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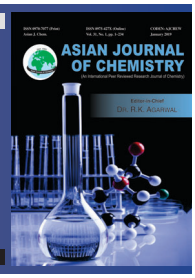

A Novel Pyridyl Ester Isolated from *Leucas cephalotes*

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From the flowers of *Leucas cephalotes*, pyridine-3-carboxylic acid ester was isolated. Its structure was established as 2′,5′-dihydroxy-3′-pyridyl-(2,5-dihydroxy)pyridinyl-3-carboxylate on the basis of spectroscopic analysis. To out best of knowledge, this is the first report from *Leucas cephalotes* in particular and *Labiatae* family in general.

Keywords: Lamiaceae, *Labiatae*, *Leucas cephalotes*, pyridyl ester.

INTRODUCTION

Leucas cephalotes (Roth) Spreng (Family: *Lamiaceae*) known as "Dronapushpi" in sanskrit. It is a weed and grows in rainy season. Two protostane type triterpenoids named leucastrins A and B and oleanolic acid were isolated from *Leucas cephalotes* [1]. Triterpenoids β-sitosterol [2], stigmasterol [3], lupeol [4,5], were isolated labellinic acid was also reported from *Leucas cephalotes* [6]. Aliphatic esters [7], essential oils [8-10], flavones [10] were isolated from *Leucas cephalotes*.

Dronapushpi is widely used as a homeopathic drug in indigenous system of medicine. It has been used for the diagnosis of several diseases like edema, diaphoretic, inflammatory and obstinate urinary troubles. The plant is a valuable drug in the treatment of snake bite. Antifilarial, antibacterial, antidiabetic and hepato-protective activities were reported from the whole plant of *L. cephalotes* [11]. The syrup of flowers mixed with honey are useful in diagnostic remedies of cough and colds [12,13]. The plant has been reported to exert multiple biological effects pharmacologically like antioxidant [14], analgesic, anthelmintic [15] and antimicrobial [16] activities. *Leucas cephalotes* shows pronounced effect on carbohydrate and fat metabolism [17]. Presence of glycoside of β-sitosterol [18], iridoid glycosides [19] and labellenic esters [20] were revealed by the phyto-chemical investigations of *Leucas cephalotes*. The present study was carried out on the flowers of *Leucas cephalotes* expecting many unidentified chemical constituents in the plant.

EXPERIMENTAL

The plant material of *Leucas cephalotes* of about 4 kg was collected from Garikapadu village of Guntur district and Alur village of Kurnool district, India along with roots during October 2018 and the material was air dried under shade. Flowers were separated from other parts of the plants.

General procedure: Flowers of *Leucas cephalotes* of about 800 g was powdered and extracted in a 5 L Soxhlet apparatus using ethanol. The ethanolic extract was impregnated on minimum amount of silica gel of 100-200 mesh size and washed successively with hexane, benzene, chloroform, ethylacetate and methanol, respectively. They are named as hexane extract, benzene extract, chloroform extract, ethylacetate extract and methanolic extract, respectively. The hexane extract is concentrated and observed on TLC. It showed four spots with considerable difference in R_f values. Therefore, it is subjected to PTLC using silica gel of 60-120 mesh size coated on plates of about 19 cm × 16 cm. The developing solvents used were benzene:ethyl acetate (8:2). Four bands were identified using UV chamber and the bands were separated and washed repeatedly with chloroform and concentrated. The second band with R_f 0.53 is separated, washed and recrystallized using methanol.

Light blue coloured compound A was separated and the yield was found to be 10 mg (m.p.: 175 ºC). IR (KBr, cm^{-1}, ν_{max}): 3392.993 (-OH, broad peak), 3230.91 (-OH), 1780 & 1346 (ester group), 1655, 1530, 1512, 1455, 1399, 1346 (pyridine ring vibrations); ^{13}C NMR (25.15 MHz, DMSO, δ ppm): 180.3, 160.1, 158.2, 135.6, 126.23, 114.9, 115, 126.23; PMR (400 MHz, $CDCl_3$, δ ppm): 7.09-7.14 (multiplet) and 7.33-7.36 (multiplet). UV spectrum (MeOH, λ_{max}, nm): 278. Mass fragmentation, [EI-MS (DIP-350)], *m/z* (I_{rel}, %): 57, 69, 75, 83, 89, 95, 111 (100 %, base peak), 121, 127, 153, 154, 155, 230, 231, 264 (35 %, molecular ion peak). ESIMS: 265 $[M+H]^+$, 287 $[M+Na]^+$. On the basis of spectral analysis, compound A is identified as 2′,5′-dihydroxy-3′-pyridyl-(2,5-dihydroxy)-pyridinyl-3-carboxylate.

