SHORT PAPER

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Triterpene glycosides from the bark of *Anthocephalus cadamba*†

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Two triterpenoid glycosides, glycosides A and B were isolated from the bark of *Anthocephalus cadamba* and defined as 3-*O*-[α-L-rhamnopyranosyl]-quinovic acid-28-*O*-[β-D-glucopyranosyl] ester and 3-*O*-[β-D-glucopyranosyl]-quinovic acid-28-*O*-[β-D-glucopyranosyl] ester respectively.

Anthocephalus cadamba (Roxb.) Miq. *Syn A. chinensis* (Lamk) A. Rich (Rubiaceae) is widely distributed throughout the greater part of India and is used as a folk medicine in the treatment of fever and anemia, as antidiuretic, and for improvement of semen quality[1]. Earlier phytochemical investigations[2-4] were limited to the isolation and characterization of few triterpenes and alkaloids only. In continuation of our work on isolation and characterization of naturally occurring saponins[5-10], our attention was drawn to the saponins of the bark of the plant since saponins of triterpene acids are drawing much attention in recent years for their cholesterol lowering[11] and other biological activities[12,13]. The present paper reports the isolation and characterization of two triterpenoid glycosides from the bark of the plant.

The *n*-butanol soluble fraction of the methanol extract of the bark of the plant on repeated chromatographic purification over Sephadex LH-20 and silica gel followed by HPLC separation furnished two homogeneous glycosides A (**1**) and B (**2**). Both the saponins gave positive Liebermann-Burchard test for triterpenes and Molisch test for sugar. Glycoside A (**1**) displayed peaks at 817 and 833 in its positive ion MALDI-TOF mass spectrum which has been assigned to $[M+Na]^+$ and $[M+K]^+$ ions suggesting the molecular weight of **1** to be 794 and the molecular formula $C_{42}H_{66}O_{14}$ in combination with ^{13}C NMR spectrum including DEPT experimental results. Acidic hydrolysis of **1** furnished an aglycone identified as quinovic acid (**3)** from its ^{13}C NMR spectral data and comparison with an authentic sample[14]. The monosaccharides were characterised as L-rhamnose and D-glucose in the ratio 1:1 by GLC analysis and comparison with authentic samples. Furthermore, the ^1H and ^{13}C NMR spectra of **1** displayed two sugar anomeric protons and carbon signals at δ 6.37 (J = 7.9 Hz), 5.18 (br s) and at δ 95.7 and 104.2 respectively. The upfield shift of one anomeric carbon signal (δ 95.7) and down field shift of the anomeric proton signal at δ 6.37 together with an absorption at 1730 cm^{-1} in its IR spectrum suggested the presence of an ester glycosidic linkage[15] in **1.** Treatment of **1** with aq MeOH-KOH yielded a prosapogenin which on acid hydrolysis furnished quinovic acid (**3**) as the aglycone and only L-rhamnose as the sugar component suggesting that L-rhamnose is linked to the C-3 hydroxy group of quinovic acid. The downfield shift of +10.3 ppm for C-3 carbon signal compared to the aglycone[14] and exhibition of two cross peaks between the anomeric proton (δ 5.18) of rhamnose and the hydroxy methine carbon (δ_c 88.2, C-3) of quinovic acid also supported a 3-*O* -glycosidic linkage in **1**. Therefore, it was concluded that glycoside A was a bisdesmoside, L-rhamnose being linked to the C-3 of quinovic acid and glucose was attached through a carboxyl group either at C-27 or C-28 of quinovic acid. The attachment of the glucose unit to C-28 carboxyl group of the genin was established using ^{13}C, HMQC and HMBC NMR experiments. The HMQC sequence indicated the connectivity between C-18 (δ 54.7) and H-18 (δ 2.68, d, J = 11.6 Hz). and in the HMBC experiment long range connectivity (^3J) was observed between H-18 and the carboxyl resonance of the genin C-28 (δ 176.5) which in turn showed a correlation peak with the anomeric proton of the glucose H-1 (δ 6.37) indicating esterification of the carboxylic group (C-28) rather than C-27 (δ 178.0) with the glucose moiety. Furthermore, the signal at δ 178.0 in the ^{13}C NMR of **1** was assigned to the carboxyl group while free one at C-28 usually resonated further down field[16]. The β anomeric configuration for the glucose was based on their large $^3J_{H1,H2}$ coupling constant (7.9 Hz) and small $J_{C1,H1}$ coupling constant (159-160 Hz) whereas the ^1H non-splitting pattern, the ^{13}C chemical shift and large $J_{C1,H1}$ coupling constant (167-169) of the rhamnose indicated α configuration[10]. The absolute configurations of the monosaccharides were chosen in keeping with those mostly encountered among plant glycosides. From the foregoing evidences glycoside A (**1**) has been concluded to be 3-*O*-[α-L-rhamnopyranosyl]-quinovic acid-28-*O*-[β-D-glucopyranosyl] ester.

