Cantleyoside-dimethyl-acetal and Other Iridoid Glucosides from *Pterocephalus perennis* – Antimicrobial Activities

Konstantia Graikou, Nektarios Aligiannis, Ioanna B. Chinou* and Catherine Harvala

Laboratory of Pharmacognosy, Department of Pharmacy, University of Athens Panepistimiopolis Zografou, GR-15771 Athens, Greece

* Author for correspondence and reprint requests

Z. Naturforsch. **57c,** 95–99 (2002); received June 19/October 10, 2001

Pterocephalus perennis subsp. perennis, Cantleyoside-dimethyl-acetal, Antimicrobial Activities

Cantleyoside-dimethyl-acetal (**6**), was isolated from the endemic Greek plant *Pterocephalus perennis* subsp. *perennis* in addition to five other known iridoid glucosides, loganin, loganic acid, cantleyoside, secologanin, and secologanin-dimethyl-acetal. The structure of these compounds was determined by all spectroscopic means mainly by NMR and MS techniques. The above compounds as well as their acetyl derivatives were tested against six Gram positive and negative bacteria and three pathogenic fungi.

Introduction

Dipsacaceae is considered as the most advanced family within dicotyledones from a phylogenetic point of view (Greuter *et al.*, 1985). Most of the taxa are widespread over the Mediterranean region and the Middle East (Ferguson, 1972). *Pterocephalus perennis* subsp. *perennis* belonging to the family Dipsacaceae, is an endemic but abundant species of Greek peninsula and can be found in rocky and bushy places (Greuter *et al.*, 1985). The aerial parts of the plant have been used traditionally, all over Greece, for their antiseptic activities and also are used to possess astringent properties (Perdetzoglou, 1994). No phytochemical work has been reported on this species and from the genus *Pterocephalus*, only phytochemical works from *P. bretscheidri* and *P. hookeri* growing in China, have been referred until now (Tian *et al.*, 1993a; Tian *et al.*, 1993b, Tian *et al.* 1995).

In the course of our investigation of the chemical constituents of Greek plants belonging to the family Dipsacaceae, we isolated the iridoids: loganin (**1**), loganic acid (**2**), cantleyoside (**3**), secologanin (**4**), secologanin-dimethyl-acetal (**5**), and cantleyoside-dimethyl-acetal (**6**).

Especially compound **6,** was isolated as an amorphous powder. The ^{1}H-NMR spectrum showed two singlets at δ7,44 and 7,46 which are characteristic of H-3 protons of dimmers of iridoids and secoiridoids. From the COSY it was shown that the signals at δ5,27 and 5,76 attributed to the coupling of the methylene protons at H-10 and H-8. Two singlets at δ3,31 corresponded to the two methoxy groups at position-7 of secoiridoid and the coupling is obvious in HMBC. From the same spectra we observed that the singlet at δ3,7 (OCH_3) has coupling with the C-11 at δ168 and the coupling of H-3 protons (δ7,44 and 7,46) with the anomeric protons H-1 (δ5,3 and 5,53) respectively. For the iridoid part the COSY spectrum showed a signal at δ3,15 (H-5) which has correlation with multiplet at δ1,7 and 2,3 for the methylene group at H-6 and in the same way for secoiridoid at δ2,9 (H-5) and δ2,04 and 1,6 for the methylene group (H-6). At δ1,08 there is a douplet which corresponded to the methyl group at position-10 of the iridoid and coupled with the H-8 at δ2,09. The signals for the two glucosides are almost together and it is not possible to give the exact signal for each position.

