Antifungal Ursene-Type Triterpene from the Roots of *Alhagi camelorum*

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A new ursene-type triterpene, (3β)-30-norurs-11-en-3-ol (**1**), was isolated from the MeOH extract of the roots of *Alhagi camelorum*. Its structure was elucidated on the basis of extensive spectroscopic investigations by 1D- and 2D-NMR studies. In addition, **1** was evaluated for antifungal activities. Its application on prepared cultures of two fungi, *i.e.*, *Aspergillus niger* and *Saccharomyces cerevisiae*, showed noticeable antifungal activity, suggesting that **1** could be used as a new promising agent in antifungal drug formulations.

1. Introduction. – The choice of plants as a source of drugs in the form of bioactive constituents has been in practice since the ancient times of civilizations [1]. Secondary metabolites are also included in plant constituents, which have had a wide contribution in this regard. Terpenes belong to those secondary metabolites, which have shown a variety of actions against different ailments, including antifungal activity [2]. It is a matter of fact that no drug is free from adverse effects and certain other limitations as well. Consequently, it is believed that plants are capable of curing diseases but simultaneously, they contain many useless substances, which may prove harmful in certain cases. Hence, there has been felt a great need for separation and isolation of new drugs from plants for making new drug formulations [3][4].

Among a large variety of biomedicinal plants in the Sindh region of Pakistan, *A. camelorum* has largely been used in folk medicine as purgative, laxative, diaphoretic, expectorant, and diuretic drug. Besides, its flowers are used to treat piles, migraine, and warts, and the oil obtained from the leaves is used in the treatment of rheumatism [5][6]. Water extracts of roots have also been used to enlarge the ureter and to remove kidney stones [7]. Isolation of bioactive constituents from the genus *Alhagi* has been carried out by various research groups [8–10]. Nevertheless, to the best of our knowledge, no antifungal constituent has been reported from *A. camelorum* to date. Thus, for the first time herein, we report the isolation of a new, antifungal ursene-type triterpene from *A. camelorum* (*Fig. 1*). Its structure **1** was elucidated with the help of different analytical and spectroscopic techniques including FT-IR, NMR, and MS. The antifungal activity of this compound was also addressed.

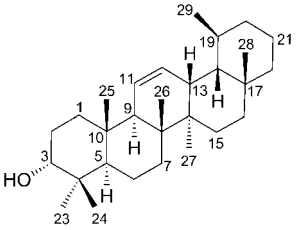

Fig. 1. *Structure of (3β)-30-norurs-11-en-3-ol* (**1**), *isolated from* Alhagi camelorum

2. Experimental. – 2.1. *General.* All of the reagents used were purchased from *Merck* (Darmstadt, Germany) and used as supplied. TLC: pre-coated silica-gel plates (SiO_2, PF_{254}; *Merck*). Column chromatography (CC): silica gel (70–230 mesh; *Merck*). M.p.: *Gallenkamp* apparatus (UK) in a sealed glass cap. tube; uncorrectd. FT-IR Spectra: *Thermo-Nicolet-Avatar-5700* FT-IR spectrometer; spectral range 4000–400 cm^{-1}; $\tilde{\nu}$ in cm^{-1}. ^{1}H- and ^{13}C-NMR Spectra: *Bruker-Avance-400* spectrometer; in $CDCl_3$ at r.t.; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. EI-MS: *Thermo-Finnigan-LCQ spectrometer*; in *m*/*z* (rel. %).

2.2. *Plant Material.* The roots of *A. camelorum* were collected in February 2010 at Malkani, a small town in the District Badin (Sindh Province, Pakistan). This plant was identified by Prof. Dr. *Tahir Rajpoot*, former Director of the Institute of Botany, University of Sindh, Jamshoro, Pakistan, where a voucher specimen has been deposited with the herbarium (*Alhagi camelorum* 15460).

