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NEW TRITERPENOID SAPONIN FROM *ACACIA AURICULIFORMIS* CUNN.

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Abstract: A new triterpenoid saponin 3-O-[α-L-rhamnopyranosyl-(1 → 4)]-α-L-arabinopyranosyl-(1 → 6)-β-D-galactopyranosyl-3β,16α,21β,22α,28-pentahydroxy-olean-12-ene along with known compound corosolic acid has been isolated from the methanolic extract of the stems of the plant by various colour reactions, chemical degradation and spectral analysis.

Keywords: *Acacia auriculiformis*, Leguminosae, stems, triterpenoid saponin, monodesmosidic

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INTRODUCTION

Acacia Auriculiformis A. Cunn.[1-5] belongs to family Leguminosae, which is commonly known as "Australian acacia". It is widely distributed in India on road sides and in parks. It is reported to have as central nervous system depressant activity . Earlier workers [6-11] have reported various active constituents from this plant.This paper deals with the isolation and structural elucidation of a new triterpenoid saponin,3-O-[α-L-rhamnopyranosyl-(1→4)]-β-D-arabinopyranosyl-(1→6)-β-D-galactopyranosyl-3β,16α,21β,22α,28-pentahydroxy-olean-12-ene along with known compound corosolic acid from the methanolic extract of the stems of the plant.

EXPERIMENTAL

General experimental procedure

All the melting points were determined on a thermo electrical melting point apparatus and are uncorrected. The IR spectra were measured on shimadzu 84005 FTIR spectrophotometer using KBr matrix.The NMR spectral data were recorded at 300 MHz for ^{1}H NMR and 75 MHz for ^{13}C NMR on Bruker DRX 300 NMR spectrometer using TMS as internal standard and CD_3OD as solvent. The FABMS were recorded on a Jeol SX (102) Mass spectrometer.

Plant material

The stems of the plant were collected locally around Sagar region and were taxonomically authenticated by Taxonomist, Department of Botany, Dr. H. S. Gour Central University, Sagar (M.P.) India.A voucher specimen (Bot/Her/A/3140) has been deposited in the Natural Products Laboratory, Department of Chemistry of this university.

Extraction and isolation

Air-dried and powdered stems (3 kg) of the plant were extracted with 95% ethanol (60-80^{o}C) in Soxhlet apparatus for 72 hour. The total ethanolic extract was concentrated under reduced pressure and successively partitioned with petroleum ether (40-60%), $CHCl_3$, $CH_3COOC_2H_5$, CH_3COCH_3 and CH_3OH. The methanol soluble fraction of the plant was concentrated under reduced pressure to give brown viscous mass. It give two spots on TLC examination using chloroform: methanol: water (6:4:2) as solvent and I_2 vapours as visualizing agent indicating it to be mixture of two compounds 1 and 2. These were separated by column chromatography over a silica gel column using $CHCl_3$: MeOH (3:6) as eluent and studied separately.

Study of compound 1

It was crystallized from acetone to give light brown crystalline compound(1.25 gm), m.p. 256-257^{o}C,m.f.$C_{47}H_{78}O_{18}$ $[M]^{+}$ *m/z* 930(FABMS). Found (%): C 60.62, H 8.33,O 30.93 Calcd. for $C_{47}H_{78}O_{18}$: C 60.64, H 8.38 O 30.96 %, IR (KBr): 3445, 2934, 1661, 1452, 1038 cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD): δ 1.01 (3H, s, Me-23), 0.87 (3H, s, Me-24), 0.98 (3H, s, Me-25), δ 1.02 (3H, s, Me-