Loganin (**1**) ($[\alpha]^{20}_D$ = −83.4°, MeOH, c 1.3) (Kawai *et al.*, 1988), loganic acid (**2**) ($[\alpha]^{20}_D$ = −87.5°, MeOH, c 0.6) (Tomita and Mouri, 1996), cantleyoside (**3**) ($[\alpha]^{20}_D$ = −90.2°, MeOH, c 0.5) (Jensen *et al.*, 1979), secologanin (**4**) ($[\alpha]^{20}_D$ = −101.2°, MeOH, c 0.9) (Souzu and Mitsuhashi, 1970), and secologanin-dimethyl-acetal (**5**) ($[\alpha]^{20}_D$ = −103.5°, MeOH, c 1.0) (Tomita and Mouri, 1996) were identified by comparison of their

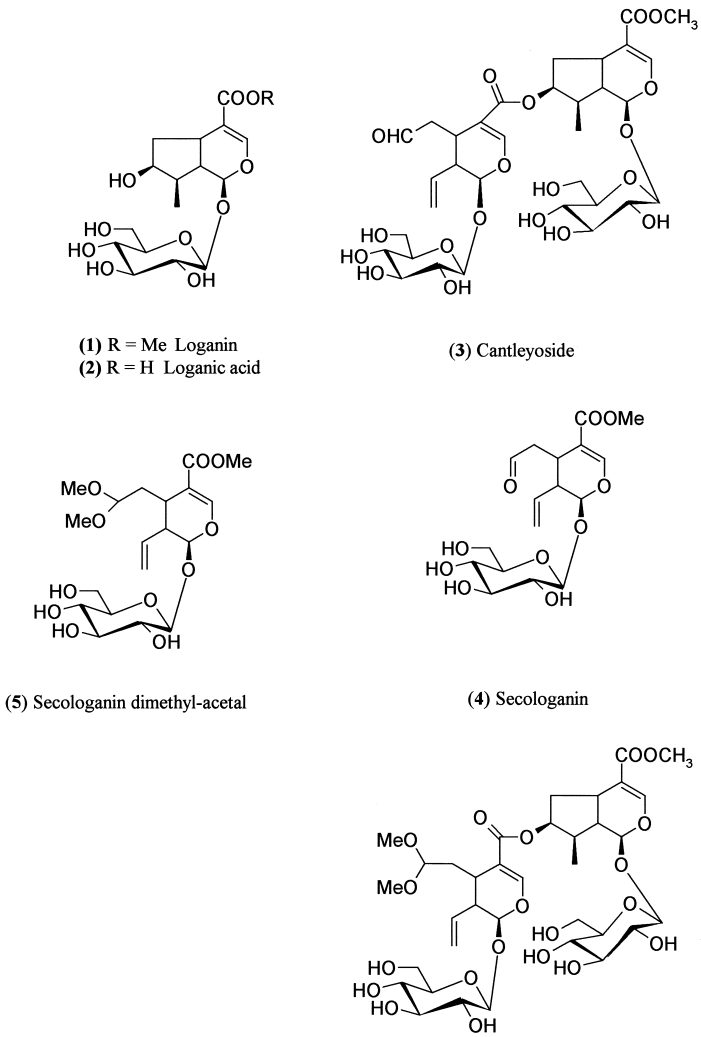

Fig. 1. The isolated iridoids from *Pterocephalus perennis.*

spectral data (ES-MS, ^{1}H-, ^{13}C-NMR, and DEPT) with those published in the literature, as well as of their acetyl derivatives (**7–11**), respectively. Iridoids (**1–3**) characterize the family Dipsacaceae and could be used as a chemotaxonomic marker of the family (Perdetzoglou, 1994). Iridoids **4–6** are reported for the first time in the family. Secologanin-dimethyl-acetal and loniceroside have been previously isolated only from Caprifoliaceae family (Kawai *et al.*, 1988; Jensen *et al.*, 1979).

Results and Discussion

Cantleyoside-dimethyl-acetal (**6**) has been isolated for the first time in the family as well as in the ordo Dipsacales. It has been reported once before, from the plant *Scaeveola montana* (Skaltsounis *et al.*, 1989) from the tropical Goodinaceae family, but it has not been assigned as a new natural product, so the search in literature data does not give any information for this molecule as a new natural product (Dictionary of Natural Compounds, 2000; Beilstein Informationsysteme GmbH 2001, Frankfurt). Besides, it has been de-

termined only by ^{1}H-NMR, so the structures of cantleyoside-dimethyl-acetal (**6**), as well as its acetylated derivative (**12**), were determined, by all spectroscopic means for the first time.

