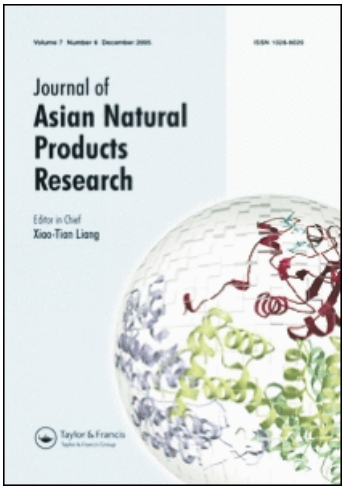

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Two new *trans*-clerodane diterpenoids from *Otostegia limbata*

V. U. Ahmad[a]; A. Khan[a]; U. Farooq[a]; F. Kousar[a]; S. S. Khan[a]; J. Hussain[a]
[a] International Centre for Chemical Sciences, H.E.J. Research Institute of Chemistry, University of Karachi, Karachi, Pakistan

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Two new *trans*-clerodane diterpenoids from *Otostegia limbata*

V. U. AHMAD*, A. KHAN, U. FAROOQ, F. KOUSAR, S. S. KHAN and J. HUSSAIN

International Centre for Chemical Sciences, H.E.J. Research Institute of Chemistry, University of Karachi, Karachi 75270, Pakistan

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Two new tricyclic clerodane-type diterpenoids, limbatolide D (**1**) and limbatolide E (**2**), have been isolated from the roots of *Otostegia limbata*. Their structures and the relative configuration were established on the basis of spectral methods, especially two-dimensional (2D) NMR techniques.

Keywords: Lamiaceae; *Otostegia limbata*; Clerodane diterpenoids

1. Introduction

The genus *Otostegia* (Lamiaceae) comprises about 33 species, mainly occurring in the Mediterranean region [1]. In Pakistan, only two species have been found, namely *Otostegia aucheri* and *Otostegia limbata*. Locally, *O. limbata* is called 'Bui' or 'Phut kandu' [2]. It is widely distributed in the North–West Frontier Province and lower hills of West Punjab in Pakistan. It is used, traditionally, in the treatment of children gum diseases and for ophthalmia in man [3]. Moreover, the species of genus *Otostegia* are widely used by the traditional practitioners against various diseases, and its constituents have shown to possess antiulcer, antispasmodic, antidepressant, anxiolytic and sedative activities [4]. Here, we report the isolation and structure elucidation of two new clerodane-type diterpenoids (figure 1).

2. Results and discussion

The $CHCl_3$ fraction of the air dried roots of *O. limbata* was subjected to silica gel chromatography to give two new tricyclic clerodane-type diterpenoids limbatolide D (**1**) and limbatolide E (**2**).

Limbatolide D (**1**) was isolated as gummy solid. Its molecular formula $C_{20}H_{26}O_3$ was established by HREIMS, which showed the $[M]^+$ peak at *m/z* 314.1893. The peaks at *m/z* 95,

*Corresponding author. Email: vuahmad@cyber.net.pk

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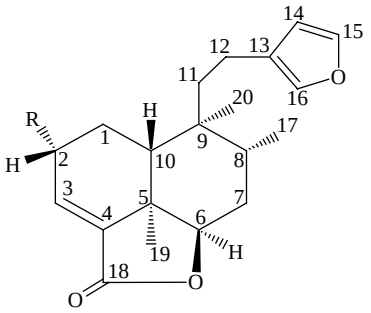

1 : R = H
2 : R = OH

Figure 1. Structures of compounds **1** and **2**.

94, and 81 suggested the presence of a furan ring with an alkyl chain in **1** [5], while the ion peaks at *m/z* 219, 201, and 173 showed the presence of diterpenoid skeleton [6]. The IR spectrum of **1** showed the presence of α,β-unsaturated lactone (1763 cm^{-1}), unsaturation (1683 cm^{-1}), and a furan ring (1510 and 870 cm^{-1}), while the UV spectrum revealed an absorption at λ_{max} 212 nm.

The ^{1}H NMR spectrum of **1** closely resembled those of *trans*-clerodanes, revealing the same substitution pattern in rings A and B [6]. It exhibited signals for two tertiary methyls at δ 0.75 and 1.20 (each 3H, s), one secondary methyl at δ 0.85 (d, $J = 7.4$ Hz), and for an olefinic proton at δ 6.90 (dd, $J = 3.2$, 4.9 Hz, H-3). A β-monosubstituted furan ring was indicated by characteristic ^{1}H NMR resonances at δ 6.30 (dd, $J = 0.8$, 1.8 Hz, H-14), 7.31 (br. s, H-16), and 7.35 (t, $J = 1.8$ Hz, H-15), and the corresponding ^{13}C NMR resonances at δ 111.3, 139.7, and 143.4 as revealed by the HMQC experiment. The large $^{1}J_{C-H}$ couplings observed for C-15/H-15 (205 Hz) and C-16/H-16 (202 Hz) are a diagnostic feature of oxygen attachment at these two centres.

