Available online at www.sciencedirect.com
SCIENCE DIRECT®
PERGAMON
Phytochemistry 62 (2003) 1231–1234
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

# Acetylated phenolic glycosides from *Harpagophytum procumbens*

Namboole Moses Munkombwe

*Chemistry Department, University of Botswana, Private Bag UB 00704, Gaborone, Botswana*

Received in revised form 6 October 2002

**Abstract**

Two acetyl phenolic glycosides, 6-acetylacteoside and 2,6-diacetylacteoside, were obtained from commercially available secondary roots of *Harpagophytum procumbens* and were identified using spectroscopic methods.


*Keywords:* *Harpagophytum procumbens*; Pedaliaceae; Acetylated phenolic glycosides

## 1. Introduction

The secondary roots of *Harpagophytum procumbens* are commercially available in Southern African, European and Canadian markets, and are widely used as rheumatic and arthritis remedies (Baghdikian et al., 1997; Wenzel and Wegener, 1995; Moussard et al., 1992). In Botswana, the root tuber of the devil's claw is harvested, processed and packaged by an umbrella nongovernmental organisation called Thusano lefatsheng. The plant is commercially farmed in South Africa and Namibia. There are many reports on the phytochemical (Lichti and Von Wartburg, 1966; Tunmann and Hammer, 1968; Bianco et al., 1971; Czygan et al., 1977; Kikuchi et al., 1983) and biological aspects (Eichler and Koch, 1970; Sticher, 1977; Czygan and Krueger, 1977; Fontaine et al., 1981) of this plant. In 1987, three phenolic glycosides, acteoside, isoacteoside and a bioside were isolated from *H. procumbens* (Burger et al., 1987). This paper reports on two additional phenolic glycosides from the secondary roots.

## 2. Results and discussion

Two acetylphenolic glycosides were obtained along with the known acteoside, isoacteoside, harpagoside, procumbide, harpagide and 8-*O*-parahydroxy-*E*-cinnamoylharpagide from the roots of *H. procumbens*. These acetyl derivatives had very similar spectroscopic features to acteoside. Like acteoside, they are made up of two aromatic moieties, 2,3 dihydroxyphenylpropenoyl and 2,3-dihydroxyphenylethyl, and two sugars, glucose and rhamnose. Compound **1** had one acetyl group while compound **2** had two acetyl groups.

Under positive electrospray mass spectrum, 6-acetyl acteoside **1** gave an $[M+Na]^+$ peak at $m/z$ 689.3 while the negative electrospray peak, $[M-H-Na]^+$, was at $m/z$ 665.3, thus the molecular mass was 666.3. The molecular formula was found to be $C_{31}H_{38}O_{16}$ from NMR data and MS. The $^1H$ NMR peak for the methyl part of the acetoxy group was a singlet resonating at $\delta$ 2.05 with a corresponding peak at 21.1 in the $^{13}C$ NMR spectrum. The carbonyl peak was resonating at 173.0 ppm as revealed by HMBC and HMQC data. The acetyl group was positioned on the methylene carbon (C-6) of glucose, because the resonance of $H_2$-6 appeared at $\delta$ 4.17 and 4.20. Furthermore, these protons showed HMBC correlations to carbons at 73.5 (C-5) and 173.0 (acetoxy carbonyl). The anomeric configurations ($\alpha$ for L-rhamnose and $\beta$ for D-glucose) were established from the coupling constants of the anomeric protons. The rest of the data matched well with acteoside as it is shown in the Tables 1 and 2. All the assignments were based on the results of COSY, HMQC and HMBC experiments. Hence compound **1** was established as 6-acetyl acteoside: *O*-α-L-rhamnopyranosyl(1→ 3)-6-*O*-acetyl-1-(3,4-dihydroxyphenylethyl)-4-(3,4-dihydroxy-*E*-cinnamoyl)-β-D glucopyranoside.

The positive and negative electrospray MS peaks for 2,6-diacetylacteoside, (**2**), were at $m/z$ 731.3 and 707.3,

*E-mail address:* munkomb@mopipi.ub.bw (N.M. Munkombwe).

