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New ursane-type triterpenic esters from the stem bark of *Thevetia peruviana*

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Phytochemical studies on the stem bark of *Thevetia peruviana* resulted in the isolation of six new ursane-type triterpenes, named peruvianursenyl acetate A, peruvianursenyl acetate B, isolupenyl acetate, peruvianursenyl acetate C, lupedienyl acetate and peruvianursenyl glucoside along with two known triterpenoids, namely α-amyrin acetate and lupeol acetate. The structures of the new phytoconstituents have been established as 23 – nor methyl urs-12-en-4α-ethylenic-18α-H-3β-yl acetate, urs-5.21-dien-18α-H-3β-yl acetate, lup-20 (29)-en-3α-yl acetate, urs-12 en-18α- H-3β-yl acetate, lup-5,20 (29)-dien-3β-yl acetate, and urs-12-en-18α-H-3-O-β-D-glucopyranoside, respectively.

1. Introduction

Thevetia peruviana (Pers.) Schum. Ait. f. (Apocynaceae) is an evergreen leafy plant with elegant and shady foliage and scented handsome flowers. The shrub is found on any type of soil throughout India. Its stem bark resembles kurchi bark in appearance and acts as bitter cathartic, emetic, purgative, abortifacient, bactericidal, cardiotonic, alexiteric and rodenticide [1]. It is useful in dropsy, rheumatism, skin diseases, boils, blisters and cancer [1, 2]. All forms of cardiac insufficiency can be successfully treated with it. Peruvoside has already been marketed in Germany under the trade name of Endocordin [3] in the treatment of congestive heart failure. Some triosides, cardenolides, flavonols, phenyl propanolides, iridioids, carbohydrates and monoterpenoids have been reported from the plant [1, 4–10]. The present paper describes the isolation and characterization of six new and two known pentacyclic triterpenic esters from the stem bark of the plant.

2. Investigations, results and discussion

Compound **1**, named as peruvianursenyl acetate A, was obtained as colourless amorphous powder from petroleum ether-chloroform (9 : 1) eluants. It gave a positive Liebermann-Burchard test and exhibited a strong IR absorption band at 1735 cm^{-1} due to an ester group. It had a molecular ion peak in its MS at m/z 480 corresponding to homotriterpenic ester molecule, $C_{33}H_{52}O_2$. The ^1H NMR spectrum of **1** displayed three one-proton each downfield doublets at δ 5.30 (J = 6.20 Hz), 5.06 (J = 3.52 Hz), and 5.04 (J = 3.52 Hz) assigned to H-12, H-31a and H-31b, respectively, and a triplet at δ 5.11 (J = 6.38 Hz) due H-23 vinylic proton. A one-proton double doublet for carbinol proton appeared at δ 4.43, placed at C-3 on the basis of biogenetic analogy, and its coupling interactions of 6.03 and 9.78 Hz indicated its α-orientation. A one-proton doublet at δ 2.28 (J = 4.52 Hz) suggested that the compound **1** is an ursane-type triterpene containing 18 α-proton (ring D/E trans). Five three-proton each singlets at δ 0.95 (Me-24), 0.84 (Me-25), 0.71 (Me-26), 0.93 (Me-27) and 0.90 (Me-27) and two three-proton doublets at δ 0.80 (J = 6.62 Hz, Me-29) and 0.78 (J = 6.62 Hz, Me-30) were attributed to methyl groups of the ursane-type compound, all located on saturated carbons. The remaining methylene and methine proton signals appeared between δ 1.89–1.18 (Table 1). The MS of **1** demonstrated the existence of characteristic ion fragments at at m/z 465 [M-Me]$^+$, 453 [M−CH=CH$_2$]$^+$ and 405 [465-AcOH]$^+$. The base peak at m/z 218 arose due to retro-Diels-Alder fragmentation pattern of ring C [11]. The important ion peaks were observed at m/z 95 [$C_{3,4}$-$C_{5,10}$-$C_{7,8}$ fission; ion a]$^+$, 81 [ion a-CH$_2$]$^+$, 67 [ion a-2 × CH$_2$]$^+$, 154 [$C_{1,10}$-$C_{4,5}$ fission; ion b]$^+$, 222 [$C_{9,10}$-$C_{7,8}$ fission; ion c]$^+$, 286 [$C_{5,6}$-$C_{9,10}$ fission; ion d]$^+$, 272 [ion d-CH$_2$]$^+$, 208 [222-CH$_2$]$^+$, 194 [208-CH$_2$]$^+$, 134 [198-AcOH]$^+$, 148 [208-AcOH]$^+$, 161 [222-AcOH]$^+$, 152 [$C_{14,15}$-$C_{13,18}$ fission; ion g]$^+$, 84 [$C_{17,22}$-$C_{18,19}$ fission; ion h]$^+$, 55 [$C_{19,20}$-$C_{17,22}$ fission, ion H]$^+$, 134 [ion f – ion h]$^+$, 232 [ion e-2 × Me]$^+$, 217 [232-Me]$^+$, 219 [ion e-Ac], 204 [219-Me]$^+$, 189 [204-Me]$^+$, 235 [ion e-CH = CH$_2$]$^+$ and 175 [235-AcOH]$^+$. This fragmentation pattern suggested the saturated nature of ring A and B and acetoxyl group in ring A. Its ^{13}C NMR spectrum (Table 2) showed the presence of 33 carbon atoms. The assignment of the carbon chemical shift were made by comparison with the δ values of the corresponding carbon atoms of urs-12-enes [12]. The olefinic carbons appeared at δ 124.37 (C-12), 139.68 (C-13), 121.54 (C-23) and 98.81 (C-31). The presence of carbon signals at δ 171.31 and 21.18 confirmed the location of the acetyl group in the molecule. Alkaline hydrolysis of the peruvianursenyl acetate **1** produced free alcohol **1a**. Oxidation of **1a** with Jones reagent gave the 3-oxo derivative **1b** which responded positively to Zimmermann test [13] for 3-oxo terpenoids suggesting the presence of the secondary hydroxyl group at C-3. On the basis of these findings compound **1** was identified 23-nor methyl urs-12-en-4α-ethylenic-18α-H-3β-yl acetate. This is a new pentacyclic triterpene of the ursane-series and is being reported from any natural source for the first time.