26), 1.46 (3H, s, Me-27), 0.89 (3H, s, Me-29), 1.06 (3H, s, Me-30),1.56 (1H, m, H-1_a), 1.08 (1H, m, H-1_b), 1.67 (1H, m, H-2_a), 1.98 (1H, m, H-2_b), 2.89(1H, m, H-3), .0.98 (1H, d, *J* 7.2 Hz, H-5), 1.52 (1H, m, H-6_a), 1.43 (1H, m, H-6_b), 1.69 (1H, m, H-7_a), 1.38 (1H, m, H-7_b), 1.73 (1H, m, H-9), 1.96 (2H, m, H-11), 5.39 (1H, br s, H-12), 1.63 (1H, m, H-15_a), 1.39 (1H, m, H-15_b), 3.78 (1H,br s, H-16), 2.46 (1H, dd, *J* 14.0, 3.2 Hz, H-18), 2.54 (2H, dd, *J* 14.0, 14.0 Hz, H-19), 4.62 (1H, d, *J* 10.0 Hz, H-21), 4.20(1H, d, *J* 10.0 Hz, H-22), 2.83 (1H, d, *J* 11.2 Hz, H-28_a), 3.14 (1H, d, *J* 11.2 Hz, H-28_b), δ 5.43 (1H, d, *J* 7.4 Hz, H-1′), 4.02 (1H, dd, *J* 7.4,9.6 Hz, H-2′), 3.58 (1H, dd, *J* 7.2,3.6 Hz, H-3′), 3.98 (1H, dd, *J* 3.5,1.6 Hz, H-4′), 3.49 (1H, ddd, *J* 1.5,5.5,7.5 Hz, H-5′), 3.76 (1H, dd, *J* 11.5,6.0 Hz, H-6_a′), 4.00 (1H, dd, *J* 11.5,5.0 Hz, H-6_b′;

δ 4.53 (1H, d, *J* 7.0 Hz, H-1′′), 3.63 (1H, dd, *J* 8.2,6.4 Hz, H-2′′), 3.79 (1H, dd, *J* 8.2,3.0 Hz, H-3′′), 3.85 (1H, m, H-4′′), 3.93 (1H, m, H-5_a′′), 3.48 (1H, m, H-5_b′′);δ 4.89 (1H, d, *J* 1.6 Hz, H-1′′′), 4.16 (1H, dd, *J* 2.3,1.4 Hz, H-2′′′), 3.78 (1H, dd, *J* 3.4,8.5 Hz, H-3′′′), 3.42(1H,t, *J* 8.5,H-4′′′), 3.84 (1H, dd, *J* 9.1,6.0 Hz, H-5′′′), 1.32 (3H,d, *J* 6.2 Hz, H-6′′′,CH_3);

^{13}C NMR (75 MHz, CD_3OD) δ 36.38 (C-1), 25.72 (C-2), 89.16 (C-3), 38.12 (C-4), 54.58 (C-5), 18.97 (C-6), 31.92 (C-7), 39.98 (C-8), 47.85 (C-9), 35.68 (C-10), 24.25 (C-11), 123.95 (C-12), 144.89 (C-13), 41.68(C-14), 35.27 (C-15), 69.01 (C-16), 48.13 (C-17), 38.95 (C-18), 45.81 (C-19), 34.98 (C-20), 78.19 (C-21), 71.89 (C-22), 27.36 (C-23), 17.97 (C-24), 18.78 (C-25), 18.01 (C-26), 26.45 (C-27), 63.32 (C-28), 29.93 (C-29), 21.80 (C-30), 170.76; δ 104.2 (C-1′), 72.1 (C-2′), 84.4 (C-3′), 70.0 (C-4′), 76.2 (C-5′), 64.2 (C-6′); δ 104.2 (C-1′′), 72.8 (C-2′′), 76.8 (C-3′′), 69.7 (C-4′′), 65.9(C-5′′); δ 101.5 (C-1′′′), 70.8 (C-2′′′), 68.7 (C-3′′′), 74.5 (C-4′′′), 66.7 (C-5′′′),17.23 (C-6′′′ CH_3).

