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Stigmasterol from *Eichhornia crassipes* (Water Hyacinth): Isolation, Characterization and X-ray Structure

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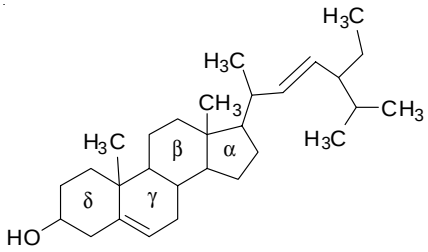
Chemical investigation of *Eichhornia crassipes* (water hyacinth) collected from the back waters of Kochin, southern India lead to isolation of an oxygenated sterol, stigmasterol (**1**). The molecular formula of **1** was determined to be $C_{29}H_{48}O$ by combination of NMR and mass spectroscopic data. The sterol was fully characterized by FTIR, NMR (^1H and ^{13}C) and mass spectral data. Solid state structure of the sterol was determined by single crystal X-ray diffraction. The compound crystalizes in the chiral monoclinic space group $P2_1$ with a solution optical rotation $[\alpha]_D$ = -35° (c 0.16, $CHCl_3$).

Keywords: *Eichhornia crassipes*, Stigmasterol, Phytochemicals, Water hyacinth, Crystal structure.

INTRODUCTION

Eichhornia crassipes (water hyacinth) is an aquatic macrophyte native to the Amazon basin[1]. It is considered to be one of the deadliest invasive plants and among the most productive weeds on earth. Its rapid spread imposes a serious threat to native species, impacting the surrounding ecosystem and hampering the socio-economics of tropical and sub-tropical regions. But even such a destructive plant may find a useful application. Main benefits associated with such plants are in displaying extraordinary bioabsorptive properties[2] and in bioactive phytochemicals associated with them[3]. The phytochemicals are reported for antibacterial[3a], antioxidant[3b] and anticancer[3c] properties. Among the list of potential bioactive compounds, stigmasterol in particular was described to possess potent antioxidant, hypoglycemic and thyroid inhibiting properties[4].

Furthermore, a systematic chemical investigation of abundant plants, if leading to the discovery of valuable compounds, could in turn help to some extent in controlling weed spread from a routine industrial harvest for extracting useful compounds. Previous investigations revealed that *Eichhornia crassipes* contained oxygenated sterol[5], phenylphenalene[6] and terpenes[3a]. The presence of sterol in *Eichhornia crassipes* has been reported as well[7], however, those studies lack a proper structural characterization of sterol by spectroscopic data and X-ray analysis. Herein, we complement previous work with description of isolation, spectroscopic and X-ray characterization of the oxygenated sterol, stigmasterol (**1**) from *Eichhornia crassipes*, which we believed could further the pathway to a dissemination of the weed.



Chemical structure stigmasterol (**1**)

EXPERIMENTAL

Column chromatography was carried out on Merck silica gel (60-120 mesh). Optical rotation was measured in $CHCl_3$ on a Perkin-Elmer polarimeter. Infrared spectra were recorded in a diffused reflection spectroscopy (DRS) assembly on a Shimadzu-8201PC spectrometer with the sample prepared in KBr. ^1H and ^{13}C NMR spectra were recorded at 300 MHz for ^1H and 75.47 MHz for ^{13}C in $CDCl_3$ containing $SiMe_4$ as internal reference on a Bruker-Avance 300 MHz spectrometer. Mass spectral data were recorded on QSTAR-XL (MS/MS) Applied Biosystem mass spectrometer.

Extraction and isolation: Minced specimens of the plant, dried using lyophilizer (dried weight approx. 10 g), were dissolved in chloroform. The chloroform extract was subjected to flash column chromatography over silica gel mesh 230-

400. Ethyl acetate: petroleum ether (v/v) solvent was used as eluent with increasing polarity of ethyl acetate (0:100 to 100:0) and finally 200 mL of methanol to remove polar compounds from the column, leading to collection of 32 fractions. Fractions 15-22 were combined based on TLC analysis and further separated on silica gel column using increasing amount of ethyl acetate in petroleum ether yielding a pale white solid of stigmasterol (127 mg). Crystals suitable for X-ray analysis were obtained by recrystallization in an ethyl acetate/methanol solvent mixture.

Chemical and spectroscopic characterization of compound 1 (stigmasterol): White solid; $[\alpha]_D$:-35° (*c* 0.16, $CHCl_3$); IR (KBr, ν_{max}, cm^{-1}): 3307, 2954, 2916, 1699, 1463; ^1H NMR (300 MHz, $CDCl_3$): 5.37 (m, 1H), 5.16 (m, 1H), 5.01 (m, 1H), 3.54 (sept, 1H), 2.36-2.28 (m, 4H), 1.84 (m, 6H), 1.49 (m, 11H), 1.27-0.89 (m, 8H), 0.86 (m, 6H), 0.82 (m, 3H), 0.81 (m, 3H), 0.71 (m, 3H); ^{13}C NMR (75.47 MHz, $CDCl_3$): 140.74, 138.29, 129.29, 121.71, 71.82, 56.86, 55.97, 51.23, 50.15, 42.28, 40.46, 39.68, 37.25, 36.51, 31.89, 31.64, 29.62, 29.22, 28.89, 25.38, 24.70, 24.35, 22.66, 21.19, 21.05, 19.38, 18.96, 14.08, 12.22, 12.03; HRMS-ESI: *m/z* $[M + H]^+$ calcd. for $C_{29}H_{49}O$: 413.3783; found: 413.2876.