0031-9422/03/$ - see front matter 
doi:10.1016/S0031-9422(02)00700-8

respectively. Like 6-acetylacteoside **1**, compound **2** had an acetyl group at position six of glucose and another acetyl group was located at C-2 of glucose. In fact the H-2 of glucose appeared deshielded at $\delta$ 4.90, overlapped to the solvent ($CD_3OD$) peak, and was easily located using COSY, HMBC, and HMQC experiments.

## 3. Experimental

### 3.1. General procedures

$^1H$ and $^{13}C$ NMR were recorded at 300.13 and 75.47 MHz, respectively, using a Brüker instrument with TMS as internal standard. 2D spectra were obtained using XWIN-NMR Version 2.6 software. Electrospray mass spectra were obtained using Finnigan LCQDECA. Silica $GF_{254}$ were used for TLC, vacuum liquid chromatography and flash chromatography. Sephadex LH-20 was also used for gel filtration chromatography.

### 3.2. Plant material

Two hundred grams of commercially available *Harpagophytum procumbens* or Kalahari Devil's claw [*Sengaparile* in Setswana] was bought on 27 September 2000, at P8.95 from The Chemist, African Mall, Gaborone. The packaging is by Thusano Lefatsheng, Private 00251, Gaborone, Botswana.

Table 1
$^1H$ (300 MHz) and $^{13}C$ (75.47) spectral data for 6-acetyl acteoside in $CD_3OD$

| $^{13}C$ NMR | $\delta_C$ | $^1H$ NMR | $\delta_H$ (*m*, *J*, Hz) |
|---|---|---|---|
| Glucose | ppm | | |
| 1 | 104.7 | 1 | 4.42 (*d*, 7.9) |
| 2 | 76.5 | 2 | 3.41 (*dd*, 8.1 and 8.9) |
| 3 | 81.8 | 3 | 3.84 (*t*, 9.2) |
| 4 | 70.8 | 4 | 5.01 (*t*, 9.8) |
| 5 | 73.5 | 5 | 3.51–3.65 (*m*) |
| 6 | 64.3 | 6a | 4.20 (*dd*, 5.0 and 12.1) |
| | | 6b | 4.17 (*dd*, 2.5 and 12.1) |
| 6-Acetyl | 173.0 | | |
| | 21.1 | | 2.05 (*s*) |
| Phenylethyl | | | |
| 1′ | 131.8 | | |
| 2′ | 117.5 | 2′ | 6.70 (*d*, 2.1) |
| 3′ | 146.6 | | |
| 4′ | 145.1 | | |
| 5′ | 116.7 | 5′ | 6.69 (*d*, 7.9) |
| 6′ | 121.6 | 6′ | 6.59 (*dd*, 1.9 and 8.1) |
| 7′ | 72.9 | 7a′ | 2.81 (*bt*) |
| 8′ | 37.0 | 8a′ | 3.97 (*dt*) |
| | | 8b′ | 3.75 (*dt*) |
| Rhamnose | | | |
| 1″ | 103.5 | 1″ | 5.20 (*d*, 1.5) |
| 2″ | 72.7 | 2″ | 3.93 (*dd*, 1.5 and 3.2) |
| 3″ | 72.5 | 3″ | 3.51–3.65 (*m*) |
| 4″ | 74.2 | 4″ | 3.32 overlaps with solvent |
| 5″ | 70.9 | 5″ | 3.51–3.65 (*m*) |
| 6″ | 18.8 | $CH_3$ | 1.10 (*d*, 6.0) |
| Phenylpropenoyl | | | |
| 1‴ | 128.1 | | |
| 2‴ | 115.7 | 2‴ | 7.07 (*d*, 1.9) |
| 3‴ | 148.5 | | |
| 4‴ | 150.2 | | |
| 5‴ | 116.9 | 5‴ | 6.79 (*d*, 8.1) |
| 6‴ | 123.6 | 6‴ | 6.97 (*dd*, 2.0 and 8.2) |
| 7‴ | 147.2 | 7‴ | 7.60 (*d*, 15.8) |
| 8‴ | 115.0 | 8‴ | 6.29 (*d*, 16.0) |
| 9‴ | 168.4 | | |

