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Sterols and Sterol Glycosides from *Cuscuta Reflexa*

E. Anis[1], G. Mustafa[1], S. Ahmed[1], Nisarullah[1], A. Malik[1]*, N. Afza[2] and Y. Badar[2]

[1]*HEJ Research Institute of Chemistry, University of Karachi, Karachi-75270, Pakistan*
[2]*Pharmaceutical and Fine Chemical division, PCSIR Karachi complex, Karachi, Pakistan*

Abstract – A new natural product stigmast-5-en-3-*O*-β-D-glucopyranoside tetraacetate (**1**) along with known compounds stigmast-5-en-3-*O*-β-D-glucopyranoside (**2**), stigmast-5-en-3-yl-cetate (**3**) and β-sitosterol (**4**) have been isolated from the stems of *Cuscuta reflexa*. Their structures were elucidated on the basis of chemical and spectroscopic evidence.
Key words – *Cuscuta reflexa,* Convolvulaceae, steroids, steroidal glycosides.

Introduction

The genus *Cuscuta* belonging to the family Convolvulaceae comprises of three species. All of these are yellow parasitic leafless, twining herbs (Theodore, 1982) distributed in tropical and temperate region *Cuscuta reflexa* Roxb. is commonly found in Karachi and other parts of Pakistan.

This plant is anthelmintic, carminative, alterative, purgative and is used in jaundice, pains of joints, paralysis and vomitting. Stems are used in bilious disorders (Shastri, 1962). The literature survey revealed that no chemical work has so far been carried out on this species. The methanolic extract of the stems of this plant showed positive cytotoxic activity in Brineshrimp test. Further pharmacological screening revealed a strong antimicrobial activity in the methanolic extract. This prompted us to carry out bioassay directed isolation studies on this species. Herein we report a new natural product (1) along with known compounds (2), (3) and (4). Their structures have been elucidated on the basis of chemical and spectral evidence.

Experimental

General – The melting points were determined on glass capillary on Buchi-535 melting point apparatus. Optical rotations were measured on Schmidt & Haensch and DIP-360 JASCO polarimeters. The IR spectra were recorded on a Finnigan MAT 112 spectrometer. The NMR spectra were recorded on Brukers instrument ($CDCl_3$: 300 MHz for ^1H and 125 MHz for ^{13}C nuclei) using TMS as int. standard. TLC: silica gel PF_{254}; CC: silica gel, 70-230 mesh.

Plant Material – The plant material was collected from Karachi region and identified as *Cuscuta reflexa* Roxb. by Prof. M. Qaiser, Department of Botany, University of Karachi. A voucher specimen is deposited in Herbarium of the Department of Botany, University of Karachi.

Extraction and Isolation – The shade dried plant material was extracted twice at room temperature with MeOH. The methanolic extract was partitioned between ethyl acetate and water. The ethyl acetate fraction was evaporated and divided into hexane soluble and insoluble fractions. The hexane soluble fraction was evaporated and the residue was chromatographed over silica gel using various mixtures of hexane, chloroform and methanol. The fr. which eluted in $CHCl_3$-MeOH (9.8:02) from CC was further chromatographed over silica gel using $CHCl_3$: MeOH (9.5:0.5) as eluent to obtain crystalline compound. (1) (30 mg).

Stigmast-5-en-3-*O*-β-D-glucopyranoside tetraacetate (1) – Crystallized from methanol, m.p.168-170°C; $[\alpha]_D$+39°; IR (KBr); 2900, 1640 (C=C), 1756, 1223 (C=O acetate), 815 (trisubstituted double bond) cm^{-1}; ^1H-NMR (300 MHz chloroform-d_1 δ) 5.37 (1H, d, J=4.4, H-6), 5.30 (1H, t, $J_{3',2'}=J_{3',4'}$=9.7 Hz-3′) 5.10 (1H, t $J_{4',3'}=J_{4',5'}$ =9.68 Hz H-4′) 4.82 (1H, d, $J_{2',3'}$ =9.6 Hz, $J_{2',1'}$ =8.1 Hz, H-2′) 4.57 (1H, d, $J_{1',2'}$ =7.91 Hz, 1.1-1′), 4.28 (1H, dd, $J_{6a'6b}$=12.2 Hz, $J_{6'a,5}$=2.5 Hz, H_a-C(6′)-H_b), 3.68 (1H, m, $J_{5'4'}$ =9.7 Hz, $J_{5'6b}$=4.9 Hz, J_5,

*Author for correspondence.