The compounds mentioned above, as well as their acetylated derivatives have been tested for their antimicrobial activities against two Gram-positive bacteria, *Staphylococcus aureus* and *S. epidermidis*, four Gram-negative bacteria *Escherichia coli, Enterobacter cloacae, Klebsiella pneumoniae, Pseudomonas aeruginosa* and three pathogenic fungi by the disc diffusion method (Chinou *et al.*, 1994; Cruickshank *et al.*, 1975).

The antibacterial studies showed that the crude extract of the plant exhibited an interesting antimicrobial profile as well as the constituents **1** and **6** which appeared as the most active against all tested bacteria and fungi (Table I). Both showed activities comparable with those of the crude extract. All the other compounds exhibited a weak activity only against the Gram (+) bacteria *S. aureus* and *S. epidermidis* while compound **3** had an interesting antibacterial profile, expressing activity against Gram negative bacteria. The total antimicrobial profile of the plant is in accordance with its external use as an antiseptic in traditional Greek medicine (Perdetzoglou *et al.*, 1993).

Experimental

General experimental procedures

Optical rotations were measured with a Perkin-Elmer 341 polarimeter. ^{1}H-NMR (400 MHz, $CDCl_3$ or CD_3OD) and ^{13}C-NMR (50 MHz, $CDCl_3$ or CD_3OD) were recorded on Bruker DRX400 and a Bruker AC200 spectrometer, respectively. TMS was used as an internal standard. COSY, HMQC and HMBC were performed using standard Bruker microprograms. ES-MS were recorded with a Finnigan MAT triple quadruple apparatus. Column chromatography was carried out using silica gel 60H (Merck, 0.015–0.040 mm), with an applied pressure of 300 mbar. MPLC was performed with a Büchi model 688 apparatus on columns containing Si gel RP-18 (Merck, 0.015–0.040 mm). TLC analyses were carried out using glass pre-coated silica gel 60 F_{254} and RP-18 F_{254} sheets (Merck).

Plant material

The aerial parts of *P. perennis* subsp. *perennis* were collected in November 1997, from Mount Parnitha, in Athens. The plant was identified by Dr. D. Perdetzoglou and a voucher specimen was deposited at the Herbarium of the Laboratory of Pharmacognosy, Department of Pharmacy, University of Athens (ATPH 307).

Table I. Results of the antimicrobial activity (zones of inhibition in mm) of the tested compounds by disk diffusion method.

Tested bacteria fungi	Studied cpds-extract	Extr	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	AMC	NET	AB
S.aureus		12	12	Nt	12	10	10	11	9	11	10	10	11	8	21	22	Nt
S.epidermidis		11	10	Nt	11	12	10	12	8	10	9	11	9	8	21	24	NT
P.aeruginosa		9	9	Nt	10	–	–	10	–	–	–	–	–	–	25	20	Nt
K.pneumoniae		9	9	Nt	9	–	–	10	–	–	–	–	–	–	22	23	Nt
E.cloacae		–	9	Nt	9	–	–	8	–	–	–	–	–	–	23	25	Nt
E.coli		10	10	Nt	8	–	–	10	–	–	–	–	–	–	24	22	Nt
Candida albicans		10	9	Nt	–	–	–	9	–	–	–	–	–	–	Nt	Nt	23
Candida tropicalis		11	9	Nt	–	–	–	10	–	–	–	–	–	–	Nt	Nt	24
Candida glabrate		12	11	Nt	–	–	–	10	–	–	–	–	–	–	Nt	Nt	25

[a] The tested extract was assayed at a concentration of 100 mg/ml, while the pure compounds were assayed at concentrations of 0.1 mg/ml each, on 6 mm discs. The results were reported as the diameter of the zone of inhibition around each disk (in mm) of the tested compounds on the surface of the Petri dishes where the tested microorganisms were cultured. The evaluation of inhibition corresponds ab < 7 mm (–), 7–10 mm (+), 11–16 mm (++), > 16 mm (+++).
Amoxicillin with clavulanic acid (AMC), netilmicin (NET), amphotericin (AB) at concentration of 0.02 mg/ml.
Nt: Not tested.

