Iridoids from *Stachys grandidentata* (Labiatae)

Orlando Muñoz[a*], Raúl C. Peña[b] and Gloria Montenegro[b]

[a] Departamento de Química, Facultad de Ciencias, Universidad de Chile. Las Palmeras 3425. Santiago, Chile. Fax: 56 (2) 271 3888. E-mail: omunoz@uchile.cl

[b] Facultad de Agronomía e Ingeniería Forestal. Pontificia Universidad Católica, P. O. Box 306. Santiago-22, Chile

* Author for correspondence and reprint request

Z. Naturforsch. **56 c,** 902–903 (2001);
received May 10/July 3, 2001

Stachys grandidentata Lindl., Labiatae, Structure Elucidation

Monomelittoside, melittoside, 8-acetyl-harpagide, harpagide, ajugol, 5-desoxy-harpagide, 5-desoxy-8-acetyl-harpagide and catalpol were isolated from the aerial parts of *S. grandidentata*.

Introduction

Stachys, one of the largest genera of Labiatae, contains *ca.* 275 species. It is a subcosmopolitan genus centered in warm temperate regions of Mediterranean Europe and in almost all types of habitat and at all altitudes (Heywood,1978). Many *Stachys* species have been investigated for their capability to produce bioactive secondary metabolites, and a number of compounds exhibiting a variety of structures as well as chemical and biological properties have been described so far (Skaltsa *et al.*,1999).

The genus *Stachys* is well represented in Chile (Epling, 1934; Marticorena and Quezada, 1985). Plant of this genus are used in folk medicine for their antispasmodic and sedative properties (Muñoz *et al.*, 1981).

As part of a research program on secondary metabolites from species of the Labiatae used in Chilean folk medicine we report on a chemical study of the aerial parts of *Stachys* species. To the best of our knowledge, no report has appeared on the constituents of Chilean *Stachys*.

Material and Methods

General experimental procedures

Melting points are uncorrected, solvents used for NMR, were $CDCl_3$, and DMSO-d6. The measurements of NMR spectra were carried out on a Bruker AMX-300 (H^1 NMR (300MHz), ^{13}CNMR (75 MHz) and Bruker AM 400 spectrometer .

Plant material

Aerial parts of *Stachys grandidentata* Lindl. were collected in October 1992, at La Serena, Chile and identified by Mrs. Gina Arancio. A voucher sample is kept in Universidad de La Serena, and duplicates specimens in the Herbarium of O. Muñoz, Chemistry Department, Faculty of Sciences, University of Chile, Santiago.

Extraction and isolation

Dried and finely powdered aerial parts (2 kg) were extracted with EtOH (2.5 l × 3) at room temperature for one week. After removal of the solvent *in vacuo*, and the pH adjusted to neutrality by addition of calcium carbonate, 20 ml of water were added to increase the solubility, and decolourising charcoal (105 g) until no visible reaction occurred with vanillin reagent. The resulting suspension was stirred for 30 min at room temperature to allow for the complete adsorption of the substances and then stratified on a gooch funnel, in which a thin layer of silica gel has previously been deposited in order to avoid the obstruction of the gooch.

The charcoal-silica gel pad was eluted with distilled water (500 ml) then with EtOH-H_2O 5% 30%, 50%, 70% v/v aqueous ethanolic solution and tested with vanillin reagent.

The 30–70% fraction was subjected to repeated chromatography on silica gel 60 (Macherey Nagel; Kieselgel 60, 50–200 μm) with *n*-BuOH/MeOH/H_2O gradient (69:10:30 v/v) resulting in 29 fractions (7 ml each). Analysis by TLC with *n*-BuOH/MeOH/H_2O (60:10:30 v/v) allowed the isolation of eleven almost pure fractions and twenty complex fractions. Fractions 2, 3, 5, and 8 directly yielded **1** (15 mg), **2**(10 mg), **4** (20 mg) and **3** (2 mg), respectively. The remaining fractions 9, 10 and 11 were applied to column chromatography on silica gel with *n*-BuOH/MeOH/H_2O (60:5:35 v/v) gradient yielding **8** (2.1 mg), **6** (1 mg), **5** (3 mg) and **7** (1 mg). Eighteen remaining frac-

0939–5075/2001/0900–0902 $ 06.00

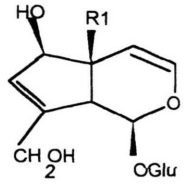
tions were complex mixtures and were not purified further.

The products of above were desiccated and identified by comparison of spectral (^{1}HNMR ^{13}C NMR) and chromatography properties with authentic samples obtained as natural products.

