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# CHEMICAL CONSTITUENTS OF *CAESALPINIA BONDUC*

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Four known triterpenoids (1-4) α-amyrin (**1**), β-amyrin (2), lup-20(29)-en-3β-ol (**3**), lup-20(20)-en-3β-yl acetate (**4**) and two known sterols (**5,6**) β-sitosterol (**5**) and β-sitosteryl galactoside (**6**) were isolated first time from the *Caesalpinia bonduc.*

***Key words:*** *Caesalpinia bonduc,* Triterpenoids,

## Introduction

The seeds of *Caesalpinia bonduc* (L.) Roxb. belonging to the family Caesalpiniaceae were used as a folk medicine to treat asthma and chronic fever. They were found to be very potent as an antiperiodic, antipyretic and utilized as febrifuge, antispasmodic, antirheumatic and mild purgative [1-2]. Literature survey revealed the presence of diterpenoids such as α-caesalpin [3-4], β-caesalpin [3,4], δ-caesalpin [4,5], γ-caesalpin [3-6] and ε-caesalpin [7] from the seeds of *Caesalpinia bonduc* (L.) Roxb. [syn. *C. bonducella* (L.) Fleming]. Our phytochemical investigations have led to the isolation of four triterpenoids α-amyrin [12-ursen-3β-ol] (**1**), β-amyrin [12-oleanen-3β-ol] (**2**), lup-20(29)-en-3β-ol (**3**), lup-20(29)-en-3β-yl acetate (**4**), and two sterols β-sitosterol [24(R)-stigmast-5-en-3β-ol] (**5**), β-sitosteryl galactoside [3-O-β-D-galactopyranosyl-stigmasta-5-ene] (**6**).

## Experimental

The seeds of *Caesalpinia bonduc* were collected from Karachi during the months of November-December. The fresh seeds (6.5 kg) were ground in a homogenizer and soaked in MeOH for two weeks. It was filtred and the filtrate was concentrated under reduced pressure. The concentrated crude extract (95 g) was partitioned between EtOAc and $H_2O$. The aqueous layer was again shaked with *n*-BuOH. The EtOAc extract was condensed by removing the solvent under reduced pressure and the material thus obtained was subjected to column chromatography using solvent systems *n*-hexane; *n*-hexane; $CHCl_3$; $CHCl_3$ : MeOH.

Compound **1** was eluted with *n*-hexane : $CHCl_3$ (9:1) and crystalized from EtOH, yielded 40 mg of **1**.

*α-Amyrin* (**1**) : Mp 184-185°C; $[\alpha]_D$ + 90° ($CHCl_3$; C, 0.30); EIMS *m/z* (rel. intens. %) : 426 [$C_{30}H_{50}O$, $M^+$] (20), 411 [M-Me]$^+$ (16), 408 [M-$H_2O$]$^+$ (25), 393 [M-Me-$H_2O$]$^+$ (22), 257 [M-$C_{11}H_{21}O$]$^+$ (20), 218 [M-$C_{14}H_{24}O$]$^+$ (100), 207 [M-$C_{16}H_{27}O$]$^+$ (12), 203 [M-$C_{15}H_{27}O$]$^+$ (50) and 189 [M-$C_{16}H_{29}O$]$^+$ (65); IR ($CHCl_3$) $\nu_{max}$ cm$^{-1}$ : 3510 (OH), 3055, 1635 and 820 (trisubstituted double bond); $^1$H-NMR (300 MHz, $CDCl_3$) [8]: δ 5.23 (1H, m, H-12), 3.21 (1H, dd, $J_{ax,ax}$ = 10.2 Hz, $J_{ax,eq}$ = 4.3 Hz, H-3), 1.15 (3H, s, Me-27), 1.01 (3H, s, Me-26), 0.99 (3H, s, Me-23), 0.95 (3H, s, Me-25), 0.88 (6H, s, Me-29 and Me-30), 0.83 (3H, s, Me-28) and 0.80 (3H, s, Me-24); 13C-NMR (75 MHz, $CDCl_3$) [9] : δ38.51 (C-1), 27.00 (C-2), 77.00 (C-3), 37.99 (C-4), 55.10 (C-5), 18.32 (C-6), 32.65 (C-7), 40.00 (C-8), 47.56 (C-9), 37.00 (C-10), 23.00 (C-11), 122.52 (C-12), 143.50 (C-13), 41.52 (C-14), 28.32 (C-15), 26.23 (C-16), 32.52 (C-17), 47.20 (C-18), 46.81 (C-19), 31.12 (C-20), 34.81 (C-21), 37.20 (C-22), 26.01 (C-23), 15.56 (C-24), 15.50 (C-25), 16.80 (C-26), 26.00 (C-27), 27.32 (C-28), 33.20 (C-29) and 23.69 (C-30).

