A Triterpenoid Saponin from the Seeds of *Ricinus communis*

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A novel oleanen type triterpenoid, has been isolated from butanolic extract of the seeds of *Ricinus communis*. Its structure was elucidated as 3-O-[β -D-glucoronopyranosyl-(1→ 3)-α –L-rhamnopyranosyl-(1→ 2)β-D-glucopyranosyl]-4α,20α-hydroxy methyl olean-12-ene-28-oic acid on the basis of spectral evidences i.e. FTIR, ^{1}H NMR, ^{13}C NMR and FAB-MS data. The isolated saponin was tested for its antibacterial and antifungal activity, where the growth of microbes was found to be inverse function of concentration of saponin. The MIC value for *E.coli* was found to be 260µg/mL and that of *Staphylococcus aureus* was 350µg/mL.

Key words: *Ricinus communis*, Triterpenoid saponin, Euphorbiaceae, Antimicrobial activity

Introduction

Ricinus communis Linn.(Euphorbiaceae) grows as weed even along the road sides in the tropical warm regions[1]. It is also cultivated in different parts of the country for its oilseeds [2-3]. Oil is used externally for dermatitis and ailments of eye. Leaves have been reported to have flavones and tannins[4], whereas roots of this plant have been found to contain alkaloids also along with flavonoids and tannins[5]. Seeds, which yield 45–50% of a fixed oil, also contain the alkaloids ricinine and toxalbumin ricin. It is considered to be purgative, counter-irritant in scorpion-sting and fish poison[6]. In continuation of our interest in investigation of triterpenoid saponins[7], we have investigated the seeds and report here a new oleanen type triterpenoid for the first time.

Results and Discussion

Saponin **I**: Amorphous powder (70mg) m.p. 281ºC $[\alpha]_D$ +26º [MeOH; *c* 1.36]; ν_{max}3755.8, 3419.2, 2938.1, 1735.0, 1651.1, 1411.6, 1381.9, 1251.0, 1043.9 and 754.8 cm^{-1}. ^{1}H NMR δ4.89

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(1H d, J=7.6 Hz. H-1 glc), 6.34 (1H d, J=7.2 Hz. H-1 rham) and 4.60 (1H d, J=7.8 Hz.H-1 glcA), 1.13, 1.01, 1.04, 1.279, 0.889 (3H s for 5 CH_3 at 24, 25, 26, 27, and 30), 1.56 (3H d, J=6Hz rham), 5.35 (t like for 1H at C-12); FAB-MS m/z 996$[M+Na]^+$ 819, 673, 511.

Acid hydrolysis **1**; Saponin 1 (25mg) was refluxed with 10% H_2SO_4 on a boiling water bath for 4 hrs the usual work of the reaction mixture afforded sapogenin **2** m.p. 192ºC $[\alpha]_D$ +17.6º [MeOH; *c* 1.15]; FAB-MS m/z 488 $[M]^+$,303,263,222,223,192,165,154.

Identification of sugar moiety of **1**: The aqueous layer separated after the removal of sapogenin was neutralized with barium carbonate, filtered and concentrated under reduced pressure. The residue obtained was compared with standard sugar on TLC and paper chromatography (BAW 4:1:5) indicating them to be D-glucose, L-rhamnose and D- glucoronic acid.

*Premethylation of **1** and acid hydrolysis of the product:* A solution of 1 (15mg) in DMSO was treated with NaH (0.2g) and CH_3I (5mL) at room temperature for 6hrs. The usual work up of the reaction mixture yield a residue, which was purified by prep-TLC in *n*-hexane-EtOAc (1:1). Hydrolysis of premethylated **1** was performed by refluxing with 10mL of 3% methanolic HCl. Paper chromatography of the neutralized and concentrated hydrolysate in benzene – Acetone (3:1) showed the presence of 3,4,6-O-methyl-D-glucose, 2,4-di-O-methyl-L-rhamnose and 2,3,4-tri-O-methyl-D-glucoronic acid (co-paper chromatography).

