

Structure elucidation of a newly isolated saponin from *Clerodendrum serratum* (L) Moon

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SUMMARY

Plant saponins are widely distributed amongst plants and have a wide range of biological properties. Icosahdropicenic acid, C₅₁H₈₀O₁₉ ((4S,6bS)-8a-((4,5-dihydroxy-6-methyl-3-((3R)-3,4,5-trihydroxy-6-methyl-tetrahydro-2H-pyran-2-yloxy)-tetrahydro-2H-pyran-2-yloxy)carbonyl)-2-hydroxy-4, 6a, 6b, 11, 14b-pentamethyl-11-(2-methylprop-1-enyl)-3-(3,4,5-trihydroxy-6-(hydroxymethyl) - tetrahydro-2H-pyran-2-yloxy)-1, 2, 3, 4, 4a, 5, 6, 6a, 6b, 7, 8, 8a, 9, 10, 11, 12, 12a, 14, 14a, 14b-icosahdropicene-4-carboxylic acid), a new saponin was first time isolated from the roots of *Clerodendrum Serratum* (L) Moon (Verbenaceae). The structure elucidation of the compound was carried out by ¹H NMR and DART-MS studies.

Key words: *Clerodendrum serratum*; DART-MS; Icosahdropicenic acid; plant saponin

INTRODUCTION

Saponins are a vast group of glycosides, widely distributed in higher plants and also been sought after in the pharmaceutical industry because some form the starting point for the semi-synthesis of steroidal drugs. Many have pharmacological properties and are used in phytotherapy and in the cosmetic industry. They are also believed to form the main constituents of many plant drugs and folk medicines, and are considered responsible for numerous pharmacological properties (Estrada *et al.*, 2000). *Clerodendrum serratum* (L) Moon (Verbenaceae) is a deciduous shrub distributed in the forests of

Western Ghats of India (Manjunatha *et al.*, 2004). In Indian system of medicine the plant is well known as Bharangi (Sanskrit) and commonly known as Blue glory (English). As per the traditional claims, roots are potential source of drugs for ailments such as asthma, cholera, rheumatism etc. (Keshavamurthy *et al.*, 1994). Phytochemically the root bark extract contain D-mannitol, stigmasterols and three triterpenoids-oleanolic acid, queretarinic acid and cerratagenic acid (Banarjee *et al.*, 1969). This communication reports the isolation and characterization studies of a novel saponin isolated from the roots of the plant.

MATERIALS AND METHODS

General

Melting point was measured by using Buchi 545 B

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apparatus and uncorrected. An IR spectrum was recorded on ParkinElmer FT-IR spectrophotometer. DART-MS (Direct Analysis in Real Time-Mass Spectroscopy) was recorded on a Jeol SX 102/Da-600 mass spectrophotometer and $^1\text{H-NMR}$ spectra was recorded on Varian Mercury YH-300 MHz.

Extraction and Isolation

Roots of *C. Serratum* were collected from foothills of Sinhagad Pune (India), and authenticated by Botanical Survey of India, Pune with voucher specimen no. SSBC1. A herbarium was also deposited for future reference.

Roots were shade dried for a week and powdered. Powdered material (500 g) was extracted using soxhlet apparatus with 95% ethanol for about 36 h. The extract was filtered and concentrated in vacuum under reduced pressure using rotary flash evaporator. A dark brown colored viscous mass weighing 50 g (10.0%w/w) was obtained. 15 g of this extract was taken into the minimum amount of methanol and was stirred until it gets well dissolved into the methanol. Cold ether was slowly added into the methanolic solution and crude saponins were obtained as yellow colored ppt which was then separated by filtration and dried under vacuum to get brownish yellow powder (8.20 g).

Further purification was done on silica gel column chromatography (60 - 120) (column dimension (3 × 120 cm) using solvent system CHCl_3 : MeOH with increasing polarity. Total 10 fractions (A - J) were collected. The fraction I gave a brown colored amorphous compound (2.87 g), which was dried and subjected to spectral analysis.

RESULTS AND DISCUSSION

Icosahdropipenic acid (Fig. 1) was obtained as brown colored amorphous compound having the melting point 295 - 298°C. FT-IR: (KBR) (cm^{-1}); 2935, 2904 and 1700 cm^{-1} . $^1\text{H-NMR}$ (300 MHz, TMS, DMSO d_6); 1.21 [d, 3-H, CH_3 (67)], 1.27 [s, 24-H,

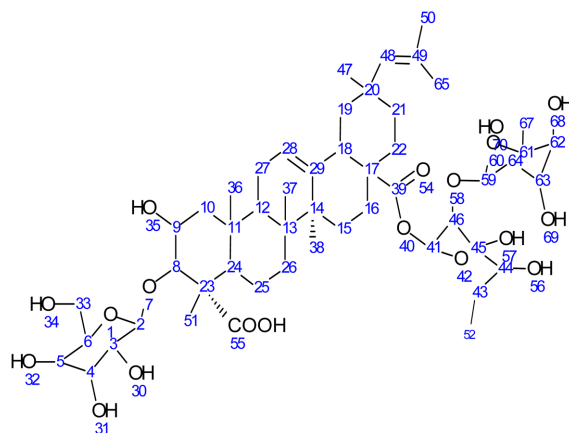


Fig. 1. Structure of Icosahdropipenic acid. ($\text{C}_{51}\text{H}_{80}\text{O}_{19}$).

CH_3 (36, 37, 38, 47, 50, 51, 52, 65)], 1.3 [s, 1-H, COOH (55)], 1.4 [q, 6-H, CH_2 (21, 22, 25)], 1.5 [t, 8-H, CH_2 (15, 16, 26, 27)], 2.1 [d, 4-H, CH_2 (10, 19)], 2.74 [t, 3-H, CH (12, 18, 28)], 3.3 [q, 1-H, CH (58)], 3.4 [t, 9-H, CH (3, 4, 5, 44, 45, 46, 61, 62, 64)], 3.5 [d, 1-H, CH (8)], 3.5 [q, 2-H, CH_2 (33)], 3.7 [t, 1-H, OH (34)], 3.75 [d, 5-H, CH (26, 41, 59, 63)], 3.9 [q, 1-H, CH (9)], 4.1 [s, 2-H, CH (24, 48)], 4.8 [d, 9-H, OH (30, 31, 32, 35, 56, 57, 68, 69, 70)]. (Numbers in bracket represents the respective protons in structure). Molecular formula: $\text{C}_{51}\text{H}_{80}\text{O}_{19}$; Cal. C-61.43%, H-8.09%, found; C-61.26%, H-7.89%. DART-MS spectra of the compound showed a quassimolecular ion peak at m/z 997.70 ($[\text{M}+\text{H}]^+$) indicating a molecular weight of 998.0 Da. From these spectral studies the compound isolated thus confirms of Icosahdropipenic acid, a new Pentacyclic triterpenoid saponin.

CONCLUSION

In conclusion the present work work confirms that Icosahdropipenic acid, a new pentacyclic triterpenoid saponin was first time isolated from the plant. Biological studies of this compound revealed that it possess significant anti asthmatic activity (Bhujbal et al., 2010). Thus this study highlights important areas of research in this field and the opportunity which exists for further research on the phytochemistry and biological activity of

this group of compounds. (Balandrin *et al.*, 1993) estimate that at least 85% of the world's estimated 250000 species of higher plants have not been adequately surveyed for potentially useful biological activity. As a result, the chances of discovering new plant constituents, including novel saponins, which may be biologically active are promising.

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