The ^{13}C NMR spectrum corroborated the presence of three CH_3, five CH_2, seven CH, and five quaternary C-atoms. The chemical shift of CH_3-19 was observed at δ 18.1 and the α-positioned axial CH_3-20 appeared at δ 19.3, while the α-positioned equatorial CH_3-17 resonated at δ 16.6. These values revealed the *trans* configuration at the A/B ring junction in **1** [7].

The positions of γ-lactone moiety and furan ring in the molecule were confirmed from $^{1}H-^{1}H$ COSY. The HMBC experiment was very informative in the structure elucidation of **1**. In the HMBC experiment of **1** (figure 2), the olefinic proton at δ 6.90 (H-3) showed correlations to the carbons at δ 174.1 (C-18), 140.5 (C-4), 40.6 (C-5), and 27.1 (C-2). In addition, the HMBC of **1** revealed that α-oriented CH_3-19 at δ 1.20 was correlated to the carbons at δ 140.5 (C-4), 82.4 (C-6), and 45.1 (C-10), which established the presence of a lactone moiety joining the A/B rings of *trans*-clerodane through C-4 and C-6 carbons [8,9]. The AB pattern centered at δ 1.50 and 2.05 could be assigned to CH_2-11 the HMBC of which showed correlations to the carbons at δ 45.1 (C-10), 41.3 (C-9), 39.1 (C-8), 19.3 (C-20), and 18.4 (C-12), confirming the attachment of the alkyl chain at C-9.

A series of NOE experiments carried out on **1** established NOEs between CH_3-19 and CH_3-20, and between CH_3-17 and CH_3-20, consistent with a *cis* relationship between these CH_3 groups. These results, and the fact that irradiation of H-10 did not cause any increase in the intensities of either the CH_3-19 or CH_3-20 signals, confirmed the *trans* configuration of

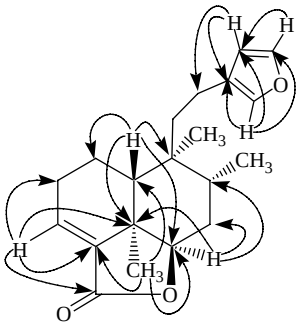

Figure 2. Important HMBC correlations of **1**.

A and B rings of the decalin system of **1** [6]. The NOEs were also observed between H-6 and CH_3-19, indicating their *cis* relationship, and establishing the β-configuration and axial conformation of lactone at C-6. This was also confirmed by the coupling constants of H-6 and the inspection of the model. Based on the foregoing evidence, the structure of limbatolide D was thus established as **1**.

Limbatolide E (**2**) was also isolated as gummy solid. Its molecular formula $C_{20}H_{26}O_4$ was established by HREIMS, which showed the $[M]^+$ peak at *m*/*z* 330.1840. As for **1**, the peaks at *m*/*z* 95, 94 and 81 were typical of an alkyl-substituted furan moiety [5], while the ions at *m*/*z* 219, 201, and 173 showed the presence of diterpenoid skeleton [6]. The IR spectrum of **2** showed the presence of an OH group (3460 cm^{-1}), α,β-unsaturated lactone (1755 cm^{-1}), unsaturation (1680 cm^{-1}), and a furan ring (1506 and 875 cm^{-1}), while the UV spectrum revealed an absorption at λ 210 nm.

A comparison of the ^1H and ^{13}C NMR spectra of **2** with those of **1** revealed a close similarity between the two compounds. The ^1H NMR of **2** showed a signal at δ 4.28 (1H, m) indicating that one of the two protons at C-2 of **2** has been substituted by an OH group [10]. The ^{13}C NMR corroborated the presence of three CH_3, four CH_2, eight CH, and five quaternary carbons. The ^{13}C NMR showed the downfield shift of the C-2 signal at δ 72.3 due to the OH group.

The position of the OH group at C-2 was further confirmed by HMBC experiments which showed correlations of the signal at δ 6.80 (H-3) to the carbons at δ 172.1 (C-18), 139.6 (C-4), 72.3 (C-2), and 42.3 (C-5). Further confirmation of the structure of **2** was provided by NOEs establishing the spatial proximity of H-2 and H-10 (figure 3). The OH group at C-2 was thus disposed to be equatorially α-oriented. Other features of 1D and 2D NMR spectra were similar to those of **1**. Based on the above evidence the structure of limbatolide E was established as **2**.

3. Experimental

3.1 General experimental procedures

Optical rotations were measured on a Jasco DIP-360 digital polarimeter using a 10-cm cell tube. UV spectra were recorded on Hitachi-UV-3200 spectrophotometer. IR spectra were recorded on Jasco-320-A spectrophotometer. ^1H and ^{13}C NMR spectra were recorded on a

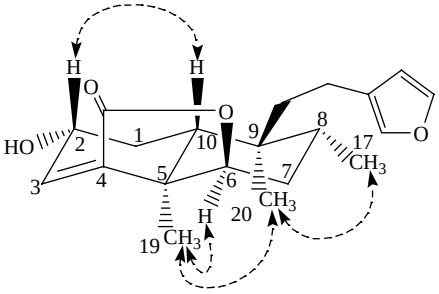

Figure 3. Important NOE correlations of **2**.