2.3. *Extraction and Isolation.* Dried root barks (100 g; coarsely powdered and shade dried) were dipped in MeOH (2 l) for a period of one week three times. The resulting combined extract was filtered and concentrated. The viscous red brown residue (21 g) was dissolved in hexane (1.5 l) and filtered. The undissolved part was again triturated in hexane and shaken vigorously. The combined hexane fraction was concentrated to afford an oily fraction (3.5 g), which was subjected to CC (flash grade SiO_2, column 1×15 inch, gradient hexane/AcOEt with gradual increase of AcOEt). With 15% of AcOEt in the mobile phase, a fraction was obtained containing a single component, *i.e.*, **1**, as seen in TLC (AcOEt/hexane 2 :3, detection as a dark violet spot by spraying with 1% $Ce(SO_4)_2$ in 10% aq. soln. of H_2SO_4).

2.4. *Data of (3β)-30-Norus-11-en-3-ol* (**1**). Off-white amorphous solid. M.p. 210°. IR (KBr): 3300, 2849, 2919, 1457, 1609, 3310, 1620, 830. NMR: *Table 1*. Anal. calc. for $C_{29}H_{48}O$ (412.71): C 84.40, H 11.72; found: C 84.37, H 11.69.

2.5. *Antifungal Activity.* The antifungal activity of **1** against two selected fungi, *A. niger* and *S. cerevisiae*, was determined following previously reported assay methods, *i.e.*, the well-diffusion-plate and streak-plate methods [11].

2.6. *Well-Diffusion-Plate Method*. In a test tube already containing 3 ml of dist. H_2O, fungi *A. niger* or *S. cerevisiae* were inoculated by using a flamed loop. Few drops of fungus and water culture were mixed with warm, melted, autoclaved PDA (potatoe dextrose agar) and poured into separate *Petri* dishes under aseptic conditions. The *Petri* dishes were covered and allowed to cool. As soon as the agar was partially solidified, the *Petri* dishes were inverted and left for 2 h. After cooling, a well was made at the centre of the *Petri* dishes by using a 6-mm cork borer that was sterilized with alcohol and flame. Compound **1** was dissolved in H_2O or MeOH at a final concentration of 5 mg/20 ml and pipetted into the wells in a sterilized environment at different volumes (0.1, 0.3, and 0.6 ml) in separate *Petri* dishes by means of a microliter syringe. Both neat solvents (H_2O and MeOH) were used as control at different volumes. The plates were labeled, covered, inverted, and placed in a fume hood for 48 h. Finally, the zone was checked for activity as shown in *Fig. 2*.

2.7. *Streak-Plate Method.* The nutrient agar (10 ml) was poured into *Petri* dishes. Compound **1** dissolved in H_2O or MeOH at a final concentration of 5 mg/20 ml was transferred into three sterilized plates under aseptic conditions at different volumes (0.1, 0.3, and 0.6 ml) by means of a micropipette. The *Petri* dishes were allowed to cool, and then the fungi were streaked onto the surface of the solidified soln. of agar and compound **1**. A flame loop was applied to inoculate the bacteria from their respective cultures. The *Petri* dishes were kept in a desiccator for 24 h. The *Petri* dishes with inhibition were used for

further experiments. Controls were also made by using neat solvents, *i.e.*, MeOH or H_2O, at the different volumes. The compound-applied areas were checked for activity (*Fig. 3*).

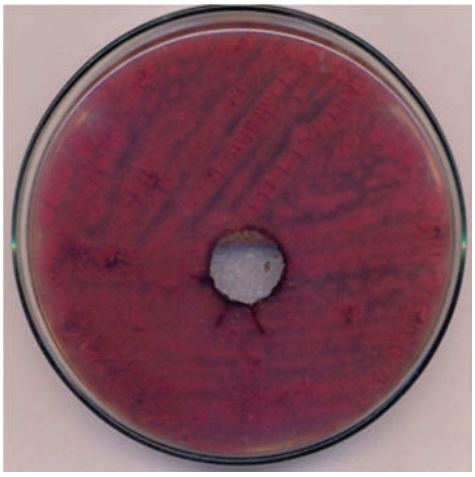

Fig. 2. *Representative plate of the antifungal activity of* ***1*** *obtained by the well-diffusion-plate method* (0.6 ml of a soln. of **1** (5 mg/20 ml) against fungal species)

