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Isolation and characterization of a steroidal compound from the benzene:acetone extract of the leaves of artabotrys odoratissimus

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ABSTRACT

Artabotrys odoratissimus has been investigated by many workers for its constituents.Previous phytochemical studies have revealed this genus to be rich in secondary metabolites including phenylcoumarins, xanthones and triterpenoids. Our recent study on the benzene:acetone extract of the leaves of *A. odoratissimus*, have led to the isolation of steroidal compound. The structure of the compound has been established by modern spectroscopic techniques such as Infrared Spectrometry (IR), Hydrogen-Nuclear Magnetic Resonance Spectrometry (1H-NMR), Carbon-Nuclear Magnetic Resonance Spectrometry (13C-NMR) and Mass-Spectroscopy and identified as *25-acetyl-24β-ethyl-cholest-4-en-3β-ol*.

KEYWORDS

Artabotrys odoratissimus;
Medicinal plant;
New compound;
Spectral analysis.

INTRODUCTION

Artabotrys odoratissimus, commonly known as Kantili Champa, is an ornamental shrub distributed throughout the country. Leaves are oblong, lanceolate, glabrus, shining acute at the base, petioles are 6 to 10 mm long. The size of the leaves is up to 18 by 3.8 to 5.0 cm. Flowers are acrid, bitter and yellowish white in colour. It is available in Bangladesh and India[1]. Ayurvedic and Yunani doctors use the leaves and flowers as a remedy for cholera, vomiting, thirst, headache and volatile oils from the leaves show antifungal and antimicrobial activity. The antifertility activity of *A. odoratissimus*, plant has been reported in albino rats[2]. The fruit extracts showed cardiac stimulatory effects on some animals and cardiac depressant effects on others[3]. In this paper, we describe the isolation and structural elucidation of the isolated compound from the benzene:acetone extract of the leaves of *A. odoratissimus*. The isolated compound is identified by its spectral data and has not been reported before.

MATERIALS AND METHODS

General

Freshly distilled solvents were used for extraction, isolation and purification. Evaporations were performed under reduced pressure on a Buchii rotary evaporator. Infrared (IR) spectra were recorded (KBr discs) on a Shimadzu UV-168A Spectrophotometer, validation (Vmax in cm-1). Hydrogen-Nuclear Magnetic Resonance Spectrometry (1H-NMR) were recorded on a Bruker R-32 (300 MHz) instrument in CDCl3 and DMSO-d6 with TMS as an internal standard (Chemical Shifts in delta, ppm). All solvents used were of analytical grade. Thin layer chromatography (TLC) was performed using

TABLE 1 : Column chromatography processing of benzene:acetone extract of *A. odoratissimus* (leaves)

Frac.No	Eluent	Ratio,v/v of eluent	Volume collected(ml)	TLC spots	Yield(gm)
1	Hexane	-	2500ml	3 spotswithstreak	11
2	Hexane:benzene	1:1	3000ml dark streak	7	
3	Hexane:benzene	2:3	2000ml unresolved streak	8	
4	Hexane:benzene	1:4	5000ml single spot	13	
5	Benzene:ETOAC	3:1	3000ml	2 spots with streak	18
6	Benzene:ETOAC	1:1	3000ml	single spot with streak	30
7	Hexane:MeOH	2:1	3000ml	single spot with streak	5
8	MeOH	-	3000ml	unresolved streak	3.2

Weight of Silica Gel = 900 gm; Weight of extract = 100 gm.

TABLE 2 : Re-column chromatography processing of fraction No.7 (From TABLE 1)

Fraction No.	Eluent	Ratio, v/v of eluent	Volume collected(ml)	TLC spots	Yield(mg)
1	Benzene	-	2500ml	2 spots with streak	19
2	Benzene:EtOAC	9:1	5000ml	unresolved streak	13
3	Benzene:EtOAC	3:1	3000ml	compound S1	80

Weight of Silica gel = 200 gm; Weight of extract = 30 gm.

Silica gel GF254.

Plant materials

The leaves of *A. odoratissimus* were collected from the gardens of Ujjain city and university campus and were identified by the authorities of IEMPS, Vikram University Ujjain (M.P).

