Two New Rare-Class Tetracyclic Diterpenoids from *Otostegia limbata*

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Two new tetracyclic diterpenoids trivially named as limbatenolide D (1) and limbatenolide E (2) have been isolated from *Otostegia limbata*. The structure elucidation of the compounds was based primarily on two-dimensional (2D) NMR techniques and on comparison with the literature data.

Key words Lamiaceae; *Otostegia limbata*; tetracyclic diterpenoid

The genus *Otostegia* (Lamiaceae) comprises *ca.* 33 species, mainly occurring in the Mediterranean region.[2] In Pakistan, only two species have been found, namely *Otostegia aucheri* Boiss. and *Otostegia limbata* (Bth.) Boiss. (Syn. *Ballota limbata* Bth.; Labiatae). *Otostegia limbata* is locally called "Bui" or "Phut kandu."[3] It is widely distributed in the North-West Frontier Province and lower hills of West Punjab in Pakistan. The species of genus *Otostegia* are widely used by the traditional practitioners against various diseases, and their constituents have shown to possess anti-ulcer, antispasmodic, antidepressant, anxiolytic and sedative activities.[4] Traditionally, *O. limbata* has been used in the treatment of children gum diseases and for ophthalmia in man.[5] Here, we report the isolation and structure elucidation of two new rare-class tetracyclic diterpenoids (Fig. 1) from *O. limbata*.

Results and Discussion

The $CHCl_3$ extract of the air-dried whole plant of *Otostegia limbata* was subjected to silica gel chromatotogarphy to give two new rare-class tetracyclic diterpenoids provisionally named as limbatenolide D (**1**) and limbatenolide E (**2**).

Compound **1** was isolated as a gummy solid from moderately polar fraction eluted with $CHCl_3$. The molecular ion peak of compound **1** at *m/z* 326 both in FD-MS and HR-EI-MS spectra combined with 22 hydrogen and 20 carbon signals seen in the ^{1}H- and ^{13}C-NMR spectra led to the molecular formula $C_{20}H_{22}O_4$ consistent with ten degrees of unsaturation. The basic skeleton of compound **1** was identified on the basis of its ^{1}H-, ^{13}C-NMR, and characteristic IR data.[6] The IR spectrum showed an absorption band for furan ring at 865 cm^{-1}, whereas a strong carbonyl absorption at 1620 cm^{-1} was attributed to an α,β-unsaturated ketone which was also conjugated to the furan ring; five-membered lactone absorption appeared at 1760 cm^{-1}. In agreement with this hypothesis, the UV spectrum of **1** showed multiple absorptions at λ_{max} 315, 308, 290, and 275 nm which were attributed to furan ring and α,β-unsaturated carbonyl group. In ^{1}H-NMR spectrum of **1** only two downfield methyl singlets were observed which were unusual for clerodanes previously isolated from *Otostegia limbata*,[6,7] whereas ^{13}C-NMR spectra showed several low field carbons, again unusual for the said class of diterpenoids. Apart from UV and IR spectra, the presence of furan was also confirmed from the methine signals in ^{1}H-NMR at δ_H 6.40 (1H, d, J=1.9 Hz) and δ_H 7.43 (1H, d, J=1.9 Hz) characteristic for two furan protons. The presence of two methyl groups attached to quaternary carbons was revealed by two singlets at δ_H 1.25 and δ_H 1.15. The ^{1}H-NMR data suggested the presence of a furan ring fused to tetracyclic clerodane skeleton indicating tetra-substituted olefinic bond and a five-membered lactone ring. The ^{13}C-NMR spectra showed the presence of 20 carbon atoms, including two methyl, six methylene, four methine, and eight quaternary carbons. The ^{13}C-NMR chemical shift of Me-18 was observed at δ_C 25.2; the β-positioned Me-20 appeared at δ_C 18.5. From empirical formula of **1** and DEPT measurement of ^{13}C-NMR, six high field methylenes were suggestive of the presence of a seven-membered ring in the compound. Whereas, the tetra-substituted olefinic bond and characteristic IR absorptions indicated α,β:α',β'-diunsaturation with carbonyl absorption at 1620 cm^{-1}, and in ^{13}C-NMR the peak at δ_C 184.3. This together with the typical quaternary signals at δ_C 152.6, 150.6, 136.1, 135.9, and two methine signals at δ_C 144.1 and δ_C 113.4 revealed the presence of an α,β-fused furan ring and an olefinic bond. It was evident from HMBC correlations (Fig. 2) that the furan ring was fused with the tetracyclic clerodane skeleton as the peak at δ_H 6.40 (H-14) showed cross peaks with downfield olefinic quaternary carbons C-13 (δ_C 135.9), C-16 (δ_C 150.6), and methylene car-

