PERGAMON

Phytochemistry 54 (2000) 771–775

PHYTOCHEMISTRY

www.elsevier.com/locate/phytochem

Labdane diterpenes from *Otostegia fruticosa*☆

Nawal M. Al-Musayeib, Fawkeya A. Abbas, M. Shamim Ahmad, Jaber S. Mossa, Farouk S. El-Feraly*

Department of Pharmacognosy, College of Pharmacy, King Saud University, PO Box 2457, Riyadh, 11451, Saudi Arabia

Received 5 January 2000; received in revised form 11 May 2000

Abstract

The new labdane diterpenes otostegin A (**2**), otostegin B (**6**) and 15-*epi*-otostegin B (**7**) were isolated from the aerial parts of *Otostegia fruticosa*, besides the previously known labdanes preleoheterin (**1**), leoheterin (**3**), leopersin C and 15-*epi*-leopersin C (**4**, **5**), ballonigrin (**9**) and vulgarol (**11**), along with the iridoid glucoside 8-*O*-acetylharpagide (**10**). The structure elucidation of all the isolated compounds was based on their spectral data and chemical derivatization. © 2000 Published by Elsevier Science Ltd.

Keywords: Otostegia fruticosa; Labdane diterpenes; Otostegin; Harpagide; Ballonigrin; Vulgarol

1. Introduction

The genus *Otostegia* (Labiatae) comprises 20 species (Shaw, 1985), of which *Otostegia fruticosa* Forssk (Briq.) is the only one found in Saudi Arabia. There is a single report in the literature on the composition of the essential oil of the fresh aerial parts of *O. fruticosa* cultivated in Egypt. Thirty-six compounds representing 98.8% of the oil were identified by analysis using the technique of gas chromatography/mass spectrometry (GC/MS). The main components of the oil were thymol, γ-terpinene and *p*-cymene. The oil was reported to exhibit significant antimicrobial activity against Gram-positive and Gram-negative bacteria as well as a number of fungi (Aboutabl et al., 1995). Phytochemical work on aerial parts of *O. fruticosa* collected in Saudi Arabia was done for the first time. The isolation and characterization of the new labdane diterpenes otostegin A (**2**), otostegin B (**6**) and 15-*epi*-otostegin B (**7**) and the previously known labdanes preleoheterin (**1**) (Hon et al., 1993), leoheterin (**3**) (Hon et al., 1993), leopersin C and 15-*epi*-leopersin C (**4**, **5**) (Tasdemir et al., 1996), ballonigrin (**9**) (Brian and James, 1977) and vulgarol (**11**) (Popa and Pasechnik, 1975), together with the iridoid glucoside 8-*O*-acetylharpagide (**10**) (Scarpati et al., 1965), are the subject of the present note.

2. Results and discussion

Otostegin A (**2**), $C_{22}H_{32}O_5$, mp 216.2–217°C, with an IR spectrum that exhibited absorption bands typical of an acetate group (1745 cm^{-1}), ketone (1708 cm^{-1}) and an olefinic double bond (1632 cm^{-1}). Its ^{1}H- and ^{13}C-NMR data (Table 1) were consistent with a pre-furanic partial structure, as it showed two one-proton olefinic doublets, at δ 5.08 and 6.40 ($J = 2.6$ Hz) (H-14 and H-15, respectively) and a two-proton AB system at δ 4.44 and 4.03 ($J = 10.5$ Hz), ascribed to the methylene group of a β,β-disubstituted dihydrofuran ring (H-16). The ^{1}H- and ^{13}C-NMR data (Table 1) contained resonances associated with three tertiary methyl singlets (δ_H 0.99, 1.06, 1.41; δ_C 32.6, 23.9, 19.4, respectively), one secondary methyl group (δ_H 0.98, *d*, $J = 6.5$ Hz; δ_C 9.2), six methylenes and

☆ Taken in part from

* Corresponding author. Fax: +966-1-467-7245.
E-mail address: fferaly@ksu.edu.sa (F.S. El-Feraly).