Compound **2**, designated peruvianursenyl acetate B, was obtained as colourless granules from petroleum ether-chloroform (9 : 1) fractions. Its IR spectrum showed the presence of an ester group (1733 cm^{-1}). It had a molecular ion peak at m/z 466 consisting to a molecular formula, $C_{32}H_{50}O_2$. Its ^1H NMR spectrum showed two one-proton each downfield triplets at δ 5.18 (J = 3.26 H_z) and 5.11 (J = 3.35 H_z) assigned to H-12 and H-6, respectively. A one-proton double doublet at δ 4.52, showing coupling interaction of 8.97 and 6.00 Hz was associated with a 3α-carbinol proton. A doublet at δ 2.28 (J = 5.10 Hz) and two methyl signals as doublets at δ 0.96 (J = 6.29 Hz) and 0.86 (J = 6.12 Hz) suggested that the compound **2** is an ursane type triterpene containing 18 α-proton (ring D/E trans). The six tertiary methyl signals resonated as three-proton singlets at δ 1.12 (Me-23), 1.06 (Me-24), 1.00 (Me-25), 0.92 (Me-26) and 0.76 (Me-28). The presence of a three-proton singlet at δ 2.04 supported acetyloxy group in the molecule and from the biogenetic point of view it was placed at C-3. The remaining methine and methylene signals appeared between δ 1.99 and 1.25 (Table 1). The MS of **2** was characteristic of pentacyclic triterpenes of the ursane series in which rings B and C were unsaturated [10]. The retro-Diels-Alder fragmentation of **2** generated the base peak at m/z 218 [ion a]$^+$ and an ion at m/z 248 [ion b]$^+$. Elimination of different groups from these ions yielded fragments at m/z 135 [ion a-C_6H_{12}, 84]$^+$, 203 [ion

Table 1: ^{1}H NMR chemical shifts of compounds 1, 2, 3, 5 and 6 ($CDCl_3$)

H	1	2	3	5	6
1a	1.32 dddd (4.88, 8.76, 8.54, 5.30)	1.31 dddd (5.21, 9.10, 9.25, 4.36)	1.33 dddd (4.72, 5.34, 5.50, 9.22)	1.31 dddd (5.26, 8.98, 9.32, 4.62)	1.31 dddd (4.92, 9.12, 9.26, 5.24)
1b	1.74 dddd (3.92, 11.32, 8.76, 8.54)	1.68 dddd (4.26, 0.25, 5.21, 12.14)	1.63 dddd (5.34, 5.50, 11.72, 8.62)	1.68 dddd (4.62, 9.32, 11.48, 5.66)	1.65 dddd (4.12, 8.70, 11.50, 5.24)
2a	1.61 m	1.60 m	1.61 m	1.62 m	1.61 m
2b	1.54 m	1.52 m	1.60 m	1.52 m	1.52 m
3	4.43 dd (6.03,9.78)	4.52 dd (8.97,6.00)	4.48 dd (5.05, 6.10)	4.50 dd (5.90,8.48)	4.49 dd (5.10, 9.52)
5	1.58 t (7.30)	–	1.40 t (4.70)	1.29 t (4.52)	–
6a	1.45 m	5.11 t (3.35)	1.38 m	1.36 m	5.12 t (7.09)
6b	1.24 m	–	1.28 m	1.52 m	–
7a	1.47 m	1.90 d (6.20)	1.37 dddd (8.66, 5.38, 9.14, 4.62)	1.38 dddd (4.60, 8.56, 11.32, 5.4)	1.89 d (5.82)
7b	1.18 m	1.63 d (3.35)	1.60 dddd (9.14, 8.78, 11.41, 4.62)	1.33 dddd (5.24, 9.50, 11.62, 5.78)	2.00 d (11.20)
9	1.56 t (3.90)	1.56 t	1.35 t (4.12)	1.54 dd (4.5, 8.96)	1.41 dd (4.50, 8.27)
11a	1.89 dd (3.90, 6.20)	1.99 dd (4.34, 5.26)	1.89 m	1.90 m	1.28 m
11b	1.82 dd (3.90, 12.8)	1.86 dd (5.26, 6.18)	1.68 m	1.65 m	1.61 m
12a	5.30 d (6.20)	5.18 t (3.26)	1.20 m	5.12 dd (3.56, 3.56)	1.25 m
12b	–	–	1.92 m	–	–
13b	–	–	1.93 m	–	1.31 dd (8.60, 9.26)
15a	1.18 m	1.29 dddd (4.98, 8.78, 9.20, 4.52)	1.89 m	1.28 dddd (5.20, 8.84, 9.52, 4.56)	1.31dddd (5.60, 9.10, 8.78, 4.90)
16a	1.84 dddd (4.38, 8.86, 9.52, 5.32)	1.89 dddd (4.52, 8.78, 11.26, 5.78)	1.89 m	1.89 dddd (4.56, 8.84, 11.39, 50)	1.40 dddd (8.78, 4.90, 11.38, 5.62)
16b	1.30 dddd (8.86, 5.86, 12.62, 9.80)	1.35 dddd (5.78, 9.10, 9.34, 4.72)	1.31 dddd (9.72, 5.54, 9.08, 11.84)	1.63 dddd (8.84, 9.50, 5.52, 11.28)	1.63 dddd (84.90, 9.16, 11.32, 8.64)
18	2.28 d (4.52)	2.28 d (5.10)	2.36 m	2.34 d (5.87)	2.36 m
19	1.56 dd (4.52, 9.54)	1.56 dd (5.10, 9.26)	1.64 m	1.65 dd (10.50, 9.24)	1.63 m
20b	1.90 m	1.92 m	–	1.62 m	–
21 a	1.18 m	1.33 m	2.04 m	1.99 m	2.17 m
21b	1.54 m	1.54 m	1.64 m	1.90 m	1.61 m
22a	1.24 dddd (4.66, 8.72, 8.90, 5.38)	1.25 m	1.28 m	1.52 m	1.36 dddd (5.60, 9.88, 8.48, 4.98)
22b	1.28 dddd (6.32, 8.90, 5.38, 12.16)	1.41 dddd (9.20, 8.78, 5.38, 11.86)	1.31 dddd (9.72, 5.54, 9.08, 11.84)	1.12 dddd (4.52, 9.45, 11.36, 8.58)	1.91 dddd (4.98, 11.32, 9.88, 8.64)
23	5.11 t (6.38)	1.12 s	1.06 s	1.25 s	1.06 s
24	0.95 s	1.06 s	0.96 s	1.06 s	1.02 s
25	0.84 s	1.00 s	0.85 s	1.00 s	0.88 s
26	0.71 s	0.92 s	0.78 s	0.97 s	0.97 s
27	0.93 s	0.82 s	0.93 s	0.83 s	0.93 s
28	0.90 s	0.76 s	0.87 s	0.79 s	0.91 s
29	0.80 d (6.62)	0.96 d (6.29)	4.68 d, 4.57 d (2.20) (1.35)	0.96 d (6.22)	4.68 d, 4.56 d (2.07) (2.06)
30	0.78 d (6.62)	0.86 d (6.12)	1.68 s	0.86 d (6.50)	1.68 s
31a	5.06 d (3.52)	–	–	–	–
31b	5.04 d (3.52)	–	–	–	–
OAc	1.98 s	2.04 s	2.04 s	2.04 s	2.04 s

