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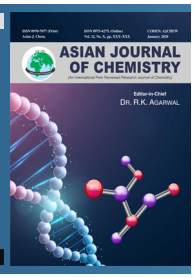

A New Steroid Isolated from *Leucas cephalotes*

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A new steroidal triterpenoid was isolated from hexane washed ethanolic extract of flowers of *Leucas cephalotes*. Its structure was elucidated as (*Z*)-17-(4,4-dimethylhept-2en-2-yl)-8,10,14-trimethyl-2,3,4,5,6,7,8,10,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopent[*a*]phenanthren-3-ol (**1**). The structure of the new compound was determined by 1D and 2D NMR and MS and DEPT experiments as well as by comparison of their data with the reported values.

Keywords: Triterpenoid, *Leucas cephalotes*, Steroid, Leucasterenol.

INTRODUCTION

Leucas cephalotes (Roth) spreng (Family: Lamiacae) is a herb having many medicinal properties. It grows to about 30-100 cm height and flowers are sessile, globus, dense, terminal whorl flowers. Varieties of chemical constituents were reported from the plant. Earlier reports revealed the isolation and characterization of essential oils [1], alkaloids and sterols like 7-oxositosterol [2,3], 7-oxostigmasterol [3], 7α-hydroxysitosterol, 7α-hydroxy stigmasterol [4] and stigmasterol [5]. Triterpenoids like oleanolic acid and ursolic acid were also reported from the plant. Flavones like pillion [6], gonzalitosin I [7], cosmosin [8], apigenin-7-O -β-D-(6-O-*p*-coumaroyl)glucopyranoside [9], anisofolin A [10] and luteolin-4′-O-β-D-glucuronopyranoside [11] were reported from the plant. Labellenic acid [12], lauric acid, glutamic acid, tridecanonic acid and adipic acid [13] are reported from the seed oil of *Leucas cephalotes*. β-Sitosterols and stigmasterols were reported in major percentages [14] in *Leucas cephalotes*. Presence of many medicinal properties may be attributed to variety of chemical constituents reported from the plant.

Anthelminthic activity [15], antimicrobial activity [16], antioxidant, analgesic, anti-inflammatory activities [17], anti-filarial activity [18], anti-diabetic activity [19] and anti-bacterial activity [20] were also reported from *Leucas cephalotes*. Some fatty acids isolated from *Leucas cephalotes* are bioactive fatty acids and they can be used in various pharmaceutical products [21].

EXPERIMENTAL

UV spectra were run on instrument Shimadzu (Internal ID: QC1-029S.No:A10834802158) spectrophotometer. IR spectra were measured on Shimadzu FT-IR spectrometer (Internal ID: QC1-030; S.No.: A2137902140). PMR spectroscopic data was recorded on 300 MHz, Bruker Avance, ^{1}H & ^{13}CNMR & DEPT were recorded on 400 and 100 MHz Bruker Avance spectrometers, respectively. 2D (1H-1H COSY) NMR was also recorded on a Bruker AVANCE-III 400 MHz instrument and mass spectra were recorded on VG Micromass 7070 H (EI). QSTAR XL (Bruker Compass) high resolution mass spectrometer (HRESIMS), Thermofinnigan ESI ion frag mass spectrometer at University of Hyderabad, Hyderabad, India. TLC plates for analysis used were of 0.25 mm thick (Silica gel-G with 15% of calcium for binding) glass-backed plates. Column chromatography was carried out with silica gel of (200 mesh, Finar).

Plant material: Whole plant of *Leucas cephalotes* of about 4 kg was collected from Garikapadu village of Guntur district, India along with roots during October 2018 and the material was air-dried under shade. The plant was identified by L. Rasingam (Scientist-in-charge), Botanical Survey of India, Deccan Regional Centre, Hyderabad, India. Flowers were separated from other parts of the plants.

