Acta Poloniae Pharmaceutica – Drug Research, Vol. 68 No. 3 pp. 393–401, 2011 ISSN 0001-6837
Polish Pharmaceutical Society

NATURAL DRUGS

NEW STEROIDAL LACTONES AND HOMOMONOTERPENIC GLUCOSIDE FROM FRUITS OF *MALVA SYLVESTRIS* L.

AKHLAQ MUSTAFA and MOHAMMED ALI[1]*

Drug Standardization Research Unit, Department of Chemistry, Faculty of Science,
Jamia Hamdard, Hamdard Nagar, New Delhi-110062, India
[1]Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy,
Jamia Hamdard, Hamdard Nagar, New Delhi-110062, India

Abstract: Phytochemical investigation of the ethanolic extract of defatted fruits of *Malva sylvestris* Linn. (Malvaceae) led to the isolation of six new steroidal lactones and a homomonoterpenic glucoside along with β-sitosterol-3-β-D-glucopyranoside. The structures of new phytoconstituents have been elucidated as cholest-5-en-3a-ol-18(21)-olide (sylvestrosterol A), cholest-9(11)-en-3α-ol-18(21)-olide (sylvestrosterol B), cholest-4,6,22-trien-3α-ol-18(21)-olide (sylvestrosterol C), 2-methyl-6-methylene-n-decan-2-olyl- 3β-D-glucopyranoside (malvanoyl glucoside), cholest-7-en-18(21)-olide-3α-olyl-3β-D-glucopyranoside (sylvestrogenin A), cholest-9(11)-en-18(21)-olide-3α-olyl-3β-D-glucopyranoside (sylvestrogenin B) and cholest-5-en-8(21)-olide-3α-olyl-3β-D-glucopyranoside (sylvestrogenin C).The structures of all these phytoconstituents have been established on the basis of spectral data analysis and chemical reactions.

Keywords : *Malva sylvestris,* fruits, steroidal lactones, homomonoterpenic glucoside

Malva sylvestris L. (Malvaceae), commonly known as gulkhair or vilaytti Kangani, is an erect, branched, woody perennial or biennial herb up to 120 cm high. It is native to Europe mainly to Italy ; distributed in western Asia, North America, temperate western Himalayas from Kashmir to Kumaon between 700–2700 m, southern India, Siberia, Australia and China (1). All parts of the plant are rich in mucilage. The herb is antiseptic, demulcent, emollient and refrigerant and prescribed to treat pulmonary diseases, blepharitis, abscesses, inflammation, sore throat, chronic bronchitis, jaundice, enlarged spleen; strangaury, urinary discharges and scorpion sting (2). The flowers and immature fruits are efficacious to cure whooping cough and are official in French and Swiss pharmacopoeias (1). Malvin (2), 8–hydroxyflavonoid glucuronides (3), malvidin-3-O-(6''-malonyl glucoside)-5-O-glucoside (4), terepenoids and phenolic derivatives (5), acidic polysaccharides (6), chlorophylls, carotenoides (7) and malvone-A (8) in the plant have been reported. This manuscript describes the isolation and characterization of six new steroidal lactones and a homomonoterpenic glucoside along with β-sitosterol-3-β-D-glucopyranoside from the fruits of the plant.

EXPERIMENTAL

General procedure

Melting points of the compounds were determined on a Perfit melting point apparatus and are uncorrected. UV spectra were recorded on Beckman DU-6 spectrophotometer in methanol. IR spectra were measured on Jasco FT / IR-5 5000 spectrophotometer using KBr discs. ^{1}H-NMR spectra were screened on Bruker spectrospin 400-MHz instrument using $CDCl_3$ as solvent and tetramethylsilane (TMS) as an internal standard. ^{13}C-NMR spectra were recorded on Bruker spectrospin 100-MHz with TMS as an internal standard. Mass spectra were scanned using electron impact (EI) ionization at 70 eV on a JEOL- JMS-DX 303 instrument. Column chromatography was performed on silica gel (Qualigens, Mumbai, India) 60–120 mesh. TLC was run on silica gel G (Qualigens, Mumbai, India). Spots were visualized by exposure to iodine vapors, UV radiation and by spraying reagents.

* Corresponding author: e-mail: maliphyto@gmail.com; mali_hamdard@yahoo.co.in

Plant material

The fruits of *M. sylvestris* were procured from Delhi market. The collection was matched with reference herbarium of Drug Standardisation Research Unit, and was identified by the botanist Dr. Raisuddin Ahmed, Drug Standardization Research Unit, Faculty of Science, Jamia Hamdard, Hamdard Nagar, New Delhi. Fruit parts were air dried in shade for 78 h and then oven dried for 72 h below 50°C.

Extraction

The dried fruits (3 kg) were powdered coarsely and extracted with petroleum ether (60–80°C) in a Soxhlet apparatus to leach out all the petroleum ether soluble matters, 206 g (6.88%). The defatted material was extracted with ethanol exhaustively for 48 h. The extract was concentrated to get a dark brown colored viscous mass, 148 g (4.93%).

Isolation and characterization

The ethanol extract was dissolved in a minimum amount of methanol and adsorbed in silica gel to form a slurry. The slurry was air-dried and chromatographed over silica gel column prepared in petroleum ether. The column was eluted with petroleum ether : chloroform (1:1, 1:3, 0:1 v/v) and chloroform : methanol (99:1, 24:1 v/v) to isolate the following compounds :

Compound 1

Elution of the column with petroleum ether : chloroform (1:1) afforded colorless flakes of **1,** recrystallized from acetone; yield : 124 mg (0.0413%), R_f : 0.79 (petroleum ether : chloroform, 1:1 v/v); m.p.: 89–90°C. UV (MeOH, λ_{max}): 290 nm (log ε 3.8), IR (KBr, cm^{-1}): 3450, 2918, 2850, 1725, 1640, 1465, 1295, 1180, 960, 722. ^{1}H-NMR ($CDCl_3$, δ, ppm): 5.34 (d, 1 H, *J* = 5.2 Hz, H-6), 4.18 (brm, 1 H, H_2-18α), 4.15 (brs, 1 H, H_2-18β), 3.66 (brm, 1 H, $w_{1/2}$ = 8.5 Hz, H-3β), 2.36 (dd, 2 H, *J* = 7.41, 6.93 Hz, H-4), 2.31 (dd, 2 H, *J* = 7.4, 6.9 Hz, H-7), 2.02 (m, 1 H, H-20), 1.05 (brs, 3 H, Me-19), 0.87 (d, 3 H, *J* = 6.7 Hz, Me-26), 0.82 (d, 3 H, *J* = 7.3 Hz, Me-27). ^{13}C-NMR: (Tab.1.). EIMS m/z (rel. int.)**:** 414 $[M]^+$ ($C_{27}H_{42}O_3$) (10.1), 399 (9.9), 396 (56.2), 384 (13.1), 281 (11.0), 192 (17.3), 178 (9.6), 174 (10.8), 164 (17.4), 156 (21.5), 142 (15.0), 135 (25.6), 126 (18.3), 109 (40.7), 97 (60.1), 95 (73.1), 85 (31.3), 83 (81.7), 72 (42.3), 71 (70.3), 69 (7.36), 57 (100), 43 (94.3).