$J_{5',6a}$=2.5Hz, H-5′), 3.47 (1H, m, J_{aa}=J_{aa}=10.1 Hz; J_{ae}=J_{ae}=5.5 Hz, H_a-3), 2.08, 2.07, 2.02, 2.01 (4×3H, each s, OCOC$\underline{H}_3$′), 0.98 (3H, s, Me-19), 0.93 (3H, d, J=6.5 Hz, Me-21) 0.89 (3H, d, J=6.8 Hz, Me-26) 0.87 (3H, t, J=7.3 Hz, Me-29, 0.83 (3H, d, J=6.8, Me-27), 0.68 (3H, s, Me-18); ^{13}C-NMR (125 MHz, $CDCl_3$); δ 140.47(C-5), 122.17(C-6), 80.11(C-3), 56.86(C-14), 56.20(C-17), 50.31(C-9),45.96(C-24), 42.42(C-13), 39.83(C-4), 38.99(C-12), 37.27(C-1), 36.80(C-10), 36.17(C-20), 33.78(C-22), 31.99(C-8), 31.97(C-7), 29.52(C-23), 29.34(C-2), 29.30(C-25), 26.31(C-16), 25.39(C-15), 23.18(C-28), 21.11(C-11), 19.81(C-26), 19.38(C-19), 19.10(C-21) 18.83(C-27), 12.23(C-29), 12.03(C-18); Sugar: 170.58, 170.28, 169.36, 169.22 (4×O$\underline{C}$OCH$_3$), 99.71(C-1′), 73.83(C-5′), 71.82(C-3′), 71.59(C-2′), 68.73(C-4′), 62.22(C-6′), 22.82,20.71, 20.67,20.58 (4×OCO$\underline{C}$H$_3$) These assignments were made by comparison with the previously published ^{13}C NMR data of related compounds (Carvalho and Sata,. 1993) and confirmed in each case by ^{1}H-^{13}C correlated (HMQC). EIMS *m/z* (rel. int.) 396 (100), 381 (3), 255 (8), 230 (2).

Alkaline hydrolysis of (1) – Compound (**1**) (25 mg) was refluxed with freshly prepared sodium methoxide (0.04 mg Na in 10 ml Abs MeOH) for 3h. The solution was neutralized on cation exchange resin, filtered and freed of solvent. The residue was passed through Si gel column using $CHCl_3$-MeOH (9:1) as eluent to afford β-sitosterol-3-*O*-β–D-glucopyranoside **1a** (20 mg). Its physical and spectral data showed complete agreement to those reported in literature (Iribarren and Pomilio, 1978).

Acid hydrolysis of (1) – Compound (**1**) (20 mg) was refluxed for 4 h with 1N methanolic HCl (5 ml). The solution was concentrated under reduced pressure and diluted with 5 ml H_2O. It was extracted with $CHCl_3$ and the residue recovered from the organic phase was subjected to preparative TLC to obtain β-sitosterol. The aqueous phase was concentrated and D-glucose was identified by PC using chromatography paper of Schleicher and Schuell 2043b; solvent system n-BuOH-HOAc-H_2O (4:1:5) detection with aniline- phthalic acid. It was further confirmed by comparing retention time of its TMS ether with standard sample of D-glucose in GC.

β-Sitosterol-3-*O*-β-D-glucoside (2) – The fr. which eluted in $CHCl_3$- MeOH (9.5:5) from CC over silica gel was further chromatographed over silica gel and eluted with solvent system $CHCl_3$: MeOH (9:1) to afford (2), m.p: 279-280°C; $[\alpha]_D$-41.5° ; EIMS *m/z* 577 $[M]^+$. The physical and spectral data identified it as β-Sitosterol glucoside (Iribarren and Pomilio, 1978).

β-Sitosterol acetate (3) – The fr. which eluted in hexane-$CHCl_3$ (0.5:9.5) from CC over silica gel provided (3), m.p. 124-126° $[\alpha]_D$+39° ($CHCl_3$); EIMS *m/z* 456 $[M]^+$. The physical and spectral data identified it as β-sitosterol acetate (Carvalho and Sata, 1993).

β-Sitosterol (4) – The fr. which eluted with hexane-$CHCl_3$ (1:1) was further purified through crystillization from chloroform, m.p 135°; $[\alpha]_D$+40° ($CHCl_3$); EIMS *m/z* 414 $[M]^+$. The physical and spectral data were in accordance with literature (Mulomoto *et al.*, 1983).