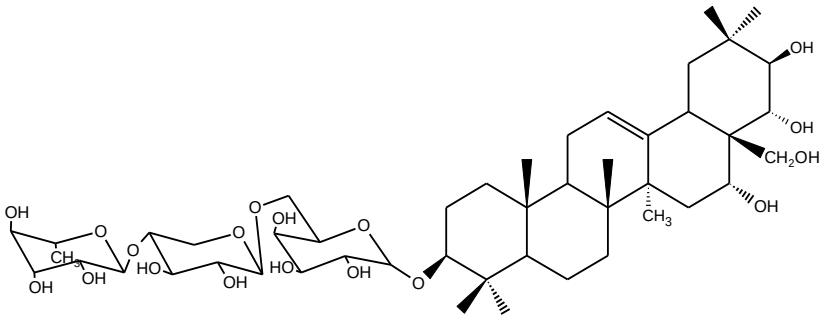

Figure 1: Compound 1

Study of compound 2

It was crystallized from acetone to give light yellow crystalline compound(1.25 gm), m.p. 244-245°C,m.f. $C_{30}H_{48}O_4$ $[M]^+$ *m/z* 472 (FABMS). Found (%):C 76%, H 10 %,O 13.2%, Calcd. for $C_{30}H_{48}O_4$: C 76.23%, H 10.24 %,O 13.54%, IR (KBr): 3443, 2936, 1659, 1449 cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD): δ 1.03 (3H, s, Me-23), 0.85 (3H, s, Me-24), 1.01 (3H, s, Me-25), δ 0.89 (3H, s, Me-26), 1.16 (3H, s, Me-27), 0.91 (3H, s, Me-29), 0.98 (3H, s, Me-30),1.52 (1H, m, H-1_a), 1.06 (1H,

m, H-1_b), 3.62 (1H, ddd, J 4.5, 9.7, 11.4 Hz, H-2), 2.06 (1H, d,J 9.3 Hz, H-3), 1.48 (1H, d, *J* 7.1 Hz, H-5), 5.23 (1H, t, J 3.4 Hz, H-12), 2.19(1H, d J 11.3 Hz, H-18), 1.06 (3H, s, H-23), 0.85(3H, s, H-24), 1.02 (3H, s, H-25), 0.84 (3H, s, H-26),1.14 (3H, s, H-27), 0.93 (3H, d, J = 6.5 Hz, H-29), 0.97(3H, s, H-30).^{13}C NMR (75 MHz, CD_3OD) δ 44.87 (C-1), 67.10 (C-2), 80.02 (C-3), 38.12 (C-4), 52.21 (C-5), 20.32 (C-6), 33.01(C-7), 42.10 (C-8), 45.83 (C-9), 38.82 (C-10), 24.23 (C-11), 124.81 (C-12), 140.31 (C-13), 43.81(C-14), 29.13 (C-15), 25.01 (C-16), 46.53 (C-17), 51.42 (C-18), 38.02 (C-19), 37.03 (C-20), 29.34 (C-21), 39.02 (C-22), 26.32 (C-23), 18.02 (C-24), 17.68(C-25), 19.23 (C-26), 21.69 (C-27), 173.32 (C-28), 16.32 (C-29), 19.75 (C-30),

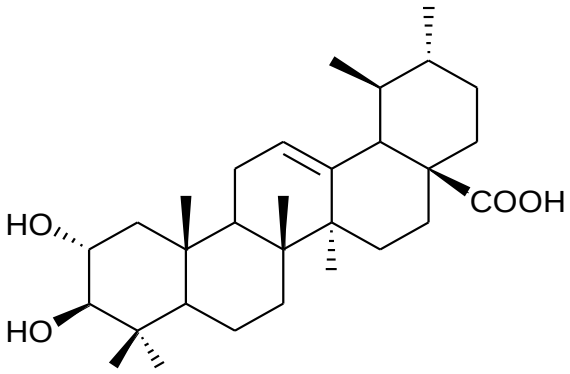

Figure 2: Compound 2

Acid hydrolysis of compound 1:

Compound 1 (300 mg) was dissolved in ethanol (20 mL) and refluxed with 10% H_2SO_4 (15 mL) on water bath for 8 hr. The contents were concentrated and allowed to cool and residue was extracted with diethyl ether. The ether layer was washed with water and evaporated to dryness. The residue was subjected to column chromatography over silica gel column using $CHCl_3$:MeOH (5:10) as solvent to yield compound 1a, which was identified as 3β,16α,21β,22α,28-pentahydroxy-olean-12-ene by comparison of its spectral data with reported literature values. The aqueous hydrolysate was neutralized with $BaCO_3$ and the $BaSO_4$ was filtered off. The filtrate was concentrated and subjected to paper chromatography examination using *n*-Butanol:Acetic Acid:Water [*n*BAW, 4:1:5] as solvent and aniline hydrogen phthalate as spraying reagent which showed the presence of D-Galactose (R_f 0.17), L-rhamnose (R_f 0.36) and L-arabinose (R_f 0.22) by (Co-Pc and Co-TLC).

Study of compound 1a:

It has m.p. 228-229^{o}C, molecular formula $C_{30}H_{50}O_5$ $[M]^+$ *m/z* 490 (FABMS). Found: C, 73.42; H, 10.21%;O 16.34 Calcd. for $C_{30}H_{50}O_5$,C 73.46%; H, 10.20%,O 16.32. IR (KBr): 3442, 2932, 1663,

1449 cm $^{-1}$, ^{1}H NMR (300 MHz, CD_3OD): δ 1.02 (3H, s, Me-23), 0.86 (3H, s, Me-24), 0.97 (3H, s, Me-25), δ 1.01 (3H, s, Me-26), 1.43 (3H, s, Me-27), 0.91 (3H, s, Me-29), 1.05 (3H, s, Me-30),1.54 (1H, m, H-1_a), 1.06 (1H, m, H-1_b), 1.65 (1H, m, H-2_a), 1.96 (1H, m, H-2_b), 2.87(1H, m, H-3), .0.97 (1H, d, *J* 7.0 Hz, H-5), 1.49 (1H, m, H-6_a), 1.41 (1H, m, H-6_b), 1.67 (1H, m, H-7_a), 1.39 (1H, m, H-7_b), 1.71 (1H, m, H-9), 1.92 (2H, m, H-11), 5.32 (1H, br s, H-12), 1.59 (1H, m, H-15_a), 1.41 (1H, m, H-15_b), 3.76 (1H,br s, H-16), 2.43 (1H, dd, *J* 14.2, 3.2 Hz, H-18), 2.44 (2H, dd, *J* 14.2, 14.1 Hz, H-19), 4.59 (1H, d, *J* 10.1 Hz, H-21), 4.24(1H, d, *J* 10.2 Hz, H-22), 2.79 (1H, d, *J* 11.1 Hz, H-28_a), 3.21 (1H, d, *J* 11.1 Hz, H-28_b);^{13}C NMR (75 MHz, CD_3OD) δ 36.34 (C-1), 25.69 (C-2), 89.14 (C-3), 38.08 (C-4), 54.54 (C-5), 18.94 (C-6), 31.90 (C-7), 39.91 (C-8), 47.88 (C-9), 34.68 (C-10), 24.20 (C-11), 122.95 (C-12), 143.89 (C-13), 41.64(C-14), 36.26 (C-15), 68.01 (C-16), 48.17 (C-17), 38.85 (C-18), 45.79 (C-19), 34.98 (C-20), 78.27 (C-21), 72.09 (C-22), 28.06 (C-23), 17.97 (C-24), 19.79 (C-25), 18.11 (C-26), 26.43 (C-27), 63.28 (C-28), 29.89 (C-29), 21.78 (C-30),