The eluent obtained with *n*-hexane : $CHCl_3$ (9 : 1, v/v) was again subjected to silica gel column chromatography to obtain **2** which was crystallized from EtOH, yielded 30 mg of **2**.

*β-Amyrin* (**2**) : Mp 197-198°C ; $[\alpha]_D$ + 99° ($CHCl_3$; C, 0.40); EIMS *m/z* (rel. intens. %) : 426 [$C_{30}H_{50}O$, $M^+$] (15), 411 [M-Me]$^+$ (18), 408 [M-$H_2O$]$^+$ (16), 393 [M-Me-$H_2O$]$^+$ (32), 257 [M-$C_{11}H_{21}O$]$^+$ (20), 218 [M-$C_{14}H_{24}O$]$^+$ (100), 207 [M-$C_{16}H_{27}O$]$^+$ (10), 203 [M-$C_{15}H_{27}O$]$^+$ (40) and 189 [M-$C_{16}H_{29}O$]$^+$ (55); IR ($CHCl_3$) $\nu_{max}$ cm$^{-1}$ : 3510 (OH), 3055, 1635 and 820 (trisubstituted double bond); $^1$H-NMR (300 MHz, $CDCl_3$) [9] : δ 5.11 (1H, m, H-12), 3.19 (1H, dd, $J_{ax,ax}$ = 10.0 Hz, $J_{ax,eq}$ = 4.5 Hz, H-3), 1.08 (3H, s, Me-27), 1.02 (3H, s, Me-26), 1.01 (3H, s, Me-23), 0.96 (3H, s, Me-25), 0.91 (3H, d, J=6.6 Hz, Me-30), 0.81 (6H, s, Me-24) and 0.80 (3H, d, J=6.8 Hz, Me-29); $^{13}$C-NMR (75 MHz, $CDCl_3$) [10] : δ39.00 (C-1), 27.31 (C-2), 78.99 (C-3), 39.00 (C-4), 55.23 (C-5), 18.30 (C-6), 33.00 (C-7), 41.01 (C-8), 47.80 (C-9), 37.00 (C-10), 17.44 (C-11), 124.30 (C-12), 139.34 (C-13), 42.00 (C-14), 28.71 (C-15), 26.62 (C-16), 33.76 (C-17), 59.00 (C-18), 39.61 (C-19), 39.56 (C-20), 31.20 (C-21), 41.52 (C-22), 28.12 (C-23), 15.62 (C-24), 15.95 (C-25), 16.81 (C-26), 23.32 (C-27), 28.00 (C-28), 23.39 (C-29) and 21.34 (C-30).

The fraction eluted with *n*-hexane : $CHCl_3$ (9 : 1, v/v) was finally purified by re-column chromatography using *n*-hexane

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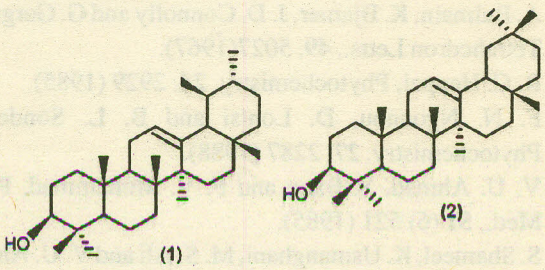
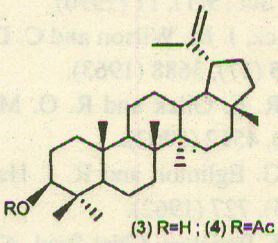
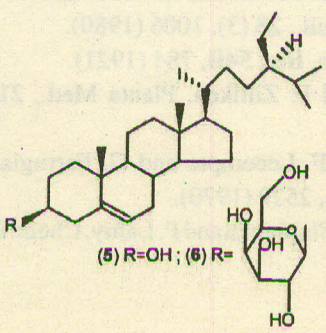
: $CHCl_3$ (8.5 : 1.5, v/v) and crystallized from $Me_2CO$-MeOH, yielded 100 mg of **3**.