X-ray crystallographic studies: Crystals of suitable quality for X-ray analysis were grown from ethyl acetate in petroleum ether (2:1). Fig. 1 shows an ORTEP diagram[8a] of compound **1**, details of data collection is presented in Table-1. X-ray structural data was obtained using a Bruker Apex II single crystal X-ray diffractometer employing MoK_α radiation (λ = 0.71073 Å). Crystal-to-detector distance was 45 mm and exposure time was 30 seconds per frame for all sets collected at steps of 0.5°. Data collection was 99.9 % complete to 25° in θ. Data was integrated and scaled using SAINT, SADBAS, within the APEX2 software packages[8b]. The structure was solved by direct methods SHELXS & SIR[8c] and refined by full matrix least squares based on F^2 using SHELXL-97[8d]. All Non hydrogen atoms were refined anisotropically and all hydrogen atoms were initially located in a difference Fourier map and were refined with a "riding" model".

TABLE-1
CRYSTAL STRUCTURE DETERMINATION AND STRUCTURE REFINEMENT OF COMPOUND **1**

Empirical formula	$C_{58}H_{98}O_3$
Formula weight	843.36
Temperature	100(2) K
Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	P 2_1
Unit cell dimensions	a = 9.3420(8) Å α = 90°
	b = 7.4969(6) Å β = 93.014 (5)°
	c = 36.853(3) Å γ = 90°
Volume	2577.4(4) $Å^3$
Z	2
Density (calculated)	1.087 Mg/m^3
Absorption coefficient	0.064 mm^{-1}
Crystal size	0.20 × 0.15 × 0.10 mm^3
Theta range for data collection	2.18 to 28.31°
	-12 ≤ h ≤ 12
Index ranges	-9 ≤ -k ≤ 9
	-48 ≤ l ≤ 49
Reflections collected	47534
Independent reflections	12598 [R_{int} = 0.0479]
Completeness to theta = 25.00°	99.9 %
Max. and min. transmission	0.9936 and 0.9873
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	12598/10/597
Goodness-of-fit on F^2	1.075
Final R indices [I > 2σ(I)]	R_1 = 0.0638, wR_2 = 0.1571
R indices (all data)	R_1 = 0.0710, wR_2 = 0.1614
Absolute structure parameter	Undetermined in absence of heavy atoms
Largest diff. peak and hole	0.448 and -0.345 e.$Å^{-3}$

RESULTS AND DISCUSSION

Stigmasterol (3β-hydroxy-24-ethyl-5,22-cholestadiene) has been isolated from the chloroform extract of *Eichhornia crassipes* by repeated column chromatography over silica gel using a petroleum ether/ethyl acetate solvent mixture. Approximately 10 g of plant material, following column chromatography yielded 127 mg of pure stigmasterol, which is considered a fairly good concentration and commercially relevant. Single crystal X-ray analysis revealed that the compound crystallized in the monoclinic space group $P2_1$. Two molecules are grouped with a water molecule resembling the shape of a tuning fork, where one site is fixed *via* hydrogen bonds to the water molecule whereas the two independent molecules are allowed to move freely at the other end. Some disorder is observed at C25-C26 and C54-C55. The bond length and bond angles in the two molecules are closely related (Table-2). The solution optical density measurement was found to be $[\alpha]_D$ = -35° (*c*. 0.16, $CHCl_3$).

TABLE-2
SELECTED BOND LENGTHS (Å) AND ANGLES (°) OF THE COMPOUND

Molecule A		Molecule B	
Bond lengths			
C1-O1	1.438(3)	C30-O2	1.438(3)
C1-C10	1.521(3)	C23-C25	1.510(4)
C22-C23	1.335(3)	C51-C52	1.318(4)
C8-C9	1.331(3)	C37-C38	1.335(3)
C24-C25	1.516(3)	C53-C54	1.517(7)
C15-C20	1.537(3)	C44-C49	1.539(3)
Bond angles			
C28-C27-C29	110.6(3)	C57-C56-C58	108.7(4)
C15-C20-C22	110.64(18)	C44-C49-C51	109.3(2)
C17-C16-C15	107.64(18)	C46-C45-C44	106.92(18)
C2-C1-C10	109.43(19)	C31-C30-C39	109.76(18)
C3-C4-C9	108.30(17)	C32-C33-C38	107.89(17)
C20-C22-C23	123.4(2)	C49-C51-C52	124.4(3)

