Isolation and study of a Saponin (Echinocystic acid-3-o-α-l-Rhamnopyranosyl (1→5)-o-β-d-xylofuranosyl (1→5)-o-β-d-Arabinofuranosyl (1→4)-o-β-d-Glucopyranoside) from the leaves of Clematis nepaulensis D.C.

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ABSTRACT

A saponin (echinocystic acid-3-o-α-l – rhamnopyranosyl (1 → 5) – o – β – d – xylofuranosyl (1 → 5) – o – β – d – arabinofuranosyl (1 → 4) – o – β - d – glucopyranoside) have been isolated and identified from the leaves of clematis nepaulensis. Repeated chromatographic manipulations and spectral analysis (IR, 1H-NMR, and Mass) suggested the structure of saponin.

Keywords: Saponin, Echinocystic acid, Isolation, spectral analysis

INTRODUCTION

Clematis nepaulensis D.C. (N.O. Ranunculaceae). It is known as Birri and wandak in Punjab and Banjuli in Kumaon.2,3 This plant occurs in temperate Himalayas region.3,4 Plant is acridic and poisonous, these properties are probably due to the presence of an acrid principle which acts deleteriously on the skin, used for purposes of visitation. Plant has been evaluated for its anti-inflammatory, cytotoxic and antimicrobial effects.3,4

METHOD OF ISOLATION

Air dried, powered and defatted leaves of clematis nepaulensis were extracted exhaustively with 95% ethanol and the extract filtered and concentrated under reduced pressure to a brown viscous mass (2.15%), which was successively extracted with benzene, chloroform, acetone and methanol.

Removal of the solvents yielded residues out of which residues from benzene and chloroform extracts were in very small amounts and could not be taken up for further studies for want of materials while residue form methanol soluble part was worked up further to get the saponin. On TLC appeared one spot concluded presence of one compound in methanolic residue.

It crystallized from methanol, had m.p. 211-212 °C and analyzed for molecular formula; C_{52}H_{49}O_{25}, \alpha \text{ D}^{28} + 36.8^\circ\text{ (in MeOH)} and M^+ = 1044 (by mass spectroscopy). It was insoluble in benzene and petroleum ether but soluble in chloroform, methanol, ethanol and pyridine. It responded to positive Molisch’s test 5 and gave characteristic haemolysis, honey comb form and other tests of saponin(s) 6,7,8

RESULTS AND DISCUSSION

Identified on the basis of following spectral analysis:

IR:

The important peaks obtained in its IR spectrum and the structural units inferred with the help of available literature 9,10 are recorded in the Table-I.

Table-I

| S. No. | Peaks cm\(^{-1}\) | Assignments |
|-------|-----------------|-------------|
| 1     | 3328            | -OH         |
| 2     | 2042-2938       | -CH_3, CH_2 Stretching. |
| 3     | 1270            | Unsaturation. |
| 4     | 1368, 1330, 1275, 1112 | tri-terpenoidal nature. |
| 5     | 1716            | +COOH group |
| 6     | 860             | Cyclo hexane ring. |
| 7     | 1445            | -CH_3 group |

The position of the various methyl, hydroxyl and that of the carboxylic group and the structure of the saponin was
established by its hydrolysis and separately studying the sapogenin and sugar moieties.

The saponin was therefore, hydrolyzed by 7% H₂SO₄ when the sapogenin precipitated out which was separated by filtration.

**Structural study of the sapogenin**

The sapogenin on TLC examination showed single spot thereby confirming its homogenous nature. It crystallized from ethanol and analyzed for molecular formula; C₃₀H₄₄O₄, M⁺ = 472 (by mass spectroscopy) m.p. 307-9°C, (decompose) (a) D₂O + 40.6° in EtOH and gave various characteristic color reactions of triterpenes e.g. Salkowski¹¹ Liebermann Burchard ¹² and Tschugajew ¹³ reaction.

**IR spectrum of the sapogenin**

The characteristic peaks obtained in the IR spectrum of the sapogenin and the structural assignments made with the help of available literature ¹⁴,¹⁵ are recorded in the Table-II.

![Table II](image)

|= S. No. | Peaks cm⁻¹ | Assignments |
|---|---|---|
|1 | 3328 | -OH group |
|2 | 2950 | -CH Stretching vibration of CH₃ |
|3 | 2840 | -CH stretching, |
|4 | 1628 | CH₂-CH group |
|5 | 1276 | Vinylidine type double bond. |
|6 | 1430, 1112 | CO- stretching of secondary -OH group. |
|7 | 862 | -C=CH₂ group |
|8 | 1720 | -COOH group. |
|9 | 1464, 1368, 1334, 1320 | Triterpenoidal nucleus. |

¹H-NMR spectrum of the methyl ester of the sapogenin

The significant signals obtained in the ¹H-NMR spectrum of the mono methyl ester of the sapogenin and the structural units inferred with the help of available literature are given in the Table III.