Glycoside B (**2**) in its positive MALDI-TOF mass spectrum gave quasi-molecular ion peaks at 833 and 814 corresponding to $C_{42}H_{60}NaO_{15}$ and $C_{42}H_{60}KO_{15}$ respectively suggesting the M^r 810 and molecular formula of **2** to be $C_{42}H_{66}O_{15}$. The IR spectrum of **2** showed similar absorption pattern to that of glycoside A (**1**). Acidic hydrolysis of **2** furnished quinovic acid (**3**) as aglycone and only D-glucose as monosaccharide from the GLC analysis. The anomeric proton signals were doublets at δ 6.35 (d, J = 8.3 Hz) and 4.77 (d, J = 7.6 Hz) respectively indicating β-configuration ($^4C_1$ conformation) for both the glucopyranosyl units, and of which, one bonded as a glycoside (δ 4.77) and other as a glycosyl ester (δ 6.35). On comparing the ^{13}C NMR data of **2** with that of **1** and analysis of HMQC and HMBC spectra of **2** it was concluded that the structure of glycoside B (**2**) is 3-*O*-[β-D-glucopyranosyl]-quinovic acid-28-*O*-[β-D-glucopyranosyl] ester.

Experimental

IR spectra were determined using a JASCO 7300 FTIR spectrometer. Optical rotations were measured using a JASCO DIP-370 digital polarimeter. MALDI-TOF-MS were conducted using PerSeptive Biosystems with 2,5-dihydroxybenzoic acid as matrix. ^1H and ^{13}C NMR spectra were recorded using JEOL α-500 FT NMR (500 MHz for ^1H and 125.65 MHz for ^{13}C NMR) spectrometer in pyridine-d_5 with TMS as internal standard. Preparative HPLC was performed using an ODS column (Capcell pak ODS, Shiseido, 10 x 250 mm, detector UV 210 nm, flow rate 1 ml/min).

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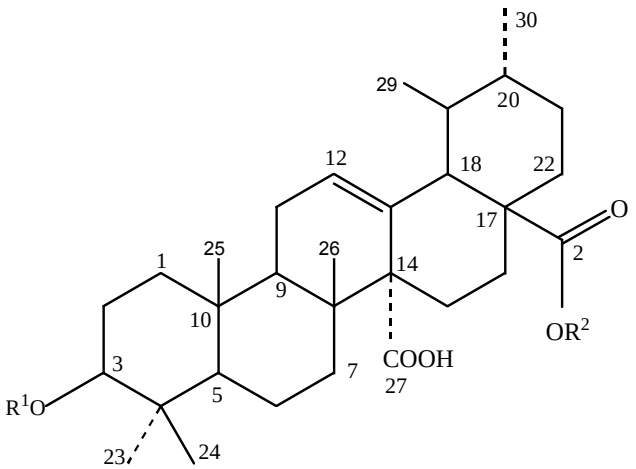
† This is a Short Paper, there is therefore no corresponding material in *J. Chem. Research (M)*.



