Phytochemistry Letters 2 (2009) 93–95

Contents lists available at ScienceDirect

Phytochemistry Letters

journal homepage: www.elsevier.com/locate/phytol

Melampolides from Argentinean *Acanthospermum australe*

Marianela Sánchez [a], Fernando Kramer [b], Severino Bargardi [b], Jorge A. Palermo [a,*]

[a] *UMYMFOR, Departamento de Química Orgánica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, Pabellón 2 (1428), Buenos Aires, Argentina*
[b] *Departamentos de Farmacia y Microbiología, Facultad de Ciencias Exactas, Químicas y Naturales, Univ. Nacional de Misiones, Av. Mariano Moreno 1375 (3300), Posadas, Misiones, Argentina*

ARTICLE INFO

Article history:
Received 24 November 2008
Received in revised form 29 December 2008
Accepted 30 December 2008
Available online 15 January 2009

Keywords:
Acanthospermum australe
Sesquiterpene lactones
Melampolides
Acanthospermolides

ABSTRACT

The investigation of the ethanol extract of *Acanthospermum australe*, collected in the province of Misiones, Argentina, yielded eight melampolides (**1**–**8**) of the acanthospermal type. Two of them, 8β-hydroxy-9α-(2-methylbutyryloxy)-14-oxo-acanthospermolide (**3**) and 9α-hydroxy-8β-(2-methylbutyryloxy)-14-oxo-acanthospermolide (**7**) are new compounds. Two other compounds (**4** and **8**) have been previously reported, and the NMR data of **4** are corrected. Compounds **1**, **2**, **5** and **6** have not been previously reported, but are probably artifacts formed during extraction. Compounds **3**, **6** and **7** showed slight antibiotic activity against Gram-positive bacteria.

1. Introduction

Acanthospermum australe (Loefl.) Kuntze is an annual shrub widely distributed in South America. It is commonly known as "tapekué" and is used in folk medicine as antiseptic, antiinflamatory and diuretic. As part of a systematic survey of the native flora of the province of Misiones (Argentina) we decided to investigate this plant as a source of secondary metabolites which could be subsequently transformed into new bioactive substances. *A. australe*, as many species belonging to the tribe Heliantheae, subtribe Melampodiinae is well known for producing a variety of melampolides, which can be useful as chemotaxonomic markers (Herz and Kalyanaraman, 1975; Seaman et al., 1980). Previous investigations of *A. australe* have led to the isolation of germacranolides, melampolides, diterpene lactones and 6-methoxyflavonoids (Bohlmann et al., 1979; Bohlmann et al., 1981; Matsunaga et al., 1996). The only previous work on argentinean samples of *A. australe* was that of Debenedetti et al., 1987, in which only 6-methoxyflavonoids were reported and no mention was made on the melampolide composition of this species.

In our present work, we were able to isolate and fully characterize eight melampolides from *A. australe* collected in Misiones, Argentina. Two of these compounds (**3** and **7**) are new, while other two (**4** and **8**) have been previously reported. Compounds **1**, **2**, **5** and **6**, all bearing an ethoxy group at C-9, have not been previously reported but are probably artifacts formed during extraction.

2. Results and discussion

Ethanol extraction of fresh plant material, followed by partition between MeOH/H_2O (9:1) and cyclohexane, afforded a polar subextract which, after fractionation on Sephadex LH-20, silica gel and reversed phase HPLC (see Section 3), yielded eight melampolides (**1**–**8**, Fig. 1) of the acanthospermal type. All compounds were identified by careful interpretation of complete sets of 2D NMR spectra.