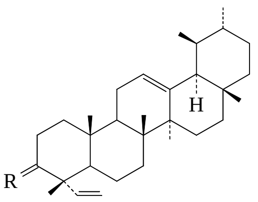
The coupling constant (s) in Hertz are given in parenthesis; a = α, b = β.

a-Me]$^+$, 233 [ion b-Me]$^+$, 205 [ion b-Ac]$^+$, 189 [205-Me]$^+$ and 188 [ion b-AcOH]$^+$. The ions peaks at m/z 81 [$C_{3,4}$-$C_{5,10}$-$C_{7,8}$ fission, ion c]$^+$, 67 [ion c-CH_2]$^+$, 142 [$C_{1,10}$-$C_{4,5}$-fission, ion d]$^+$, 257 [$C_{9,10}$-$C_{7,8}$ fission, ion e]$^+$, 208 [M-ion e, ion f]$^+$, 194 [ion f-CH_2]$^+$, 165 [ion f-Ac]$^+$, and 148 [ion f-AcOH]$^+$, indicated the existence of an olefinic linkage in ring B at Δ^5. The saturated nature of rings D and E was inferred from the ion peaks appearing at m/z 152 [$C_{13,18}$-$C_{14,15}$ fission, ion c]$^+$, 138 [ion c-CH_2]$^+$, 124 [138-CH_2]$^+$, 84 [$C_{18,19}$-$C_{17,22}$ fission, ion h]$^+$, 69 [ion h-CH_2]$^+$ and 55 [69-CH_2]$^+$. The ^{13}C NMR spectrum of **2** showed 32 carbon atoms and the values were compared with that of ursane-type molecules [12]. The signals of vinylic carbons appeared at δ 145.53 (C-5), 121.53 (C-6), 124.57(C-12) and 139.78 (C-13). Alkaline hydrolysis of **2** provided free alcohol, peruvianursenol B (**2a**). Oxidation of **2a** with Jones reagent gave peruvianursenone B (**2b**). The latter gave a positive Zimmermann test [13] indicating the presence of the 3-oxo group. On the basis of these data, the structure of **2** was elucidated as urs-5, 12-dien-18 α-H-3β-yl acetate. This is a new member of ursane-type triterpenes.

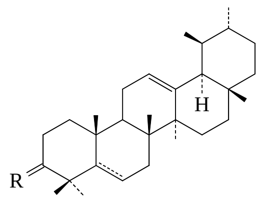
Compound **3**, named isolupenyl acetate, was isolated from the petroleum ether-chloroform (9 : 1) eluants of the column. Its molecular formula was determined to be $C_{32}H_{52}O_2$ (m/z 468 [M]$^+$) by electron impact mass and ^{13}C NMR spectra. It responded to Burchard-Liebermann test positively and showed the presence of an ether group (1735 cm^{-1}) in its IR spectrum. The ^{1}H NMR spectrum of **3** accounts for six tertiary methyl singlets, all attached to saturated carbons, at δ 1.06 (Me-23), 0.96 (Me-24), 0.85 (Me-25), 0.78 (Me-26), 0.93 (Me-27) and 0.87 (Me-28), a methyl group singlet attached at olefinic carbon at δ 1.68 (Me-30), acetyl group singlet at δ 2.04, a β-carbinol proton as a double doublet at δ 4.48 having coupling interactions of 5.05 and 6.10 Hz and two one-proton each C-29 exo cyclic methylene doublets at δ 4.68 (J = 2.20 Hz) and 4.57 (J = 1.35 Hz). The MS of **3** was characteristic of pentacylic triterpenes of the lupene-series in which all the rings were saturated [11]. The base peek at m/z 218

Table 2: ^{13}C NMR chemical shifts of compounds 1–7 ($CDCl_3$)