RESULTS AND DISCUSSION

An isolated novel pyridyl ester (2′,5′-dihydroxy-3′-pyridyl-(2,5-dihydroxy)pyridinyl-3-carboxylate) from *L. cephalotes* was recrystallized as blue coloured product having m.f. $C_{11}H_8O_6N_2$ $[M^+]$ *m/z* = 264. Compound-A showed presence of one ester group, two pyridine nuclei and four hydroxyl groups. The presence of ester group was suggested by IR absorption at 1780 cm^{-1} (C=O) and 1346 cm^{-1} (C-O) are characteristic peaks for esters [21]. Presence of ester group was further supported by ^{13}C NMR at δ 180.3 [22]. Two pyridine nuclei in compound-A was suggested by four IR bands at 1655 , 1530-1512, 1458-1396 and 1346 cm^{-1} indicating ring stretching vibrations of pyridine ring [23]. This was further supported by UV absorption at λ_{max} 278 nm [24,25]. This suggests the presence of pyridine nucleus and is further supported by EI mass at *m/z* = 75. The molecular formula shows presence of six oxygen atoms in compound-A, two of them are attributed to ester group and remaining four oxygen atoms are in the form of hydroxyl groups. The IR peaks at 3230.91 and 3392.93 (broad peak) suggesting the presence of bonded hydroxyl group (-OH). The ^1H NMR spectrum showed only two multiplets at δ 7.09-7.14 and 7.33-7.36 ppm each corresponding to four protons, present in two pyridine nuclei of two each. Two multiplets corresponding to four hydrogens and even mass suggests the presence of two pyridine nuclei each with two hydroxyl substituents. Thus, compound-A has two pyridine nuclei each with two hydroxy substituents each.

In pyridine, 2,4,6-protons appears at downfield due to adjacent nitrogen showing inductive effect and mesomeric effect. Since there is no downfield signal (δ 8.0 ppm) therefore either a substitution at these positions or mesomeric or inductive effect of substituents at *ortho* to these positions is expected. Due to the mesomeric effect of hydroxyl group, *ortho* protons are shielded and shows upfield shift in PMR spectrum. Similarly, hydroxy substitution at 2nd position leads to upfield shift of H-3 and H-5 protons to 6.60 ppm while H-4 and H-6 to 7.23-7.30 ppm [26]. In the nmr spectrum of compound A, there is