Extraction of the Compound S1

Dried leaves of the plant (5 kg) were milled into powder and then extracted with hexane (8 L) in a Soxhlet extractor for 36 h. The hexane extract was evaporated in a rotator evaporator and dried by vacuum pump. The hexane extract (200 gm) was extracted successively with hexane, benzene, benzene:acetone, benzene:EtOAC and ethanol to yield hexane (17.5 gm), benzene (14 gm), benzene:acetone (100 gm), benzene:EtOAC (11 gm) and ethanol (2.3 gm) soluble fractions, respectively. The benzene:acetone (100 gm) soluble fraction on TLC examination showed several spots. To isolate different compounds, the benzene:acetone extract (100 gm) was subjected to column chromatography using Silica gel as adsorbent. The column was eluted with different solvents in their increasing order of polarity. Various elutes showed different pattern of spots on TLC examination and therefore, were further separated by rechromatography on Silica gel columns and the results are being reported in TABLES 1 and 2.

Thus, the Benzene: EtOAC (3:1,v/v) fraction revealed the presence of single Compound S1 with clear spot on TLC plate.

RESULTS AND DISCUSSIONS

Finally the compound S1 was crystallized from Benzene : Acetone extract to give white crystals (80mg), m.p. 155^0C. IR. λmax (kBr): 3438,2919,2850,1713,1616-1602, 845,831,759 and 730-720cm^{-1}; ^{1}H- NMR (300MHz, $CDCL_3$, TMS) and ^{13}C- NMR (75 MHz, $CDCL_3$, TMS) are shown as under TABLE 3.

EIMS(m/z, rel, int):M^+458(5.50), 444(10.09), 436(8.71), 430(16.05), 384(21.10), 373(24.31), 352(16.97), 331(21.33), 303(14.67), 300(13.07), 289(17.43), 273(24.31), 271(34.86), 253(37.84), 246(10.55), 226(28.44), 199(28.36), 185(8.94), 180(15.60), 171(10.55), 159(18.81), 153(24.77), 145(36.47), 141(11.01), 129(29.82), 113(26.15), 107(50.46), 95(55.50), 81(70.64), 60(100), 43(14.22), 15(19.72).

The TLC examination of the isolated compound from Benzene: Acetone extract of the leaves of Artabotrys Odoratissimus showed a single spot upon

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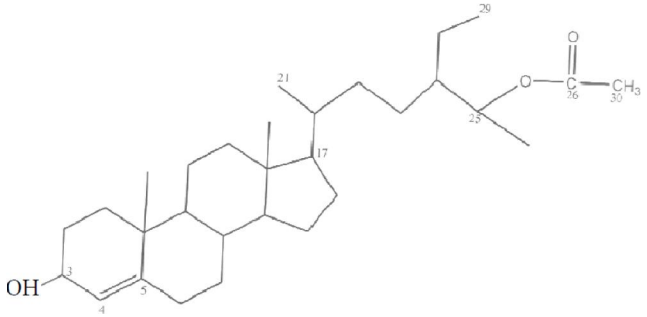
TABLE 3 : Crystallization of Compound S1

Carbon NO.	Type of Carbon	^{1}H-NMR, δppm ($CDCL_3$, 300MHz)	^{13}CNMR, δppm ($CDCL_3$, 75MHz)
1	CH_2	1.33(ov),2.28(ov)	37.5
2	CH_2	1.86,1.82	31.5
3	CH	3.51(m)	72.1
4	CH	5.36(d,J=4.8Hz)	122.0
5	C	-	143.8
6	CH	1.12	24.6
7	C	33.9	
8	CH	1.17	29.9
9	C	1.44	50.4
10	C	-	36.4
11	CH_2	2.05	21.3
12	CH_2	1.08	40.9
13	C	-	42.6
14	CH	1.15	57.2
15	CH_2	0.92	22.9
16	CH_2	-	28.7
17	CH	1.44	56.3
18	CH_3	0.68	12.2
19	CH_3	1.01	19.8
20	CH	2.12	36.0
21	CH_3	0.98	19.0
22	CH_2	1.55	40.9
23	CH_2	1.257	26.4
24	CH	1.20	45.8
25	CH	3.75(m)	73.3
26	CO	-	172.1
27	CH_3	0.80(d)	19.3
28	CH_2	1.15(m)	20.1
29	CH_3	0.84(t)	12.1
30	CH_3	2.05(s)	21.3

