NOVEL NOR AND SECO TRITERPENOIDS FROM *Koelpinia linearis*

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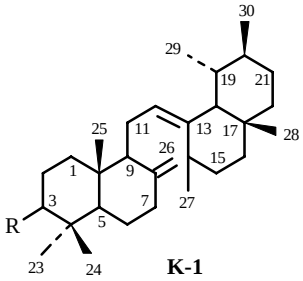
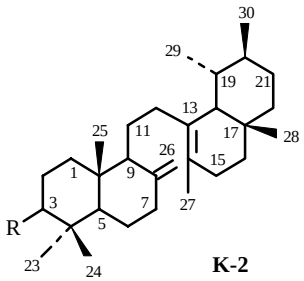
Three novel triterpenoids, C:D seco (8→14) urso-8(26),12(13)-dien-3β-ol, C:D seco (8→14) urso-8(26),13(14)-dien-3β-ol, and C:D seco (8→14),27 nor-urso-12-ene-3β-20α-diol were isolated from Koelpinia linearis *and characterized by spectral and chemical methods.*

Key words: *Koelpinia linearis*, compositeae, triterpenoids, ursane, C:D seco(8→14) urso-8(26),12(13)-dien-3β-ol, C:D seco (8→14) urso-8(26),13(14)-dien-3β-ol, C: D seco (8→14), 27 nor- urso-12-ene-3β,20α-diol.

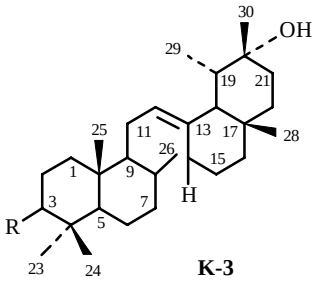
Koelpinia linearis is a subalpine plant found in the Ladakh region of the state Jammu and Kashmir, India [1]. The plant grows wild and has a long lanceolate leaves. It flowers during August–September. It is a rich source of triterpenoids and steroids [2]. From the methanolic extract, three steroids were isolated, namely stigmasterol, β-sitosterol, and a new steroid namely 5β,24α-Stigmasta-8,22-dien-3β-ol [3]. Ten triterpenoids were isolated and characterized from the methanolic extract of the leaves of the plant. Three of these, **K-1**, **K-2**, and **K-3**, were new. Characterisation of these compounds is based on exhaustive spectral and chemical studies.



K-1



K-2



K-3

K-1, K-2, K-3: R = OH, H

K$_a$-1, K$_a$-2, K$_a$-3: R = OCOCH$_3$, H

K$_k$-1, K$_k$-2, K$_k$-3: R = O

Compound **K-1** (mp 170°C) is a colorless crystalline solid. High resolution MS shows M^+ at *m/z* 426, analyzed for $C_{30}H_{50}O$ - 426.7234. Its IR-spectrum exhibited absorption bands at 3490 cm^{-1} for -OH; 1480, 1400, 880 cm^{-1} for carbon–carbon double bonds. It gives a pink coloration with concentrated H_2SO_4 and responds positively to the Liebermann Burchard test [4]. It gives rose a pink color on heating with trichloroacetic acid at 80°C [5] and a yellow coloration with tetranitromethane. The PMR of the compound contained four upfield resonance signals for four methyls at 0.75, 0.78, 0.95, and 0.97 ppm and two secondary methyl doublets (J = 7.5 Hz) at δ 0.83 and 0.84 (3H each). One proton multiplet δ 3.6 was due to a secondary carbinolic proton which can be assigned to the usual C-3 position. In the downfield region, the spectrum displayed two doublets at δ 4.55 and δ 4.84 each, integrating for one proton with a coupling constant of 8 Hz which is characteristic of an exocyclic double bond. A downfield vinylic proton resonated at δ 5.17 dd (J = 12.6 Hz) arising from its coupling with C-11 protons. The chemical shift of the highest methyl δ 0.75 [6] together with the presence of two secondary methyls and their chemical shifts and coupling constants were in conformity with the ursane skeleton. The mass spectrum contained prominent fragment ion peaks at *m/z* 208 (a) and 218 (b) arising from the cleavage of the allylic 9,11, single bond; the fragment ion peak at *m/z* 203 arising by the loss of methyl from the fragment (b) is a characteristic feature of the ursanes [7].

