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5β,24α-STIGMASTA-8,22-DIEN-3β-OL, A STEROL FROM *KOELPINIA LINEARIS*

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Key Word Index—*Koelpinia linearis*; Compositae; sterol; 5β,24α-stigmasta-8,22-dien-3β-ol.

Abstract—A sterol 5β,24α-stigmasta-8,22-dien-3β-ol was isolated from *Koelpinia linearis* and characterized by spectral and chemical methods.

INTRODUCTION

Koelpinia linearis Pall. is a toxic alpine xerophyte from Ladakh (India) [1] which is a rich source of triterpenoids [2]. Chemical examination of the methanolic extract of the plant led to the isolation of a new sterol **1**. The sterol **1** responded positively to Liebermann–Burchard colour and TNM tests, showed the presence of 29 carbons in its ^{13}C NMR spectrum and exhibited $[M]^+$ at *m/z* 412.374, in its mass spectrum, indicating a molecular formula of $C_{29}H_{48}O_1$. The R infrared spectrum indicated the presence of a hydroxyl group (3500 cm^{-1}) and a disubstituted double bond (1605, 980 cm^{-1}). The presence of an axial hydroxyl group was evident from the methine proton resonance signal at δ3.60 (1H, *m*); δ_C72.3 (*d*, C-3). The compound formed a monoacetate **2** (δ2.03, 3H, *s*, $-OCO\ CH_3$) whose ^1H NMR displayed the methine proton signal at δ4.21 (1H, *m*). On oxidation with CrO_3-pyridine, the sterol slowly transformed into sterone **3** which responded positively to Zimmermann's test for 3-keto steroids [3] confirming the presence of 3β hydroxyl in **1**. The mass spectrum of **1**, **2** and **3** contained the base peak at *m/z* 55. The prominent peaks at *m/z* 273 $(M - C_{10}H_{19})^+$ and 255 $(M - C_{10}H_{19} - H_2O)^+$; in the mass spectrum of **1** indicated clearly the presence of one double bond in the side chain. The corresponding peaks in the mass spectrum of **2** and **3** were observed at *m/z* 315, 255 and 271. The abundant ion peak at *m/z* 272 in the mass spectrum of **1** results from the loss of a hydrogen and a side chain from ring D. The presence of a *cis*-disubstituted double bond in the side chain was suggested by the vinylic proton resonance signals at δ5.18 and 5.22 (^1H each, *dd*, J = 10.8 Hz and 10.1 Hz) indicating the *Z*-orientation of these protons; this was also supported by ^{13}C NMR signals of δ_C122.2 and 122.7 and the upfield shift of one of the methyl groups (18-CH_3) to δ0.68.

The presence of abundant ion peaks at *m/z* 327, 313 and 271, along with other prominent peaks in the mass spectrum of **1** placed the side chain double bond at C-22 and also showed the presence of an ethyl group at C-24 in the side chain. A multiplet at δ2.30 (2H) in ^1H NMR spectrum was attributed to the side-chain allylic protons. The lack of an $M\text{-}70^+$ peak in the mass spectrum ruled out the presence of an α-H at C-5 [4]. A base peak at *m/z* 55 in Δ^{22} cholestanones is generally observed with a 5β-epimer [5, 6]. The intense fragment ion peaks at *m/z* 255 and 257 in the mass spectrum of **1** is a characteristic feature of (24*S*)-24 ethyl-Δ^{22}-sterols [7, 8]. The absence of vinylic resonance signals of the ring system in the ^1H NMR spectrum of **1** and its derivatives, together with the positive Tortelli–Jaffe test (green colouration) [9] indicated that the sterol had a tetra-substituted nuclear double bond, δ_C139.7 and 142.3, either between C-8 and C-9 or C-8 and C-14. The ^{13}C NMR showed the C-18 and C-19 signals at δ_C34.8 and 56.9, respectively. This was within the range of calculated values δ_C35.0 and 55.9 for C-18 and C-19 because, for a sterol with a $\Delta^{8(14)}$ double bond the resonance signals due to C-18 and C-19 are observed at δ_C52.0 and 41.0, respectively [10]. The methyl resonance signals at δ1.20 (3H, *s*) and δ_C56.9, due to C-19 were also in agreement with the $\Delta^{8(9)}$ double bond position.

