Phytochemical Studies on *Alstonia scholaris*

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Reinvestigation of the flowers of *Alstonia scholaris* of Pakistan origin have resulted in the isolation of three new triterpenoids, two of the ursane type, 3β-acetate-24-nor-urs-4,12-diene ester triterpene (**1**) and 3β-hydroxy-24-nor-urs-4,12,28-triene triterpene (**2**), and one of the oleanane type, 3,28-β-diacetoxy-5-olea-triterpene (**3**), together with two known triterpenes, α-amyrin acetate (**4**) and ursolic acid (**5**). This is the first report of the isolation of **4** and **5** from the flower part of this species. The structures of **1** – **5** were elucidated with the aid of extensive NMR-spectroscopic studies.

Key words: Alstonia scholaris, Apocynaceae, Triterpene, Flowers, Spectroscopic Studies

Introduction

The genus *Alstonia* comprises about twelve species. *Alstonia scholaris* Linn. R. Br. belongs to the family Apocynaceae [1] and grows throughout India, also in plains [2, 3]. Its timber is a non-durable hardwood, used in pulp and paper production. Because the wood has also been used for school blackboards, the name 'scholaris' was given. The plants are milk-bearing shrubs or trees, with large, entire, generally whorled leaves and terminal cymes of white flowers.

The plant *Alstonia scholaris* has been used in different systems of traditional medication for the treatment of diseases and ailments of human beings. It is reported to contain various alkaloids, flavonoids and phenolic acids. The flowers of *Alstonia scholaris* are known to contain lupeol and β-amyrin [4]. The bark contains alkaloids, including ditaine, echitenine, echitamine (ditamine) and echitamidine together with the triterpenes β-amyrin and lupeol [5 – 9]. It has been reported as antimicrobial, anti-amoebic, antidiarrhoeal, antiplasmodial, hepatoprotective, immunomodulatory, anti-cancer, antiasthmatic, free radical scavenging, antioxidant, analgesic, anti-inflammatory, anti-ulcer, anti-fertility agent, and is used for treatment of anaemia, chronic diarrhea, dysentery, menstrual disorders, malarial fever, colic and acute arthritis, showing also wound healing activities [14 – 18]. There are also reports available for the traditional use of this plant for its cardiotonic, anti-diabetic and anti-arthritic properties. The bark yields a tonic and antiseptic medicine. A concentrated decoction of trunk bark is used as a wash in furunculosis and impetigo, and as a gargle in dental caries. The bark, leaves and milky exudates of *Alstonia scholaris* are used in India [1 – 3].

Results and Discussion

3β-Acetate-24-nor-urs-4,12-diene ester triterpene (**1**), $C_{31}H_{48}O_2$, was obtained as a colorless solid mass. The UV spectrum recorded in methanol showed terminal absorptions only, while the infrared spectrum displayed absorptions for ester C=O (1732 cm^{-1}) and C=C (1630 cm^{-1}) groups [10 – 13].

The molecular ion peak of this compound was determined with the aid of electron impact mass spectrometry (EIMS) which showed the molecular ion at *m/z* = 452, and the high-resolution electron impact mass spectrum (HREIMS) of **1** showed the M^+ ion at *m/z* 452.3652 corresponding to the molecular formula $C_{31}H_{48}O_2$ (calcd. 452.3654). This indicated eight double bond equivalents in **1**. The high-resolution electron impact mass measurements also showed the characteristic fragment ions at *m/z* = 234.1618, 218.2032, 174.1406 (calcd. 174.1408 for $C_{13}H_{18}$), indicative of the molecule being a pentacyclic triterpene of the oleanane/ursane series. The presence of a double bond in the ursane series has proved to be readily recognizable by mass spectrometry, because the molecular ion undergoes retro-Diels-Alder fragmentation of ring C,

Table 1. ^{1}H-NMR data of compounds **1**, **2** and **3**.