Extraction and isolation: Air dried flowers of *Leucas cephalotes* were powdered and extracted using ethanol in a Soxhlet apparatus. The ethanolic extract (50 g) obtained was distilled to remove ethanol and impregnated on silica gel. This was washed by using hexane (5 L),benzene (2 L), chloroform (2 L), ethyl acetate (2 L) and methanol (1 L), respectively on a Büchner Funnel. Hexane wash was concentrated and subjected to fracational crystallization using methanol. Solid was separated and filtered and the filtrate obtained was concentrated (32 g) subjected to column chromatography (CC: 10 cm, 200 g, silica gel of 200 mesh) using hexane-ethyl acetate mixtures. Hexane:ethyl acetate (100:1 to 0:100) yielded 82 fractions. Fraction 36 (9:1) fraction showed single spot on TLC. This fraction on crystallization with methanol yielded compound **1**.

Leucasterenol (1): Colourless needles (MeOH); m.p.: 240-242 ºC. UV spectra (λ_{nm}): 204. IR (KBr, ν_{max}, cm^{-1}): 3437 (brs, OH), 2946.39 (=CH), 2330.0 (C-H), 1648 (C=C), 1367 (OH, def.), 1056 (cycloalkane). Mass fragmentation (EI): 413 [M+1], 271, 231, 242, 97, 84, 69, 55. HRESIMS: 413.2645 $[M + H]^+$ (calcd. for $C_{29}H_{48}O$+H, 413.3783).

RESULTS AND DISCUSSION

Compound (**1**) was obtained as white colourless needles on recrystallization using MeOH and the molecular formula $C_{29}H_{48}O$ was deduced from the positive ion *m/z* 413.2645 $[M + H]^{+\bullet}$ (calcd. for $C_{29}H_{48}O$ + H, 413.3783) in the HR-ESI-MS spectrum. The compound **1** gave positive test for Salkowski reaction [22] and Libermann-Burchard reaction [23] suggesting compound **1** to be a steroid. The HR-ESI-MS, COSY and ^{13}C NMR spectral data suggested compound **1** to be a tetracyclic compound. Molecular formula $C_{29}H_{48}O_2$ indicates that it is a stigmastane type of steroid [24]. Seven methyl groups, one hydroxyl group and a side-chain are associated with tetracyclic carbon skeleton. IR showed the presence of double bond at 1648.24 cm^{-1} corresponds to (C=C) stretching, signal at 745.52 cm^{-1} in IR supports compound **1** as *cis*-isomer [25]. The PMR, 2D (1H-1HCOSY) NMR, ^{13}CNMR, DEPT, IR showed presence of olefinic protons. PMR showed the olefinic protons at δ 5.1927 (1H) & δ 5.3442 (1H) ppm indicating two substituted double bonds in compound **1**. ^{13}C NMR showed four different sp^2 carbons at δ values 140.73, 138.29, 129.23 and 121.69, which further supports presence of two double bonds [26,27]. One of the olefinic proton was placed outside the ring between C-21 & C-22 *i.e.* in the side-chain as it appeared as a singlet at δ 5.3442 ppm, the same signal was observed in 2D (1H-1H COSY) NMR also, ^{13}C NMR at δ 138.29 & 129.23 ppm corresponds to this double bond at C-21 & C-22, respectively [26,27]. No further correlation of this double bond was observed in 2D (1H-1H COSY) NMR. The second double bond was placed between C-9 & C-11 and it appeared as a multiplet at δ 5.1927 obtained due to allylic (with H-12 protons) & homoallylic (methyl at positions C-10 & C-18) [28], correlation of this olefinic proton with methylenic protons at C-12 was clearly observed in 2D (1H-1H-COSY) NMR. ^{13}CNMR showed signal at δ 140.73 & 121.69 ppm, DEPT at δ 121.74 corresponding to C-9 & C-11, respectively [26,27]. The presence of double bond between C-9 and C-11 was also shown in some steroids [29,30]. The peak at 55 (100%) in EI mass fragmentation also suggests the presence of double bond in the side chain corresponding to isobutenyl, the side-chain was built based on the EI-mass fragment ion peaks at 69, 84 and 97 obtained by adding alkyl groups to the base peak 55. The side chain was attached at the 17th carbon position of compound **1** as it was the characteristic position in many steroids having side chain [31]. Out of the seven methyl groups, four were placed in the side-chain and remaining three as angular methyl based on literature [30,32]. Two methyls were placed at position C-23 by considering down-field δ value at 1.01 ppm (s, 6H, H-27 & H-28) and downfield was due to olefinic proton at C-22. One of the methyl group was placed at C-21 as it appeared at downfied 1.97 (s, 3H, H-29) by the effect of double bond between C-21 & C-22. One of the methyl group was present at the end of side-chain *i.e.* at position C-25 (m, 3H, H3-26). The position of remaining three methyl groups was placed according to the literature of Leucastrin-A, which was isolated from *L. cephalotes* [32] *i.e.* at positions C-8, C-10 and C-14 of compound **1** [3, 33]. Some of δ ppm values of carbon atoms of structure were compared with literature as shown in Table-1.