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TABLE 1. PMR Spectral Data of Triterpenoids (δ, ppm, J/Hz)

Atom	Compounds		
	K-1	**K-2**	**K-3**
H-3	3.61 (m)	3.20 (m)	3.21 (dd, J = 7.4)
H-12	5.71 (dd, J = 6.12)	-	5.23 (d, J = 18)
H-18	2.31 (d, J = 4.6)	2.31 (d, J = 4.6)	2.31 (d, J = 4.6)
H-19	1.95 (m)	1.95 (m)	1.95 (m)
H-20	1.95 (m)	1.95 (m)	-
H-23	0.78 (3H, s)	0.78 (3H, s)	0.80 (3H, s)
H-24	0.95 (3H, s)	0.95 (3H, s)	0.95 (3H, s)
H-25	0.97 (3H, s)	0.97 (3H, s)	0.96 (3H, s)
H-26	-	-	0.98 (3H, d, J = 6.4)
H-26(a)	4.57 (d, J = 8)	4.57 (d, J = 8)	-
H-26 (b)	4.84 (d, J = 8)	4.62 (d, J = 8)	-
H-27	1.02 (3H, s)	1.62 (3H, s)	-
H-28	0.75 (3H, s)	0.75 (3H, s)	0.85 (3H, s)
H-29	0.84 (3H, d, J = 6.4)	0.88 (3H, d, J = 6.4)	0.87 (3H, d, J = 6.4)
H-30	0.83 (3H, d, J = 6.4)	0.87 (3H, d, J = 6.4)	1.57 (3H, s)
	K_a-1	**K_a-2**	**K_a-3**
H-3	4.58 (m)	4.58 (m)	4.14 (m)
OCOC**H**$_3$	2.03 (3H, s)	2.03 (3H, s)	2.03 (3H, s)
	K_k-1	**K_k-2**	**K_k-3**
H-2	2.40 (m)	2.40 (m)	2.41 (m)

The loss of a molecule of water from the fragment **K-1** (a) resulted in the fragment ion peak at *m/z* 190 which further undergoes RDA fragmentation of ring A to give ion peaks at *m/z* 109 and 82. The molecular formula suggests that the index of unsaturation is six, out of which five could be attributed to the pentacyclic triterpenoid skeleton and the sixth to one double bond. However, the PMR indicates the presence of two double bonds, one the exocyclic methylene and the other the double bond in the nucleus, which indicates that the triterpenoid skeleton is tetracyclic with a seco nature. This alone is in conformity with PMR and MS data.

The compound **K-1** formed a monoacetate (**K_a-1**), mp 185°C, M^+ 468, analyzed for $C_{32}H_{52}O_2$; IR of the **K_a-1** exhibited an acetoxyl signal at 1740 cm^{-1}. The PMR of **K_a-1** contained a methyl resonance signal at δ 2.03 and the secondary C-3 proton was shifted as expected to downfield at δ 4.58 (1H; m). The MS of **K_a-1** contained peaks at *m/z* 218 involving ring D and E and at *m/z* 250 involving ring A and B.

