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Short communication

Isolation and characterization of β-sitosterol-D-glycoside from petroleum extract of the leaves of *Ocimum sanctum* L

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Abstract. β-sitosterol-D-glycoside was isolated from the petroleum ether extract of the leaves of Ocimum sanctum L. This compound has not been previously isolated or reported from the leaves of this variety. The structures of β-sitosterol-D-glycoside are elucidated with the help of UV, IR, ^{1}H-NMR, ^{13}C-NMR, spectral data.

Keywords: biochemistry, bioactive compounds, isolation, basil, Bangladesh, spectral analysis.

Introduction

Ocimum sanctum L. plant is a shrub reaching a height of 0.5 to 1.5m. The plant is locally known as Tulshi throughout the Indo-Bangla subcontinent, while the English name is Holy Basil. The leaves are 2-4cm in length. Among the several varieties of the plant, one of the most commonly used has dark leaves. The inflorescence is a long spike with tiny purple flowers and the plant has a stronger smell. Different parts of the plant have been traditionally used for the treatment of various diseases such as reducing the blood glucose level as well as reduction of total cholesterol in the blood [1]. Its antioxidant properties have also been reported [2]. It has also been reported [3, 4] that the leaves and seeds of this variety of *Ocimum basilicum* L. contain essential oils [5, 10] and the volatile oil of the seed is composed of fatty acids and β-sitosterol, while in addition the seed mucilage has some level of sugars [5, 10].

In the present investigation, we describe the isolation of β-sitosterol-D-glycoside from the petroleum ether extract of the leaves of *Ocimum sanctum* L. To the best of our knowledge this compound has not been previously isolated or reported from the leaves of this variety.

Materials and Methods

Plant material

The plant materials (including the root) of *Ocimum sanctum* L. were collected from the nursery of the Bangladesh Agricultural Development Corporation at Gazipur. The plant was identified from the Department of Botany of Dhaka University. The collected fresh plant materials were cleaned thoroughly and after separation, the leaves from the stem were initially dried under sunlight, followed by controlled drying in an electrical oven at 40^{o}C. The dried plant materials were chopped into small pieces followed by grinding through a Cyclotec grinder (200 mesh). These fine powdered materials were used in the present investigation.

Spectroscopic investigation

Melting point was determined by an electrochemical micro-melting point apparatus (Gallenkamp). The UV, IR (KBr) spectra were recorded on a Shimadzu UV-168A and Shimadzu IR-470A spectrophotometer, respectively. The ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker R-32 (400 MHz) in deuterated methanol (CD_3OD) with TMS as an internal standard (chemical shifts in δ, ppm). TLC was performed with silica gel GF_{254}. All solvents were analytical reagent grade.

Extraction and isolation

The leaf powder (80g) was exhaustively extracted in a soxhlet apparatus with methanol. After filtration the crude extract was concentrated under reduced pressure at 40^{o}C in a rotary evaporator. After the removal of methanol the dried mass was suspended in water and further extracted successively with hexane, chloroform, ethyl acetate and n-butanol, respectively. All these extracts were collected separately and preserved for analysis. The hexane soluble fraction was concentrated to dry mass under reduced pressure at 40^{o}C in a rotary evaporator. The concentrated extract was dried by vacuum pump to yield dry mass (18.0g). The dry mass was mixed with a small amount of silica gel (60-120 mesh) maintaining the ratio (2:1) and dried in air. After drying the mixture was powdered in a mortar and applied to vacuum liquid chromatography (VLC) over TLC grade silica gel (GF_{254}). The column was initially eluted with petroleum ether (40-60^{o}C) followed by gradient elution with the mixture of petroleum ether with an increasing amount of dichloromethane. These elutes were collected in a series of test tubes (more than 170 tubes) with 20ml in each fraction. All of these fractions were monitored by TLC (over silica gel GF_{254}). The elutes of similar behaviour (similar R_f values) were combined together to afford seven fractions F_1 (1-12), F_2 (17-19), F_3 (32-35), F_4 (62-64), F_5 (92-100), F_6 (101-103), F_7 (119-121). All of these fractions were concentrated separately and allowed to stand at room temperature for a few weeks. A yellowish semi-solid amorphous substance (10.0 mg) settled out from fraction F_4 and this fraction was marked as SP_1.