0031-9422/00/$ - see front matter
PII: S0031-9422(00)00185-0

four nonoxygenated methines. Included among the methine signals were two vinylic carbons at δ_C 106.9 and 148.2 (C-14 and C-15, respectively). In addition, the ^{13}C-NMR (Table 1) spectrum also showed five quaternary carbon signals, including a ketonic carbonyl (δ_C 204.2) and an ether function (δ_C 96.6, 93.7, due to C-9 and C-13, respectively). The correlations between the carbon and proton signals were confirmed by HETCOR experiments.

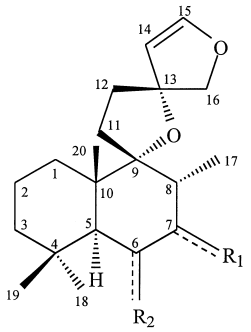
By analyzing the ^{1}H–^{1}H COSY spectrum, it was observed that a one-proton quartet signal at δ 3.30 ($J = 6.5$ Hz) was correlated with the secondary methyl doublet at δ 0.98 ($J = 6.5$ Hz, H-17) and was assigned to H-8. The proton resonance at δ 1.76 (*d*, $J = 2.4$ Hz, H-5) was coupled to H-6 (δ 5.36, *d*, $J = 2.4$ Hz). This suggested that the ketone group should be located at C-7, and the acetoxy group at C-6. The relatively downfield position of H-6 was in agreement with the presence of an acetoxy group at C-6. The presence of the latter functional group in the molecule was substantiated from the molecular formula and also from the characteristic NMR signals that can be attributed to an acetate group (δ_H 2.07, *s*, H-22; δ_C 169.4, *s*, and 21.3, *q*, due to C-21 and C-22, respectively).

The stereochemical assignment of H-6 was deduced to be α (relative stereochemistry) based on the small coupling constant between H-6 and H-5 ($J = 2.4$ Hz), suggesting that H-6 must be equatorial and, therefore, α-oriented. This notion was further confirmed by studying a two-dimensional Nuclear Overhauser Effect spectrum (NOESY) of otostegin A (**2**) as a solution in benzene-d_6. The NOESY spectrum revealed the fact that H-5, H-6 and H-18 were on the α-side of the molecule, while H-8 and H-20 were both on the β-side, thus confirming the relative stereochemistry at these positions. It is interesting to note that both H-16 protons were found to correlate with H-17, thus making it possible to assign the stereochemistry of C-13 to be the same as in preleoheterin (**1**) (Hon et al., 1993). Otostegin A (**2**) is a new labdane diterpene that has not been previously reported from any other source.

Otostegin B (**6**, **7**), $C_{22}H_{34}O_6$, was isolated as a colorless oil that was homogeneous on TLC. Its ^{1}H- and ^{13}C-NMR spectra (Table 1) indicated that this compound was likely to be a mixture of epimers, as suggested by the numerous duplicate peaks observed. Also, the NMR signals (Table 1) were in accord with those observed earlier for otostegin A (**2**), apart from the tetrahydrofuran moiety. The presence of a hemiacetal group was unambiguously pointed out by the downfield methine carbon signal at δ 101.2. To further clarify the NMR data, it was decided to convert otostegin B (**6**, **7**) into a single entity by oxidation with Jones reagent to the corresponding lactone. The reaction proceeded smoothly and yielded **8** as a pure crystalline product.

