Pak. J. Sci. Ind. Res. 2005 **48**(3) 180-183

Phytochemical Studies on *Adhatoda vasica*

Nighat Sultana*, Muhammad Aijaz Anwar, Yousuf Ali and Nighat Afza
Pharmaceutical Research Centre, PCSIR Laboratories Complex, Karachi, Pakistan

(received October 14, 2004; revised April 5, 2005; accepted April 12, 2005)

Abstract. Reinvestigation of the aerial parts of *Adhatoda vasica* led to the isolation of a new triterpenoid, 3α-hydroxy-oleanane-5-ene (**1**). The structure of this compound was elucidated and identified by spectral studies.

Keywords: triterpene, Acanthaceae, *Adhatoda vasica*, 3α-hydroxy-oleanane-5-ene

Introduction

Adhatoda vasica, one of the two Indian species of the genus *Adhatoda* (Family: Acanthaceae), is well known for its anthelmintic and herbicidal properties (Ikram *et al.,* 1965). Its extracts have been used against various chest ailments, while a number of bioactive constituents have been isolated from this plant (Mehta *et al.,* 1963). A new triterpenoid, 3α-hydroxy-oleanane-5-ene **(1)** has now been isolated from the aerial parts of this plant, along with a known triterpenoid, taraxerone (**2**), which are reported here.

Materials and Methods

General experimental procedures. Optical rotations were measured on JASCO DIP-360. IR spectra were recorded on JASCO 302. UV spectra were recorded on a Hitachi U 3200 spectrophotometer. EIMS, FABMS and HREIMS were recorded on JMS HX 110 with data system DA 5000 and on MAT 112S mass spectrometers. The ^{1}H- and ^{13}C-NMR spectra were recorded on Bruker 400 MHz and 500 NMR spectrometers.

Plant material. *Adhatoda vasica* (whole plant; 200 kg) was collected from the suburban areas of Karachi, Pakistan, in December. The plant was identified by Mr. Tahir Ali, Plant Taxonomist, Department of Botany, University of Karachi, Pakistan and a voucher specimen (KUH number 53882) was deposited in the herbarium of the department.

Extraction and fractionation. The EtOH extract of the whole plant of *A. vasica* was concentrated to a gum (8 kg). This gum was dissolved in distilled H_2O. The aqueous extract was first extracted with pet ether (40-60 °C) and then with $CHCl_3$. The pet ether extract (168.21 g) was loaded on a silica gel column and eluted first with pet ether and then with pet ether-acetone mixtures. Several fractions were obtained. The identical fractions were combined into larger fractions 1-12. Compound **1** was isolated from these fractions.

*Author for correspondence

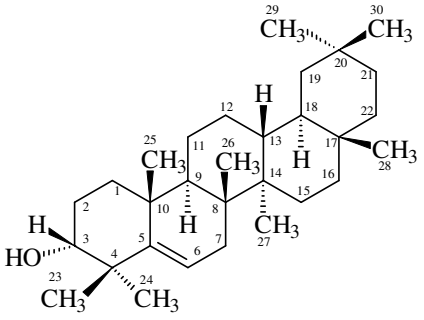
3α-Hydroxy-oleanane-5-ene (1). The new compound **1** isolated from *A. vasica* was 19.5 mg (yield, 9.75x10^{-6} %): white crystals $[a]^{23}_{D} = 24^{o}$ ($c = 0.05$, $CHCl_3$ + MeOH); EIMS *m/z* 426 (M^+, 41%), 274 (20%), 259 (16%), 205 (8%), 134 (18%), 83 (100%); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 0.83 (3H, s, Me-25), 0.94 (3H, s, Me-30), 1.12 (3H, s, Me-24), 1.15 (3H, s, Me-28), 1.85 (1H, ddd, $J_{2\alpha, 1\alpha} = 6.9$, $J_{2\alpha, 1\beta} = 4.8$, $J_{2\alpha, 3\beta} = 2.2$ Hz, H-2a), 2.00 (1H, ddd, $J_{2\beta, 1\beta} = 6.0$, $J_{2\beta, 1\beta} = 4.2$, $J_{2\beta, 1\alpha} = 1.9$, H-2b), 1.82 (1H, m; H-7α), 1.95 (1H, m; H-7β), 3.45 (1H, dd, $J_{3\beta, 2\beta} = 3.3$ Hz, $J_{3\beta, 2\alpha} = 2.4$ Hz; H-3), 5.62 (1H, dd, $J_{6\alpha, 7\alpha} = 4.0$ Hz, $J_{6\alpha, 7\beta} = 2.4$ Hz; H-6); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ (Table 1).

