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A Pentacyclic Triterpenoid from *Asteracantha longifolia* Ness.

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A new pentacyclic triterpenoid 3β-acetoxyurs-18-ene (**4**) has been isolated from the aerial parts of *Asteracantha longifolia* Ness., alongwith lupeol (**1**), 16-hydroxy-26-methyltheptacosan-2-one (**2**) and stigmasterol (**3**). Their structures were elucidated by spectral analysis and chemical studies.

Key Words: *Asteracantha longifolia* Ness., 16-Hydroxy-26-methyltheptacosan-2-one, Stigmasterol, Triterpenoid, Lupeol, 3β-Acetoxyurs-18-ene.

INTRODUCTION

Asteracantha longifolia Ness. (Acathaceae) is a small spiny herb used as herbal medicine in dropsy, rheumatism, jaudice, urinogenital tract diseases[1], *etc*. Aliphatic compounds, flavonoids, terpenoids and sterols have been reported from this plant[2-5]. This work deals with isolation and structure elucidation of a new pentacyclic triterpenoid 3β-acetoxyurs-18-ene (**4**) alongwith lupeol (**1**), aliphatic hydroxyt ketone (**2**) and stigmasterol (**3**) from the *n*-hexane insoluble ethanolic extract of aerial parts of this plant.

EXPERIMENTAL

All reported m.p.s are uncorrected. IR (KBr) spectra were recorded on a Perkin Elmer 177 spectrometer. ^{1}H NMR spectra in $CDCl_3$ on a WM-400 (Bruker-FT) instrument using TMS as internal standard and EIMS spectra on a Jeol D-300 mass spectrometer. Silica gel (qualigens) was used for TLC and column chromatography. Spots on TLC plates were visualized by UV light, I_2, vapours as well as by heating the plates after spraying with 10 % H_2SO_4.

Aerial parts (leaves and stem) of *Asteracantha longifolia* Ness. were collected from nearby area of Gorakhpur, India in November 2003 and identified by Prof. S.C. Tripathi, Department of Botany, DDU Gorakhpur, India. A voucher specimen has been deposited at the Herbarium of K.B. Postgraduate College, Mirzapur, India.

Extraction and isolation: Air dried and powdered plant material (3 kg) was extracted with hot ethanol. The solvent was removed under

reduced pressure to give a dark brownish semi-solid mass (250 g). Its *n*-hexane insoluble fraction (110 g) was chromatographed over a column of silica gel (2 kg) and the column was eluted with hexane, hexane-chloroform mixture (9:1, 3:1, 1:1, 1:3), chloroform, chloroform-ethyl acetate mixture (3:1, 1:1, 1:3) and ethyl acetate. The progress of elution of the column was monitored by intermittent co-TLC of effluent fraction (*ca.* 200 mL). Fraction showing similar spots on TLC plates were mixed together and solvent removed under reduced pressure.

Lupeol 1: Fractions 14-42 of hexane eluate gave a solid mass which recrystallized from methanol into white crystals (85 mg), m.p. 211ºC, m.f. $C_{30}H_{50}O$, M^+ at m/z 426. Its identity was finally confirmed by comparison of melting point of **2** and its monoacetate derivative **2a** with literature value[4]. IR (KBr, cm^{-1}): 3340, 2900, 1640, 1450, 1370, 1190, 1100, 1040, 1017, 986, 960 and 880; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.76 (3H, s, CH_3), 0.84 (3H, s, CH_3), 0.86 (3H, s, CH_3), 0.87 (3H, s, CH_3), 0.90 (3H, s, CH_3), 1.00 (3H, s, CH_3), 1.60 (3H, brs, >C=C–CH_3), 3.35 (1H, m, >CH–OH), 4.58 (2H, m, >C–CH_2) and 5.00 (1H, s, –OH); MS: m/z 426 [M^+] (3.2 %) for $C_{30}H_{50}O$ 411 (2.3), 408, (10.2), 385 (11.3), 383 (9.1), 207 (25.3), 203 (15.1) and 189 (100.0).

Acetylation of 1: A mixture of **1** (20 mg), AC_2O and pyridine (1 mL each) was allowed to stand overnight at room temperature. On usual work-up, themixture afforded colourless crystals (15 mg), m.p. 188-189ºC.

16-Hydroxy-26-methylheptacosan-2-one[6] (2): Fractions 17-26 of hexane-chloroform (3:1) eluate yielded a residue which on recrystallization from methanol yielded white crystals (30 mg), m.p. 70-71ºC, m.f. $C_{28}H_{56}O_2$, M^+ at m/z 424. IR (KBr, cm^{-1}): 3450, 2900, 2810, 1725, 1575, 1490, 1395, 1385, 1255, 1100, 1050, 985, 725 and 715; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.81 (6H, s, J = 6.5 Hz, $(CH_3)_2$CH-), 1.21 (36H, brs, 18 CH_3), 1.55 (4H, m, -CH_2-CH(OH)-CH_2), 1.71 (2H, m, -CH_2-CH_2-C=O-), 1.80 (1H, m, $(CH_3)_2$CH-), 2.00 (3H, s, CH_3-CO-), 2.24 (2H, t, J = 6.0 Hz, -CH_2-CO-Me) and 3.57 (1H, t, J = 7.0 Hz, >CH-OH); MS : m/z 424 [M^+] (1.2 %) for $C_{28}H_{56}O_2$, 409 (1.0), 380 (1.5), 370 (2.7), 355 (2.6), 295 (3.0), 269 (1.2), 255 (34.0), 227 (1.2), 225 (2.0), 213 (16.0), 211 (1.3), 199 (6.0), 197 (1.2), 169 (1.5), 1.55 (1.5), 141 (3.0), 127 (7.0), 113 (8.0), 99 (1.5), 85 (12.0), 71 (21.0), 57 (43.0) and 43 (100).

