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Two novel seco triterpenoids from *Koelpinia linearis*

Wajaht A.Shah*, Mushtaq A.Qurishi

Deptt. of Chemistry, University of Kashmir, Srinagar-190006 (J&K), (INDIA)

Tel : 09419054484; Fax: 0194-2421357

E-mail : wajaht_shah@yahoo.com

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ABSTRACT

Several triterpenoids were isolated from methanolic extract of the plant *Koelpinia linearis.* Three of them have been already reported. Other two novel triterpenoids C:D seco (12→13) Urso-8(9),12(26)-dien-3β-ol (1) and C:D seco (12→13) Urso-12(26),13(14)-dien-3β,20α-diol (2) are being reported first time. Their structures were identified by spectral and chemical methods.

KEYWORDS

Koelpinia linearis;
Compositeae;
Triterpenoids.

INTRODUCTION

Koelpinia linearis is a sub-alpine plant found in ladakh region of the J&K state, India[1]. The plant grows wild and has long lanceolate leaves. It flowers during August-September. It is a rich source of triterpenoids and steroids[2]. From the methanolic extract of the plant three steroids, one of them being novel, has been reported by us[3]. Several triterpenoids were isolated three of them have been already reported[4]. Other two novel triterpenoids, C:D seco (12→13) Urso-8(9),12(26)-dien-3β-ol, C:D seco (12→13) Urso-12(26),13(14)-dien-3β,20α-diol, are being reported first time. The compounds have been characterized on the basis of exhaustive spectral and chemical analysis.

RESULTS AND DISCUSSION

Compound **(1)** (m.p 165^{0}C) is a colourless crystalline solid. High resolution MS shows M^+ at m/z 426, analysed for $C_{30}H_{50}O$ 426.7324.Its IR-spectrum exhibited 3490cm^{-1} for –OH : 1605,1480,1400,880cm^{-1} for carbon-carbon double bonds. It gives pink coloration with concentrated H_2SO_4 and responds positively to the Liebermann Burchard test[5]. It gives rose pink color on heating with tricholoroacetic acid at 80^{0}C[6] and a yellow coloration with tetranitromethane. The PMR of the compound **(1)** contained five up-field resonance signals for five tertiary methyls at δ 0.75,0.77, 0.96, 0.98 and 1.02 ppm and two secondary methyl doublets (J= 7.5 Hz) at δ0.82 and δ0.83 (3H each). One proton multiplet at δ3.6 was due to a secondary carbinolic proton which can be assigned to the usual C-3 position. Its ^{13}CNMR value at δ_c79.46 is in conformity at C-3 position. In the down field region, the spectrum displayed two doublets at δ4.57 and δ4.78 each, integrating for one proton with a coupling constant of 8Hz which is characteristic of an exocyclic ethylenic double bond. A downfield vinylic proton resonated δ5.16 (dd,J=6.12 Hz.) arising from its coupling with C-11 protons. The chemical shift of the highest methyl δ0.75 together with the presence of two secondary methyls and their chemical shifts and coupling constants were in conformity with the ursane skeleton[7].

Prominent ion peaks at m/z 234 1(a) , m/z 192 1(b) arising from the cleavage of 8,14 single bond of compound **(1)**. The fragment ion peak at m/z 177 arising by the loss of methyl from fragment 1(b) is a characteristic feature of the ursanes[8]. The loss of water molecule from the fragment 1(a) resulted in the fragment ion peak at

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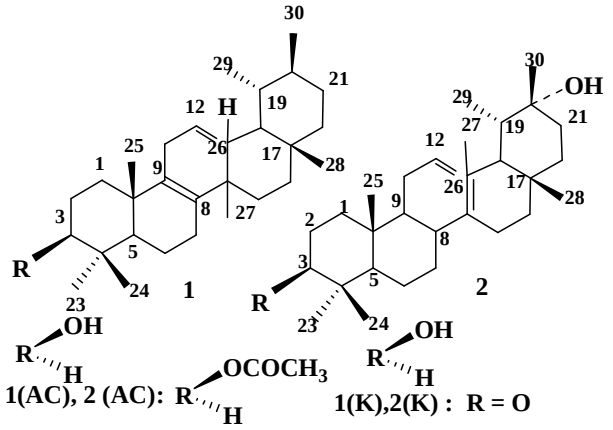

Figure 1: Structures of compound (1) and (2)

m/z 216.After the loss of water molecule by the fragment 1(a) its further fragmentation occurs to give fragment 1(a/) with ion peak at m/z 176 and fragment 1 (a//) with ion peak at m/z 41.

