.4 mg). The resulting powder (215.7 g) of *C. graminicola* (CG) was extracted following the protocol used for *C. buphanoides*. The organic extract, which presented as a yellow residue (9.0 g) was fractionated by column chromatography and eluted with a gradient eluent of CHCl_3 and *i*-PrOH of increasing polarity. The first fraction (CGF₁) obtained with CHCl_3 gave apocynin **1** (2.6 mg). The compounds were visualised by exposure to iodine vapours and UV radiation and identified by NMR and MS studies. ^1H and ^{13}C NMR spectra were recorded at 600/125 MHz in CDCl_3 on Bruker spectrometers. The same solvent was used as internal standard. DEPT, COSY-45, HSQC and HMBC experiments (Berger and Braun, 2004) were performed using Bruker microprograms. ESI MS spectra were recorded on an Agilent 6230 LC MSTOF. Analytical and preparative TLC were performed on silica gel plates (Merck, Kieselgel 60 F254, 0.25) Column chromatography was performed on silica gel (Merck, Kieselgel 60, 0.063–0.200 mm).

3. Results

Acetovanillone **1** was isolated as white crystalline solid with a typical sweet vanilla-like smell. Its ESIMS spectrum showed the protonated form $[\text{M} + \text{H}]^+$ at m/z 167 as the base peak. The proton spectrum showed the typical pattern of a 1,3,4-trisubstituted benzene ring with two overlapping signals for H-6 and H-2 at δ 7.50 and a doublet ($J = 8.9$ Hz) for H-5 at δ 6.92. The singlets of the methoxy and methyl groups resonated at δ 3.93 and 2.53 respectively. The HSQC and HMBC spectra facilitated assignment of the chemical shifts to all the carbons, namely the signals at δ 196.7, 150.3, 146.6, 130.2, 123.9, 113.7, 109.7, 56.0 and 26.1 to C = O, C-4, C-3, C-1, C-6, C-5, C-2, OMe and Me respectively. The spectroscopic data were in agreement with those reported for the same metabolite recently re-isolated from *Boophone disticha* (Tagwireyi and Majinda, 2017).

Piceol **2** was isolated as a white amorphous solid with a similar sweet vanilla-like smell. Its ESIMS spectrum showed the protonated form $[\text{M} + \text{H}]^+$ at m/z 137 as the base peak. Its ^1H NMR spectrum showed the presence of a system typical of a *para*-disubstituted benzene ring with two doublets ($J = 9.0$ Hz) at δ 7.87 and 6.85 for H-2,6 and H-3,5 respectively, together with the singlet for the methyl group at δ 2.53. The carbon spectrum indicated the carbonyl signal at δ 196.7 and the signals of the aromatic ring carbons were evident at δ 159.8, 130.9, 132.5 and 115.2 for C-4, C-2,6, C-1 and C-3,5 respectively, while the methyl group resonated at δ 26.3. These spectroscopic data were in agreement with those previously reported (Borah et al., 2017).

4. Discussion

Isolation of acetovanillone **1** from both *C. buphanoides* and *C. graminicola* and piceol **2** from *C. buphanoides* is described here for the first time. The metabolites were identified essentially by MS and NMR spectroscopic data which were compared with those previously reported in the literature (Borah et al., 2017; Tagwireyi and Majinda, 2017). Acetovanillone **1** has previously been reported as being isolated from *Boophone disticha* (Tutin, 1911) and more recently re-isolated from the same species. In addition, the important anti-inflammatory activity of **1** has been reported (Van den Worm et al., 2001). Piceol **2** was isolated for the first time from *Crinum buphanoides*, and was previously isolated together with its glucoside, picein, from the needles of the Norway spruce (*Picea abies*) (Løkke, 1990). Interestingly, derivatives of **2** viz., 2-hydroxy-4,6-dimethoxyacetophenone and 2,4,6-trimethoxyacetophenone were isolated together with fifteen alkaloids, including phenanthridine-, benzylphenethylamine-, crinine-, pyrrolphenanthridine-, licorenane- and galanthamine-type alkaloids from *Lycoris albiflora* whose aqueous extract showed potent anticancer activity against HL-60 cells (Jitsuno et al., 2011). Of considerable interest was that two new isomeric glycosides viz., 4,6-dimethoxyacetophenone-2-*O*- β -D-glucoside and 2,6-dimethoxyacetophenone-4-*O*- β -D-glucoside were isolated together with the known 2,4,6-trimethoxyacetophenone from *Pancreaticum biflorum* (Ghosal et al., 1989). In this research, the allelopathic activity of the two glucosides and their aglycones were evaluated on the growth on the bulbs of two other Amaryllidaceae species. Furthermore, the glucosides showed anticancer activity while their aglycones exhibited the ability to modulate prostaglandine synthetase activity. These interesting results encourage the further investigation of the biological activities of **1** and **2**. In addition, in our opinion it would also be worthwhile to prepare a range of diverse derivatives in order to perform structure activity relationship (SAR) studies with respect to a variety of bioassays.

The isolation for the first time of acetovanillone **1** and piceol **2** is of noteworthy taxonomic value and these two metabolites could be used as potential markers for quality control in medicinal and other preparations using extracts of both *C. buphanoides* and *C. graminicola* as ingredients.

List of abbreviations

^{13}C NMR	Carbon-13 Nuclear Magnetic Resonance
^1H NMR	Proton Nuclear Magnetic Resonance
ESI MS	Electrospray Mass Spectrometry
TLC	Thin Layer Chromatography

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