New Triterpenoid Glycosides from the Stem Bark of Samanea Saman

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Abstract: ***From the stem bark of Samanea saman two new triterpenoid glycoside have been isolated and characterized as 3β, 22β – dihydroxyolean -12-ene-24-0-β-D-xylopyranoside 1 and 1β, 2α, 3β-trihydroxy-16β-0-acetylolean-12-ene-28-oic-0 [-β-D-arabinopyranosyl (1→3)] -β-D-arabinopyranoside 2 by spectral and chemical studies.***

Keyword: ***Samanea saman, triterpenoid, glycoside***

I. INTRODUCTION

Samanea saman (Mimosaecae) commonly called "Raintree" is a useful medicinal plant distributed from Yucatan Peninsula, Guatemala to Peru, Bolivia, Brazil, throughout the West Indies, in old world tropics and also in Southern Florida[1]. It is used as an alcoholic source[2]. It is used for cold, diarrhea, headache, intestine ailments, stomachach[3], stomach cancer[4], sore throat[5]. Earlier alkaloids, lupeol, lupeone, octaconsanolic acid, hexacosanol, flavonoids, kaempferol[6] have been isolated from the different parts of *Samanea saman.*

`In continuation of our research on the chemical investigation of medicinal plants, we report herein isolation and structure elucidation of two new triterpenoid glycosides from *Samanea saman*.

The water insoluble portion of the hot ethanol extract of the air-dried crushed and defatted stem barks of *S. saman* on column chromatography yield compound **1** and **2**.

II. RESULT AND DISCUSSION :

Compound **1** $C_{35}H_{58}O_7$, a glycoside on acid hydrolysis gave D-xylose and an aglycone**1a**. The aglycone gave colour reactions[7-10] of unsaturated pentacyclictriterpenoid. It formed triacetate on acetylation showing the presence of three hydroxyl group. The IR spectrum of **1a** showed absorption for hydroxyl (3450 cm^{-1}), trisubstituted double bond (1630 cm^{-1}), primary and secondary alcoholic group (1250, 1100, 1050 cm^{-1}) and C-methyl (2900, 1385 and 1330 cm^{-1}) groups. The ^{1}HNMR spectrum of **1a** showed signals for seven tertiary methyl groups at (δ0.85, 0.95, 0.99, 1.03, 1.17, 1.31 and 1.35 (each 3H, S) and a vinyl proton at δ5.25 (1H, t, *J*=3.3Hz) all of which suggested **1a** to be an olean-12-ene derivative.

The mass spectrum of **1a** revealed important peaks at m/z 234, 224, 210, 216, 204, 201 and 175 typical of retro-Diels-Alder fragmentation of ring-C of olean-12-ene derivative containing a hydroxyl group in ring A or B[11]. Compound **1a** also gave positive Zimmermann test[12] suggesting the C-3 position for this hydroxyl group which was also biogenetically favoured.

The aglycone showed signal at δ3.83 (1H, dd, *J*=9.6 Hz) with higher value of coupling constant confirming the *β*–orientation for hydroxyl group at C-3 (α or axial H)[13].

The ion peak at m/z 216[234-H_2O) due to ready loss of water molecule showed the position of –OH group at C-22 which was also supported by the ^{13}C-NMR signal at δ75.6(d) for C-22. The ^{1}H-NMR signals at δ4.35 and 4.52 (each 1H, d, *J*=12.1 Hz) were attributed to the methylene protons of hydroxymethyl group (AB system)[14]. Signal at δ64.5 in ^{13}C-NMR spectrum of **1a** confirmed the presence of CH_2OH group at C-24. Thus, aglycone**1a** was identified as 3*β*, 22*β*, 24-trihydroxyolean-12-ene.