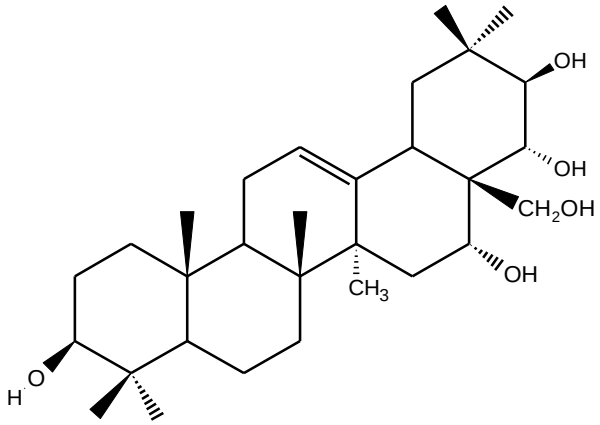

Figure 3:Compound 1a

Permethylation followed by hydrolysis of Compound 1

Compound 1 (20 mg) was dissolved in DMF (30 mL) and treated with MeI (5 mL) and Ag_2O (15 mg) in 150 ml round bottomed flask fitted with condenser and refluxed for 2 days. The contents were filtered and washed with DMF. The filtrate was concentrated under reduced pressure and treated with $CHCl_3$ (25 mL) and washed with water. After removal of solvent a syrupy mass was found which was hydrolysed with 7% H_2SO_4 (5 mL) to give methylated aglycone, identified as 3β-hydroxy-16α,21β,22α,28-tetramethoxy-olean-12-ene and methylated sugars. The aqueous hydrolysate obtained after the removal of aglycone was neutralized with $BaCO_3$ and the $BaSO_4$ was filtered off. The filtrate was concentrated under reduced pressure and subjected to paper chromatography on Whatmann filter paper No.1 using *n*-Butanol:Ethanol:Water (5:1:4) as solvent system and aniline hydrogen phthalate as spraying agent. The methylated sugars were

identified as 2,4-di-O-methyl-D-galactose (R_G 0.41), 2,3-di-O-methyl-L-arabinose (R_G 0.63) and 2,3,4-tri-O-methyl-L-rhamnose (R_G 1.02).

Enzymatic hydrolysis of compound 1

Compound 1 (40 mg) was dissolved in MeOH (25 mL) and hydrolyzed with equal volume of enzyme takadiastase. The reaction mixture was allowed to stay at room temperature for 2 days and filtered. The hydrolysate was concentrated and subjected to paper chromatography examination using nBAW (4:1:5) as solvent system and aniline hydrogen phthalate as a spraying reagent which showed the presence of L-rhamnose, L-arabinose suggesting the presence of α linkage between L-rhamnose (R_f 0.36) and L-arabinose (R_f 0.22) and also between L-arabinose and Proaglycone . Proaglycone on further hydrolysis with enzyme almond emulsin yielded D-galactose (R_f 0.17) and aglycone suggesting the presence of β linkage between D-galactose and aglycone.

RESULTS AND DISCUSSION

The methanolic extract of the stems of the plant yielded a new compound **1**, m.p. 256-257^{o}C,m.f. $C_{47}H_{78}O_{18,}$ $[M]^+$ *m/z* 930(FABMS). It gave positive Liebermann Burchard test for triterpenoid saponin [12-15]. In the IR spectrum two peaks at 3445, 1661 cm^{-1}were assigned for hydroxyl group and double bond respectively. In ^{1}H NMR spectrum (300 MHz, CD_3OD) showed signals of an olean-12-ene-type triterpene [seven singlet methyl resonances at δ_H 1.01, 0.87 , 0.98 , 1.02, 1.46 ,0.89, 1.06 (each 3H, s, Me x7) and trisubstituted olefinic proton signal at δ_H 5.39 (br, s),A multiplet at 2.89(1H, m) were assigned to H-3 and 3.78 (1H,br s) for H-16, and three anomeric proton signal at δ_H 5.43, 4.53,4.89 were assigned for H-1',H-1'',H-1''' of D-Galactose , L-arabinose and L-rhamnose respectively. In ^{13}C NMR spectrum (75 MHz, CD_3OD) two signals at δ 123.95 (C-12), 144.89 (C-13) indicated the presence of double bond between C-12 and C-13. A signal at δ 69.01 revealed the presence of hydroxyl group at C-16. The ^{13}C NMR spectrum of compound 1 exhibited 47 signals, 30 of which could be assigned to the aglycone and 17 signals to the sugar moieties revealing compound 1'nature as monodesmosidic.