*Lupeol (3):* Mp 214-215°C; $[\alpha]_D$ + 27° ($CHCl_3$; C, 1.00); EIMS *m/z* (rel. intens. %) : 426 [$C_{30}H_{50}O$, $M^+$] (20), 411 [M-Me]$^+$ (25), 408 [M-$H_2O$]$^+$ (30), 393 [M-Me-$H_2O$]$^+$ (35), 385 [M-41]$^+$ (15), 220 [M-$C_{15}H_{26}$]$^+$ (80), 218 [M-$C_{14}H_{24}O$]$^+$ (55), 207 [M-$C_{16}H_{27}$]$^+$ (25) 189 [M-$C_{16}H_{29}O$]$^+$ (100) and 139 [M-$C_{21}H_{35}$]$^+$ (70); IR ($CHCl_3$) $\nu_{max}$ $cm^{-1}$ : 3455 (OH), 3075, 1645 and 880 (exomethylene group); $^1$H-NMR (300 MHz, $CDCl_3$) [10] : δ 4.75 and 4.62 (2H, br. s, 1H each, H-29), 3.21 (1H, dd, $J_{ax,ax}$ = 9.9 Hz, $J_{ax,eq}$ = 4.5 Hz, H-3), 1.65 (3H, br. s, Me-30), 1.05 (3H, s, Me-26), 0.97 (3H, s, Me-23), 0.94 (3H, s, Me-27), 0.85 (3H, s, Me-25) 0.79 (3H, s, Me-28) and 0.77 (3H, s, Me-24); $^{13}$C-NMR (75 MHz, $CDCl_3$) [10] : δ38.62 (C-1), 27.55 (C-2), 78.81 (C-3), 38.76 (C-4), 55.33 (C-5), 18.30 (C-6), 34.25 (C-7), 40.85 (C-8), 50.44 (C-9), 37.11 (C-10), 20.95 (C-11), 25.20 (C-12), 38.10 (C-13), 42.80 (C-14), 27.39 (C-15), 35.51 (C-16), 92.91 (C-17), 48.22 (C-18), 47.79 (C-19), 150.65 (C-20), 29.89 (C-21), 39.88 (C-22), 28.02 (C-23), 15.42 (C-24), 16.11 (C-25), 15.99 (C-26), 14.52 (C-27), 18.11 (C-28), 109.25 (C-29) and 19.20 (C-30).

The fraction obtained by *n*-hexane : $CHCl_3$ (1 : 1, v/v) was subjected to repeated chromatographic purifications using *n*-hexane : EtOAc (9 : 1, v/v) afforded compound **4** which was crystallized from $Me_2CO$ : MeOH (yield: 32 mg).




*Lupeol Acetate (4):* Mp 214-216°C; $[\alpha]_D$ + 41° ($CHCl_3$; C, 0.32); EIMS *m/z* (rel. intens. %) : 468 [$C_{23}H_{52}O_2$, $M^+$] (50), 453 [M-Me]$^+$ (15), 427 [M-$C_3H_5$]$^+$ (5), 408 [M-AcOH]$^+$ (20), 393 [(453)-AcOH]$^+$ (3), 249 [M-$C_{16}H_{27}$]$^+$ (22), 218 [M-$C_{16}H_{26}O_2$]$^+$ (37), 189 [(249)-AcOH]$^+$ (63), 181 [M-$C_{21}H_{35}O$]$^+$ (10) and 121 [(181)-AcOH]$^+$ (48); IR ($CHCl_3$) $\nu_{max}$ $cm^{-1}$ : 1710 (ester carbonyl) and 3075, 1645 and 880 (exomethylene group); $^1$H-NMR (400 MHz, $CDCl_3$) : δ 4.72 and 4.62 (2H, br. s, 1H each, H-29), 4.25 (1H, dd, $J_{ax,ax}$ = 9.8 Hz, $J_{ax,eq}$ = 4.2 Hz, H-3), 2.11 (3H, s, $CH_3COO$), 1.65 (3H, dd, J=1.25 Hz, Me-30), 1.05 (3H, s, Me-26), 0.97 (3H, s, Me-23), 0.94 (3H, s, Me-27) 0.85, (3H, s, Me-25) 0.79 (3H, s, Me-28) and 0.77 (3H, s, Me-24); $^{13}$C-NMR (100 MHz, $CDCl_3$) : δ 38.41 (C-1), 23.75 (C-2), 81.05 (C-3), 37.81 (C-4), 55.43 (C-5), 18.22 (C-6), 34.30 (C-7), 40.91 (C-8), 50.43 (C-9), 37.11 (C-10), 21.06 (C-11), 25.12 (C-12), 38.10 (C-13), 42.94 (C-14), 27.52 (C-15), 35.65 (C-16), 43.08 (C-17), 48.07 (C-18), 48.31 (C-19), 150.95 (C-20), 29.99 (C-21), 40.00 (C-22), 28.05 (C-23), 16.51 (C-24), 16.22 (C-25), 16.03 (C-26), 14.50 (C-27), 18.06 (C-28), 19.33 (C-29), 109.41 (C-30), 21.32 ($CH_3COO$) and 170.81 ($CH_3COO$).