Fragment 1(a/) undergoes further RDA fragmentation of ring A to give ion peaks at m/z 95 and m/z 82.

The molecular formula suggests that the index of un-saturation 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 ethylenic double bond and the other in the nucleus, which indicates that the triterpenoid skeleton is tetra cyclic with a seco nature. This alone is in conformity with PMR and MS data. The compound **(1)** formed a mono acetate 1(AC), m.p.182^{0}c, M^+ 468 analyzed for the molecular formula $C_{32}H_{52}O_2$; IR of 1(AC) exhibited an acetoxyl signal at 1740cm^{-1}. The PMR of 1(AC) contained a methyl resonance signal at δ2.03 and the secondary C-3 proton was shifted as expected to down field at δ4.58 (1H,m). The MS of 1(AC) contained fragment ion peaks at m/z 276 containing ring A , B and fragment ion peak at m/z 192 containing ring D,E.

Oxidation of the compound 1 with CrO_3-pyridine gave a ketone 1(K), m.p. 185^{0}C analyzed for molecular formula $C_{30}H_{48}O_2$ with M^+ 424. The IR of 1(K) exhibited a band at 1700cm^{-1}. The PMR of 1(K) displayed a signal at δ2.40 (2H,m) due to methylene protons. The compound also responded to Zimmerman's test[9]. The mass spectrum of 1(K) was in consistent with a 3-keto derivative. The MS of 1(K) contained peaks at m/z 232 fragment containing ring A,B and m/z 192 fragment containing ring D,E.

TABLE 1: ^{1}HNMR and ^{13}CNMR data of compound (1) and (2)

No.	1		2	
	δ_c	δ_H (J= Hz)	δ_c	δ_H (J= Hz)
1	36.35	-	36.34	-
2	27.86	-	27.84	-
3	79.4	3.6 m	79.46	3.5 m
4	38.49	-	38.45	-
5	55.70	0.76 dd (11.5,1.4)	55.70	0.76dd(11.5,1.4)
6	18.50	1.56, m	18.48	1.54,m
7	30.17	-	30.15	-
8	158.71	-	40.98	-
9	140.2	-	48.5	-
10	40.42	-	40.42	-
11	25.52	-	25.52	-
12	119.31	5.16 dd (6.12)	120.31	5.12 dd (6.5)
13	26.2	-	141.31	-
14	41.2	-	140.20	-
15	26.4	-	26.8	-
16	23.3	-	22.3	-
17	40.74	-	40.74	-
18	38.28	2.31 dd (4.6)	39.26	-
19	50.87	1.95 m	50.87	1.95 m
20	55.74	1.95 m	78.80	-
21	34.72	-	40.96	-
22	36.75	-	40.05	-
23	28.40	0.75 s	28.40	0.75 s
24	16.40	0.96 s	16.40	0.96 s
25	15.77	0.98 s	15.77	0.98 s
26	109.72	(a) 4.57 d (8) (b) 4.78 d (8)	110.25	(a) 4.61d (8) (b) 4.74 (8)
27	16.52	1.02 s	16.40	2.03 s
28	14.97	0.77 s	14.97	0.75 s
29	21.4	0.82 d (7.5)	36.75	1.12 d (6.4)
30	17.5	0.83 d (7.5)	23.50	1.57 s
	1(AC)		1(K)	
2	-	-	-	2.40 m
3	-	4.58, m	-	-
OCOC<u>H</u>$_3$	-	2.03 , s	-	-
	2(AC)		2(K)	
2	-	-	-	2.40 m
3	-	4.34, m	-	-
OCOC<u>H</u>$_3$	-	2.03, s	-	-