In the mass spectrum of the compound 1,characteristsic ion peak at $[M^+]$m/z 930,784,652 and 490 were found by subsequent losses from the molecular ion of each molecular of L-rhamnose,L-arabinose and D-galactose showing L-rhamnose as terminal sugar,D-galactose was linked at C-3 position of aglycone and L-arabinose linked with L-rhamnose and D-galactose.

Acid hydrolysis of compound 1 with ethanol and 10% H_2SO_4 gave compound 1a. It has m.p. 210-212 ^{o}C, molecular formula $C_{30}H_{50}O_5$ $[M]^+$ *m/z* 490 (FABMS).It responded to all the colour reactions of triterpenoids[16-19]. It was identified as 3β,16α,21β,22α,28-pentahydroxy-olean-12-ene by comparison of its spectral data with reported literature values.The aqueous hydrolysate obtained after acid hydrolysis of the compound 1 was neutralized with $BaCO_3$ and the $BaSO_4$

was filtered off. The filtrate was concentrated under reduced pressure and subjected to paper chromatography examination using *n*BAW (4:1:5) as solvent and aniline hydrogen phthalate as detecting agent yielding D-galactose (R_f 0.17),L-arabinose (R_f 0.22) and L-rhamnose (R_f 0.36) (Co-PC) [20] and Co-TLC).

Permethylation [21]of compound 1 followed by acid hydrolysis yielded methylated aglycone identified as 3β-hydroxy-16α,21β,22α,28-tetra-O-methyl-olean-12-ene confirming that glycosidation was involved at C-3 and methylated sugars were identified as 2,4-di-O-methyl-D-galactose (R_G 0.41), 2,3-di-O-methyl-L-arabinose (R_G 0.63) and 2,3,4-tri-O-methyl-L-rhamnose (R_G 1.02) suggesting that C-1‴of L-rhamnose was attached with C-4″of L-arabinose and C-1″ of L-arabinose attached to C-6′ D-galactose and C-1′of D-galactose attached to C-3 position of aglycone thus the interlinkage (1→4) was found between L-rhamnose and L-arabinose and interlinkage (1→6) was found between L-arabinose and D-galactose This linkage and the linkage between D-galactose and aglycone's was further confirmed by ^{13}C NMR spectral data. Periodate oxidation [22] of compound 1 confirmed that three sugars were present in pyranose form.

Enzymatic hydrolysis [23] of compound 1 with enzyme takadiastase liberated L- rhamnose (R_f 0.36) first followed by L- arabinose (R_f 0.22) and proaglycone identified as 3-O-[β-D-galactopyranosyl-3β,16α,21β,22α,28-pentahydroxy-olean-12-ene suggesting the presence of α-linkage between between L-rhamnose and L-arabinose and also between L-arabinose and Proaglycone.Proaglycone was hydrolysed with enzyme almond emulsin liberating D-galactose (R_f 0.17) and sapogenin (aglycone) revealing the presence of β-linkage between D-galactose and aglycone.

On the basis of above evidences, the structure of compound 1 was established as 3-O-[α-L-rhamnopyranosyl-(1 → 4)]-β-D-arabinopyranosyl-(1 → 6)-β-D-galactopyranosyl-3β,16α,21β,22α,28-pentahydroxy-olean-12-ene.

Quantitative examination [24] of the sugars present in the hydrolysate revealed that the three sugars were present in equimolecular proportions indicating that the triterpenoid saponin consists of one molecule of each, aglycone (sapogenin), D-galactose, L-rhamnose and L-arabinose.

Conclusion

Thus above evidences establish the presence of a new triterpenoid saponin 3-O-[α-L-rhamnopyranosyl-(1 → 4)]-α-L-arabinopyranosyl-(1 → 6)-β-D-galactopyranosyl-3β,16α,21β,22α,28-pentahydroxy-olean-12-ene along with known compound corosolic acid has been isolated from the methanolic extract of the stems of the plant.

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