Diterpenoids of *Otostegia persica* (Burm.) Boiss

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Received 16 Sept 2008; Revised 21 Apr 2009; Accepted 5 May 2009

ABSTRACT

Background and propose of the study: As there is no significant work on *O. persica,* a phytochemical investigation of this species was carried out in this study.
Methods: The structures of the compounds **1-4** were established by their spectral data and their relative configuration was determined by 2D NMR.
Results and major conclusion: Four known diterpenoids belonging to the clerodane and tetracyclic diterpene types were isolated for the first time from *Otostegia persica* (Burm.) Boiss. These compounds are known to occur only in genus *Otostegia.*

Keywords: Lamiaceae, *Otostegia persica*, Diterpenoids

INTRODUCTION

The genus *Otostegia* (Lamiaceae) consists of about 33 species growing mainly in the Mediterranean region and adjoining Asia Minor (1). In Iran only three species are available, *Otostegia aucheri, O. michauxi and O. persica*, of which the last two ones are endemic to Iran (2).
O. persica is locally called "Golder". It is widely distributed in south and south east of Iran. It is traditionally used for treatment of malaria, fever and diabetes. The aerial part of *O. persica* is reported to have high antioxidant activity which is related to the flavonoids (3) and its hydro-alcoholic extract is effective on morphine withdrawal syndrome in mice (4). Methanolic extract of the aerial parts of *Otostegia persica* has shown positive activity in the brine shrimp lethality test. Additional biological screening of the methanolic extract has revealed strong antioxidant as well as antibacterial activities against various strain of Gram-positive and Gram-negative bacteria (5-7). A literature survey revealed that no significant Phytochemical studies have been carried out so far on *O. persica*. This prompted us to carry out a phytochemical investigation of this species. In this article the isolation and structural elucidation of four known clerodane and tetracyclic deterpenoids which were isolated for the first time from *O. persica* is described.

MATERIALS AND METHODS

General experimental procedure
The FT IR spectra were recorded on a vector 22 instrument. The ^{1}H-NMR was recorded on a Bruker AMX 500 NMR (Avance) instruments using the UNIX data solvent. ^{1}H-^{13}C HMBC and HMQC were recorded at 500 MHz (proton) and 125 MHz (carbon), respectively. EI MS spectra were recorded on a Finnigan MAT 312. FAB mass measurements were performed on Jeol JMS HX 110 mass spectrometer using glycerol as the matrix. HREI MS was carried out on Jeol JMS 600 mass spectrometer. Column chromatography were carried out on silica gel (M&N), 70-230 mesh. Compounds on the TLC (M&N) were detected at 254 and 366 nm and by ceric sulfate as spraying reagent.

Plant material
The plant *Otostegia persica* (Lamiaceae) was collected from Bandar Abbas, Hormozgan province, Iran, in May 2005, and identified by Mr.M.Kamalinezhad at the Department of Pharmacognosy, Faculty of Pharmacy, Shaheed Beheshti University of Medical Sciences, Tehran, Iran. A voucher specimen (NO. 1719) has been deposited in the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Shaheed Beheshti University of Medical Sciences, Tehran, Iran.

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Table 1. ^{1}H-NMR (CDCl3) Data of Compounds **1-4**.δ in ppm, J in Hz.

