A New Ketone and a Known Anticancer Triterpenoid from the Leaves of *Onosma limitaneum*

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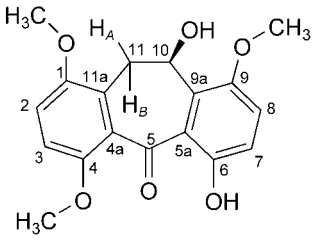
A new ketone, onosmone (**1**), and a known anticancer triterpenoid, bauerenone, besides β-sitosterol glycoside were isolated for the first time from the $CHCl_3$ extract of the leaves of *Onosma limitaneum*. The structures of these compounds were established on the basis of spectral and chemical evidence.

Introduction. – *Onosma* is a genus of hairy herbs and under-shrubs distributed in the Mediterranean region and Central Asia. About nine species occur in Pakistan. The dried leaves and flowers of *O. bracteatum* constitute the drug Gazoban, imported from Iran, which is used as tonic, demulcent, diuretic, and refrigerant. It is reported to be useful as spasmolytic. *O. hispidum* has been reported to be the source of Ratanjot, a red dye used for coloring food stuffs, oils, and medicinal preparations. The flowers of this plant are used as stimulant and cardiac tonic [1]. A deep red oil with molecular formula $C_{17}H_{18}O_5$ and an orange oil with molecular formula $C_{22}H_{24}O_6$ were isolated from this genus [2].

Results and Discussion. – From the $CHCl_3$ extract of *O. limitaneum,* a new compound, onosmone (**1**), was isolated along with the known anti-cancer agent bauerenone [3], β-sitosterol, and β-sitosterol glycoside. These compounds were isolated for the first time from this plant.



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Compound **1** was isolated as white powder. A molecular formula $C_{18}H_{18}O_6$ was deduced from the HR-FAB-MS data. FAB-MS: $[M-1]^-$ at *m/z* 331). The UV maxima at 222, 288, and 310 nm suggested a considerable conjugation in the molecule. In the IR spectrum, absorption bands at 3330 (OH), 1575, and 1577 cm^{-1} (aromatic ring) were

apparent. Detailed analysis of the NMR data (*Table*) established the structure of (10*R*)-10,11-dihydro-6,10-dihydroxy-1,4,9-trimethoxy-5*H*-dibenzo[*a*,*d*]cyclohepten-5-one for **1**.

Table 1. *^{13}C-* (100 Mz) *and ^{1}H-NMR* (400 Mz) *Data* ($CDCl_3$) *of Compound* **1**. Assignments by COSY, HMQC, HMBC, and DEPT (135°) experiments. δ in ppm, J in Hz.

	δ(H)	δ(C)
C(1)	1.00	150.1
*Me*O–C(1)	3.98	55.9
*Me*O–C(4)	3.95	55.6
*Me*O–C(9)	3.89	55.8
H–C(2)	7.09 (*dd*, J = 8.9, 2.3)	118.4
H–C(3)	6.91(*dd*, J = 6.4, 2.3)	111.9
C(4)	–	162.8
C(4a)	–	130.8
C(5)	–	195.9
C(5a)	–	149.3
C(6)	–	167.9
H–C(7)	6.05(*dd*, J = 6.4, 2.3)	118.1
H–C(8)	6.89 (*dd*, J = 6.4, 2.3)	110.8
C(9)	–	164.1
C(9a)	–	149.5
H–C(10)	5.36 (*dd*, J = 13, 3)	79.23
C(11a)		144.1
H_A–C(11)	2.91 (*dd*, J = 13, 17)	–
H_B–C(11)	3.12 (*dd*, J = 17, 13)	–

The ^{1}H-NMR spectrum of compound **1** showed three downfield *singlets*, which were assigned to the MeO groups at C(1), C(4), and C(9) (*Table*). These 3 MeO groups were attached to aromatic rings. The aromatic protons H–C(2) at δ 7.09 (*dd*, J = 8.9, 2.3 Hz) and H–C(3) at δ 6.91 (*dd*, J = 6.4, 2.3 Hz) showed *ortho* coupling with each other. The aromatic protons H–C(8) at δ 6.89 (*dd*, J = 6.4, 2.3 Hz) and H–C(7) at δ 6.05 (*dd*, J = 6.4, 2.3 Hz) also showed *ortho*-coupling with each other. The geminal protons CH_2(11) exhibited each a *dd* at δ 2.91 (J = 17.0, 13.0 Hz) and 3.12 (J = 17.0, 3.0 Hz), respectively, and H–C(10), bearing the OH group, showed a *dd* at δ 5.36 (*dd*, J = 13.0, 3.0 Hz). The larger coupling constants between H_A–C(11) and H–C(10) indicated that they have diaxial orientation; therefore, the OH group is equatorially positioned.

