A new iridoid diglucoside has been isolated from an aqueous extract of *Harpagophytum procumbens* secondary roots, together with six known compounds. Its structure has been assigned as 6'-O-glucopyranosyl-8-O-trans-coumaroylharpagide by spectroscopic means.

**Keywords:** *Harpagophytum procumbens*; iridoid glucosides; 6'-O-glucopyranosyl-8-O-trans-coumaroylharpagide; harpagoside; phenylpropanoid glycosides

1. Introduction

*Harpagophytum procumbens* D.C. (Pedaliaceae), very well known with the common name ‘Devil’s claw’ for the characteristic shape of its fruits, is widely distributed in southern African countries, where its secondary roots have been used by native people to treat digestive disorders, arthritis and rheumatic diseases (Stewart & Cole 2005).

The *H. procumbens* roots hydro-alcoholic extracts have been used in a great number of studies and there are abundant data supporting its clinical efficacy in inflammation and pain reduction (Chrubasik et al. 1996, 2004; Chantre et al. 2000). The pharmacological effects of *H. procumbens*, in particular the anti-inflammatory activity, have been attributed to its major constituents, mainly iridoid glycosides based on the structure of harpagide (McGregor et al. 2005; Qi et al. 2006), leading to a wide utilisation of the extracts in medicine (Wichtl 2004; Gagnier et al. 2007). However, depending on the extraction procedure of the crude drug, the proportions of constituents in *H. procumbens* extract (in particular, the ratio iridoid/phenylpropanoid glycosides) can differ greatly with potentially varied pharmacological effects (Boje et al. 2003; Bae et al. 2014). Due to these considerations, since the great majority of the
ethnomedicine remedies are prepared with water, we decided to re-examine the content of the aqueous extract obtained from *H. procumbens* raw material.

## 2 Results and discussion

The secondary roots of *H. procumbens* D.C. were first extracted with H$_2$O and the aqueous solution was successively fractionated with EtOAc and then with n-BuOH. The dried EtOAc fraction was then separated by column chromatography (CC), affording pure harpagoside (1) and 8-trans-coumaroylharpagide (2). The dried n-BuOH fraction was separated by reverse-phase CC, affording pure 8-trans-coumaroylharpagide (2), acteoside (3) and isoacteoside (4). A further CC separation, directly obtained from another portion of the dried aqueous extract, allowed to isolate 3,4-dimethoxybenzoic aldehyde (5), trans-cinnamic acid (6), together with harpagoside (1) and a more polar iridoid glucoside which was identified as the new 6'-O-glucopyranosyl-8-O-trans-coumaroylharpagide (7). All the structures (Figure 1) have been determined by spectroscopic means.

In particular, regarding compound 7, it was observed that its $^1$H and $^{13}$C NMR spectra appeared almost identical to those of 8-O-trans-coumaroylharpagide (2), with the presence of the signals of an additional glucose unit (H-1'' at $\delta$ 4.37 and C-1'' at $\delta$ 101.6). The two glucose units appeared linked through a β-1,6 glycosidic bond, as evidenced by the characteristic low field shift of the C-6' signal ($\delta$ from 62.6 to 69.4). Further confirmation was obtained by two-dimensional NMR spectra $^1$H–$^1$H COSY and $^1$H–$^{13}$C HMQC. Finally, nOe-difference NMR experiments allowed to obtain the relative configurations for the stereogenic centres C-6 and C-8, assigning the configurations 6'-β-OH and 8-β-OH/8-α-CH$_3$, analogously to the harpagide structure and in complete agreement with the $^{13}$C NMR spectrum data interpretation, according to Damtoft et al. (1982). The structure of 7 was therefore assigned as 6'-O-glucopyranosyl-8-O-trans-coumaroylharpagide.

## 3 Experimental

### 3.1. General analytical procedures

Optical rotations were measured on a JASCO DIP-370 polarimeter (Jasco, Easton, MD, USA). NMR spectra were recorded in CD$_3$OD with a Bruker Avance 400 NMR spectrometer (Bruker, Karlsruhe, Germany). ESI-MS were run on a Thermofisher LCQDeca XP Plus ion-trap mass spectrometer (Thermofisher, Waltham, MA, USA). MPLC was carried out on a reverse phase Merck Lobar RP-18 column. Thin layer chromatography was performed on silica gel SiF$_{254}$ and plates were visualised using 2N H$_2$SO$_4$ as spray reagent.