Table 2
$^1H$ (300 MHz) and $^{13}C$ (75.47) spectral data for acteoside in $CD_3OD$

| $^{13}C$ NMR | $\delta_C$ | $^1H$ NMR | $\delta_H$ (*m*, *J*, Hz) |
|---|---|---|---|
| Glucose | ppm | | |
| 1 | 104.6 | 1 | 4.39 (*d*, 7.7) |
| 2 | 76.7 | 2 | 3.41 (*dd*, 8.1 and 9.0) |
| 3 | 82.1 | 3 | 3.85 (*t*, 9.2) |
| 4 | 76.6 | 4 | 5.00 under water peak |
| 5 | 76.4 | 5 | 3.51–3.65 (*m*) |
| 6 | 62.8 | 6a | 3.51–3.65 (*m*) |
| 2-Acetyl | 171.9 | 6b | 3.51–3.65 (*m*) |
| | 21.2 | | 2.00 (*s*) |
| 6-Acetyl | 173.0 | | |
| | 21.3 | | 2.06 (*s*) |
| Phenylethyl | | | |
| 1′ | 131.9 | | |
| 2′ | 117.5 | 2′ | 6.71 (*d*, 2.1) |
| 3′ | 146.0 | | |
| 4′ | 145.1 | | 3.31 (*t*, 9.6) |
| 5′ | 116.7 | 5′ | 6.69 (*d*, 8.3) |
| 6′ | 121.7 | 6′ | 6.58 (*dd*, 2.0 and 8.0) |
| 7′ | 72.7 | 7′ | 2.81 (*m*) |
| 8′ | 37.0 | 8a′ | 4.07 (*m*) |
| | | 8b′ | 3.74 (*m*) |
| Rhamnose | | | |
| 1″ | 103.5 | 1″ | 5.20 (*d*, 1.7) |
| 2″ | 72.8 | 2″ | 3.94 (*dd*, 1.7 and 3.2) |
| 3″ | 72.5 | 3″ | 3.51–3.65 (*m*) |
| 4″ | 74.2 | 4″ | 3.28 (*t*, 9.4) |
| 5″ | 70.9 | 5″ | 3.51–3.65 (*m*) |
| 6″ | 18.9 | $CH_3$ | 1.11 (*d*, 6.2) |
| Phenylpropenoyl | | | |
| 1‴ | 128.1 | | |
| 2‴ | 115.5 | 2‴ | 7.07 (*d*, 2.1) |
| 3‴ | 148.5 | | |
| 4‴ | 150.2 | | |
| 5‴ | 116.9 | 5‴ | 6.80 (*d*, 8.1) |
| 6‴ | 123.6 | 6‴ | 6.97 (*dd*, 2.1 and 8.3) |
| 7‴ | 147.3 | 7‴ | 7.61 (*d*, 15.8) |
| 8‴ | 115.1 | 8‴ | 6.29 (*d*, 16.0) |
| 9‴ | 168.7 | | |

### 3.3. Extraction and isolation

*H. procumbens* secondary roots (500 g) were extracted with $CH_2Cl_2$/MeOH (1:1) for 24 h followed by MeOH for 1 h. The combined extracts were evaporated and a slurry prepared. Using vacuum liquid chromatography (PTLC silica gel), the slurry was resolved eluting with a gradient from hexane to EtOAc, and to $CH_3COCH_3$, to MeOH. Fractions (200 ml each) were collected as follows: hexane (one); hexane/EtOAc, 1:1 (one); EtOAc (two); EtOAc/$CH_3COCH_3$; 1:1 (two); $CH_3COCH_3$ (two); $CH_3COCH_3$/MeOH, 9:1 (two); and $CH_3COCH_3$/MeOH; 1:1 (four). Further separations were achieved using flash chromatography (eluting with $CHCl_3$/MeOH gradients saturated with $H_2O$) in conjunction with Sephadex LH-20 (eluting with $CHCl_3$/MeOH, 2:1).