Saponin **1** M.P.281ºC $C_{48}H_{77}O_{20}$ $[M]^+$-973 obtained by repeated CC purification of n-BuOH fraction followed by preparative TLC. Its UV spectra contained absorption maxima at 242 nm., while the IR spectrum exhibit peak at 3400-3756(OH), 2938.1(C-H), 1651.1(C=C) and 1043.9(C-O)cm^{-1}. Broadband at 3419.2cm^{-1} indicate its glycosidic nature.

Saponin **1** on acid hydrolysis yields sapogenin **2** m.p.192ºC as the aglycone, which was identified by comparison of physical and spectral properties[8-9]. Direct comparisons with authentic samples (m.p., co-TLC) also establish the identity. The sugar components in the hydrolysate were identified as D-glucose, L-rhamnose and D-glucoronic acids in the ratio 1:1:1, indicating **1** to be a sapogenin triglycoside. The ratio of sugar was established by comparing with the HPLC chromatogram of the standard. The position of FAB-MS showed a molecular ion peak at m/z 996 $[M+Na]^+$ indicating a molecular mass of 973 which is in good agreement with the molecular formula $C_{48}H_{77}O_{20}$. The fragment at m/z 819 consistent with the loss of a terminal glucoronic acid unit from the molecular ion, where as the other fragments at m/z 673 $[M+Na-(178+146)]^+$ and 511 $[M+Na- (178+ 146 + 162)]^+$ were attributed to the loss of rhamnose and glucose unit respectively. The results obtained by FAB-MS indicated the sugar sequence in **1.** Interglycosidic linkages in l were suggested by permethylation followed by acid hydrolysis, which liberated as 3,4,6-O-methyl-D-glucose, 2,4-di-O-methyl-L-rhamnose and 2,3,4-tri-O-methyl-D-glucoronic acid one molecule each as obtained by HPLC chromatogram. The presence of glucronic acid as the terminal sugar was conformed by detection on partial hydrolysis of **l** on TLC in HCl atmosphere. Partial hydrolysis of l with methanolic H_2SO_4 also yield glucronic acid and a prosaponin which display an $[M+Na]^+$ ion at m/z 819 in FAB-MS. The presence of glucronic acid in the hydrolysate was conformed by co-TLC with authentic sample and by HPLC chromatogram.

The proton noise decoupled ^{13}C NMR spectrum of **l** displayed 48-carbon resonance. The no. of attached hydrogen to each carbon were determined by DEPT technique[10],which suggested the presence of 6 quaternary C atom, 19xCH, $13xCH_2$, $6xCH_3$ and 4 sp^2 hybrid carbon atom (for aglycone CH=, C=, C=O and C=O for sugar) indicating the presence of a trisaccharide moiety in

triterpene(Table-1). The presence of three-anomeric carbon signal at δ104.3, 104.8, 101.4 was in accordance with the presence of trisaccharide moiety in **l**. On the basis of analysis of DEPT spectrum the molecular formula of **l** could be assigned as $C_{48}H_{77}O_{20}$. A comparison of ^{13}C NMR spectral data of the aglycone moiety of **l** with those of aglycone of triterpene further confirmed its identity[11-12].

The inter glycosidation assignment were further conformed by the chemical shift of glycosylated carbon atom-82.7 and 84.5. The C-3 signal of rhamnose was observed at 82.7 where as C-2 signal of glucose at 84.5 reveal the deshielding of carbon by 4 and 6 ppm for these carbon resonance; hence C-2 in glucose and C-3 in rhamnose were concluded to be the glycosidation site. In addition, the presences of a trisaccharide moiety in **l** were suggested by absorbance of proton signal at 4.89 (d, J=7.6 Hz.1H), 6.34(d, J=7.2 Hz.1H) and 4.60(d, J=7.8 Hz.1H) for glucose, rhamnose and glucoronic acid. The chemical shift and coupling constant of these signal suggested the β-anomeric configuration for glucose, glucoronic acid and α for rhamnose when compared with the reported values. The trisaccharide moiety in l was linked at C-3 of the aglycone as C-3 showed a significant downfield shift (δ C 87.1 ppm) in ^{13}C NMR spectra indicating the glycosidation position[13]. Further the glycoside was hydrolyzed with 10% sulphuric acid, which is specific reagent for hydrolyzing only β-glycosidic linkage without attacking other sugar ester linkage. Thus sugars are attached through glycosidic linkage. The ^{13}C NMR spectral data of aglycone is in good agreement with the ^{13}C NMR data of saponin **l** and other related saponin.