	1	**2**	**3**	**4**
CH_2 (1)	1.50-1.67(*m*)[a]	0.74[a]	0.74[a]	2.15-2.4(*m*)[a]
CH_2(2)	2.43-2.49(*m*)	2.39-2.43(*m*)	2.20-2.31(*m*)	1.53-1.55(br. *m*)
H–C(3) or CH_2(3)	6.46(br. *s*)	6.83(br. *t, J*=3.1)	6.83(br. *t, J*=2.1)	1.95-2.0(*m*)
				2.07(*dd, J*=5.17)
H–C(5)	-	-	-	1.36(br. *d, J*=12)
H–C(6) or CH_2(6)	3.69(*dd, J*=3.5, 7.5)	2.32-1.13(*m*)	2.30(*m*)	1.69-1.79(*m*)
			1.12(*dd, J*=5.5, 12)	1.95-2.0(*m*)
CH_2(7)	1.99-2.03(*m*)	1.37-1.50(*m*)[a]	1.40-1.51(*m*)[a]	1.79-1.95(*m*)[a]
H–C(8)	1.78(*m*)	1.59-1.69(*m*)	1.44[a]	-
H–C(10)	1.43(br. *d, J*=12)	1.31(br. *d, J*=11.5)	1.30(br. *d, J*=11.5)	-
CH_2(11)	1.44-1.60(*m*)[a]	1.42-1.53(*m*)[a]	1.40-1.5[a]	1.79-1.88(br. *m*)
	2.10(*ddd, J*=4.8, 11.6)			2.41-2.47(*m*)
CH_2(12)	2.15-2.21(*m*)	2.01(br. *t, J*=12.5)	2.02(br. *t, J*=14)	2.32-2.41(*m*)[a]
		2.15(br. *t, J*=13)	2.19(*m*)	
H–C(14)	6.75(*t, J*=1.4)	7.07(br. *s*)	6.74(br. *s*)	5.63(*s*)
H–C(15) or CH_2(15)	5.70(br. *s*)	4.74(*d, J*=1.5)	5.70(br. *s*)	-
Me(17) or H–C(17)	0.96(*d, J*=6.5)b	0.80(*d, J*=7)	0.79(*d, J*=6)b	5.65(*s*)
	0.98(*d, J*=6.5)b		0.78(*d, J*=6)b	
Me(18)	-	-	-	1.24(*s*)
Me(19) or CH_2(19)	1.01(*s*)	1.22(*s*)	1.22(*s*)	-
Me(20)	0.86(*s*)	0.74(*s*)	0.74(*s*)	0.83(*s*)
MeO	3.56(*s*), 3.55(*s*)b	-	3.55(*s*), 3.54(*s*)b	-

[a]Overlapped [b]Epimer mixture

Extraction and isolation

The air dried flowering aerial parts of *Otostegia persica* (8 kg) was exhaustively extracted by maceration with 80% EtOH (3×80 lit).

The extract was evaporated to yield the residue (1.112 kg) which was partitioned between water (72 ml), petroleum ether (188 ml), CH_2Cl_2 (380 ml), EtOAc (276 ml) and n-butanol (132 ml).

The CH_2Cl_2 fraction was subjected to silica gel chromatography using petroleum ether with a gradient of CH_2Cl_2 up to 100% followed by methanol. Six fractions were collected.

Fraction No. 4 of the first column was loaded on silica gel and eluted with petroleum ether-EtOAc in increasing order of polarity to provide two fractions by using petroleum ether/EtOAc 76:24 and 81:19.

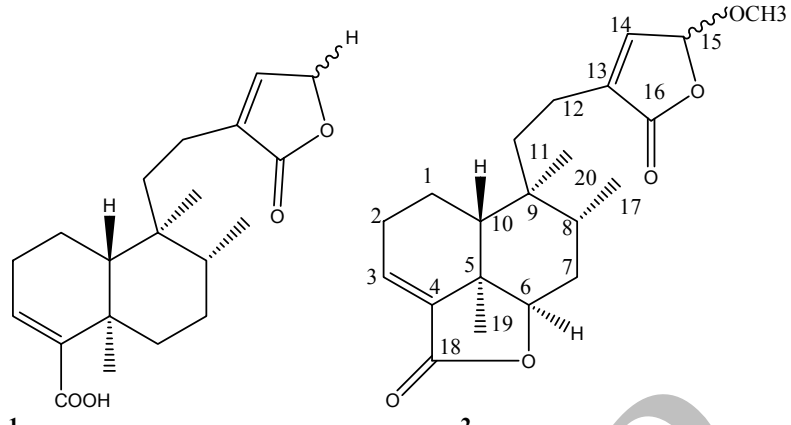
The first fraction was subjected to column chromatography and eluted with petroleum ether/EtOAc (46:54) to give pure compound **1** (8.2 mg). Similarly, the second fraction was re-chromatographed over silica gel and eluted with petroleum ether/EtOAc (58:42 and 52:48) to give pure compound **2** (7.7 mg) and compound **3** (7 mg).