Compound 2

Elution of the column with petroleum ether : chloroform (1:3 v/v) gave colorless amorphous powder of **2** recrystallized from acetone; yield: 280 mg (0.0093 %), R_f: 0.61 (petroleum ether : chloroform, 1:3 v/v); m.p.: 81–83°C. UV (MeOH, λ_{max}): 280 nm (log ε 3.1). IR (KBr, cm^{-1}): 3450, 2924, 2850, 1725, 1640, 1465, 1350, 1280, 1105; ^{1}H-NMR ($CDCl_3$, δ, ppm): 5.27 (dd, 1 H, *J* = 7.4, 7.6 Hz, H-11), 4.08 (brs, 1 H, H-18α), 4.01 (brs, 1 H, H-18β), 3.59 (brm, 1 H, $w_{1/2}$ = 8.5 Hz, H-3β), 2.29 (d, 1 H, *J* = 7.4 Hz, H_2-12a), 2.21 (1 H, d, *J* = 7.6 Hz, H_2-12 b), 2.04 (m, 1 H, H-20), 1.94 (m, 1 H, H-8), 1.55 (m, 1 H, H-5), 1.52 (m, 1 H, H-14), 1.08 (brs, 3 H, Me-19), 0.86 (d, 3 H, *J* = 6.9 Hz, Me-26), 0.80 (d, 3H, *J* = 6.9 Hz, Me-27); ^{13}C-NMR ($CDCl_3$): (Tab. 1); EIMS m/z (rel. int.): 414 $[M]^+$ ($C_{27}H_{42}O_3$) (10.7), 394 (10.7), 379 (4.29), 255 (11.1), 212 (14.1), 184 (10.6), 156 (7.1), 142 (10.8), 128 (36.1), 126 (9.3), 112 (10.8), 110 (28.2), 95 (31.5), 85 (48.6), 83 (51.1), 73 (79.3), 57 (100), 43 (91.5).

Compound 3

Elution of the column with chloroform gave colorless amorphous powder of **3**, recrystallized from acetone; yield: 36 mg (0.0012%); R_f: 0.65 (chloroform); m.p.: 120–121°C; UV (MeOH, λ_{max}): 281 nm (log ε 3.9); IR (KBr, cm^{-1}): 3450, 2950, 2865, 1730, 1645, 1460, 1355, 1055, 948; ^{1}H-NMR (DMSO-d_6 + $CDCl_3$, δ, ppm): 5.36 (ddd, 1 H, *J* = 6.3, 7.5, 8.3 Hz, H-23), 5.26 (d, 1 H, *J* = 6.3 Hz, H-6), 5.15 (d, 1 H, *J* = 6.3 Hz, H-4), 5.07 (dd, 1 H, *J* = 6.3, 8.1 Hz, H-7), 5.01 (dd, 1 H, *J* = 9.9, 8.3 Hz, H-22), 3.84 (brs, 1 H, H_2-18α), 3.82 (brs, 1 H, H_2-18β), 3.27 (brm, 1 H, $w_{1/2}$ = 8.5 Hz, H-3β), 1.20 (brs, 3 H, Me-19), 0.88 (d, 3 H, *J* = 6.5 Hz, Me-26), 0.84 (3 H, *J* = 6.3 Hz, Me-27); ^{13}C-NMR: (Tab. 1); EIMS m/z (rel. int.): 410 $[M]^+$ ($C_{27}H_{38}O_3$) (1.1), 395 (8.3), 327 (51.5), 312 (18.3), 260 (11.4), 193 (46.8),190 (10.2), 176 (13.6), 175 (10.8), 165 (10.1), 162 (10.1), 151 (10.9), 144 (8.5), 137 (13.2), 136 (14.7), 110 (43.8), 96 (31.3), 85 (63.8), 83 (48.3), 71 (51.8), 58 (100), 43 (51.6).

Compound 4

Elution of the column with $CHCl_3$: MeOH (49:1 v/v) furnished colorless amorphous powder of **4,** recrystallized from MeOH; yield: 42 mg (0.0014%); R_f : 0.51 (chloroform : MeOH, 45:2 v/v); m.p.: 248–249°C; UV (MeOH, λ_{max}): 300 nm (log ε 3.2); IR (KBr, cm^{-1}): 3500, 3350, 2923, 2853, 1640, 1278, 795; ^{1}H-NMR ($CDCl_3$, δ, ppm): 5.30 (d, 1H, *J* = 7.1 Hz, H-1') 4.77 (brs, 2 H, H-12), 4.42 (m, 1 H, H-5') 3.64 (dd, 1 H, *J* = 7.7, 6.5 Hz H-2'), 3.56 (m, 1 H, H-3'), 3.44 (m, 1 H, H-4'), 3.29 (d, H, *J* = 6.1 Hz, H_2-6'a), 3.26 (d, 1 H, *J* = 6.1 Hz, H_2-6'b), 2.26 (m, 2 H, H-5), 1.97 (m, 2 H, H-7), 1.61 (m, 2 H, H-3), 1.48 (m, 2 H, H-4), 1.25 (brs, 4 H, H-8, H-9),

Table 1. ^{13}C-NMR spectral data of compounds **1**–**3** and **5**–**8**.