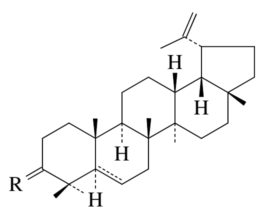
Carbon	1	2	3	4	5	6	7
1	38.53	38.36	40.07	38.08	38.03	38.12	38.79
2	26.61	26.63	23.43	27.52	27.31	23.73	27.47
3	81.07	81.08	80.97	80.68	80.62	81.01	80.98
4	37.72	37.83	38.53	37.88	37.83	38.44	38.49
5	55.04	145.53	55.37	55.40	55.47	139.65	55.41
6	18.21	121.53	17.95	18.40	18.13	124.35	18.27
7	32.80	32.65	34.15	32.66	32.16	18.25	34.24
8	41.08	40.13	40.95	41.26	40.05	40.04	40.04
9	47.66	47.83	50.79	47.84	47.89	50.38	50.38
10	36.81	36.86	37.16	37.14	37.03	37.18	37.17
11	23.22	23.35	21.31	23.25	23.41	21.38	21.34
12	124.37	124.57	25.78	122.84	122.36	25.13	25.12
13	139.68	139.78	38.42	139.52	139.85	35.60	38.07
14	41.63	41.68	42.77	41.64	41.87	41.98	42.10
15	28.81	28.80	27.55	28.62	28.65	27.48	27.99
16	23.65	23.81	28.52	27.24	27.31	28.11	35.60
17	33.72	33.82	55.25	33.08	33.03	55.42	43.03
18	59.01	59.19	48.06	59.02	59.51	48.32	48.03
19	39.83	39.89	47.46	39.42	39.96	47.85	47.66
20	39.56	39.75	151.91	38.16	38.42	150.98	150.97
21	29.70	29.71	29.12	31.48	31.06	29.87	29.86
22	31.27	31.30	39.83	37.01	37.14	39.66	39.66
23	121.54	28.46	28.47	28.78	28.47	27.99	28.10
24	15.87	15.76	16.00	17.26	16.47	16.01	16.01
25	14.18	15.48	16.45	16.08	16.00	16.21	16.53
26	16.70	16.80	16.37	17.30	17.33	16.52	16.22
27	23.30	23.72	14.45	24.02	23.55	14.54	15.77
28	27.89	28.15	17.89	29.10	29.15	19.25	18.03
29	17.53	17.73	109.81	17.62	17.83	109.39	109.39
30	21.34	23.21	19.77	23.98	21.39	20.97	20.97
31	98.81	–	–	–	–	–	–
OAc	171.31	170.91	170.53	171.03,	171.73	171.04	171.04
	21.18	21.37	21.45	21.36	21.37	21.34	21.34



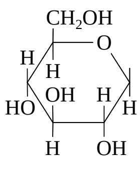
1 R = α-H, β-OAc
1a R = α-H, β-OH
1b R = O



2 R = α-H, β-OAc, 18α-H, Δ^5
2a R = α-H, β-OH, 18α-H, Δ^5
2b R = O, 18α-H, Δ^5
4 R = α-H, β-OAc, 18β-H
4a R = α-H, β-OH, 18β-H
5 R = α-H, β-OAc, 18α-H
5a R = α-H, β-OH, 18α-H
5b R = O, 18α-H
8 R = α-H, β



3 R = 3β-H, α-OAc
3a R = 3β-H, α-OH
3b R = O
6 R = α-H, β-OAc, Δ^5
6a R = α-H, β-OH, Δ^5
6b R = O, Δ^5
7 R = α-H, β-OAc
7a R = α-H, β-OH



18α-H

[ion a]$^+$ and another ion at m/z 250 [ion b]$^+$ were arose due to cleavage of Ca, 11, 14 linkages. The ion fragments at m/z 258 [$C_{9,10}$-$C_{7,8}$ fission, ion c]$^+$, 83 [$C_{3,4}$-$C_{5,10}$, $C_{7,8}$ fission, ion d]$^+$, 69 [83-CH_2]$^+$, 55 [69-CH_2]$^+$, 177 [ion a- C_3H_5]$^+$, 162 [177-Me]$^+$, 203 [ion a-Me]$^+$, 189 [$C_{12,13}$-$C_{8,14}$ fission, ion e]$^+$, 175 [ion e-Me]$^+$, 160 [ione-2 × Me]$^+$, 150 [$C_{13,18}$-$C_{14,15}$ fission ion f]$^+$, 109 [ion f-C_3H_5]$^+$, 135 [ion f-Me]$^+$, 120 [ion f-2 × Me]$^+$, 136 [ion f-CH_2]$^+$, 95 [136-C_3 H_5]$^+$, 121 [136-Me]$^+$, 106 [136-2 × Me]$^+$, 122 [ion f-2 × CH_2]$^+$, 81 [122-C_3H_5]$^+$, 107 [122-Me]$^+$ and 82 [$C_{18,19}$-$C_{17,22}$ fission, ion g]$^+$ supported the saturated nature of carbocylic rings. The ^{13}C NMR spectrum displayed the presence of acetate carbons (δ 170.53, 21.45), vinylic carbons at δ 151.91 (C-20) and 109.81 (C-29) typical of lupenes and carbinol carbon at δ 80.97 (C-3). Treatment of **3** with ethanolic potassium hydroxide at reflux temperature afforded a free alcohol (**3a**) which on further treatment with Jones reagent yielded the ketone **3b** responding positively to the Zimmermann test [13] for 3-oxo triterpenoids. On the basis of these findings, compound **3** was identified as lup-20(29)-en-3α-yl acetate. This is a new isomer of lupenyl acetate.

Compound **4**, α- amyrin acetate, was obtained as a colourless amorphous powder from petroleum ether-chloroform (9 : 1) eluants. It was identified as urs-12-en-3β-yl acetate by comparing melting and mixed melting points, Co-TLC, specific rotation and analysis of spectral data. This is a known phytoconstituent.