Positions	**1** δ ^{1}H (ppm)[a,b]	**2** δ ^{1}H (ppm)[a,b]	**3** δ ^{1}H (ppm)[a,b]
1	1.23 (m), 1.25 (m)	1.23 (m), 1.25 (m)	1.45 (m), 2.00 (m)
2	1.61 (m), 1.81 (m)	1.61 (m), 1.81 (m)	1.65 (m), 1.85 (m)
3	4.50 (dd, $J_{3\alpha,2\alpha}$ = 6.12, $J_{3\alpha,2\beta}$ = 14.5 Hz)	3.54 (dd, $J_{3\alpha,2\alpha}$ = 4.5, $J_{3\alpha,2\beta}$ = 12.4 Hz)	4.50 (dd, $J_{3\alpha,2\alpha}$ = 6.12, $J_{3\alpha,2\beta}$ = 14.5 Hz)
4	–	–	–
5	0.92 (m)	0.92 (m)	–
6	1.42 (m), 1.50 (m)	1.42 (m), 1.50 (m)	5.62 (dd, $J_{6,7\alpha}$ = 12.5, $J_{6,7\beta}$ = 4.0 Hz)
7	1.30 (m), 1.40 (m)	1.30 (m), 1.40 (m)	1.82 (m), 1.95 (m)
8	–	–	–
9	1.56 (m)	1.56 (m)	2.03 (m)
10	–	–	–
11	1.52 (m), 1.62 (m)	1.52 (m), 1.62 (m)	1.46 (m), 1.52 (m)
12	5.11 (t, $J_{12,11}$ = 3.26 Hz)	5.11 (t, $J_{12,11}$ = 3.26)	1.38 (m)
13	–	–	0.89 (m)
14	–	–	–
15	1.33 (m), 1.14 (m)	1.33 (m), 1.14 (m)	1.30 (m), 1.13 (m)
16	1.40 (m), 1.52 (m)	1.40 (m), 1.52 (m)	1.38 (m), 1.51 (m)
17	–	–	–
18	2.20 (d, $J_{18,19}$ = 11.2 Hz)	2.20 (d, $J_{18,19}$ = 11.2 Hz)	1.51 (m)
19	1.99(m)	1.99 (m)	1.50–1.58 (m)
20	1.95(m)	1.95(m)	–
21	1.01, 1.11(m)	1.00, 1.11(m)	1.00, 1.21(m)
22	1.02, 1.21(m)	1.02, 1.21(m)	1.20–1.31 (m)
23	4.57 (d, $J_{23\alpha,23\beta}$ = 2.4 Hz) 4.68 (d, $J_{23\beta,23\alpha}$ = 2.4 Hz)	4.57 (d, $J_{23\alpha,23\beta}$ = 2.4) 4.68 (d, $J_{23\beta,23\alpha}$ = 2.4)	1.07 (s)
24	–	–	0.85 (s)
25	0.85 (s)	0.85 (s)	0.95 (s)
26	0.95 (s)	0.95 (s)	0.84 (s)
27	0.85 (s)	0.84 (s)	0.97 (s)
28	1.23 (s)	–	–
29	0.80 (d, $J_{29,19}$ = 6.8 Hz)	0.80 (d, $J_{29,19}$ = 6.8)	0.94 (s)
30	0.91 (d, $J_{30,20}$ = 6.6 Hz)	0.91 (d, $J_{30,20}$ = 6.6)	1.08 (s)
31	– –	4.54 (d, $J_{31\alpha,31\beta}$ = 2.5) 4.65 (d, $J_{31\beta,31\alpha}$ = 2.5)	2.10 (s)
32	2.11 (s)	1.64 (s)	–
33	–	–	2.13 (s)

[a] Multiplicity and coupling constants in parentheses; [b] one-bond heteronuclear correlations determined by HMQC experiment.

thereby bisecting the molecule into two major fragments at *m/z* =234.1618 ($C_{15}H_{22}O_2$) and *m/z* = 218. 2030 (calcd. 218.2034 for $C_{16}H_{26}$).

The ^{1}H-NMR spectrum [15, 16] ($CDCl_3$, 500 MHz) of **1** (Table 1) showed five three-proton singlets at δ = 2.11, 0.85, 0.95, 0.84, and 1.23 and two methyls as doublets (δ = 0.80, $J_{29,19}$ = 6.5 Hz, 0.91, $J_{30,20}$ = 6.5 Hz) indicating the presence of five tertiary methyls and two secondary methyls in the molecule as expected in a pentacyclic triterpenoidal skeleton. The downfield region of the spectrum contained only three signals *i. e.* a broad double doublet at δ = 4.50 ($J_{3\alpha,2\beta}$ = 14.5 Hz, $J_{3\alpha,2\alpha}$ = 6.12 Hz), two close doublets (δ = 4.57, $J_{23\alpha,23\beta}$ = 2.4 Hz, 4.68, $J_{23\beta,23\alpha}$ = 2.4 Hz) and a triplet at δ = 5.11 (t, $J_{12,11}$ = 3.26 Hz), which could be assigned to the acetate-bearing C-3 methine, C-4 methylene protons and the vinylic C-12 protons, respectively. The chemical shift and coupling constants of the C-3 methine signal indicated an equatorial (β) orientation of the acetate group.

The ^{13}C-NMR spectrum ($CDCl_3$, 125 MHz) [15, 16] of **1** exhibited signals for all 31 carbon atoms. DEPT spectra showed the presence of seven methyl, ten methylene, seven methine and (by difference from the broad-band decoupled spectrum) seven quaternary carbons. The downfield signals at δ = 80.98 and 124.34 were due to the acetate-bearing C-3 and vinylic C-12, respectively. The seven methyl carbons resonated at

Table 2. ^{13}C-NMR data (δ in ppm) of compounds **1**, **2** and **3**[a,b].