Extraction and isolation

The plant material (1.6kg) was extracted with MeOH at room temperature (3 × 3 liters). After concentration of the combined extracts under reduced pressure, the residue (50 g) was chromatographed over a Silica gel 60H column and eluted with CH_2Cl_2-MeOH (100:0 → 0:100) to give fourteen fractions (1.5–2 l each). Fraction 8 (1.5 g), eluted with CH_2Cl_2-MeOH 80:20 (v/v 1.5 l), was re-chromatographed over silica gel 60H column eluted with CH_2Cl_2-MeOH (90:10 → 80:20, 250 ml) to give compounds **5** (143 mg) and **4** (40.8 mg). Fraction 9 (2.4 g), eluted with CH_2Cl_2-MeOH 75:25 (2 l), was purified over a silica gel 60H eluted with CH_2Cl_2-MeOH 80:20 (350 ml), and gave the compound **1** (19 mg). Fraction 10 (735 mg), eluted with CH_2Cl_2-MeOH 60:40 (1.5 l), was re-chromatographed over a silica gel RP-18 eluted with H_2O-MeOH (80:20 → 50:50), to give the compounds **3** (50 mg) and **6** (42.6 mg). Fraction 13 (650 mg), eluted with CH_2Cl_2-MeOH 50:50 (v/v 1.5 l), was purified over a silica gel RP-18 eluted with H_2O (350 ml) and gave the compound **2** (30 mg).

Acetylation of compounds **1** (9 mg), **2** (10 mg), **3** (10 mg), **4** (8 mg), **5** (12 mg), and **6** (7 mg) with acetic anhydride (0.5 ml) and pyridine (0.5 ml) gave the compounds **7** (8.3 mg, 92%), **8** (9.4 mg, 94%), **9** (9.0 mg, 90%), **10** (7.7 mg, 96%), **11** (11.2 mg, 93%), and **12** (6.4 mg, 92%), respectively.

Spectroscopic data

Cantleyoside-dimethyl-acetal (**6**): $[\alpha]^{20}_D$: −110.0° (*c* 0.35, MeOH); ^{1}H-NMR (MeOD, 400MHz): δ = 7.46 (1H, s, H-3′), 7.44 (1H, s, H-3), 5.76 (1H, m, H-8′), 5.53 (1H, d, *J* = 5.4 Hz, H-1′), 5.35–5.28 (3H, m, H-1 / H-10a / H-10b), 5.22 (1H, t , *J* = 3.7 Hz, H-7), 4.71 (1H, d, *J* = 7.8 Hz, H-1″), 4.70 (1H, d, *J* = 7.9 Hz,H-1‴), 4.54 (1H, dd, *J* = 5.0 Hz/7.0Hz, H-7′), 3.90 (2H, d, *J* = 11.6 Hz, H-6a′′ / H-6a′‴), 3.72 (3H, s, CH_3O-11), 3.65 (2H, m, H-6b″ / H-6b‴), 3.40–3.18 (14H, m, H-2″ / H-2‴ / H-3″ / H-3‴ / H-4″ / H-4‴ / H-5″ / H-5‴ / 2 × OMe-7′), 3.15 (1H, m, H-5), 2.93 (1H, m, H-5′), 2.70 (1H, m, H-9′), 2.32 (1H, m, H-6a), 2.15–2.05 (3H, m, H-8 / H-9 / H-6a′), 1.78 (1H, m, H-6b), 1.65 (1H, m, H-6b'), 1.09 (3H, d, *J* = 6.2 Hz, H-10); ^{13}C-NMR (MeOD, 50MHz): δ = 170.3 (C-11), 169.2 (C-11′), 154.2 (C-3′), 153.4 (C-3′), 136.7 (C-8′), 120.7 (C-10′), 114.2 (C-4), 112.9 (C-4′), 105.7 (C-7′), 101.1 (C-1″ / C-1‴), 98.7 (C-1′), 98.3 (C-1), 79.3 (C-7 / C-5″ / C-5‴), 78.9 (C-3″ / C-3‴), 75.5 (C-2″ / C-2‴), 72.5 (C-4″ / C-4‴), 63.6 (C-6″ / C-6‴), 54.5 (CH_3O-7′), 53.6 (CH_3O-7′), 52.6 (CH_3O-11), 47.9 (C-9), 46.3 (C-9′), 41.9 (C-8), 41.2 (C-6), 34.1 (C-6′), 33.4 (C-5), 30.4 (C-5′), 14.7 (C-10), ES-MS *m/z* : $[M+H^+]$ = 793.