The ^{1}H-NMR spectrum of **1** showed a signal for an anomeric proton at δ4.98 (1H, d, *J*=6.5 Hz, H-1, xylose) and sugar protons at δ3.2-3.8 (br, xylosyl protons) which was consistent with configuration of D-xylose. The *β*-type of glycosidic linkage was also confirmed by hydrolysis of **1** with the enzyme emulsin. The position of attachment of D-xylose at C-24 hydroxyl group was confirmed by down field signal at δ74.5 (t) in the 13C-NMR of **1** for C-24. Thus the compound **1** was characterized as 3*β*, 22*β* –dihydroxyolean -12-ene-24-O-*β*-D-xylopyranoside.

Compound **2**, $C_{42}H_{68}O_{16}$ (M^+ 828), mp280^{O}C, a non-reducing glycoside gave aglycone**2a**on acid hydrolysis with 7% H_2SO_4anda sugar arabinose (Co-chromatographed with an authentic sample). Compound **2a**, $C_{32}H_{50}O_7$ (M^+ 546), gave colour reactions characteristic of unsaturated pentacyclic triterpenoid. Its IR spectrum showed absorption peaks for hydroxyl groups (3300 cm^{-1}), trisubstituted double bond (1640 cm^{-1}) and carboxylic group (3200 & 1700 cm^{-1}) and peak for –$OCOCH_3$ group (1690 & 1100 cm^{-1}). The ^{1}HNMR spectrum of **2a** sowed signals for seven tertiary methyl groups between δ0.85-1.31 (each 3H, S), a vinylic proton at δ5.34 (1H, t, *J* = 3.4H_2) all of which suggested **2a** to be an olean-12-ene derivative.

In ^{1}HNMR singlet at δ2.03 (3H, S) shows the presence of –$OCOCH_3$ group. Which was also confirmed by singlet at δ169.4 and quartet at δ20.4 in ^{13}C-NMR. On acetylation aglycone furnished tetraacetylated product and further methylation with diazomethane provided its monomethyl ester. These results clearly suggested the presence of three hydroxyl, one –$OCOCH_3$ and one acidic group in the aglycone **2a**.

Acetylated product of **2a** showed signals for three hydroxymethine protons signals in its ^{1}HNMR spectrum at δ3.22 (1H, d, *J*=10.7 Hz), 3.41 (1H, d, *J*=9.5 Hz) for H-1 and H-3, and at 4.03 (dd, 1H, J_1=9.5 Hz and J_2=10.7 Hz) for H-2 and for three acetoxymethine protons at δ4.80 (1H, d, J_1=10.5 Hz and J_2=9.3 Hz) which were assigned as C-1 (or C-3), C-3 (or C-1) and C-2.

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The *J* values of these signals indicated its trans-diaxial correlated protons of these three hydroxyl groups must be equatorial[15].

The mass spectrum of aglycone showed the presence of both the groups (-COOH and $-OCOCH_3$) in ring D/E, peaks at m/z 306, 240 obtained due to retro Diels Alder fragmentation and subsequent fragments 261 (306-COOH), 247 (306-$OCOCH_3$), 202 (-COOH-$OCOCH_3$) and –COOH group must be present at C-16 and C-17, respectively. On compairing the ^{13}C-NMR values of aglycone with 16β-O-acetyl-28 oicacid[16] confirmed the above result. The larger *J* value (δ3.32, t, *J*=17.0 Hz) showed equatorial orientation of $-OCOCH_3$ group at C-16. On the basis of above facts **2a** was identified as 1β, 2α, 3β- trihydroxy-16β-*O*-acetylolean-12-ene-28-oic acid.

Easy hydrolysis of glycoside with acid as well as base[17] [5N-NH_4OH] confirmed that sugar was attached by ester linkage. Quantitative estimation and molecular weight difference suggested the presence of two moles of sugar per mole of aglycone. ^{1}H-NMR spectrum of compound showed signals of two anomeric protons compound showed signals of two anomeric protons at δ4.90 (1H, d, *J*=5.2 Hz) and 5.76 (1H, d, *J*=7.5 Hz). This confirmed that glycoside was dissacharide. On permethylation followed by acid hydrolysis permethylated derivative gave 2,4 di-*O*-methyl-D-arabinose and 2,3,4-trimethyl-*O*-D-arabinose. This confirmed that interglycosidic linkage was (1→3).