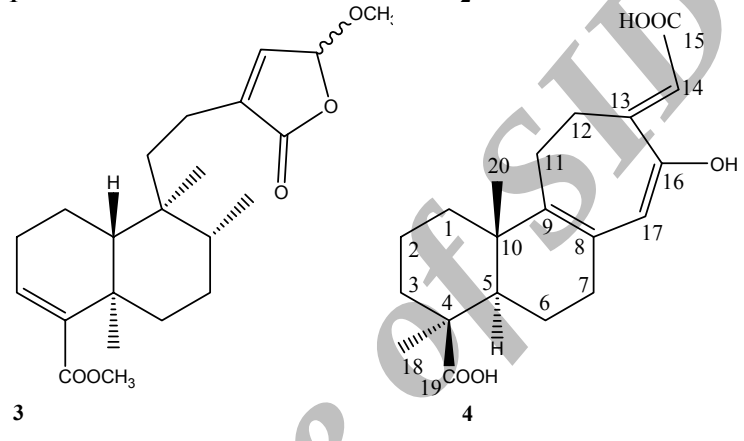
Fraction No.5 of the first column was loaded on silica gel and eluted with petroleum ether/ acetone in increasing order of polarity to provide a fraction with petroleum ether/acetone 75:25 which was subjected to silica gel column chromatography and eluted with petroleum ether/acetone 30:70 to give pure compound **4** (8.4 mg).

RESULT AND DISCUSSION

Compounds **1-3** were identified as clerodane-type diterpenoids. Their UV and IR data indicated the presence of an α,β-unsaturated γ-lactone, and their structures were established by a detailed analysis of their spectral data and comparison with those of the related known compounds.

The α,β-unsaturated γ-lactone and the α,β-unsaturated carbonyl group of **1-3** gave rise to absorptions at 1752-1769 cm^{-1} and 1678-1681 cm^{-1}. An absorption at 2980 cm^{-1} suggested the presence of a COOH group in **2** (9). The tricyclic clerodane skeleton of **1-3** was identified by their unique ^{1}H-NMR spectrum (Table 1) in which the two tertiary Me groups appeared as *s* at δ 1.01-1.22 and 0.86-0.74, respectively and one secondary Me group as *d* at δ 0.8-0.96 (*J*=6.0 Hz). Apart from these typical highfield Me signals, the downfield region showed two olefinic CH signals at δ 6.46-6.83 and 6.75-7.07 with very small *J* values, which are typical for most clerodanes. These compounds were distinguished as *β*-substituted analogues of clerodanes (9) since both of these individual downfield olefinic H-atoms showed correlation with a downfield quaternary olefinic C-atom at δ(C) 134-140.4 and a C=O at δ(C) 171-174.3 in the HMBC spectrum. The high-resolution mass spectrum of compound **1** exhibited the highest peak at *m/z* 342 ([*M*-H_2O]$^+$, $C_{21}H_{28}O_5^+$). The ^{13}C-NMR DEPT experiment disclosed the presence of six quaternary C-atoms including the two olefinic C-atoms and the two C=O groups (Table 2). The presence of two γ-lactone moieties,





configuration of the lactone ring at C (4) and C (6), the presence of a MeO substituent at C(15), the substitution at the lactone ring and the relative configuration of **1** was deduced by the aid of 2D NMR experiments and the reported literature data (1).

Compound **2** (FAB-MS: *m/z* 425 ([M^+ glycerol $^+1]^+$) was devoid of the MeO substituent at C(15) and instead had a CH_2 group (δ(H) 4.74, δ(C) 70.2). Comparison of its NMR data with literature data revealed that compound **3** was identical to the compound reported by *Choudhary et al*. (1).

The clerodane structure of **3** (FAB-MS: *m/z* 425 ([M^+ glycerol – 1] $^-$) was suggested by its UV, IR and ^{1}H-NMR spectra, which were similar to those discussed above.