The molecular formula of compound **3** was shown to be $C_{20}H_{26}O_6$ on the basis of its HRMS ESI/APCI (*m*/*z*: 385.1630 $[M+Na]^+$), which indicated eight degrees of unsaturation. By observation of the NMR spectra (see Table 1), six of these could be readily identified as: an aldehyde, two ester carbonyls and three double bonds. This suggested the presence of a bicyclic sesquiterpene lactone such as a melampolide. The ^1H NMR spectrum showed some characteristic features of acanthospermal melampolides: an aldehyde group (δ: 9.42 (1H, d, 2 Hz, H-14)) conjugated with a 1-10-*cis* double bond (6.73 (1H, dd, 10, 8 Hz, H-1)); an α-methylene-γ-lactone showing two one-proton doublets (3 Hz) at δ: 6.35 and 5.64, both coupled to a multiplet at δ: 2.44 (H-7) and showing long- range H-C correlations in the HMBC spectrum to the lactone carbonyl at δ: 169.3 (C-12). The presence of a single 2-methylbutanoate moiety was evident from the ^1H NMR spectrum. A long range HMBC correlation between the proton at δ: 5.05 (1H, dd, J8-9 = 8 Hz, J9-14 = 2 Hz, H-9) and the 2-methylbutanoate

* Corresponding author. Fax: +5411 4576 3385.
E-mail address: palermo@qo.fcen.uba.ar (J.A. Palermo).

1874-3900/$ – see front matter © 2009 Phytochemical Society of Europe. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.phytol.2008.12.007

Table 1
NMR data of compounds **3** and **7** (^{1}H 500 MHz; ^{13}C 125 MHz; $CDCl_3$).

	3		**7**	
	δ^{13}C	δ^1H	δ^{13}C	δ^1H
1	159.4	6.73 dd (10, 8)	153.8	6.65 dd (10, 8)
2	26.8	2.93 m 2.62 m	24.9	2.65 m 2.41 m
3	36.9	2.44 m 2.08 m	35.4	2.44 m 2.10 m
4	138.5	–	135.7	–
5	126.9	4.87 d (10)	125.6	4.91 d (10)
6	74.2	5.19 dd (10, 10)	73.8	5.06 dd (10, 10)
7	51.3	2.44 m	49.9	2.57 dd (7, 3)
8	68.0	5.31 d (7)	69.8	6.39 d (8)
9	72.7	5.05 dd (8, 2)	69.3	3.91 d (8)
10	141.5	–	144.6	–
11	136.0	–	167.4	–
12	169.3	–	132.9	–
13	120.3	6.35 d (3) 5.64 d (3)	120.1	6.26 d (3) 5.65 d (3)
14	194.2	9.42 d (2)	195.1	9.47 d (2)
15	17.0	1.97 brs	15.6	1.91 s
2-MeBu	176.3	–	174.8	–
	41.0	2.37 m	39.9	2.42 m
	26.7	1.66 m 1.47 m	25.5 –	1.67 m 1.49 m
	16.3	1.09 d (7)	15.4	1.13 d (7)
	11.5	0.88 t (7)	11.4	0.92 t (7)

carbonyl at δC: 176.3 clearly defined the substituted position as C-9. A relatively large (2 Hz) W-coupling was observed between H-9 and the aldehyde. Two additional oxidized carbons were observable in the HSQC spectrum: one of them (δC: 74.2; δH: 5.19, t, 10 Hz) was assigned to C-6 due to the HMBC correlation of its proton with the lactone carbonyl together with the COSY correlation between H-6 and H-7. The remaining signal (δC: 68.0; δH: 5.31, d, 8 Hz) was attributed to C-8 due to the COSY correlation between H-8 and H-9. A hydroxyl group was located at C-8 in order to account for the molecular formula. The third double bond was located between C-4 and C-5 due to the observed coupling between the typically upfield shifted olefinic proton H-5 (δ: 4.87, d, 10 Hz) and H-6. The remaining substituent on this double bond was identified as a methyl group (δ: 1.95, brs) from its allylic coupling (COSY) to H-5 and the observed long range H–C correlations. The relative stereochemistry at C-7, C-8 and C-9 was obtained by interpretation of the coupling constants and the correlations observed in a ROESY experiment. In this way, compound **3** was identified as 8β-hydroxy-9α-(2-methylbutyryloxy)-14-oxo-acanthospermolide.