The attachment of two sugar moieties were confirmed by comparing the 13C-NMR spectral data with **2** and **2a** which showed the attachment of sugar to C-28 by higher δ value at C-28 for **2** and lower δ value of **2a**. Hydrolysis of **2** with emulsion gave D-arabinose confirming the β-nature of glycosidic linkage.

From the above evidences the structure of compound **2** was determined as 1β, 2α, 3β-trihydroxy-16β-*0*-acetylolean-12-ene-28-oic-*0* [-β-D-arabinopyranosyl (1→3)] -β-D-arabinopyranoside.

III. EXPERIMENTAL SECTION :

The stem bark of *Samanea saman* was collected in April, 1999 from the Botany Department, University of Allahabad, Allahabad, U.P., India. M.ps measured in open capillary tube and are corrected. ^{1}HNMR and ^{13}CNMR spectra were recorded on a JEOLUNK-A 500 spectrometer in $CDCl_3$ using TMS as an internal standard at 300 MHz and 100 MHz, respectively. IR spectra were run in KBr pellets. Mass spectra were recorded on JEOLSX 102/DA-6000 mass spectrophotometer.

The air-dried and finely crushed stem bark (5kg) of *Samanea saman* was repeatedly extracted with boiling EtOH (4x10*l*), concentrated under reduce pressure in a rotatory evaporator and poured into an excess of ice-cold distilled water with constant stirring to give reddish brown aqueous solution (fraction-I) and light brown residue (fraction-II) were obtained. The water insoluble portion was extracted successively with different solvents of increasing polarity over a sintered Column. Elution with solvent system C_6H_6 : EA (9:1, v/v) followed by runing in preparative TLC using benzene:chloroform (9:1, v/v) marked with the help of UV lamp gave compounds **1** (0.69g), and from pure ethyl acetate followed by runing in preparative TLC using ethylacetate : methanol(9:1, v/v), compond **2** (0.35g) were obtained.

Compound **1,** mp. 136^{O}C, yield 690 mg, homogenous on TLC, R_f 0.46 solvent C_6H_6:$CHCl_3$ (9:1, v/v); Anal. Found: C, 71.10; H, 9.82; Calcd. for $C_{35}H_{58}O_7$: C, 71.15; H, 9.89% . IR (KBr) 3450, 2923, 1630, 1100, 1050. ^{1}HNMR ($CDCl_3$, 300 MHz) : δ0.85 (s, 3H, H-25), 0.95 (s,3H, H-26), 0.99 (s,3H, H-29), 1.03 (s,3H, H-30), 1.17 (s,3H, H-27), 1.31 (s,3H, H-28), 1.35 (s,3H, H-23), 2.59 (brs,1H, *J*=13Hz), 3.83 (dd, 1H, *J*=9.6 Hz, β-OH at C-3), 5.25 (t, 1H, *J*=3.3 Hz), 4.35 and 4.52 (d, 1H, *J*=12.1 Hz for $-CH_2OH$ group proton), 4.98 (d, 1H, *J*=7 Hz for β linkage) and 3.2-3.8 (m, 5H, sugar protons); MS : m/z 588[M^+], 458, 427, 409, 391, 234, 224, 219, 216, 204, 201, 175, 119; ^{13}C-NMR data are given in **Table-I**.

