Phytochemical Studies on *Asphodelus tenuifolius*

[1]MUHAMMAD SAFDER, [2]NAHEED RIAZ, [1]MUHAMMAD IMRAN, [1]HAQ NAWAZ, [1]ABDUL MALIK* AND [2]ABDUL JABBAR

[1]*International Centre for Chemical and Biological Sciences, HEJ Research Institute of Chemistry, University of Karachi, Karachi-75270, Pakistan.*

[2]*Department of Chemistry, Baghdad Campus, Islamia University of Bahawalpur, Bahawalpur, Pakistan.*

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Summary: Eight compounds have been isolated for the first time from *Asphodelus tenuifolius* namely, β-sitosterol (**1**), 1-octacosanol (**2**), 1-triacontanol (**3**), hexadecanoic acid (**4**), tetracosanoic acid (**5**), triacontanoic acid (**6**), 3-hydroxybenzoic acid (**7**) and β-sitosterol 3-*O*-β-D-glucopyranoside (**8**), respectively. Their structures have been elucidated by EIMS, HREIMS, HRFABMS and NMR spectroscopic data.

Introduction

The genus *Asphodelus* belongs to the family Lillaceae which comprises 187 genera and 2500 species. The genus *Asphodelus* has seven species which are distributed in South Europe, India and Pakistan [1]. *Asphodelus tenuifolius* is a small erect annual herb found in Karachi, Sindh and Makran [2]. Various species of genus *Asphodelus* are used as anti-ulcer and anti-inflammatory agents [3]. *A. tenuifolius* is used in preventing atherosclerosis, having diuretic properties [4]. Literature survey revealed that no phytochemical studies have so far been carried out on this species and only twelve compounds are reported from the whole genus. This prompted us to carry out the phytochemical studies on this plant. Herein we report the isolation and structure elucidation of β-sitosterol (**1**), 1-octacosanol (**2**), 1-triacontanol (**3**), hexadecanoic acid (**4**), tetracosanoic acid (**5**), triacontanoic acid (**6**), 3-hydroxybenzoic acid (**7**) and β-sitosterol 3-*O*-β-D-glucopyranoside (**8**), respectively.

Results and Discussion

The methanolic extract of the whole plant of *Asphodelus tenuifolius* was subsequently divided into *n*-hexane, chloroform, ethylacetate, *n*-butanol and water-soluble fractions. The chloroform-soluble fraction was subjected to a series of column and flash chromatographic techniques as described in the experimental to obtain eight compounds reported for the first time from this species. These could be identified as β-sitosterol (**1**), 1-octacosanol (**2**), 1-triacontanol (**3**), hexadecanoic acid (**4**), tetracosanoic acid (**5**), triacontanoic acid (**6**), 3-hydroxybenzoic acid (**7**) and β-sitosterol 3-*O*-β-D-glucopyranoside (**8**), respectively, on the basis of their respective spectral data.

Experimental

General

Column and flash chromatography was carried out using silica gel of 70-230 and 230-400 mesh. Aluminium sheets precoated with silica gel 60 F_{254} (20 × 20 cm, 0.2 mm thick; E-Merck) were used for TLC to check the purity of the compounds and were visualized under UV light (254 and 366 nm) followed by ceric sulfate as spraying reagent. Optical rotations were measured on a Jasco DIP-360 digital polarimeter. The UV spectra were recorded on a Hitachi UV-3200 spectrometer (λ_{max} in nm). IR spectra were recorded on Shimadzu IR-460 spectrophotometer (ν in cm^{-1}). EIMS, HREIMS and HRFABMS were recorded on Jeol JMS-HX 110 spectrometer with data system. The ^{1}H-NMR spectra were recorded on Bruker AMX-400 MHz instruments using TMS as an internal reference. The chemical shift values are reported in ppm (δ) units and the scalar coupling constants (*J*) are in Hz. The ^{13}C-NMR spectra were recorded at 100 MHz on the same instrument.

Plant Material

The whole plant of *Asphodelus tenuifolius* Cav. (8 kg) was collected from Cholistan Desert (Bahawalpur) in May 2006 and identified by Dr.

*To whom all correspondence should be addressed.

Muhammad Arshad, Plant Taxonomist, Cholistan Institute for Desert Studies (CIDS), The Islamia University of Bahawalpur, where a voucher specimen (28/CIDS/06) is deposited.

