New Antiproliferative Tricyclic Sesquiterpenoid from the Leaves of *Ocimum sanctum*

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A new tricyclic sesquiterpenoid 2-(hydroxymethyl)-5,5,9-trimethyltricyclo[7.2.0.0^{3,6}]undecan-2-ol (**1**), was identified from the leaves of *Ocimum sanctum*, and its structure was elucidated by extensive NMR and ESI-MS analyses. Compound **1** was found to have potent antiproliferative activity against the MCF-7 cell line.

Introduction. – *Ocimum sanctum* (Lamiaceae family) commonly known as *Tulsi* in India is an annual herb distributed throughout the Indian subcontinent and is well-recognized in the Ayurvedic system of medicine [1]. *O. sanctum* is reported to possess antioxidant, anti-inflammatory, and antidiabetic properties [2]. The essential oil of *O. sanctum* is primarily composed of monoterpenoids and sesquiterpenoids and reported to exhibit antipyretic, analgesic, antiarthritic, and anti-inflammatory activities [3]. In the present study, the oil of *O. sanctum*, obtained from hexane-soluable fraction, was purified by fractional distillation to yield a colorless oil and a low-melting solid residue. The solid residue on further purification, gave a viscous compound which was identified as 2-(hydroxymethyl)-5,5,9-trimethyltricyclo[7.2.0.0^{3,6}]undecan-2-ol (**1**; *Fig.*) by an extensive 1D- and 2D-NMR study. Compound **1** showed potent antiproliferative activity against MCF-7 cells in the MTT (= 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) assay.

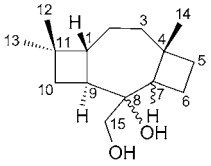

Figure. *Structure of compound* **1**. Arbitrary atom numbering following caryophyllene.

Result and Discussion. – Compound **1** was isolated as a yellow viscous solid and found to be homogeneous on TLC with different solvents. Compound **1** showed a single peak with retention time (t_R) of 38.20 min in GLC and a pseudomolecular ion peak at *m*/*z* 277.2 ([M + K]$^+$) and 515.4 ([2M + K]$^+$) in its ESI-MS, corresponding to the molecular formula $C_{15}H_{26}O_2$, with M^+ at *m*/*z* 238. The IR spectrum (KBr) of **1** showed

absorption bands for OH groups (3405 cm^{-1}) and C–H stretchings (2933 cm^{-1}). In its ^{1}H-NMR spectrum (*Table*), three Me groups appeared as *singlet*s at δ(H) 0.91, 0.99, and 1.03 ppm which were further corroborated by their chemical shift values at δ(C) 20.87, 28.61, and 33.87, respectively, in its ^{13}C-NMR spectrum. In addition, one broad *singlet* at δ(H) 3.48 indicated the presence of a $HOCH_2$ group in compound **1**. The ^{13}C-NMR spectrum along with DEPT-135 showed 15 C-atom signals including those of three tertiary Me, six CH_2, three CH groups, and three quaternary C-atoms, together with the signals of an O-bearing quaternary C-atom at δ(C) 71.10 and of a $HOCH_2$ group at δ(C) 72.60. Hence, compound **1** is a hydroxylated sesquiterpenoid. Further, ^{13}C-NMR spectrum corroborated its molecular formula of $C_{15}H_{26}O_2$ and the degree of unsaturation, indicating its tricyclic nature without a C=C bond. The correlation of a ^{13}C-NMR signal at δ(C) 72.60 with a 2-H *singlet* at δ(H) 3.48 in its HSQC confirmed the presence of a $HOCH_2$ group attached to an adjacent quaternary C-atom. The signals of CH_2 and CH H-atoms of compound **1** gave rise, in the region δ(H) 1.38–2.22, to *multiplet*s, which were similar to the characteristic signals of a caryophyllene derivative with an additional primary OH group (δ(H) 3.48 (br. *s*)) and a tertiary OH group (δ(C) 71.10). The signals at δ(C) 42.90 and 35.87 were assigned to C(1) and C(9) of compound **1**, respectively, and were shifted upfield compared to those of β-caryophyllene (δ(C) 53.6 (C(1)); 48.5 (C(9))) due to the presence of a seven-membered ring formed by C(4),C(7) bond formation [4]. The nine-membered ring of β-caryophyllene is considerably strained and well-known for its susceptibility to undergo rearrangements by ring contraction, forming more stable bi- and tricyclic systems [5]. The C-atom connectivities of the cyclobutane rings were deduced from HMBCs of C(2) with H–C(1), of C(3) with H–C(2), of C(3) with the angular Me(14), and of Me(14) with H–C(5), and evidenced the presence of a tricyclic system. Further connectivities of C(8) and C(7), and C(3) and C(4) in the HMBC, along with a DEPT-135 study, confirmed the ring junction across C(4)/C(7) of the β-caryophyllene skeleton

Table. *^{1}H- and ^{13}C-NMR* (300 and 75 MHz, resp.; in $CDCl_3$). *Data of* **1**. δ in ppm. Arbitrary atom numbering as indicated in the *Figure*.