EXPERIMENTAL

Herbarium specimen No. 4093 was deposited in the Herbarium of the Univ. of Kashmir. Mps are uncorr., IR spectra was recorded in KBr. ^1H and ^{13}C NMR data were recorded in CCl_4 and $CDCl_3$ at 90 and 100 MHz respectively with TMS as int. ref.

Isolation of 5β,24α-*stigmasta*-8,22-*dien*-3β-*ol* (**1**). The elution of silica gel column carrying the nonsaponifiable

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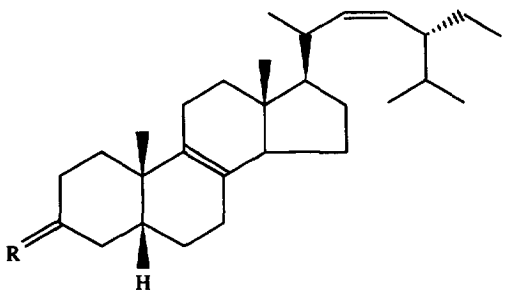

	R
1	β-OH, α-H
2	β-OAc, α-H
3	=O

MeOH extract of the aerial parts of *K. linearis* was carried out with petrol. The fractions recorded were monitored by TLC on silica gel. The second fraction contained a mixture which was rechromatographed on a neutral Al_2O_3 column and eluted with benzene–EtOAc and EtOAc, respectively. The EtOAc fraction was a mixture of four compounds and was resolved on argentite silica gel (10%) to yield two compounds. The first compound was found to be β-sitosterol by comparison with an authentic sample (mp, co-tlc) and the sterol **1** was recovered as a crystalline substance from petrol–MeOH.

Sterol **1**. mp 153.54° $[\alpha]_D^{25}$ + 23.8° (*c* 1.0 MeOH) IR ν_{max}^{KBr} cm^{-1} 3500, 2950, 2870, 1605, 1490, 1400, 1030, 980. ^1H NMR; δ0.68 (3H, *s*, H-18), 0.80 (3H, *d*, *J* = 6.5 Hz, H-26), 0.82 (3H, *d*, *J* = 6.5 Hz, H-27). 0.95 (3H, *t*, *J* = 5.7 Hz, H-29), 1.0 (3H, *d*, *J* = 5.0 Hz, H-21), 1.20 (3Ha, *s*, H-19), 2.30 (1H each, *m*, H-20 and H-24), 3.60 (1H, *m*, H-3) 5.18 and 5.22 (1H each, *dd*, *J* = 10.8 and 10.1 Hz, H-22 and 23) MS *m/z* 412.3740 $[M]^+$ $C_{29}H_{48}O$, 410, 397 $[M^+ - CH_3]$, 379 $[M^+ - CH_3 - H_2O]$, 327, 313, 302, 273, 272, 271, 270, 255, 254, 230, 228, 212, 198, 192, 191, 186, 178, 177, 176, 172, 165, 162, 160, 158, 144, 133, 115, 106, 80, 65, 55 (100%) ^{13}C NMR (100 MHz): See Table 1.

Acetylation of **1**. 70 mg of **1** 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 **2** (60 mg) mp 168° $[\alpha]_D^{25}$ + 21.9° (*c* 1.0 MeOH). IR ν_{max}^{KBr} 1730 and 1235 cm^{-1} ($OCOCH_3$). ^1H NMR δ0.62 (3H, *s*, H-18), 0.80 (3H, *d*, *J* = 6.5 Hz, H-26), 0.82 (3H, *d*, *J* = 6.5 Hz, H-27), 0.90 (3H, *t*, *J* = 5.7 Hz, H-29), 0.96 (3H, *s*, H-19), 1.06 (3H, *br s*, H-21), 2.32 (1H each, *m*, H-24 and H-20), 4.20 (1H, *m*, H-3), 5.18 and 5.22 (1H each, *dd*, *J* = 10.8 and 10.1 Hz, H-22 and 23). MS *m/z* 454.3938, $C_{31}H_{50}O_2$, 452, 412, 411, 410, 394, 369, 327, 302, 273, 272, 271, 255, 254, 230, 228, 212, 198, 192, 186, 178, 177, 158, 144, 115, 106, 80, 55 (100%).