![Table III](image)

|= S. No. | Value | Pattern | J Value | No. of protons | Assignments |
|---|---|---|---|---|---|
|1 | 0.70 | s | - | 3 | -CH₃ |
|2 | 0.85 | s | - | 3 | -CH₃ |
|3 | 0.97 | s | - | 9 | 3xCH₃ |
|4 | 1.22 | s | - | 6 | 2xCH₃ |
|5 | 1.8-2.04 | Couple Pattern | - | 15 | Polyethylene CH₂ and CH |
|6 | 2.7 | s | - | 3 | -OCH₃ |
|7 | 3.0-3.40 | m | - | 2 | C₅-OH, C₆-OH |
|8 | 4.3 | bs | - | 2 | C₅-H and C₆-H |
|9 | 5.45 | bs | - | 1 | Ethylene 4-H |
|10 | 3.60 | s | - | 3 | COOCH₃ |

Mass spectrum of the sapogenin¹⁶,¹⁷;

The important fragmentation patterns obtained in the electron impact mass spectrum of the sapogenin are given below.

M⁺ = 472 and m/e 440, 427, 248, 133, 207, 203, 190, 189, 175, 133.

On the basis of the interpretation of above data following structure has been proposed.

![Structure of the sapogenin](image)

Identified as: Echinocystic acid

I – R = H, II – R = Ac

The above facts and a survey of literature ¹⁸, when put together identified the sapogenin as the well known compound Echinocystic acid.

**Nature of the glycosidic linkage:**

The glycoside was hydrolysable with Tokadiastase¹⁰ solution affording prosapogenin SC3and L-rhamnose(by CO-PC & TLC with authentic sample) The prosapogenin SC3 when hydrolyzed with almond emulsion ¹⁹ yielded sapogenin D-xylose, D-arabinose and D-glucose (by CO-PC and TLC with authentic samples) Confirming the presence of α-linkage between L-rhamnose and D-xylose and β-linkage between D-xylose, D-arabinose and D-glucose and also β linkage between D-glucose and the sapogenin.

Thus the structure to the saponin was assigned as; Echinocystic acid-3-0-α-L-rhamnopyranosyl1 (1 → 5)-0-β-D-xylofuranosyl1 (1 → 5) 0-β-D-arabinofuranosyl1 (1 → 4)-0-β-D-glucopyranoside.
Finally the proposed structure of the saponin was confirmed by 1H-NMR spectral studies.

**1H-NMR spectrum of deca acetyl derivative of saponin**

The 1H-NMR spectrum of the deca acetyl derivative of the saponin was found to be in complete conformity with the above structure.

The significant signals obtained in the 1H-NMR spectrum of the saponin (Fig. IV) and structural units inferred with the help of available literature21,22 are given below:

**TABLE – IV**

| S. No. | (S) Value | Pattern | J value (Hz) | No. of protons | Assignments |
|--------|-----------|---------|--------------|----------------|-------------|
| 1.     | 0.70      | s       | -            | 3              | -CH$_3$     |
| 2.     | 0.85      | s       | -            | 3              | -CH$_3$     |
| 3.     | 0.97      | s       | -            | 9              | 3xCH$_3$    |
| 4.     | 1.22      | s       | -            | 6              | 2xCH$_3$    |
| 5.     | 1.8-1.04  | Couple pattern | - | 15 | Polymethylene CH$_3$ and CH |
| 6.     | 2.1       | s       | -            | 6              | 2x-OCH$_3$ |
| 7.     | 3.0-3.40  | m       | 2            | 2              | C$_2$-OH, C$_16$-OH |
| 8.     | 4.3       | bs      | -            | 2              | C$_3$H and C$_15$-H |
| 9.     | 5.45      | bs      | -            | 1              | Ethylenic 4-H |
| 10.    | 3.60      | s       | -            | 3              | CoocH$_3$   |
| 11.    | 4.30      | d       | 7.8          | 1              | 1’anomeric proton |
| 12.    | 5.45      | d       | 2            | 1              | 1’anomeric proton |
| 13.    | 4.29      | d       | 7.0          | 1              | 1”anomeric proton |
| 14.    | 4.2       | d       | 7.5          | 1              | 1”anomeric proton |
| 15.    | 2.08      | s       | -            | 6              | 2’OAc, 6’OAc |
| 16.    | 2.15      | s       | -            | 6              | 3’OAc       |
| 17.    | 2.00      | s       | -            | 6              | 2”OAc, 3’OAc |
| 18.    | 2.06      | s       | -            | 6              | 2”OAc, 3”OAc |
| 19.    | 2.02      | s       | -            | 3              | 5”- and 6”OAc |
| 20.    | 1.02      | s       | -            | 2              | 2”’OAc      |
| 21.    | 1.02      | s       | -            | 6              | 3”’OAc      |

**Mass spectrum of saponin 22**

The important fragmentation pattern obtained in the electron impact mass spectrum of the saponin is given below. The presence of fragmentation due to the cleavage of sugar units provided considerable assistance in the identification of sugars.

M + = 1044,911,895,779,763,633,617,440,248,233,207,190,175,113.

On the basis of the above data and comparison with the authentic sample the structure of the compound has been elucidated as:-

**Echinocystic acid-3-O-α-L-rhamnopyranosyl(1 → 5)-O-β-D-xylufuranosyl(1 → 5) O-β-D-arabinofuranosyl(1 → 4)-O-β-D-glucopyranoside**

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