Fractions 30-60 (5.0 g) were eluted with pet ether : acetone (99 : 1), combined and were again subjected to column chromatography on a small silica gel column, and eluted with pet ether. The first fractions (5-16), collected on elution with pet ether : acetone (99 : 1) from this column, were combined, evaporated and further purified by using TLC plates (silica gel, 0.25 mm) [pet ether : acetone (98 : 2) to yield compound **1** (19.5 mg, 9.75 x 10^{-6} % yield, with $R_f = 0.58$].

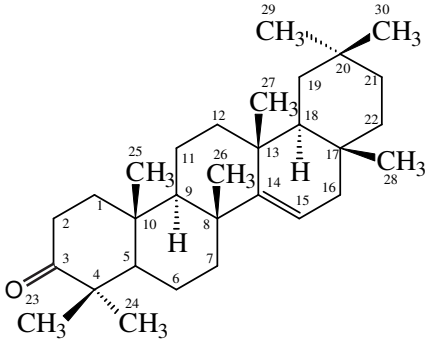
Results and Discussion

3α-Hydroxy-oleanane-5-ene **(1)**, $C_{30}H_{50}O$, was obtained as a crystalline solid (m.p. 197-199 °C) by column and preparative thin-layer chromatography of the ethanolic extracts of *A. vasica*. The molecular composition was determined as $C_{30}H_{50}O$ by high-resolution electron-impact mass measurements of the M^+ (*m/z* 426.3861), which indicated six degrees of unsaturation in the molecule. The UV spectrum recorded in methanol showed terminal absorption only, while the infrared spectrum displayed absorptions for OH (3400 cm^{-1}) and C = C (1630 cm^{-1}) groups.

The characteristic fragment ions were found at *m/z* 166 (fragment **a**), 260 (fragment **b**), 148 (fragment **c**), 205 (fragment **d**), and 287 (fragment **e**), indicative of the molecule being a pentacyclic triterpene of the oleanane series. The presence of a double bond in the oleanane series has proved to be readily recognizable by



3-α-Hydroxy-oleanane-5-ene (**1**)



Taraxerone-14-ene (**2**)

Fig. 1. The new triterpenoid, 3-α-hydroxy-oleanane-5-ene (**1**) and the known triterpenoid, taraxerone-14-ene (**2**) isolated from *Adhatoda vasica.*

mass spectrometry, since the molecular ion undergoes retro-Diels-Alder fragmentation of ring 'B' bisecting the molecule into two major fragments at *m/z* 166.1236 ($C_{11}H_{18}O$, fragment **a**) and *m/z* 260.2215 ($C_{19}H_{32}$, fragment **b**) as depicted in Fig. 2.

The ^{1}H-NMR spectrum ($CDCl_3$, 500 MHz) of **1** showed eight three-proton singlets at δ 0.83, 0.94, 0.97, 0.99, 1.03, 1.08, 1.13 and 1.15, indicating the presence of eight tertiary methyls in the molecule as expected in a pentacyclic triterpenoidal skeleton. The downfield region of the spectrum contained only two signals, indicating a close double doublet at δ 3.45 (J = 3.3 Hz, J = 2.4 Hz) and a broad double doublet at δ 5.62 (J_1 = 4.0 Hz, J_2 = 2.0 Hz), which could be assigned to the hydroxy-bearing C-3 methine and the vinylic C-6 protons, respectively. The chemical shift and coupling constants of the C-3 methine signal indicated an axial (α) orientation of the OH group (Ahmad and Rahman, 1994).

The ^{13}C-NMR spectra (broad-band decoupled and DEPT CD_3OD, 100 MHz) exhibited signals for all 30 carbons and further supported the formula derived by mass spectrometric observations. DEPT spectra showed the presence of eight methyl, ten methylene, five methine and (by difference from the broad-band decoupled spectrum) seven quaternary carbons. The downfield signals at δ 76.0 and 120.5 were due to the hydroxy-bearing C-3 and vinylic C-6, respectively. The eight methyl carbons resonated at δ 34.7, 32.7, 32.4, 29.4, 25.9, 19.9, 18.8 and 16.6