Compound **5** which was eluted with *n*-hexane: $CHCl_3$ (8 : 2, v/v) was finally purified by crystallization from MeOH (yield : 50 mg).

*β-Sitosterol* (**5**): Mp 134.5°C; $[\alpha]_D$ + 40° ($CHCl_3$; C,0.50); EIMS *m/z* (rel. intens. %) : 414 [$C_{29}H_{50}O$, $M^+$] (30), 399 [M-Me]$^+$ (10), 396 [M-$H_2O$]$^+$ (15), 381 [M-Me-$H_2O$]$^+$ (75), 329 [M-$H_2O$-$C_5H_7$]$^+$ (26), 303 [M-$H_2O$-$C_7H_9$]$^+$ (20), 275 [M-$H_2O$-$C_9H_{13}$]$^+$ (14), 273 [M-side chain at C-17]$^+$ (35) and 255 [M-side chain-$H_2O$]$^+$ (35); IR ($CHCl_3$) $\nu_{max}$ $cm^{-1}$: 3450 (OH), 3050, 1650 and 815 (trisubstituted double bond); $^1$H-NMR (300 MHz, $CDCl_3$) [11] : δ 5.23 (1H, m, H-6), 3.32 (1H, m, H-3), 1.01 (3H, s, Me-19), 0.92 (3H, d, J = 6.2 Hz, Me-21), 0.84 (3H, t, J = 7.0 Hz, Me-29), 0.83 (3H, d, J = 6.5 Hz, Me-26), 0.81 (3H, d, J = 6.5 Hz, Me-27) and 0.68 (3H, s, Me-18), 13C-NMR (75 MHz, $CDCl_3$) [11] : δ37.31 (C-1), 31.81 (C-2), 71.90 (C-3), 42.40 (C-4), 140.90 (C-5), 121.87 (C-6), 32.07 (C-7), 32.00 (C-8), 50.81 (C-9), 36.61 (C-10), 21.12 (C-11), 40.00 (C-12), 42.61 (C-13), 56.78 (C-14), 24.32 (C-15), 28.24 (C-16), 56.20 (C-17), 11.90 (C-18), 19.44 (C-19), 36.26 (C-20), 19.10 (C-21), 34.00 (C-22), 29.31 (C-23), 45.80 (C-24), 26.21 (C-25), 18.80 (C-26), 19.80 (C-27), 23.10 (C-28) and 11.92 (C-29).

The eluate obtained from $CHCl_3$ : MeOH (9 : 1, v/v) was further purified through flash chromatography using various ratios of $CHCl_3$ and MeOH. The aliquot obtained by pure $CHCl_3$ was finally purified by washing with $Me_2CO$ and crystallized from mixture of $CHCl_3$ and MeOH to get **6** (yield : 45 mg).