Compound **5**, designated as peruvianursenyl acetate C, was obtained as a colourless amorphous powder from petroleum ether-chloroform (9 : 1) eluants. It responded positively to the Liebermann-Burchard test and showed characteristic IR absorption band for an ester group (1732 cm^{-1}). Its molecular formula was established as $C_{32}H_{52}O_2$ (M^+ m/z 468) corresponding to a pentacylic triterpene on the basis of MS and ^{13}C NMR spectral data. The spectrum showed ion peaks of diagnostic importance at m/z 218 and 249 generated due to retro-Diels Alder fragmentation of ring C characteristic of α-and β-amyrins type triterpenes [11] and other important ions at m/z 409 [M-AcOH]$^+$, 394 [409-Me]$^+$, 379 [394-Me]$^+$, 364 [379-Me]$^+$, 203 [218-Me]$^+$, 187 [203-Me]$^+$, 189 [249-AcOH]$^+$, 174 [189-Me]$^+$ and 159 [174-Me]$^+$. The cleavage of rings A and B through $C_{5,10}$-$C_{2,3}$-$C_{7,8}$/$C_{6,7}$/$C_{5,6}$ fission, $C_{1,10}$-$C_{4,5}$ fission and $C_{9,10}$-$C_{7,8}$/$C_{6,7}$/$C_{5,6}$ fissions formed fragments at m/z 142,182, 196, 210, 286, 272 and 258. The ions at m/z 152, 124, 84, 137 [152-Me]$^+$, 122 [137- Me]$^+$, 107 [122-Me]$^+$ and 109 [124-Me]$^+$ were arose due to fission of rings D and E. The ^{1}H NMR spectrum of **5** exhibited signals for a C-12 vinylic proton as double doublet at δ 5.12 (J = 3.56, 3.56), an equatorial acetoxy group (double doublet at δ 4.50 for 3α-H having coupling interactions of 5.90 and 8.48 Hz), a three proton singlet at δ 2.04 for acetoxyl group, six three-proton each singlets for tertiary methyls between δ 1.25–0.83 and two three-proton doublets at δ 0.96 (J = 6.22 Hz) and 0.86 (6.50) associated with C-29 and C-30 secondary methyls, respectively. The resonance of all these methyls in the range δ 1.25–0.83 indicated the attachment of these groups to saturated carbons. A one-proton doublet at δ 2.34 having coupling constant of 5.87 Hz supported that **5** was an ursane type triterpene containing 18α-proton (rings D/E trans). The remaining methylene and methine protons resonated between δ 1.90–1.28. In the ^{13}C NMR spectrum the olefinic carbons appeared at δ 122.36 (C-12) and 139.85 (C-13). The δc values were compared with the corresponding carbons of the ursane-type molecules [12]. Alkaline hydrolysis of 5 produced the free alcohol, peruvianursenol C (**5a**), which on treatment with Jones reagent yielded a 3-oxo derivative (**5b**). The keto compound **5b** responded positively to the Zimmermann test [13] for 3-oxo terpenoids suggesting the presence of a secondary hydroxyl group at C-3 in **5a** and, hence, acetoxyl group at C-3 in **5**. From these results the structure of **5** has been formulated as urs-12-en-18α-H-3β-yl acetate. This is a new 8α-H ursane-type triterpene.

Compound **6**, named lupedienyl acetate, was obtained as a colourless crystalline mass from petroleum ether-chloroform (9 : 1) eluants. It was analyzed for $C_{32}H_{52}O_2$ (m/z 466 $[M]^+$). Its IR spectrum demonstrated an absorption band for acetate group (1735 cm^{-1}). The ^1H NMR spectrum of **6** exhibited signals for vinylic protons on C-6 at δ 5.12 as triplet (J = 7.09 Hz) and on C-29 as one-proton doublets at δ 4.68 (J = 2.07 Hz), and 4.56 (J = 2.06 Hz), 3α-carbinol proton as a double doublet at δ 4.49 (5.10, 9.52 Hz), a methyl group attached to unsaturated carbon at δ 1.68 (Me-30) and six tertiary methyls at δ 1.06 (Me-23), 1.02 (Me-24), 0.88 (Me-25), 0.97 (Me-26), 0.93 (Me-27) and 0.91 (Me-28). These data suggested that **6** was a pentayclic triterpene of lupene series containing one olefinic linkage in the carbocylic framework. The MS of **6** showed the important ions associated with lupenes [11]. The significant peaks at m/z 81 [$C_{3,4}$-$C_{5,10}$-$C_{7,8}$ fission, ion a]$^+$, 67 [ion a-CH_2]$^+$, 258 [$C_{7,8}$-$C_{9,10}$ fission, ion b]$^+$, 248 [$C_{9,11}$-$C_{8,14}$ fission, ion d]$^+$, and 218 [M-ion, e]$^+$, generated due to cleavage of $C_{8,14}$-$C_{9,11}$ linkage, 203 [ion e- Me]$^+$, indicated the location of the olefinic linkage at C-5. The ions at m/z 189 [$C_{8,14}$-$C_{12,13}$ fission, ion]$^+$, 150 [$C_{14,15}$-$C_{13,18}$ fission, ion g]$^+$, 136 [ion g-CH_2]$^+$, 122 [$C_{16,17}$-$C_{13,18}$ fission, ion h]$^+$, 107 [ion h-Me]$^+$, and 95 suggested the saturated nature of rings C, D and E. The ^{13}C NMR spectrum of **6** exhibited 32 carbon atoms (acetate carbon signals, δc 171.0, 80, 21.34). The olefinic carbons resonated at δ 139.65 (C-5), 124.35 (C-6), 150.98 (C-20) and 109.39 (C-29). The signals for C-20 and C-29 were characteristic of lupenes. Alkaline hydrolysis of **6** furnished a free alcohol **6a**. Jones oxidation of **6a** yielded a 3-oxo derivative **6b**) which showed the positive Zimmermann test [13] for 3-oxo triterpenes. These data led to assign the structure of **6** as lup-5, 20(29)-dien-3β-yl acetate. This is a new lupene-type triterpenes.

Compound **7**, lupeol acetate, was isolated as a colourless amorphous powder from petroleum ether – chloroform (9 : 1) eluants. It has molecular ion peak at m/z 468 ($C_{32}H_{52}O_2$), in its MS and yielded lupeol (**7a**) on alkaline hydrolysis. Its structure was established on the basis of m.p. specific rotation, spectral data analysis and Co-TLC of the deacetylated product with lupeol as lup-20(29)-en-3β-y1 acetate.