Isolation

The shade dried, ground whole plant of *A. tenuifolius* (8 kg) was extracted with MeOH (3×33 L). The combined MeOH extract was concentrated, and the residue (450 g) was divided into *n*-hexane, chloroform, ethylacetate, *n*-butanol and water-soluble sub-fractions. The chloroform-soluble sub-fraction (95 g) was subjected to column chromatography over silica gel eluting with *n*-hexane, *n*-hexane-chloroform chloroform, chloroform-methanol and methanol in increasing order of polarity. The fractions obtained from *n*-hexane-chloroform (8:2) were combined and chromatographed over silica gel eluting with *n*-hexane-chloroform (7.8:2.2) to obtain β-sitosterol (**1**). The fractions obtained from *n*-hexane-chloroform (7.5:2.5) was a binary mixture, which was resolved by flash chromatography using the same solvent system to obtain 1-octacosanol (**2**), and 1-tricontanol (**3**) from the top and tail fractions, respectively. The fractions obtained from *n*-hexane-chloroform (7:3) showed three spots on TLC, were combined and chromatographed over silica gel using *n*-hexane-chloroform (6.8:3.2) to obtain hexadecanoic acid (**4**), tetracosanoic acid (**5**) and triacontanoic acid (**6**) from the top, middle and tail fractions, respectively. The fractions obtained from *n*-hexane-chloroform (5.5:4.5) were flash chromatgraphed using same solvent system to obtain 3-hydroxybenzoic acid (**7**). The fraction obtained from chloroform-methanol (9.8:0.2) was chromatographed over silica gel using chloroform-methanol (9.7:0.3) as eluent to provide β-sitosterol 3-*O*-β-D-glucopyranoside (**8**).

β-Sitosterol (1)

Crystallized from acetone (22 mg), m.p. 136-137 °C; $[\alpha]_D^{25}$-36 (*c* 0.002, $CHCl_3$); IR (KBr) ν_{max} cm^{-1}: 3444, 3054, 1655; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 5.23 (1H, m, H-6), 3.35 (lH, m, H-3), 1.03 (3H, s, Me-19), 0.93 (3H, d, J = 6.5 Hz, Me-21), 0.86 (3H, t, J = 7.0 Hz, Me-29), 0.84 (3H, d, J = 6.5 Hz, Me-26), 0.82 (3H, d, J = 6.5 Hz, Me-27), 0.69 (3H, s, Me-18); HREIMS, *m/z:* 414.3840 (calcd. for $C_{29}H_{50}O$, 414.3849). The physical and spectral data showed complete agreement with those reported in the literature [5-7].

1-Octacosanol (2)

Crystallized from chloroform (11 mg), m.p. 83-84 °C; IR (KBr) ν_{max} cm^{-1}: 3285, 2890, 1280; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 3.61 (2H, t, J = 6.5 Hz, H-1), 1.52 (4H, m, CH_2-2,4), 1.23 (48H, br. s, CH_2-3,5–27), 0.85 (3H, t, J = 6.5 Hz, H-28); HREIMS, *m/z:* 410.4480 (calcd. for $C_{28}H_{58}O$, 410.4487). The physical and spectral data corresponded to the reported values [8, 9].

1-Tricontanol (3)

Crystallized from *n*-hexane (16 mg), m.p. 87-87°C; IR (KBr) ν_{max} cm^{-1}: 3280, 2880, 1282; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 3.62 (2H, t, J = 6.6 Hz, H-1), 1.54 (4H, m, CH_2-2,4), 1.23 (52H, br s, CH_2-3,5–29), 0.86 (3H, t, J = 6.6 Hz, H-30); HREIMS, *m/z:* 438.4790 (calcd. for $C_{30}H_{62}O$, 430.4800). The physical and spectral data showed close similarities to the literature values [10, 11].