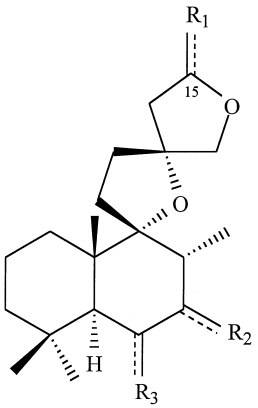
Table 1
^{1}H- and ^{13}C-NMR assignments of compounds **2** and **6**–**8**

Proton/carbon	**2**		**6**, **7**		**8**	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
1	1.58 (*m*)	34.4	1.45 (*m*)	34.9, 34.8	1.46 and 1.31 (*m*)	35.0
2	1.41 (*m*)	18.7	1.52 and 1.70 (*m*)	19.1	1.73 and 1.56 (*m*)	19.0
3	1.42 and 1.34 (*m*)	43.7	1.40 (*m*)	44.3, 44.1	1.33 and 1.42 (*m*)	44.1
4	–	34.9	–	35.2	–	35.3
5	1.76 (*d*, 2.4)	50.2	1.75 (masked *d*)	49.3, 49.1	1.71 (*d*, 2.4)	50.7
6	5.36 (*d*, 2.4)	77.1	5.45 (masked *d*)	76.1, 76.0	5.36 (*d*, 2.4)	77.2
7	–	204.2	–	204.5	–	204.3
8	3.30 (*q*, 6.5)	46.7	3.27 (*dq*, 6.8, 7.0)	45.8, 45.5	3.33 (*q*, 6.6)	46.5
9	—	96.6	–	98.1, 98.2	–	98.5
10	—	43.1	–	43.8, 43.9	–	43.8
11	2.27 and 1.83 (*m*)	30.4	2.20 and 1.80 (*m*)	30.4, 30.1	2.27 and 1.93 (*m*)	30.0
12	2.19 and 2.04 (*m*)	37.6	2.12 and 2.10 (*m*)	38.9, 38.8	2.17 and 2.04 (*m*)	38.1
13	–	93.7	–	90.7, 90.6	–	87.0
14	5.08 (*d*, 2.6)	106.9	1.51 (*m*)	42.2, 42.1	2.83 (*d*, 17.1) 2.46 (*d*, 17.1)	43.0
15	6.40 (*d*, 2.6)	148.2	5.37 (*m*)	101.2	–	174.7
16	4.44 (*d*, 10.5) 4.03 (*d*, 10.5)	80.5	4.03 (*d*, 9.0) 3.75 (*d*, 9.0)	71.0	4.34 (*d*, 9.2) 4.17 (*d*, 9.2)	78.5
17	0.98 (*d*, 6.5)	9.2	0.95 (*d*, 6.8)	9.5	0.97 (*d*, 6.6)	9.6
18	0.99 (*s*)	32.6	0.97 (*s*)	33.1	0.99 (*s*)	33.1
19	1.06 (*s*)	23.9	1.04 (*s*)	24.2, 24.9	1.06 (*s*)	24.3
20	1.41 (*s*)	19.4	1.41 (*s*)	20.3	1.44 (*s*)	20.3
21	–	169.4	–	169.8	–	169.8
22	2.07 (*s*)	21.3	2.05 (*s*)	21.8	2.07 (*s*)	21.8