*β-Sitosterol Galactoside* (**6**) : Mp 275-277°C; $[\alpha]_D$ + 63° (Pyridine; C, 0.52); EIMS *m/z* (rel. intens. %) : 577 [$M^+$, (M+H), FAB-$MS^+$], Aglycone : 414 $[C_{29}H_2O]^+$ (20), 396 $[M\text{-}H_2O]^+$ (15), 381 $[M\text{-}Me\text{-}H_2O]^+$ (26), 371 $[M\text{-}C_3H_7]^+$ (35), $[M\text{-}C_3H_7\text{-}H_2O]^+$ (75), 329 $[M\text{-}H_2O\text{-}C_5H_7]^+$(55), 303 $[M\text{-}C_7H_9\text{-}H_2O]^+$ (20), 302 $[M\text{-}C_8H_{16}]^+$ (65), 275 $[M\text{-}H_2O\text{-}C_9H_{13}]^+$ (35); IR $\nu_{max}$ $cm^{-1}$ : 3440-3410 (OH), 3025, 1650 (C=C) and 1440 (gemdimethyls); $^1$H-NMR (300 MHz, $CDCl_3$) [11] : δ Aglycone : 5.23 (1H, m, H-6), 3.72 (1H, m, H-3), 1.01 (3H, s, Me-19), 0.92 (3H, d, J = 6.2 Hz, Me-21), 0.84 (3H, t, J = 7.0 Hz, Me-29), 0.83 (3H, d, J = 6.5 Hz, Me-26), 0.81 (3H, d, J = 6.5 Hz, Me-27) and 0.68 (3H, s, Me-18). Sugar : 4.20 (1H, d, J = 7.8 Hz, H-1), 3.65 (1H, dd, J = 1.0, 3.0 Hz, H-4) 3.55 (1H, dd, J = 8.0, 9.0 Hz, H-2), 3.52 (1H, dd, J = 7.0, 11.0 Hz, H-6), 3.30 (1H, dd, J = 5.0, 11.0 Hz, H-6), 3.15 (1H, dd, J = 3.0, 9.0 Hz, H-3) and 3.03 (1H, dd, J = 1.0, 5.0 Hz, H-5); $^{13}$C-NMR (75 MHz, $CDCl_3$) [11] : δ Aglycone : 37.31 (C-1), 31.81 (C-2), 78.85 (C-3), 42.40 (C-4), 140.90 (C-5), 121.87 (C-6), 32.07 (C-7), 32.00 (C-8), 50.81 (C-9), 36.61 (C-10), 21.12 (C-11), 40.00 (C-12), 42.61 (C-13), 56.78 (C-14), 24.32 (C-15), 28.24 (C-16), 56.20 (C-17), 11.90 (C-18), 19.44 (C-19), 36.26 (C-20), 19.10 (C-21), 34.00 (C-22), 29.31 (C-23), 45.80 (C-24), 26.21 (C-25), 18.80 (C-26), 19.80 (C-27), 23.10 (C-28) and 11.92 (C-29). Sugar : 100.51 (C-1), 71.90 (C-2), 74.00 (C-3), 69.90 (C-4), 76.53 (C-5) and 62.58 (C-6).

*Acid hydrolysis of 6*: Compound **6** (12 mg) was hydrolysed with 2N HCl in dilute MeOH (9mL methanol + 1 mL $H_2O$) at 100°for 3 hrs. The solvent of the reaction mixture was evaporated under reduced pressure. The residue was diluted with water and neutralized with $Ag_2CO_3$. The reaction mixture was then extracted with ethylacetate to furnish the aglycone moiety, while the aqueous layer was evaporated under reduced pressure. The residue obtained showed the presence of β-D-galactose when compared with the authentic sample by TLC using the solvent system EtOAc : MeOH : AcOH : $H_2O$ (6 : 2 : 1 : 1). The spots were detected by spraying with sugar reagent (Solution A: 1gm $FeCl_3$ in 100 ml of 10% $H_2SO_4$ ; Solution B: 6% orcine in EtOH. Solutions 1 & 2 were mixed in the ratio of 10:1, respectively).

## Results and Discussion

The four triterpenoids (**1-4**) α-amyrin [12-ursen-3β-ol] (**1**) [12-14], β-amyrin [12-oleanex-3β-ol](**2**) [12-13], lup-20(29)-en-3β-ol (**3**) [10, 12, 16-17], lup-20(29)-en-3β-yl acetate (**4**) [17-19] and two sterols (**5,6**) β-sitosterol [24(R)-stigmast-5-en-3β-ol] (**5**) [19-20] and β-sitosteryl galactoside [3-O-β-D-galactopyranosyl-stigmasta-5-enen] (**6**) [21] were isolated from the said source. Compounds **1-5** were isolated from the ethylacetate soluble part of the methanolic extract whereas compound **6** was isolated from the butanolic part of the methanolic extract of the seeds of *Caesalpinia bonduc.* These compounds were first time isolated from the source but these have been already reported in the literature, so, they were not discussed extensively in the Results and Discussion section. They were just identified by direct comparison with published data [12-20].

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