Compound **7** had the same molecular formula as **3**, $C_{20}H_{26}O_6$, obtained by HRMS ESI/APCI (*m*/*z*: 385.1633 $[M+Na]^+$). The ^{1}H NMR spectra of compounds **7** and **3** were very similar, suggesting that **7** had also the structure of an acanthospermal melampolide with a single 2-methylbutanoate substituent (see Table 1). The difference between both compounds was the substitution pattern. In the case of compound **7**, H-9 was strongly shifted upfield (δ: 3.91, d, 8 Hz) when compared to compound **3**. This suggested that in **7**, C-9 was not esterified. On the other hand, H-8 (δ: 6.39, d, 8 Hz) showed a marked downfield shift. The identity of H-9 was verified by observation of the characteristic W-coupling to the aldehyde and the correlations observed in the HMBC spectrum. A strong correlation of H-8 to the 2-methylbutanoate carbonyl in the HMBC spectrum confirmed that in compound **7**, C-9 was hydroxylated and C-8 was esterified. Compound **7** was thus identified as 9α-hydroxy-8β-(2-methylbutyryloxy)-14-oxo-acanthospermolide.

The structures of compounds **4** and **8** had been previously reported and were identified by comparison of their NMR spectra with published data (Bohlmann et al., 1984; Saleh et al., 1980). In the case of compound **4**, there is a significant difference in the chemical shift of H-1 (our data = δH-1: 6.65 (dd, 10; 7); Saleh et al. = δH-1: 6.93 (11; 6.4)), while the rest of the reported shifts are quite similar. The structure of **4** was confirmed as identical to that reported and in our case was supported by 2D NMR (HSQC, COSY, ROESY) at 500 MHz, while in the previous reference only incomplete ^{1}H NMR data at 90 MHz were reported. It is possible that the compound isolated by Saleh et al. might have a different stereochemistry at the 1-10 double bond, leading to a germacranolide skeleton instead of a melampolide.

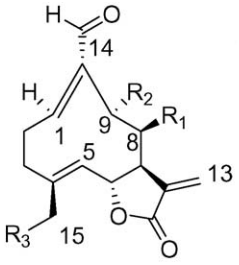
Compounds **1**, **2**, **5** and **6** have not been previously reported, and all have an ethoxy substituent at C-9. ^{1}H NMR spectral data are shown in Supplementary data. These compounds are probably artifacts formed with the solvent during the extractive process. A similar finding had been previously described in melampolides (Xiang et al., 2005). Taking into account that extraction with methanol is a widespread practice in many research groups, the presence of several reported lactones bearing a methoxy group at C-9 should raise some concern as to whether these compounds may also be artifacts formed by a similar process.

Compounds **6** and **7** showed mild antibiotic activity against *Staphylococcus aureus*, (growth inhibition zones of 7 and 9 mm respectively) at 50 μg/6 mm disk. Compound **3** showed a growth inhibition halo of 9 mm against *Bacillus subtilis*, while none of the tested compounds were active against *Escherichia coli*. Gentamicin was used as positive control (25-30 mm halo at 25 μg/6 mm disk).