Compound **8**, named peruvianursenyl glucoside, gave a positive test for a triterpenic glycosides. Its IR spectrum exhibited absorption bands for a glycoside (3480, 3365, 1015 cm^{-1}). Its MS showed a molecular ion peak at m/z 588 consistent to a triterpenic glycoside, $C_{36}H_{60}O_6$. The ion fragments at m/z 408 [M-$C_6H_{12}O_6$]$^+$, 180 [$C_6H_{12}O_6$]$^+$ and 163 [$C_6H_{11}O_5$]$^+$ supported the presence of a glucose moiety in the molecule. The fragments generated at m/z 218 due to retro-Diels-Alder fragmentation and at m/z 258 [$C_{7,8}$-$C_{9,10}$ fission]$^+$ and 152 [$C_{14,15}$-$C_{13,18}$ fission]$^+$ indicated the presence of olefinic linkage in ring C at Δ^{12} and carbinol proton in ring A which was placed at C-3 on the basis of biogenetic analogy. The ^1H NMR spectrum of **8** showed a one-proton downfield triplet at δ 5.18 (J = 3.59 Hz) assigned to H-12, a one-proton double doublet at δ 4.47 (J = 9.68, 5.81 Hz) ascribed to H-3α and six tertiary methyl signals at δ 1.25 (Me-23), 1.18 (Me-24), 1.00 (Me-25), 0.91 (Me-26), 0.84 (Me-27) and 0.79 (Me-28). Two three-proton doublets at δ 0.97 (J = 6.50 Hz) and 0.87 [J = 6.0 Hz) associated with C-29 and C-30 secondary methyls and a one- proton doublet at δ 2.75 with coupling interaction of 6.35 Hz accounted to H-18α, suggested an ursane-type carbon framework of the molecule possessing a D/E trans system. The signals for a glucose moiety appeared at δ 4.60 (anomeric), 4.56 (H-2′), 3.49 (H-3′), 3.63 (H- 4′), 4.50 (H-5′) and 4.13, 4.05 (H-6′). Acid hydrolysis of **8** yielded D-glucose and an aglycone (**8a**) which was identified as peruvianursenol C by the direct comparison with the sample (Co-TLC, melting point). On the basis of these findings, **8** was identified as urs-12-en-18α-H-3-0-β-D-glucopyranoside. This is a new triterpenic glycoside and the first report of occurrence of a triterpenic glycoside in *T. peruviana.*

3. Experimental

M.p.s: uncorr.; $[\alpha]_D^{22}$: Abes Polarimeter, $CHCl_3$; IR: Jasco FT/IR-5000, KBr; UV: Beckman DU-64,MeOH; ^1H NMR Avance DRY 400, Bruker (400-MHz) VarianT 400 A, $CDCl_3$, with TMS as int. standard; ^{13}C NMR: 100-MHz Varian T 400 A, $CDCl_3$ MS: JEOL-JMS-DX.303; CC: silica gel (Qualigen), 60−120 mesh; TLC: silica gel G (Merck). The spots were visualised by exposure to I_2 vapours, UV radiation and by spraying with ceric ammonium sulphate and perchloric acid. For ^1H NMR data see Table 1, for ^{13}C NMR data Table 2.

3.1. Plant material

The bark of *T. peruviana* was freshly collected from the plants in September 1997 from the Hamdard University campus and identified by Dr. M. P. Sharma, Department of Botany, Jamia Hamdard. A voucher specimen has been deposited in the herbarium of the department.

3.2. Extraction

The bark was dried for 48 h under shadow and another 48 h in an oven at 50 °C, coarsely powdered (3 kg) and extracted exhuastively with alcohol in a Soxhlet apparatus. The total extract was concentrated under reduced pressure to get a dark brown viscous mass (650 g).

3.3. Isolation of chemical constituents

The extract (400 g) was dissolved in minimum amount of methanol and adsorded on silica gel. The dried slurry was chromatographed over a silica gel column prepared in petroleum ether. The column was eluted with petroleum ether, chloroform and methanol in order of increasing polarity to isolate the following compounds :

3.3.1. Peruvianursenyl acetate A (**1**)

Elution of the column with petroleum ether-$CHCl_3$ (9 : 1) (fractions 15 to 19) furnished colourless amorphous powder of **1**, recrystallized from $CHCl_3$ (1 : 1), 0.61g (0.02% yield). R_f : 0.3829 (EtoAc-$CHCl_3$-petroleum ether 0.2 : 1 : 9); m.p.: 157–158 °C, $[\alpha]_D^{22}$ + 3.8° ($CHCl_3$); UV λ_{max} (MeOH) 214 nm (log ∈ 5.3); IR ν_{max} (KBr) 2940, 2830, 1735, 1465, 1375, 1242, 1100, 1020, 905, 795 cm^{-1}; EIMS m/z (ret. int.) 480 [M]$^+$ ($C_{33}H_{52}O_2$) (3.2), 465(39.1), 453(12.8), 405(10.5), 369(3.1), 386(2.9), 273(17.4), 366(2.9), 236(2.9), 273(17.4), 257(14.1), 249(18.6), 235(6.1), 232(12.3), 222(3.7), 221(9.8), 219(9.8), 218(100), 217(10.3), 208(2.9), 204(25.8), 203(84.5), 194(2.5), 189(92.4), 175(30.7), 161(39.8), 154(2.3), 152(4.2), 135(69.3), 134(51.4), 122(55.9), 109(51.0), 95(58.6), 84(7.6), 81(51.8), 69(57.4), 67(17.2), 55(38.8).

3.3.1.1. Hydrolysis of **1**

Compound **1** (50 mg) was refluxed with 0.5 N ethanolic KOH solution (10 ml) for 3 h to obtain peruvianursenol, (**1a**) TLC comparable, mp 161 to 162 °C, ν_{max} 3410 cm^{-1}.

3.3.1.2. Oxidation of **1a**

Compound **1a** (10 mg) was dissolved in acetone (20 ml). Freshly prepared Jones reagent (3 ml) was added slowly until a brown colour persisted. After usual work-up, a 3-oxo derivative of (**1b**) was produced, TLC comparable.