Results and Discussion

The compound 1 was assigned the molecular formula $C_{43}H_{68}O_{10}$ by HRMS which showed the $[M]^+$ peak at *m/z* 744.1032 (calc. 744.0832). The molecular ion peak was confirmed by positive FABMS which showed $[M + H]^+$ and $[M + Na]^+$ peaks at *m/z* 745 and 767, respectively. It gave positive Carr-Price test for steroidal glycosides. The I.R. spectrum showed the peaks at 2900, 1640 (C=C), 1756, 1223, (C=O acetate), 815 (trisubstituted) C=C cm^{-1}. The presence of tetraacetylated sugar moiety was indicated by both NMR and mass spectra. The anomeric proton was observed in ^{1}H NMR at d 4.57 and its coupling of the magnitude of 7.9 Hz. confirmed the presence of sugar moiety in b-linkage. Other signals of sugar component were observed at δ 4.82 ($J_{2',3'}$=9.6 Hz, $J_{2'1'}$=8.1 Hz dd), δ 5.30 and 5.10 ($J_{3'2'}$=$J_{3'4'}$ =9.7 Hz, $J_{4'3'}$ =$J_{4',5'}$ =9.68 Hz), δ 4.25 (J=12.2, 4.8 Hz) and 4.12 (J=12.3, 2.5 Hz) (Ramaih *et al.*, 1984). The ^{13}C NMR spectrum showed the signals of anomeric carbon at 99.71. It also showed four signals of ester carbonyl at 169.2, 169.3, 17.1 and 170.5 and signals of OC-CH_3, at 20.5, 20.6, 20.7 and 20.7 respectively. The mass spectrum showed the base peak at *m/z* 396 due to the loss of tetraacetyl hexose moiety along with an additional hydrogen. Further peak at *m/z* 381 resulted from the loss of angular methyl group from the ion at *m/z* 396. The subtraction of signals of tetraacetyl hexose moiety from B.B ^{13}C NMR spectrum left twenty nine carbon signals revealing the presence of stigmastane nucleus. The peaks at 255 and 230 in the mass spectrum were characteristic of Δ^5 sterols (Aizawa *et al.*, 1974). The olefinic protons was observed in ^{1}H NMR spectrum at d 5.37 (J=4.4 Hz). The multiplet at δ 3.47 ($J_{aa'}$ =10.1 Hz & $J_{ae'}$ =J_{ae} 5.5 Hz) was due to proton geminal to the oxygen in axial configuration.

The signals of methyl groups at δ 0.68 (s, M-18), 0.98 (s, Me-19), 0.93 (d, Me-21), δ: 87 (d, Me-26), 0.83 (d, Me-27), 0.87 (t, Me-27) corresponded exactly to those of β-sitosterol. The hexose moiety was assigned to C-3 on biogenetic grounds and compound 1 was identified as stigmast-5-*en*-3-*O*-β-D-glucopyranoside tetraacetate. On deacetylation with NaOMe the compound 1 was converted into 1a whose physical and spectral data showed complete agreement to those of stigmast 5-*en*-3-*O*-β-D-glucoside reported in literature (Iribarren and Pomilio, 1978). The acid hydrolysis of **1** provided β-sitosterol and the corresponding sugar moiety which was identified as D-glucose by comparison of R_f value with an authentic sample of glucose on PC as well as retention time of its TMS ether in GC.

The compound **1** is described in literature as a synthetic product and this is the first report of its occurrence as a natural product.

References

Aizawa, K.,Yoshida, S., and Takahashi, T., The applicability of the defocussing technique in organic mass spectrometry. *Org. Mass spectrom.* **9**, 470-479 (1974).

Carvalho, L. M. and Sata, J, A new oleanolic acid derivative from *Securinega tinchoria. Planta Medica*, **59**, 369-372 (1993).

Iribarren, A. M. and Pomilio, A B, Sitosterol 3-*O*-β-D-Xylopyranoside. *Phytochemistry*, **23**, 2087-2088 (1984).

Muloumoto, T., Shigemoto, T., and Itoh, T., Occurence of 24-Ethyl -α-sterols and 24-Ethyl-α-sterols as C-24 epimeric mixture in seeds of *Cucumis sativus. Phytochemistry*, **22**, 2622-2624 (1983).

Ramaih, P. A., Lavie, D., Budhiraja, R. D., Sudher, S., and Garg, K. N., Spectroscopic studies on a withanolide from *Withania coagulans. Phytochemistry*, **23**, 143-149 (1984).

Shastri, B. N., *Wealth of India Raw Material*, Council of Scientific and Industrial Research, Delhi (1962).

Theodore, C., *The Flora of Presidency of Bombay*, Bishen Singh Mahendra Pal Singh, New Connaught Dehradun (1982).

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