C	Compound						
atom	**1**	**2**	**3**	**5**	**6**	**7**	**8**
1.	37.21	37.40	37.15	36.84	36.82	36.87	37.23
2.	31.89	31.89	31.62	31.42	31.38	31.43	31.87
3.	69.24	73.71	81.21	78.70	70.77	73.25	72.08
4.	45.82	45.82	121.18	44.07	45.42	45.24	44.89
5.	139.15	50.02	145.00	140.48	54.32	51.04	141.05
6.	128.03	19.00	127.30	121.22	18.29	18.55	128.79
7.	31.49	32.75	115.27	33.36	120.97	33.33	33.93
8.	34.06	34.37	45.91	35.02	139.41	35.57	34.82
9.	51.22	139.96	48.95	49.64	51.39	140.43	51.41
10.	36.96	37.07	33.63	36.23	35.65	36.23	37.18
11.	36.12	122.55	24.47	31.42	33.58	121.11	33.93
12.	39.75	39.71	32.18	41.30	39.33	41.86	39.33
13.	42.29	45.40	42.17	45.17	41.32	49.67	42.18
14.	56.04	56.02	53.11	53.75	55.61	56.23	52.27
15.	24.66	24.96	23.16	25.49	24.42	24.47	24.68
16.	29.64	29.06	28.83	28.73	29.11	29.09	29.05
17.	56.74	56.21	55.23	55.45	56.33	55.99	54.16
18.	62.10	63.15	61.79	11.79	65.16	61.13	62.07
19.	14.05	14.06	14.31	18.62	13.29	13.85	14.04
20.	28.22	28.22	28.16	35.58	28.62	28.91	27.14
21.	173.77	173.64	169.37	18.95	172.22	173.06	174.03
22.	29.64	29.66	113.22	29.27	30.82	31.43	31.46
23.	29.40	29.42	110.82	29.06	29.11	29.09	29.62
24.	29.32	29.33	28.71	28.41	28.60	28.77	29.40
25.	29.22	29.22	30.16	33.36	28.36	33.33	29.30
26.	26.08	26.71	25.21	25.49	24.38	24.47	24.68
27.	22.65	22.65	22.15	22.63	22.10	22.13	22.63
28.	—-	—-	—-	23.86	—-	—-	—-
29.	—-	—-	—-	12.07	—-	—-	—-
1’	—-	—-	—-	100.81	99.6	100.73	105.11
2’	—-	—-	—-	76.70	71.23	75.97	71.81
3’	—-	—-	—-	73.49	67.31	73.47	67.12
4’	—-	—-	—-	70.14	64.47	70.08	64.98
5’	—-	—-	—-	76.76	78.63	79.34	78.89
6’	—-	—-	—-	61.13	61.67	60.83	60.17

0.89 (brs, 3 H, Me-1), 0.86 (brs, 3 H, Me-11), 0.84 (t, 3 H, J = 6.5 Hz, Me-10); ^{13}C-NMR ($CDCl_3$, δ, ppm): 11.34 (C-10), 22.34 (C-9), 23.51 (C-4), 25.35 (C-8), 28.89 (C-11), 28.92 (C-1), 31.17 (C-5), 33.20 (C-7), 36.56 (C-3), 61.69 (C-6’), 68.26 (C-2’), 70.21 (C-4’), 71.36 (C-3’), 73.05 (C-5’), 75.18 (C-2), 100.54 (C-1’), 108.96 (C-12), 144.35 (C-6); EIMS m/z (rel. int.): 346 $[M]^+$ ($C_{18}H_{34}O_6$) (5.2) 183 (26.6), 168 (100), 152 (69.1), 134 (1.0), 125 (9.2), 79 (17.2), 43 (15.1).

Hydrolysis of **4**

Compound **4** (20 mg) was dissolved in ethanol (5 mL), 1 M KOH solution added and the reaction mixture heated for 3 h. It was acidified with dilute HCl and extracted with chloroform (3 × 5 mL). The aqueous phase was concentrated and chromatographed on silica gel TLC along with samples of standard sugars using n-BuOH : AcOH : H_2O (4:1:5 v/v/v) as a developing solvent. R_f 0.12, comparable with D-glucose.

Compound 5

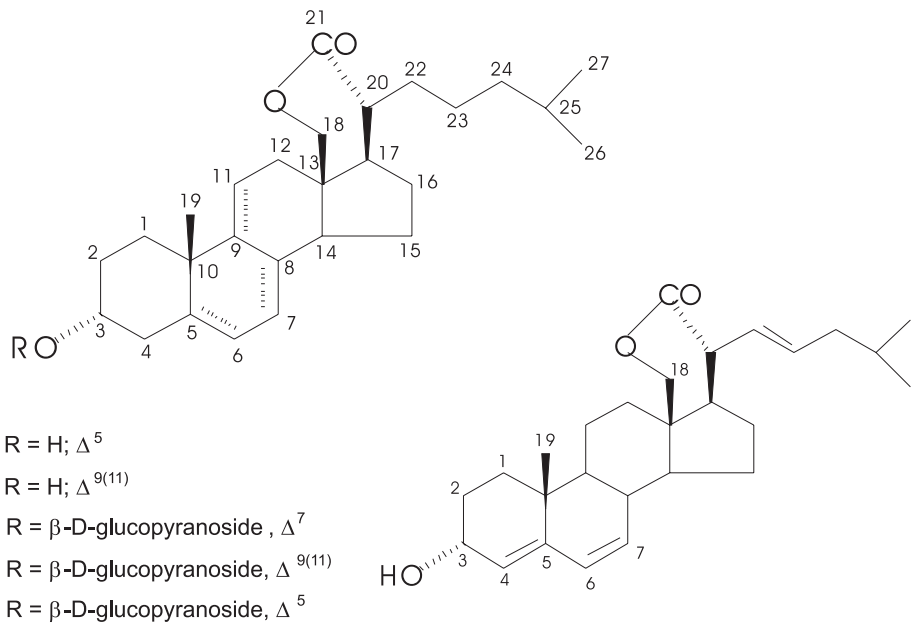
Elution of the column with chloroform : methanol (19:1 v/v) yielded colorless amorphous powder of **5,** recrystallized from MeOH; yield: 450 mg (0.015%), R_f : 0.68 (chloroform), m.p.: 135–136°C, UV (MeOH, λ_{max}): 285 nm (log ε 3.2), IR (KBr, cm^{-1}): 3450, 2923, 2853, 1640, 1465, 1350, 1160, 1085; ^{1}H-NMR (DMSO-d_6, δ, ppm): 5.32 (d, 1 H, *J* = 5.3 Hz, H-6), 4.85 (d, 1 H, *J* = 7.2 Hz, H-1), 4.42 (m, 1 H, H-5'), 4.23 (dd, 1 H, *J* = 7.5, 7.2 Hz, H-2'), 4.05 (m, 1 H, H-3'), 3.62 (brm, 1 H, $w_{1/2}$ = 18.6 Hz, H-3a), 3.51 (m, 1 H, H-4'), 3.09 (brs, 1 H, H_2-6'a), 3.04 (brs, 1 H, H_2-6'b), 0.98 (brs, 3 H, Me-19), 0.95 (d, 3 H, *J* = 6.0 Hz, Me-21), 0.90 (d, 3 H, *J* = 6.1 Hz, Me-26), 0.86 (d, 3 H, *J* = 6.0 Hz, Me-27), 0.82 (d, 3 H, *J* = 6.5 Hz, Me-29), 0.64 (brs, 3 H, Me-18). EIMS m/z (rel. int.): 576 $[M]^+$ ($C_{35}H_{60}O_6$) (9.8). ^{13}C-NMR: (Tab. 1).