Rings α, β and γ are considered to be rigid and in both of the independent molecules, the ring δ is out-of-plane while the other three rings remain in plane. The carbon oxygen bond length C1-O1 and C30-O2 for both molecules are 1.438(3) Å (Table-2). This bond length is comparable to C-O bond distance of carbon and hydroxyl groups of related sterols[9]. The bond lengths corresponding to double bond C8-C9 and C22-C23 as well as C37-C38 and C51-C52 are comparable and the values fall within the range of carbon-carbon double bonds. The mean

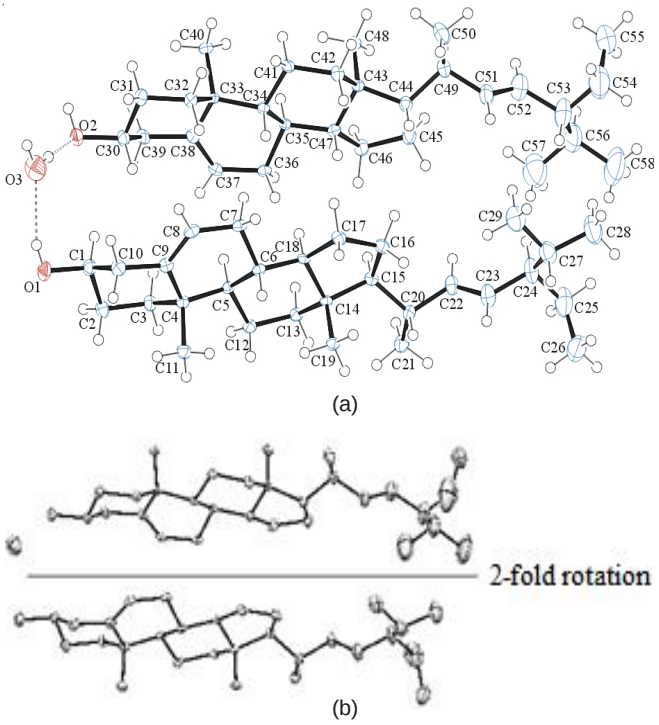


Fig. 1. (a) ORTEP diagram of sterol with thermal ellipsoids at the 50 % probability level (b) ORTEP diagram shows two independent molecules approximately related by a twofold rotation. Hydrogen atoms have been omitted for clarity

values of C-C-C bond angles for five membered and six membered rings in both the molecules are comparable and values are close to the reported values of other related sterols[9].

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REFERENCES

1. S.C.H. Barrett and I.W. Forno, *Aquat. Bot.*, **13**, 299 (1982).
2. (a) S. Dixit and S. Dhote, *Environ. Monit. Assess.*, **169**, 367 (2010); (b) J.S. Weis and P. Weis, *Environ. Int.*, **30**, 685 (2004).
3. (a) S.M.M. Shanab, E.A. Shalaby, D.A. Lightfoot and H.A. El-Shemy, *Plus One*, **5**, e13200 (2010); (b) C.C. Liu, G.L. Zhao, Y.N. Li, Z.P. Ding, Q.G. Liu and J. L.Li, *Adv. Mater. Res.*, **156-157**, 1372 (2010); (c) H. Ali and N. Lata, *Drug Invention Today*, **2**, 212 (2010).
4. (a) S. Panda, M. Jafri, A. Kar and B.K. Meheta, *Fitoterapia*, **80**, 123 (2009); (b) T. Ghosh, T.K. Maity and J. Singh, *Orient. Pharm. Exp. Med.*, **11**, 41 (2011).
5. M.D. Greca, P. Monaco and L. Previtera, *Tetrahedron Lett.*, **47**, 7129 (1991).
6. M.D. Greca, L. Previtera and A. Zarrelli, *Tetrahedron*, **65**, 8206 (2009).
7. (a) P. Jayanthi, P. Lalitha and K.S. Shubasini, *J. Pharm. Res.*, **3**, 1240 (2011); (b) P.C. Goswami, B. Nag, A.K. Sharma and A. Borthakur, *Curr. Sci.*, **52**, 806 (1983).
8. (a) L.J. Farrugia, *J. Appl. Cryst.*, **30**, 565 (1997); (b) Bruker:APEX2 Version 2.1-4, SAINT Version 7.34A, SADABS Version 2007/4, Bruker AXS Inc, Madison, Wisconsin, USA (2007); (c) A. Altomare, C. Burla, M. Camalli, L. Cascarano, C. Giacovazzo, A. Guagliardi, A.G.G. Moliterni, G. Polidori and R. Spagna, *J. Appl. Cryst.*, **32**, 115 (1999); (d) G.M. Sheldrick, SHELXL-97: Program for the Refinement of Crystal Structures, University of Göttingen, Germany (1997).
9. K.S. Singh and W.H. Kaminsky, *Nat. Prod. Commun.*, **6**, 1237 (2011).