1 R_1=β-OH, R_2=O

2 R_1=O, R_2= β-OAc



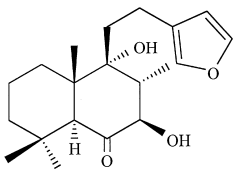
4 R_1=β-OH, R_2=β-OH, R_3=O

5 R_1=α-OH, R_2=β-OH, R_3=O

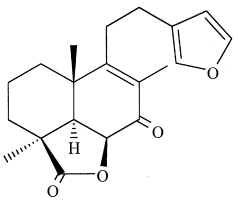
6 R_1=α-OH, R_2=O, R_3=β-OAc

7 R_1=β-OH, R_2=O, R_3=β-OAc

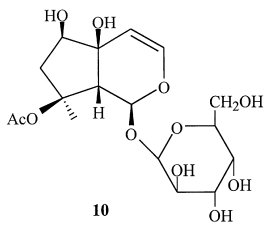
8 R_1=O, R_2=O, R_3=β-OAc



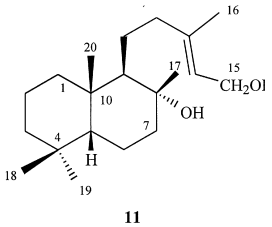
3



9



10



11

The NMR data (Table 1) obtained for **8** showed an additional carbonyl function at δ_C 174.7, due to C-15. The data also confirmed its relationship with otostegin A (**2**) by showing the characteristic one-proton quartet due to H-8 at δ 3.33 ($J = 6.6$ Hz) and the one-proton doublet due to H-5 at δ 1.71 (*d*, $J = 2.4$ Hz), which was coupled to H-6 (δ 5.36, *d*, $J = 2.4$ Hz). Furthermore, it was possible to assign the pair of doublets at δ 2.83 and 2.46 ($J =$ 17.1 Hz) to the methylene group at C-14 (next to a carbonyl), while the other more deshielded pair of doublets at δ 4.34 and 4.17 ($J = 9.2$) was assigned to the methylene at C-16 (next to the heterocyclic oxygen). Therefore, otostegin B was assigned structure (**6**, **7**), while its Jones oxidation product was given structure **8**. The stereochemical assignments at positions 5–8 followed the same argument adopted in case of otostegin A (**2**) (vide supra).

In addition to the new diterpenes **2** and epimeric mixture (**6**, **7**), leopersin C and 15-*epi*-leopersin C (**4**, **5**) (Tasdemir et al., 1996), leoheterin (**3**) and preleoheterin (**1**) (Hon et al., 1993), and the iridoid glucoside 8-*O*-acetylharpagide (**9**) (Brian and James, 1977) were also isolated from this source. Their identity was established by comparing their physical and spectral data with those previously reported. Furthermore, the structural correlations between **4** and **5** on one hand, and between **1** and **3** on the other hand, was confirmed for the first time by acid treatment of the mixture of **4** and **5**. The reaction went smoothly to provide **1** and **3** in a yield of 43 and 50%, respectively.

Pre-furanic and furanic labdane diterpenes are commonly encountered in many species of family labiatae (Lamiaceae), such as *Leonurus heterophyllus* (Hon et al., 1993), *Marrubium vulgare* (Bergeron et al., 1995) and *Ballota aucheri* (Rustaiyan et al., 1992). However, *O. fruticosa* is the only member, so far, in which not only pre-furanic and furanic labdanes are encountered, but also their C-15 hydroxylated derivatives. Numerous labdanes have been reported to occur in *Leonurus persicus*, including furanic and pre-furanic ones, but in addition to those, one finds C-15 acetals, but not the C-15 hydroxylated analogs of the same series (Tasdemir et al., 1996, 1998).

3. Experimental

3.1. General

The plant material used in this study was collected in the pre-flowering stage, in April 1997, near the city of Abha, located in the southern region of Saudi Arabia and voucher specimens were kept at the herbarium of the Research Center for Medicinal, Aromatic and

Poisonous Plants of College of Pharmacy, King Saud University. Melting points were determined on a Mettler FP 80 Central Processor supplied with a Mettler FP 81 MBC Cell apparatus, and were uncorrected. IR spectra were recorded in KBr unless otherwise specified, using a Perkin-Elmer IR, Model 783, or a Perkin-Elmer FTIR, Model 1600 spectrophotometers. Specific rotations were measured as solutions in chloroform, unless specified otherwise, on a Perkin-Elmer 242 MC polarimeter. The ^{1}H- and ^{13}C-NMR spectra were recorded in $CDCl_3$ or benzene-d_6 on a Varian VXR-300 FT spectrometer at 300 and 75 MHz, respectively, and on Bruker NMR spectrometer at 300, 400 or 500 MHz. The chemical shift values are reported as ppm referenced to TMS and the coupling constants are in Hz.