Stigmasterol[7] (3): Fractions 1-13 of hexane chloroform (1:1) eluate yielded a solid mass which recrystallized from acetone into white crystals (90 mg), m.p. 170ºC. It is unsaturated steroid confirmed by colour test (LB, noller and tetranitro methane). IR (KBr, cm^{-1}): 3350, 2960, 2890, 1640, 1465, 1380, 1370, 325, 1260, 1100, 1050, 1040, 950, 840 and 790.

3β-Acetoxyurs-18-ene (4): Fraction 41-60 of hexane-chloroform (1:3) eluate gave a residue which on recrystallization from chloroform afforded

white crystals (75 mg), m.p. 218-219ºC, $[\propto]_D^{22}$-24ºC (c 0.25, $CHCl_3$), m.f. $C_{32}H_{55}O_2$, M^+ at m/z 468. The compound is found to be an unsaturated tri-terpenoid by positive colour test (LB, noller and tetranitro methane. IR (KBr, cm^{-1}): 2910, 2800, 1725, 1645, 1460, 1390, 1380, 1260, 1210, 1025, 990 and 880. The absence of bands at 960 or 840 cm^{-1} indicated the unsaturation to be a tetrasubstituted double bond[8]. The lone acetoxy group was located at the 3β-position as a sharp band recorded at 1260 cm^{-1} which is characteristics of 3β-acetoxy grouping in A/B *trans*-triterpenoids[9,10]. ^1H NMR (300 MHz, $CDCl_3$): δ 0.78 (6H, s, 2 CH_3), 0.85, (3H, s, CH_3), 0.93 (3H, s, CH_3), 0.95 (3H, s, CH_3), 1.00 (3H, s, CH_3), 1.20 (3H, d, -J = 5.0 Hz, -HC_{20}-CH_3), 1.60 (3H, s, >C=C-CH_3), 1.90 (3H, s, -$OCOCH_3$) and 4.60 (1H, m, >CH-OAc), MS: m/z 468 [M^+] (20 %) for $C_{32}H_{52}O_2$, 453 (5.0), 408 (15.5), 440 (15.0), 438 (12.0), 380 (22.0), 250 (8.0), 249 (26.5), 219 (21.0), 218 (22.0), 205 (15.0), 204 (23.0), 203 (18.0), 191 (30.0), 190 (35.0), 180 (100).

Hydrolysis (4): Compound **4** (30 mg) was refluxed with 3 % alcoholic KOH for 7 h and the reaction content was then poured into ice-cold water to give a white solid. It was filtered and recrystallized from $CHCl_3$ into white crystals of hydrolysis product **4a** (22 mg), m.p. 230-231ºC, m.f. $C_{30}H_{50}O$, M^+ at m/z 426. IR (KBr, cm^{-1}): 3400, 2920, 2810, 1640, 1460, 1380, 1370, 1050, 1000, 980 and 885; ^1H NMR (300 MHz $CDCl_3$): δ 0.76 (3H, d, J = 5.5 Hz)-HC_{20}-CH_3), 1.60 (3H, s, >C=C-CH_3) and 3.50 (1H, m, >CH-OH); MS: m/z 426 [M^+] (12 %) for $C_{30}H_{50}O$, 411 (5.0), 408 (10.5), 398 (10.0), 380 (15.5), 219 (18.0), 218 (14.0), 208 (7.5), 207 (22.0), 205 (11.5), 203 (21.0), 192 (24.0), 189 (100).

RESULTS AND DISCUSSION

A high resolution ^1H NMR (300 MHz) spectrum of compound **4** displayed signals for 8 methyl groups at δ 0.78 (6H, s), 0.85 (3H, s), 0.93 (3H, s), 0.95 (3H, s), 1.00 (3H, s), 1.20 (3H, d) and 1.60 (3H, s). Thereby suggesting the pentacyclic nature of the triterpenoid. A careful study of these methyl signals showed that one methyl out of eight recorded as a doublet at δ 1.20 while another appeared as a singlet at comparatively low field δ 1.60. In view of these typical signals the compound **4** was concluded to be a member of the ursane series of triterpenoids. As one of the two secondary methyls of the ursane skeleton appeared downfield as a signlet, it could be attached with an olefinic carbon. These diagnostic methyl signals further indicated the possibility of the double bond being located either at C-18 or C-20 in ring E. As IR has already suggested the double bond to be tetrasubstituted and ^1H NMR spectrum has no signal for olefinic proton, the double bond was assigned at C-18. In view of this, the compound was concluded to possess urs-18-ene skeleton[11]. A sharp singlet

appearing at δ 1.90 was assigned to the acetoxy methyl group and the proton attached to acetoxy bearing carbon (C-3) resonated as a multiplet at δ 4.60. ^{1}H NMR spectrum of the hydroxy derivative **4a** showed the appearance of a one proton multiplet at δ 3.50 for a proton attached to C-3 bearing β-OH group and the disappearnace of signal for acetoxy methyl as well as signal for a proton attached to acetoxyl bearing carbon.