Positions	1	2	3
1	41.55 (CH_2)	41.55 (CH_2)	39.79 (CH_2)
2	27.12 (CH_2)	27.12 (CH_2)	28.23 (CH_2)
3	80.98 (CH)	79.01 (CH)	80.94 (CH)
4	150.94 (C)	156.12 (C)	40.45 (C)
5	55.41 (CH)	55.41 (CH)	140.78 (C)
6	18.26 (CH_2)	18.26 (CH_2)	121.70 (CH)
7	32.88 (CH_2)	32.88 (CH_2)	24.36 (CH_2)
8	38.51 (C)	38.51 (C)	35.88 (C)
9	47.65 (CH)	47.65 (CH)	51.24 (CH)
10	37.11 (C)	37.11 (C)	34.86 (C)
11	21.09 (CH_2)	21.09 (CH_2)	21.09 (CH_2)
12	124.34 (CH)	124.34 (CH)	37.28 (CH_2)
13	139.64 (C)	139.64 (C)	40.46 (CH)
14	42.86 (C)	42.86 (C)	40.05 (C)
15	29.87 (CH_2)	29.87 (CH_2)	31.68 (CH_2)
16	23.71 (CH_2)	23.71 (CH_2)	24.36 (CH_2)
17	40.82 (C)	40.82 (C)	48.67 (C)
18	59.09 (CH)	59.09 (CH)	50.11 (CH)
19	39.62 (CH)	39.62 (CH)	42.32 (CH_2)
20	39.66 (CH)	39.66 (CH)	29.34 (C)
21	28.11 (CH_2)	28.11 (CH_2)	33.98 (CH_2)
22	38.06 (CH_2)	38.06 (CH_2)	29.68 (CH_2)
23	109.35 (CH_2)	109.31 (CH_2)	12.05 (CH_3)
24	–	–	29.20 (CH_3)
25	15.72 (CH_3)	15.72 (CH_3)	18.04 (CH_3)
26	17.48 (CH_3)	17.48 (CH_3)	19.04 (CH_3)
27	28.06 (CH_3)	28.06 (CH_3)	31.49 (CH_3)
28	28.74 (CH_3)	150.96 (C)	170.97 (C)
29	23.53 (CH_3)	23.53 (CH_3)	19.39 (CH_3)
30	28.11 (CH_3)	28.11 (CH_3)	31.87 (CH_3)
31	170.88 (C)	105.93 (CH_2)	20.00 (OCH_3)
32	21.29 (CH_3)	19.74(CH_3)	170.96 (C)
33	–	–	21.15 (CH_3)

[a] Multiplicity assignments based on DEPT experiments; [b] one-bond heteronuclear correlations determined by HMQC experiment.

δ = 15.72, 17.48, 28.06, 28.74, 23.53, 28.11, and 21.29 in the ^{13}C-NMR spectrum. The assignments to the various carbon atoms in the molecule are presented in Table 2.

Two-dimensional NMR techniques such as COSY-45°, HOHAHA, HMQC and HMBC [16 – 18] were used to obtain more structural information. The C-3 methine proton resonating at δ = 4.50 showed cross-peaks with the geminally coupled C-2 methylene protons at δ = 1.61 and 1.81 in the COSY-45° spectrum. The C-12 vinylic proton resonating at δ = 5.11 showed vicinal couplings with the C-11 methylene protons resonating at δ =1.52 and 1.62. The Homonuclear Hartmann Hahn spectrum [16 – 19] recorded with a 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 =1.23/1.25 and 1.61/1.81), respectively.

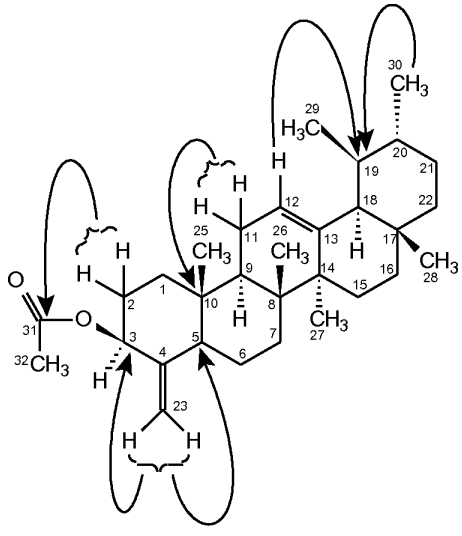


Fig. 1. Selected multiple bond interactions in **1** as observed in the HMBC experiment.

It was now left to define the exact position of the exocyclic double bond. The two downfield exocyclic methylene protons appearing in the COSY-45° spectrum [19 – 22] (δ = 4.57 and 4.68) displayed geminal coupling interactions. This exocyclic double bond was needed to be placed in the ursane series of triterpenes in such a way that it should follow the regular fragmentation pattern of the series. This was accomplished by placing this functionality at the C-4 position, which was also in accordance with cross signals observed in the HMBC spectrum. The C-23 olefinic protons gave strong cross peaks with the C-3 α proton (δ = 4.50/4.57, 4.68). In the HMBC spectrum, C-4 exhibited cross-peaks with H-3α (δ = 150.94/4.50, 2J), H-5α (δ = 150.94/0.92), H-6α (δ = 150.94/1.42,1.50), and H-2α (δ = 150.94/1.61, 1.81). Therefore the HMBC experiment revealed the attachment of the exocyclic methylene group at C-4 (Fig. 1) [23 – 27].