3.2. Extraction and isolation of diterpenes

The air-dried powdered aerial parts of the plant (2 kg) were exhaustively percolated with neutral, acetic acid-free, ethyl acetate. The solvent was evaporated and the residue (74 g) was partitioned between acetonitrile, (1850 ml) and *n*-hexane (4 × 460 ml), pre-saturated with each other. The combined hexane phases were back-washed with 100 ml of acetonitrile and the combined acetonitrile phases were evaporated in vacuo to leave 30 g of a greenish oily residue. A portion of the acetonitrile fraction obtained above (5 g) was flash chromatographed on a column (40 × 2.5 cm) of silica gel and eluted using increasing concentrations of ether in *n*-hexane. Further purification of the resulting fractions using column chromatography led to the isolation of preleoheterin (**1**) (0.0033% yield), otostegin A (**2**) (0.0594% yield), leoheterin (**3**) (0.0363% yield), leopersin C and 15-*epi*-leopersin C (**4**, **5**) (0.0081% yield), otostegin B (**6**, **7**) (0.0063% yield), ballonigrin (**9**) (0.0047% yield) and vulgarol (**11**) (0.00155% yield).

3.3. Otostegin A (2)

Otostegin A (**2**), colorless needles (ether), R_f 0.45; mp 216.2–217.2°C; $[\alpha]_D^{22}$ −117° (*c* 0.08, $CHCl_3$); IR ν_{max} (cm^{-1}): 1745 ($CH_3\underline{CO}$), 1708 (ketone) as shoulder, 1632 (C=C); UV λ_{max} (methanol) nm ($\log \varepsilon$): 220 (3.24); ^{1}H- and ^{13}C-NMR ($CDCl_3$): Table 1; EI MS, *m*/*z* (%): 376 (9) $[M]^+$, with the base peak at *m*/*z* 55, 95 (88), 81 (93).

3.4. Otostegin B (6, 7)

Otostegin B (**6**, **7**) was isolated as colorless oil that was homogeneous on TLC, R_f 0.36 (50% toluene in ethyl acetate); IR ν_{max} (cm^{-1}): 3450 (OH), 1740 ($CH_3\underline{CO}$), 1715 (ketone) as shoulder; UV λ_{max} (methanol) nm ($\log \varepsilon$): 212 (3.06); ^{1}H- and ^{13}C-NMR ($CDCl_3$): Table 1; electrospray HRMS: M^+ at *m*/*z* 394.23520. Calculated for $C_{22}H_{34}O_6$: 394.23553.

3.5. Oxidation of otostegin B (6, 7) to dehydro-otostegin B (8)

Otostegin B (40 mg) was dissolved in acetone (3 ml) and 0.20 ml of Jones reagent was added and the mixture was stirred for 15 min. Methanol was added to quench the red color then the solution was diluted with water and extracted with $CHCl_3$, 3 × 30 ml. The combined chloroform extracts were washed with water (5 ml), 5% sodium bicarbonate (5 ml) and finally water again (5 ml). The chloroform extract was dried over anhydrous sodium sulfate and evaporated to yield 20 mg of a crystalline residue. Re-crystallization from ether–*n*-hexane gave colorless crystals, mp 160–161°C (with decomposition); $[\alpha]_D^{22}$ −46° (*c* 0.092, $CHCl_3$); IR ν_{max} (cm^{-1}): 1788 ($\underline{CO}O$), 1743 ($CH_3\underline{CO}$), 1723 (ketone) as shoulder; UV λ_{max}: (methanol) nm ($\log \varepsilon$): 208 (3.13); ^{1}H- and ^{13}C-NMR ($CDCl_3$): Table 1; EI MS, *m*/*z* (%): 392 (less than 1%), with the base peak at *m*/*z* 43.