Compound 6

Elution of the column with chloroform : MeOH (19:1 v/v) yielded colorless amorphous powder of **6,** recrystallized from $CHCl_3$: MeOH (1:1 v/v); yield: 140 mg (0.005%); m.p.: 195–197°C; UV (MeOH, λ_{max}): 290 nm (log ε 3.2); IR (KBr, cm^{-1}): 3450, 3360, 2920, 2850, 1725, 1635, 1460,1375, 1280, 1110, 980; ^{1}H-NMR ($CDCl_3$ δ, ppm): 5.08 (d, 1 H, *J* = 5.3 Hz, H-7), 4.97 (d, 1 H, *J* = 7.1 Hz, H-1'), 4.33 (brs, 2 H, H-18), 4.02 (m, 1 H, H-5'), 3.92 (dd, 1 H, *J* = 6.5, 7.1 Hz, H-2'), 3.87 (m, 1 H, H-3'), 3.62 (m, 1 H, H-4'), 3.41 (brm, 1 H, $w_{1/2}$ = 7.5 Hz, H-3ß), 3.08 (brs, 2 H, H_2-6'), 2.08 (m, 2 H, H-6), 2.07 (m, 1 H, H-20), 2.00 (m, 1 H, H-9), 1.97 (m, 1 H, H-14), 1.52 (m, 1 H, H-5), 1.35 (m, 1 H, H-17), 1.30 (m, 1 H, H-25), 1.01 (brs, 3 H, Me-19), 0.85 (d, 3 H, *J* = 6.5 Hz, Me-26), 0.82 (d, 3 H, *J* = 6.5 Hz, Me-27); ^{13}C-NMR ($CDCl_3$): (Tab. 1); EIMS m/z (rel int.): 576 $[M]^+$ ($C_{33}H_{52}O_8$) (2.3), 413 (7.3), 396 (100), 381 (21.2), 287 (5.4), 256 (10.5), 254 (11.7), 214 (12.0), 174 (13.2), 162 (15.4), 160 (18.0), 148 (23.6), 146 (16.6), 144 (18.6), 134 (20.7), 120 (21.2), 107 (19.6), 93 (31.5), 85 (29.4), 73 (48.1), 57 (30.6), 43 (19.2).

Hydrolysis of 6

Compound 6 (25 mg) was dissolved in ethanol (5 mL), dil. HCl (2 mL) was added and the mixture was heated for 3 h. The solvent was evaporated



1. R = H; Δ^5

2. R = H; $\Delta^{9(11)}$

6. R = β-D-glucopyranoside , Δ^7

7. R = β-D-glucopyranoside, $\Delta^{9(11)}$

8. R = β-D-glucopyranoside, Δ^5

Figure 1. Structures of the new compounds from *Malva sylvestris* (fruits)

under reduced pressure and the residue was dissolved in $CHCl_3$ to separate the sterol, m.p. 115–116°C. The residue was dissolved in water and chromatographed on silica gel TLC with the samples of reference sugars. The sugar was identified as β-D-glucose (R_f: 0.12) in mobile phase n-BuOH : AcOH : H_2O, 4:1:5 v/v/v, top layer).

Compound 7

Elution of the column with chloroform : MeOH (9:1 v/v) furnished colorless amorphous powder of **7,** recrystallized from $CHCl_3$: MeOH (1:1 v/v), yield: 450 mg (0.015%), m.p.: 220–221°C, UV (MeOH, λ_{max}): 282 nm (log ε 4.1), IR (KBr, cm^{-1}): 3380, 3295, 3445, 2923, 2853, 1737, 1640, 1464, 1377, 1174, 1018; ^{1}H-NMR (DMSO-d_6, δ, ppm): 5.16 (m, 1 H, H-11), 4.89 (d, 1 H, *J* = 7.1 Hz, H-1'), 4.01 (brs, 1 H, H_2-18α), 3.98 (brs, 1 H, H_2-18β), 3.85 (m, 1 H, H-5'), 3.54 (dd, 1 H, *J* = 7.1, 5.2 Hz, H-2'), 3.47 (m, 1 H, H-3'), 3.44 (brm, 1 H, $w_{1/2}$ = 8.5 Hz, H-3β), 3.34 (brs, 1 H, H-4'), 3.05 (brs, 1 H, H_2-6α), 3.01(brs, 1 H, H_2-6β), 2.32 (d, 1 H, *J* = 5.3 Hz, H_2-12α), 2.13 (d, 1 H, *J* = 5.5 Hz, H_2-12β), 2.09 (m, 1 H, H-8β), 2.01 (m, 1 H, H-20α), 1.85 (m, 1 H, H-14) 1.67 (m, 1 H, H-5), 1.37 (m, 2 H, H-17, H-25), 1.09 (brs, 3 H, Me-19), 0.82 (d, 3 H, *J* = 6.0 Hz, Me-26), 0.79 (d, 3 H, *J* = 6.3 Hz, Me-27). ^{13}C-NMR: (Tab. 1). EIMS m/z (rel. int.): 576 $[M]^+$ ($C_{33}H_{52}O_8$) (2.1), 394 (12.8), 379 (9.9), 256 (16.8), 254 (12.1), 238 (9.4), 214 (12.1), 198 (7.4), 184 (9.9), 170 (14.8), 154 (15.6), 134 (17.2), 124 (21.0), 110 (37.3), 96 (58.2), 85 (52.8), 83 (69.3), 71 (78.6), 69 (55.1), 57 (100), 55 (51.4), 43 (73.5).

Hydrolysis of **7**

Compound **7** (20 mg) was dissolved in ethanol (5 mL), dil. HCl (2 mL) was added and the reaction mixture was heated for 1 h on the steam bath. The solvent was evaporated under reduced pressure and the residue was dissolved in $CHCl_3$ to separate the sterol, m.p. 81–82°C. The residue was dissolved in water and chromatographed on silica gel TLC using n-BuOH : AcOH : H_2O (4:1:5 v/v/v) as a developing solvent along with standard samples of sugars, R_f: 0.12, comparable with β-D-glucose.