Position	δ(H)	δ(C)	DEPT-135
1	1.40–1.50 (*m*, 1 H)	42.90	CH
2	1.36–1.40 (*m*, 1 H), 1.50–1.60 (*m*, 1 H)	21.21	CH_2
3	1.61–1.69 (*m*, 2 H)	27.03	CH_2
4	–	39.74	C_q
5	1.83–1.90 (*m*, 2 H)	44.39	CH_2
6	2.14–2.19 (*m*, 2 H)	30.92	CH_2
7	1.45–1.55(*m*, 1 H)	34.46	CH
8	–	71.10	C_q
9	1.40–1.45 (*m*, 1 H)	35.87	CH
10	2.17–2.25 (*m*, 2 H)	38.59	CH_2
11	–	35.47	C_q
12	0.99 (*s*, 3 H)	20.87	Me
13	0.91 (*s*, 3 H)	28.61	Me
14	1.03 (*s*, 3 H)	33.87	Me
15	3.48 (br. *s*, 2 H)	72.60	CH_2

[7.2.0]. The structure of compound **1** was, therefore, deduced as 2-(hydroxymethyl)-5,5,9-trimethyltricyclo[7.2.0.0^{3,6}]undecan-2-ol (*Fig.*) from its HMBC and HSQC spectra.

Compound **1** appeared to be formed biogenetically from β-caryophyllene by ring closure of C(4)/C(7) with hydroxylation at the C(8)=C(15) bond. The configuration at the C(4)/C(7) ring juncture was determined as *cisoid* from its conformation study through *Dreiding* models. A tricyclic compound with *cisoid*-configuration at C(7)/C(8) of cyclobutane ring was already reported [6]. The present report on the isolation and identification of compound **1** documents its first natural occurrence to the best of our knowledge.

Compound **1** showed potent antiproliferative activity against MCF-7 (30 ± 0.5 μM) cell line in MTT assay. The other known compounds identified are β-caryophyllene, elemene, α-humulene, α-caryophyllene, germacrene-A, and *trans*-α-bergamotene, along with 5β-hydroxycaryophyllene identified for the first time in *O. sanctum*.

Experimental Part

General. TLC: Precoated silica gel *60 F_{254}* plates (SiO_2; *Merck*, Germany). Flash chromatography (FC): silica gel (SiO_2, 230–400 mesh; *SRL*, India). Optical rotation: *HORIBA*, *SEPA-300* polarimeter. IR Spectra: *FT-IR PerkinElmer spectrum BX* in KBr pellets; $\tilde{\nu}$ in cm^{-1}. ^{1}H- and ^{13}C-NMR ($CDCl_3$): *Bruker AV-400* spectrometer at 300 (^{1}H) and 75 MHz (^{13}C); δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. GC/MS: *PerkinElmer Turbomass*; autosystem *XLGC*. ESI-MS: *API 3000*, *Applied Biosystems* (Canada) Mass spectrometer; in *m*/*z* (rel. %). Elemental analysis: *Vario EL III*.

Plant Material. The leaves of *Ocimum sanctum* cultivated at CIMAP farm, Lucknow, India, were collected during October, 2006 and shade-dried.

Extraction and Isolation. The shade-dried leaves of *O. sanctum* (5 kg) were extracted with MeOH, and the MeOH extract was concentrated and partitioned with hexane. Hexane extract was chromatographed over SiO_2 (100–200 mesh) followed by FC to give an oil, which was purified further by fractional distillation to yield colorless oil and a pale-yellow low-melting solid residue under low pressure. The low-melting solid residue on repeated chromatography over SiO_2 led to the identification of a new tricyclic sesquiterpenoid **1** on the basis of NMR and MS studies. The known compounds β-caryophyllene, elemene, α-humulene, α-caryophyllene, germacrene A, *trans*-α-bergamotene, and 5β-hydroxycaryophyllene were identified by NMR, GLC, and GC/MS.

(3S,6R,9R)-2-(Hydroxymethyl)-5,5,9-trimethyltricyclo[7.2.0.0^{3,6}]undecan-2-ol (**1**). Yield: 0.0003%. Yellow viscous solid. $[\alpha]_D = +0.36$ ($c = 0.2$, MeOH). IR (KBr): 3405, 2933, 1709, 1457, 1379, 1255, 1084, 559. ^{1}H- and ^{13}C-NMR: see the *Table*. ESI-MS (pos.): 238 (M^+, $C_{15}H_{26}O_2^+$), 277.2 ($[M+K]^+$). Anal. calc. for $C_{15}H_{26}O_2$: C 75.63, H 10.92; found: C 75.58, H 10.98.

Bioassay. The IC_{50} values of compound **1** was determined by MTT assay as described by *Woerdenbag et al.* [7]. 2×10^3 cells/well were incubated in the 5% CO_2 incubator for 24 h to enable them to adhere properly to the 96-well polystyrene microplates (*Grenier*, Germany). Test compound dissolved in 100% DMSO was added at least in 5 doses and left for 6 h, after which the compound plus media was replaced with fresh media and the cells were incubated for another 48 h in the CO_2 incubator at 37°. Then, 10 μl of MTT was added, and plates were incubated at 37° for 4 h. DMSO (100 μl) was added to all wells and mixed thoroughly to dissolve the dark-blue crystals. After a few min at r.t., to ensure that all crystals were dissolved, the plates were read on a *Spectra Max 190 microplate ELISA* reader (*Molecular Devices Inc.*, USA) at 570 nm. Plates were normally read within 1 h after adding the DMSO. The IC_{50} value is the concentration [μM] required for 50% inhibition of cell growth as compared to that of untreated control. The results were expressed as mean ± SD of triplicate determinations from a representative experiment. Doxorubicin was used as the positive control.

The authors are grateful to the Director, *CIMAP*, for his encouragement, and *Deepika Singh* is grateful to the Director for a fellowship. This work is a part of *PINNGP* project (CSIR-TFYP) during 2002-07.

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Received July 4, 2013