The characteristic retro-Diels-Alder fragmentation of **1** along with the characteristic ^{13}C-NMR chemical shifts of C-12 and C-13 at δ 124.34 and 139.64, respectively, suggested that **1** belongs to the $^{\Delta}$12-α amyrin series of triterpenoids with methylene and ester groups in the A/B rings. These observations led to define the structure of **1** as 3β-acetate-24-nor-urs-4,12-diene ester triterpene.

3β-Hydroxy-24-nor-urs-4,12, 28-triene triterpene (**2**), $C_{31}H_{48}O$, was obtained as a colorless amorphous substance by column and preparative thin-layer chro-

matography of the ethanolic extracts of *A. scholaris*. The UV spectrum recorded in methanol showed terminal absorptions only, while the infrared spectrum displayed absorptions for hydroxyl (ν = 3415 cm^{-1}) and C=C (ν =1630 cm^{-1}) groups. The molecular composition was determined as $C_{31}H_{48}O$ by high-resolution electron impact mass measurements of the $[M]^+$ peak (*m/z* = 436.3703) which indicated eight degrees of unsaturation in the molecule. In the EIMS, the characteristic fragment ions were found at *m/z* = 192, 244 and 174, indicative of the molecule being a pentacyclic triterpene of the oleanane/ursane series. The presence of a double bond in the ursane series has proved to be readily recognizable by mass spectrometry, since the molecular ion undergoes retro-Diels-Alder fragmentation of ring C, thereby bisecting the molecule into two major fragments at *m/z* = 192.1513 (calcd. 192.1514 for $C_{13}H_{20}O$) and *m/z* = 244.2192 (calcd. 244.2190 for $C_{18}H_{28}$).

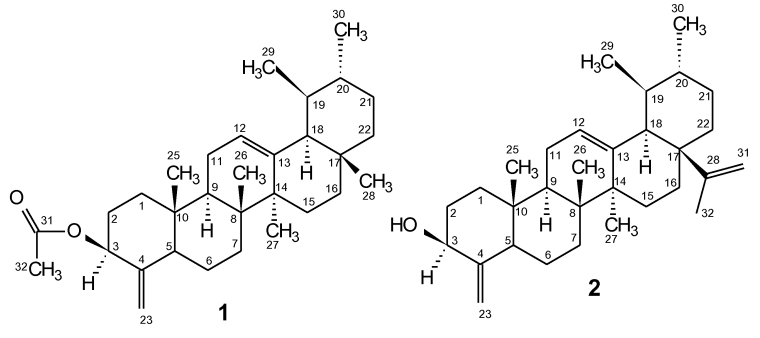
The ^{1}H-NMR spectrum ($CDCl_3$, 500 MHz) [15, 16] of **2** (Table 1) showed four three-proton singlets at (δ = 0.84, 0.95, 0.85, 1.64) and two methyls as doublets (δ = 0.80, d, J = 6.8 Hz, 0.91, J = 6.6 Hz) indicating the presence of four tertiary and two secondary methyl groups in the molecule. The downfield region of the spectrum contained only three signals *i. e.* a broad double doublet at δ = 3.54 ($J_{3\alpha,2\beta}$ = 12.4 Hz, $J_{3\alpha,2\alpha}$ = 4.5 Hz), four close doublets, two at δ = 4.57 (d, $J_{23\alpha,23\beta}$ = 2.4 Hz), 4.68 (d, $J_{23\beta,23}$ = 2.4 Hz) and another two at δ = 4.54 (d, $J_{31\alpha,31\beta}$ = 2.5 Hz), 4.65 (d, $J_{31\beta,31\alpha}$ = 2.5 Hz) and a triplet at δ = 5.11 (t, J = 3.26 Hz), which could be assigned to the hydroxy-bearing C-3 methine, C-23, 31 methylene and the vinylic C-12 protons, respectively. The chemical shift and coupling constants of the C-3 methine signal indicated an equatorial (β) orientation of the OH group [11].

The ^{13}C-NMR spectrum of **2** [15, 16] (CD_3OD, 100 MHz) exhibited signals for all 31 carbon atoms. DEPT spectra showed the presence of six methyl, eleven methylene, seven methine and (by difference from the broad-band decoupled spectrum) seven quaternary carbons. The downfield signals at δ = 79.01 and 124.34 were due to the hydroxy-bearing C-3 and the vinylic C-12, respectively. The six methyl carbons resonated at δ = 15.72, 17.48, 28.06, 23.53, 28.11, and 19.74 in the ^{13}C-NMR spectrum. Two carbons, C-23/C-31, have close chemical shift (δ = 109.31 and 105.93). Similarly other two carbons, C-4/C-28, also have close chemical shift (δ = 156.12 and δ = 150.96). The assignments to the various carbons in the molecule are presented in Table 2.