Compound **2**, mp. 280^{O}C, yield 350mg, homogenous on TLC, Rf 0.64 solvent $CHCl_3$:MeOH (8:2, v/v); Anal. found : C, 59.99; 8.23; Calcd for $C_{42}H_{68}O_{16}$: C, 60.85; H, 8.24%; IR (KBr) 3300, 3200, 1700, 1690, 1640, 2933, 1381, 1362, 1100, 970; ^{1}HNMR ($CDCl_3$, 300 MHz) : δ0.85 (s, 3H, H-25), 0.93 (s, 3H, H-26), 0.95 (s, 3H, H-29), 1.04 (s, 3H, H-30), 1.17 (s, 3H, H-27), 1.24 (s, 3H, H-28), 1.31 (s, 3H, H-23), 2.03 (s, 3H, -$OCOCH_3$), 5.34 (t, 1H, J=3.4 Hz), 4.80 (d, 1H, *J*=10.5 Hz), 4.88 (d, 1H, *J*=9.3Hz), 520 (dd, 1H, *J*=10.5 and 9.3 Hz), 3.22 (d, 1H, *J*=10.7Hz, H-1), 3.41 (d, 1H, *J*=9.5 Hz, H-3), 4.03 (dd, 1H, *J*=10.7 and 9.5 Hz, H-2), 4.90 (d, 1H, *J*=5.2 Hz), 5.76 (d, 1H, *J*=7.5Hz), 3.25-3.85 (m, 10H, sugar protons); MS : m/z 828 [M^+], 546, 306, 261, 247, 240, 202; ^{13}CNMR data are given in **Table-II**.

IV. CONCLUSION:

In conclusion *Samanea saman* is a rich source of trirepenoid. Trirepenoid are of great interest due to many biological activity and several parts of the plants have a particular pleasant smell. This smell is due to the presence of certain volatile oil known as essential oil. Among the chief constituent of essential oil are triterpenoid upto C_{15} . Due to their biological activity and characteristic smell, plants are used in pharmacy and perfumery industry respectivety.