The presence of OH group was showed by IR absorptions at 3437 and 1056 cm^{-1} (OH, def.). Hydroxyl group presence was further supported by PMR δ value at 3.52 ppm (m,1H, H-3) corresponding to the methine proton to which hydroxyl group was attached. It was further confirmed by 2D (1H-1H COSY) NMR showing correlation of δ 3.5 ppm (H-3) with two methylene protons at δ 2.2 ppm and δ 1.5 ppm (one on each at positions H-2 & H-4). The presence of hydroxyl group was further supported by ^{13}C NMR signal at δ 71.7 ppm, DEPT at δ 71.83 [34]. The hydroxyl group was placed at C-3 position due to its characteristic position of monohydroxy group containing steroids [35]. The ^{13}C NMR and DEPT spectra of compound **1** supported the assigned structure. The PMR values and ^{13}C NMR, DEPT values are shown in Table-2 and 2D (1H-1H COSY) NMR (Table-2) also supports the assigned structure.

On the basis of PMR, 2D (1H-1H COSY) NMR, ^{13}C NMR, DEPT, UV, IR and mass spectral analysis [36], the structure of compound **1** is assigned in Fig. 1. Its IUPAC name is (*Z*)-

TABLE-1
COMPARISON OF ^{13}C NMR VALUES WITH SIMILAR COMPOUNDS

Compound	Position of carbon	δ (ppm) value	Position of carbon	δ (ppm) value	Position of carbon	δ (ppm) value	Position of carbon	δ (ppm) value	Position of carbon	δ (ppm) value	Position of carbon	δ (ppm) value	Ref.
Leucastrin-A	19	22.5	18	22.1	30	17.4	–	–	–	–	–	–	[32]
Compound-A	19	14.3	–	–	–	–	9	147.5	–	–	22	130.4	[27]
Compound-B	19	17.2	–	–	–	–	9	142.4	11	124.0	22	130.4	[27]
Leucasterenol (**1**)	18	17.8	19	22.0	20	19.3	9	140.7	11	121.6	22	129.2	Present work

TABLE-2
PMR (300 MHz), ^{13}C NMR (100 MHz), COSY AND DEPT DATA FOR COMPOUND (**1**) ($CDCl_3$, δ, ppm, *J*/Hz)

C atom	δ_C (100 MHz)	δ_H (300 MHz)	2D (1H-1H-COSY) NMR (400 MHz)	DEPT (100 MHz)	C atom	δ_C (100 MHz)	δ_H (300 MHz)	DEPT (100 MHz)
1	37.24	H-1a - 1.71(m) H-1b - 1.31(m)		37.26	16	26.06	H-16a - 1.53(m) H-16b - 1.43(m)	26.04
2	28.903	H-2a - 2.2(m) H-2b - 1.8(m)	a*	28.95	17	56.85	H-17 - 2.22(m)	56.87
3	71.78	H-3 - 3.52(m)	a*	71.83	18	19.39	H-18 - 0.84(s)	19.42
4	39.76	H-4a - 2.2(m) H-4b - 1.8(m)	a*	39.77	19	19.02	H-19 - 0.82(s)	19.03
5	56.75	H-5 - 1.48(m)		56.77	20	19.81	H-20 - 0.80(s)	19.84
6	24.35	H-6a - 1.56(m) H-6b - 1.18(m)		24.32	21	138.29		
7	31.65	H-7a - 1.18(m) H-7b - 1.96(m)		31.67	22	129.23	H-22 - 5.34(s)	129.27
8	42.29				23	36.137		
9	140.73				24	25.39	H-24a - 1.28(m) H-24b - 1.21(m)	25.44
10	39.67				25	23.06	H-25a - 0.93(m) H-25b - 0.82(m)	23.06
11	121.69	H-11 - 5.19(m)	a*	121.74	26	40.48	H-26 - 0.80(m)	40.53
12	28.23	H-12a - 2.27(m) H-12b - 2.00(m)		28.26	27	29.14	H-27 - 1.01(s)	29.13
13	45.82	H-13 - 2.02(m)		45.83	28	31.89	H-28 - 1.01(s)	31.90
14	55.94				29	21.08	H-29 - 1.97(s)	21.08
15	33.93	H-15a - 1.44(m), H-15b- 1.23(m)		33.94				

