Prayogik Rasayan

First isolation of stigmasterol from the indian medicinal plant *Roscoea purpurea* Sm. (Kakoli)

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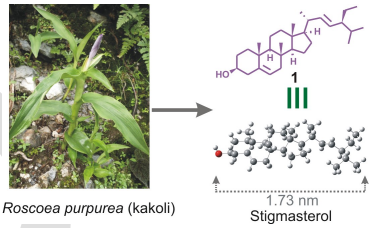
Received- February 10, 2019 | Accepted: February 24, 2019 | Published online – April 18, 2019

Kakoli *(Roscoea purpurea)* is an important ingredient of *Chyawanprash* which is well known for its anti-ageing activities since the pre-historic period. While investigating the chemical constituents of Kakoli, herein we report the first isolation and spectroscopic characterization of a phytosterol *stigmasterol* **1** from the leaves of *Roscoea purpurea*. The molecule constitute a rigid 6-6-6-5 tetracyclic backbone with one secondary hydroxyl group attached at the 'A'-ring and one branched C10 hydrocarbon chain in the other end of the molecule. Energy minimized structure revealed that the molecule is 1.73 nm long having a polar 'OH' group attached at the end of an almost planar lipophilic steroidal skeleton making it useful as a functional nano-entity.



Roscoea purpurea (kakoli)

Keywords: Astavarga, *Chyawanprash, Roscoea purpurea*, Stigmasterol

1. Introduction

Ayurveda (a Sanskrit word, '*Ayus*' means life and '*Veda*' means science or knowledge) is a '*science of long life*' practiced in India since the prehistoric period. *Astavarga*, a set of eight medicinal plants formulated by the reputed *Ayurvedic* wonder healers *Ashwini Kumars*, could rejuvenate the old, frail and emaciated body of the Rishi Chyavan and miraculously restored his youth.[1] Since then this *Ayurvedic* preparation containing the set of eight medicinal plants has been known as *Chyavanprash* after the name of Rishi Chyavan and has been an important and demanding *Ayurvedic* health tonic for the Kings and the rich people. The eight *Astavarga* plants namely *Kakoli, Kshrikakoli, Jeevak, Rishvok, Meda, Mahameda, Riddhi* and *Vriddhi* grow naturally in small patches in particular ecological environments in the Himalaya especially in the North-Western Himalaya at an elevation of 1200 – 4000 m above the sea level.[2] Recent attempts by a group scientists and sages have enabled the proper identification of the eight Astavarga plants. Thorough literature search by us have revealed that there is no report of the active chemical constituents of most of the Astavarga plants. To our knowledge, stigmasterol is the first compound isolated from the medicinal plant *Roscoea purpurea*. The compound was obtained as a white crystalline solid and it has the molecular formula $C_{29}H_{48}O$. The structure of this compound (**1**) was determined by ^{1}H, ^{13}C, DEPT NMR techniques and HRMS. The NMR data obtained for the compound matched with the data reported previously in the literature.[3,4]

Figure 1: Photograph of Kakoli (*Roscoea purpurea*) taken in the month of July, 2016 at Barlowganj, India.

2. Experimental Section

2.1 Plant Material. The *Roscoea purpurea* sample used in this study was collected from Barlow ganj, Mussoorie, Uttarakhand, India, in July 2016. The details of the sample collection is given in the supporting information.

2.2 Extraction and isolation

The leaves of Kakoli was shade dried and finely powdered using a grinder. Powdered leaves of *Roscoea purpurea* (Kakoli) (16 g) were extracted with ethyl acetate (250 mL) by using an extraction apparatus (capacity 500 mL) during 24 h at room temperature. The volatiles were removed under reduced pressure to afford a brownish solid material (0.636 g). The crude extract was purified by successive column chromatography (thrice, Si-gel, 100–200 mesh) using 10-20 % ethyl acetate/ petroleum ether as the eluant. The product appeared as a white crystalline solid (0.038 g, 0.23% yield). MP = 160 – 165°C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ: 5.377 (1H, t, J=4.7 Hz), 5.20 (1H, dd, *J* = 8.4,15.1 Hz), 5.04 (1H, dd *J* = 8.4,15.1 Hz) 3.546 (1H, m), 2.036 (1H,m), 1.03 (3H, d), 1.01 (3H,s) 0.843 (3H, d), 0.825 (3H, d), 0.807 (3H, d), 0.718 (3H, s), 2.31-1.01 (25H: phytosterol proton, m) ppm. 13C NMR (CDCl3, 100 MHz): δ: 140.76, 138.30, 129.29, 121.70, 71.81, 56.88, 55.97, 51.23, 50.18, 42.21, 42.22,