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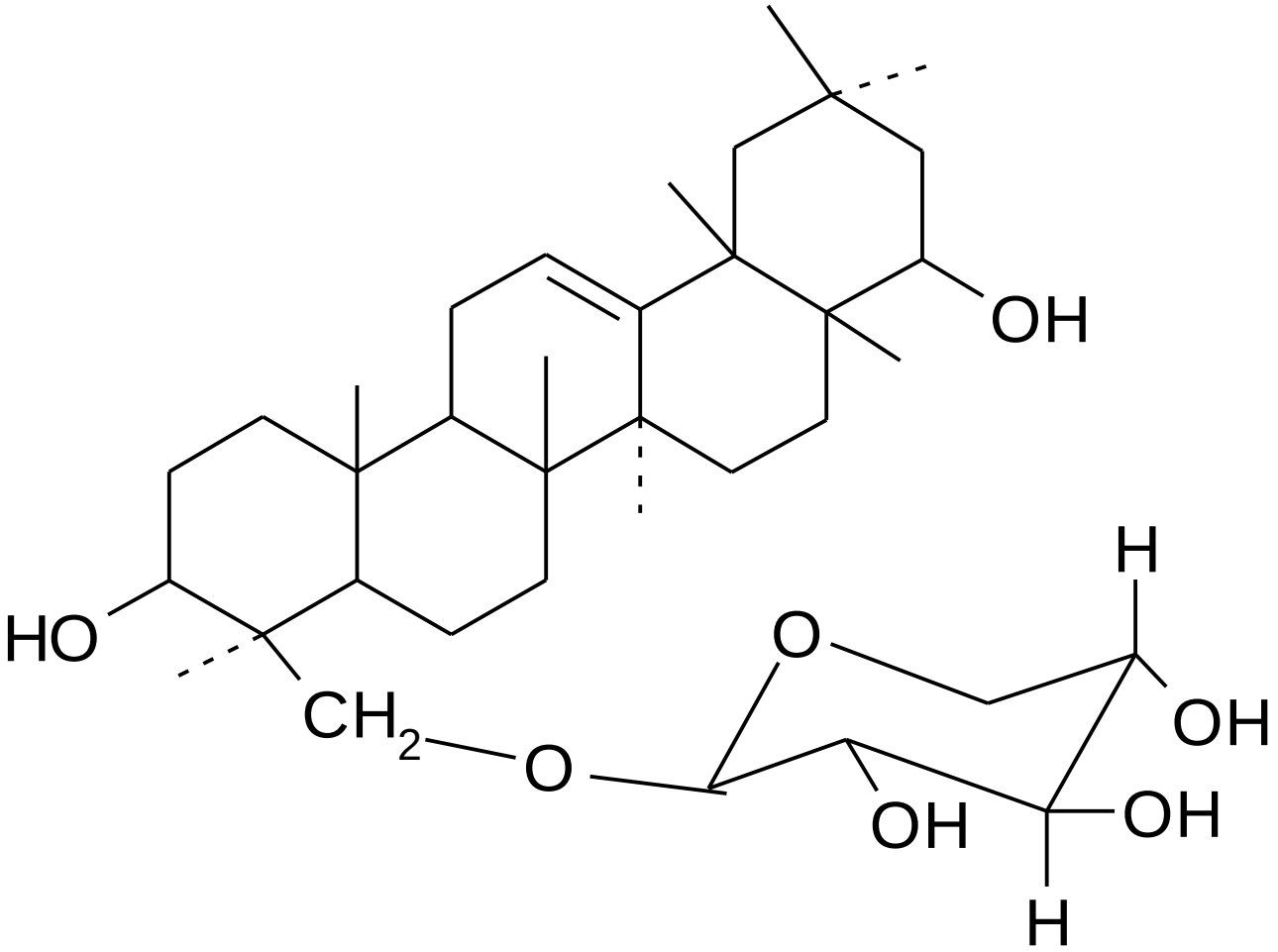
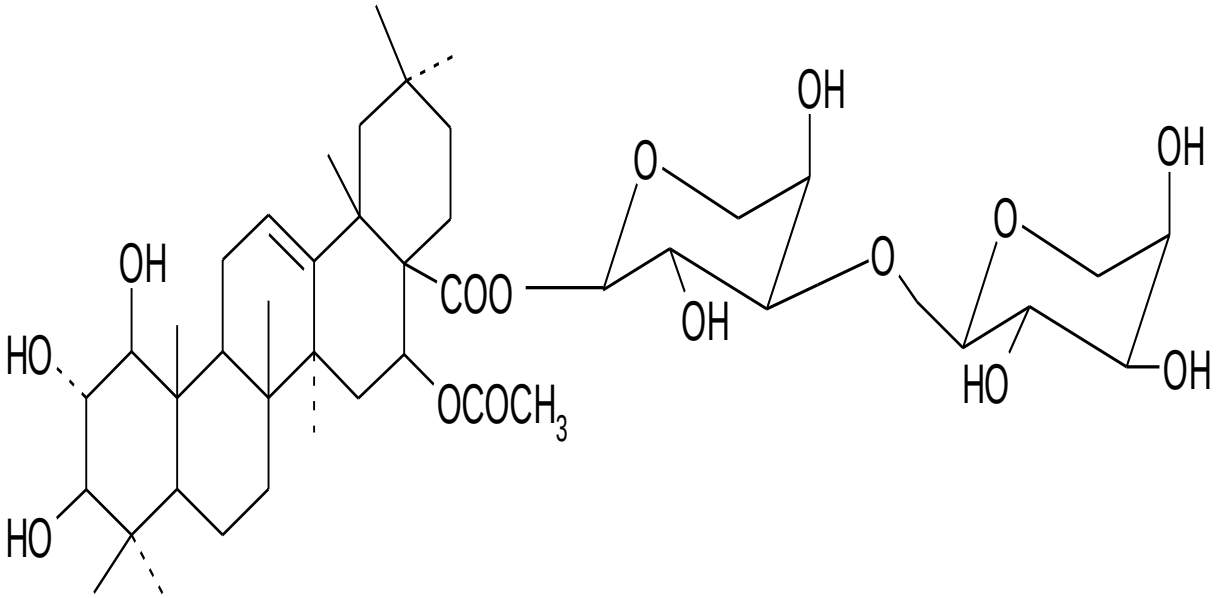