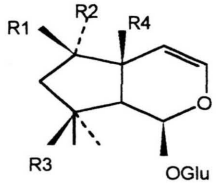
Results

Eight compounds were isolated and identified by ^{1}H NMR: monomelittoside **1** (15 mg), ^{1}H NMR (300 MHz, D_2O), 5.70 (d, J = 2.5 Hz; H-1), 6.17 (d, J = 6.4 Hz; H-3), 5.06 (dd, J = 1.2 Hz; H-4), 4.25 (m, H-6), 5.78 (m, H-7), 3.2 (m; H-9), 4.25 (m, H-10), ^{13}C NMR (D_2O), 93.6(1), 142.4(3), 108.4(4), 72.8(5), 80.5(6), 127.7(7), 148.3(8), 53.6(9), 60.8(10), 99.4(1′), 74.4(2′), 78.2(3′), 71.6(4′), 77.4(5′), 62.6(6′) coincided with those reported (Nicoletti, 1989); melittoside **2** (10 mg), 8 acetyl-harpagide **3** (2 mg), harpagide **4** (20 mg), ajugol **5** (3 mg), ^{1}H NMR (500 MHz, D_2O), 5.52 (1H, d, J = 2.1 H-1), 6.22 (1H, dd, J= 6.3, 1.9 Hz; H-3), 4.90 (1H, dd, J = 6.3; 2.4 Hz; H-4), 2.72 (m, H-5), 4.04 (dt, J = 5.2, 2,9; H-6), 2.04 (dd, J-13.1; 5.0 Hz; H-7), 2.50 (dd, J = 9.6; 2.0 Hz; H-9), 1.31 (s) H-10); coincided with those reported (Boros and Stermitz, 1990). 5-desoxy-harpagide **6** (1 mg), 5-desoxy-8-acetyl-harpagide **7** (1 mg) and catalpol **8** (2.1 mg): ^{1}H NMR (CD_2O, 300 Hz), 5.53 (d, J = 10 Hz; H-1), 6.85 (dd, J = 6.0; 4.0 Hz; H-4), 5.61 (dd, J = 6.0; 4.0 Hz; H-4), 2.64–2.86 (m, H-5), 4.51 (dd, J= 10; 8 Hz; H-6), 4.10 (S; H-7), 3.12 (dd, J = 10; 10Hz; H-9), 4.22–4.72 (AB, J= 13Hz; H-10), ^{13}C NMR (CD_3OD; 25.2 MHz), 95.33 (1), 141.78(3), 104.03 (4), 39.10(5), 78.58(6), 62.55(7), 66.23 (8), 43.60(9), 61.60(10), 99.74(1′), 74.82 (2′), 78.54 (3′), 71.74(4′), 77.70(5′), 62.90(6′) coincided with those reported (Inouye, 1991), (Fig. 1).

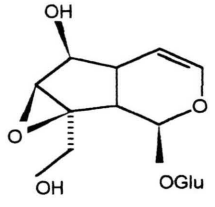
Ajugol, 5-desoxy-harpagide, 5-desoxy-8-acetyl-harpagide and catalpol were further elucidated by



	R1
1:	OH
2:	OGlu



	R1	R2	R3	R4
3:	OH	H	OAc	OH
4:	OH	H	OH	OH
5:	H	OH	OH	H
6:	OH	OH	OH	H
7:	OH	OH	OAc	H



8

Fig. 1.

^{13}C NMR. The structure of **1**, **2** and **6** were established also by comparison with authentic samples by TLC and NMR.

Although none of the compounds isolated in this study were new natural products, this is the first time that their occurrence is reported in aerial parts of a Chilean *Stachys*.

Acknowledgements

The authors are grateful to Dr. M. Nicoletti (University La Sapienza, Rome, Italy) for his kind supply of authentic samples.

The authors thank for financial support from N° 2 TW 00316–08 NIH (B. N. Timmermann).

Boros C. A. and Stermitz F. R. (1990), Iridoids. An updated review. Part 1. J. Nat. Prod. **53**, 1055–1147.

Epling C. (1934), Labiatae of Chile Rev. Universitaria **22**, 169–174.

Heywood V. H. (1978), Flowering Plants of the World. Oxford University Press, London.

Inouye H. (1991), Iridoids. In (ed.) Methods in Plant Biochemistry **7**, 99–143.

Marticorena C. and Quezada M. (1985), Catálogo de la flora vascular de Chile. Gayana (Bot.) **42**, 13–114.

Muñoz M., Barrera E. and Meza I. (1981), El uso medicinal y alimenticio de plantas nativas y naturalizadas en Chile. Museo Historia Natural, Publicación Ocasional N° 3, 91 pp. 48.

Nicoletti M. 1989. Iridoids. Rev. Lat. Quím. Suppl. **1**,131–159.

Skaltsa H. D., Lazari D. M., Chinou I. B. and Loukis A. E. (1999), Composition and antibacterial activity of the essential oils of *Stachys candida* and *S. chrysantha* from southern Greece. Planta Med. **65,** 255–256.