3.3.2. Peruvianursenyl acetate B (**2**)

Further elution of the column with petroleum ether-$CHCl_3$ (9 : 1), fractions (20–27), gave colourless granular powder of **2**, recrystallized from $CHCl_3$-MeOH (1 : 1), 0.2 g (0.007% yield); R_f: 0.3756 (EtOAc-$CHCl_3$-petroleum ether; 0.2 : 1 : 9); m.p.: 173–175 ºC; $[\alpha]_D^{22}$ + 24.35° ($CHCl_3$); UV λ_{max} (MeOH) 213 nm (log ∈ 3.7); IR ν_{max} (KBr) 2940, 2830, 1733, 1660, 1460, 1375, 1245, 1020, 995 cm^{-1}; EIMS m/z (ret. Int.) 466[M]$^+$($C_{32}H_{50}O_2$) (36.5), 451(11.2), 407(15.7), 392(8.4), 367(2.3), 286(2.9), 284(2.0), 270(9.8), 257(13.2), 248(14.8), 233(7.5), 218(100), 208(2.3), 205(33.4), 203(99.8), 194(2.0), 189(93.2), 188(11.2), 175(29.4), 165(2.9), 160(31.4), 152(2.9), 148(33.9), 142(4.5), 138(24.5), 135(57.1), 124(34.5), 121(42.9), 119(41.0), 109(44.6), 106(40.6), 95(50.3), 84(12.0), 81(41.5), 69(43.9), 67(17.2), 55(34.6).

3.3.2.1. Hydrolysis of **2**

Compound **2** (50 mg) was refluxed with 0.5 N ethanolic KOH solution (10 ml) for 3 h to obtain **2a**, TLC comparable, m.p. 181–183 °C, ν_{max} 3420 cm^{-1}.

3.3.2.2. Oxidation of **2a**

The compound **2a** (15 mg) was dissolved in Me_2CO (20 ml) and treated with Jones reagent (4 ml) to yield the 3-oxo-derivative **2b**, TLC comparable, m.p. 158–159°, ν_{max} 1710 cm^{-1}.

3.3.3. Isolupenyl acetate (**3**)

Elution of the column with petroleum ether-$CHCl_3$ (9 : 1), fractions 28–37, afforded colourless amorphous powder of **3**, recrystallized from $CHCl_3$-MeOH (1 : 1), 0.24 g (0.008% yield); R_f: 0.34 (EtOAc-$CHCl_3$-petroleum ether 0.2 : 1 : 9); m.p.: 113–114°C; $[\alpha]_D^{22}$ + 32.25° ($CHCl_3$); UV λ_{max} (MeOH) 215 nm (log ∈ 4.71); IR ν_{max} (KBr) 2970, 2830, 1735, 1470, 1375, 1245, 1100, 1012, 975, 880 cm^{-1}. EIMS m/z (ret. Int.) 468 $[M]^+$ ($C_{32}H_{52}O_2$) (26.6), 453(2.7), 408(7.7), 393(5.9), 356(2.5), 258(6.9), 250(12.6), 218(100), 204(21.0), 203(32.7), 189(69.7), 177(6.7), 175(17.3), 162(9.4), 160(19.7), 150(13.0), 136(27.0), 135(38.7), 122(23.5), 121(32.7), 120(22.8), 109(30.8), 107(28.8), 106(16.9), 95(34.3), 83(7.3), 82(26.1), 69(26.3), 55(21.1).

3.3.3.1. Hydrolysis of **3**

Compound **3** (25 mg) was refluxed with 0.5 N ethanolic KOH solution (10 ml) for 4 h. After usual work-up, a deacetylated product (**3a**) was obtained, IR ν_{max} (KBr) 3395 cm^{-1}, TLC comparable.

3.3.3.2. Oxidation of **3a**

The compound **3a** (10 mg) was dissolved in acetone (10 ml) and oxidized with Jones reagent (5 ml) to yield the 3-oxo-derivative **3b**. IR ν_{max} (KBr) 1705 cm^{-1}, TLC comparable.

3.3.4. α-Amyrin acetate (**4**)

The second crop of compound **3** furnished a colourless amorphous powder of **4**, recrystallized from $CHCl_3$-MeOH (1 : 1), 0.96 g (0.032% yield); R_f: 0.3948 (ethyl acetate-chloroform-petroleum ether 0.2 : 1 : 9); m.p.: 223–225 °C; $[\alpha]_D^{22}$ + 23.80° ($CHCl_3$); UV λ_{max} (MeOH) 215 nm (log ∈ 4.4.6); IR ν_{max} (KBr) 2920, 2845, 1730, 1640, 1460, 1365, 1245, 1100, 1020, 980, 900 cm^{-1}; EIMS m/z (ret. Int.) 468 $[M]^+$ ($C_{32}H_{52}O_2$) (6.9).

3.3.4.1. Alkaline hydrolysis of **4**

Compound **4** (20 mg) was refluxed with 0.5 N ethanolic KOH solution (10 ml) for 3 h. After usual work-up, α-amyrin (**4a**) was obtained; m.p. 184–185 °C.

3.3.5. Peruvianursenyl acetate (**5**)

Elution of the column with $CHCl_3$-petroleum ether (1 : 9), fractions (38 to 45), furnished colourless amorphous powder of **5**, recrystallized from $CHCl_3$-MeOH (1 : 1), 0.63 g (0.02% yield); R_f: 0.4062 (EtOAc-chloroform-petroleum ether 0.2 : 1 : 9); m.p.: 137–138 °C; $[\alpha]_D^{22}$ + 19.69° ($CHCl_3$); UV λ_{max} (MeOH) 214 nm (log ∈ 4.38); IR ν_{max} (KBr) 2945, 2850, 1732, 1650, 1465, 1375, 1245, 1010, 980, 875 cm^{-1} ; EIMS m/z (ret. int.) 468 $[M]^+$ ($C_{32}H_{52}O_2$) (2.1), 409(24.8), 394(11.2), 379(3.7), 364(2.5), 286(3.0), 272(27.9), 258(3.4), 249(3.1), 232(22.8), 218(100), 210(3.2), 203(29.1), 196(4.2), 189(55.6), 187(22.6), 182(9.0), 174(38.9), 159(19.3), 152(7.8), 147(29.4), 144(13.9), 142(5.0), 137(35.9), 124(41.5), 122(47.2), 109(55.6), 107(41.2), 95(74.3), 84(11.9), 83(45.3), 69(87.5), 55(80.7).

3.3.5.1. Hydrolysis of **5**

Compound **5** (25 mg) was refluxed with 0.5 N ethanolic KOH solution (10 ml) for 3 hr. After usual work-up, the deacetylated product **5a** was obtained, m.p. 146–147 °C, IR ν_{max} 3410 cm^{-1}.

