



Steroid from the Seeds of *Artabotrys odoratissimus*

SUBEDAR PRASAD¹ and CHITRASEN GUPTA^{2*}

¹Department of Chemistry, M.M. T.D. College, Ballia, India.

²Department of Chemistry, Kutir Post Graduate College, Chakkey, Jaunpur, India.

*Corresponding author E-mail: chitrasengupta@yahoo.co.in

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ABSTRACT

A steroid has been isolated from the seeds of *Artabotrys odoratissimus* (Annonaceae) and its structure has been established as (3 β , 22E)-ergosta-5,7,22-trien-3-ol by spectroscopic and chemical studies. By chemical correlations, it has been identified as a known compound ergosterol. This is the first report of isolation of ergosterol from this plant.

Key words: *Artabotrys odoratissimus*, Annonaceae, Ergosterol.

INTRODUCTION

Artabotrys odoratissimus, Wight & Arn. [(Syn: *A. uncinatus* (Lam.) Merr. and *Annona hexapetalus* (L. f.) Bhandari)] of the family Annonaceae is a shrub distributed in tropical and temperate Asia from the southern part of China to India. The plant, known for its essential oils, is used for the treatment of malaria and scrofula in traditional Chinese medicine¹. Isolation of aliphatic alcohols and esters², sesquiterpenes³, alkaloids⁴, anthraquinones⁵ and four new triterpenes⁶ were earlier reported from the plant. In previous works, isolation of flavonol glycosides⁷ is also reported from the leaves of the plant. In this paper we report the isolation of an ergosteroid from the n-hexane extract of defatted seeds of *Artabotrys odoratissimus*.

MATERIAL AND METHODS

Isolation procedure

500g seeds of *A. odoratissimus* were collected from the campus of Banaras Hindu University, Varanasi, India, milled and left overnight in n-hexane to defat. Then filtered and residue was extracted with n-hexane in a soxhlet extractor for 16 hours. Systematic column chromatographic fractionation of the n-hexane extract by with mixture of solvents i.e., n-hexane and benzene (8:2) resulted in isolation of an ergosteroid, named ergosterol.

Chemical and physical analysis

Melting point was taken on a Yazawa hot stage microscope. ¹H-NMR and ¹³C-NMR spectra were recorded on 300 MHz or 500 MHz/ 125 MHz on Bruker 300 or JEOL GSX-500 MHz spectrometer

using tetramethyl silane (TMS) as a internal reference. The chemical shift value are reported in delta (δ , ppm) units. Silica gel for column chromatography refers to E. Merck (India) Ltd. (60-120 mesh). Thin layer chromatography plates were prepared with silica gel-G of E. Merck (India) Ltd. and spot were visualized in iodine vapour or by 5% H_2SO_4 in ethanol and heating the plates at $110^\circ C$ for 10 minutes. Compound was routinely dried over P_2O_5 for 24 hours in vacuum and was tested for purity by thin layer chromatography. Anhydrous sodium sulphate was routinely used for drying the organic solvents.

RESULTS AND DISCUSSION

The single spot nature of compound was determined by exposure of the TLC plates to iodine vapours as well as charring the plates after spraying with 5% ethanolic sulphuric acid at $110^\circ C$ for 10 minutes. The compound responded positive to Liebermann-Burchard test, when its solution in chloroform and acetic anhydride was treated gently with concentrated sulphuric acid; it developed pink colouration at the junction of two layers which turned to be gray on prolonged keeping, which shows steroidal nature of the compound. The R_f values of compound in different solvent systems are tabulated in table-1.

Further elution of same column yielded 1.73g of compound as white crystalline solid and recrystallised from methanol, m.p.: $165-168^\circ C$, $[a]_D -135^\circ$ (c, 0.1, $CHCl_3$). EIMS : m/z (rel. int.) 396 (100) (M^+), 386 (15), 363 (71), 353 (7), 337 (29), 316 (4), 301 (5), 283 (9), 271 (25, 256 (50), 253 (22), 213 (213), 199 (14), 171 (16), 157 (20), 137 (29), 129 (29), 109 (29), 95 (46), 81 (65), 55 (61), 41 (61).

IR spectrum of compound showed broad absorption at λ_{max} 3400 cm^{-1} for hydroxyl group. It was, therefore, inferred from IR spectrum of compound that it bears hydroxyl group in the molecule. The 1H -NMR spectrum of compound was measured in $CDCl_3$ solution at 500 MHz. The spectrum showed (Table 2) signals for a number of methyl groups in upfield region, a carbonyl hydrogen signal at δ 3.642, 1H, heptet, signals for four olefinic hydrogens from δ 5.199 to 5.60 ppm and a large number of signals ranging from δ 1.2 to 2.5 ppm for methine and methylinic hydrogens.