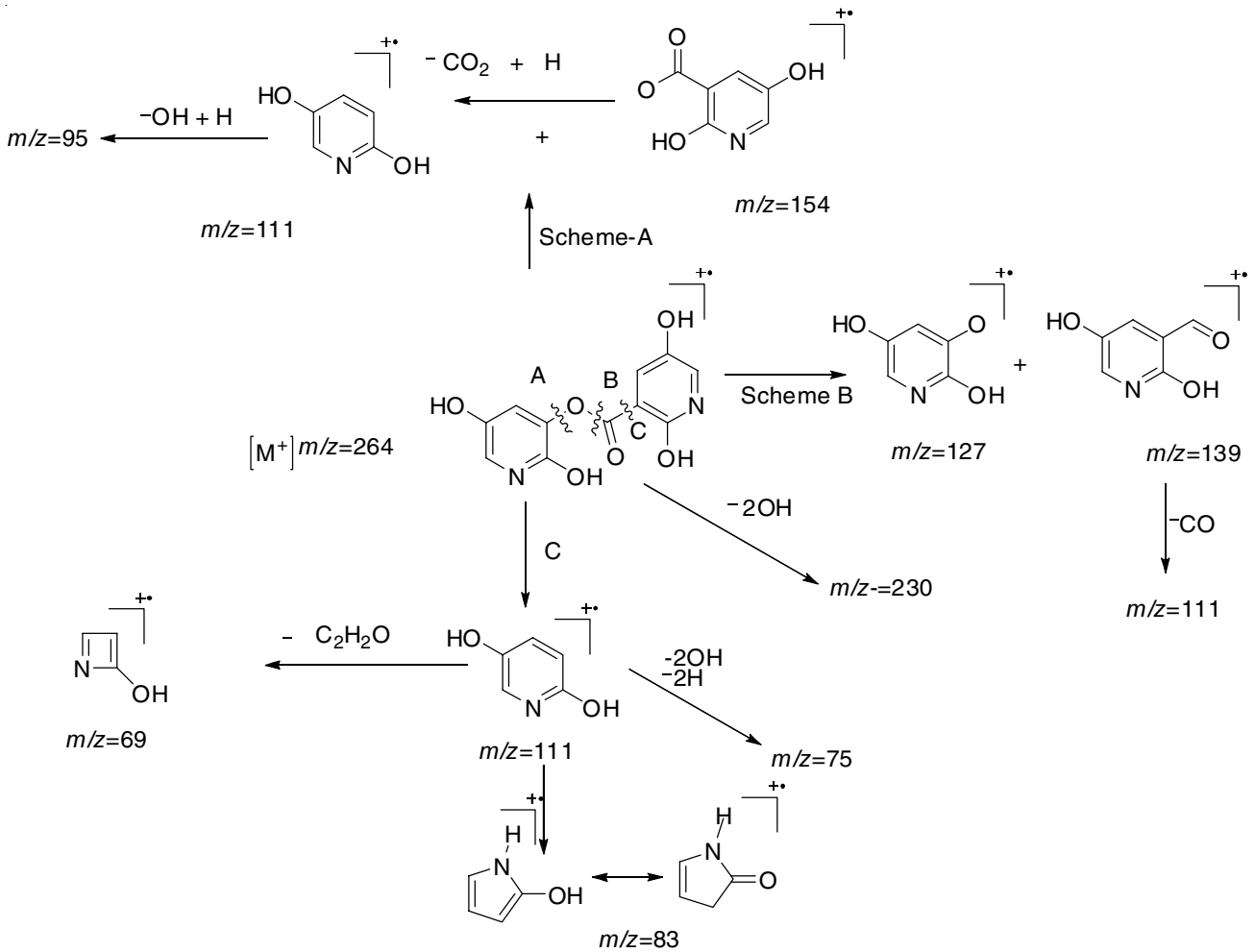

Fig. 1. Mass spectral fragmentation of isolated novel pyridyl ester from *Leucas cephalotes*

no signal in the range of 6.3-6.9 ppm. The nmr showed only two multiplets at δ 7.09-7.14 and δ 7.33-7.36 ppm suggesting substitution at H-2,H-3 and H-5. Moreover, there is no downfield proton (beyond 7.7 ppm) this suggests absence of free β-proton [27]. Thus substitution pattern in both the pyridine rings can be predicted to 2,3,5-positions. The multiplet at δ 7.09-7.14 ppm is assigned to H-6′ and H-6 and the multiplet at δ 7.33-7.36 ppm is assigned to H-4 and 4′-protons. Based on this the structure of compound A can be predicted as 2′,5′-dihydroxy-3′-pyridyl-(2,5-dihydroxy)pyridinyl-3-carboxylate. ^{13}C NMR values also suggested *meta*-substitution [28,29].

ESI-MS of novel pyridyl ester compound shows peaks at 265 corresponding to $[M+H]^+$ and 287 corresponding to $[M+Na]^+$. This structure is further supported by its mass spectral fragmentation as given in different paths (Fig. 1) [30].

Thus, the structure of compound is confirmed as 2',5'-dihydroxy-3'-pyridyl-2,5-dihydroxy pyridinyl-3-carboxylate and its structure is shown in Fig. 2.

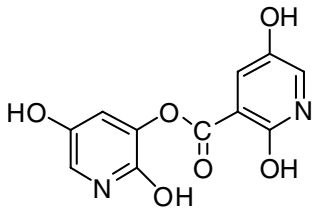

Fig. 2. Structure of 2',5'-dihydroxy-3'-pyridyl-2,5-dihydroxy pyridinyl-3-carboxylate

Conclusion

Though several triterpenoids, flavonoids and aliphatic esters are reported from *Leucas cephalotes*. To our best of knowledge, this is the first report on the isolation of novel pyridinyl ester from this plant.

CONFLICT OF INTEREST

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

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