Available online on 15.03.2019 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Open Access Research Article

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.

Kumud Shrivastava

Professor of Chemistry, Govt. M.L.B. Girls College Bhopal, (M.P.), INDIA

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, ^{1}H-NMR, and Mass) suggested the structure of saponin.

Keywords: Saponin, Echinocystic acid, Isolation, spectral analysis

Article Info: Received 31 Jan 2019; Review Completed 04 March 2019; Accepted 09 March 2019; Available online 15 March 2019

Cite this article as:

Shrivastava K, 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., Journal of Drug Delivery and Therapeutics. 2019; 9(2):255-258 **http://dx.doi.org/10.22270/jddt.v9i2.2412**

***Address for Correspondence:**

Kumud Shrivastava, Professor of Chemistry, Govt. M.L.B. Girls College Bhopal, (M.P.), INDIA

INTRODUCTION

Clematis nepaulensis D.C. (N.O. Ranunculaceae). It is known as Birri and wandak in Punjab and Banjuli in Kumaon. [1,2]. This plant occurs in temperate Himalayas region . 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 ^{0}C and analyzed for molecular formula; $C_{52}H_{84}O_{21}$, (α) D^{28} + 36.8^0 (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. N.	Peaks cm^{-1}	Assignments
1	3328	-OH
2	2842-2938	-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% H_2So_4 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; $C_{30}H_{48}O_4$, M^+ = 472 (by mass spectroscopy) m.p. 307-9^{0}C, (decompose) (α) D^{28} + 40.6^0 in EtOH and gave various characteristic color reactions of triterpenes e.g. Salkowski[11] 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

S. No.	Peaks cm-1	Assignments
1	3328	-OH group
2	2950	-CH Stretching vibration of CH_3
3	2840	-CH stretching.
4	1628	CH_2-CH group
5	1276	Vinylidine type double bond.
6	1430, 1112	CO- stretching of secondary -OH group.
7	862	-C- = CH_2 group
8	1720	-COOH group.
9	1464, 1368, 1334, 1320	Triterpenoidal nucleus.

^{1}H-NMR spectrum of the methyl ester of the sapogenin

The significant signals obtained in the ^{1}H-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.

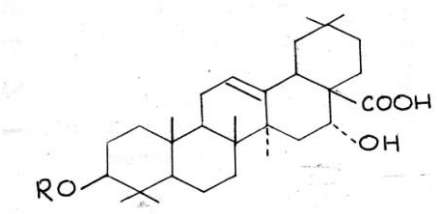
S. No.	Value	Pattern	J Value	No. of protons	Assignments
1	0.70	s	-	3	-CH_3
2	0.85	s	-	3	-CH_3
3	0.97	s	-	9	3xCH_3
4	1.22	s	-	6	2xCH_3
5	1.8-2.04	Couple Pattern	-	15	Polymethylene CH_2 and CH
6	2.7	s	-	3	-OCH_3
7	3.0-3.40	m	-	2	C_3-OH, C_{16}-OH
8	4.3	bs	-	2	C_3-H and C_{16}-H
9	5.45	bs	-	1	Ethylene 4-H
10	3.60	s	-	3	$COOCH_3$

Mass spectrum of the sapogenin[16, 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



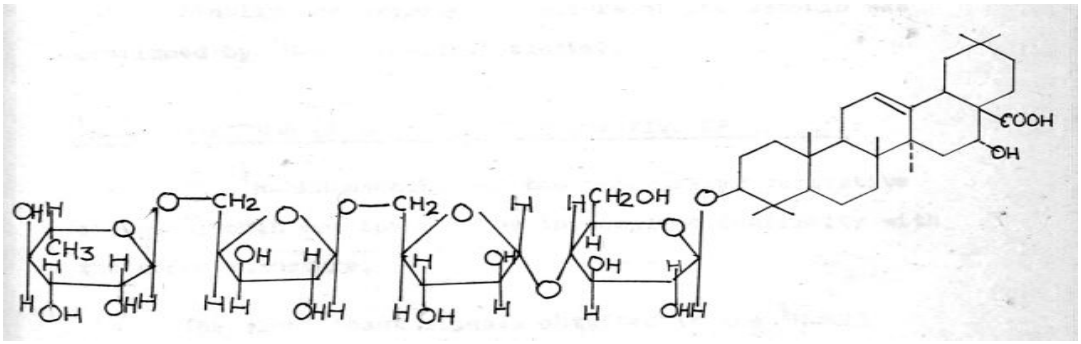
Structure of the sapogenin
Identified as: Echinocystic acid
I – R =H , II – R = Ac

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 Tokadistase[18] 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-rhamnopyransyl1 (1 → 5)-O-β-D-xylofuranosy1 (1 → 5) O-β-D-arabinofuranosy1 (1 → 4)-O-β-D-glucopyranoside



Finally the proposed structure of the saponin was confirmed by ^{1}H-NMR spectral studies.