### 3.2. Plant material, extraction and isolation

Grinded secondary roots of *H. procumbens* D.C. were supplied by the company A. Minardi (Bagnacavallo, RA, Italy), which identified and collected the plant in Namibia, Africa (lot n. 0PMP0062). Then 50 g of drug were extracted with H$_2$O at a temperature of 40°C for 24 h, filtered and concentrated to dryness, obtaining a dry residue of 20.4 g. Subsequently, a portion of the residue (9.5 g) was re-suspended in H$_2$O (400 mL) and extracted first with EtOAc (300 mL) and then with n-BuOH (300 mL). The residue obtained by evaporation of the EtOAc fraction (3.9 g) was chromatographed by CC on silica gel in CH$_2$Cl$_2$–MeOH (9:1), affording harpagoside (1) (32 mg) and 8-trans-coumaroylharpagide (2) (27 mg). The dried n-BuOH fraction (2.0 g) was subjected to reverse-phase chromatographic separation by MPLC (MeOH–H$_2$O, 4:6), affording pure 8-trans-coumaroylharpagide (2) (15 mg) and the two isomeric phenylpropanoid glycosides acteoside (3) (36 mg) and isoacteoside (4) (22 mg).
Figure 1. Structures of compounds 1–7.
A subsequent separation has been obtained by subjecting a second portion of the initial aqueous extract residue (8.2 g) to a new silica gel chromatographic column, eluted with increasingly polar mixture of CHCl$_3$–MeOH (rates from 9:1 to 7:3). The main components isolated from this column, in pure form, were the simple molecules 3,4-dimethoxybenzoic aldehyde (5) (23 mg) and trans-cinnamic acid (6) (12 mg), together with harpagoside (1) (51 mg) and the more polar iridoid diglucoside 6-O-glucopyranosyl-8-O-trans-coumaroylharpagide (7) (10 mg).

3.3 6′-O-glucopyranosyl-8-O-trans-coumaroylharpagide (7)

Amorphous powder. [α]$^20_{D} = - 10.2$ (c = 0.30, MeOH). $^1$H NMR (400 MHz, CD$_3$OD); δ 1.42 (3H, s, H$_3$-10), 1.90 (1H, dd, $J = 15.4$ and 5.0 Hz, H-7a), 2.17 (1H, bd, $J = 15.4$ Hz, H-7b), 2.83 (1H, s, H-9), 3.02 (1H, t, $J = 8.6$ Hz, H-2$^0$), 3.12 (1H, t, $J = 8.6$ Hz, H-2$^0$), 3.16–3.35 (5H, m, H-5$^0$, H-4$^0$, H-4$^00$, H-3$^0$, H-3$^00$), 3.41 (1H, dd, $J = 11.2$ and 9.1 Hz, H-6$^0$), 3.49 (1H, dd, $J = 11.2$ and 4.6 Hz, H-6$^0$), 4.37 (1H, d, $J = 8.2$ Hz, H-1$^0$), 4.53 (1H, d, $J = 8.2$ Hz, H-1$^0$), 4.84 (1H, d, $J = 6.1$ Hz, H-4), 6.08 (1H, s, H-1), 6.21 (1H, d, $J = 15.7$ Hz, H-α), 6.31 (1H, d, $J = 6.1$ Hz, H-3), 6.70 (2H, d, $J = 8.7$ Hz, H-5$^0$ and H-5$^00$), 7.35 (2H, d, $J = 8.7$ Hz, H-2$^0$ and H-6$^0$), 7.50 (1H, d, $J = 15.7$ Hz, H-β). $^{13}$C NMR (100 MHz, CD$_3$OD); δ 23.0 (C-10), 47.2 (C-7), 56.5 (C-9), 62.6 (C-6$^0$), 69.4 (C-6$^00$), 71.9 (C-4$^0$), 72.9 (C-4$^00$), 73.8 (C-2$^0$), 73.8 (C-2$^00$), 74.1 (C-5), 76.8 (C-5$^0$), 77.1 (C-5$^00$), 77.3 (C-3$^0$), 77.6 (C-3$^00$), 78.1 (C-6), 88.0 (C-8), 94.6 (C-1), 99.0 (C-1$^0$), 101.6 (C-1$^00$), 107.8 (C-4), 116.0 (C-α), 117.5 (C-3$^00$), 129.1 (C-1$^0$), 122.2 (C-2$^00$), 144.0 (C-3), 146.5 (C-β), 162.2 (C-4$^00$), 166.6 (COO$^-$). HR-ESI-MS $m/z$ 673.2348 [M $+$ H$^+$] (calcld for C$_{30}$H$_{41}$O$_{17}$$^+$, 673.2344). Elem. anal. C 53.54%, H 6.01%, calcld for C$_{30}$H$_{40}$O$_{17}$, C 53.57%, H 5.99%.

4 Conclusions

From the phytochemical study of the $H$. procumbens aqueous extract, we expected to find a predominant occurrence of phenylpropanoid glycosides, based on their high polarity and on recent literature data regarding the plant (Bae et al. 2014). On the contrary, we found iridoid glucosides are always predominant in the fractions analysed, but with the unusual absence of procumbide, which is a characteristic and abundant metabolite of this plant (Bianco et al. 1971). This peculiarity could be related to the extraction procedure, since in other studies based on aqueous extracts of $H$. procumbens, procumbide was not detected as well (Baghdikian et al. 1999; Boje et al. 2003). Among our results, the isolation of the new compound 6′-O-glucopyranosyl-8-O-trans-coumaroylharpagide (7) is very interesting, as it could play an important role by its enhanced polarity in the activity, explaining in part the effectiveness of the aqueous preparations used in folk medicine.

Supplementary material

Supplementary material relating to this article is available online.

Disclosure statement

No potential conflict of interest was reported by the authors.

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