In the light of above observation the structure of **I** was established as 3-O-[β –D - glucoronopyranosyl - (1 → 3) - α – L – rhamnopyranosyl - (1 → 2) β-D-glucopyranosyl] - 4α,

20α - hydroxymethyl olean-12-ene-28-oic acid.(Fig-1)

Figure-1 Structure of Triterpenoid saponin **I**

General Experimental Procedures

Melting points were measured on a MAC model Melting point apparatus and are uncorrected. Optical rotations were measured on Rudolf Autopol III polarimeter. UV spectra were measured on Thremospectronic UV 100 model spectrophotometer in MeOH solution. ^{1}H NMR and ^{13}C NMR were recorded on Bruker DRX 300 model operating at 300MHz and 75MHz (CD_3OD or CDC_{l3}). All the NMR spectra were recorded using TMS as internal standard. IR spectra (KBr disc) on a Perkin Elmer spectron RXI spectrophotometer having range of 4000-450cm^{-1}. FAB-MS was recorded on a Jeol SX 102/DA-6000 spectrometer using argon as FAB gas and accelerating voltage of 10KV with nitro benzyl alcohol as matrix. Column chromatography was

carried out on silica gel (B.D.H.; 60-120 mesh), TLC and preparative TLC on 20x20cm plats coated with 2mm thick silica gel (Merk; F_{254}). Spots were visualized by 10% H_2SO_4, followed by heating at 110ºC. Paper chromatography of sugars was performed on whatman no.1 paper using descending mode in *n*-BuOH-AcOH-H_2O (BAW 4:1:5) and developed with aniline hydrogen phthalate.

Plant Material: The seed of *Ricinus communis* were collected from the campus of Rani Durgawati University, Jabalpur, M.P. India. The Head, Department of Biosciences, R.D.V.V. identified the seeds and a voucher specimen was deposited in the herbarium of the Department.

Extraction and Isolation of compounds: The air dried and powdered seeds (1Kg) were extracted with petroleum ether (60-80°C) for 12-14hrs.The defatted seeds powder was then extracted with MeOH for 18-20 hrs and combined extract was concentrated in vacuum and the resulting dark yellow residue (150 g) was suspended in water. The aqueous methanolic extract was then fractionated successively with *n*-Hexane, $CHCl_3$ and *n*-BuOH to get a total of four fractions

The *n*-BuOH fraction (20 g) was subjected to column chromatography on silica gel (100 g, 60-120 mesh) using $CHCl_3$-MeOH-H_2O (v: v = 65:25:10 to 50:40:10) with a 5mL each as gradient eluent to give 48 fractions and was monitored by TLC. The fractions 25-36 showing same R_f on TLC were pooled together and recolumn chromatograph on silica gel with $CHCl_3$: MeOH (60:40 to 50:50), followed by preparative TLC in EtOAc: MeOH: H_2O (13:8:2) to yield saponin **1**.

Antimicrobial assay:

Antibacterial activity

The antimicrobial activity was performed with two bacterial strains –*Staphylococcus aureus* and *E.coli* by 'Broth dilution method'[14]. The inoculates of the micro organisms were prepared from 12hrs broth culture and suspensions were adjusted to 0.5 McFarland Standard turbidity. Tested saponin was dissolve in distilled water and its different dilutions were prepared ranging from 50µg/mL to1000 µg/mL. 0.2mL of different dilutions of saponin was added to 1.8mL of seeded broth. A set of seeded broth control was also prepared and the whole set was incubated for 24 hrs. Seeded broth control showed maximum growth of bacteria and on addition of saponin, growth decreased with increase in saponin concentration. The MIC value for *E.coli* was 260µg/mL and that of *Staphylococcus aureus* was 350µg/mL.

Antifungal activity

The fungal strains used for antifungal activity were *Aspergillus fumigatus, Alternaria alternata* and *Colletotrichum dematium*. The saponin was evaluated in triplicates in a dose response format where the final concentrations were 10, 25, 50, 100, 250, and 500µg/mL and the final fungal spores concentration was 10^4 CFU/mL. The antifungal activity was determined by 'Spore dilution method'[15]. The growth of fungus was found to be inverse function of concentration of saponin. The relationship was determined by observing the number of fungal colony developed.