Compound **3** lacked the C(4)/C(6) lactone moiety but contained an α,β-unsaturated ester function at the olefinic quaternary C(4). With the aid of 2D NMR experiments and literature data, compound **3** was found to be identical with the compound reported by Krishna et al. (9).

Limbatenolide C (**4**) had the molecular ion at 346 in the EI mass spectra corresponding to molecular formula $C_{20}H_{26}O_5$, respectively. Its structure was deduced from spectral data and by comparison with literature data. Compound **4** contained a COOH group at C (4) as deduced from the IR absorption at 1710 cm^{-1}; also the H-atoms of Me (18) showed a ^3J HMBC correlation with COOH (δ (C) 182.2). The two downfield olefinic signals of **4** at δ (H) 5.63 (H–C(14)) and 5.65 (H–C (17)) gave HMBC correlations for the seven-membered ring.

The IR data of **4** suggested the presence of a free α,β-unsaturated-acid function at C(13) rather than a lactone moiety, which was confirmed by an absorption at 1656 cm^{-1} (free acid). The C (13)=C(14) bond apparently had (*E*)-configuration because the COOH group at C(14) did not form a lactone ring with OH–C(16). The IR spectrum confirmed the presence of a free OH group at C(16). Comparison of its NMR data with literature data established that compound **4** was identical to the compound reported by choudhary et al. (1).

Compounds **1-4** have been previously reported from *Ballota limbata* and all of them have shown inhibitory activity against butyrylcholinesterase (1). It has been found that butyrylcholinesterase inhibition may be an effective approach for the treatment of Alzheimer-disease and related dementias. Compounds **1-3** have also displayed inhibitory activity against acetylcholinesterase (1). Acetylcholinesterase is known to be a key component of cholinergic brain synapses and neuromuscular junctions as it reduces memory deficiency in patients suffering from Alzheimer-disease by potentiating and affecting the cholinergic transmission process (8).

Table 2. ^{13}C-NMR (CDCl3, 125 MHz) data of compounds **1-4**. δ in ppm.

	1	2	3	4
C(1)	17.5	17.3	17.4	37.2
C(2)	27.3	27.4	27.2	19.5
C(3)	130.1	141.2	141.3	36.0
C(4)	139.7	140.4	140.4	43.5
C(5)	39.1	37.5	37.6	53.0
C(6)	86.1	35.7	35.8	20.8
C(7)	31.4	27.2	27.5	35.6
C(8)	38.1	36.2	36.4	127.5
C(9)	42.3	38.7	38.9	142.4
C(10)	44.9	46.6	46.8	40.5
C(11)	36.4	36.2	35.9	25.2
C(12)	19.9	19.2	19.0	27.8
C(13)	138.8	134.9	139.3	130.1
C(14)	141.6	143.5	141.3	115.1
C(15)	102.5	70.2	102.5	172.9
C(16)	172	174.3	172.2	143.2
C(17)	15.5	15.9	15.9	114.9
C(18)	171	172.5	171.3	28.1
C(19)	16.1	20.5	20.5	182.2
C(20)	19.7	18.2	18.2	17.2
MeO	57.1		56.9	

CONCLUSION

The preliminary general phytochemical tests showed that dichloromethane extract of aerial parts of *Otostegia persica* could be considered as a rich source of different terpenoids. The results of this investigation revealed the structures of four diterpenes which have not been reported from this plant so far. The interesting biological activities reported for these compounds by other researchers emphasize the importance of these types of studies which could lead to the discovery of new active compounds.

ACKNOWLEDGMENT

Financial support from Shaheed Beheshti University of Medical Sciences, Tehran, Iran is gratefully acknowledged. We would like to thank International Center for Chemical Sciences, H.E.J. research Institute of Chemistry, University of Karachi for performing the UV, IR, MS, 400 and 500 MHz NMR and 2D NMR spectroscopy. Thanks are also due to Mr.M. Kamalinejad for identification of the pant material.

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