Compound (2), m.p. 175^{0}C is a colorless crystalline solid. High resolution MS shows M^+ at m/z $C_{30}H_{50}O_2$, 442.3272

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 responded positively to Liebermann Burchard test. It gives a rose pink color on heating with trochloroacetic acid at 80^{0}C and a yellow coloration with tetra nitromethane. The PMR

of the compound contained up-field signals for four tertiary methyls at δ0.75, 0.78, 0.96 and 0.98 ppm. One of the secondary methyl signal C-27 was displayed downfield at δ2.03 (3H,s) indicating that the methyl was connected to carbon containing double bond. Other secondary methyl signal C-29 was displayed as doublet (J=6.4 Hz) at δ1.12.Third secondary methyl C-30 was displayed downfield at δ1.57(3H,s) indicating it was connected to carbon carrying an oxygen function. One proton multiplet at δ3.5 (1H.m) was due to a secondary carbinylic proton which can be assigned to the usual C-3 position and its location was confirmed by ^{13}C NMR at δ_c79.46. In the downfield region the spectrum displayed two doublets at δ4.61 and δ4.74 each integrating for one proton with coupling constant of 8 Hz which is characteristic of an exocyclic ethylenic double bond. A down field vinylic proton for C-12 resonated at δ5.28 (dd, J=6.12 Hz) arising from its coupling with C-11 proton.The chemical shift of the highest methyl at δ0.75 with the presence of two secondary methyls and their chemical shifts and coupling constants were in conformity with the ursane skeleton The mass spectrum of the compound 2 contained prominent fragment ion peaks at m/z 234, 2(a) and m/z 208, 2(b) arising from the cleavage of 8,14 single bond of compound 2; the fragment ion peak at m/z 194 due to the loss of methyl from fragment 2 (b) is followed by the loss of water molecule to give fragment ion peak at m/z 176, while the loss of water molecule from the fragment 2 (a) gives apeak at m/z 216. this fragmentation revealed that the A/B rings carried only one –OH function while the other -OH must be in the rings D/E. Compound **(2)** on treatment with acetic anhydride and pyridine formed a monoacetate 2 (AC) mp 185^{0}C, at m/z484, corresponding to the formula $C_{32}H_{52}O_3$. PMR of the acetate showed the carbinylic signal at δ4.34 (1H, m) which confirmed that compound 2 carried the hydroxyl at usual C-3 position δ_c79.34 (C-3). The carbon signal at δ_c41.2 and δ_c39.5 corresponding to C-8 and C-9 of compound 2 indicated that the double bond was not present between C-8 and C-9, instead carbon signal at δ_c139.5 and δ_c141.50 corresponding to C-14 and C-13 indicated the presence of double bond between C-13 and C-14.

Since the IR spectrum of compound **(2)** didn't reveal any absorption on account of carbonyl nor due to an epoxide and the compound formed only monoacetate, it was presumed that the second hydroxyl is tertiary in nature.

The presence of tertiary methyls together with the MS fragmentation pattern was supporting the presence of second –OH somewhere attached to ring D or E. Since there are three secondary methyls one is attached to C-13 containing double bond the rest of two methyls occupied C-19 and C-20 of ring E. In ursane skeleton –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 δ1.57 (3H, s) and the carbon signal at δ_c23.5, s. In view of the fact that a tertiary –OH connected to a carbon carrying a methyl group can't be oxidized and needs drastic conditions, no drastic oxidation was attempted because the molecule breaks into several fragments with CrO_3 and acetic acid. However the assigned structure was in consistent with ^{13}C NMR data of the compound (**2**).

EXPERIMENTAL

General

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, 400 MHz NMR, using tetramethyl silane as internal standard and $CDCl_3$ as solvent. ^{13}C-NMR, APT, DEPT (90^0) experiments were done on a Brucker instrument. TLC was carried out on silica gel-G layers (BDH,0.3mm).The plates were activated at 110-120^{0}C for 30 min: 10% aq. H_2SO_4 (containing 7g ceric ammonium sulfate per 100ml) spray followed by heating at 120^{0}c was used for visualization of spots. Column chromatography was carried on 60-120 mesh silica gel (BDH). The analytical samples were dried in vacuum at 35^{0}c over P_2O_5 for 25hrs.All the reactions were carried out in anhydrous conditions, unless otherwise stated.