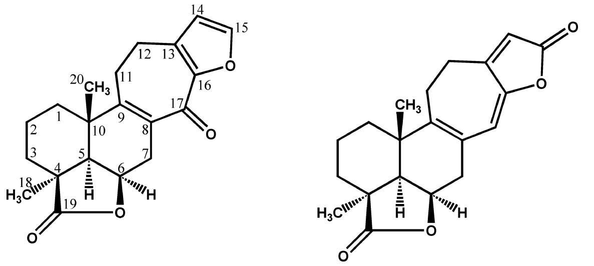

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Fig. 1. Structures of Compounds **1** and **2**

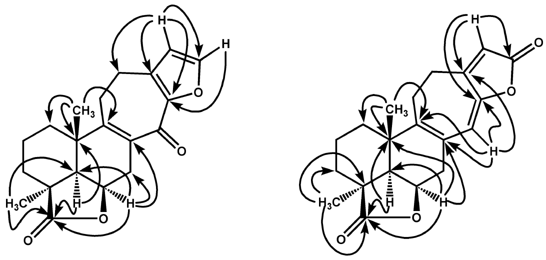

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Fig. 2. Important HMBC Correlations of **1** and **2**

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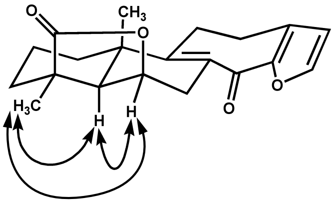

Fig. 3. Important NOE Correlations of **1**

Table 1. ^{1}H- and ^{13}C-NMR Data for Compounds **1** and **2** in CDCl$_3$[a]

Position	**1** δ_C	**1** δ_H	**2** δ_C	**2** δ_H
1	38.4	2.10—2.25 m, ovlp	37.1	1.90 m, 2.13 m
2	20.4	1.62—1.67 m	20.9	1.79—1.85 m, ovlp
3	32.4	2.01—2.07 m, ovlp	33.0	1.87—2.05 m, ovlp
4	44.1	—	42.6	—
5	53.1	1.30 ovlp	51.9	1.32 ovlp
6	83.5	3.48 dd (5.8, 11.3)	80.3	3.64 dd (6.0, 11.5)
7	29.2	1.85—1.95 m, ovlp	35.6	2.15—2.20 m, ovlp
8	136.1	—	127.1	—
9	152.6	—	145.9	—
10	42.3	—	40.3	—
11	25.4	2.55—2.60 m, ovlp	24.8	1.80 m, 2.35 m
12	28.6	2.78—2.83 m	27.2	2.36—2.45 m
13	135.9	—	150.1	—
14	113.4	6.40 d (1.9)	114.1	5.80 s
15	144.1	7.43 d (1.9)	170.6	—
16	150.6	—	156.7	—
17	184.3	—	118.2	5.90 s
18	25.2	1.25 s	27.0	1.20 s
19	180.1	—	181.9	—
20	18.5	1.15 s	19.3	0.90 s

a) Chemical shifts (δ) in ppm relative to TMS; coupling constants (J in Hz) are given in parentheses.

bon C-12 (δ_C 28.6). The identity of the fused furan and carbonyl system in the seven-membered ring was established upon comparison of the mentioned features with those of hispanonic acid.[6,8] The carbonyl carbon was established at C-17 due to γ-shielding effect for C-7 (δ_C 29.2).[6,8] The two terminal methyls were in good agreement with those of methyl hispanonate and methyl hispaninate.[8] However, the downfield methyl at δ_H 1.25 showed HMBC cross peaks with C-19 (δ_C 180.1) and C-5 (δ_C 53.1) while H-6 showed HMBC correlations with C-19 and also with those of C-5 (δ_C 53.1), C-7 (δ_C 29.2), and C-8 (δ_C 136.1). These were in accordance with the presence of five-membered C-4/C-6 lactone bridging.[9] The stereochemistry was confirmed by NOE experiments carried out on **1**, which showed NOE between Me-18 and C-5 methine proton, and between Me-18 and C-6 methine proton. These results and the fact that irradiation of Me-20 did not cause any increase in the intensities of the Me-18, C-5 or C-6 protons confirmed the *trans* relationship between Me-20 and H-5, and the lactone ring was deduced to be oriented axially at C-6 (Fig. 3).