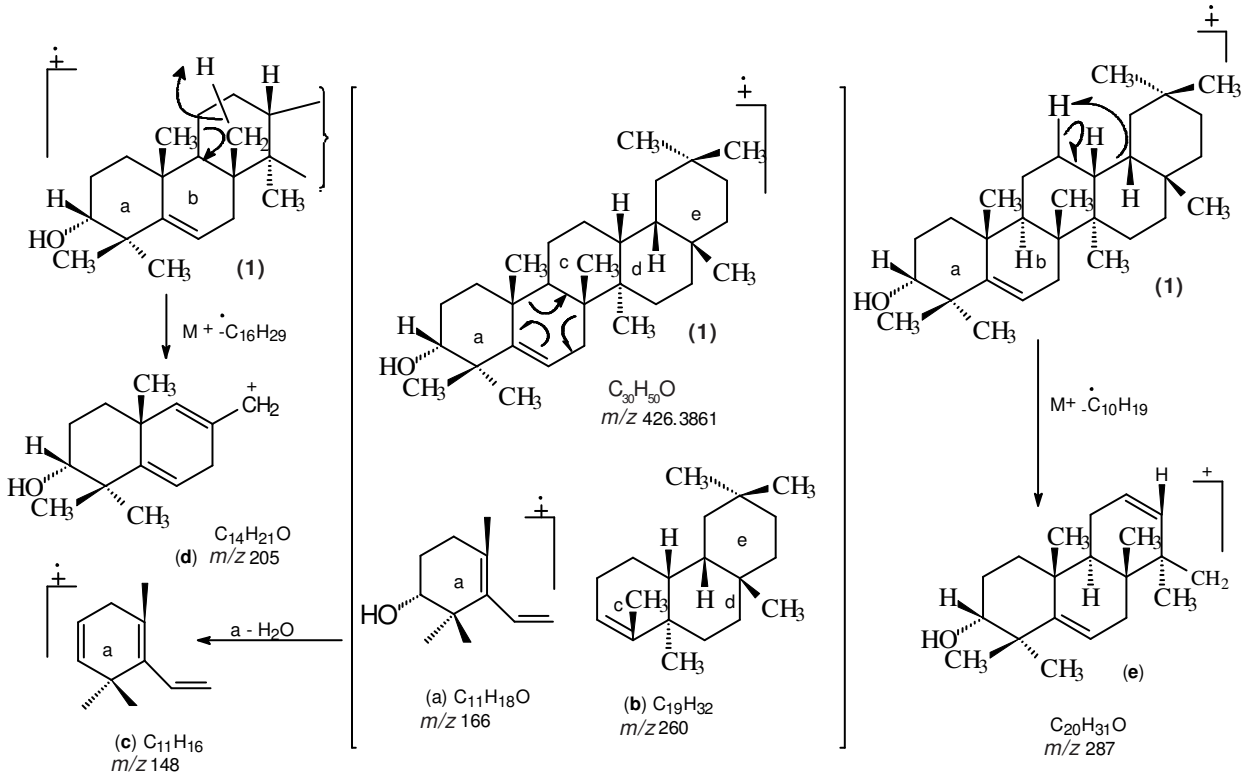


Fig. 2. Mass fragmentation pattern of 3α-hydroxy-oleanane-5-ene (**1**), a new triterpenoid isolated from *Adhatoda vasica*.

in the ^{13}C-NMR spectra. The assignments to the various carbons in the molecule are presented in Table 1.

Two-dimensional NMR techniques, such as COSY 45°, HOHAHA, HMQC and HMBC (Rahman, 1989; Rahman and Choudhary, 1996) were used to obtain more structural information. The C-3 methine proton resonating at δ 3.45 showed cross-peaks with the geminally coupled C-2 methylene protons at δ 1.60 and 1.85 in the COSY 45° spectrum. The C-6 vinylic proton resonating at δ 5.62 showed vicinal couplings with the C-7 allylic protons resonating at δ1.82 and 1.95. The Homonuclear Hartmann Hahn spectrum (HOHAHA), recorded with mixing delay of 100 ms, showed that the C-3 methine proton is coupled with four protons, i.e., with the C-2 and C-1 methylenic protons (δ 1.60/1.85 and 1.45/2.00), respectively.

The Heteronuclear Multiple Quantum Coherence (HMQC) spectrum of **1** displayed cross peaks between directly coupled carbon-proton pairs. The C-3 proton at δ 3.45 showed a cross-peak with the carbon at δ 76.0, while the vinylic C-6 proton (δ 5.62) was coupled with the carbon at δ 120.5 (C-6). Other HMQC interactions are presented in Table 1.

Table 1. ^{13}C and ^{1}H-NMR chemical shifts of 3α-hydroxy-oleanane-5-ene **(1)**