Oxidation of **K-1** with CrO_3 – pyridine gave a ketone (**K_k-1**), mp 185°C, analyzed for $C_{30}H_{48}O$ with M^+ at *m/z* 424. The IR of **K_k-1** exhibited a band at 1700 cm^{-1}. The PMR of **K_k-1** displayed a signal at δ 2.40 (2H, m) due to methylene protons. The compound also responded to Zimmerman's test [8]. The mass spectrum of **K_k-1** was consistent with a 3-keto derivative. Compound **K-2** (mp 161°C) is a colorless crystalline solid. High-resolution MS shows M^+ at *m/z* 426, analyzed for $C_{30}H_{50}O$, 426.7231. Its IR spectrum exhibited absorption bands at 3490 cm^{-1} for -OH; 1605, 1480, 1400, 880 cm^{-1} for carbon–carbon double bonds. It gives a pink coloration with concentrated H_2SO_4 and responds positively to Liebermann Burchard test. It gives a rose pink color on heating with trichloroacetic acid at 80°C and a yellow coloration with tetranitromethane. The PMR of the compound contained four upfield resonance signals for four methyls at 0.75, 0.78, 0.95, and 0.97 besides two secondary methyl doublets at δ 0.82 and 0.88 (3H each, δ, J = 6.42 Hz). One proton multiplet δ 3.20 was due to a secondary carbinylic proton which can be assigned to the usual C-3 position. In the downfield region, the spectrum displayed two doublets at δ 4.57 and δ 4.62 each, integrating for one proton with a coupling constant of 8 Hz, which is a characteristic of an exocyclic methylene. The chemical shift of the methyl δ 0.75 together with the presence of two secondary methyls and their chemical shifts and coupling constants were in conformity with the ursane skeleton. The mass spectrum contained prominent fragment ion peaks at *m/z* 208 (a′) and 218 (b′), the former involving D/E rings and the latter involving A/B rings.

TABLE 2. ^{13}C NMR Data of Triterpenoids

Atom	Compounds		
	K-1	**K-2**	**K-3**
C-1	36.35	36.34	36.35
C-2	27.86	27.84	27.86
C-3	79.46	79.42	79.09
C-4	38.49	38.45	38.49
C-5	55.70	55.70	55.47
C-6	18.50	18.50	18.50
C-7	30.15	30.12	30.15
C-8	151.37	151.31	54.45
C-9	48.50	48.50	48.50
C-10	40.42	40.42	40.42
C-11	25.52	25.50	25.50
C-12	119.31	26.20	119.96
C-13	140.20	139.5	139.09
C-14	39.28	141.50	26.20
C-15	27.80	26.80	27.85
C-16	21.36	47.24	21.36
C-17	48.74	46.25	47.82
C-18	39.28	40.02	40.98
C-19	50.87	50.23	50.87
C-20	55.74	55.72	78.80
C-21	34.72	34.70	40.96
C-22	36.75	35.72	40.05
C-23	28.40	28.40	28.40
C-24	16.40	16.20	16.40
C-25	15.77	14.95	15.77
C-26	109.72	110.25	17.72
C-27	16.52	16.40	-
C-28	14.97	15.20	14.97
C-29	30.10	30.10	36.75
C-30	34.50	34.50	23.50
	K_a-1	**K_a-2**	**K_a-3**
C-3	82.50	82.50	82.50
OCO**C**H_3	21.40	21.40	21.40
O**C**OCH_3	170.20	170.20	170.20
	K_k-1	**K_k-2**	**K_k-3**
C-3	210.4	210.05	210.4

This placed the OH and a double bond in the ring A/B. The fragment ion at *m/z* 203 resulted from the loss of methyl from fragment (b′). The loss of a water molecule from the ion peak at *m/z* 208 resulted in a very strong peak at *m/z* 190. Since the molecular formula of **K-2** was similar to that of **K-1** except for the difference in the intensities of the peaks, this suggests that **K-2** is an isomer of **K-1**, hence **K-2** is also a seco ursane, most probably with the same C-8(26) exocyclic methylene group.

The compound **K-2** formed a monoacetate (**K_a-2**), mp 165°C, M^+ 468, analyzed for $C_{32}H_{52}O_2$; the IR of **K_a-2** exhibited an acetoxyl C-3 signal at 1740 cm^{-1}. The PMR of **K_a-2** contained a methyl resonance signal at δ 2.03 and the secondary C-3 proton shifted as expected to downfield at δ 4.58 (1H; m); the MS of **K_a-3** is also a low melting point (mp 164°C) colorless crystalline solid. High-resolution MS showed M^+ at *m/z* 430 and analyzed for $C_{29}H_{50}O_2$, 430.4272. Its IR spectrum exhibited absorption bands at 3490 cm^{-1} for -OH; 1605, 1480, 1400 and 880 cm^{-1} for carbon-carbon double bonds. It gives a pink coloration with concentrated sulfuric acid, and responds positively to Liebermann Buchard test. It gives a rose pink color on heating with trichloroacetic acid at 80°C and a yellow coloration with tetranitromethane.