Compound 8

Further elution of the column with chloroform : methanol (9:1 v/v) gave colorless amorphous powder of **8,** recrystallized from MeOH; yield: 380 mg (0.013 %) m.p.: 160–161°C; UV (MeOH, λ_{max}): 260 nm (log ε 2.8) IR (KBr, cm^{-1}): 3460, 3350, 2922, 2852, 1730, 1640, 1465, 1380, 1230, 1018. ^{1}H-NMR ($CDCl_3$, δ, ppm): 5.34 (d, 1 H, *J* = 5.3 Hz, H-6), 4.95 (d, 1 H, *J* = 7.1 Hz, H-1'), 4.77 (m, 1 H, H-5'), 4.13 (brs, 1 H, H_2-18α), 4.11 (brs, 1 H, H_2-18β), 4.08 (dd, 1 H, *J* = 7.1, 7.5 Hz, H-2'), 3.66 (brm, 2 H, H-3', H-4'), 3.47 (brm, 1 H, $w_{1/2}$ = 8.5 Hz, H-3β), 3.17 (brs, 2 H, H -6'), 2.77 (m, 1 H, H-20β), 2.36 (m, 2 H, H -4), 2.31 (m, 2 H, H-7), 2.03 (m, 1 H, H-9), 1.60 (m, 1 H, H-8), 1.54 (m, 1 H, H-14) 1.47 (m, 1 H, H-17), 1.39 (m, 1 H, H-25), 1.06 (brs, 3 H, Me-19), 0.87 (d, 3 H, *J* = 6.5 Hz, Me-26), 0.83 (d, 3 H, *J* = 6,.5 Hz, Me-27). ^{13}C-NMR ($CDCl_3$): (Tab. 1). EIMS m/z (rel. int.): 576 $[M]^+$ ($C_{33}H_{52}O_8$) (3.2), 412 (12.5), 396 (32.4), 394 (100), 379 (20.3), 328 (6.0), 302 (7.3), 287 (10.4), 274 (11.3) 256 (13.8), 254 (25.0), 232 (10.4), 213 (19.3), 202 (9.3), 176 (11.8), 160 (28.8), 146 (33.0), 144 (29.6), 139 (15.1), 137 (15.0), 135 (28.1), 120 (28.8), 108 (35.8), 95 (46.2), 83 (73.8), 71 (38.2), 57 (35.1), 43 (28.7).

Hydrolysis of **8***:*

Compound **8** (20 mg) was heated with ethanol (5 mL) and dil. HCl for 1 h on a steam bath. The solvent was evaporated under reduced pressure and the residue was dissolved in $CHCl_3$ to separate the sterol, m.p. 89–90° and co-TLC comparable with **1**. The residue was dissolved in water and chromatographed on silica gel TLC along with standard samples of sugars, using n-BuOH : AcOH : H_2O (4:1:5 v/v/v) as a developing solvent, R_f: 0.12, comparable with β-D-glucose.

RESULTS AND DISCUSSION

Compound **1**, named sylvestristerol A, was obtained as colorless flakes from petroleum ether : chloroform (1:1) eluants. It responded positively to Liebermann-Burchard test and showed IR absorption bands for hydroxyl group (3450 cm^1), lactone group (1725 cm^{-1}) and unsaturated bond (1640 cm^{-1}). On the basis of mass and ^{13}C-NMR spectra, its molecular weight was established at m/z 414 consistent with the molecular formula of a steroidal lactone as $C_{27}H_{42}O_3$. It had seven double bond equivalents adjustable in the steroidal nucleus, vinylic linkage and lactone ring. The important ion fragments appeared at m/z 399 $[M - Me]^+$, 384 $[399 - Me]^+$, 396 $[M - H_2O]$, 72 $[C_{1,10} - C_{4,5}$ fission$]^+$ and 83 $[C_{2,3} - C_{5,10} - C_{7,8,}$ fission$]^+$ indicating the presence of hydroxyl group in ring A which was placed at C_3 atom on biogenetic grounds. The ion peaks at m/z 192 $[C_{12,13} - C_{8,14}$ fission$]^+$, 174 $[192 - H_2O]^+$, 178 $[192 - CH_2]^+$, 164 $[178 - CH_2]^+$, 156 $[C_{13,18} - C_{17,20}$ fission$]^+$, 142 $[156 - CH_2]^+$, 126 $[156 - CH_2O]^+$ and prominent ion peaks at m/z 85, 71, 57 and 43 supported saturated nature of the rings C and D and

location of the side chain and lactone ring involving C_{18} and C_{21} atoms. The ^{1}H-NMR spectrum of **1** exhibited a one-proton doublet at δ 5.34 ppm (*J* = 5.2 Hz) assigned to vinylic H-6 proton. Two one-proton broad signals at δ 4.18 and 4.15 ppm were ascribed to C-18 oxygenated methylene protons. A one-proton broad multiplet at δ 3.66 ppm with half-width of 8.5 Hz was due to C-3β methine proton. A three-proton broad signal at δ 1.05 ppm was associated with C-19 tertiary methyl protons. Two three-proton doublets at δ 0.87 (*J* = 6.7 Hz) and 0.82 (*J* = 7.3 Hz) ppm were associated to C-26, C-27 secondary methyl functionalities, respectively. The remaining methylene and methine protons resonated between δ 2.36 and 2.02 ppm. The appearance of all the methyl signals between δ 1.05–0.82 ppm supported their attachment at the saturated carbons. The ^{13}C-NMR spectrum of **1** displayed the existence of 27 carbon atoms in the molecule. The assignment of carbon chemical shift were made by comparing with the d values of the corresponding carbon atoms in the structurally similar compounds (8).The important signals appeared for lactone carbon at δ 173.77 ppm (C-21), oxygenated methylene carbon at δ 62.10 ppm (C-18), carbinol carbons at δ 69.24 ppm (C-3), vinylic carbons at δ 139.15 ppm (C-5) and 128.03 ppm (C-6) and methyl carbons at δ 14.05 (C-19), 26.08 (C-26) and 22.65 (C-27) ppm. The DEPT spectrum of **1** showed the presence of three methyl, twelve methylene, eight methine and four quaternary carbons. The ^{1}H – ^{1}H COSY spectrum of **1** exhibited correlations of H-3 with H-2 and H-4, H-6 with H-4, H-7 and H- 8; H -18 with H -12 and H-17; and H-20 with H-17 and H-22. The HMBC spectrum of **1** exhibited interactions of C-3 with H-2 and H-4; C-5 with H-4, H-3 and H-6; C-13 with H-12, H-18 and H-17; and C-21 with H-18 and H-20. On the basis of these evidences, the structure of **1** has been formulated as cholest-en-3α-ol-18(21)-olide. This is a new steroidal lactone isolated from natural or synthetic source for the first time.