The mass spectrum of this new isolate supported it to be a pentacyclic triterpenoid having urs-18-ene skeleton with 3β-acetoxy group (Fig. 1). It showed molecular ion peak $[M]^+$ at m/z 468 and other important fragments at m/z 453 (M-Me), 408 (M-AcOH), 440 (M-28), 380 (440-AcOH), 250,

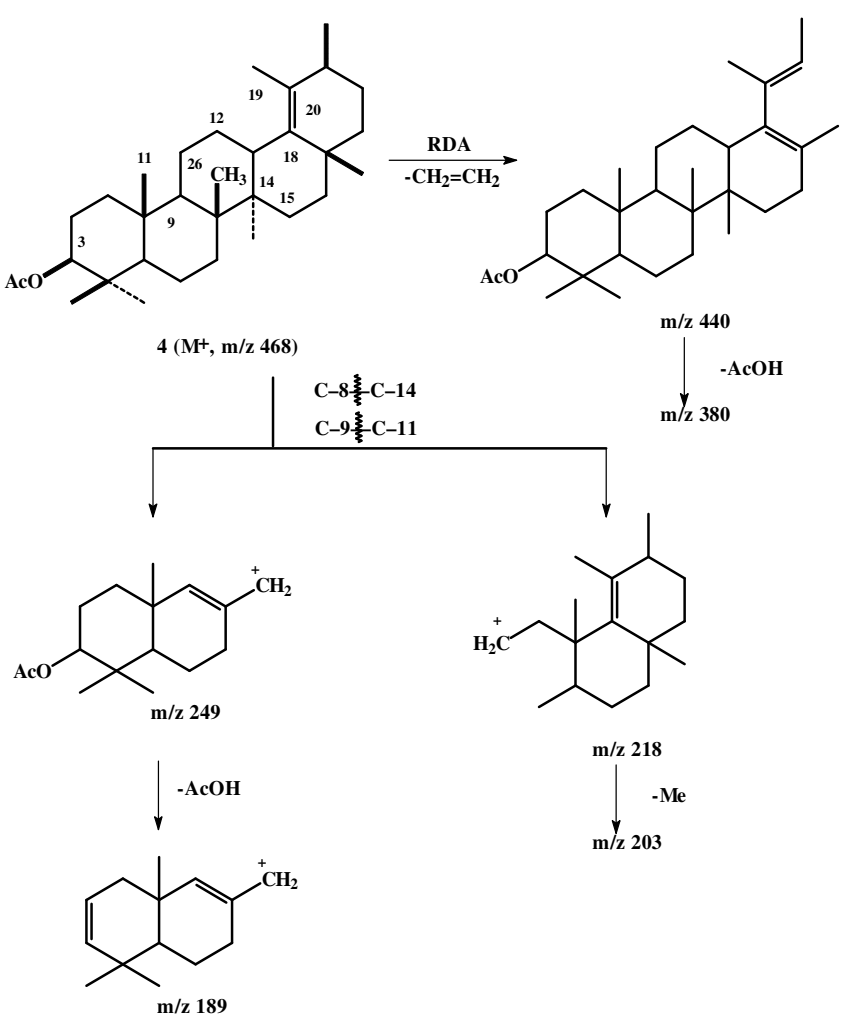

Fig. 1.

249, 219, 218, 204, (219-Me), 203 (218-Me), 190 (250-AcOH) and 189 (base peak, 249-AcOH). Cleavage of the C-8–C-14 and C-9–C-11 σ bonds of ring C followed by the transfer for a hydrogen atom from C-26 to C-11 gave a fragment ion at m/z 249 which eliminated a molecule of AcOH to produce base peak at m/z 189. Another important fragment at m/z 218 is formed by simultaneous cleavage of C-8–C-14 and C-9–C-11 σ bonds without hydrogen transfer and it loses a methyl radical to give a fragment at m/z 203. The appearance of a more stable fragment at m/z 189 than that at m/z 203 is a typical feature of triterpenoids having ursane skeleton[12]. The molecular ion (M^+ 468) undergoes RDA rearrangement to give an important fragment at m/z 440 which further supports the double bond at C-18 (Fig. 1). This assignment was also supported by the mass spectrum of hydroxy derivative (3β-hydroxyurs-18-ene) **4a** which showed molecular ion peak at m/z 426 and other important fragments at m/z 411, 418, 398, 396, 218, 207, 203, 189 (base peak), *etc.* Thus this new trilerpenoid was characterized as 3β-acetoxyurs-18-ene (**4**).

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