Characterization of the compound SP_1

A yellowish semi-solid amorphous substance (10.0 mg) was obtained from the fraction F_4. It could not be crystallized from any solvent. It was soluble in petroleum ether, ethyl acetate, chloroform and dichloromethane. It was further purified by preparative TLC over silica gel GF_{254} using petroleum ether-dichloromethane (2:3) as a developing solvent. R_f was found to be 0.60 and the compound was visualized as a yellow coloured single spot upon its exposure to iodine chamber and as a violet colour on spraying with vanillin-sulphuric acid reagent followed by heating in an electric oven at 110^{o}C. The structures according to IR were found to be as follows:

(3600-3400), 2900, 2850, 1720, 1640, 1450, 1240, 900, (830-800) cm^{-1}; ^{1}H-NMR (400 Mz, $CDCl_3$+ CD_3OD): 7.25 (s, 1-H, –OH), 6.67-6.84 (m,1H proton of sugar moiety), 5.47 (s, 1H, H-6), 5.35 (dd, 1H, J=12.5 and 8.5 Hz, H-23), 5.03-5.08 (dd, 1H, J= 12.5 and 8.5 Hz, H-22), 4.96 (s, 1H, proton of sugar moiety), 4.85 (s, 1H, anomeric proton), 3.86 (m, 1H, H-3) 2.03-3.31 (m 3H, proton of sugar moiety), 1.24 (s, 3H, H-19), 1.0 (d, 3H, J=6.5 Hz, H-21), 0.97 (t, 3H, J=7.1 Hz, H-29), 8.8 (s, 3H, H-27), 8.7 (s, 3H, H-26), and 0.85 (s, 3H, H-18) ; ^{13}C-NMR (400 Mz, $CDCl_3$+ CD_3OD): 39.9 (C-1), 29.9 (C-2), 77.3 (C-3), 39.8 (C-4), 55.8 (C-5), 21.6 (C-6),39.2 (C-7), 29.7 (C-8), 48.7 (C-9), 29.4 (C-10), 21.6 (C-11), 27.2 (C-12), 50.8 (C-13), 30.2 (C-14), 62.1 (C-15), 77.3 (C-16), 124.3 (C-17), 118.26 (C-18), 130.2 (C-19), 151.87 (C-20), 178.91 (C-21), 146.47 (C-22), 29.2 (C-23), 14.1 (C-24), 62.12 (C-25), 76.7 (C-26), 63.75 (C-27), 184.9 (C-28), 111.14 (C-29), 121.2(C-30) and the chemical shifts (210.3, 209.45, 130,24, 130.0 and 143.96) ppm are due to the carbon of the sugar moiety.