Oxidation of **1**. To 30 mg of **1** in pyridine (2 ml) was added freshly prepared CrO_3-pyridine complex (0.15 g) and left for 24 hr at room temp. to yield ketone **3** crystallized from MeOH mp 128°.

IR ν_{max}^{KBr} cm^{-1}: 1680 cm^{-1}. ^1H NMR δ0.64 (3H, *s*, H-18), 0.76 (3H, *d*, *J* = 6.3 Hz, H-26), 0.82 (3H, *d*, *J* = 6.3 Hz, H-27), 0.95 (3H, *d*, *J* = 6.5 Hz, H-29), 1.02 (3H, *d*, *J* = 5.5 Hz, H-21), 1.30 (3H, *s*, H-19), 2.18 (2H, *dd*, *J* = 7.5 Hz, H-2), 2.32 (1H, each *m*, H-24, H-20), 5.19 and 5.21 (1H each, *dd*, *J* = 10.8 and 10.0 Hz, H-22 and H-23). MS *m/z* at 410.3541, $C_{29}H_{46}O$ 395, 367, 343, 317, 312, 269, 244, 229, 185, 122, and 55 (100%).

Table 1. ^{13}C NMR of compounds **1**, **2** and **3** in $CDCl_3$

C	1	2	3
1	38.4	36.5	37.3
2	23.0	25.0	26.2
3	72.0	80.6	203.2
4	26.5	27.2	27.5
5	57.2	58.4	55.2
6	19.1	18.2	18.9
7	33.2	34.2	34.3
8	139.7	140.1	140.5
9	142.3	142.9	143.0
10	47.0	37.3	37.5
11	25.1	23.9	25.0
12	27.3	25.9	25.8
13	42.4	42.5	42.4
14	40.1	40.2	39.8
15	30.9	30.5	30.0
16	31.2	31.1	31.0
17	44.0	44.3	44.2
18	34.8	34.6	34.5
19	56.9	45.7	56.3
20	41.0	41.0	41.0
21	29.0	29.0	29.0
22	122.2	122.5	122.4
23	122.7	122.7	122.7
24	27.3	27.9	27.9
25	24.3	24.8	24.8
26	19.1	20.3	20.3
27	20.4	20.6	20.6
28	18.0	18.7	18.7
29	22.5	23.2	23.0
$OCOCH_3$	—	175.1	—
$OCOCH_3$	—	35.4	—

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REFERENCES

1. Dhar, U. (1982) in *Alpine flora of Himalayas*. Vikas Publication, New Delhi.
2. Razdan, T. K., Qurishi, M. A. and Khuroo, M. A. (1990) *Proc. XVIIth IUPAC Symp. Natural Products*, New Delhi p. 254.
3. Barton, D. H. R. and de Mayo, P. (1954) *J. Chem. Soc.* 900.
4. Khroo, M. A., Qurishi, M. A., Razdan, T. K. and Nicholas, P. (1988) *Phytochemistry* **27**, 3541.

5. Budzikiewicz, H. and Djerassi, C. (1962) *J. Am. Chem. Soc.* **84**, 1430.
6. Wyllie, S. G. and Djerassi, C. (1968) *J. Org. Chem.* **33**, 305.
7. Williams, D. H., Wilson, J. M., Budzikiewicz, H. and Djerassi (1963) *C. J. Am. Chem. Soc.* **85**, 2091.
8. Garg, V. K. and Nes, W. R. (1984) *Phytochemistry* **23**, 2919.
9. Fieser, L. F., Fieser, M. (1959) *Steroids* p. 144. Rein hold, New York.
10. Abramson, H. N. and Kim, C. S. (1973) *Phytochemistry* **12**, 951.