Two-dimensional NMR techniques such as COSY-45°, HOHAHA, HMQC and HMBC [15 – 17] were used to obtain more structural information. The C-3 methine proton resonating at δ = 3.54 showed cross-peaks with the geminally coupled C-2 methylene protons at δ =1.61 and 1.81 in the COSY-45° spectrum. The C-12 vinylic proton resonating at δ = 5.11 showed vicinal couplings with the C-11 methylene protons resonating at δ =1.52 and 1.62. The Homonuclear Hartmann Hahn spectrum [19 – 24] recorded with a 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 =1.23/1.25 and 1.61/1.81), respectively. The exact position of the two exocyclic methylene groups was also confirmed by 2D experiments. The two downfield exocyclic methylene protons appearing in the COSY-45° spectrum (δ = 4.57 and 4.68) not only displayed geminal coupling interactions but also gave strong cross peaks with the α-C-3 proton (δ = 3.54/4.57, 4.68) in the HMBC experiment. In addition, these two downfield protons (H-23) also exhibited strong interactions with H-5 (0.92/4.57, 4.68) in the same 2D experiment indicating the vicinity of the olefinic protons. This exocyclic double bond was needed to be placed in the ursane series of triterpenes in such a way that it should follow the regular fragmentation pattern of the series. This was accomplished by placing this functionality at the C-4 position. This was also in accordance with cross signals observed in the HMBC spectrum (Table 3). The other two downfield exocyclic methylene protons appearing in the COSY-45° spectrum (4.54 and 4.65) displayed geminal coupling interactions, but also gave strong cross peaks with C-32 (δ = 19.74/4.54, 4.65) and C-17 (δ = 40.82/4.54, 4.65) in the HMBC experiment indicating the presence of an isopropenyl group. The C-16 methylene protons resonating at δ = 1.40 and 1.52 showed cross-peaks with C-28 at δ =150.96 in the HMBC spectrum (Table 3). The characteristic retro-Diels-Alder fragmentation of **2** (*m/z* = 192, $C_{13}H_{20}O$ and *m/z* = 244, $C_{18}H_{28}$) along with the characteristic ^{13}C-NMR chemical shifts of C-12 and C-13 at δ = 124.34 and 139.64, respectively, suggested that **2** belongs to the $^{\Delta}12-\alpha$ ursane series of triterpenoids with one methylene and one hydroxyl group in the A/B rings and the isopropenyl group in the C/D rings. These observations led to define the structure of **2** as 3β-hydroxy-24-nor-urs-4,12,28-triene triterpene.

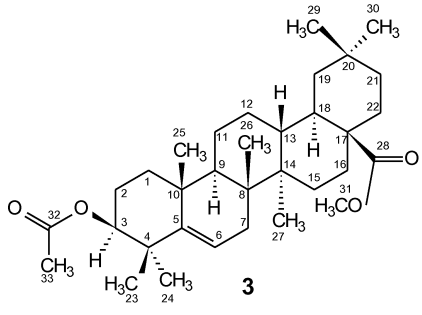
Table 3. HMBC interaction data of compound **2**.

Carbon	Proton
C-4	H-9
C-3	H-2
C-3	H-23
C-11	H-12
C-17	H-31
C-28	H-16
C-28	H-22
C-28	H-32

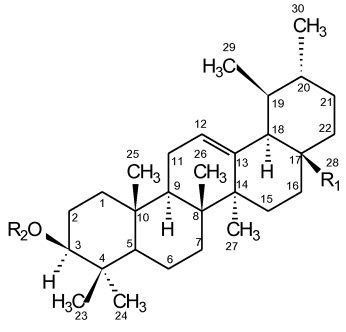


1

2



3



4 " R_1 = CH_3" : " R_2 = $COCH_3$"

5 " R_1 = COOH " : " R_2 = H "

3,28-β-Diacetoxy-5-olea triterpene (**3**), $C_{33}H_{52}O_4$, was obtained as a colorless amorphous compound. The UV spectrum recorded in methanol showed terminal absorptions only, while the infrared spectrum displayed absorptions for C=O ester (1735 cm^{-1}) and C=C (1630 cm^{-1}) groups [10 – 13]. The molecular composition was determined as $C_{33}H_{52}O_4$ by high-resolution electron impact mass measurements of the $[M]^+$ peak (*m/z* = 512.3863) which indicated eight degrees of unsaturation in the molecule. HREIMS of **3** also showed the characteristic fragment ions at *m/z* = 208.1462 (calcd. 208.1463 for $C_{13}H_{20}O_2$), 304.2403 (calcd. 304.2402 for $C_{20}H_{32}O_2$), 148.1252 (calcd. 148.1251 for $C_{11}H_{16}$), and 244.2191 (calcd. 244.2190 for $C_{18}H_{28}$), 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 mass spectrometry, since the molecular ion undergoes retro-Diels-Alder fragmentation [14] of ring B, thereby bisecting the molecule into two major fragments at *m/z* = 208.1462 (calcd. 208.1463 for $C_{13}H_{20}O_2$) and *m/z* = 304.2403 (calcd. 304.2402 for $C_{20}H_{32}O_2$).