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References

1. Ivan, A. *Chemical constituents, traditional and modern uses, in medicinal plants of the World*; Ross Humana Press Inc., Totowa, NJ, **1998**, pp.375-395.

2. Devendra, C.; Raghavan, G.V. *World Rev. Anim. Prod.* **1978**, *14(4)*, 11–27.
3. Gaydou, A.M.; Menet, L.; Ravelojaona, G.; Geneste, P. *Oleagineux* **1982**, *37(3)*,135–141.
4. Khogali, A.; Barakat,S.; Abou-zeid,H. *Delta J. of Science* **1992**,*16*,198-211.
5. Ilavarasan, R.; Mallika, M. ; Venkataraman,S. *J.of Ethnopharmacology* **2006**, *103,* 478 - 480.
6. Duke,J.A. ; Wain, K.K. Medicinal plants of world. Computer index with more than 85,000 entries.Vol: 3, **1981**.
7. Ansari, N.G. and Srivastava, A. Thesis , Rani Durgawati University, July **2005**.
8. Dodell,D.M.; Peg,D.T. ; Bendel,M.R. *J. Magn.Reson*. **1982**, *48*, 323-326.
9. Avila,A.J.G. ; Vivar,A.R.D. *Biochem. Syst. and Ecology* **2002**, *30*, 1003-1005
10. Jon,K.H.; Jung, K.Y.; Chang, H.W.; Kim, H.P. ; Kang, S.S. *Phytochemistry* **1994**, *35(4)*, 1005-1008.
11. Abdel-khader, M.S.; Bahler, B.D.; Malone, S.; Werkhoren, C.M.; Wisse, J.H.; Neddermania, K.M.; Burcuker, I. ; Kingston, D.G.I. *J. of Nat. Prod.* **2000**, *63*, 1461-1464.
12. Mitrokotsa, D.; Mitaku, S.; Demetzoc, C.; Harvala, C.; Mentis, A.; Perez, S.; Kokhinopopulos, D. *Planta Medica* **1993**, *59*,517-520.
13. Miyase, T.; Shiokawa, K.; Zhang,D.M. ; Veno,A. *Phytochemistry* **1996**, *41(5)*,1411-1418.
14. Maria IC, Rodrigue JI, Delasm, MO, Demo M, Fallaci, MS, Zygadlo AJ. *Planta Med*., **42**(4): 269 -73 (2001).
15. Favel, A.; Steinmetz, M.D.; Regli, P.; Olivier, E.V.; Elias, R.; Balansaed, G. *Planta Medica* **1994**, *60*, 50-53.

Table-1 ^{13}C NMR chemical shift and DEPT data of saponin I.

Carbon	chemical shift	DEPT	Carbon	Chemical shift	DEPT
1	39.6	CH_2	Glc 1	104.3	CH
2	27.2	CH_2	2	84.5	CH
3	87.1	CH	3	74.4	CH
4	44.2	C	4	69.5	CH
5	48.1	CH	5	79.9	CH
6	18.9	CH_2	6	62.2	CH_2
7	32.1	CH_2	Rhm 1	101.4	CH
8	40.2	C	2	72.0	CH
9	50.9	CH	3	82.7	CH
10	36.9	C	4	73.1	CH
11	24.6	CH_2	5	69.6	CH
12	125.9	CH	6	18.4	CH_3
13	131.2	C	GluA 1	104.8	CH
14	39.8	C	2	79.5	CH
15	33.1	CH_2	3	76.6	CH
16	24.0	CH_2	4	76.9	CH
17	47.5	C	5	75.5	CH
18	42.5	CH	6	175.1	C (COOH)
19	46.5	CH_2			
20	31.9	C			

21	34.6	CH_2
22	33.6	CH_2
23	62.4	CH_2
24	13.8	CH_3
25	24.7	CH_3
26	17.5	CH_3
27	22.6	CH_3
28	176.0	C
29	63.5	CH_2
30	29.0	CH_3