Compound **12**: $[\alpha]^{20}_D$: −101.0° (*c* 0.36, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400MHz): δ = 7.30 (1H, s, H-3), 7.27 (1H, s, H-3′), 5.58 (1H, m, H-8′), 5.30–5.15 (7H, m, H-1′ / H-1 / H-7 / H-10a / H-10b / H-3″ / H-3‴), 5.11–5.08 (2H, m, H-4″ / H-4‴), 5.02–4.94 (2H, m, H-2″ / H-2‴), 4.87 (1H, d, *J* = 8.3 Hz, H-1″), 4.85 (1H, d, *J* = 8.3 Hz,H-1‴), 4.47 (1H, dd, *J* = 6.0 Hz/4.6 Hz, H-7′), 4.28 (2H, m, H-6b″ / H-6b‴), 4.12 (2H, d, *J* = 12 Hz, H-6a′′ / H-6a′‴), 3.71 (2H, m, H-5″ / H-5‴), 3.67 (3H, s, CH_3O-11), 3.27 (2 × OMe-7′), 2.98 (1H, m, H-5), 2.76 (1H, m, H-5′), 2.69 (1H, m, H-9′), 2.22–1.80 (4H, m, H-6a / H-6a′ / H-8 / H-9), 1.82 (1H, m, H-6b), 1.53 (1H, m, H-6b'), 1.01 (3H, d, *J* = 6.6 Hz, H-10); ^{13}C-NMR ($CDCl_3$, 50MHz): δ = 169.3 (C-11), 169.0 (C-11′), 150.3 (C-3′), 148.9 (C-3′), 133.2 (C-8′), 120.2 (C-10′), 113.6 (C-4), 111.4 (C-4′), 102.0 (C-7′), 96.1 (C-1′), 95.8 (C-1″ / C-1‴), 94.4 (C-1), 77.2 (C-7), 72.4 (C-3″ / C-3‴), 72.1 (C-5″ / C-5‴), 70.5 (C-2″ / C-2‴), 68.1 (C-4″ / C-4‴), 61.6 (C-6″ / C-6‴), 53.1 (2 X CH_3O-7′), 51.3 (CH_3O-11), 45.5 (C-9), 43.3 (C-9′), 38.9 (C-8), 38.7 (C-6), 31.1 (C-6′), 29.6 (C-5), 26.9 (C-5′), 12.5 (C-10). ES-MS *m/z* : $[M+H^+]$ = 1129.

Antimicrobial assay

Compounds **1–12** and the crude extract (MeOH) of the plant were tested by the disk diffusion method (Chinou *et al.*, 1994; Cruickshank *et al.*, 1975) against the Gram-positive bacteria: *Staphylococcus aureus* (ATCC 25923) and *Staphylococcus epidermidis* (ATCC 12228), the Gram negative: *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922), *Enterobacter cloacae* (ATCC 13047), *Klebsiella pneumoniae* (ATCC 13883), as well as against three pathogenic fungi, *Candida albicans* (ATCC 10231), *C. tropicalis* (ATCC 13801) and *C. glabrata* (ATCC 28838). All the tested microorganisms were standard strains from ATCC (American Type Culture Collection).