The ^{1}H-NMR spectrum ($CDCl_3$, 500 MHz) of **3** (Table 1) showed nine three-proton singlets at δ = 2.13, 2.10, 1.15, 1.08, 0.97, 0.95, 0.94, 0.85, and 0.84 indicating the presence of nine tertiary methyl groups in the molecule. The downfield singlets at δ = 2.10 and 2.13 were due to the C-31, C-33 acetate methyls. The downfield region of the spectrum also contained two signals, *i. e.* a double doublet at δ = 4.50 (dd, $J_{3\alpha,2\alpha}$ = 6.12 Hz, $J_{3\alpha,2\beta}$ = 14.5 Hz) and a broad double doublet at δ = 5.62 ($J_{6,7\alpha}$ = 4.0 Hz, $J_{6,7\beta}$ = 12.5 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 δ = 4.50 (dd, $J_{3\alpha,2\alpha}$ = 6.12 Hz, $J_{3\alpha,2\beta}$ = 14.5 Hz) of the C-3 methine signal indicated an equatorial (β) orientation of the acetate group. Further, the β-orientation of acetate was assigned on the basis of a comparison with those reported in the literature [28]. A comparison of ^{1}H-NMR chemical shifts of **3** with 3α-hydroxy-D-friedoolean-5-ene was also done [14].

The ^{13}C-NMR spectrum [14, 15] ($CDCl_3$, 100 MHz) of **3** exhibited signals for all 33 carbon atoms and further supported the formula derived by mass spectrometric observations. DEPT spectra showed the presence of nine methyl, ten methylene, five methine and (by difference from the broad-band decoupled spectrum) nine quaternary carbons. The downfield signals at δ = 80.94, 121.70, and 170.96 were due to the acetate-bearing C-3, vinylic C-6 and acetate carbonyl C-28, respectively. The eight methyl carbons resonated at δ = 31.87, 31.49, 29.20, 20.00, 19.39, 19.04, 18.04, and 12.05 in the ^{13}C-NMR spectrum. The assignments to the various carbons in the molecule are presented in Table 2.

Two-dimensional NMR techniques such as COSY-45°, HOHAHA, HMQC and HMBC [17] were used to obtain more structural information. The C-3 methine proton resonating at δ = 4.50 showed cross-peaks with the geminally coupled C-2 methylene protons at δ =1.65 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 at δ =1.82 and 1.95. The Homonuclear Hartmann Hahn spectrum [25 – 27] recorded with a 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 methylene protons (δ =1.65/1.85 and 1.45/2.00, respectively).

The C-3 proton at δ = 4.50 showed a cross-peak with the carbon at δ = 80.94, while the vinylic C-6 proton (δ = 5.62) was coupled with the carbon at δ = 121.70 in the HMQC spectrum. Other HMQC interactions are presented in Table 2.

The C-6 vinylic proton (δ = 5.62) showed long-range interactions with C-4 (δ = 40.45) and C-9 (δ = 51.24) in the HMBC spectrum. The C-3 proton (δ = 4.50) showed coupling with C-5 (δ = 140.78), and the C-23 and C-24 methyl protons showed Heteronuclear Shift Correlations with C-3, C-4 and C-5 (Fig. 2).

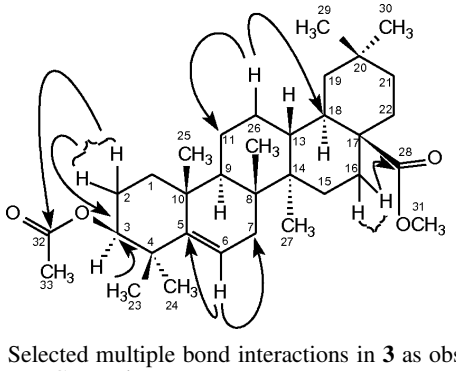


Fig. 2. Selected multiple bond interactions in **3** as observed in the HMBC experiment.

To establish that compound **3** was a genuine natural product and not an artifact, a comparative TLC of compound **3** against the crude ethanolic extract of the fresh plant was studied, thus establishing that compound **3** was a naturally occurring compound as it was present in the crude extract with the same TLC mobilities (R_f = 0.78). On the basis of this spectroscopic evidence, compound **3** was identified as the naturally occurring 3β-28 di-acetyl derivative of 3α-hydroxy-D-friedoolean-5-ene [14] with a β orientation of the C-3 acetate group, and the presence of an acetoxy group at C-17, besides seven regular methyl groups on the saturated pentacyclic triterpene skeleton. These observations led to define the structure of **3** as 3,28-β-diacetoxy-5-olea-triterpene.