3. Experimental

3.1. General

NMR experiments were performed on a Bruker Avance 2 (500 MHz) instrument at 500.13 MHz for ^{1}H and 125.13 MHz for ^{13}C. All spectra were recorded in $CDCl_3$ using TMS as internal standard. All 2D NMR experiments (COSY, HSQC, HMBC, ROESY) were performed using standard sequences. ESI/APCI HRMS experiments were recorded at UCR Mass Spectrometry Facility, California, USA, on a Micromass Ultima Global QTOF high resolution mass spectrometer. Optical rotations were measured on a PerkinElmer 343 polarimeter. Dry column flash chromatography was carried out on silica gel (Aldrich Chemical Co.). HPLC separations were performed using a Thermo Separations SpectraSeries P100 pump, a Thermo Separations Refractomonitor IV RI detector and a Thermo Separations SpectraSeries UV 100 detector, with simultaneous UV (220 nm) and RI detection. An YMC RP-18 (5 μm, 20 mm × 250 mm) column working at a flow rate of 5 mL/min was used for separations. All solvents were HPLC grade (typically acetonirile/water 1:1). Typical retention times in the previous conditions were: **1** (1 h 13 min); **2** (1 h 40 min), **3** (42.5 min), **4** (23.5 min), **5** (33 min), **6** (26.5 min), **7** (31 min), **8** (70 min). Sephadex LH-20 was obtained from Pharmacia Inc.; TLCs were carried out on Merck Silicagel 60 F_{254} plates, using CH_2Cl_2/EtOAc mixtures as mobile phase. TLC plates were sprayed with 2% vainillin in conc. H_2SO_4; all compounds gave purple-blue spots. Typical R_f values in CH_2Cl_2/EtOAc (80:20) were the following: **1** (0.74), **2** (0.73), **3** (0.73), **4** (0.40), **5** (0.53), **6** (0.53), **7** (0.34), **8** (0.43).



Compound	R_1	R_2	R_3
1	$OCOCH(CH_3)_2$	OEt	H
2	$OCOCH(CH_3)CH_2CH_3$	OEt	H
3	OH	$OCOCH(CH_3)CH_2CH_3$	H
4	$OCOCH(CH_3)_2$	OH	H
5	$OCOCH(CH_3)CH_2CH_3$	OEt	OH
6	$OCOCH(CH_3)_2$	OEt	OH
7	$OCOCH(CH_3)CH_2CH_3$	OH	H
8	$OCOCH(CH_3)CH_2CH_3$	$OCOCH(CH_3)CH_2CH_3$	OH

Fig. 1. Structure of acanthospermolides **1**–**8**.

3.2. Plant material

The aerial parts of *A. australe* were collected at Santa Ines, Gurupá, Misiones province, Argentina in March 2006. A voucher specimen (N° 1443) was identified by Prof. Manuela Rodriguez (Universidad Nacional de Misiones, Argentina) and was deposited at the herbarium (FCEQN, Universidad Nacional de Misiones).

3.3. Extraction and isolation

Fresh plant material (2.5 kg) was ground and extracted twice at room temperature for 3 days with ethanol (6 L). The combined extracts were evaporated *in vacuo* to yield a residue of 7.9 g. This residue was partitioned between MeOH/H_2O (9:1) and cyclohexane. The polar phase (7.0 g) was concentrated under reduced pressure to an aqueous suspension and partitioned between EtOAc and water. The organic phase (2.70 g) was permeated in a Sephadex LH-20 column, using MeOH as eluant, and 19 fractions of 200 mL each were collected. Fraction 5 (0.9834 g) was subjected to dry column flash chromatography on silica gel (CH_2Cl_2/EtOAc gradient). The fractions eluted with CH_2Cl_2/EtOAc 8:2 (E2 and E3) were purified by preparative HPLC with acetonitrile/H_2O (1:1) as eluant. E_2 (0.1813 g) yielded **1** (3.1 mg); **2** (7.3 mg) and a fraction (H10, 3.5 mg), which was purified by TLC with CH_2Cl_2/EtOAc 7:3 as eluant to yield **3** (2.7 mg). On the other hand, separation of E_3 (0.1066 g) by HPLC under the same conditions afforded **5** (21.2 mg), a fraction H30 (2.4 mg) which was purified by TLC (CH_2Cl_2/EtOAc 8:2) to yield **4** (1.1 mg), and fractions H32 (8.9 mg) and H35 (12 mg) which were purified by TLC in the same conditions to yield **6** (1.4 mg), **7** (5 mg) and **8** (2 mg).