a* = confirmed by 2D (1H-1H COSY) NMR

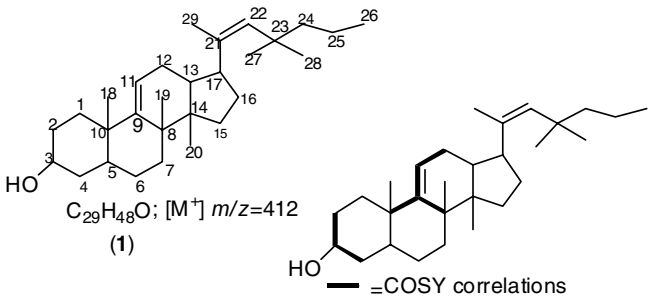

Fig. 1. Structure of leucasterenol (**1**)

17-(4,4-dimethylhept-2-en-2-yl)-8,10,14-trimethyl-2,3,4,5,6,7,8,10,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[*a*]-phenanthren-3-ol and named as leucasterenol. The assigned structure was further supported by the mass spectral fragmentation (Fig. 2).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

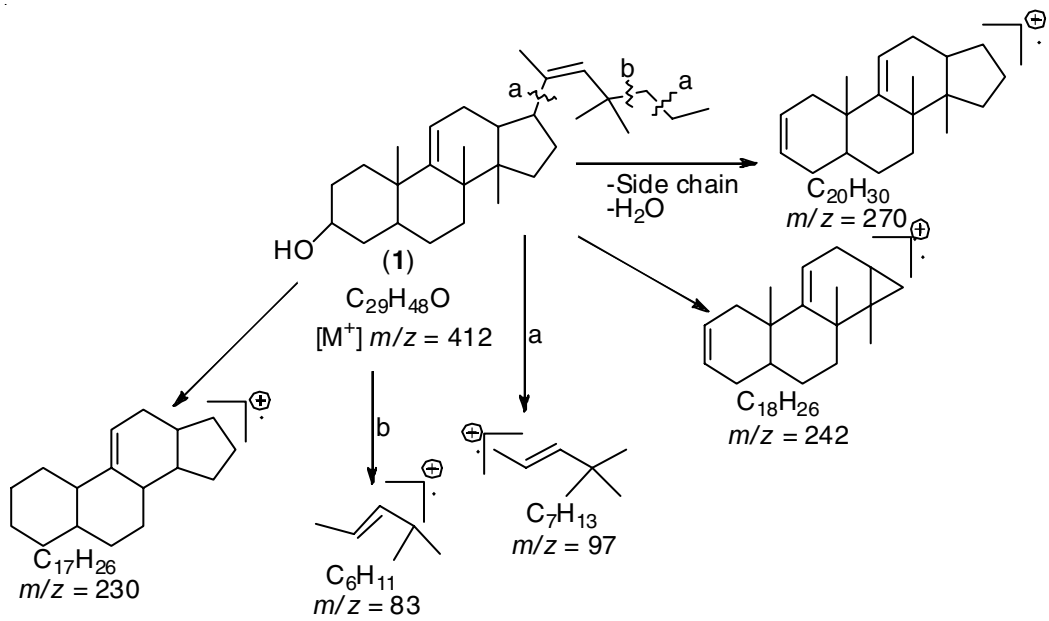

Fig. 2. Mass fragmentation scheme of leucasterenol (**1**)

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