Fractions 6 and 7 gave:

*Compound* **2**: *O*-α-L-rhamnopyranosyl(1→3)-2,6-di-*O*-acetyl-1-(3,4-dihydroxyphenylethyl)-4-(3,4-dihydroxy-*E*-cinnamoyl)-β-D glucopyranoside (2,6-diacetyacteoside) (28 mg); brown solid; $[\alpha]_D^{20}$ −87 (*c* 0.23, MeOH); $\lambda_{max}^{MeOH}$ nm (log ε) 334 (3.60) and 296 (3.53); for NMR data see Table 3

*Compound* **1**: *O*-α-L-rhamnopyranosyl(1→3)-6-*O*-acetyl-1-(3,4-dihydroxyphenylethyl)-4-(3,4-dihydroxy-*E*-cinnamoyl)-β-D glucopyranoside (6-acetyl acteoside) (125 mg) brown solid; $[\alpha]_D^{20}$ +43 (*c* 2.41, MeOH); $\lambda_{max}^{MeOH}$ nm (log ε): 333 (3.63) and 297 (3.41). For NMR data see Table 1. And acteoside (verbascoside) (81 mg) $[\alpha]_D^{20}$ −82 (*c* 1.36, MeOH); $\lambda_{max}^{MeOH}$ nm (log ε): 335 (4.06) and 297 (3.92). Electrospray MS; 647.3 ($[M+Na]^+$), 623.3 ($[M-H-Na]^+$).

Fractions 8–11 gave a mixture of acteoside and isoacteoside (300 mg), harpagoside (1.5 g), harpargide (44 mg), 8-*O*-*para*hydroxyl-*E*-cinnamoylharpagide (63 mg) and procumbide (350 mg).

Table 3
$^1H$ (300 MHz) and $^{13}C$ (75.47) spectral data for 2,6-diacetylacteoside in $CD_3OD$

| $^{13}C$ NMR | $\delta_C$ | $^1H$ NMR | $\delta_H$ (m, *J*, Hz) |
|---|---|---|---|
| Glucose | ppm | | |
| 1 | 102.2 | 1 | 4.56 (*d*, 8.1) |
| 2 | 75.4 | 2 | 4.90 under water peak |
| 3 | 80.7 | 3 | 4.03 (*t*, 9.2) |
| 4 | 71.2 | 4 | 5.09 (*t*, 9.6) |
| 5 | 73.5 | 5 | 3.81 (*m*) |
| 6 | 64.1 | 6a | 4.20 (*dd*, 4.7 and 12.2) |
| | | 6b | 4.12 (*dd*, 2.8 and 12.3) |
| 2-Acetyl | 171.9 | | |
| | 21.2 | | 2.00 (*s*) |
| 6-Acetyl | 173.0 | | |
| | 21.3 | | 2.06 (*s*) |
| Phenylethyl | | | |
| 1′ | 132.1 | | |
| 2′ | 116.2 | 2′ | 6.60 (*d*, 1.9) |
| 3′ | 146.5 | | |
| 4′ | 145.0 | | |
| 5′ | 117.0 | 5′ | 6.69 (*d*, 8.1) |
| 6′ | 121.7 | 6′ | 6.54 (*dd*, 1.9 and 8.1) |
| 7′ | 36.7 | 7′ | 2.72 (*m*) |
| 8′ | 72.4 | 8a′ | 4.03 (*dt*, 4.7 and 9.6) |
| | | 8b′ | 3.64 (*m*) |
| Rhamnose | | | |
| 1″ | 103.5 | 1″ | 4.82 (*d*, 1.7) |
| 2″ | 72.8 | 2″ | 3.64 (*dd*, 1.7 and 3.2) |
| 3″ | 72.5 | 3″ | 3.52–3.55 (*m*) |
| 4″ | 74.2 | 4″ | 3.28 (*t*, 9.4) |
| 5″ | 70.9 | 5″ | 3.48–3.53 (*m*) |
| 6″ | 18.9 | $CH_3$ | 1.08 (*d*, 6.2) |
| Phenylpropenoyl | | | |
| 1‴ | 128.0 | | |
| 2‴ | 115.7 | 2‴ | 7.07 (*d*, *2.1*) |
| 3‴ | 148.7 | | |
| 4‴ | 150.3 | | |
| 5‴ | 117.6 | 5‴ | 6.80 (*d*, 8.1) |
| 6‴ | 123.7 | 6‴ | 6.97 (*dd*, 2.1 and 8.3) |
| 7‴ | 147.3 | 7‴ | 7.61 (*d*, 15.8) |
| 8‴ | 114.9 | 8‴ | 6.28 (*d*, 15.8) |
| 9‴ | 168.3 | | |