Hexadecanoic acid (4)

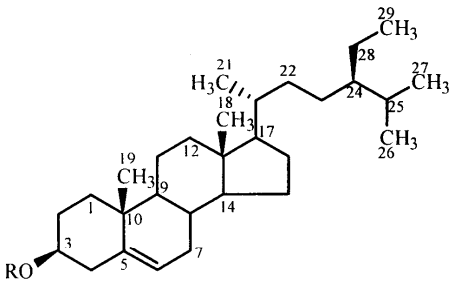
Crystallized from chloroform (18 mg), m.p. 63-64°C; IR (KBr) ν_{max} cm^{-1}: 3310-2686, 1710; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 2.32 (2H, t, J = 7.5 Hz, H-2), 1.59 (2H, m, H-3), 1.23 (24H, br. s, CH_2-4–15), 0.85 (3H, t, J = 6.5 Hz, H-34); HREIMS, *m/z:* 256.2395 (calcd. for $C_{16}H_{32}O_2$, 256.2402). The physical and spectral data showed complete resemblance with those reported in literature [12,13].

Tetracosanoic acid (5)

Crystallized from methanol (17 mg), m.p. 87-88°C; IR (KBr) ν_{max} cm^{-1}: 3286-2723, 1709; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 2.32 (2H, t, J = 7.5 Hz, H-2), 1.58 (2H, m, H-3), 1.23 (40H, br s, CH_2-4–23), 0.85 (3H, t, J = 6.6 Hz, H-30); HREIMS, *m/z:* 368.3648 (calcd. for $C_{24}H_{48}O_2$, 368.3654). The physical and spectral data showed complete agreement with those reported in literature [14, 15].

Triacontanoic acid (6)

Crystallized from acetone (13 mg), m.p. 93-94 °C; IR (KBr) ν_{max} cm^{-1}: 3310-2713, 1709; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 2.33 (2H, t, J = 7.4 Hz, H-2), 1.61 (2H, m, H-3), 1.23 (52H, br s, CH_2-4-29), 0.85 (3H, t, J = 6.4 Hz, H-16); HREIMS, *m/z:* 452.4585 (calcd. for $C_{30}H_{60}O_2$, 452.4593). The



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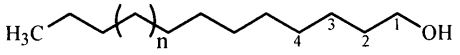
8 R = β-D-glucose



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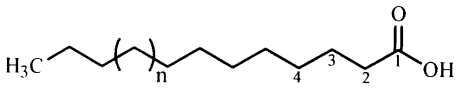

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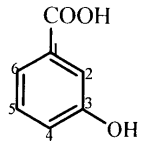

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Fig. 1: Structures of compounds isolated from *A. tenuifolius*.

physical and spectral data showed complete resemblance with the reported values [16, 17].

3-Hydroxybenzoic acid (7)

Crystallized from H_2O (16 mg), m.p. 202-203 °C; UV (CH_3OH) λ_{max} (log ε) nm: 308 (3.92), 288 (3.81), 212 (4.09); IR (KBr) ν_{max} cm^{-1}: 3481, 3250-2680, 1704; ^{1}H-NMR ($CDCl_3$+CD_3OD, 400 MHz) δ: 7.38 (1H, d, J = 7.8 Hz, H-6), 7.31 (1H, d, J = 1.8 Hz, H-2), 7.11 (1H, t, J = 7.8 Hz, H-5), 6.88 (1H, dd, J = 7.8, 1.8 Hz, H-4); HREIMS, *m/z* 138.0306 (calcd. for $C_7H_6O_3$, 138.0309). The physical and spectral data showed complete resemblance with the reported values [18, 19].

β-Sitosterol 3-O-β-D-glucopyranoside (8)

Colorless amorphous solid (29 mg), m.p. 232-235 °C, $[\alpha]_D^{25}$-45 (*c* 0.022, MeOH); IR (KBr) ν_{max} cm^{-1}: 3452, 3044, 1648. ^{1}H-NMR ($CDCl_3$+CD_3OD, 400 MHz) δ: 5.12 (1H, m, H-6), 4.56 (1H, d, J = 7.3 Hz, H-1'), 3.85 (1H, m, H-3), 3.25-3.81 (m, Glc-H), 1.02 (3H, s, Me-19), 0.93 (3H, d, J = 6.2 Hz, Me-21), 0.85 (3H, t, J = 7.0 Hz, Me-29), 0.83 (3H, d, J = 6.4 Hz, Me-26), 0.81 (3H, d, J = 6.4 Hz, Me-27) and 0.68 (3H, s, Me-18); HRFABMS, *m/z*: 577.4444 $[M+H]^+$ (calcd. for $C_{35}H_{61}O_6$, 577.4438). The physical and spectral data were in agreement with the reported values [5-7].

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