The PMR of the compound contained signals for five tertiary methyls and two secondary methyls. One of the tertiary methyls (C-29) was displaced downfield at δ 1.57 (3H, s), indicating that it was connected to a carbon carrying either a double

bond or an oxygen function. The spectrum displayed a carbinylic proton signal at δ 3.21 (1H, m) which was placed at the usual C-3 position and its location was confirmed by the ^{13}C NMR signal at δ_c 79.09. The vinylic proton signal was displaced at δ 5.23 (1H, d, J = 18 Hz), which showed that the double bond was a tri-substituted double bond.

The vinylic carbons gave resonance signals at δ_c 119.9 and 139.0 corresponding to C-12 and C-13 carbons respectively. In the mass spectrum the molecule breaks into two prominent fragments **K-3** (a) and **K-3** (b) at *m/z* 224 and 208 respectively. The **K-3** (b) fragment loses a methyl radical followed by the loss of a water molecule to give fragment ion peak at *m/z* 191 while the loss of a water molecule from **K-3** (a) gives a peak at *m/z* 190. This fragmentation revealed that the rings A/B carried only one -OH function while the other oxygen function must be present in ring D or E.

K-3 on treatment with acetic anhydride and pyridine formed a mono-acetate M^+ at *m/z* 472 corresponding to the formula $C_{31}H_{52}O_3$. Its PMR showed the carbinylic proton signal at δ 4.14 (1H, m), which confirmed that **K-3** carried the hydroxyl at the usual C-3 position (δ_c 79.09, C-3).

Since the IR spectrum did not reveal any absorption on account of a carbonyl nor due to an epoxide and since the compound formed only mono-acetate, it was presumed that the second hydroxyl is tertiary in nature. The presence of four tertiary methyls together with the MS fragmentation pattern was very similar to **K-1**, which indicated that the hydroxyl did not occupy, the position on C-18, which is the only tertiary carbon in D and E rings. Since there are only two secondary methyls, one located at C-8 and the other in the D/E ring, and because we have only one methyl doublet, in an ursane skeleton the -OH must be attached to the carbon carrying the methyl group. It was placed at C-20 in view of the downfield chemical shift of C-30 methyl at δ 1.57 (3H, s) and the carbon resonance signal at δ_c 23.5 (s).

In view of the fact that a tertiary –OH connected to a carbon carrying a methyl group cannot be easily oxidized and needs drastic conditions, no oxidation was attempted because the molecule breaks down into several fragments with chromium trioxide and acetic acid. However, the assigned structure was consistent with the ^{13}C NMR data of the compound.

EXPERIMENTAL

Melting points were recorded on a Kofler block apparatus. IR spectra were recorded on a Perkin–Elmer-350 spectrometer. NMR spectra were recorded on FT-NMR 90 MHz 250 MHz NMR, 400 MHz NMR, using tetramethyl silane as internal standard and $CDCl_3$ as solvent. ^{13}C NMR, APT, DEPT (90°) experiments were done on a Brucker intrument. TLC was carried out on silicagel-G layers (BDH, 0.3mm). The plates were activated at 110–120°C for 30 min; 10% aq. H_2SO_4 (containing 7g ceric ammonium sulfate per 100 ml) spray followed by heating at 120° was used for visualization of spots. Column chromatography was carried on 60–120 mesh silicagel (BDH). The analytical samples were dried in vacuum at 35° over P_2O_5 for 25 h. All the reactions were carried out in anhydrous conditions, unless otherwise stated.

Isolation of Compounds of *Koelpinia linearis*. *Koelpinia linearis* (Family, Compositeae) was collected from the Ladakh region of Jammu and Kashmir, India. About 10 kg of the dried plant material was powdered and defatted with petroleum ether. Exraction with methanol was carried out in a Soxhlet aspirator (10 kgs) for about 48 h. The methanol extract so obtained was vacuum dried and found to weigh 1 kg. It was analyzed by TLC and was then subjected to column chromatography in a bomb column of 6 ft. height and 4 inches diameter.