## Acknowledgements

University of Botswana is acknowledged for providing research funds. Mr. Stumbo Marape and Mr. Detsang Mosimanegape provided NMR and MS data, respectively.

## References

Baghdiapkian, B., Lanhers, M.C., Fleurentin, J., Ollivier, E., Balansard, G., Mortier, F., 1997. An analytical study, anti-inflammatory and analgesic effects of *Harpagophytum procumbens* and *H. zeyheri*. Planta Med. 63, 171–176.

Bianco, A., Esposito, P., Guiso, M., Scarpati, M.L., 1971. Iridoids. X. Procumbide, a diastereoisomer of antirrinoside. Gazz Chim. Ital. 101, 764–773.

Burger, J.F.W., Brandt, E.V., Ferreira, D., 1987. Iridoid and phenolic glycosides from *Harpagophytum procumbens*. Phytochemistry 26, 1453–1457.

Czygan, F.C., Krueger, A., Achier, W., Volk, O.H., 1977. Pharmaceutical-biological studies of the genus *Harpagophytum procumbens* (Bruch.) DC ex Meisson. Part I. Phytochemical standardisation of tubera Harpagophyti. Dtsch. Apoth.-Ztg. 117, 1431–1434.

Czygan, F.C., Krueger, A., 1977. Pharmaceutical-biological studies of the genus *Harpagophytum procumbens*. Part 3. Distribution of the iridoid glycoside harpagoside in the different organs of *Harpagophytum procumbens* DC. and *Harpagophytum zehyeri Decne*. Planta Med. 31, 305–307.

Eichler, O., Koch, C., 1970. Antiphylogistic, analgetic, and spasmolytic effects of Harpagosidea glycoside from *Harpagophytum procumbens* roots. Arzneim.-Forsch. 20, 107–109.

Fontaine, J., Elchami, A.A., Vanhaelen, M., Vanhaelen-Fatre, R., 1981. Biological analysis of *Harpagophytum procumbens* DC. II. Pharmacological analysis of the effects of harpagoside, harpagide, and harpagogenine on the isolated guinea pig *ileam*. J. Pharm. Belg. 36, 321–324.

Kikuchi, T., Matsuda, S., Kubo, Y., Namba, T., 1983. New iridoid glucosides from *Harpagophytum procumbens* DC. Chem. Pharm. Bull. 31, 2296–2301.

Lichti, H., Von Wartburg, A., 1966. Irodoids. II. Structure of harpagoside. Helv. Chim. Acta 49, 1552–1580.

Moussard, C., Alber, D., Toubin, M.M., Thevenon, N., Henry, J.C., 1992. A drug used in tradition medicine, *Harpagophytum procumbens*: no evidence for NSAID-like effect on the whole blood eicosanoid production in human. Fatty Acids 46, 283–286.

Sticher, O., 1977. The important drug: *Harpagophytum procumbens*. Dtsch. Apoth.-Ztg. 117, 1279–1284.

Tunmann, P., Hammer, H.E., 1968. Components of Harpagophytum roots. IV. Structure of procumbide. Ann. Chem. 712, 138.

Wenzel, P., Wegener, T., 1995. Devil's claw: an antirheumatic of plant origin. Dtsch. Apoth.-Ztg. 135, 15–16, 19–20, 23–24, 27–28.