α-Amyrin acetate (**4**), m. p. 166 – 170, was isolated as a colorless amorphous solid. The IR spectrum of **4** afforded intense absorptions at 2850 (C–H), 1630 (C=C), and 1136 and 1090 (C–O) cm^{-1}. The high-resolution electron-impact mass spectrum of **4** showed the molecular ion-peak at *m/z* = 468.3708, which corresponded to the molecular formula $C_{32}H_{52}O_2$ and indicated the presence of seven degrees of unsaturation in the molecule. The characteristic retro-Diels-Alder fragmentation of **4** (*m/z* = 250, $C_{16}H_{26}O_2$ and *m/z* = 218, $C_{16}H_{26}$) along with the characteristic ^{13}C-NMR chemical shifts of C-12 and C-13 at δ = 124.33 and 139.63, respectively, suggested that **4** belongs to the $^{\Delta}12 - \alpha$ amyrin series of triterpenoids with an ester group.

Ursolic acid (**5**) was isolated as a greenish powder. Compounds **4** and **5** were isolated for the first time from the flowers of this plant [4].

Experimental Section

General experimental procedures

The mass spectra were recorded on a Jeol HX-110 instrument. The ^{1}H- and ^{13}C-NMR spectra were recorded in $CDCl_3$ at 500, 400, and 125, 75 MHz, respectively, on Bruker AM-500 and 400 NMR spectrometers. The UV and IR spectra were recorded on Shimadzu UV-240 and JASCO A-320 spectrophotometers, respectively. Optical rotations were measured on a polatronic D Polarimeter. The purity of the compounds was checked on TLC (Si-gel, Merck PF_{254}, 0.25 mm thickness). Melting points were determined in glass capillary tubes using Buchi 535 and Gallenkamp 30/MF-370 melting point apparatus.

Plant material

The flowers of *Alstonia scholaris*, (5 kg) were collected from the university campus Kashmir, Pakistan, in October 2006. A voucher specimen (AKUH # 58106) was deposited in the Herbarium of Department of Botany, University of Azad Kashmir, Pakistan.

Extraction and isolation

Air-dried flowers of *Alstonia scholaris* (5 kg dry weight) were extracted with MeOH (50 L). The MeOH extract was concentrated to a gum (822 g), dissolved in distilled water and extracted thoroughly with petrol ether (25 L). The petrol ether-soluble portion was evaporated under reduced pressure to yield a gum (66.92 g) which was chromatographed on a silica gel column (Merck, 70 – 230 mesh, 2025.01 g). The elution of the column was initiated with petrol ether. The combined column sub-fractions 1 – 8 (5.91 g) obtained by elution with 5 : 95 ethyl acetate-petrol ether, which showed similar TLC behavior upon spraying with ceric sulfate reagent, were combined and again subjected to CC using silica gel (type 60, 70 – 230 mesh, 200.10 g), and the column was eluted with petrol ether-ethyl acetate (99 : 1). The sub-fractions 6 – 30 (1.86 g), which showed similar TLC behavior were combined and further purified on preparative TLC plates using a solvent system of petrol ether-ethyl acetate (98 : 2) to afford pure compound **1** (19.5 mg). The fractions obtained on elution of the column with *n*-hexane-ethyl acetate (10 : 90) were checked by TLC. Fractions 7 – 18 showing similar behavior on TLC were combined and further purified by preparative TLC (Merck PF_{254}, 0.2 mm) using $CHCl_3$ as eluent to afford pure compound **2** (28 mg). Elution of the major column which was loaded with 66.92 g of petrol ether-soluble material was eluted with 30 % ethyl acetate-petrol ether to yield an impure mixture (7.83 g). This mixture was again subjected to CC (silica gel, 70 – 230 mesh, 60.20 g). The sub-fractions 6 – 30 (1.86 g), which showed similar TLC behavior, were combined and further purified on preparative TLC plates using a solvent system of petrol ether-ethyl acetate (90 : 10) to afford pure compound **3** (19.5 mg). The fractions obtained with 30 : 70 ethyl acetate-petrol ether yielded an impure compound **4**, which was further purified by preparative TLC using a solvent system of petrol ether-ethyl acetate (70 : 30) to obtain pure **4** (20 mg). Fractions 99 – 145 (1.91 g) obtained by elution with petrol ether-ethyl acetate (35 : 65, 500 mL each) were collected and again subjected to CC (70 – 230 mesh, 60.20 gm). The sub-fractions 7 – 18 (0.92 g, 500 mL each) obtained with 80 : 20 petrol ether-ethyl acetate showed similar TLC behavior (ceric sulfate-active) and were combined and further purified on preparative TLC (Merck PF_{254}, 0.2 mm) using petrol ether-ethyl acetate 70 : 30 as eluent to obtain pure **5** (28 mg, $R_f = 0.1$).