Standard antibiotics netilmicin and amoxicillin with clavulanic acid (Sanofi, Diagnostics Pasteur, Marnes la Coquette-France) were used in order to control the sensitivity of the tested bacteria and amphotericin B was used to control the tested fungi.

Beilstein INFORMATIONsysteme GmbH, Frankfurt. License Certificate No.: L-1999/07/03–02.

Chinou I., Demetzos C., Harvala C., Roussakis C., and Verbist J. F. (1994), Cytotoxic and antibacterial labdane type diterpenes from the aerial parts of *Cistus incanus* subsp. *creticus*. Planta Medica **60**, 34–36.

Cruickshank R., Duguid I. P., Marmion B. P., and Swain R. H. A. (1975). In: Medical Microbiology, Vol. **3**. Churchill Livingstone, Edinburgh, pp. 190-208.

Dictionary of Natural compounds. Chapman & Hall. London. CD-ROM data-base 2000.

Ferguson I. K. (1972) "*Pterocephalus* Adanson" In: Flora Europaea (Tutin T. G., Heywood V. H., Burges N. A., Moore D. M., Valentine S. M., Webb D. A., eds.). Cambridge University Press, Cambridge U.K., Vol. **4**, pp. n68.

Greuter W. and Burdet H. M. (1985), Dipsacaceae In: Med. Checklist Notulae 11 (Greuter W., Raus T., eds.). Willdenowia **15**, 71–72.

Jensen S. R., Lyse-Petersen S. E. and Nielsen B. J. (1979), Novel bis-iridoid glucosides from *Dipsacus sylvestris*. Phytochemistry **18**, 273–276.

Kawai H., Kuroyanagi M. and Veno A. (1988), Iridoid glucosides from *Lonicera japonica* Thunb. Chem. Pharm. Bull. **36**, 3664–3666.

Perdetzoglou D. K. (1994) Pharmacognostic, chemotaxonomic and morphological studies on the genus *Scabiosa L.* s. l. in Crete. Ph.D. Thesis. University of Athens, Athens.

Perdetzoglou D., Kofinas C., Chinou I., Loukis A. and Gally A. (1993), Comparative chemotaxonomic study of several taxa of the Dipsacaceae family: Fatty acid and sterol composition. Antibacterial activity. Phytochemical Society of Europe Intern. Symp.: Phytochemistry of plants used in traditional Medicine. Proceedings. Lausanne, Switzerland

Skaltsounis A. L., Sbahi S., Demetzos C. and Pusset J. (1989), Plantes de Nouvelle-Calédonie. Iridoïdes de *Scaevola montana* Labill. Ann. Pharm. Fr. **47**, 249-254.

Souzu I. and Mitsuhashi H. (1970), Structures of iridoids from *Lonicera morrowii* A. Gray, Tetrahedr. Lett. **2**, 191-192.

Tian J., Wu F. E., Qiu M-H. and Nie R.-L. (1993a), Triterpenoid saponins from *Pterocephalus hookeri*. Phytochemistry **32**, 1535–1538.

Tian J., Wu F. E., Qiu M-H. and Nie R.-L. (1993b), Two triterpenoid saponins from *Pterocephalus bretschneidri* . Phytochemistry **32**, 1539–1542

Tian J., Wu F. E., Qiu M-H. and Nie R.-L. (1995), Chemical constituents from *Pterocephalus bretschneidri*. Acta Botan. Yunnan. **17**, 108–110 (In Engl.).

Tomita H. and Mouri Y. (1996), An iridoid glucoside from *Dipsacus asperoides*. Phytochemistry **42**, 239–240.