Compound **2,** designated as silvestristerol B, [M^+ 414; $C_{27}H_{42}O_3$], was obtained as a colorless amorphous powder from petroleum ether : chloroform (1:3) eluants. Its chemical reactions and spectral data analyses indicated that compound **2** was a positional isomer of **1.** The mass spectrum of **2** showed important ion fragments at m/z 72 [$C_{1,10}$ – $C_{4,5}$ fission]$^+$, 112 [$C_{5,6}$ – $C_{9,10}$ fission]$^+$, 302 [M – 112]$^+$, 126 [$C_{6,7}$ – $C_{9,10}$ fission]$^+$, 140 [$C_{7,8}$ – $C_{9,10}$ fission]$^+$ and 274 [M –140]$^+$ indicated saturated nature of rings A and B and existence of the hydroxyl group in ring A at C-3. The ion peaks arising at m/z 178 [$C_{8,14}$ – $C_{1,12}$ fission]$^+$, 236 [M – 178]$^+$ and 192 [$C_{8,14}$ – $C_{12,13}$ fission]$^+$ supported the location of vinylic linkage at C-9(11). The ^{1}H-NMR spectrum of **2** exhibited a one-proton double doublet at δ 5.27 ppm (*J* = 7.4, 7.6 Hz) assigned to vinylic H-11, two one-proton broad signals at δ 4.8 and 4.01 ppm ascribed to oxygenated H-18 methylene protons, a one-proton broad multiplet at δ 3.59 ppm with half-width of 8.5 Hz attributed to carbinol H-3ß protons, a three-proton broad signals at 1.08 accounted to tertiary C-19 methyl protons and two three-proton doublets at 0.86 ppm (*J* = 6.9 Hz) and at 0.80 ppm (*J* = 6.9 Hz) due to C-26 and C-27 secondary methyl protons, respectively. The ^{13}C-NMR spectrum showed 27 carbon signals and the important signals appeared for olefinic carbons at δ 139.96 (C-9) and 122.55 (C-11) ppm, carbinol carbon at δ 73.71 (C-3) ppm, lactone carbon at δ 173.64 (C-21) ppm and oxygenated methylene carbon at δ 63.15 (C-18) ppm. The ^{1}H – ^{1}H COSY spectrum of **2** exhibited correlations of H-11 with H-12 and H-8; H-3 with H-2, H-4 and H-5; H-18 with H-12 and H-17; and H-20 with H-17 and H-22. The HMBC spectrum of **2** exhibited interactions of C-9 with H-11, H-19 and H-8; C-13 with H-18, H-12 and H-17; and C-21 with H-18, H-20 and H-17. On the basis of above mentioned discussion, the structure of **2** has been elucidated as cholest-9(11)-en-3α-ol-18(21)-olide. This is a new phytosterol lactone.

Compound **3,** named sylvestristerol C, was obtained as a colorless amorphous powder from chloroform eluants. It gave positive tests for a sterol and had IR absorption bands at 3450 (OH), 1730 (lactone) and 1645 (C = C) cm^{-1}. On the basis of mass and ^{13}C-NMR spectra, its molecular weight was established at m/z 410 relating to the steroidal formula $C_{27}H_{38}O_3$. It indicated nine double bond equivalents which were adjusted in tetracyclic steroidal carbon framework, three double bonds and lactone ring. The prominent ion fragments arising at m/z 58 [$C_{1,10}$ – $C_{3,4}$ fission]$^+$, 110 [$C_{5,6}$ – $C_{9,10}$ fission]$^+$ and 136 [$C_{7,8}$ – $C_{9,10}$ fission]$^+$ suggested the existence of the hydroxyl group in ring A, placed at C-3 atom on biogenetic analogy and olefinic linkages at C-4 and C-6. The prominent ion peaks at m/z 83 and 327, generated due to cleavage of C_{20}–C_{22} linkage, indicated the location of one of olefinic linkages at C-22. The ion peaks at m/z 176 [$C_{11,12}$ – $C_{8,14}$ fission]$^+$, 162 [$C_{9,11}$ – $C_{8,14}$ fission]$^+$, 144 [162 – H_2O]$^+$, 190 [$C_{12,13}$ – $C_{8,14}$ fission]$^+$, 165 [M –162 – 83]$^+$, 151 [M – 176 – 83]$^+$ and 137 [M – 190 – 83]$^+$ supported saturated nature of ring C. The ^{1}H-NMR spectrum of **3** displayed one-proton doublet of double doublets at δ 5.36 (*J* = 6.3, 7.5 and 8.3 Hz) ppm assigned to vinylic H-23. Two one-proton doublets at δ 5.26 (*J*

= 6.3 Hz) and 5.15 (*J* = 6.6 Hz) ppm were ascribed to vinylic H-6 and H-4, respectively. Two double doublets at δ 5.07 (*J* = 6.3, 8.1 Hz) and 5.01 (*J* = 9.9, 8.3 Hz) ppm were associated correspondingly with H-7 and H-22. Two broad signals at δ 3.84 and 3.82 ppm, integrated for one proton each, were due to C-18 oxygenated methylene protons. A one-proton broad multiplet at δ 3.27 ppm with half width of 8.5 Hz attested the presence of C-3 carbinol proton in β-orientation. A three-proton broad signal at δ 1.20 ppm was accounted to C-19 tertiary methyl protons. The C-26 and C-27 secondary methyl protons appeared as a three-proton doublets at δ 0.88 (*J* = 6.5 Hz) and 0.84 (*J* = 6.3 Hz) ppm, respectively. The ^{13}C-NMR spectrum of **3** showed six unsaturated carbon signals at δ 121.18 (C-4), 145.0 (C-5), 127.30 (C-6), 115.27 (C-7), 113.22 (C-22) and 110.82 (C-23) ppm, C-3 carbinol carbon at δ 81.21 ppm and lactone carbon at δ 169.37 (C-21) ppm and oxygenated methylene carbon at δ 61.79 (C -18) ppm. The ^{1}H – ^{1}H COSY spectrum of **3** showed correlation of H-3 with H-2 and H-4; H-6 with H-7; and H-22 with H-20, H-23 and H-24. The DEPT spectrum of **3** indicated the presence of three methyl, eight methylene, twelve methine and four quaternary carbon atoms. The HMBC spectrum of **3** exhibited interactions of C-5 with H-4, H-3, H-6 and H-7; C-21 with H-18 and H-20; and C-23 with H-22, H-20 and H-24. On the basis of these informations, the structure of **3** has been established as cholest-4,6,22-trien-3α-ol-18(21)-olide. This is a new phytosterol isolated from a natural or synthetic source for the first time.