3.6. Conversion of leopersin C and 15-epi-leopersin C (4, 5) to preleoheterin (1) and leoheterin (3)

Leopersin C and 15-*epi*-leopersin C mixture (**4**, **5**) (100 mg) was dissolved in $CHCl_3$ (2 ml) and a drop of conc. HCl was then added. The solution was stirred for 24 h, diluted with additional $CHCl_3$, washed with H_2O then evaporated after drying over anhydrous Na_2SO_4. Column chromatography of the residue on silica gel using 8% ethyl acetate in *n*-hexane as eluent provided **1** (43 mg) and **3** (50 mg), identical, in all aspects, with the isolated compounds.

3.7. Isolation of 8-O-acetylharpagide (10) and vulgarol (11)

Compounds **10** (0.01262% yield) and **11** (0.00155% yield) were isolated from the CH_3CN fraction, and their physical and spectral data were indistinguishable from those reported for pure 8-*O*-acetylharpagide (**10**) (Scarpati et al., 1965) and crude vulgarol (**11**) (Popa and Pasechnik, 1975). The ^{13}C-NMR ($CDCl_3$) assignments for vulgarol (**11**) were not previously reported and are as follows: δ 36.8 (C-1), 19.0 (C-2), 42.6 (C-3), 33.3 (C-4), 46.9 (C-5), 21.0 (C-6), 37.8 (C-7), 74.0 (C-8), 61.2 (C-9), 39.2 (C-10), 25.8 (C-11), 43.4 (C-12), 140.9 (C-13), 123.4 (C-14), 59.5 (C-15), 16.9 (C-16), 32.3 (C-17), 33.5 (C-18), 21.7 (C-19) and 25.2 (C-20).

Acknowledgements

The authors thank Dr Charles D. Hufford (University of Mississippi) and Dr Harry S. Fong (University of Illinois-Chicago) for the NMR and MS measurements.

References

Aboutabl, E.A., Sokkar, N.M., Megid, R.M.A., de Pooter, H.L., Masoud, H., 1995. Composition and antimicrobial activity of *Otostegia fruticosa* Forssk. oil. J. Essent. Oil Res. 7, 299.

Bergeron, C., Charbonneau, J., Desroches, B., Gosselin, A., 1995. Influence of supplemental lighting and irrigation on mineral composition, growth and premarrubin content of horehound, *Marrubium vulgare* L. J. Herbs Spices Med. Plants 3, 3.

Brian, D., James, R., 1977. Studies in terpenoid biosynthesis part 19. Formation of pimara-8(9), 15-diene by *Trichothecium roseum*. J.C.S. Perkin 1, 324.

Hon, P.M., Wang, E.S., Lam, S.K.M., Choy, Y.M., Lee, C.M., Wong, H.N., 1993. Preleoheterin and leoheterin, two labdane diterpenes from *Leonurus heterophyllus*. Phytochemistry 33, 639.

Popa, D.P., Pasechnik, G.S., 1975. The structure of vulgarol — a new diterpenoid from *Marrubium vulgare*. Khim. Priorodn. Soedin., 722.

Rustaiyan, A., Mosslemin Kupaii, M.H., Zdero, C., 1992. Furolabdanes and related compounds from *Ballota aucheri*. Phytochemistry 31, 344.

Scarpati, M.L., Guiso, M., Panizzi, L., 1965. Iridoids. I. Harpagide acetate from *Melittis melissophyllum*. Tetrahedron Lett., 3439.

Shaw, A., 1985. A Dictionary of the Flowering Plants and Ferns. Cambridge University Press, Cambridge, p. 835.

Tasdemir, D., Calis, I., Sticher, O., 1998. Labdane diterpenes from *Leonurus persicus*. Phytochemistry 49, 137.

Tasdemir, D., Wright, A.D., Sticher, O., Calis, I., 1996. New furanoid and seco-labdanoid diterpenes from *Leonurus persicus*. J. Nat. Prod. 59, 131.