Results and Discussion

β-sitosterol and some level of sugar have already been reported from the seeds of one of the varieties of basil [3, 4] but β-sitosteryl-D-glycoside from the leaves of *Ocimum sanctum* L. plant was isolated and reported for the first time. Compound **SP_1** was obtained as a yellowish amorphous solid. Its IR spectrum showed an absorption peak in the region (3600-33400) cm^{-1} indicating the presence of a hydroxyl group (-OH) and the absorption bands at 2900-2850 cm^{-1} indicated the presence of –CH aliphatic asymmetric stretching of $–CH_3$, $–CH_2–$ and > CH_2 groups. The absorption band at 1720 cm^{-1} indicated the presence of (>C=O) stretching of normal aliphatic ester. The absorption band at 1240 cm^{-1} indicated the presence of C–N stretching. The absorption peak at 900 cm^{-1} indicated the aromatic stretching (out of plane bending). Finally, the absorption band at 830 and 800 cm^{-1} indicated the –CH stretching of >C=C-H group. The ^{1}H-NMR spectrum showed the chemical shift at δ 0.85 and 1.24 indicated the presence of two angular methyl signals. The proton NMR spectrum also exhibited one olefinic double bond proton as a doublet at δ 5.35, along with the two up field signals at δ 0.87 and 0.88 respectively, due to the presence of two secondary methyl groups at position 26 and 27 of the skeleton, *i.e*, the presence of an isopropenyl group of the molecular structure. The very up field chemical shift at δ 0.97 as a triplet with the intensity of 3H and coupling constant of J=7.1 Hz was assigned for the terminal methyl group of 29. Similarly, the other up field chemical shift at δ 1.0 with the coupling constant J=6.5 Hz of 3H intensity was assigned the secondary methyl group at position 21 of the molecular structure. The chemical shifts in the region δ 2.03-3.31 as a multiplet was assigned the presence of five protons of the sugar moiety and the very downfield chemical shift at δ 7.25 was assigned for the proton of OH group of glycoside. The ^{13}C-NMR spectra of the compound **SP_1** revealed the presence of 29 carbons, the chemical shift at δ 76.7 and 63.8 were assigned for the two separate terminal methyl groups linked at position 25 of the molecular structure. The three downfield chemical shifts at δ 128.3, 130.2 and 178.9 respectively, were assigned for the angular methyl carbons linked at C-18, C-19 and C-21 position. The up field signals at δ 29.7, 29.4 and 30.4 were assignable to the carbon at positions 8, 10 and 14 that was fused in the proposed β-sitosteryl-D-glycoside derivative. Similarly, the relative down field chemical shifts at δ 48.7, 50.8 and 55.8, respectively, were assigned for the carbon that was fused at positions C-9, C-13 and C-5, respectively, in the proposed skeleton. The up field chemical shift at δ 39.9, 29.9 77.3, 39.8,21.6, 39.2, 21.6, 27.2, 62.1 and 77.3 were appropriate for the cyclohexyl and cyclopentyl carbons at positions 1, 2, 3, 4, 6, 7, 11, 12, 15 and 16, respectively. The other shifts at δ 151.87, 146.47, 29.2, 14.1, 62 12 and 184.9 were assigned for the carbon numbers 20, 22, 23, 24, 25 and 28, respectively, which constitute the side

chain of six carbons which were linked at position 17 of the cyclopentyl ring. The chemical shift at δ 124.3 was assigned for the carbon number 17 which was the point of link of a side chain to the cyclopentyl ring. The very down field chemical shift at 210.3, 209.45, 130.24, 130.0 and 143.96 were assigned for the carbon of the sugar moiety.

On the basis of IR, ^{1}H-NMR, ^{13}C-NMR, spectral data and the other physical properties the isolated pure compound **SP$_1$** were identified and established as β-sitosterol-D-glycoside as shown in Fig. 1.

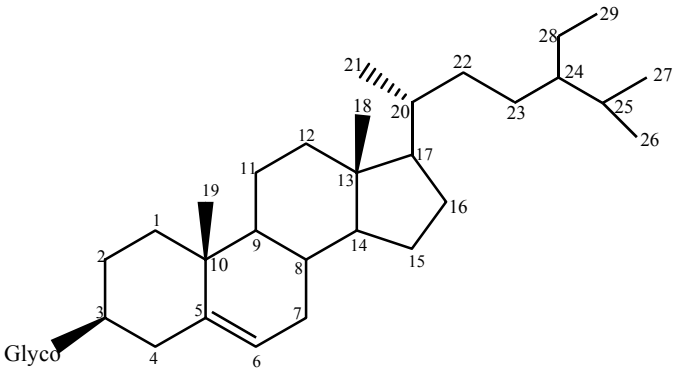

Figure: 1 β-Sitosteryl-D-glycoside

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