3*β*, 22*β* – dihydroxyolean -12-ene-24-*O*-*β*-D-xylopyranoside1



1*β*, 2*α*, 3*β*-trihydroxy-16*β*-*O*-acetylolean-12-ene-28 oic acid-28-*O*-
[-*β*-D-arabinopyranosyl (1→3)]-*β*-D-arabinopyranoside**2**

Table –I. ^{13}C-NMR (δ) values of glycoside SS-1 and its aglycone

Chemical Assigned	Glycoside Chemical Shift (ppm)	Aglycone Chemical shift (ppm)
C-1	38.6 (t)	39.0(t)
C-2	27.5(t)	28.4(t)
C-3	80.0 (d)	79.8(d)
C-4	43.0 (s)	43.0(s)
C-5	57.0(d)	56.3(d)
C-6	19.2(t)	19.4(t)
C-7	33.7(t)	33.5(t)
C-8	40.1(s)	39.9(s)
C-9	48.6(d)	48.1(d)
C-10	36.8(s)	37.0(s)
C-11	24.6(t)	24.0(t)
C-12	123.0(d)	122.4(d)
C-13	145.0(s)	144.9(s)
C-14	42.0(s)	42.3(s)
C-15	26.0 (t)	26.4(t)
C-16	29.4 (t)	29.0(t)
C-17	37.5 (s)	38.4 (s)
C-18	44.5(d)	44.8(d)
C-19	41.9 (t)	41.5 (t)
C-20	30.5(s)	30.0(s)
C-21	37.0(t)	37.3(t)
C-22	75.6(d)	75.6(d)
C-23	23.1(q)	23.5(q)
C-24	74.5 (t)	64.5 (t)
C-25	16.2(q)	16.3(q)
C-26	17.5(q)	17.1(q)
C-27	26.8(q)	25.5(q)
C-28	21.1(q)	21.1(q)
C-29	32.5(q)	32.0(q)
C-30	24.6(q)	24.4(q)
C-1′	103.6(q)	
C-2′	71.5 (q)	
C-3′	75.2 (d)	
C-4′	70.1 (d)	
C-5′	67.6 (t)	

Table –II. 13C-NMR (δ) values of glycoside SS-2 and its aglycone

Chemical Assigned	Glycoside Chemical Shift (ppm)	Aglycone Chemical shift (ppm)
C-1	74.6 (d)	74.4 (d)
C-2	74.8 (d)	74.8 (d)
C-3	76.5 (d)	76.3(d)
C-4	43.2(s)	43.2(s)
C-5	57.2 (d)	57.3(d)
C-6	19.2(t)	19.2(t)
C-7	33.6(t)	33.6(t)
C-8	40.2(s)	40.1(s)
C-9	48.4(d)	48.4(d)
C-10	36.8(s)	36.8(s)
C-11	24.6(t)	24.6(t)
C-12	124.2(d)	124.2(d)
C-13	146.1(s)	146.1(s)
C-14	42.0(s)	42.0(s)
C-15	26.3(t)	26.3(t)
C-16	81.4(d)	81.4(d)
C-17	48.5(s)	48.5(s)
C-18	42.6(d)	42.6(d)
C-19	39.6(t)	39.5(t)
C-20	36.8(s)	36.6(s)
C-21	37.3(t)	37.4(t)
C-22	38.2(t)	38.2(t)
C-23	23.2(q)	23.2(q)
C-24	17.6(q)	17.6(q)
C-25	16.1(q)	16.1(q)
C-26	17.5(q)	17.5(q)
C-27	26.8(q)	26.8(q)
C-28	181.9(s)	179.2(s)
C-29	27.4(q)	27.3(q)
C-30	17.9(q)	
$OCOCH_3$	20.5(q)	
$OCOCH_3$	168.7(s)	
C-1′	105.6(d)	
C-2′	73.8(d)	
C-3′	74.6(d)	
C-4′	70.2(d)	
C-5′	67.0(t)	
C-1′	104.5(d)	
C-2′	73.0(d)	
C-3′	72.1(d)	
C-4′	70.0(d)	
C-5′	67.5(t)	

AUTHORS PROFILE

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