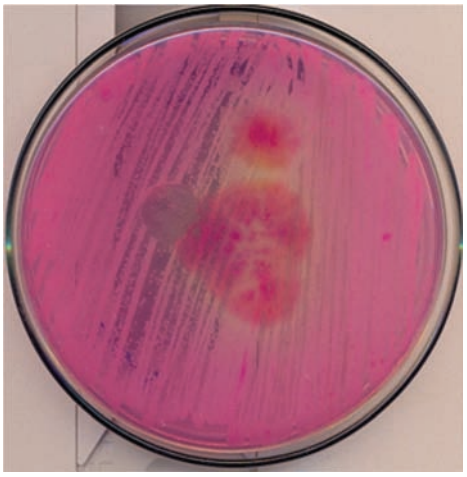

Fig. 3. *Representative plate of the antifungal activity of* **1** *obtained by the streak-plate method* (0.6 ml of a soln. of **1** (5 mg/20 ml) against fungal species)

3. Results and Discussion. – *Characterization of Compound* **1**. Compound **1**, isolated from the roots of *A. camelorum* as an off-white amorphous solid, was soluble in MeOH and H_2O. It gave a dark violet color on TLC when sprayed with $Ce(SO_4)_2$ solution which was the first clue to its triterpene nature [12]. The molecular formula of **1** as $C_{29}H_{48}O$ was supported by the observation of the molecular-ion peak at m/z 412 in its EI-MS and its elemental analysis. Besides this, the formula and its structure were further confirmed by the fragmentation pattern in the EI-MS. The IR spectrum of **1** showed bands at 3310 cm^{-1} for an OH group, at 2919 cm^{-1} for an aliphatic C–H stretching, and at 1457 and 1609 cm^{-1} for C=C bending. Moreover, the basic skeleton of **1** was found to resemble a previously reported ursene-type triterpene [13]. Therefore, the NMR data of **1** (*Table 1*) were compared with those of previously reported triterpene analogues [13][14] and found to agree, apart from some minor differences. Of those, a *t* at δ(H) 2.30 for H–C(18) was evidence for a C=C bond at C(11) as discussed by *Ahmed* and co-workers [14], not at C(12) as previously reported by *Malik* and co-workers [13] for different analogues. The ^{13}C-NMR spectrum showed 29 C-atoms for a nortriterpene molecule, *i.e.*, seven Me, nine CH_2, and eight CH groups, and five tertiary C-atoms. Only one (C(3)) of the 29 C-atoms was confirmed to be attached to an O-atom, since its signal was observed at d(C) 79.0 in the ^{13}C-NMR spectrum. The absence of any other electronegative atom in **1** was further confirmed by the appearance of all NMR signals at high field except for those of the C=C bond that appeared at δ(H) 5.14 (*dd*, $J = 11.5$ Hz), H–C(11), and 5.00–5.3 (*m*, H–C(12)) and δ(C) 119 (C(11)) and 121 (C(12)). The spatial dispositions of all Me groups, H-atoms,

Table 1. *^{1}H- and ^{13}C-NMR Data* ($CDCl_3$) *of* **1**. δ in ppm, J in Hz.