Plant material

The plant Koelpinia linearis was collected from Ladakh region of Jammu and Kashmir state India during August September 2002. It was identified by Dr. A.R. Naqshi, Head, Taxonomy centre, Department of Botany, University of Kashmir (J&K state India) A voucher (specimen No. WAS 22, 05:72 Kashmir University) has been deposited in the taxonomy centre of

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Department of Botany, University of Kashmir.

Extraction and isolation

About 10 kg of the dried plant material was powdered and defatted with petroleum ether. Extraction with methanol was carried out in a Soxhlet aspirator (10 Kgs) for about 48 hrs. 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 6ft. 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 obtained were analyzed by TLC and the compounds **(1)** and **(2)** were obtained in pure form after repeated crystallization.

C;D seco (12→13) urso-8(9),12(26)-dien-3β-ol (1) White crystalline solid; mp 165^{0}C$[\alpha]_D$ +32.860 (c 0.5 EtOH),IR (KBr,ν_{max}, cm^{-1}): 3490,1605,1480,1400 and 880: MS: M^+ at m/z 426, 234,216, 192, 177, 176,95,82 and 41. ^{1}H and ^{13}C-NMR see TABLE 1.

Acetylation of 1

70 mg of 1 in $CHCl_3$ (10ml) was treated with Ac_2O (1.5) and H_2SO_4 (0.1ml) The mixture was left overnight and the usual work up and purification yielded 1(AC) acetate. mp 182^{0}c$[\alpha]_D$ +30.350(c 0.5 EtOH), IR (KBr, ν_{max}, cm-1): 1740,1605,1480,1420 and 890: MS: M^+ at m/z 468, 276 ,216, 192,176,95,82 and 41. ^{1}H and ^{13} C-NMR see TABLE 1.

Oxidation of 1

To 50 mg of the compound 1in pyridine (3ml) was added freshly prepared CrO_3-pyridine complex (0.25g) and the mixture was left for 24 hrs at room temperature to yield 1(K) ketone crystallized from MeOH mp 185^{0}c $[\alpha]_D$ +32.250(c 0.5 EtOH),IR (KBr, ν_{max}, cm^{-1}): 1700, 1610,1450,1410 and 890: MS: M^+ at m/z 424, 232, 203,192,178,163.^{1}H and ^{13}C-NMR see TABLE 1.

C;D seco (12→13) urso-,12(26),13(14)-dien-3β,20α-diol (2) White crystalline solid mp 175^{0}c$[\alpha]_D$ +22.460 (c 0.5 EtOH), IR (KBr, ν_{max}, cm^{-1}): 3490, 1605, 1480,1400 and 880: MS: M^+ at m/z 442,234, 216, 208, 194,176,95,82, 56 and 41. ^{1}H and ^{13}C-NMR see TABLE 1.

Acetylation of 2

60 mg of 2 in $CHCl_3$ (10ml) was treated with Ac_2O (1.5ml) and H_2SO_4 (0.1ml) The mixture was left overnight and the usual work up and purification yielded 2(AC) acetate. mp 185^{0}c$[\alpha]_D$ +30.350 (c 0.5, EtOH),IR (KBr, ν_{max}, cm^{-1}): 1740,1610,1480,1420 and 890: MS: M^+ at m/z 484, 276, 216, 208, 194, 17695,82,56 and 41. ^{1}H and ^{13}C-NMR see TABLE 1.

Oxidation of 2

To 50 mg of the compound 2 in pyridine (3ml) was added freshly prepared CrO_3-pyridine complex (0.25g) and the mixture was left for 24 hrs at room temperature to yield 2(K) ketone crystallized from MeOH mp 180^{0}c $[\alpha]_D$ +21.0(c 0.5 EtOH) ,IR (KBr , ν_{max}, cm-1): 1700, 1608,1450,1400 and 890: MS: M^+ at m/z 440, 232, 203,192,178,163. ^{1}H and ^{13}C-NMR see TABLE 1.

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