	R^1	R^2
1.	rhamnose	glucose
2.	glucose	glucose
3.	H	H

Plant material. The bark of *Anthocephalus cadamba* was collected around the Institute campus during April, 1998 and identified at Indian Botanic Garden, Howrah.

Extraction and isolation. The *n*-BuOH extract (3.8 g) of the MeOH extract of the defatted air dried powdered (1 kg) bark of *A. cadamba* was chromatographed on a silica gel (SRL, India) (120 g) column with $CHCl_3$ followed by various ratios of $CHCl_3$ and MeOH mixtures as eluent. Fractions eluted with $CHCl_3$-MeOH (9:1) yielded previously reported cadambine, dihydrocadambine and isocadambine[3,4]. Fractions eluted with $CHCl_3$-MeOH (17:3) were combined according to their TLC behaviour and was applied to a column of Sephadex LH-20 (Pharmacia) (70 g) and eluted with H_2O, 30, 50, 70 and 100% MeOH to give 15 fractions. Earlier fractions eluted with H_2O-MeOH (50%) were mixed and repurified on silica gel using $CHCl_3$-MeOH-H_2O (40:9:1) as eluent. Fractions having similar R_f values on TLC ($CHCl_3$-MeOH-H_2O; 75:23:2) were combined (185 mg) and purified by HPLC on a ODS column using MeOH-H_2O (65:35) to afford glycoside A (**1**, 48 mg) and glycoside B (**2**, 55 mg).

Glycoside A (**1**): An amorphous powder, $[\alpha]_{23}^{D}$ + 16.57° (c 0.7, MeOH); IR: ν_{max} (KBr) cm^{-1} 3421, 2926, 2849, 1730, 1695, and 1075; MALDI-TOF-MS (positive) *m/z* , 817 $[M+Na]^+$, 833 $[M+K]^+$; ^{1}H NMR (500 MHz): δ 0.75 (3H, d, *J* = 6.0 Hz), 0.77 (6H, s), 0.92, 1.24 (each 3H, s), 1.17 (3H, d, *J* = 6Hz) (assigned for six methyl groups), 1.65 (3H, d, *J* = 6 Hz, methyl group of rhamnose), 2.70 (1H, d, *J* = 11.7 Hz, H-18), 3.03 (1H, dd, *J* = 4.6, 11.6 Hz, H-3), 5.18 (1H, brs, anomeric proton of rhamnose), 6.0 (1H, t-like, H-12) and 6.37 (1H, d, *J* = 8 Hz, anomeric proton of glucose); ^{13}C NMR: δ 178.0 s (C-27), 176.5 s (C-28), 133.3 s (C-13), 129.6 d (C-12), 88.2 d (C-3), 56.8 s (C-14), 55.6 d (C-5), 54.7 d (C-18), 49.0 s (C-17), 47.3 d (C-9), 40.2 s (C-10), 39.1t (C-1), 39.1s (C-20), 38.9 s (C-8), 37.6 d (C-19), 37.5 t (C-7), 37.0 t (C-22), 36.5 s (C-4), 30.3 t (C-21), 28.1 q (C-23), 26.1 t (C-2), 26.0 t (C-16), 25.5 t (C-15), 23.4 t (C-11), 21.2 q (C-30), 19.2 q (C-26), 18.5 t (C-6), 18.1 q (C-29), 16.8 q (C-24), 16.6 q (C-25); Carbohydrate moiety: C-3 rhamnose: 104.2 d (C-1), 72.5 d (C-2), 72.9 d (C-3), 74.1$^+$ d (C-4), 69.8 d (C-5), 18 .7 q (C-6); C-28-glucose: 95.7 d (C-1), 74.2$^+$ d (C-2), 79.0* d (C-3), 71.3 d (C-4), 79.3* d (C-5) and 62.4 (C-6), (*,$^+$ values may be interchanged). (Found: C, 63.31; H, 8.40; $C_{42}H_{66}O_{14}$ requires C, 63.45; H, 8.37 %).