Position	δ(H)	δ(C)	Position	δ(H)	δ(C)
CH_2(1)	1.3–1.55 (*m*)	36.1	CH_2(16)	1.3–1.55 (*m*)	28.2
CH_2(2)	1.81–1.83 (*m*)	26.1	C(17)	–	33.9
H–C(3)	3.18–3.21 (*m*)	79.0	H–C(18)	2.30 (*t*, J = 8.5)	51.2
C(4)	–	37.1	H–C(19)	1.3–1.55 (*m*)	31.6
H–C(5)	0.82–0.85 (*m*)	55.9	CH_2(20)	1.08–1.2 (*m*)	39.7
CH_2(6)	1.3–1.55 (*m*)	18.9	CH_2(21)	1.3–1.55 (*m*)	25.4
CH_2(7)	1.3–1.55 (*m*)	31.6	CH_2(22)	1.3–1.55 (*m*)	24.7
C(8)	–	39.6	Me(23)	0.66 (*s*)	28.2
H–C(9)	1.3–1.55 (*m*)	45.8	Me(24)	0.76 (*s*)	11.8
C(10)	–	36.1	Me(25)	0.78 (*s*)	12.0
H–C(11)	5.14 (*dd*, J = 11.5)	119	Me(26)	0.81 (*s*)	12.2
H–C(12)	5.00–5.3 (*m*)	121	Me(27)	0.86 (*s*)	22.6
H–C(13)	2.40 (*dd*, J = 8.5)	50.1	Me(28)	0.80 (*s*)	24.3
H–C(14)	–	40.4	Me(29)	1.01 (*s*)	28.2
CH_2(15)	1.3–1.55 (*m*)	29.3			

and the OH group were confirmed by 2D-NMR techniques, including HMBC (*Fig. 4*) and NOESY (*Fig. 5*). The HMBC experiment revealed that H–C(11) at δ(H) 5.14 correlated with C-atoms at δ(C) 45.8 (C(9)), 121 (C(12)), 36.1 (C(10)), and 50.1 (C(13)). Correlations of H–C(12) at δ(H) 5.00–5.3 with C-atoms at δ(C) 45.8 (C(9)), 119 (C(11)), 50.1 (C(13)), 40.4 (C(14)), and 51.2 (C(18)) were also observed. Besides this, the NOESY correlations supported the proposed structure of **1** as (3β)-30-norurs-11-en-3-ol.

Antifungal-Activity Assay of **1**. *Table 2* shows the antifungal activities of different volumes of solutions of **1** (concentration, 5 mg/20 ml) and of controls, obtained by the

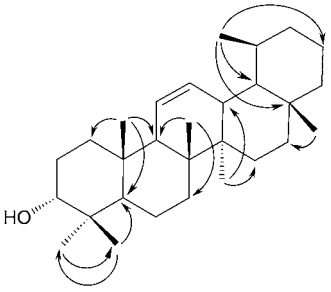

Fig. 4. *HMBCs* (H → C) *of compound* **1**

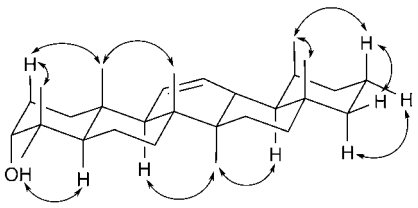

Fig. 5. *NOESY Correlations* (H ↔ H) *of compound* **1**

Table 2. *Antifungal-Activity Assay of* **1** *by the Streak-Plate Method*

Solvent	Solution of **1**[a]) [ml]	Inhibition or no growth of *A. niger*[b])	Inhibition or no growth of *S. cerevisiae*[b])
MeOH	0.0		
MeOH	0.2	–	–
MeOH	0.3	–	–
MeOH	0.6	+	+
H_2O	0.0	–	–
H_2O	0.2	+	–
H_2O	0.3	+	–
H_2O	0.6	+	–

[a]) Concentration: 5 mg of **1** in 20 ml of solvent. [b]) Sign +, inhibition or no growth; sign –, no inhibition or growth.

streak-plate method applied to the fungi *Aspergillus niger* (ATCC 16404) and *Saccharomyces cerevisiae* (ATCC 9763) (see also *Figs. 2* and *3*).

4. Conclusions. – A new compound, norursenol **1**, was isolated from the roots of *Alhagi camelorum* (Fabaceae). Spectroscopic evidences confirmed its structure to be (3β)-30-norurs-11-en-3-ol. From the experimental observations, it was established that this compound possesses remarkable antifungal activities.

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