The ^{13}C-NMR spectrum showed the aromatic atoms C(1), C(2), and C(3), at δ 150.1 (C), 118.4 (CH), and 111.9 (CH), respectivley, besides C(4a) and C(11a) at δ 130.8 (C) and 144.1 (C), respectively. The ^{13}C-NMR signals of the other aromatic ring, *i.e.*, of C(9), C(8), and C(7) appeared at δ (C) 164.1(C), 110.8 (CH), and 118.1 (CH), respectively, besides C(9a) and C(5a) at δ 149.5 (C) and 149.3 (C), respectively. The downfield signal at δ 195.9 was due to the oxo-substituted C(5) at the seven-membered ring. The three MeO groups showed peaks at δ(C) 55.6, 55.8, and 55.9, corresponding to the three downfield *singlets* at δ(H) 3.98, 3.95, and 3.89 of MeO–C(1), MeO–C(4), and MeO–C(9) in ^{1}H-NMR spectrum. The OH group at C(6) showed a broad peak at δ 11.9 due to H-bonding with the carbonyl group. All these correlations were confirmed by NOESY and HMBC experiments (*Figure*).

Experimental Part

General. Column chromatography (CC): silica gel, 70–230 mesh. Flash chromatography (FC): silica gel; 230–400 mesh. TLC: pre-coated silica gel *G-25-UV_{254}* plates; detection at 254 nm, and by ceric sulfate and *Kedde* reagent. Optical rotations: *Jasco DIP-360* digital polarimeter. UV and IR Spectra: *Hitachi UV-3200* and *Jasco-320-A* spectrophotometer, resp., ^{1}H- and ^{13}C-NMR, COSY, HMQC, and HMBC: *Bruker* spectrometers

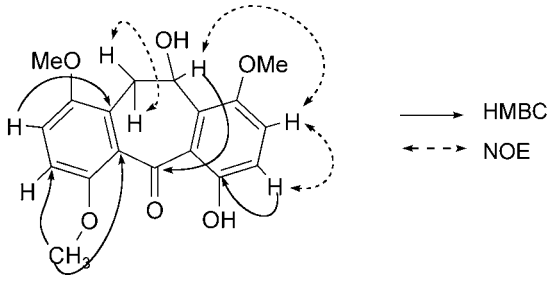

Figure. *Selected HMBC and NOE interactions in compound* **1**

operating at 500 and 400 MHz; chemical shifts δ in ppm and coupling constants J in Hz. EI CI-MS: *JMS HX-110 spectrometer* with a data acquisition system.

Plant Material. The plant *O. limitaneum* (Boraginaceae) was collected from Ziarat, Balochistan, Pakistan, in August 2002, and identified by one of us, *Rasool Bakhsh Tareen*. A voucher specimen (# 1510) has been deposited in the herbarium of Botany Department, Balochistan University, Quetta, Pakistan.

Extraction and Isolation. The air-dried ground plant (50 kg) was extracted with MeOH at r.t. After evaporation the crude MeOH extract (900 g) was again extracted with hexane, $CHCl_3$, and BuOH. The $CHCl_3$ extract (100 g) was subjected to CC (silica gel, gradient 0–100% $CHCl_3$/hexane): *Fractions 1–50*. *Fr. 20* (5 spots) was subjected to FC (silica gel, $CHCl_3$/hexane 5:95): pure **1**. *Fr. 10* (3 spots), obtained with $CHCl_3$/hexane 75:5, was subjected to FC (silica gel, $CHCl_3$/hexane 80:20). From this solvent, onosmone (**1**) was obtained as a white powder.

*Onosmone (=(10*R*)-10,11-Dihydro-6,10-dihydroxy-1,4,9-trimethoxy-5*H*-dibenzo[*a,d*]cyclohepten-5-one;* **1**): Amorphous white powder: TLC: red to orange spot with ceric sulfate. M.p. 172°, $[\alpha]_D^{25} = +160$ ($c = 0.01$, $CHCl_3$). UV ($CHCl_3$): 288.6 (5.17). ^{1}H- and ^{13}C-NMR: *Table*.

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