The fractions so obtained were monitored by TLC and those containing a mixture of various compounds were pooled. These pooled fractions were further subjected to column chromatography using a mixture of petroleum ether and EtOAc solvents in different proportions on a column impregnated with 25% $AgNO_3$. The various fraction so obtained were analyzed by TLC and the compounds **K-1**, **K-2**, and **K-3** were obtained in pure form after repeated crystallization.

K-1: mp 170°C, $[\alpha]_D$ +23° (c 0.5 EtOH). IR (KBr, ν_{max}, cm^{-1}): 3490, 2980, 2850, 1605, 1480, 1400 and 880; MS: M^+ at *m/z* 426, 218, 208, 203, 190, 177, 109, 82.

Acetylation of K-1. 70 mg of **K-1** in $CHCl_3$ (10 ml) was treated with Ac_2O (1.5 ml) and H_2O_4 (0.1 ml). The mixture was left overnight and the usual work up and purification yielded $\mathbf{K_a}$**-1** acetate. $\mathbf{K_a}$**-1** mp 185°C, $[\alpha]_D$ + 21.9° (c 0.5 EtOH); IR (KBr, ν_{max}, cm^{-1}): 1740, 1605, 1480, 1420 and 890; MS: M^+ at *m/z* 468, 250, 218, 203, 170, 177.

PMR (δ, J/Hz): 4.58 (1H, m, H-3), 5.26 (1H, dd J = 6, 12, H-12), 2.30 (1H, d, J = 4.6, H-18), 1.95 (2H, m, H-19 and H-20), 0.80 (3H, s, H-23), 0.95 (3H, s, H-24), 0.97 (3H, s, H-25), 4.01 (1H, d, J = 8, H-26 (a)), 4.07 (1H, d, J = 8, H-26 (b)), 1.04 (3H, d, J = 5.2, H-27), 0.75 (3H, s, H-28), 0.85 (3H, d, J = 7.5, H-29), 0.83 (3H, d, J = 7.5, H-30), 2.03 (3H, s, $OCOC\underline{H}_3$).

Oxidation of K-1 (K_k-1). To 50 mg of the compound **K-1** in pyridine (3 ml) was added freshly prepared CrO_3 – pyridine complex (0.25 g) and the mixture was left for 24 h at room temperature to yield **K_k-1** crystallized from MeOH. **K_k-1** mp 195°C, $[\alpha]_D$ + 23.8° (c 0.5 EtOH); IR (KBr, ν_{max}, cm^{-1}): 1700, 1610, 1450, 1410 and 890; MS: M^+ at *m/z* 424, 218, 206, 203, 178.

PMR (δ, J/Hz): 2.40 (2H, m H-2), 2.30 (1H, d, J = 4.6, H-18), 1.95 (2H, m, H-19 and H-20), 0.77 (3H, s, H-23), 0.97 (3H, s, H-24), 0.95 (3H, s, H-25), 4.50 (1H, d, J = 7.9, H-26 (a)), 4.77 (1H, d, J = 7.9, H-26 (b)), 1.02 (3H, d, J = 5.2, H-27), 0.75 (3H, s, H-28), 0.84 (3H, d, J = 7.5, H-29), 0.82 (3H, d, J = 7.5, H-30).

K-2: mp 161°C, $[\alpha]_D$ + 24° (c 0.5 EtOH). IR (KBr, ν_{max}, cm^{-1}): 3490, 1610, 1485, 1400 and 880; MS: M^+ at *m/z* 426, 218, 208, 190, 177, 109, 82.

Acetylation of K-2 (K_a-2). 60 mg of K-2 in $CHCl_3$ (10 ml) was treated with Ac_2O (1.5 ml) and H_2SO_4 (0.1 ml). The mixture was left overnight and the usual work up and purification yielded **K_a-2** mp 165°C, $[\alpha]_D$ + 21.9° (c 0.5 EtOH); IR (KBr, ν_{max}, cm^{-1}): 1740, 1610, 1480, 1420 and 880. MS: M^+ at *m/z* 468, 250, 218, 190, 177.