40.47, 39.7, 37.26, 36.52, 36.7, 31.97, 31.67, 29.2, 28.4, 25.6, 24.5, 21.4, 21.3, 22.3, 19.93, 19.15, 12.23, 12.05. HRMS: calculated for $[C_{29}H_{48}O + NH_3]^+$ = 430.2448 obtained = 430.2430.

Figure 2: Photograph of Kakoli sample used for chemical investigations.

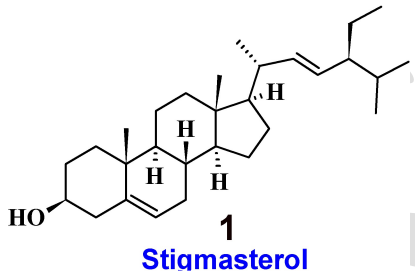


Figure 3: Structure of stigmasterol isolated from Kakoli

3 Results and discussion

3.1 Extraction, Purification & Isolation of Stigmasterol

Stigmasterol (**1**) is a tetracyclic plant based phytosterol, obtainable from the leaves of Indian medicinal plant *Roscoea purpurea*, commonly known as *Kakoli* found in Himalayan area of India. The compound was extracted from several plants ware reported previously but to our knowledge stigmasterol is the first compound isolated from the indian medicinal plant *Roscoea purpurea*. The molecule constitute a rigid tetracyclic backbone (6-6-6-5) with one secondary hydroxyl group in one end and one long hydrocarbon chain in the other end of the molecule. Energy minimized structure revealed that the molecule is 1.73 nm long. Compound **1** was isolated from the leaves of *Roscoea purpurea* and purified by following an improved method developed in our laboratory (see experimental section). Stigmasterol (**1**) was obtained as a white crystalline solid and has the molecular formula $C_{29}H_{48}O$ as established by HRMS. The IR spectrum confirmed the presence of the -OH groups (3350 cm-^{1}) and C=C (1688 cm-1). The ^{1}H NMR spectrum exhibited two methyl singlets (*δ* 1.01 and 0.71 ppm), and two-proton double doublet at δ 5.20 (*J* = 8.4, 15.1 Hz), 5.04 (*J* = 8.4, 15.1 Hz)) for one double bond system, one-proton multiplate at *δ* 3.546 an R-proton geminal to a hydroxyl group. The ^{13}C NMR spectrum showed 29 carbon signals. The multiplicity of each carbon was achieved by the DEPT experiment, which confirming the presence of one secondary hydroxyl group and five methyls group. The NMR data obtained for the compound are compatible to those reported compound.

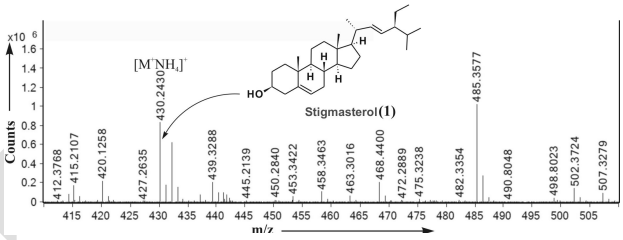


Figure 4: HRMS of compound **1**

4. Conclusion

Investigations of chemical constituents of medicinal plants will aid in understanding the chemical basis of biological and medicinal activities. With an aim to investigate the chemical constituents of various parts of the medicinal plant Kakoli (*Roscoea purpurea)*, we have isolated stigmasterol from the leaves of it and characterized by spectroscopic methods. To our knowledge, this is the first report of the isolation of stigmasterol in Kakol. Molecular modeling studies have revealed that stigmasterol can act as a functional nano-entity with tremendous potential application in supramolecular chemistry and nano-science. The details will be published in due course.

5. Acknowledgement

BGB thanks India Srilanka project (DST/INT/SL/P25/2016), Science and Engineering Research Board (SERB), India (ref. EMR/2016/0069), UGC-SAP and DST-FIST New Delhi and Vidyasagar University for financial support and infrastructural facilities. ACB thanks UGC to provide research fellowships.

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