Acid hydrolysis of glycoside A (**1**): **1** (12 mg) was heated with 1 ml 1N HCl (dioxane-water 1:1) at 80˚C under nitrogen atmosphere for 2 h. After removal of dioxane, the solution was extracted with EtOAc (2 ml x 3). The EtOAc layer was washed with water and then distilled off to give a colourless residue (6 mg), which was found to be identical with quinovic acid[14] (**3**) by ^{13}C NMR comparisons. The aqueous part was worked up as described[10] and the monosaccharides were identified as D-glucose and L-rhamnose in the ratio 1:1 from the GLC analysis.

Alkaline hydrolysis of glycoside A (**1**): 15 mg of **1** was hydrolysed with 2 ml of 5% methanolic KOH for 3 h. The prosapogenin thus obtained on further hydrolysis with 1N HCl-MeOH furnished quinovic acid as the aglycone and only rhamnose as the sugar component.

Glycoside B (**2**): Colourless powder, $[\alpha]_{23}^{D}$ + 24.85° (c, 4.0 MeOH); IR: ν_{max} (KBr) cm^{-1} 3423, 2926, 2849, 1730, 1680, 1066; MALDI-TOF-MS (positive) *m/z*, 833 $[M+Na]^+$, 849 $[M+K]^+$; ^{1}H NMR (500 MHz): δ 0.76 (d, *J* = 6.1 Hz 3H-30), 0.89 (s, 3H-23), 0.95 (s, 3H-26), 1.13 (s, 3H-25), 1.18 (d, *J* = 6.1 Hz, 3H-29), 1.21 (s, 3H-24), 2.65 (dd, *J* = 5.5, 11 Hz, H-9), 2.68 (d, *J* = 11.6 Hz, 18-H), 3.22 (*J* = 4, 12 Hz, H-3), 4.77 (d, *J* = 7.6 Hz, anomeric proton of glucose at C-3), 5.98 (t-like, H-12) and 6.35 (d, *J* = 8.3 Hz, anomeric proton of glucose at C-28); ^{13}C NMR: δ 178.0 s (C-27), 176.5 s (C-28), 133.2 s (C-13), 129.6 d (C-12), 88.7 d (C-3), 56.8 s (C-14), 55.8 d (C-5), 54.7 d (C-18), 49.0 s (C-17), 47.2 d (C-9), 40.2 s (C-10), 39.4 d (C-20), 39.1 t (C-1), 39.0 s (C-8), 37.5 d (C-19), 37.5^a t (C-7), 37.0^a t (C-22), 36.4 s (C-4), 30.3 t (C-21), 28.0 q (C-23), 26.8 t (C-2), 26.0 t (C-16), 25.5 t (C-15) , 23.4 t (C-11), 21.2 q(C-30), 19.2 q (C-26), 18.6 t (C-6), 18.1 q (C-29), 17.0 q (C-24), 16.6 q (C-25); Carbohydrate moiety: C-3 glucose: 106.0 d (C-1), 75.8 d (C-2), 78.9^b d (C-3), 71.3^c d (C-4), 78.2^b d (C-5), 63.1 t (C-6); C-28-glucose: 95.7 d (C-1), 74.2 d (C-2), 79.3^b d (C-3), 71.9^c d (C-4), 78.7^b (C-5) and 62.4 (C-6), (^{a,b,c,} values may be interchanged). (Found: C, 62.17; H, 8.24; $C_{42}H_{46}O_{15}$ requires C, 62.20; H, 8.20 %).

Acidic hydrolysis of glycoside B (**2**): Glycoside B (2, 10 mg) was treated with 1N HCl (dioxane-water, 1:1) at 80˚C under nitrogen atmosphere for 2 h. Similar work up as described for glycoside A (1) furnished quinovic acid as the aglycone and only glucose as the monosaccharide.

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