Compound **2** was also isolated as gummy solid. It also belonged to the rare-class of tetracyclic diterpenoids which are known to occur only in genus *Otostegia*. The EI-MS of **2** provided the $[M]^+$ peak at *m/z* 326, and the HR-EI-MS indicated the molecular formula $C_{20}H_{22}O_4$, consistent with ten degrees of unsaturation. The IR spectra showed an absorption at 1750 cm^{-1} for α,β-unsaturated γ-lactone with the absence of extended carbonyl conjugation discussed for compound **1**. The UV spectra were suggestive of the absence of furan ring as λ_{max} were observed at 352, 301, 260, and 235 nm. The structure of compound **2** was deduced on the basis of spectral data (see Experimental) and comparison with those of methyl hispaninate,[10] limbatane B,[6] and compound **1**. The stereochemistry was confirmed by NOE experiments carried out on **2** which showed NOE between Me-18 and C-5 methine proton, and between Me-18 and C-6 methine proton. These results and the fact that the irradiation of Me-20 did not cause any increase in the intensities of the Me-18, C-5 or C-6 protons confirmed the *trans* relationship between Me-20 and H-5, and the lactone ring was deduced to be oriented axially at C-6.

Experimental

General Experimental Procedures UV and IR spectra were recorded on Hitachi-UV-3200 and Jasco-320-A spectrophotometers, respectively. ^{1}H- and ^{13}C-NMR were recorded on a Bruker AM-400 spectrophotometer with tetramethylsilane (TMS) as an external standard. 2D NMR spectra were recorded on a Bruker AMX 500 NMR spectrophotometer. Optical rotations were measured on a Jasco DIP-360 digital polarimeter using a 10 cm cell tube. Mass spectra (EI- and HR-EI-MS) were measured in an electron impact mode on Finnigan MAT 12 or MAT 312 spectrophotometers, ions are given in *m/z* (%). FAB-MS were measured on a JEOL HX 11 mass spectrometer. TLC was performed with pre-coated silica gel G-25-UV$_{254}$ plates and detection was done at 254 nm, and by ceric sulphate in 10% H_2SO_4 solution. Silica gel (E. Merck, 230—400 mesh) was used for column chromatography.

Plant Material The plant *Otostegia limbata* (Lamiaceae) was collected from Abbottabad, Pakistan, in June 2001, and identified by Dr. Manzoor Ahmad (Taxonomist) at the Department of Botany, Post Graduate-College, Abbottabad, Pakistan. A voucher specimen (#6872) has been deposited in the herbarium of the Botany Department of Post-Graduate College, Abbottabad, Pakistan.

Extraction and Purification The air dried whole plant (35 kg) was exhaustively extracted with methanol (40 l×3) at room temperature. The extract was evaporated to yield the residue (315 g), which was partitioned between hexane (47 g), chloroform (95 g), ethyl acetate (69 g), butanol (33 g) and water (59 g). The chloroform extract was subjected to silica gel chromatography using hexane with a gradient of $CHCl_3$ up to 100% and followed by methanol. Twelve fractions were collected. Fraction no. 6 of the first column was loaded on silica gel (230—400 mesh) and eluted with EtOAc : hexane (20 : 80) to purify compound **1** (5.2 mg). Similarly, compound **2** (4.8 mg) was purified from the same fraction with EtOAc : hexane (28 : 72). The purity of the compounds was checked on TLC and HPTLC plates.

Compound **1**: Gummy solid. ^{1}H-NMR (CDCl$_3$, 400 MHz), see Table 1. ^{13}C-NMR (CDCl$_3$, 100 MHz), see Table 1. IR ν_{max} (CHCl$_3$) cm^{-1}: 1760, 1620, 865. UV λ_{max} (MeOH) nm (log ε): 315 (3.2), 308 (4.3), 290 (4.6), 275 (5.1). EI-MS *m/z* (rel. int.): 326 (85), 308 (75), 312 (60), 284 (50), 238 (40), 214 (20), 177 (55). HR-EI-MS *m/z*: 326.1523 (Calcd for $C_{20}H_{22}O_4$: 326.1512). $[\alpha]_D^{25}$ +86.2° (c=0.30, CHCl$_3$).

Compound **2**: Gummy solid. ^{1}H-NMR (CDCl$_3$, 400 MHz), see Table 1. ^{13}C-NMR (CDCl$_3$, 100 MHz), see Table 1. IR ν_{max} (CHCl$_3$) cm^{-1}: 1750 br. UV λ_{max} (MeOH) nm (log ε): 352 (4.2), 301 (3.2), 260 (5.1), 235 (4.6). EI-MS *m/z* (rel. int.): 326 (80), 308 (73), 298 (65), 282 (30), 256 (40), 238 (50), 212 (30), 177 (25), 150 (98). HR-EI-MS *m/z*: 326.1519 (Calcd for $C_{20}H_{22}O_4$: 326.1512). $[\alpha]_D^{25}$ +48.2° (c=0.25, CHCl$_3$).

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