3.4. 8β-Hydroxy-9α-(2-methylbutyryloxy)-14-oxo-acanthospermolide (3)

Yellow pale oil, [α]: −79.4 (c = 0.18, CH_2Cl_2). ESI-APCI-MS: m/z: 385.1630, calculated for $C_{20}H_{26}O_6Na$ $[M+Na]^+$ 385.1627. ^1H and ^{13}C NMR spectral data are shown in Table 1.

3.5. 9α-Hydroxy-8β-(2-methylbutyryloxy)-14-oxo-acanthospermolide (7)

Yellow pale oil, [α]: −36.3 (c = 0.22, CH_2Cl_2). ESI-APCI-MS: m/z: 385.1633, calculated for $C_{20}H_{26}O_6Na$ $[M+Na]^+$ 385.1627. ^1H and ^{13}C NMR spectral data are shown in Table 1.

4. Antimicrobial assays

The antibiotic activity was determined by the agar diffusion method using 50 μg of sample/6 mm disk against *B. subtilis* ATCC 6633, *S. aureus* ATCC 25923 and *E. coli* ATCC 25922. All samples were dissolved in CH_2Cl_2 and applied to the disks. The incubations were carried out at 37 °C in 10% CO_2, and were started immediately after drying of the sample under nitrogen. Gentamicin was used as positive control, showing grown-inhibition halos of 25–30 mm at 25 μg/disk. A negative control (only CH_2Cl_2, no halo) was run in parallel.

Acknowledgements

This research was supported by grants from CONICET (PEI 6478), UBA (X 260 Programación 2004–2007) and ANPCyT (PICT (2003) 14321). We thank Mr. José Gallardo and Lic. Gernot Eskuche (UMYMFOR–CONICET) for recording some of the NMR spectra. M. Sánchez thanks CONICET for a postdoctoral fellowship.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.phytol.2008.12.007.

References

Bohlmann, F., Jakupovic, J., Zdero, C., King, R.M., Robinson, H., 1979. Neue Melampolide und cis,cis-Germacranolide aus Vertretern der Subtribus Melampodiinae. Phytochemistry 18, 625–630.
Bohlmann, F., Jakupovic, J., Dhar, A.K., King, R.M., Robinson, H., 1981. Two sesquiterpene and three diterpene lactones from *Acanthospermum australe*. Phytochemistry 20, 1081–1083.
Bohlmann, F., Schmeda-Hirschmann, G., Jakupovic, J., 1984. New melampolides from *Acanthospermum australe*. Planta Med. 50, 37–39.
Debenedetti, S., Martino, V., Palacios, P., Coussio, J.D., 1987. 6-Methoxyflavonoids from *Acanthospermum Australe*. J. Nat. Prod. 50, 325–1325.
Herz, W., Kalyanaraman, P.S., 1975. Acanthospermal A and Acanthospermal B, two new melampolides from Acanthospermum species. J. Org. Chem. 40, 3486–3491.
Matsunaga, K., Sayito, M., Ohizumi, Y., 1996. Acanthostral, a novel antineoplastic *cis-cis-cis*-germacranolide from *Acanthospermum australe*. Tetrahedron Lett. 37, 1455–1456.
Saleh, A.A., Cordell, G.A., Farnsworth, N.R., 1980. Potential anticancer agents. Part 13. Cytotoxic constituents of *Acanthospermum glabratum* (Asteraceae). J. Chem. Soc. Perkin Trans. 1 (5), 1090–1097.
Seaman, F.C., Fischer, N.H., Stuessy, T.F., 1980. Systematic implications of sesquiterpene lactones in the subtribe melampodiinae. Biochem. Syst. Ecol. 8, 263–271.
Xiang, Y., Fan, C.Q., Yue, J.M., 2005. Novel sesquiterpenoids from *Siegesbeckia orientalis*. Helv. Chim. Acta 88, 160–170.