Bruker AM-400 spectrophotometer at 400 and 100 MHz, respectively, in $CDCl_3$ using TMS as internal standard. 2D NMR spectra were recorded on a Bruker AMX 500 spectrophotometer. MS spectra were measured on Finnigan MAT 12 or MAT 312 spectrophotometers. Silica gel 230–400 mesh (Merck, Germany) was used for column chromatography. TLC was performed on pre-coated silica gel 60 F_{254} plates and detection was achieved by spraying with ceric sulphate in 10% H_2SO_4 solution followed by heating.

3.2 Plant material

The root parts of *Otostegia limbata* (Lamiaceae) were collected in July 2002 from Abbottabad, Pakistan, and identified by Dr. Manzoor Ahmad (taxonomist) at the Department of Botany, Post-Graduate College, Abbottabad, Pakistan. A voucher specimen (No. 6872) has been deposited in the herbarium of the Botany Department of Post-Graduate College, Abbottabad, Pakistan.

3.3 Extraction and isolation

The air-dried roots of *Otostegia limbata* (30 kg) were extracted repeatedly with methanol (50 L) three times, at room temperature. The solvent was evaporated under reduced pressure to give a dark residue (450 g), which was partitioned between hexane (50 g), chloroform (80 g), ethyl acetate (120 g), butanol (170 g) and water (30 g). The chloroform extract was subjected to silica gel chromatography using hexane with a gradient on $CHCl_3$ up to 100% and followed by methanol. Eleven fractions were collected. Fraction 9 (10.5 g) of the first column was loaded on silica gel and eluted with EtOAc–hexane (3:7) to give compound **1** (8 mg). Fraction 11 (13 g) was subjected to column chromatography and eluted with EtOAc–hexane (4:6) to give compound **2** (6.5 mg).

3.3.1 Limbatolide D (1). Gummy solid, $[\alpha]_D^{25}$ − 38 (*c* 0.423, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ): 214 (5.2) nm; IR ($CHCl_3$) ν_{max} (cm^{-1}): 1763, 1683, 1510, 870; EIMS *m/z* (rel. int.) 314 $[M]^+$(88), 299 (60), 298 (50), 271 (34), 219 (55), 201 (25), 173 (60), 95 (80), 94 (18), 81 (100); HREIMS *m/z* 314.1893 $[M]^+$ (calcd for $C_{20}H_{26}O_3$, 314.1882). ^1H and ^{13}C NMR data, see table 1.

3.3.2 Limbatolide E (2). Gummy solid, $[\alpha]_D^{25}$ − 60 (*c* 0.040, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ): 210 (4.1) nm; IR ($CHCl_3$) ν_{max} (cm^{-1}): 3460, 1755, 1680, 1506, 875; EIMS *m/z* (rel. int.) 330 $[M]^+$(70), 314 (40), 312 (35), 299 (40), 219 (15), 201 (28), 173 (50), 95 (75), 94 (60), 81 (100). HREIMS *m/z* 330.1840 $[M]^+$ (calcd for $C_{20}H_{26}O_4$, 330.1831); ^1H and ^{13}C NMR data, see table 1.

Table 1. NMR spectral data of **1** and **2**.

	1		***2***	
No.	δ_C	δ_H *(J, Hz)*	δ_C	δ_H *(J, Hz)*
1	18.2	1.50 m, 1.78 m	30.5	1.80 m, 2.60 m
2	27.1	2.20 m, 2.35 m	72.3	4.28 m
3	132.6	6.90 dd (3.2, 4.9)	125.6	6.80 br. s
4	140.5	––	139.6	––
5	40.6	––	42.3	––
6	82.4	3.75 dd (4.1, 9.6)	84.5	3.68 dd (3.5, 9.8)
7	32.6	1.80 m	33.1	1.92 m
8	39.1	1.92 m	40.5	1.70 m
9	41.3	––	44.3	––
10	45.1	1.46 br. d (11.5)	46.7	1.50 br. d (10.9)
11	38.2	1.50 m, 2.05 m	37.1	1.40–1.50 m
12	18.4	2.20 m, 2.30 m	20.1	2.28 m, 2.35 m
13	131.5	––	130.1	––
14	111.3	6.30 dd (0.8, 1.8)	111.7	6.25 dd (0.8, 1.8)
15	143.4	7.35 t (1.8)	143.6	7.37 t (1.7)
16	139.7	7.31 br. s	140.2	7.34 br. s
17	16.6	0.85 d (7.4)	15.8	0.80 d (7.8)
18	174.1	––	172.1	––
19	18.1	1.20 s	19.3	1.16 s
20	19.3	0.75 s	19.8	0.76 s

[a] 100 and 400 MHz for ^{13}C and ^1H NMR ($CDCl_3$), respectively.

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