*3β-Acetate-24-nor-urs-4,12-diene ester triterpene (**1**)*

Colorless solid. – $[\alpha]_D^{29}$ = 84 (c = 0.18, $CHCl_3$). – IR ($CHCl_3$): ν_{max} = 1732 (C=O), 1630 (C=C) cm^{-1}. – HRMS (EI): *m/z* = 452.3652 (calcd. 452.3654 for $C_{31}H_{48}O_2$), 234.1618 (calcd. 234.1619), 218.2032 (calcd. 218.2034 for $C_{16}H_{26}$), 174.1406 (calcd. 174.1408 for $C_{13}H_{18}$). – ^{1}H-NMR ($CDCl_3$, 500 MHz) and ^{13}C-NMR ($CDCl_3$, 125 MHz): see Tables 1, 2.

*3β-Hydroxy-24-nor-urs-4,12,28-triene triterpene (**2**)*

Colorless amorphous solid. – $[\alpha]_D^{29}$ = 70.19 (c = 0.10, $CHCl_3$). – IR ($CHCl_3$): ν_{max} = 415 (OH), 1630 (C=C) cm^{-1}. – HRMS (EI): *m/z* = 436.3703 (calcd. 436.3705 for $C_{31}H_{48}O$), 244.2191 (calcd. 244.2190 for $C_{18}H_{28}$), 192.1513 (calcd. 192.1514 for $C_{13}H_{20}O$), 174.1406 (calcd. 174.1408 for $C_{13}H_{18}$). – ^{1}H-NMR ($CDCl_3$, 500 MHz) and ^{13}C-NMR (CD_3OD, 100 MHz): see Tables 1, 2.

*3,28-β-Diacetoxy-5-olea-triterpene (**3**)*

Colorless amorphous compound. – $[\alpha]_D^{29}$ = 62.5 (c = 0.08, $CHCl_3$). – IR ($CHCl_3$): ν_{max} = 1735 (C=O), 1630 (C=C) cm^{-1}. – MS (EI): *m/z* = 512. – HRMS (EI): *m/z* = 512.3863 (calcd. 512.3865 for $C_{33}H_{52}O_4$), 304.2403 (calcd. 304.2402 for $C_{20}H_{32}O_2$), 244.2191 (calcd. 244.2190 for $C_{18}H_{28}$), 208.1462 (calcd. 208.1463 for $C_{13}H_{20}O_2$), 148.1252 (calcd. 148.1251 for $C_{11}H_{16}$). – ^{1}H-NMR (500 MHz, $CDCl_3$) and ^{13}C-NMR (100 MHz, CD_3OD): see Tables 1, 2.

*α-Amyrin acetate (**4**)*

Colorless amorphous solid. – M. p. = 166 – 170°C. – $[\alpha]_D^{29}$ = +83 ($CHCl_3$). – IR ($CHCl_3$): ν_{max} = 2850 (C–H), 1630 (C=C), 1715 (C=O), 1136, 1369 (C–O) cm^{-1}. – MS (EI): *m/z* (%) = 469 ($C_{32}H_{52}O_2$, $[M+H]^+$), 454 $[M+H–CH_3]^+$, 409 (42), 394 (28), 357 (15), 298 (11), 273 (39), 267 (38), 249 (68), 232 (24), 218 (100), 203 (73), 189 (75), 175 (66). – ^{1}H-NMR ($CDCl_3$, 500 MHz): δ = 0.78 (s, 1× CH_3), 0.86 (s, 2 × CH_3), 0.87 (s, 2 × CH_3), 0.97 (s, 1 × CH_3), 1.00 (s, 1 × CH_3), 1.06 (s, 1 × CH_3), 2.02 (s, $COCH_3$), 4.50 (m, 1-3β-H), 5.11 (t, J = 3.6 Hz, C-12H). – ^{13}C-NMR ($CDCl_3$, 125 MHz) [30 – 32]: δ = 171.0, 139.71, 124.41, 81.02, 59.16, 55.35, 47.73, 42.82, 39.73, 39.72, 39.62, 38.56, 36.89, 34.82, 33.36, 31.32, 28.79, 28.45, 28.18, 26.68, 23.68, 23.44, 23.29, 23.29, 21.34, 18.32, 17.55, 16.94, 16.79, 15.77.

*Ursolic acid (**5**)*

Greenish powder. – M. p. = 283 – 285 °C. – $[\alpha]_D^{29}$ = 59 (c = 0.3, pyridine). – IR ($CHCl_3$) ν_{max} = 3493 (OH), 1703 (C=O), 1666 (C=C), 1381 (doublet, gem-dimethyl group) cm^{-1} [32]. – MS (EI): *m/z* = 456.4 $[M]^{+\bullet}$, 438.4, 423.4, 300.3, 248.3, 203.2, 133.1. – HRMS, ^{1}H, ^{13}C-NMR: see ref. [29].

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