3.3.5.2. Oxidation of **5a**

Compound **5a** (10 mg) was oxidized with Jones reagent (5 ml) in acetone to obtain the 3-oxo-derivative (**5b**). TLC comparable.

3.3.6. Lupedienyl acetate (**6**)

Elution of the column with $CHCl_3$-petroleum ether (1 : 9), fractions 46 to 57, furnished colourless granular powder of **6**, recrystallized from $CHCl_3$-MeOH (1 : 1), 0.2 g (0.007% yield); R_f: 0.416 (EtOAc-$CHCl_3$-petroleum ether 0.2 : 1 : 9); m.p.: 133–134 °C; $[\alpha]_D^{22}$ + 77.27° ($CHCl_3$); UV λ_{max} (MeOH) 213 nm (log ∈ 4.8); IR ν_{max} (KBr) 2965, 2830, 1735, 1460, 1375, 1245, 1015, 890 cm^{-1}; EIMS m/z (ret. int.) 466 $[M]^+$ ($C_{32}H_{52}O_2$) (33.6), 4541(7.2), 407(9.2), 392(6.4), 258(6.2), 248(16.8), 218(100), 207(6.6), 204(26.8), 203(36.0), 189(79.5), 161(21.4), 150(183), 147(23.3), 146(7.1), 155(39.5), 133(24.1), 122(38.2), 107(34.8), 95(41.4), 81(31.0), 61(27.7), 67(14.3), 55(22.4).

3.3.6.1. Hydrolysis of **6**

Compound **6** (15 mg) was refluxed with 0.5 N ethanolic KOH solution (10 ml) for 4 h. After usual work-up, the deacetylated product **3a** was obtained, IRν_{max} (KBr) 3395 cm^{-1} TLC comparable.

3.3.6.2. Oxidation to **6a**

The deacetylated compound **3a** (10 mg) was dissolved in acetone (10 ml) and oxidized with Jones reagent (5 ml) to yield the 3-oxo derivative **6a**. IRν_{max} (KBr) 1705 cm^{-1}, TLC comparable.

3.3.7. Lupeol acetate (**7**)

The second crop of compound **6** gave a colourless powder of **7**, recrystallized from $CHCl_3$-MeOH (1 : 1) 0.45 g (0.015% yield); R_f: 0.4218 (EtOAc-petroleum ether-$CHCl_3$; 0.2 : 1 : 19); m.p.: 215–217 °C (Lit m.p. 218 °C); $[\alpha]_D^{22}$ + 21.15° ($CHCl_3$); IR ν_{max} (KBr) 2940, 2850, 1738, 1400, 1375, 1245, 1020, 990 cm^{-1}; EIMS m/z (ret. int.) 468$[M]^+$; $C_{32}H_{52}O_2$ (11.0).

3.3.7.1. Alkaline hydrolysis of **7**

Compound **7** (20 mg) on hydrolysis with 0.5 N ethanolic KOH solution yielded lupeol (**7a**), m.p. 213–215 °C, m.m.p. 213–215 °C, $[\alpha]_D^{22}$ + 27.5 °C (c 4.6, $CHCl_3$), TLC comparable.

3.3.8. Peruvianursenyl acetate (**8**)

Elution of the colunm with petroleum ether-$CHCl_3$ (1 : 1), (fractions 58–66), gave a yellow coloured mass of 8, recrystallized from $CHCl_3$-MeOH (1 : 2), 0.066 g (0.002% yield); R_f : 0.488 (EtOAc-$CHCl_3$-petroleum ether; 0.2 : 1 : 1); m.p.: 92–93 °C; $[\alpha]_D^{22}$ + 51.65° ($CHCl_3$); UV λ_{max} (MeOH) 213 nm (log ∈ 4.17); IR ν_{max} (KBr) 3480, 3365, 2940, 2855, 1650, 1465, 1370, 1245, 1015, 985 cm^{-1}. ^1H NMR δ 5.18 (1 H, t, J = 3.59 Hz, H-1^1), 4.60 (1 H. J = 10.8 Hz, H-1^{11}) 4.56 (^1H, m, H-2^1), 4.50 (1 H, m, H-5^1), 4.47 (1 H, dd, J = 9.68, 5.81 Hz, H-32), 4.13 (1 H, d, J = 7.14 Hz, H-6^1a), 4.05 (1 H, d, J = 6.66 Hz, H-6^1b), 3.63 (1 H, dd, J = 9.38, 7.08 Hz, H-4^1), 3.49 (1 H, dd, J = 7.08, 2.50 Hz, H-3^1), 2.75 (1 H, d, J = 6.35 Hz, H-18α), 1.25 (3 H, s, Me-23), 1.18 (1 H, s, Me-24), 1.00 (3 H, s, Me-25), 0.97 (1 H, d, J = 6.50 Hz, Me-29), 0.91 (3 H, s, Me-26), 0.87 (3 H, d, J = 6.0 Hz, Me-30), 0.84 (3 H, s, Me-27), 0.79 (3 H, s, Me-28); EIMS m/z (ret. int.) 588 $[M]^+$ ($C_{36}H_{60}O_6$) (4.6), 408(11.7), 395(25.1), 393(4.70), 258(4.60), 218(85.02), 203(15.6), 189(20.9), 180(3.1), 163(5.3), 152(5.9), 147(13.3), 138(19.0), 135(18.5), 124(27.0), 111(35.8), 103(100), 85(40.9), 82(60.30), 69(63.2), 55(59.8).

3.3.8.1. Acid hydrolysis of **8**

Compound **8** (15 mg) was refluxed with 2 N HCl in 80% MeOH (10 ml) for 4 hr. After cooling, the reaction mixture was powdered into crushed ice, and the hydrolysate was then extracted with EtOAc to give the aglycone, m.p. 145–147 °C, Co-TLC comparable with peruvianursenol (**5a**). The neutralized ($AgCO_3$) aqueous hydrolysate showed the presence of glucose on comparison with authentic sugar on silica gel TLC, R_f 0.416 (EtOAc-HOAc-H_2O-MeOH, 6 : 1:1 : 2).

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