Carbon numbr	δ^{13}C ♣	δ^{1}H # (J in Hz) $J_{1\alpha,1\beta}$
C-1	36.8 (CH_2)	1.45 (ddd, $J_{1\alpha,1\beta}$ = 10.3, $J_{1\alpha,2\alpha}$ = 6.3, $J_{1\alpha,2\beta}$ = 4.2, 1H) 1.65 (ddd, $J_{1\beta,1\alpha}$ = 10.5, $J_{1\beta,2\beta}$ = 6.6, $J_{1\beta,2\alpha}$ = 4.0, 1H)
C-2	30.3 (CH_2)	1.85 (ddd, $J_{2\alpha,1\alpha}$ = 6.9, $J_{2\alpha,1\beta}$ = 4.8, $J_{2\alpha,3\beta}$ = 2.2, 1H) 2.00 (ddd, $J_{2\beta,1\beta}$ = 6.0, $J_{2\beta,1\beta}$ = 4.2, $J_{2\beta,1\alpha}$ = 1.9, 1H)
C-3	76.0 (CH)	3.45 (dd, 1H, $J_{3\beta,2\beta}$ = 3.3, $J_{3\beta,2\alpha}$ = 2.4)
C-4	41.0 (C)	-
C-5	141.0 (C)	-
C-6	120.5 (CH)	5.62 (dd, $J_{6\alpha,7\alpha}$ = 4.0, $J_{6\alpha,7\beta}$ = 2.0)
C-7	24.3 (CH_2)	1.82 (m, 1H), 1.95 (m, 1H)
C-8	35.8 (C)	-
C-9	51.0 (CH)	2.03 (m, 1H)
C-10	34.0 (C)	-
C-11	19.0 (CH_2)	1.46 (m, 1H), 1.52 (m, 1H)
C-12	35.7 (CH_2)	1.38 (m, 2H)
C-13	44.1 (C)	-
C-14	41.0 (CH)	0.92 (m, 1H)
C-15	29.2 (CH_2)	1.30 (m, 1H), 1.14 (m, 1H)
C-16	35.4 (CH_2)	1.40 (m, 1H), 1.52 (m, 1H)
C-17	32.9 (C)	-
C-18	48.4 (CH)	1.51 (m. 1H)
C-19	39.6 (CH_2)	1.50 (m, 1H), 1.63 (m, 1H)
C-20	26.0 (C)	-
C-21	33.9 (CH_2)	1.00 (m, 1H), 1.20 (m, 1H)
C-22	31.1 (CH_2)	1.21 (m, 1H), 1.30 (m, 1H)
C-23	29.4 (CH_3)	1.03 (s)
C-24	25.9 (CH_3)	1.12 (s)
C-25	16.6 (CH_3)	0.83 (s)
C-26	18.8 (CH_3)	1.15 (s)
C-27	32.4 (CH_3)	0.97 (s)
C-28	32.7 (CH_3)	0.99 (s)
C-29	34.7 (CH_3)	0.94 (s)
C-30	19.9 (CH_3)	1.08 (s)

♣ multiplicity assignments based on DEPT study

one-bond heteronuclear correlations determined by heteronuclear multiple quantum coherence (HMQC) study

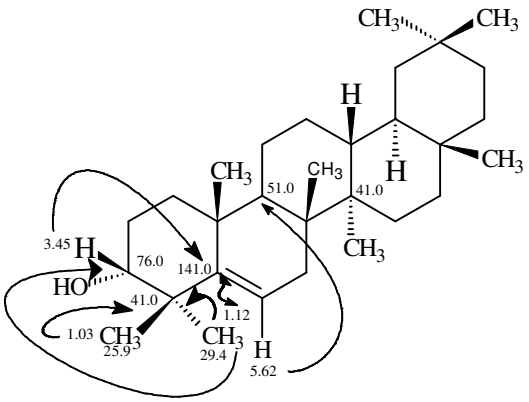


Fig. 3. Some selected multiple bond interactions in 3-α-hydroxy-oleanane-5-ene (**1**) observed in the heteronuclear multiple bond connectivity (HMBC) study.

The Heteronuclear Multiple Bond Connectivity (HMBC) spectrum was also very informative in determining the position of the double bond between C-5 and C-6, since the C-6 vinylic proton (δ 5.62) showed long-range interactions with C-4 (δ 41.0) and C-9 (δ 51.0). The C-3 proton (δ 3.45) showed coupling with C-5 (δ 141.0). The C-23 methyl protons showed heteronuclear shift correlations with C-3, C-4 and C-5, while the C-24 methyl protons showed coupling interactions with C-3, C-4 and C-5 (Fig. 3).

References:

Ahmad, V.U., Rahman, A.U. 1994. *Handbook of Natural Products Data*: *Pentacyclic Triterpenoids,* vol. **2,** p. 509, Elsevier Science Publishers, Amsterdam, Netherlands.

Ikram, M., Huq, M.E., Warsi, S.A., Ahmad, V.U. 1965. Chemical composition of *Adhatoda vasica. Pak. J. Sci. Ind. Res.* **8:** 76.

Mehta, D.R., Naravane, J.S., Desai, R.M. 1963. Vasicinone. A bronchodilator principle from *Adhatoda vasica* Nees. (N.O. Acanthaceae). *J. Org. Chem.* **28:** 445-447.

Rahman, A.U. 1989. *One- and Two-Dimensional NMR Spectroscopy,* Elsevier Science Publishers, Amsterdam, Netherlands.

Rahman, A.U., Choudhary, M.I. 1996. *Solving Problems with NMR Spectroscopy*, Academic Press, New York, USA.