Compound **4,** named malvanoyl glucoside, was obtained as colorless amorphous powder from chloroform : methanol (49:1) eluants. It responded to tests for glycosides and unsaturation. Its IR spectrum showed absorption bands for hydroxyl groups (3500, 3350 cm^{-1}) and unsaturation (1640 cm^{-1}). On the basis of mass and ^{13}C-NMR spectra, the molecular weight of **4** was established at m/z 346 corresponding to the molecular formula of a homomonoterpenic glucoside – $C_{18}H_{34}O_6$. The mass spectrum showed important ion peaks at m/z 183 [M – $C_6H_{11}O_5$]$^+$, 168 [183 – Me]$^+$, 152 [168 – Me]$^+$ and 125 [C_2 – C_3 fission]$^+$ suggesting it is a homomonoterpene type glycoside. The ^{1}H-NMR spectrum of **4** exhibited a one-proton doublet at δ 5.30 (*J* = 7.1 Hz) ppm assigned to anomeric H-1'. A two-proton broad signal at δ 4.77 ppm was associated with methylene H-12. Two three-proton broad signals at δ 0.89 and 0.86 ppm were ascribed to C-1 and C-11 tertiary methyl protons. A three-proton triplet at δ 0.84 (*J* = 6.5 Hz) ppm was assigned to terminal C-10 primary methyl protons. The sugar protons appeared as one-proton multiplets at δ 4.42, 3.56 and 3.44 ppm accounted to H-5', H-3' and H-4', respectively, as one-proton double doublets at δ 3.64 (*J* = 7.1, 6.5 Hz) ppm due to (H-2') and as one-proton doublets at δ 3.29 (*J* = 6.1 Hz) and 3.26 (*J* = 6.1 Hz) ppm associated with hydroxyl methylene H-6' protons. The remaining methylene protons resonated between δ 2.26–1.25 ppm. The ^{13}C-NMR spectrum of **4** exhibited eighteen carbon signals and the important signals appeared for vinylic carbons at δ 144.35 (C-6) and 108.96 (C-12) ppm, oxygenated quaternary carbon at δ 75.18 (C-2) ppm, methyl carbons at δ 28.92 (C-1), 11.34 (C-10) and 28.89 (C-11) ppm and sugar protons between δ 100.54–61.69 ppm. The ^{1}H – ^{1}H COSY spectrum of **4** showed correlations of H-12 with H-5 and H-7; H-1 with H-3 and H-11; and H-1' with H-2' and H-3'. The HMBC spectrum of **4** exhibited interactions of C-6 with H-5, H-7 and H-12; C-2 with H-1, H-11 and H–1'; and H-5' with H-6' and H-4'. Alkaline hydrolysis of **4** yielded ß-D-glucose. Based on these evidences the structure of malvanoyl glucoside has been elucidated as 2-methyl-6-methylene-*n*-undecan-2-olyl-β-D-glucopyranoside. This is a new homomonoterpenic constituent isolated from a natural or synthetic source for the first time.

Compound **5**, obtained as a colorless amorphous powder from chloroform : methanol (19:1) eluants, was a known compound characterized as β-sitosterol-3β-D-glucopyranoside.

Compound **6,** designated sylvestrogenin A, was obtained as a colorless amorphous powder from chloroform : methanol (19:1) eluants. It gave positive tests of steroidal glycosides and showed IR absorption bands for hydroxy groups (3450, 3360 cm^{-1}), lactone ring (1725 cm^{-1}) and unsaturation (1635 cm^{-1}). On the basis of mass and ^{13}C-NMR spectra, its molecular weight was established at m/z 576 consistent with the molecular formula of a steroidal lactone glycoside, $C_{33}H_{52}O_8$. The ion peaks arising at m/z 413 [M – $C_6H_{11}O_5$]$^+$ 396 [M – $C_6H_{12}O_6$]$^+$ and 381 [396 – Me]$^+$ supported the glycosidic nature of the molecule. The ion peaks generated due to fragmentation of mass unit 396 at m/z 93 [$C_{5,6}$ – $C_{9,10}$ fission]$^+$, 107 [$C_{6,7}$ – $C_{9,10}$ fission]$^+$, 146 [$C_{8,14}$ – $C_{9,11}$ fission]$^+$, 160 [$C_{8,14}$ – $C_{11,12}$ fission]$^+$, and 174 [$C_{8,14}$ – $C_{12,13}$ fission]$^+$, suggested the location of the vinylic linkage at C-7 and saturated nature of ring C. The ^{1}H-NMR spectrum of **6** showed two one-proton doublets at δ 5.08 (*J* = 5.3 Hz) and 4.97 (*J* = 7.1 Hz) ppm assigned to vinylic H-7 and anomeric H-1', respectively, a two-proton broad signal at 4.33 ppm ascribed to oxygenated methylene

H-18 protons, a one-proton broad multiplet δ 3.41 ppm with half-width of 7.5 Hz accounted to ß-oriented H-3 carbinol proton, sugar protons between δ 4.02–3.08 ppm, a three-proton broad singlet at δ 1.01 ppm and two three-proton doublets at δ 0.85 (J = 6.5 Hz) and 0.82 (J = 6.5 Hz) ppm associated correspondingly to tertiary C-19 and secondary C-26 and C-27 methyl protons. The ^{13}C-NMR spectrum of **6** exhibited signals for lactone carbon at δ 172.22 (C-21) ppm and vinylic carbons at δ 120.97 (C-7) and 139.41 (C-8) ppm, carbinol carbon at δ 70.77 (C-3) ppm, oxygenated methylene carbon at δ 65.16 (C-18) ppm, anomeric carbon at δ 99.6 (C-1') ppm and other sugar carbons from δ 78.63 to d 61.67 ppm. ^{1}H – ^{1}H COSY spectrum of **6** showed correlations of H-3 with H-1', H-2 and H-4; H-7 with H-6 and H-5; and H-18 with H-12 and H-17. The HMBC spectrum of **6** exhibited interactions of C-1' with H-2' and H-3; C-8 with H-7, H-6 and H-9; and C-21 with H-18 and H-20. Acid hydrolysis of **6** yielded D-glucose and a sterol. On the basis of these evidences, the structure of **6** has been formulated as cholest-7-en-18(21)-olide-3α-olyl-3β-D-glucopyranoside. This is a new steroidal glycoside.