exposure to iodine vapour. The compound was obtained as white Crystals (m.p. 155^0C). It was readily soluble in $CHCL_3$. To the best of our knowledge this compound has not been previously isolated or separated from any other sources. 25-acetyl-24β-ethyl-cholest-4-en-3β-ol was isolated from this plant for the first time in our laboratory. The IR spectrum showed the presence of a carbonyl group 1713cm^{-1}, C=C and an isopropyl group(1616-1602,1380-1341cm^{-1} respectively)[10, 12, 14]. The peak corresponds to hydroxyl group at 3438cm^{-1}. The absorptions at 759,831 and 845cm^{-1} revealed the characteristic"4-unsaturated and skeletal vibrations of steroidal moiety. The ^{1}H-NMR spectrum of S1 exhibited a doublet at δH 5.36 (J=4.8Hz) was assigned to vinylic H-4 proton[16, 17] and a one proton broad multiplet at δH 3.51 with $W_{1/2}$ 15.64Hz showed the presence of 3α- methine proton (axial) interacting with C-2 equatorial, C-2 axial and C-4 protons, confirmed the β orientation of the hydroxyl group. A multiplet at δH 3.75 was account for the oxygenated Methine H-25. Three proton singlet at δH 2.05 was assigned to acetyl group at C-26[16, 17, 15]. Two broad signals integrating for three protons each, at δH 0.68 and 1.01 assigned to two angular Methyl groups. Two doublets at δH 0.98, 0.80 and triplet at δH 0.84 all inte-

grated for three protons, for H-21, H27 and H-29 methyl protons respectively. The presence of all the methyl signals in the range of δH 1.01-0.67 supported their attachment on the saturated carbons. The remaining methylene and methine protons resonated between δH 2.32-1.01 except H-25[16,11]. The downfield shift of the 3- carbinol proton at δH 3.51 in comparison to the 3-proton of β-sitosterol (δH 3.25), and the appearance of the olefinic proton at δH 5.36 as a doublet suggested a "$^{4(5)}$- double bond in the ring A[16, 11]. The multiplicity of carbon atoms in the^{13}C-NMR($CDCL_3$, 75MHz) spectrum was confirmed the suggested structure. Compound S1 showed the sharp peaks at δc 12.2 and 19.8 were assigned to the two angular methyl carbons and a signal at δc 19.0 was due to the presence of methyl carbon at C-21[16, 11, 15]. The deshielded signals at δc143.8 and 122.85 were assigned to vinylic C-5 and C-4. The signals at δc 72.1 and 73.3 were accounted to C-3 carbinol and C-25 oxygenated methine carbons, respectively. The methyl carbons appeared at δc 12.2 (C-18), 19.8(C-19), 19.0(C-21), 19.3(C-27) and 12.1(C-29)[16, 11, 15, 17]. The upfield resonances of C-18 and C-29 at δc 12.2 and 12.1 suggested the location of the acetyl group at C-25. The remaining chemical shift values were characteristic of a steroid, when compared with lawsaritol, but a peak at δc 172.1 for C-26 indicated the presence of carbonyl group, which is in turn supported by a signal for carbonyl methyl at δc 21.3 for C-30[16, 11, 15, 17]. The molecular ion peak of S1 was found at [M^+ +2] 458, which suggested its molecular formula as $C_{30}H_{48}O_3$(Cal.458.6885). The fragmentation pattern showed peaks at m/z 393[M-CH_3-$OCOCH_3$], 444[M^+H-CH_3], 441[M-H_2O]. The base peak at m/z60 was formed by the McLafferty rearrangement.



Scheme 1 : 25-acetyl-24β-ethyl-cholest-4-en-3β-ol

This can be possible only if the- $OCOCH_3$ group attached at the end of the side chain. Peaks at m/z 429,273,246 were formed due to [M-2* Me], [M-side chain], [M-side chain + ring D cleavage][16, 17]. Thus the IR, ^{1}HNMR, ^{13}CNMR and Mass spectral analysis with physical properties established the identity of compound as *25-acetyl-24β-ethyl-cholest-4-en-3β-ol*[11]*(Scheme1).*

CONCLUSION

The results of the present investigation constitute the occurrence of *25-acetyl-24β-ethyl-cholest-4-en-3β-ol,* type compound in plant kingdom. The title compound has been isolated from this plant for the first time.

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