A careful analysis of the signals in upfield region from δ 0.62 to 1.05 ppm revealed that a compound possess two tertiary methyl groups at δ 0.631 and 0.947, 3H singlet, each which is probably due to 18 and 19-methyl groups of a steroid molecule. The four methyl doublets at δ 0.824 (J = 6.6Hz); 0.834 (J = 6.6 Hz) 0.918 (J = 6.6 Hz) and

Table 1: R_f value of compound in different solvent system

Solvent system	Ratio	R_f value
Benzene	-	0.14
Benzene : Chloroform	8.5 : 1.5	0.42
Benzene : Ethyl acetate	9 : 1	0.56
Benzene : Methanol	5 : 5	0.60

Table 2: 1H -NMR in $CDCl_3$ at 500 MHz compound (Ergosterol)

S. N.	No. of Carbons	Chemical Shift	Coupling constant J (Hz)
1.	3 H	0.631 s	-
2.	3 H	0.824 d	6.6
3.	3 H	0.834 d	6.6
4.	3 H	0.918 d	6.6
5.	3 H	0.947 s	—
6.	3 H	1.037 d	7.2
7.	1 H	3.642 heptet	

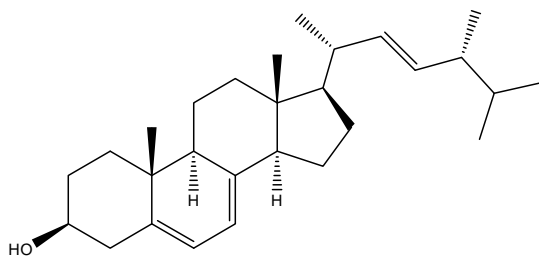


Fig. 1: Ergosterol

1.037 (J = 7.2 Hz) suggests that these are due to secondary methyl groups present in the side chain of an ergostane. Amongst these four secondary methyl groups one at d 1.037 was attributed to be due to C-21 methyl group and two secondary methyl groups at d 0.824 (6.6 Hz) and d 0.834 (6.6 Hz) were due to terminal isopropyl group of the side chain present in normal ergostane type of steroid.

The carbonyl hydrogen signal at d 3.642 heptet was obviously considered for the hydrogen bound to C-3-position and is \pm to C₃-b-hydroxyl group. It is a general characteristic of C-3-a-H, which appears as heptet in nearly all C-3-hydroxylated steroids. The presence of C-3-hydroxyl is further supported by acetylation of compound with acetic anhydride and pyridine at room temperature which yielded monoacetate of compound, analyzed for C₃₀H₄₆O₂. The IR spectrum, of acetylated compound showed the presence of ester carboxyl absorption at 1736 cm⁻¹ with simultaneous disappearance of band for hydroxyl absorption around 3400 cm⁻¹. This showed that only oxygen present in compound is acylable secondary hydroxyl group.

The presence of four olefinic protons in the molecule of compound is evidence by the

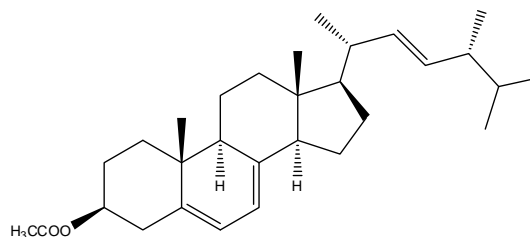


Fig. 2: Ergosterol-3-acetate

signals at d 5.199, 2H, multiplet, d 5.395, and d 5.569, 1H each. The one at d 5.199, 2H multiplet was considered to be for the hydrogens associated with the double bond present in the side chain of the molecule whereas the two signals at 5.395 and d 5.569 were attributed to two conjugated double bonds which can only be possible in the B ring of a steroid nucleus and on the basis of the data presented so for the structure of compound can be written as:

A search of literature has revealed that this is a known ergosteroid and has earlier reported from: *Engleromyces goetzei*⁸, *Polyporus umbellatus*⁹, *Phycomyces blakesleeianus*¹⁰ and *Agelas sceptrum*¹¹.

This is the first report of isolation of ergosterol from the seeds of *Artabotrys odoratissimus*.

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