PMR (δ, J/Hz): 4.58 (1H, m H-3), 2.31 (1H, d, J = 4.6, H-18), 1.95 (2H, m, H-19 and H-20), 0.80 (3H, s, H-23), 0.98 (3H, s, H-24), 0.95 (3H, s, H-25), 4.48 (1H, d, J = 8, H-26 (a)), 4.65 (1H, d, J = 8, H-26 (b)), 1.62 (3H, s, H-27), 0.75 (3H, s, H-28), 0.88 (3H, d, J = 6.4, H-29), 0.83 (3H, d, J = 6.4, H-30), 2.03 (3H, s, OCOC$\underline{H}_3$).

Oxidation of K-2. To 60 mg of the compound **K-2** in pyridine (3 ml) was added freshly prepared CrO_3 – pyridine complex (0.25 g) and the mixture was left for 24 h at room temperature to yield **K_k-2** crystallized from MeOH. **K_k-2**: mp 170°C, $[\alpha]_D$ + 21.8° (c 0.5 EtOH); IR (KBr, ν_{max}, cm^{-1}): 1700, 1608, 1480, 1400 and 880; MS: M^+ at *m/z* 424, 218, 206, 203, 178.

PMR (δ, J/Hz): 2.40 (2H, m, H-2), 2.31 (1H, d, J = 4.6, H-18), 1.95 (2H, m, H-19 and H-20), 0.76 (3H, s, H-23), 0.98 (3H, s, H-24), 4.50 (1H, d, J = 8, H-26 (a)), 4.65 (1H, d, J = 8, H-26 (b)), 1.62 (3H, s, H-27), 0.75 (3H, s, H-28), 0.88 (3H, d, J = 6.4, H-29), 0.82 (3H, d, J = 6.4, H-30).

K-3: mp 163°C, $[\alpha]_D$ + 24° (c 0.5 EtOH); IR (KBr, ν_{max}, cm^{-1}): 3490, 1605, 1480, 1400, 984 and 880; MS: M^+ at *m/z* 432, 224, 209, 190, 188, 109, 106, 56.

Acetylation of K-3 (K_a-3). 60 mg of **K-3** in $CHCl_3$ (10 ml) was treated with Ac_2O (1.5 ml) and H_2SO_4 (0.1 ml). The mixture was left overnight and the usual work up and purification yielded **K_a-3**, mp 164°C, $[\alpha]_D$ + 22.9° (c 0.5 EtOH); IR (KBr, ν_{max}, cm^{-1}): 1740, 1610, 1480, 1420 and 880; MS: M^+ at *m/z* 472, 250, 224, 209, 191, 190, 106, 56.

PMR (δ, J/Hz): 4.14 (1H, m H-3), 5.24 (1H, dd, J = 6.12, H-12), 2.32 (1H, d, J = 4.6, H-18), 1.95 (1H, m, H-19), 0.82 (3H, s, H-23), 0.95 (3H, s, H-24), 0.96 (3H, s, H-25), 0.98 (3H, d, J = 6.4, H-26),0.85 (3H, s, H-28), 0.87 (3H, d, J = 6.4, H-29), 1.57 (3H, s, H-30), 2.03(3H, s, OCOC$\underline{H}_3$).

Oxidation of K-3 (K_k-3). To 60 mg of the compound **K-3** in pyridine (3 ml) was added freshly prepared CrO_3 – pyridine complex (0.25 g) and the left for 24 h at room temperature to yield **K_k-3** crystallized from MeOH; mp 180°C, $[\alpha]_D$ + 21° (c 0.5 EtOH); IR (KBr, ν_{max}, cm^{-1}): 1700, 1608, 1480, 1400 and 880; MS: M^+ at *m/z* 432, 224, 209, 206, 178, 106, 56.

PMR (δ, J/Hz): 2.41 (2H, m, H-2), 5.24 (1H, d, J = 18, H-12), 2.32 (1H, d, J = 4.6, H-18), 1.95 (1H, m, H-19), 0.80 (3H, s, H-23), 0.95 (3H, s, H-24), 0.96 (3H, s, H-25), 0.97 (3H, d, J = 6.4, H-26), 0.85 (3H, s, H-28), 1.57 (3H, s, H-30).

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