Compound **7,** named sylvestrogenin B, was obtained as a colorless amorphous powder from chloroform : MeOH (9:1) eluants. It responded positively to steroidal glycoside tests. Its IR spectrum showed absorption bands for lactone ring (1737 cm^{-1}), hydroxyl groups (3445, 3380, 3295 cm^{-1}) and unsaturation (1640 cm^{-1}). On the basis of mass and ^{13}C-NMR spectra, the molecular weight of **7** was established at m/z 576 ($C_{33}H_{52}O_8$).The mass fragmentation pattern of **7** was almost identical to that of **6**. The ^{1}H-NMR spectrum of **7** showed a one-proton multiplet at δ 5.16 ppm assigned to vinylic H-11. The anomeric H-1' proton appeared as a one-proton doublet at d 4.89 (J = 7.1 Hz). Three one-proton broad signals at d 4.01, 3.98 and δ 3.44 ($w_{1/2}$ = 8.5 Hz) ppm were associated with C-18 oxygenated methylene protons and C-3β carbinol proton. The sugar protons resonated as double doublet at δ 3.54 (J = 7.1, 5.5 Hz, H – 2') and as broad signals at δ 3.34 (H-4'), 3.05 and 3.01 (H-6') ppm and as a multiplet at δ 3.85 (H-5'). A three-proton singlet at d 1.09 was ascribed to C-19 tertiary methyl protons. Two three-proton doublets at δ 0.82 (J = 6.0 Hz) and 0.79 (J = 6.0 Hz) ppm were associated with C-27 and C-26 secondary methyl protons. The ^{13}C-NMR spectrum of **7** displayed carbon signals for lactone ring at δ 173.06 (C-21) and 60.83 (C-18) ppm, olefinic carbons at δ 140.3 (C-9) and 121.11 (C-11) ppm, oxygenated methine carbon at δ 73.25 (C-3) ppm, anomeric carbon at δ 100.73 (C-1') ppm and other sugar carbons between δ 79.34–60.83 ppm. The ^{1}H – ^{1}H COSY spectrum of **7** showed correlations of H-3 with H-2, H-4 and H-1'; H-11 with H-12 and H-8; H-18 with H-12 and H-17; and H-20 with H-17 and H-22. The HMBC spectrum of **7** exhibited interactions of C-1' with H-2' and H-3; C-9 with H-11, H–8, H-7 and H–19; and C-21 with H-18, H-20 and H-22. Acid hydrolysis of **6** yielded D-glucose. From the foregoing account the structure of **7** has been elucidated as cholest-9(11)-en-18-(21)-olide-3α-olyl-3β-D-glucopyranoside. This is a new sterol glucoside isolated from a natural source for the first time.

Compound **8,** named sylvestrogenin C, [M^{+} 576, $C_{33}H_{52}O_3$], was obtained as a colorless amorphous powder from chloroform : methanol (9:1) eluants. It responded positively to steroidal glycoside tests and showed IR absorption bands for hydroxyl groups (3460, 3350 cm^{-1}), lactone group (1730 cm^{-1}) and unsaturation (1640 cm^{-1}). The mass fragmentation pattern of steroidal nucleus was identical to that of compound **1.** The ^{1}H NMR spectrum of **8** established two one-proton doublets at δ 5.34 (J = 5.3 Hz) and 4.95 (J = 7.1 Hz) ppm assigned to vinylic H-6 and anomeric H-1', respectively. A one-proton multiplet at δ 3.47 ppm with half-width multiplet of 8.5 Hz was ascribed to oxygenated methine H-3β, two one-proton broad signals at δ 4.13 and 4.11 ppm were attributed to oxygenated methylene H-18 protons, sugar protons from δ 4.27 to 3.17 ppm, a three-protons broad signal at δ 1.06 due to tertiary C-19 protons and two three-proton doublets at δ 0.87 (J = 6.5 Hz) and 0.83 (J = 6.5 Hz) ppm accounted to secondary C-26 and C-27 methyl protons. The ^{13}C-NMR spectrum of **8** showed signals for lactone carbon at δ 174.3 ppm, vinylic carbons at δ 141.05 (C-5) and 128.79 (C-6) ppm, anomeric carbon at δ 105.11 (C-1') ppm, oxygenated methine carbon at δ 72.08 (C-3) ppm, oxygenated methylene carbon at δ 62.07 (C-18) ppm, and other sugar carbons from δ 78.89 to 60.17 ppm. The ^{1}H – ^{1}H COSY spectrum of **8** showed correlations of H-1' with H-2' and H-3; H-6 with H-4 and H-7; H-18 with H-12 and H-17; and H-20 with H-17 and H-22. The HMBC spectrum of **8** exhibited interactions of C-3 with H-2, H-4 and H-1'; C-5 with H-4 and H-6; and C-21 with H-18 and H-20. Acid hydrolysis of **8** yielded D-glucose. On the basis of the foregoing account, the structure of **8** has been established as cholest-5-en-18(21)-olide-3α-olyl-β-D-glucopyranoside. It is a new steroidal glucoside.

Conclusion

We have isolated six new steroidal lactones and a new monoterpenic glucoside from the fruits of

the drug together with known compound β-sitosterol-3-β-D-glucoside. This is first report concerning isolation of the phytoconstituents from the fruits of *M. sylvestris* L. The existing knowledge about the natural products may be increased by present investigation.

Acknowledgment

Authors are thankful to the Head, SAIF, Central Drug Research Institute, Lucknow, for scanning mass spectra of the compounds.

REFERENCES

1. Anonymous: Wealth of India, Raw materials, National Institute of Science Communication and Information Resources, Vol. 6, p. 250–251, CSIR, New Delhi 2003.
2. Mhaskar K.S., Blatter E., Caius J.F.: Kirtikar and Basu's Indian Medicinal Plants, Vol. 1, p. 300, Shiva Offset Press, Dehradoon 1993.
3. Billeter M., Meier B., Sticher O.: Phytochemistry 30, 987 (1991)
4. Farina A., Doldo A., Cotichini V., Rajevie M., Queglia M.G., Mulinacci N., Vinicieri F.F.: J. Pharm. Biomed. Anal. 14, 203 (1995).
5. Cutillo F., D'Abrosca B., Dellagreca M., Fiorentino A., Zarrelli A.: Phytochemistry 67, 481 (2006).
6. Classen B., Blaschek W.: Planta Med. 64, 640 (1998).
7. Redzic S., Hodzic N., Tuka M.: Bosn. J. Basic Med. Sci. 5, 53 (2005).
8. Veshkurova O., Golubenko Z., Pshenichnov E., Arzanova I., Uzbekov V., Sultanova E. et al.: Phytochemistry, 67, 2376 (2006).
9. Ali M.: Techniques in terpenoid identification, p. 471–530, Birla Publications, Delhi 2001.

Received: 31. 03. 2010