^{1} H-NMR spectrum of deca acetyl derivative of sapobnin

The ^{1} H-NMR spectrum of the decal acety1 derivative of the saponin was found to be in complete conformity with the above structure.

The significant signals obtained in the ^{1}H-NMR spectrum of the saponin (Fig. IV) and structural units inferred with the help of available literature[21,22] 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_2 and CH
6.	2.1	s	-	6	2x-OCH_3
7.	3.0-3.40	m	2	2	C_3-OH, C_{16}-OH
8.	4.3	bs	-	2	C_3H and c_{16}-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'' '' OAc
20.	1.02				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^{+} = 1044,911,895,779,763,633,617,471,440,427,248,233,207,190,175,113.

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

Echinocystic acid-3-0-α-L-rhamnopyransyl1 (1 → 5)-O-β-D-xylofuranosy1 (1 → 5) O-β-D-arabinofuranosy1 (1 → 4)-O-β-D-glucopyranoside

ACKNOWLEDGEMENT

Author thanks to prof, p, patil of Botany for authentication of plant. And UGC for financial assistance as an MRP, and also to the chemistry department of the college for providing infrastructure. CDRI for the spectral analysis.

REFERENCES

1. Chopra, R.N., Nayar,S.L. and Chopra, I.C., "Glossary of Indian Medicinal Plants" CSIR publication. New Delhi (1981),p.,248
2. Kirtikar K.R. and Basu B.D." Indian Medicinal Plants", Lalit Mohan Basu and co., Allahabad 1988; 2:936-959.
3. Shawaze H., Dati, "Indian J. of Pharm Sci,, 2012; 74(1):29-35.
4. Areej M., Al T, Kndariya M S.," Saudi Pharmaceutical Journal 2008; 16(2).
5. Ckark H.T., "Organic Analysis", Orient Longmans Limited, New Delhi 1966, 91-92.
6. Sannie C.H., Annual Biochem. Med., 1948; 9:175.
7. Guggotz and Vantt, G.R., J. Agr. Food. Chem., 1958; 6:849.
8. Kolosapithes E., Gyogyszereszet, (1960), 4, 839.
9. Saxena V.K. D.sc. Thesis, sagar University sagar (1981)

10. Conley R.T., "Infrared spectroscopy", 2nd ed., Allyn and Bacon, Boston, (1972)
11. Salkowski E., Hoppe Seylers. Z., 1908; 57:52.
12. Liebermann, C., Ber, dt, chem. Ges. (1885), 18, 1804.
13. Tschugajew Chew. Zig., 1900; 24:542.
14. Rao, C.N.R., "Chemical Applications of Infra Red Spectroscopy", Academic Press, London, (1963).
15. Joshi, K.C., Singh, P. and Mishra, A., Planta Medica, 1979; 37:64-66.
16. John, R. Dyer, "Applications of absorption Spectroscopy of organic compounds", Prentice Hall of India PVT. Ltd. New Dellhi., (1971).
17. Bible R.H. "J.r. Interpretation of NMR Spectra", Plenum Press, New York, (1965)
18. Jain B, Sarin GK, Khanna JPS, Indian J. Chem., 1977; 15 B:1139.
19. Mann F.G., and Saunder B.C., " PRACTICAL Organic Chemistry" Longmans , New York, (1966) p. 365.
20. Mathieson D.W. (ed.), "Nuclear magnetic Resonance for organic Chemists" Academic press, New York, (1967).
21. Jeckman L.M., Sternhell, S., "Applications of Nuclear magnetic, Resonance Spectroscopy in organic chemistry", 2nd Ed., Pergamon Press, London (1969).
22. Budzikiewicz H., Djerassi C. and williams D.H., "Structure Elucidation of Natural Products by Mass Spectometry" HOlden – Day, 1964; 2:107.